IARC Handbooks of Cancer Prevention

International Agency for Research on Cancer
World Health Organization

Volume 4

Retinoids

1999

WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC Handbooks of Cancer Prevention

Retinoids

Volume 4

This publication represents the views and expert opinions
of an IARC Working Group on the
Evaluation of Cancer-preventive Agents,
which met in Lyon,

24–30 March 1999

Published by the International Agency for Research on Cancer,
150 cours Albert Thomas, F-69372 Lyon cedex 08, France

Distributed by Oxford University Press, Walton Street, Oxford, OX2 6DP, UK (Fax: +44 1865 267782) and in the USA by Oxford University Press, 2001 Evans Road, Carey, NC 27513, USA (Fax: +1 919 677 1303).
All IARC publications can also be ordered directly from IARC*Press*
(Fax: +33 4 72 73 83 02; E-mail: press@iarc.fr).

IARC Library Cataloguing in Publication Data

Retinoids/
IARC Working Group on the Evaluation of
Cancer Preventive Agents (1999 : Lyon, France)

(IARC handbooks of cancer prevention ; 4)

1. Retinoids – congresses. I. IARC Working Group on the Evaluation of Cancer Preventive Agents II Series

ISBN 92 832 3004 3 (NLM Classification: W1)
ISSN 1027-5622

Printed in France

International Agency For Research On Cancer

The International Agency for Research on Cancer (IARC) was established in 1965 by the World Health Assembly, as an independently financed organization within the framework of the World Health Organization. The headquarters of the Agency are in Lyon, France.

The Agency conducts a programme of research concentrating particularly on the epidemiology of cancer and the study of potential carcinogens in the human environment. Its field studies are supplemented by biological and chemical research carried out in the Agency's laboratories in Lyon and, through collaborative research agreements, in national research institutions in many countries. The Agency also conducts a programme for the education and training of personnel for cancer research.

The publications of the Agency contribute to the dissemination of authoritative information on different aspects of cancer research. A complete list is printed at the back of this book. Information about IARC publications, and how to order them, is also available via the Internet at: **http://www.iarc.fr/**

Note to the Reader

Anyone who is aware of published data that may influence any consideration in these *Handbooks* is encouraged to make the information available to the Unit of Chemoprevention, International Agency for Research on Cancer, 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France

Although all efforts are made to prepare the *Handbooks* as accurately as possible, mistakes may occur. Readers are requested to communicate any errors to the Unit of Chemoprevention, so that corrections can be reported in future volumes.

Acknowledgements

The Foundation for Promotion of Cancer Research, Japan, is gratefully acknowledged for its generous support to the meeting of the Working Group and the production of this volume of the *IARC Handbooks of Cancer Prevention.*

Contents

List of participants

Volume 4. Retinoids

Lyon, 24–30 March 1999

Members[1]

J.S. Bertram
Cancer Research Center of Hawaii
University of Hawaii
1236 Lauhala Street
Honolulu, HI 96813
USA

W.S. Blaner
Hammer Health Sciences Building
Room 502, Department of Medicine
Columbia University
701 West 168th Street
New York, NY 10032
USA

R.A. Chandraratna
Vice-President, Retinoid Research
Allergan
2525 Dupont Drive
PO Box 19534
Irvine, CA 92623–9534
USA

C. Chomienne
Laboratoire de Biologie Cellulaire Hématopoïétique
Hôpital Saint Louis
1 avenue Claude Vellefaux
75010 Paris
France

J.A. Crowell
Chemoprevention Branch
Division of Cancer Prevention and Control
National Institutes of Health
National Cancer Institute
Bethesda, MD 20892
USA

M.I. Dawson
Molecular Medicine Research Institute
325 East Middlefield Road
Mountain View, CA 94043
USA

F. Formelli
Istituto Nazionale per lo Studio e la Cura dei Tumori
Via Venezian 1
20133 Milan
Italy

E.R. Greenberg *(Vice-Chairman)*
Norris Cotton Cancer Center
Dartmouth-Hitchcock Medical Center
One Medical Center Drive
Lebanon, NH 03756
USA

H. Gronemeyer
Institut de Génétique et de Biologie Moléculaire et Cellulaire
BP 163
67404 Illkirch Cedex
France

D.L. Hill
Chemoprevention Center
University of Alabama at Birmingham
1675 University Boulevard
Webb Building #310
Birmingham, AL 35294–3361
USA

R.J. Kavlock
Reproductive Toxicology Division
Environmental Protection Agency
Research Triangle Park, NC27711
USA

R. Lotan
Department of Thoracic/Head and Neck Medical Oncology
University of Texas
M.D. Anderson Cancer Center
1515 Holcombe Boulevard
Houston, TX 77030
USA

---

[1] **Unable to attend:** F.R. Khuri, Associate Professor of Medicine, University of Texas, M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Box 80, Houston, TX 77030, USA

M. Maden
Division of Biomedical Sciences
King's College
26–29 Drury Lane
London WC2B 5RL
United Kingdom

R.G. Mehta
University of Illinois at Chicago
Department of Surgical Oncology and Pharmacology
840 South Wood Street
Chicago, IL 60912–7322
USA

T.E. Moon
Clinical Research Department
Ligand Pharmaceuticals Inc.
10275 Science Center Drive
San Diego, CA 92121
USA

H. Nau
Department of Food Toxicology
Centre of Food Sciences
School of Veterinary Medicine Hannover
Bischofsholer Damm 15
30173 Hannover
Germany

P. Nettesheim
National Institute of Environmental Health Sciences
Research Triangle Park, NC 27709
USA

J.A. Olson
Iowa State University
Biochemistry, Biophysics and Molecular Biology Department
Ames, IA 50011
USA

M.P. Rosin
British Columbia Cancer Agency
Cancer Control Research Program
600 West 10th Avenue
Vancouver, British Columbia
Canada V5Z 1E6

B.W. Stewart *(Chairman)*
Cancer Control Program
South Eastern Sydney Area Health Service
PO Box 88
Randwick, NSW 2031
Australia

C.C. Willhite
State of California
Department of Toxic Substances Control
700 Heinz Street, Suite 200
Berkeley, CA 94710–2737
USA

R.A. Woutersen
Department of General Toxicology
TNO-Nutrition and Food Research Institute
Toxicology Division
PO Box 360
3700 AJ Zeist
The Netherlands

Secretariat
R. Baan
E. Heseltine *(Editor)*
C. Malaveille
A.B. Miller (*Responsible Officer*)
C. Partensky
R. Sankaranarayanan
A.L. Van Kappel-Dufour
J. Wilbourn

Technical assistance
A. Meneghel
D. Mietton
J. Mitchell
E. Perez
S. Reynaud
S. Ruiz
J. Thévenoux

Preamble to the *IARC Handbooks of Cancer Prevention*

The prevention of cancer is one of the key objectives of the International Agency for Research on Cancer (IARC). This may be achieved by avoiding exposures to known cancer-causing agents, by increasing host defences through immunization or chemoprevention or by modifying lifestyle. The aim of the *IARC Monographs* programme is to evaluate carcinogenic risks of human exposure to chemical, physical and biological agents, providing a scientific basis for national or international decisions on avoidance of exposures. The aim of the series of *IARC Handbooks of Cancer Prevention* is to evaluate scientific information on agents and interventions that may reduce the incidence of or mortality from cancer. This preamble is divided into two parts. The first addresses the general scope, objectives and structure of the *Handbooks*. The second describes the procedures for evaluating cancer-preventive agents.

Part One

Scope

Cancer-preventive strategies embrace chemical, immunological, dietary and behavioural interventions that may retard, block or reverse carcinogenic processes or reduce underlying risk factors. The term 'chemoprevention' is used to refer to interventions with pharmaceuticals, vitamins, minerals and other chemicals to reduce cancer incidence. The *IARC Handbooks* address the efficacy, safety and mechanisms of cancer-preventive strategies and the adequacy of the available data, including those on timing, dose, duration and indications for use.

Preventive strategies can be applied across a continuum of: (1) the general population; (2) subgroups with particular predisposing host or environmental risk factors, including genetic susceptibility to cancer; (3) persons with precancerous lesions; and (4) cancer patients at risk for second primary tumours. Use of the same strategies or agents in the treatment of cancer patients to control the growth, metastasis and recurrence of tumours is considered to be patient management, not prevention, although data from clinical trials may be relevant when making a *Handbooks* evaluation.

Objective

The objective of the *Handbooks* programme is the preparation of critical reviews and evaluations of evidence for cancer-prevention and other relevant properties of a wide range of potential cancer-preventive agents and strategies by international working groups of experts. The resulting *Handbooks* may also indicate when additional research is needed.

The *Handbooks* may assist national and international authorities in devising programmes of health promotion and cancer prevention and in making benefit–risk assessments. The evaluations of IARC working groups are scientific judgements about the available evidence for cancer-preventive efficacy and safety. No recommendation is given with regard to national and international regulation or legislation, which are the responsibility of individual governments and/or other international authorities. No recommendations for specific research trials are made.

IARC Working Groups

Reviews and evaluations are formulated by international working groups of experts convened by the IARC. The tasks of each group are: (1) to ascertain that all appropriate data have been collected; (2) to select the data relevant for the evaluation on the basis of scientific merit; (3) to prepare accurate summaries of the data to enable the reader to follow the reasoning of the Working Group; (4) to evaluate the significance of the available data from human studies and experimental models on cancer-preventive activity, carcinogenicity and other beneficial and adverse effects; and (5) to evaluate data relevant to the understanding of mechanisms of action.

Working Group participants who contributed to the considerations and evaluations within a particular *Handbook* are listed, with their addresses, at the beginning of each publication. Each participant serves as an individual scientist and not as a representative of any organization, government or industry. In addition, scientists nominated by national and international agencies, industrial associations and consumer and/or environmental organizations may be invited as observers. IARC staff involved in the preparation of the *Handbooks* are listed.

Working procedures

Approximately 13 months before a working group meets, the topics of the *Handbook* are announced, and participants are selected by IARC staff in consultation with other experts. Subsequently, relevant clinical, experimental and human data are collected by the IARC from all available sources of published information. Representatives of producer or consumer associations may assist in the preparation of sections on production and use, as appropriate.

About eight months before the meeting, the material collected is sent to meeting participants to prepare sections for the first drafts of the *Handbooks*. These are then compiled by IARC staff and sent, before the meeting, to all participants of the Working Group for review. There is an opportunity to return the compiled specialized sections of the draft to the experts, inviting preliminary comments, before the complete first-draft document is distributed to all members of the Working Group.

Data for *Handbooks*

The *Handbooks* do not necessarily cite all of the literature on the agent or strategy being evaluated. Only those data considered by the Working Group to be relevant to making the evaluation are included. In principle, meeting abstracts and other reports that do not provide sufficient detail upon which to base an assessment of their quality are not considered.

With regard to data from toxicological, epidemiological and experimental studies and from clinical trials, only reports that have been published or accepted for publication in the openly available scientific literature are reviewed by the Working Group. In certain instances, government agency reports that have undergone peer review and are widely available are considered. Exceptions may be made on an ad-hoc basis to include unpublished reports that are in their final form and publicly available, if their inclusion is considered pertinent to making a final evaluation. In the sections on chemical and physical properties, on production, on use, on analysis and on human exposure, unpublished sources of information may be used.

The available studies are summarized by the Working Group, with particular regard to the qualitative aspects discussed below. In general, numerical findings are indicated as they appear in the original report; units are converted when necessary for easier comparison. The Working Goup may conduct additional analyses of the published data and use them in their assesment of the evidence; the results of such supplementary analyses are given in square brackets. When an important aspect of a study, directly impinging on its interpretation, should be brought to the attention of the reader, a comment is given in square brackets.

Criteria for selection of topics for evaluation

Agents, classes of agents and interventions to be evaluated in the *Handbooks* are selected on the basis of one or more of the following criteria.

- The available evidence suggests potential for significantly reducing the incidence of cancers.
- There is a substantial body of human, experimental, clinical and/or mechanistic data suitable for evaluation.
- The agent is in widespread use and of putative protective value, but of uncertain efficacy and safety.
- The agent shows exceptional promise in experimental studies but has not been used in humans.
- The agent is available for further studies of human use.

Outline of data presentation scheme for evaluating cancer-preventive agents

1. Chemical and physical characteristics

2. Occurrence, production, use, analysis and human exposure

- 2.1 Occurrence
- 2.2 Production
- 2.3 Use
- 2.4 Human exposure
- 2.5 Analysis

3. Metabolism, kinetics and genetic variation

- 3.1 Humans
- 3.2 Experimental models

4. Cancer-preventive effects

- 4.1 Humans
  - 4.1.1 Epidemiological studies
  - 4.1.2 Intervention trials
  - 4.1.3 Intermediate end-points
- 4.2 Experimental models
  - 4.2.1 Cancer and preneoplastic lesions
  - 4.2.2 Intermediate biomarkers
  - 4.2.3 *In-vitro* models
- 4.3 Mechanisms of cancer-prevention

5. Other beneficial effects

6. Carcinogenicity

- 6.1 Humans
- 6.2 Experimental models

7. Other toxic effects

- 7.1 Adverse effects
  - 7.1.1 Humans
  - 7.1.2 Experimental models
- 7.2 Reproductive and developmental effects
  - 7.2.1 Humans
  - 7.2.2 Experimental models
- 7.3 Genetic and related effects
  - 7.3.1 Humans
  - 7.3.2 Experimental models

8. Summary of data

- 8.1 Chemistry, occurrence and human exposure
- 8.2 Metabolism and kinetics
- 8.3 Cancer-preventive effects
  - 8.3.1 Humans
  - 8.3.2 Experimental models
  - 8.3.3 Mechanisms of cancer prevention
- 8.4 Other beneficial effects
- 8.5 Carcinogenicity
  - 8.5.1 Humans
  - 8.5.2 Experimental models
- 8.6 Other toxic effects
  - 8.6.1 Humans
  - 8.6.2 Experimental models

9. Recommendations for research

10. Evaluation

- 10.1 Cancer-preventive activity
  - 10.1.1 Humans
  - 10.1.2 Experimental animals
- 10.2 Overall evaluation

11. References

Part Two

Evaluation of cancer-preventive agents

A wide range of findings must be taken into account before a particular agent can be recognized as preventing cancer. On the basis of experience from the *IARC Monographs* programme, a systematized approach to data presentation is adopted for *Handbooks* evaluations.

1. Chemical and physical characteristics of the agent

The Chemical Abstracts Services Registry Number, the latest Chemical Abstracts Primary Name, the IUPAC Systematic Name and other definitive information (such as genus and species of plants) are given as appropriate. Information on chemical and physical properties and, in particular, data

relevant to identification, occurrence and biological activity are included. A description of technical products of chemicals includes trade names, relevant specifications and available information on composition and impurities. Some of the trade names given may be those of mixtures in which the agent being evaluated is only one of the ingredients.

2. Occurrence, production, use, analysis and human exposure

2.1 Occurrence

Information on the occurrence of an agent or mixture in the environment is obtained from data derived from the monitoring and surveillance of levels in occupational environments, air, water, soil, foods and animal and human tissues. When available, data on the generation, persistence and bioaccumulation of the agent are included. For mixtures, information is given about all agents present.

2.2 Production

The dates of first synthesis and of first commercial production of a chemical or mixture are provided; for agents that do not occur naturally, this information may allow a reasonable estimate to be made of the date before which no human use of, or exposure to, the agent could have occurred. The dates of first reported occurrence of an exposure are also provided. In addition, methods of synthesis used in past and present commercial production and methods of production that may give rise to different impurities are described.

2.3 Use

Data on production, international trade and uses and applications are obtained for representative regions. Some identified uses may not be current or major applications, and the coverage is not necessarily comprehensive. In the case of drugs, mention of their therapeutic applications does not necessarily represent current practice, nor does it imply judgement as to their therapeutic efficacy.

2.4 Analysis

An overview of current methods of analysis or detection is presented. Methods for monitoring human exposure are also given, when available.

2.5 Human exposure

Human uses of, or exposure to, the agent are described. If an agent is used as a prescribed or over-the-counter pharmaceutical product, then the type of person receiving the product in terms of health status, age, sex and medical condition being treated are described. For nonpharmaceutical agents, particularly those taken because of cultural traditions, the characteristics of use or exposure and the relevant populations are described. In all cases, quantitative data, such as dose–response relationships, are considered to be of special importance.

3. Metabolism, kinetics and genetic variation

In evaluating the potential utility of a suspected cancer-preventive agent or strategy, a number of different properties, in addition to direct effects upon cancer incidence, are described and weighed. Furthermore, as many of the data leading to an evaluation are expected to come from studies in experimental animals, information that facilitates interspecies extrapolation is particularly important; this includes metabolic, kinetic and genetic data. Whenever possible, quantitative data, including information on dose, duration and potency, are considered.

Information is given on absorption, distribution (including placental transfer), metabolism and excretion in humans and experimental animals. Kinetic properties within the target species may affect the interpretation and extrapolation of dose–response relationships, such as blood concentrations, protein binding, tissue concentrations, plasma half-lives and elimination rates. Comparative information on the relationship between use or exposure and the dose that reaches the target site may be of particular importance for

extrapolation between species. Studies that indicate the metabolic pathways and fate of the agent in humans and experimental animals are summarized, and data on humans and experimental animals are compared when possible. Observations are made on inter-individual variations and relevant metabolic polymorphisms. Data indicating long-term accumulation in human tissues are included. Physiologically based pharmacokinetic models and their parameter values are relevant and are included whenever they are available. Information on the fate of the compound within tissues and cells (transport, role of cellular receptors, compartmentalization, binding to macromolecules) is given.

Genotyping will be used increasingly, not only to identify subpopulations at increased or decreased risk for cancers but also to characterize variation in the biotransformation of, and responses to, cancer-preventive agents.

This subsection can include effects of the compound on gene expression, enzyme induction or inhibition, or pro-oxidant status, when such data are not described elsewhere. It covers data obtained in humans and experimental animals, with particular attention to effects of long-term use and exposure.

4. Cancer-preventive effects

4.1 Human studies

Types of study considered. Human data are derived from experimental and non-experimental study designs and are focused on cancer, precancer or intermediate biological end-points. The experimental designs include randomized controlled trials and short-term experimental studies; non-experimental designs include cohort, case–control and cross-sectional studies.

Cohort and case–control studies relate individual use of, or exposure to, the agents under study to the occurrence of cancer in individuals and provide an estimate of relative risk (ratio of incidence or mortality in those exposed to incidence or mortality in those not exposed) as the main measure of association. Cohort and case–control studies follow an observational approach, in which the use of, or exposure to, the agent is not controlled by the investigator.

Intervention studies are experimental in design — that is, the use of, or exposure to, the agent is assigned by the investigator. The intervention study or clinical trial is the design that can provide the strongest and most direct evidence of a protective or preventive effect; however, for practical and ethical reasons, such studies are limited to observation of the effects among specifically defined study subjects of interventions of 10 years or fewer, which is relatively short when compared with the overall lifespan.

Intervention studies may be undertaken in individuals or communities and may or may not involve randomization to use or exposure. The differences between these designs is important in relation to analytical methods and interpretation of findings.

In addition, information can be obtained from reports of correlation (ecological) studies and case series; however, limitations inherent in these approaches usually mean that such studies carry limited weight in the evaluation of a preventive effect.

Quality of studies considered. The *Handbooks* are not intended to summarize all published studies. It is important that the Working Group consider the following aspects: (1) the relevance of the study; (2) the appropriateness of the design and analysis to the question being asked; (3) the adequacy and completeness of the presentation of the data; and (4) the degree to which chance, bias and confounding may have affected the results.

Studies that are judged to be inadequate or irrelevant to the evaluation are generally omitted. They may be mentioned briefly, particularly when the information is considered to be a useful supplement to that in other reports or when it provides the only data available. Their inclusion does not imply acceptance of the adequacy of the study design, nor of the analysis and interpretation of the results, and their limitations are outlined.

the adequacy of the data for each treatment group: (1) the initial and final effective numbers of animals studied and the survival rate; (2) body weights; and (3) tumour incidence and multiplicity. The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose. In particular, the statistical methods should be appropriate for the characteristics of the expected data distribution and should account for interactions in multifactorial studies. Consideration is given as to whether the appropriate adjustment was made for differences in survival.

4.2.2 Intermediate biomarkers

Other types of study include experiments in which the end-point is not cancer but a defined preneoplastic lesion or tumour-related, intermediate biomarker.

The observation of effects on the occurrence of lesions presumed to be preneoplastic or the emergence of benign or malignant tumours may aid in assessing the mode of action of the presumed cancer-preventive agent. Particular attention is given to assessing the reversibility of these lesions and their predictive value in relation to cancer development.

4.2.3 In-vitro models

Cell systems *in vitro* contribute to the early identification of potential cancer-preventive agents and to elucidation of mechanisms of cancer prevention. A number of assays in prokaryotic and eukaryotic systems are used for this purpose. Evaluation of the results of such assays includes consideration of: (1) the nature of the cell type used; (2) whether primary cell cultures or cell lines (tumorigenic or nontumorigenic) were studied; (3) the appropriateness of controls; (4) whether toxic effects were considered in the outcome; (5) whether the data were appropriately summated and analysed; (6) whether appropriate quality controls were used; (7) whether appropriate concentration ranges were used; (8) whether adequate numbers of independent measurements were made per group; and (9) the relevance of the end-points, including inhibition of mutagenesis, morphological transformation, anchorage-independent growth, cell–cell communication, calcium tolerance and differentiation.

4.3 Mechanisms of cancer prevention

Data on mechanisms can be derived from both human studies and experimental models. For a rational implementation of cancer-preventive measures, it is essential not only to assess protective end-points but also to understand the mechanisms by which the agents exert their anticarcinogenic action. Information on the mechanisms of cancer-preventive activity can be inferred from relationships between chemical structure and biological activity, from analysis of interactions between agents and specific molecular targets, from studies of specific end-points *in vitro*, from studies of the inhibition of tumorigenesis *in vivo*, from the effects of modulating intermediate biomarkers, and from human studies. Therefore, the Working Group takes account of data on mechanisms in making the final evaluation of cancer prevention.

Several classifications of mechanisms have been proposed, as have several systems for evaluating them. Cancer-preventive agents may act at several distinct levels. Their action may be: (1) extracellular, for example, inhibiting the uptake or endogenous formation of carcinogens, or forming complexes with, diluting and/or deactivating carcinogens; (2) intracellular, for example, trapping carcinogens in non-target cells, modifying transmembrane transport, modulating metabolism, blocking reactive molecules, inhibiting cell replication or modulating gene expression or DNA metabolism; or (3) at the level of the cell, tissue or organism, for example, affecting cell differentiation, intercellular communication, proteases, signal transduction, growth factors, cell adhesion molecules, angiogenesis, interactions with the extracellular matrix, hormonal status and the immune system.

Many cancer-preventive agents are known or suspected to act by several mechanisms, which

cancer-preventive activity; and (4) the number and structural diversity of carcinogens whose activity can be reduced by the agent being evaluated.

An important variable in the evaluation of the cancer-preventive response is the time and the duration of administration of the agent in relation to any carcinogenic treatment, or in transgenic or other experimental models in which no carcinogen is administered. Furthermore, concurrent administration of a cancer-preventive agent may result in a decreased incidence of tumours in a given organ and an increase in another organ of the same animal. Thus, in these experiments it is important that multiple organs be examined.

For all these studies, the nature and extent of impurities or contaminants present in the cancer-preventive agent or agents being evaluated are given when available. For experimental studies of mixtures, consideration is given to the possibility of changes in the physicochemical properties of the test substance during collection, storage, extraction, concentration and delivery. Chemical and toxicological interactions of the components of mixtures may result in nonlinear dose–response relationships.

As certain components of commonly used diets of experimental animals are themselves known to have cancer-preventive activity, particular consideration should be given to the interaction between the diet and the apparent effect of the agent being studied. Likewise, restriction of diet may be important. The appropriateness of the diet given relative to the composition of human diets may be commented on by the Working Group.

Qualitative aspects. An assessment of the experimental prevention of cancer involves several considerations of qualitative importance, including: (1) the experimental conditions under which the test was performed (route and schedule of exposure, species, strain, sex and age of animals studied, duration of the exposure, and duration of the study); (2) the consistency of the results, for example across species and target organ(s); (3) the stage or stages of the neoplastic process, from preneoplastic lesions and benign tumours to malignant neoplasms, studied and (4) the possible role of modifying factors.

Considerations of importance to the Working Group in the interpretation and evaluation of a particular study include: (1) how clearly the agent was defined and, in the case of mixtures, how adequately the sample composition was reported; (2) the composition of the diet and the stability of the agent in the diet; (3) whether the source, strain and quality of the animals was reported; (4) whether the dose and schedule of treatment with the known carcinogen were appropriate in assays of combined treatment; (5) whether the doses of the cancer-preventive agent were adequately monitored; (6) whether the agent(s) was absorbed, as shown by blood concentrations; (7) whether the survival of treated animals was similar to that of controls; (8) whether the body and organ weights of treated animals were similar to those of controls; (9) whether there were adequate numbers of animals, of appropriate age, per group; (10) whether animals of each sex were used, if appropriate; (11) whether animals were allocated randomly to groups; (12) whether appropriate respective controls were used; (13) whether the duration of the experiment was adequate; (14) whether there was adequate statistical analysis; and (15) whether the data were adequately reported. If available, recent data on the incidence of specific tumours in historical controls, as well as in concurrent controls, are taken into account in the evaluation of tumour response.

Quantitative aspects. The probability that tumours will occur may depend on the species, sex, strain and age of the animals, the dose of carcinogen (if any), the dose of the agent and the route and duration of exposure. A decreased incidence and/or decreased multiplicity of neoplasms in adequately designed studies provides evidence of a cancer-preventive effect. A dose-related decrease in incidence and/or multiplicity further strengthens this association.

Statistical analysis. Major factors considered in the statistical analysis by the Working Group include

the adequacy of the data for each treatment group: (1) the initial and final effective numbers of animals studied and the survival rate; (2) body weights; and (3) tumour incidence and multiplicity. The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose. In particular, the statistical methods should be appropriate for the characteristics of the expected data distribution and should account for interactions in multifactorial studies. Consideration is given as to whether the appropriate adjustment was made for differences in survival.

4.2.2 *Intermediate biomarkers*

Other types of study include experiments in which the end-point is not cancer but a defined preneoplastic lesion or tumour-related, intermediate biomarker.

The observation of effects on the occurrence of lesions presumed to be preneoplastic or the emergence of benign or malignant tumours may aid in assessing the mode of action of the presumed cancer-preventive agent. Particular attention is given to assessing the reversibility of these lesions and their predictive value in relation to cancer development.

4.2.3 *In-vitro models*

Cell systems *in vitro* contribute to the early identification of potential cancer-preventive agents and to elucidation of mechanisms of cancer prevention. A number of assays in prokaryotic and eukaryotic systems are used for this purpose. Evaluation of the results of such assays includes consideration of: (1) the nature of the cell type used; (2) whether primary cell cultures or cell lines (tumorigenic or nontumorigenic) were studied; (3) the appropriateness of controls; (4) whether toxic effects were considered in the outcome; (5) whether the data were appropriately summated and analysed; (6) whether appropriate quality controls were used; (7) whether appropriate concentration ranges were used; (8) whether adequate numbers of independent measurements were made per group; and (9) the relevance of the end-points, including inhibition of mutagenesis, morphological transformation, anchorage-independent growth, cell–cell communication, calcium tolerance and differentiation.

4.3 Mechanisms of cancer prevention

Data on mechanisms can be derived from both human studies and experimental models. For a rational implementation of cancer-preventive measures, it is essential not only to assess protective end-points but also to understand the mechanisms by which the agents exert their anticarcinogenic action. Information on the mechanisms of cancer-preventive activity can be inferred from relationships between chemical structure and biological activity, from analysis of interactions between agents and specific molecular targets, from studies of specific end-points *in vitro*, from studies of the inhibition of tumorigenesis *in vivo*, from the effects of modulating intermediate biomarkers, and from human studies. Therefore, the Working Group takes account of data on mechanisms in making the final evaluation of cancer prevention.

Several classifications of mechanisms have been proposed, as have several systems for evaluating them. Cancer-preventive agents may act at several distinct levels. Their action may be: (1) extracellular, for example, inhibiting the uptake or endogenous formation of carcinogens, or forming complexes with, diluting and/or deactivating carcinogens; (2) intracellular, for example, trapping carcinogens in non-target cells, modifying transmembrane transport, modulating metabolism, blocking reactive molecules, inhibiting cell replication or modulating gene expression or DNA metabolism; or (3) at the level of the cell, tissue or organism, for example, affecting cell differentiation, intercellular communication, proteases, signal transduction, growth factors, cell adhesion molecules, angiogenesis, interactions with the extracellular matrix, hormonal status and the immune system.

Many cancer-preventive agents are known or suspected to act by several mechanisms, which

may operate in a coordinated manner and allow them a broader spectrum of anticarcinogenic activity. Therefore, multiple mechanisms of action are taken into account in the evaluation of cancer-prevention.

Beneficial interactions, generally resulting from exposure to inhibitors that work through complementary mechanisms, are exploited in combined cancer-prevention. Because organisms are naturally exposed not only to mixtures of carcinogenic agents but also to mixtures of protective agents, it is also important to understand the mechanisms of interactions between inhibitors.

5. Other beneficial effects

This section contains mainly background information on preventive activity; use is described in Section 2.3. An expanded description is given, when appropriate, of the efficacy of the agent in the maintenance of a normal healthy state and the treatment of particular diseases. Information on the mechanisms involved in these activities is described. Reviews, rather than individual studies, may be cited as references.

The physiological functions of agents such as vitamins and micronutrients can be described briefly, with reference to reviews. Data on the therapeutic effects of drugs approved for clinical use are summarized.

6. Carcinogenicity

Some agents may have both carcinogenic and anticarcinogenic activities. If the agent has been evaluated within the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, that evaluation is accepted, unless significant new data have appeared that may lead the Working Group to reconsider the evidence. When a re-evaluation is necessary or when no carcinogenic evaluation has been made, the procedures described in the Preamble to the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* are adopted as guidelines.

7. Other toxic effects

Toxic effects are of particular importance in the case of agents that may be used widely over long periods in healthy populations. Data are given on acute and chronic toxic effects, such as organ toxicity, increased cell proliferation, immunotoxicity and adverse endocrine effects. If the agent occurs naturally or has been in clinical use previously, the doses and durations used in cancer-prevention trials are compared with intakes from the diet, in the case of vitamins, and previous clinical exposure, in the case of drugs already approved for human use. When extensive data are available, only summaries are presented; if adequate reviews are available, reference may be made to these. If there are no relevant reviews, the evaluation is made on the basis of the same criteria as are applied to epidemiological studies of cancer. Differences in response as a consequence of species, sex, age and genetic variability are presented when the information is available.

Data demonstrating the presence or absence of adverse effects in humans are included; equally, lack of data on specific adverse effects is stated clearly.

Findings in human and experimental studies are presented sequentially under the headings 'Adverse effects', 'Reproductive and developmental effects' and 'Genetic and related effects'.

The section 'Adverse effects' includes information on immunotoxicity, neurotoxicity, cardiotoxicity, haematological effects and toxicity to other target organs. Specific case reports in humans and any previous clinical data are noted. Other biochemical effects thought to be relevant to adverse effects are mentioned.

The section on 'Reproductive and developmental effects' includes effects on fertility, teratogenicity, foetotoxicity and embryotoxicity. Information from nonmammalian systems and *in vitro* are presented only if they have clear mechanistic significance.

The section 'Genetic and related effects' includes results from studies in mammalian and nonmammalian systems *in vivo* and *in vitro*. Information on

whether DNA damage occurs via direct interaction with the agent or via indirect mechanisms (e.g. generation of free radicals) is included, as is information on other genetic effects such as mutation, recombination, chromosomal damage, aneuploidy, cell immortalization and transformation, and effects on cell–cell communication. The presence and toxicological significance of cellular receptors for the cancer-preventive agent are described.

The adequacy of epidemiological studies of toxic effects, including reproductive outcomes and genetic and related effects in humans, is evaluated by the same criteria as are applied to epidemiological studies of cancer. For each of these studies, the adequacy of the reporting of sample characterization is considered and, where necessary, commented upon. The available data are interpreted critically according to the end-points used. The doses and concentrations used are given, and, for experiments *in vitro*, mention is made of whether the presence of an exogenous metabolic system affected the observations. For studies *in vivo*, the route of administration and the formulation in which the agent was administered are included. The dosing regimens, including the duration of treatment, are also given. Genetic data are given as listings of test systems, data and references; bar graphs (activity profiles) and corresponding summary tables with detailed information on the preparation of genetic activity profiles are given in appendices. Genetic and other activity in humans and experimental mammals is regarded as being of greater relevance than that in other organisms. The *in-vitro* experiments providing these data must be carefully evaluated, since there are many trivial reasons why a response to one agent may be modified by the addition of another.

Structure–activity relationships that may be relevant to the evaluation of the toxicity of an agent are described.

Studies on the interaction of the suspected cancer-preventive agent with toxic and subtoxic doses of other substances are described, the objective being to determine whether there is inhibition or enhancement, additivity, synergism or potentiation of toxic effects over an extended dose range.

Biochemical investigations that may have a bearing on the mechanisms of toxicity and cancer-prevention are described. These are carefully evaluated for their relevance and the appropriateness of the results.

8. Summary of data

In this section, the relevant human and experimental data are summarized. Inadequate studies are generally not summarized; such studies, if cited, are identified in the preceding text.

8.1 Chemistry, occurrence and human exposure

Human exposure to an agent is summarized on the basis of elements that may include production, use, occurrence in the environment and determinations in human tissues and body fluids. Quantitative data are summarized when available.

8.2 Metabolism and kinetic properties

Data on metabolism and kinetic properties in humans and in experimental animals are given when these are considered relevant to the possible mechanisms of cancer-preventive, carcinogenic and toxic activity.

8.3 Cancer-preventive effects

8.3.1 Human studies

The results of relevant studies are summarized, including case reports and correlation studies when considered important.

8.3.2 Experimental models

Data relevant to an evaluation of cancer-preventive activity in experimental models are summarized. For each animal species and route of administration, it is stated whether a change in the incidence of neoplasms or preneoplastic lesions was observed, and the tumour sites are indicated. Negative findings are also summarized. Dose– response relationships and other quantitative data may be given when available.

8.3.3 Mechanism of cancer-prevention

Data relevant to the mechanisms of cancer-preventive activity are summarized.

8.4 Other beneficial effects

When beneficial effects other than cancer prevention have been identified, the relevant data are summarized.

8.5 Carcinogenic effects

The agent will has reviewed and evaluated within the *IARC Monographs* programme, that summary is used with the inclusion of more recent data, if appropriate.

8.5.1 Human studies

The results of epidemiological studies that are considered to be pertinent to an assessment of human carcinogenicity are summarized.

8.5.2 Experimental models

Data relevant to an evaluation of carcinogenic effects in animal models are summarized. For each animal species and route of administration, it is stated whether a change in the incidence of neoplasms or preneoplastic lesions was observed, and the tumour sites are indicated. Negative findings are also summarized. Dose–response relationships and other quantitative data may be mentioned when available.

8.6 Other toxic effects

Adverse effects in humans are summarized, together with data on general toxicological effects and cytotoxicity, receptor binding and hormonal and immunological effects. The results of investigations on the reproductive, genetic and related effects are summarized. Toxic effects are summarized for whole animals, cultured mammalian cells and non-mammalian systems. When available, data for humans and for animals are compared.

Structure–activity relationships are mentioned when relevant to toxicity.

9. Recommendations for research

During the evaluation process, it is likely that opportunities for further research will be identified. These are clearly stated, with the understanding that the areas are recommended for future investigation. It is made clear that these research opportunities are identified in general terms on the basis of the data currently available.

10. Evaluation

Evaluations of the strength of the evidence for cancer-preventive activity and carcinogenic effects from studies in humans and experimental models are made, using standard terms. These terms may also be applied to other beneficial and adverse effects, when indicated. When appropriate, reference is made to specific organs and populations.

It is recognized that the criteria for these evaluation categories, described below, cannot encompass all factors that may be relevant to an evaluation of cancer-preventive activity. In considering all the relevant scientific data, the Working Group may assign the agent or other intervention to a higher or lower category than a strict interpretation of these criteria would indicate.

10.1 Cancer-preventive activity

The evaluation categories refer to the strength of the evidence that an agent prevents cancer. The evaluations may change as new information becomes available.

Evaluations are inevitably limited to the cancer sites, conditions and levels of exposure and length of observation covered by the available studies. An evaluation of degree of evidence, whether for a single agent or a mixture, is limited to the materials tested, as defined physically, chemically or biologically. When the agents evaluated are considered by the Working Group to be sufficiently closely related, they may be grouped for the purpose of a single evaluation of degree of evidence.

Information on mechanisms of action is taken into account when evaluating the strength of

evidence in humans and in experimental animals, as well as in assessing the consistency of results between studies in humans and experimental models.

10.1.1 Cancer-preventive activity in humans

The evidence relevant to cancer prevention in humans is classified into one of the following categories.

- *Sufficient evidence of cancer-preventive activity*
  The Working Group considers that a causal relationship has been established between use of the agent and the prevention of human cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence.
- *Limited evidence of cancer-preventive activity*
  The data suggest a reduced risk for cancer with use of the agent but are limited for making a definitive evaluation either because chance, bias or confounding could not be ruled out with reasonable confidence or because the data are restricted to intermediary biomarkers of uncertain validity in the putative pathway to cancer.
- *Inadequate evidence of cancer-preventive activity*
  The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding a cancer-preventive effect of the agent, or no data on the prevention of cancer in humans are available.
- *Evidence suggesting lack of cancer-preventive activity*
  Several adequate studies of use or exposure are mutually consistent in not showing a preventive effect.

The strength of the evidence for any carcinogenic effect is assessed in parallel.

Both cancer-preventive activity and carcinogenic effects are identified and, when appropriate, tabulated by organ site. The evaluation also cites the population subgroups concerned, specifying age, sex, genetic or environmental predisposing risk factors and the relevance of precancerous lesions.

10.1.2 Cancer-preventive activity in experimental animals

Evidence for cancer prevention in experimental animals is classified into one of the following categories.

- *Sufficient evidence of cancer-preventive activity*
  The Working Group considers that a causal relationship has been established between the agent and a decreased incidence and/or multiplicity of neoplasms.
- *Limited evidence of cancer-preventive activity*
  The data suggest a cancer-preventive effect but are limited for making a definitive evaluation because, for example, the evidence of cancer prevention is restricted to a single experiment, the agent decreases the incidence and/or multiplicity only of benign neoplasms or lesions of uncertain neoplastic potential or there is conflicting evidence.
- *Inadequate evidence of cancer-preventive activity*
  The studies cannot be interpreted as showing either the presence or absence of a preventive effect because of major qualitative or quantitative limitations (unresolved questions regarding the adequacy of the design, conduct or interpretation of the study), or no data on cancer prevention in experimental animals are available.
- *Evidence suggesting lack of cancer-preventive activity*
  Adequate evidence from conclusive studies in several models shows that, within the limits of the tests used, the agent does not prevent cancer.

10.2 Overall evaluation

Finally, the body of evidence is considered as a whole, and summary statements are made that encompass the effects of the agents in humans with regard to cancer-preventive activity, carcinogenic effects and other beneficial and adverse effects, as appropriate.

General Remarks

1. Introduction and definitions

This is the fourth handbook in the series of *Handbooks of Cancer Prevention*. Previous volumes represented the views and expert opinions of working groups that met in 1997 and 1998 which considered non-steroidal anti-inflammatory drugs (IARC, 1997), carotenoids (IARC, 1998a) and vitamin A (IARC, 1998b). The present volume completes the consideration of vitamin A-related compounds — so-called retinoids — some of which, like all-*trans*-retinoic acid, the prototypic family member, exert potential cancer preventive effects.

Although it is not the intention that the *IARC Handbooks of Cancer Prevention* be restricted to chemoprevention, the drugs and micronutrients that have been considered until now are potential chemopreventive agents. All of the retinoids discussed in the present volume are the result of a deliberate attempt to identify or synthesize compounds that prevent cancer and simultaneously reduce toxicity and teratogenicity, thus increasing the therapeutic ratio.

Chemoprevention can be defined as the inhibition or reversal of any stage of carcinogenesis by intervention with chemical agents before an overt malignancy is detectable. Chemoprevention can theoretically act at any of the multiple stages of carcinogenesis, although most of the agents evaluated in this handbook do so at relatively early stages of cancer development.

As the efficacy of a chemopreventive agent that is not in use in the general population cannot be evaluated by conventional, observational, analytical epidemiology studies (case–control and cohort studies), the evidence considered in this volume is derived almost exclusively from randomized clinical trials which have either development of cancer as the end-point or some intermediate biomarker of carcinogenesis, usually precancerous lesions. Thus, almost all of the data considered to date in this series (with the exception of that for sulindac in Volume 1) are derived from experimental situations or from trials involving small groups of individuals. In considering the trials that had cancer as the end-point, the Working Group had to satisfy themselves that the end-point considered was a second primary tumour rather than a recurrence of the original tumour, because agents that are effective in preventing recurrence of a cancer are therapeutic and therefore outside the purview of this series. The distinction between chemoprevention and therapy was sometimes difficult to make, especially for organs that show multiple primary tumours. For example, in this handbook, of the studies that relate to the potential prevention of recurrences of superficial tumours of the urinary bladder, only those in which the patients no longer had any evidence of overt neoplasia at the time of initiation of the trial were retained.

The availability of data from clinical trials was of considerable value to the Working Group, since in such studies bias and confounding have largely been eliminated; however, the conclusions are necessarily restricted to the population studied. Since many of the clinical trials cited here relate to patients known to be at substantial risk of a second primary tumour, at the same or a different site but with similar etiology, extrapolation to other groups, whether at high risk for the relevant cancer or more like members of the general population, would be tenuous at best and potentially in serious error.

Other considerations arise when markers of intermediate steps in the carcinogenesis process are considered, either as a means of selecting patients for study or, more problematically, as an end-point of the investigation. Studies that involve precancerous lesions as an end-point allow more rapid identification of putative chemopreventive agents than studies in which cancer is the end-point, but they cannot be given as much weight since the proportion of lesions that progress to cancer is usually small. It is anticipated that biomarkers that can be used in studies in both animals and humans will be the subject of a more detailed evaluation by a special group convened by IARC in the near future.

1.1 Use of biomarkers in studies of chemoprevention

Many malignancies develop over a period of one to two decades, during which time the multistep process of carcinogenesis evolves, culminating in the appearance of invasive and metastatic cancer. While this 'latent period' of cancer development offers a chance to interrupt the process at various stages by chemical or physical means, it poses a challenge to trials of cancer chemopreventive agents, because the outcome may not be known for many years. Therefore, investigators are searching for intermediate or surrogate biomarkers of cancer, in order to identify individuals at increased (or high) risk of developing cancer and to have an early indication of the effectiveness of intervention strategies.

Biomarkers can be used to detect exposure to carcinogenic influences, to identify individuals susceptible to cancer and as indicators of prognosis or diagnosis. Much of the initial work on the identification and validation of biomarkers that characterize the neoplastic process was carried out in cellular or whole-animal models, and the results may not reflect the usefulness of a marker in humans. Biomarkers that are useful in chemoprevention have measurable biological or chemical properties that are highly correlated to cancer incidence in humans and can therefore serve as indicators of the incidence or progression of cancer.

Several types of biomarkers are being considered:

- gene mutations, activated oncogenes and inactivated tumour suppressor genes;
- markers indicative of exposure of molecular targets, such as DNA adducts;
- biochemical changes, such as high plasma concentrations of insulin-like growth factor in breast cancers; and
- cytological and tissue lesions considered to be precancerous.

Although numerous oncogenes and tumour suppressor genes are being identified that help to define early events in the process of carcinogenesis, subsequent events are critical in the actual occurrence of cancer, and interventions at all stages of carcinogenesis may lead to cancer prevention.

Precancerous lesions (dysplasia and carcinoma *in situ*) have been used as the starting point for intervention in a number of studies of the efficacy of chemical agents in inhibiting progression of lesions to frank malignancy. Because not all such lesions progress to carcinoma, identifying the critical events in progression may permit identification of strategies to reverse the process that would otherwise lead to the occurrence of invasive cancer, even at relatively late stages of carcinogenesis.

An alternative approach is to use precancerous lesions as the end-point of an intervention, i.e. to determine the extent to which the earlier stages of carcinogenesis that eventually result in the development of a precancerous lesion can be terminated. Unfortunately, this approach has two practical drawbacks. The first is that the earliest stages of carcinogenesis may begin early in life, when the subjects who would be studied would not be regarded as being at substantial risk for cancer. The more critical problem is that precancerous lesions almost invariably occur in a much higher proportion of the population than does frank malignancy. Thus, prevention of the development of some of these lesions may not guarantee that those that eventually progress and result in the occurrence of cancer have been ablated.

Recent advances in our understanding of the molecular and tissue lesions that occur during the development of hereditary and sporadic colon cancer indicate that a combination of molecular and morphological end-points could be useful predictive measures of neoplastic development in other organ systems as well.

The genetic markers of precancerous lesions that are currently undergoing phase II trials include cytogenetic manifestations such as chromosomal aberrations and micronuclei, abnormal DNA content, DNA and protein adducts and genetic alteration in oncogenes, as well as markers of abnormal cell proliferation, growth regulation and differentiation. These biomarkers have not yet been validated in relation to subsequent cancer occurrence, and they are not yet being tested as the definitive end-points in phase III trials.

1.2 Data from animal models

Data from studies of chemoprevention in animal models are useful for evaluating the potential role of chemopreventive agents in humans but are not sufficient of themselves. A number of studies designed to evaluate whether retinoids inhibit the development of malignant tumours have been conducted on transplanted tumours, often of human origin, as xenografts in nude mice. Studies with this model were not considered in this handbook because it is considered to be a model of the treatment of established cancer rather than of chemoprevention of cancer.

Collectively, the results of the studies reviewed in this handbook suggest that the chemopreventive effects of retinoids are species- and tissue-dependent because of differences in variables such as tissue distribution, retinoid metabolism and cognate receptor levels and activity.

1.3 Agents considered in this handbook

The retinoids are a class of compounds structurally related to vitamin A. Synthesis of retinoids by chemical modification of the vitamin A molecule was begun in 1968, with the objective of identifying retinoids with a better risk–benefit ratio than vitamin A (Bollag & Holdener, 1992). More than 2500 retinoids have since been synthesized and tested biologically. all-*trans*-Retinoic acid and 13-*cis*-retinoic acid were synthesized and then recognized as occurring naturally. The second generation of retinoids included the aromatic compounds etretinate and acitretin, which had an enhanced therapeutic ratio, and the third generation included the polyaromatic retinoids with or without polar end groups.

The retinoids selected for evaluation in this handbook are those for which there were sufficient data in humans and/or animals to permit evaluation. Thus, a number of retinoids at various stages of development or being considered for use in humans were left out, were some that have been studied but are no longer under consideration for use.

In studies of retinoids, care must be taken over their handling in various media to ensure that what is being measured clearly reflects the compound of interest. When blood is collected for assay of retinoids, it should be heparinized or clotted in the dark, centrifuged to obtain plasma or serum and then protected from light and oxygen. Other anticoagulants such as oxalate, citrate and ethylene diamine tetraacetic acid should be avoided because they reduce the recovery of retinoid (Nierenberg, 1984). Serum or plasma samples for analysis should be stored at – 20 °C or less. An internal standard is usually used to correct for incomplete extraction. To minimize oxidation of retinoids during their extraction, an antioxidant such as butylated hydroxytoluene is added to all the solvents at a final concentration of approximately 100 mg/ml.

The relationship between the toxicity of an agent and its beneficial effects (the therapeutic index) is critical in chemoprevention because the people subjected to this form of cancer prevention are healthy and the probability that they will not develop cancer is usually substantially greater than the probability that they will develop cancer, except for carriers of the rare dominant cancer susceptibility genes. These considerations are less germane for patients at risk for second primary tumours, as they may be prepared to tolerate side-effects of chemoprevention as an extension of their therapy. Toxicity is also likely to be less of a problem when the agent is used as therapy for a malignancy, such as all-*trans*-retinoic acid in the treatment of acute promyelocytic leukaemia. Section 7 of each handbook therefore addresses the toxicity of these compounds. A disappointing outcome of the review is that the therapeutic index of the retinoids considered in this volume appears to be too strongly balanced to the risk side, suggesting that any role of these agents in chemoprevention should be restricted to people at high risk for cancer, who are willing to suffer the almost inevitable side-effects.

1.4 Nomenclature of the retinoids

The natural retinoids consist of four isoprenoid units joined in a head-to-tail manner (IUPAC–IUB Joint Commission on Biochemical Nomenclature, 1983). The term 'vitamin A' is a generic descriptor of retinoids that qualitatively

Table 1. Abbreviations and standardized expressions

all-*trans*-*N*-Ethylretinamide (NER)
Etretinate, acitretin, *N*-(4-hydroxyphenyl)retinamide, *N*-ethyl retinamide, and LGD1550 are assumed to have the all-*trans* configuration unless denoted otherwise.
all-*trans*-*N*-(4-Hydroxyphenyl)retinamide (4-HPR)
all-*trans*-4-Hydroxyretinoic acid (all-*trans*-4-hydroxy-RA)
all-*trans*-4-Oxoretinoic acid (all-*trans*-4-oxo-RA)
all-*trans*-Retinoic acid (all-*trans*-RA; ATRA)
13-*cis*-Retinoic acid (13-*cis*-RA)
9-*cis*-Retinoic acid (9-*cis*-RA)
9,13-Di-*cis*-retinoic acid
Retinoic acid, if an isomeric designation is not given, is a generic indicator or is assumed to be a mixture of isomers.
all-*trans*-Retinol

have the biological activity of retinol. Retinoic acid contains a carboxyl group in place of the primary alcohol group of retinol.

The initial numbering system of the IUPAC (International Union of Pure and Applied Chemistry) for the 20 carbon atoms in retinol and retinoic acid, in keeping with the systematic convention of organic chemistry, started with 1 at the carboxyl group. This terminology proved to be awkward, however, inasmuch as the IUPAC-approved number of the corresponding carbon atom in the biological precursor of retinol and retinoic acid, all-*trans*-β-carotene, was 15 (IUPAC Commission on the Nomenclature of Biological Chemistry, 1960). A nomenclature in which the trimethylcyclohexane ring of vitamin A was considered as the parent compound (IUPAC–IUB Commission on Biochemical Nomenclature, 1966) suffered from the same drawback. Thus, in more recent recommendations of the IUPAC–IUB (International Union of Biochemistry) Joint Commission on Biochemical Nomenclature, the oxygen-bearing carbon in retinol and retinoic acid was labelled C-15 and the *gem*-dimethyl carbon atom in the β-ionone ring was called C-1 (IUPAC–IUB Joint Commission on Biochemical Nomenclature, 1983), in keeping with carotenoid numbering. That nomenclature has been retained to the present (e.g. Anon., 1990).

The IUPAC–IUBMB (International Union of Biochemistry and Molecular Biology) Joint Commission on Biochemical Nomenclature (1998) has suggested in its newsletter that aromatic acidic compounds that control epithelial differentiation and prevent metaplasia without having the full range of activities of vitamin A be termed 'retinoate analogues' rather than 'retinoids'. This suggestion is pending. Although a reasonable argument was presented in favour of this change, the term 'retinoids' is used to refer to the compounds considered in this handbook, as there is no single universally accepted definition of retinoids. Usage and abbreviations of retinoids are shown in Table 1.

2. Endogenous metabolism of retinoids

Since retinoic acid is both a naturally occuring retinoid and a pharmacological agent that may be effective in preventing and/or treating cancer, both its endogenous or physiological metabolism and its metabolism after administration as a pharmacological agent must be considered. The endogenous metabolism of all-*trans*-, 13-*cis*- and 9-*cis*-retinoic acid is considered mainly in this section, whereas the metabolism of each isomer that is relevant to its pharmacological use is considered in the individual handbooks.

Most of the information on the synthesis and oxidation of retinoic acid is derived from studies carried out in animal models. The studies in humans consist mainly of metabolic studies of the uptake, plasma turnover and/or metabolism of physiological or pharmacological doses or biochemical studies of the enzymes or processes responsible for the formation or oxidation of retinoic acid in human tissues or cultured cells. Comparisons of the results of metabolic studies in humans and in animal models show marked differences in metabolite profiles, but biochemical investigations of enzymes and enzymatic processes that are

important for retinoic acid formation and/or metabolism suggest that they do not differ widely in humans and other higher animals. Thus, although humans and species such as the rat and mouse probably have similar enzymatic pathways for retinoic acid synthesis and oxidation, there are probably important differences in how the pathways are regulated and how balances between them are maintained.

2.1 Studies in humans

2.1.1 Metabolism

It is generally accepted that all-*trans*-retinoic acid facilitates most of the gene-modulating actions of vitamin A in humans. Other natural forms of retinoic acid, including all-*trans*-3,4-didehydroretinoic acid and all-*trans*-4-oxo-retinoic acid, can also induce retinoic acid receptor (RAR) *trans*-activation *in vitro*, and these forms of retinoic acid are probably important for facilitating the effects of retinoids in birds and amphibians *in vivo* (Hofmann & Eichele, 1994; Mangelsdorf *et al.*, 1994). Moreover, 9-*cis*-retinoic acid is the putative physiological ligand for the retinoid X receptor (RXR) nuclear receptors. 13-*cis*-Retinoic acid, which only weakly activates transcription (Mangelsdorf *et al.*, 1994), is found in human and animal tissues and blood (Blaner & Olson, 1994). All of these forms of retinoic acid are in large part derived from all-*trans*-retinol (vitamin A or vitamin A alcohol). A hypothetical metabolic scheme for the formation of these active retinoic acid forms from all-*trans*-retinol is shown in Figure 1, which provides a framework for the metabolism and transport of all-*trans*-, 13-*cis*- and 9-*cis*-retinoic acid.

It is generally believed that the synthesis of retinoic acid initially involves the oxidation of retinol to retinal, catalysed by a number of alcohol dehydrogenases which, because they can use retinol as a substrate, are referred to subsequently as 'retinol dehydrogenases'. The retinal formed through the action of a retinol dehydrogenase undergoes subsequent oxidation to retinoic acid with one of several aldehyde dehydrogenases, which are also referred to as retinal dehydrogenase because of their substrate preference. The oxidative metabolism of retinoic acid is not solely deactivating since both the 4-hydroxy and 4-oxo derivatives of retinoic acid show activity in *trans*-activation assays. The metabolism is, however, catabolic, since many of the oxidized metabolites of all-*trans*-retinoic acid are excreted.

Numerous enzymes have been proposed to be involved in the synthesis and oxidative metabolism of retinoic acid (Table 2).

*2.1.1.1 Biosynthesis of all-*trans*-retinoic acid — Oxidation of retinol*

The identity of the enzymes in humans that catalyse the formation of all-*trans*-retinal from all-*trans*-retinol, the first of the two oxidative steps in the formation of retinoic acid, has not yet been established unequivocally, and it is unknown whether only some or all of the enzymes described in the literature as important are indeed physiologically essential in humans. *In vitro*, all-*trans*-retinol is oxidized to all-*trans*-retinoic acid by multiple enzymes present in the cytosol or in microsomes (Duester, 1996; Napoli, 1996). The cytosolic enzymes that catalyse retinol oxidation have been identified as alcohol dehydrogenases (ADHs) with 40-kDa subunits (Boleda *et al.*, 1993). These medium-chain ADHs are encoded in humans by nine genes. The ADH isoenzymes have been grouped into six classes on the basis of their catalytic properties and primary structures. All the members of this enzyme family are dimeric zinc metalloenzymes that require NAD^+ to catalyse the oxidation of a variety of primary, secondary and cyclic alcohols (Jörnvall & Höög, 1995). Several human and rat ADH isozymes (classes I, II and IV) oxidize retinol to retinal *in vitro* (Duester, 1996; Napoli, 1996). The class IV ADH human isozyme (ADH4) is the most efficient catalyser of retinol oxidation (Yang *et al.*, 1994) and consequently has been proposed to contribute importantly to retinoic acid biosynthesis *in vivo*.

all-*trans*-Retinol and 9-*cis*-retinol, but not 13-*cis*-retinol, are good substrates for human ADH4, and the apparent K_m values for the reverse (reduction) reactions were very similar to those determined for the two retinol isomers (Allali-Hassani *et al.*, 1998). Kedishvili *et al.* (1998) used purified recombinant human ADH4 and purified recombinant rat cellular

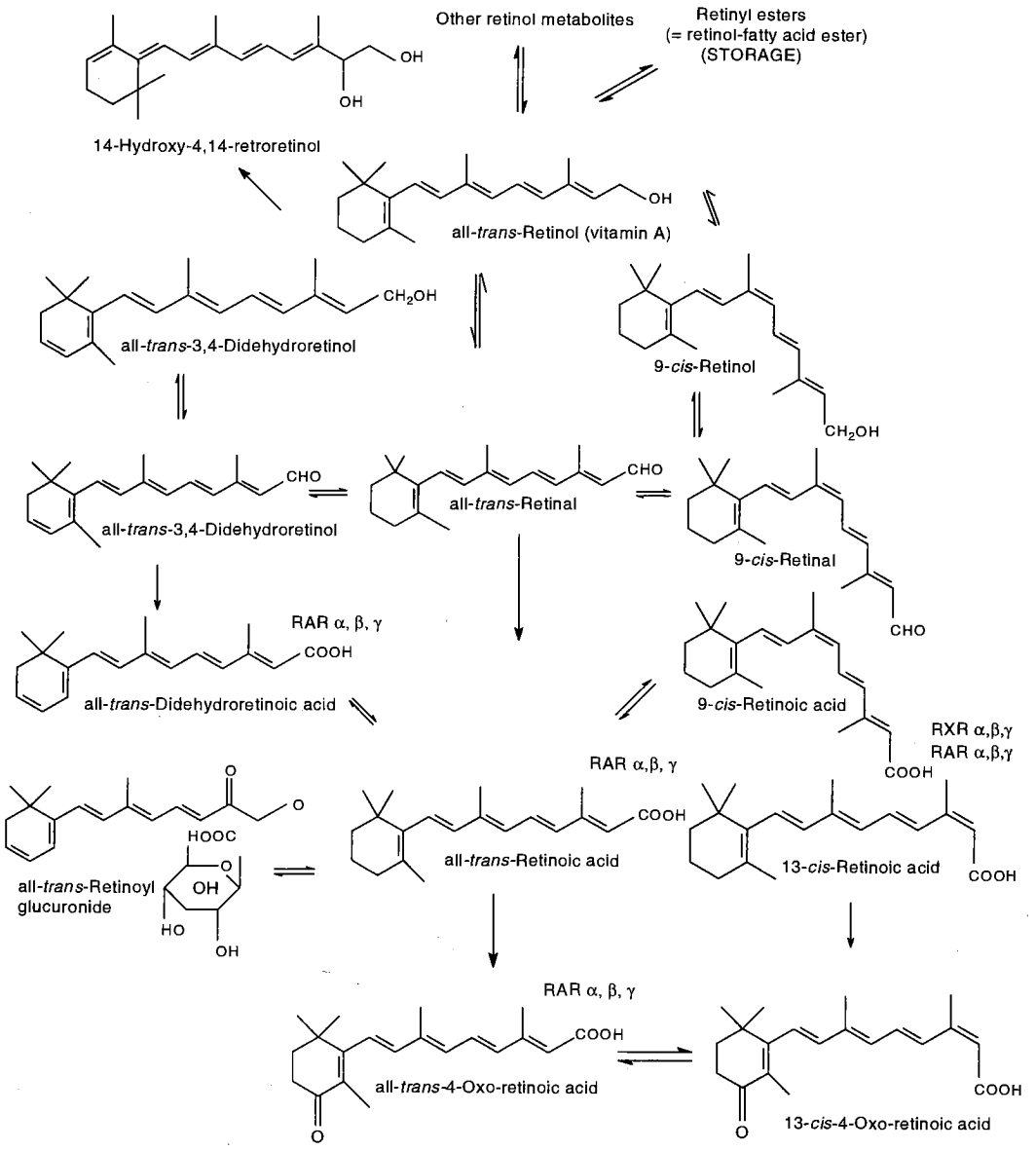

Figure 1. Hypothetical scheme for the metabolism of all-*trans*-retinol

Although all of the metabolic interconversions of vitamin A species indicated in this scheme have not been unequivocally demonstrated experimentally, they have been postulated to take place in some living organism. The reader should focus on the metabolic transformations described in the text, which are those most extensively studied and involve oxidative metabolism of retinol to retinoic acid and oxidative and conjugative transformation of retinoic acid to more polar metabolites. The *cis–trans* isomerizations proposed in the figure are less well understood.

Table 2. Nomenclature and abbreviations of enzymes proposed to be involved in the metabolism of retinoic acid

Enzyme	Abbreviation	Reference
Oxidation of retinol		
Short-chain dehydrogenase/reductase	SCDR	Jörnvall & Höög (1995)
Medium-chain alcohol dehydrogenases	ADH	Jörnvall *et al.* (1995)
Retinol dehydrogenase, type I	RoDH(I)	Boerman & Napoli (1995)
Retinol dehydrogenase, type II	RoDH(II)	Chai *et al.* (1995)
Retinol dehydrogenase, type III	RoDH(III)	Chai *et al.* (1996)
Retinol dehydrogenase, type IV	RoDH-4	Gough *et al.* (1998)
Retinol short-chain dehydrogenase/reductase	RetSDR1	Haeseleer *et al.* (1998)
9-*cis*-Retinol dehydrogenase	9cRDH	Mertz *et al.* (1997)
11-*cis*-Retinol dehydrogenase	11cRDH	Saari (1994)
cis-Retinol/3α-hydroxysteroid dehydrogenase, I	CRADI	Chai *et al.* (1997)
cis-Retinol/3α-hydroxysteroid II dehydrogenase, II	CRADII	Su *et al.* (1998)
Alcohol dehydrogenase, class I	ADH1	Jörnvall & Höög (1995)
Alcohol dehydrogenase, class III	ADH3	Jörnvall & Höög (1995)
Alcohol dehydrogenase, class IV	ADH4	Jörnvall & Höög (1995)
Oxidation of retinal		
Liver aldehyde dehydrogenase	ALDH1	Bhat & Samaha (1999)
Retinal dehydrogenase, type I	Ra1DH(I)	El Akawi & Napoli (1994)
Retinal dehydrogenase, type II	Ra1DH(II)	Wang *et al.* (1996a); Niederreither *et al.* (1997)
Oxidative metabolism		
Cytochrome P450 26	CYP26	Ray *et al.* (1997); White *et al.* (1997a); Fujii *et al.* (1997)

This list is not comprehensive. The abbreviations given here and used in the text are those coined in the original citation.

retinol-binding protein, type I (CRBP I), to demonstrate that retinol bound to CRBP I cannot be channelled to the active site of ADH4. They concluded that the contribution of ADH isozymes to retinoic acid biosynthesis depends on the amount of free retinol present in a cell. Since most retinol within a cell is thought to be bound to CRBP I and since human ADH4 catalyses the oxidation of free retinol and not retinol bound to CRBP I, the physiological relevance of ADH4 in the formation of retinoic acid is questionable (Duester, 1996; Napoli, 1996).

Other reports indicate that the oxidation of all-*trans*-retinol to all-*trans*-retinal is catalysed by microsomal enzymes that can use all-*trans*-retinol bound to CRBP I as a substrate. These enzymes are members of the short-chain dehydrogenase/reductase (SCDR) family, over 60 members of which have been identified. They consist of peptides of 28–32 kDa and do not require zinc for activity. Many of the members of this family are reported to catalyse oxidation or reduction of hydroxysteroids and prostaglandins (Baker, 1994; Jörnvall *et al.*, 1995; Baker, 1998). Several of the SCDRs that oxidize retinol prefer retinol bound to CRBP I or cellular retinaldehyde-binding protein as a substrate rather than unbound retinol (Saari, 1994; Duester, 1996; Napoli, 1996).

A human liver SCDR with all-*trans*-retinol dehydrogenase activity has been cloned by Gough *et al.* (1998). This NAD^+-dependent enzyme comprises 317 amino acids and has strong primary sequence similarity to rat

retinol dehydrogenase (RoDH)(I), RoDH(II) and RoDH(III). This microsomal protein, designated RoDH-4, is expressed strongly in adult liver and weakly in the lung and not in heart, brain, placenta, skeletal muscle or pancreas. RoDH-4 has also been found to be expressed in human fetal liver and lung but not in fetal brain or kidney. The apparent K_m values of RoDH-4 for 5α-androstene-3α,17β-diol, androsterone and dihydrotestosterone were reported to be well under 1 mmol/L, similar to those of RoDH(I) for these hydroxysteroids (Biswas & Russell, 1997). Gough *et al.* (1998) further reported that all-*trans*-retinol is a much better substrate for RoDH-4 than 13-*cis*-retinol. all-*trans*-Retinol and 13-*cis*-retinol bound to CRBP I were relatively potent inhibitors of RoDH-4-catalysed androsterone oxidation, whereas apo-CRBP I had no effect. Thus, Gough *et al.* (1998) suggested that one enzyme may be involved in the metabolism of both steroids and retinoids.

Links between the metabolism of retinoids and steroids were first suggested by Biswas and Russell (1997), who studied 17β- and 3-hydroxysteroid dehydrogenases cloned from rat and human prostate and found that the human prostate hydroxysteroid dehydrogenase shares a high degree of primary sequence homology with rat RoDH(I). This observation led the investigators to hypothesize that the microsomal retinol dehydrogenases might also use hydroxysteroids as substrates. Both rat and human recombinant RoDH(I) catalyse the oxidation of 5α-androstan-3,17-diol to dihydrotestosterone, with apparent K_m values of 0.1 mmol/L, as compared with approximately 2 mmol/L for rat RoDH(I) for retinol-CRBP I (Boerman & Napoli, 1995).

Chai *et al.* (1997) and Su *et al.* (1998) identified two previously unknown members of the SCDR enzyme family that can use both sterols and retinols as substrates. These rat enzymes, *cis*-retinol/3α-hydroxysteroid dehydrogenases (CRADs), catalyse the oxidation of *cis*-retinols. The existence of the CRADs adds further weight to the suggestion that steroid and retinoid metabolism intersects at key multifunctional enzymes.

Haeseleer *et al.* (1998) cloned a human SCDR that can catalyse oxidation of all-*trans*-retinol and reduction of all-*trans*-retinal. This enzyme, called 'retinol short-chain dehydrogenase/reductase' (retSDR1), was cloned from human, bovine and mouse retinal tissue cDNA libraries, and the human, bovine and murine enzymes show approximately 35% amino acid similarity to rat RoDH(I), RoDH(II) and RoDH(III). Human retSDR1 was found by northern blot analysis to be expressed in heart, liver, kidney, pancreas and retina. Recombinant retSDR1 catalyses the reduction of all-*trans*-retinal but not 11-*cis*-retinal, indicating that it is specific for all-*trans*-retinoids. The authors proposed that one physiological action of retSDR1 is to reduce the bleached visual pigment, all-*trans*-retinal, to all-*trans*-retinol.

*2.1.1.2 Biosynthesis of all-*trans*-retinoic acid — Oxidation of retinal*

Several distinct cytosolic aldehyde dehydrogenases have been identified that catalyse the oxidation of retinal to retinoic acid. These enzymes show strong preference for NAD^+ as a substrate. The irreversible nature of the reaction catalysed by these enzymes indicates why retinoic acid cannot restore vision in retinol-deficient animals. Only a few studies of retinal oxidation by human aldehyde dehydrogenases have been reported.

The kinetics of a human liver cytosolic aldehyde dehydrogenase (ALDH1) for retinal isomers has been explored (Bhat & Samaha, 1999). Human ALDH1 shares 87% amino acid identity with a rat kidney retinal dehydrogenase described previously (Bhat *et al.*, 1995). The human isozyme catalyses the oxidation of all-*trans*-, 9-*cis*- and 13-*cis*-retinal, and all-*trans*-retinol was found to be a potent, noncompetitive inhibitor of retinal oxidation. Unlike human ALDH1, all-*trans*-retinol also inhibits the rat kidney retinal dehydrogenase homologue, but competitively (Bhat *et al.*, 1995). It was therefore suggested that human ALDH1 and rat kidney aldehyde dehydrogenases have different actions in retinal metabolism (Bhat & Samaha, 1999).

*2.1.1.3 Oxidative metabolism of all-*trans*-retinoic acid*

Duell *et al.* (1996) demonstrated that application of all-*trans*-retinoic acid onto human skin markedly increases the activity of retinoic acid 4-hydroxylase. The inducible 4-hydroxylase activity was present in microsomes and could catalyse 4-hydroxylation of all-*trans*-retinoic acid but not of 9-*cis*- or 13-*cis*-retinoic acid *in vitro*. Neither all-*trans*-retinol nor all-*trans*-retinal could compete with all-*trans*-retinoic acid as a substrate for 4-hydroxylase. The inducible 4-hydroxylase activity was inhibited by addition of the cytochrome P450 (CYP) inhibitor ketoconazole to skin microsomes.

Han and Choi (1996) reported that treatment of human breast cancer T47-D cells with all-*trans*-retinoic acid induced the activity of all-*trans*-retinoic acid 4- and 18-hydroxylases. The induction was time- and dose-dependent and appeared to be regulated at the transcriptional level. The retinoic acid-inducible 4- and 18-hydroxylase activities present in microsomes from T47-D cells showed high specificity for all-*trans*-retinoic acid and could be inhibited by treatment with the CYP inhibitor liarazole. On the basis of these and other data, the authors suggested that these enzymes are new CYP isozymes.

The metabolism and isomerization of all-*trans*- and 9-*cis*-retinoic acid were studied in primary cultures of human umbilical cord endothelial cells and in primary human and HepG2 hepatocytes by Lansink *et al.* (1997), who observed that all-*trans*-retinoic acid was quickly metabolized by both cell types. all-*trans*-Retinoic acid induced its own metabolism in endothelial cells but not hepatocytes. Liarazole and ketoconazole at 10 mmol/L inhibited oxidation of all-*trans*-retinoic acid in both endothelial cells and hepatocytes. Interestingly, 9-*cis*-retinoic acid was degraded slowly by endothelial cells, whereas hepatocytes metabolized this isomer very quickly.

White *et al.* (1997a) cloned a specific human CYP isoform that is very rapidly induced within cells and tissues after exposure to all-*trans*-retinoic acid. This novel isoform, called CYP26, catalyses the oxidative metabolism of the retinoic acid. Like the zebrafish enzyme (White *et al.*, 1996), with which human CYP26 shares 68% amino acid identity, CYP26 catalyses hydroxylation of all-*trans*-retinoic acid to its 4-hydroxy-, 4-oxo- and 18-hydroxy forms (White *et al.*, 1997a), and CYP26 mRNA expression in human LC-T, MCF-7, HB4 and HepG2 cells was strongly induced by retinoic acid. Ray *et al.* (1997) cloned CYP26 from human liver mRNA and found that 195 of 200 amino acids were identical to those of mouse CYP26. CYP26 is expressed in adult human liver, brain and placenta (Ray *et al.*, 1997) and also in fetal liver and brain (Trofimova-Griffin & Juchau, 1998). The highest levels of CYP26 transcription were observed in adult liver, heart, pituitary gland, adrenal gland, placenta and regions of the brain, whereas the fetal brain showed the highest level of expression, comparable to that of mRNA in adult tissues.

Sonneveld *et al.* (1998) demonstrated that CYP26 is induced within 1 h of all-*trans*-retinoic acid treatment in retinoic acid-sensitive T47-D human breast carcinoma cells but not in retinoic acid-resistant MDA-MB-231 human breast cancer cells or HCT 116 human colon cancer cells. Stable transfection of RARα and RARγ and to a lesser extent RARβ into HCT 116 cells showed that CYP26 induction is dependent on these retinoic acid nuclear receptors. Retinoic acid-induced CYP26 is highly specific for the hydroxylation of all-*trans*-retinoic acid and does not recognize either 13-*cis*- or 9-*cis*-retinoic acid.

Induction of oxidative metabolism of all-*trans*-retinoic acid in MCF-7 mammary carcinoma cells within 1 h of treatment with all-*trans*-retinoic acid at 10^{-9} to 10^{-6} mol/L was reported by Krekels *et al.* (1997). The apparent K_m value of the induced activity for all-*trans*-retinoic acid was 0.33 mmol/L, and the activity was observed after treatment of MCF-7 cells with 13-*cis*- and 9-*cis*-retinoic acid and several keto forms of retinoic acid.

all-*trans*-Retinoic acid induced its own oxidative metabolism in four of eight human squamous-cell carcinoma lines examined (Kim *et al.*, 1998). Induction was blocked by actinomycin D or cyclohexamide and was inhibited by the addition of ketoconazole, suggesting involvement of a CYP isozyme. The authors

further reported that the metabolism was first detectable within 4 h of retinoic acid treatment and that 13-*cis*- and 9-*cis*-retinoic acid and all-*trans*-retinal also effectively induced CYP-mediated oxidative metabolism. Like Krekels *et al.* (1997), Kim *et al.* (1998) were probably exploring the actions of CYP26 or a closely related isoform.

A link between the CYP-catalysed oxidation of all-*trans*-retinoic acid and human disease was proposed by Rigas *et al.* (1993, 1996), who observed marked interindividual differences in the pharmacokinetics of all-*trans*-retinoic acid in patients with non-small-cell lung cancer. Initial studies indicated that patients who rapidly cleared all-*trans*-retinoic acid from their plasma also had significantly lower endogenous plasma concentrations of this compound than persons who cleared it from their circulation more slowly. Since administration of ketoconazole, a CYP inhibitor, attenuated all-*trans*-retinoic acid plasma clearance, these authors concluded that rapid plasma clearance was probably catalysed by a CYP-dependent oxidative pathway.

*2.1.1.4 Other metabolism of all-*trans*-retinoic acid*

Other metabolites of all-*trans*-retinoic acid generated *in vivo* include 13-*cis*-retinoic acid, 9-*cis*-retinoic acid, all-*trans*-retinoyl-β-glucuronide and all-*trans*-3,4-didehydroretinoic acid (Blaner & Olson, 1994). Some of these metabolites mediate retinoic acid function, whereas others are probably catabolic products destined for export from the body. This section focuses primarily on the conjugated metabolite all-*trans*-retinoyl-β-glucuronide, which is water-soluble and appears rapidly in the bile of animals given the parent compound, suggesting that it is a catabolic product of all-*trans*-retinoic acid destined for elimination from the body. all-*trans*-Retinoyl-β–glucuronide also induces retinoic acid-dependent differentiation of some human cell lines in culture. It is less toxic than the parent compound. Its synthesis appears to be catalysed by several distinct microsomal UDP-dependent glucuronyl transferases. As most tissues contain β-glucuronidases that can hydrolyse retinoyl-β-glucuronide to retinoic acid, the water-soluble all-*trans*-retinoyl-β-glucuronide may in some instances serve as a precursor for retinoic acid.

all-*trans*-Retinoyl-β-glucuronide is present in fasting human plasma at a concentration of 5–17 nmol/L (Barua & Olson, 1986). Eckhoff *et al.* (1991) reported the presence of both all-*trans*-4-oxoretinoic acid and 13-*cis*-4-oxoretinoic acid in the plasma of volunteers given retinyl palmitate at 0.88 mmol/kg body weight orally for 20 days and showed that 4-oxygenated derivatives of retinoic acid were present in the human plasma before, during and after daily treatment. Similar observations were made after administration of 13-*cis*-retinoic acid (Creech Kraft *et al.*, 1991a).

Although some retinoyl-β-glucuronides are destined for excretion from the body, retinoyl-β-glucuronide can induce human promyelocytic HL-60 cell differentiation *in vitro* (Janick-Buckner *et al.*, 1991). Although it is a good inducer of cellular differentiation *in vitro* (Gallup *et al.*, 1987; Zile *et al.*, 1987; Janick-Buckner *et al.*, 1991) and *in vivo* (Sietsema & DeLuca, 1982), it does not bind to cellular retinoid-binding proteins or to nuclear retinoid receptors (Mehta *et al.*, 1992; Sani *et al.*, 1992). It is either hydrolysed to retinoic acid or induces differentiation by transferring its retinoyl moiety to a retinoid nuclear receptor (Olson *et al.*, 1992).

The metabolic basis of the limited toxicity of all-*trans*-retinoyl-β-glucuronide to the skin, to embryonic development and to cells in tissue culture in comparison with that of all-*trans*-retinoic acid may be due in part to its solubility in water (Janick-Buckner *et al.*, 1991; Gunning *et al.*, 1993). Whereas retinoyl-β-glucuronide undergoes slow hydrolysis *in vivo*, retinoyl-β-glucose, a synthetic conjugate, is hydrolysed rapidly to retinoic acid *in vivo* and is therefore both cytotoxic and teratogenic (Barua *et al.*, 1991; Janick-Buckner *et al.*, 1991). Thus, β-glycosidases appear to be less compartmentalized than β-glucuronidases.

*2.1.1.5 Biosynthesis of 13-*cis*-retinoic acid*

Figure 1 summarizes the hypothetical metabolic pathways that can give rise to 13-*cis*-retinoic acid. Since there is little evidence that 13-*cis*-retinol or 13-*cis*-retinal is present in tissues or

cells, it is generally assumed that 13-*cis*-retinoic acid is formed from isomerization of all-*trans*-retinoic acid or 9-*cis*-retinoic acid. The absence of data on the biosynthesis of this compound cannot, however, be taken as evidence that it is formed only by isomerization of other retinoic acid isomers.

13-*cis*-Retinoic acid is formed in humans after consumption of vitamin A. The concentrations of all-*trans*-, 13-*cis*- and 13-*cis*-4-oxo-retinoic acid in the plasma of 20 volunteers after a single oral dose of retinyl palmitate at 0.87 mmol/kg body weight rose two- to four-fold, with maximal concentrations 1.5–6 h after dosing. When the volunteers were dosed daily for 20 days, the plasma concentrations of all-*trans*- and 13-*cis*-retinoic acid rose transiently but returned to the initial concentrations after 20 days, whereas the plasma concentrations of 13-*cis*-4-oxo-retinoic acid increased gradually over the 20-day period to a steady-state concentration which was approximately three times that present at day 0 (Eckhoff *et al.*, 1991). In human subjects given physiological or pharmacological doses of retinyl palmitate orally, the plasma concentrations of all-*trans*- and 13-*cis*-retinoic acid rose 1.3- and 1.9-fold, respectively (Tang & Russell, 1991).

2.1.1.6 Biosynthesis of 9-cis-*retinoic acid*

Any factor that influences the availability of 9-*cis*-retinoic acid to or within a cell affects retinoic acid signalling pathways and cellular responses. Little is known about how 9-*cis*-retinoid isomers are formed, although the mechanism of isomerization of all-*trans*-retinoids to 11-*cis*-retinoid isomers in the visual process is now well established to be catalysed by a specific enzyme. In the visual cycle, *trans* to 11-*cis* isomerization takes place at the level of retinal and not of retinaldehyde (Saari, 1994). Since the first reports in 1992 that 9-*cis*-retinoic acid is a ligand for the RXRs, possible pathways for 9-*cis*-retinoic acid formation have been explored, and three pathways have been proposed (Figure 2): isomerization of all-*trans*-retinoic acid, probably through non-enzymatic processes; enzymatic oxidation of 9-*cis*-retinol by a pathway similar to the oxidation of all-*trans*-retinol to all-*trans*-retinoic acid; and cleavage of 9-*cis*-β-carotene or other 9-*cis*-carotenoids either to 9-*cis*-retinal and all-*trans*-retinal followed by oxidation or possibly directly to 9-*cis*-retinoic acid.

Humans appear to form 9-*cis*-retinoic acid after eating a meal rich in preformed vitamin A (Arnhold *et al.*, 1996). Significant quantities of both 9-*cis*-retinoic acid and 9,13,-di-*cis*-retinoic acid were present in the circulation after consumption of a retinoid-rich meal (Table 3). The mean maximum concentration at peak absorption (at 4 h) was 9 ± 3.7 nmol/L for 9-*cis*-retinoic acid and 57 ± 19 nmol/L for 9,13-di-*cis*-retinoic acid; the integrated area under the curve of concentration–time was 11 ± 3.4 ng-h/ml for 9-*cis*-retinoic acid and 68 ± 22 ng-h/ml for 9,13-di-*cis*-retinoic acid. Intravenous doses of 9,13-di-*cis*-retinoic acid substantially increase the concentration of 9-*cis*-retinoic acid in rats (see Handbook 3, section 3.2), which suggests that 9,13-di-*cis*-retinoic acid can serve as a precursor for 9-*cis*-retinoic acid rather than solely as a catabolic product destined for excretion from the body.

9-*cis*-Retinoic acid can be formed non-enzymatically in isolated human cells and homogenates from all-*trans*-retinoic acid. Lansink *et al.* (1997) studied the metabolism of this compound in primary cultures of human umbilical cord endothelial cells, primary human hepatocytes and human HepG2 hepatocytes and found that hepatocytes and HepG2 cells but not endothelial cells isomerized all-*trans*-retinoic acid to 9-*cis*-retinoic acid. Mertz *et al.* (1997) identified and cloned from a human mammary tissue an NAD^+-dependent retinol dehydrogenase (9-*cis*-retinol dehydrogenase) which specifically oxidizes 9-*cis*-retinol and not all-*trans*-retinol. 9-*cis*-Retinol dehydrogenase, a member of the SCDR family, is expressed in adult human mammary tissue, kidney, liver, and testis and during the first trimester of pregnancy in several human embryonic tissues, including brain, kidney and adrenals.

9-*cis*-β-Carotene can be converted via 9-*cis*-retinal to 9-*cis*-retinoic acid (Ben-Amotz *et al.*, 1988; Levin & Mokady, 1994; Nagao & Olson, 1994; Hébuterne *et al.*, 1995). You *et al.* (1996) found that very little radiolabel from oral doses of [^{13}C]9-*cis*-β-carotene was present in

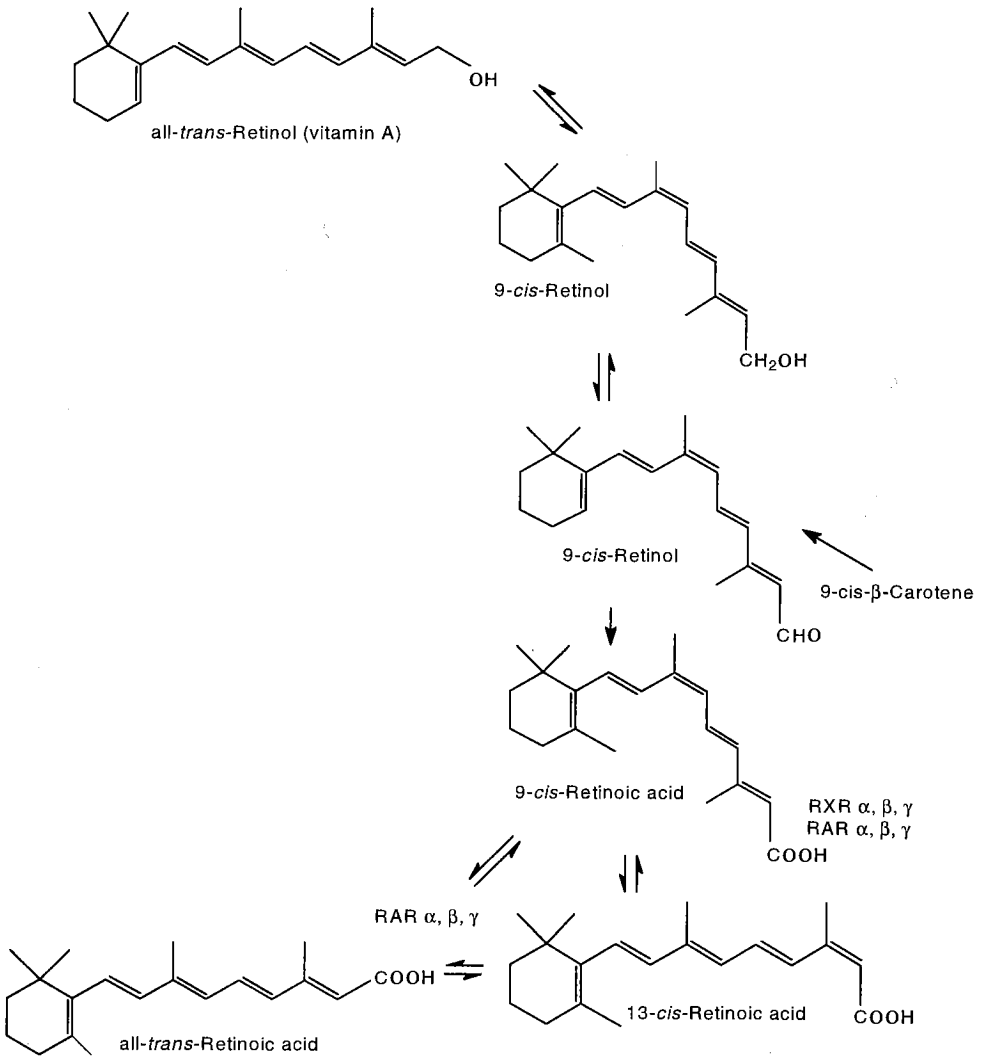

Figure 2. Hypothetical scheme for the synthesis of 9-*cis*-retinoic acid
As outlined in the text, three pathways have been proposed for the formation of 9-*cis*-retinoic acid: isomerization from all-*trans*-retinoic acid, oxidation of 9-*cis*-retinol and 9-*cis*-retinal and cleavage of 9-*cis*-β-carotene. It is not known to what extent each of these three routes contributes to 9-*cis*-retinoic acid formation in tissues.

postprandial plasma, and nearly all of the administered compound was found in the circulation as all-*trans*-β-carotene; *cis* to *trans* isomerization took place exclusively before uptake by the intestinal mucosa.

2.1.2 Plasma transport and kinetics

A small percentage of dietary vitamin A is converted to all-*trans*- and 13-*cis*-retinoic acid in the intestine and is absorbed through the portal system as retinoic acid bound to albumin

Table 3. Plasma retinoid concentrations after consumption of fried turkey liver by healthy male volunteers[a]

Retinoid	C_{end} (ng/mL)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0-24\ h}$ (ng x h/mL)
Retinol	641 ± 99	800 ± 105*	9	16 822 ± 1982
Retinyl palmitate[b]	32.2 ±19.1	3540 ± 1736*	4	21 114 ± 7952
14-Hydroxy-4,14-retroretinol	[c]	3.7 ± 0.9	4	61.7 ± 9.0
all-*trans*-Retinoic acid	0.8 ± 0.2	2.0 ± 0.5*	2	19.7 ± 1.7
all-*trans*-4-Oxoretinoic acid	[c]	0.8 ± 0.2	10	14.7 ± 6.4
13-*cis*-Retinoic acid	1.1 ± 0.2	21.5 ± 4.3*	4	204 ± 35.3
13-*cis*-4-Oxoretinoic acid	2.4 ± 0.6	32.1 ± 4.9*	10	435 ± 68.5
9-*cis*-Retinoic acid	ND[d]	2.7 ± 1.1	4	10.7 ± 3.4
9,13-Di-*cis*-retinoic acid	ND[d]	17.1 ± 5.8	4	68.2 ± 21.6

From Arnhold *et al.* (1996)

[a] Value for C_{end}, C_{max} and $AUC_{0-24\ h}$ are means ± SD; those for T_{max} (n = 10) are medians.

[b] Data calculated with n = 9 owing to one outlier (C_{max} = 14 106 ng/mL, and $AUC_{0-24\ h}$ = 104 858 ng x h/mL).

[c] Endogenously detectable in three samples only; 1.3 ± 0.2 ng/mL (for 14-hydroxy-4,14-retroretinol) and 0.6 ± 0.3 ng/mL (for all-*trans*-4-oxo retinoic acid)

[d] Not detectable; detection limit; 0.3 ng/mL for 9-*cis*-retinoic acid and 0.5 ng/mL for 9,13-di-*cis*-retinoic acid

* Significantly greater than C_{end} ($p < 0.001$, Student's t test for paired data)

(Olson, 1990; Blaner & Olson, 1994). The plasma concentration of all-*trans*-retinoic acid in fasting humans is 4–14 nmol/L, which is 0.2–0.7% of the plasma concentration of retinol (De Leenheer *et al.*, 1982; Eckhoff & Nau, 1990). Retinoic acid can be taken up efficiently from the circulation by cells, although no specific cell surface receptor is known. all-*trans*-Retinoic acid is fully ionized in free solution at pH 7.4 but is uncharged in a lipid environment (Noy, 1992a,b). It can traverse cell membranes rapidly.

2.1.3 Tissue distribution and variations within human populations

Little information is available about the concentrations and distribution in human tissues of all-*trans*-, 13-*cis*- and 9-*cis*-retinoic acid, primarily because of the difficulty in measuring the relatively low concentrations of all-*trans*-retinoic acid in tissues. The extent to which diet, sex or other factors influence the physiological transport or metabolism of these compounds in humans is unclear. The work of Muindi *et al.* (1992) and Rigas *et al.* (1993, 1996) suggests that some cancer patients clear pharmacological doses of all-*trans*-retinoic acid from their circulation more rapidly than controls because of differences in the CYP-mediated oxidative metabolism of the retinoic acid.

2.2 Experimental models

2.2.1 Overview

Most studies of the transport and metabolism of retinoic acid have been carried out in rats; others have been carried out in mice, rabbits and primates. None of these animal models fully mimics the human situation.

2.2.2 Metabolism

Much of the early information on the enzymes responsible for retinol oxidation was obtained by studying enzymatic activity in tissue homogenates or fractionated homogenates or in cells in culture. Since 1990, an increasing

number of enzymes have been studied *in vitro* and proposed to act *in vivo* as retinol dehydrogenases. The increase is due in part to the development of cloning strategies based on searches for sequence homology to identify previously unknown retinol dehydrogenases. Because nearly all of the enzymes that have been identified by either classical or more modern approaches as retinol dehydrogenases do not show absolute substrate specificity for retinol, it is difficult to demonstrate unequivocally that a 'retinol dehydrogenase' actually catalyses retinol oxidation *in vivo*.

As only a few enzymes that catalyse the oxidation of retinal to retinoic acid have been described, there is generally greater agreement on their physiological significance in retinoic acid formation than is the case for retinol dehydrogenases.

The oxidative metabolism of retinoic acid was actively investigated nearly two decades ago, when many oxidative metabolites of retinoic acid were identified. Renewed interest in this area stems from the finding that the oxidative metabolism of retinoic acid plays an important role in strictly maintaining its tissue and blood concentrations. It is now believed that specific CYP isoenzymes are involved in the metabolism of retinoic acid and are important in maintaining it. Moreover, it is now generally assumed that the oxidative metabolism of retinoic acid is a significant factor in its chemopreventive activity.

*2.2.2.1 Biosynthesis of all-*trans*-retinoic acid — Oxidation of retinol*

Enzymes of two distinct families, the ADHs and the SCDRs, are considered to be important in catalysing the oxidation of retinol (see section 2.1.1.1).

Although the relatively nonspecific cytosolic ADH of liver can catalyse the oxidation of retinol to retinal (Zachman & Olson, 1961; Mezey & Holt, 1971), the physiological role of this enzyme in the conversion of retinol to retinoic acid is still debated (Duester, 1996; Napoli, 1996). Early research on retinol oxidation focused mainly on cytosolic retinol dehydrogenases, but by the mid-1980s microsomal retinol dehydrogenases were also being investigated (Frolik, 1984; Blaner & Olson, 1994). Leo and Lieber (1984) described a strain of deermice which genetically lack cytosolic ADH in the liver and testis and reported that cytosolic preparations from these organs were unable to oxidize retinol to retinal at a significant rate, even though the testes of these animals were morphologically normal and the animals could reproduce normally. Later studies by these investigators indicated that the livers of this strain have a microsomal retinol dehydrogenase (Leo *et al.*, 1987). When cytosolic fractions from the testis of these ADH-deficient animals were incubated with microsomal fractions of liver, all-*trans*-retinoic acid was formed from all-*trans*-retinol (Kim *et al.*, 1992), and retinal was shown to be an intermediate in this process. In contrast, Posch and Napoli (1992) found that cytosolic preparations from the testis of the deermice could convert all-*trans*-retinol into all-*trans*-retinoic acid. When these cytosolic preparations were fractionated by anion-exchange fast-proton liquid chromatography followed by size-exclusion fast-proton liquid chromatography, the peaks for retinol and retinal dehydrogenase activity co-migrated. Thus, the enzymes that can synthesize retinoic acid from exogenous retinol seemed to reside in a tightly bound protein–protein complex.

Because inhibitors of alcohol and acetaldehyde metabolism do not block retinoic acid synthesis from retinol in LLC-PK1 porcine kidney cells, Napoli (1986) concluded that the enzymes involved in the formation of retinoic acid in these cells are distinct from ADH, ALDH and aldehyde oxidase. Napoli and Race (1988) demonstrated the oxidation of free retinol (not bound to CRBP I) to retinal by cytosolic, but not by microsomal, preparations of rat liver and kidney. In soluble extracts of hairless-mouse epidermis, retinol is oxidized to retinoic acid in two steps, which are catalysed by two NAD^+-dependent enzymes (Connor & Smit, 1987). The retinol oxidizing enzyme had characteristics of a cytosolic ADH, whereas the retinaldehyde oxidizing activity was not further characterized. An $NADP^+$-dependent oxidase in rat liver microsomes converts retinol to retinal (Shih & Hill, 1991); this oxidase was

induced by 3-methylcholanthrene and inhibited by citral, ketoconazole and α-naphthoflavone, but was unaffected by the dehydrogenase inhibitor pyrazole.

Several membrane-bound dehydrogenases catalyse this oxidation–reduction reaction in ocular tissue, and one such enzyme, present in the rod outer segments, catalyses the interconversion of all-*trans*-retinol and all-*trans*-retinal (Lion *et al.*, 1975; Blaner & Churchich, 1980; Nicotra & Livrea, 1982). In the retinal pigment epithelium, a different membrane-bound dehydrogenase was reported to catalyse the stereospecific interconversion of 11-*cis*-retinol and 11-*cis*-retinal (Lion *et al.*, 1975; Nicotra & Livrea, 1976), and 11-*cis*-retinal bound to cellular retinal-binding protein is reduced by this enzyme to 11-*cis*-retinol (Saari & Bredberg, 1982). Although soluble ADHs are present in the eye, they may not play a major role in retinoid metabolism (Nicotra & Livrea, 1976; Julia *et al.*, 1986). The eye needs 11-*cis*-retinal as the visual pigment and all-*trans*-retinoic acid to maintain normal retinoid-regulated gene expression. Cultured rabbit Müller cells can synthesize retinoic acid from [^{3}H]retinol (Edwards *et al.*, 1992). Thus, some cells of the adult vertebrate retina can synthesize retinoic acid from retinol and release retinoic acid into the extracellular environment.

(i) Cytosolic retinol dehydrogenases

In rat and mouse tissues, all-*trans*-retinol can be oxidized to all-*trans*-retinoic acid by multiple enzymes present in the cytosol or in microsomes (Duester, 1996; Napoli, 1996; see section 2.1.1.1), and it has been proposed that ADH4 contributes importantly to retinoic acid biosynthesis *in vivo*. Ang *et al.* (1996a,b) demonstrated in developing mouse embryos that the pattern of expression of ADH4 overlaps both temporally and spatially with the distribution of retinoic acid in the embryo. Investigations of the expression of ADH1 and ADH4 in developing mouse embryos demonstrated that both isoforms are present in day-11.5 adrenal blastemas (Haselbeck & Duester, 1998a). The presence of both ADH1 and ADH4 during the earliest stages of adrenal gland development and the observation of high concentrations of retinoic acid in embryonic adrenal glands (Haselbeck *et al.*, 1997) suggest that an early function of ADH1 and ADH4 is to provide an embryonic source of retinoic acid (Haselbeck & Duester, 1998a). Investigations with ADH4-*lacZ* transgenic mice showed that ADH4 expression is located in the brain and craniofacial region of the embryo as early as days 8.5–9.5, during neuroregulation. At day 8.5, ADH4-*lacZ* expression was seen in several dispersed regions throughout the head, but by day 9.5 expression was evident in regions that corresponded to the otic vesicles and migrating neural crest cells, particularly the mesencephalic, trigeminal, facial and olfactory neural crest (Haselbeck & Duester, 1998b).

(ii) Microsomal dehydrogenases

Oxidation of all-*trans*-retinol to all-*trans*-retinal is catalysed by microsomal enzymes that can use all-*trans*-retinol bound to CRBP I as a substrate. These microsomal enzymes are members of the SCDR family of enzymes (see above). Three retinol dehydrogenases, RoDH(I), RoDH(II) and RoDH(III), have been cloned and characterized from rat microsomes (Posch *et al.*, 1991; Boerman & Napoli, 1995; Chai *et al.*, 1995, 1996), each of which recognizes all-*trans*-retinol bound to CRBP I as a substrate. Since most retinol within cells is bound to CRBP I, this substrate specificity suggests that these enzymes are physiologically relevant for retinol oxidation. The three enzymes are 82% identical and very similar to other members of the SCDR family. Each requires $NADP^+$ and is expressed most prominently in liver. RoDH(I) is the best studied isoform and is present in kidney, brain, lung and testis but at concentrations less than 1% that in liver. RoDH(II) is also expressed in kidney, brain, lung and testis, at concentrations 25, 8, 4 and 3%, respectively, of that in liver, whereas RoDH(III) is expressed only in liver (Chai *et al.*, 1996). RoDH(I) does not use 9-*cis*-retinol as a substrate (Posch *et al.*, 1991; Boerman & Napoli, 1995); the substrate specificities of RoDH(II) and RoDH(III) for different retinol isomers have not been reported. These three enzymes are probably involved in the oxidation of all-*trans*-retinol to all-*trans*-retinal, the first step in retinoic acid formation.

Zhai *et al.* (1997) demonstrated that CRBP I mRNA and mRNA for RoDH(I) and RoDH(II) are co-expressed in adult rat hepatocytes and in the proximal tubules of rat renal cortex. CRBP I and RoDH(I) and RoDH(II) were also co-expressed in rat testicular Sertoli cells with weaker co-expression in spermatogonia and primary spermatocytes. Since CRBP I and RoDH(I) and/or RoDH(II) are expressed in the same cellular loci *in vivo*, the authors suggested that their data support the hypothesis that holo-CRBP I serves as a substrate for RoDH isozyme-catalysed retinoic acid synthesis.

Rat RoDH(II) has been characterized by Imaoka *et al.* (1998) as a binding protein that is associated with CYP2D1 in the liver. After isolating and cDNA cloning the protein, these workers purified the same protein described by Chai *et al.* (1995) and demonstrated that recombinant RoDH(II) binds tightly to CYP2D1 even in the presence of 1% sodium cholate. Since CYP2D1 contributes to steroid metabolism and can hydroxylate testosterone, oestrogen and cortisol, it was suggested that the binding of RoDH(II) to CYP2D1 is important in the metabolism of diverse bioactive substances including retinoids and steroids. Links between the metabolism of retinoids and steroids were first reported by Biswas and Russell (1997; see section 2.1.1.1).

Members of the ADH family can also catalyse both retinol and hydroxysteroid oxidation (Kedishvili *et al.*, 1997). cDNAs encoding for a class III ADH and a previously unknown ADH were cloned from chick embryo limb bud and heart RNA. The previously unknown ADH cDNA clone exhibited 67 and 68% sequence identity with chicken class I and III ADHs, respectively, and had less identity with mammalian class II and IV ADH isozymes. Expression of this cDNA yielded an active ADH species that was stereospecific for the 3β,5α-hydroxysteroids as opposed to 3β,5β-hydroxysteroids, and this cytosolic enzyme catalysed retinol oxidation with an apparent K_m of 56 mmol/L for all-*trans*-retinol, as compared with a K_m of 31 mmol/L for epiandrosterone. Thus, like members of the SCDR family, ADH enzymes can catalyse reactions involving both steroids and retinoids.

2.2.2.2 *Biosynthesis of all-*trans*-retinoic acid—Oxidation of retinal*

Moffa *et al.* (1970) partially purified an enzyme from intestinal mucosa that converted retinal to retinoic acid and found that it was stimulated by glutathione, NAD^+ and $FADr^+$; it had an apparent K_m of 0.3 mmol/L for retinal. Since cellular retinal concentrations are less than 0.1 mmol/L (McCormick & Napoli, 1982; Williams *et al.*, 1984), this enzyme may have limited physiological importance. Cytosols of rat kidney, testis and lung cells also can catalyse the oxidation of retinal to retinoic acid (Bhat *et al.*, 1988a,b). Leo *et al.* (1989a) demonstrated that a cytosolic NAD^+-utilizing aldehyde dehydrogenase activity in rat tissues catalysed the oxidation of retinal to retinoic acid. Hupert *et al.* (1991) reported that an enzyme present in rat liver cytosol is responsible for the formation of retinoic acid from retinal.

Lee *et al.* (1991) explored the ability of the 13 ALDHs in mouse tissues to catalyse oxidation of all-*trans*-retinal to all-*trans*-retinoic acid. Three of the six ALDHs present in mouse liver cytosol, ALDH-2, ALDH-7 and xanthine oxidase, catalysed this oxidation. ALDH-2 was estimated to catalyse about 95% of retinaldehyde oxidation to retinoic acid in the liver. The apparent K_m of ALDH-2 for all-*trans*-retinal was 0.7 mmol/L. Since none of the ALDH present in the particulate fractions of mouse liver could catalyse significant retinaldehyde oxidation, the authors concluded that the enzymes responsible for retinoic acid formation from retinal are cytosolic, NAD^+-linked, non-substrate-specific dehydrogenases.

Bhat *et al.* (1988a,b) and Labrecque *et al.* (1993, 1995) purified the cytosolic retinal dehydrogenase present in rat kidney. The purified enzyme (subunit relative molecular mass of 53 kDa) is NAD^+-dependent and catalyses the oxidation of both all-*trans*- and 9-*cis*-retinal to the corresponding retinoic acid isomer. The rat kidney cytosolic ALDH had typical Michaelis–Menten kinetics towards all-*trans*-retinal, with an apparent K_m of 8–10 mmol/L, whereas the apparent K_m for 9-*cis*-retinol is 5.7 mmol/L. The rat kidney ALDH was subsequently cloned by Bhat *et al.* (1995), who found that its amino acid sequence was very

similar to those of other cytosolic ALDHs cloned from rat, mouse and human livers. The enzyme is also strongly expressed in rat lung, testis, intestine, stomach and trachea. Initially, the relevant cDNA was used to define the pattern of expression of this ALDH in fetal and adult rat kidney (Bhat *et al.*, 1998), but Bhat (1998) subsequently investigated its expression in the stomach and small intestine of rats during postnatal development and in vitamin A deficiency. Two days before birth, expression was high in the small intestine but was not detectable in the stomach, whereas after birth expression in the intestine decreased progressively, while expression in the stomach increased and reached its highest concentration at postnatal day 42. Vitamin A deficiency was found to upregulate enzyme expression in the stomach and small intestine while administration of retinoids downregulated expression in these tissues.

Two distinct ALDHs, ALDH-1 and ALDH-2, that can catalyse retinal oxidation have been purified from bovine kidney. They have relatively low apparent K_m values of 6.4 and 9.1 mmol/L, respectively, for all-*trans*-retinal (Bhat *et al.*, 1996). ALDH-1 is proposed to be the primary enzyme in the oxidation of retinal to retinoic acid in this tissue. A retinal dehydrogenase (designated RalDH(I)) purified from rat liver cytosol by Posch *et al.* (1992) and El Akawi and Napoli (1994) is the predominant ALDH isoform in rat liver, kidney and testis (Posch *et al.*, 1992). all-*trans*-Retinal concentrations greater than 6 mmol/L were reported to inhibit RalDH(I). RalDH(I) also recognizes all-*trans*-retinol bound to CRBP I as a substrate. El Akawi and Napoli (1994) demonstrated that RalDH(I) catalyses the oxidation of both all-*trans*- and 9-*cis*-retinal in an NAD^+-dependent manner, but 13-*cis*-retinal was not an effective substrate. Oxidation of retinal was not inhibited by all-*trans*- or 9-*cis*-retinoic acid or by holo-CRBP I. RalDH(I) may serve as a common enzyme in the conversion of all-*trans*- and 9-*cis*-retinal into their acids.

Wang *et al.* (1996a) cloned another ALDH from rat testis and called it RalDH(II). The amino acid sequence of RalDH(II) from rat testis is 85% identical to that of RalDH(I), 85% identical to mouse AHD-2, 87% identical to human ALDH1 and 87% identical to bovine retina retinal dehydrogenase. Recombinant RalDH(II) recognizes as substrate both unbound all-*trans*-retinal and all-*trans*-retinal in the presence of CRBP I (Wang *et al.*, 1996a). In addition, RalDH(II) can use as a substrate all-*trans*-retinal generated *in situ* by the action of rat liver microsomal retinol dehydrogenase(s) from holo-CRBP I.

An ALDH present at high concentrations in the basal forebrain of mice catalyses the formation of retinoic acid (McCaffery & Dräger, 1995; Zhao *et al.*, 1996). This enzyme, now called RalDH-2, is expressed very early in embryonic development and at lower levels later in development (Niederreither *et al.*, 1997). Expression of this enzyme in mouse embryos given a teratogenic dose of all-*trans*-retinoic acid on day 8.5 results in downregulation of expression. Labrecque *et al.* (1993, 1995) purified a cytosolic retinal dehydrogenase from rat kidney which is NAD^+-dependent and catalyses the oxidation of both all-*trans*- and 9-*cis*-retinal to the corresponding retinoic acid isomer. This retinal dehydrogenase is either identical or very similar to the retinal dehydrogenase partially purified from rat liver cytosol by El Akawi and Napoli (1994). Both enzymes catalyse the oxidation of all-*trans*- and 9-*cis*-retinaldehyde in an NAD^+-dependent manner. The presence of CRBP I reduces the rate of all-*trans*-retinoic acid synthesis by rat liver retinaldehyde dehydrogenase. These and other studies (Blaner & Olson, 1994) strongly indicate that cytosolic retinaldehyde dehydrogenases play a role in the formation of retinoic acid *in vivo*. CYP1A2 and CYP3A6 from rabbit liver microsomes can oxidize retinaldehyde to retinoic acid (Roberts *et al.*, 1992).

2.2.2.3 *Synthesis of all-*trans*-retinoic acid from β-carotene*

A small percentage of the retinal formed from dietary β-carotene can be oxidized to all-*trans*-retinoic acid and taken into the circulation bound to albumin (Goodman & Blaner, 1984; Blaner & Olson, 1994; Wang *et al.*, 1996b). A large percentage of the retinoic acid of dietary origin appears to be removed from the circulation by tissues. Dietary intake of pre-

formed vitamin A and/or provitamin A carotenoids can give rise to increased circulating concentrations of all-*trans*- and 13-*cis*-retinoic acid (Folman *et al.*, 1989; Tang *et al.*, 1995). Rabbits maintained for several weeks on a β-carotene-supplemented diet had a markedly higher plasma concentration of all-*trans*- and 13-*cis*-retinoic acid than rabbits maintained on a control diet (Folman *et al.*, 1989). Thus, dietary intake of β-carotene (and presumably other provitamin A carotenoids) contributes directly to the synthesis of all-*trans*-retinoic acid and consequently to the concentrations of all-*trans*- and 13-*cis*-retinoic acid in the circulation.

Cytosol preparations from rat tissues catalyse the formation of all-*trans*-retinoic acid from β-carotene (Napoli & Race, 1988). Retinol that was generated during β-carotene metabolism was not the major substrate, and all-*trans*-retinal was not detected as a free intermediate in this process. Thus, it might be tightly bound by the enzyme, or β-carotene might be oxidized to a 15,15'-enediol before dioxygenase cleavage, by analogy to the conversion of catechol to *cis,cis*-muconic acid. Homogenates of liver, lung, kidney and fat from monkeys, ferrets and rats incubated with β-carotene generate all-*trans*-retinoic acid (Wang *et al.*, 1991) through a biochemical process which does not involve all-*trans*-retinal as an intermediate (Wang *et al.*, 1992).

2.2.2.4 *Oxidative metabolism of all-*trans*-retinoic acid*

The most abundant oxidized metabolites produced from all-*trans*-retinoic acid are its 4-hydroxy and 4-oxo derivatives, with some all-*trans*-5,6-epoxy-retinoic acid.

(i) Metabolites

Roberts *et al.* (1979) demonstrated in hamster liver microsome preparations that all-*trans*-retinoic acid is first converted to all-*trans*-4-hydroxyretinoic acid, which in turn is oxidized to the 4-oxo derivative. The formation of 4-hydroxyretinoic acid required NADPH, whereas the subsequent formation of 4-oxoretinoic acid is NAD^+-dependent (Roberts *et al.*, 1980). Frolik *et al.* (1980) established that both all-*trans*-4-hydroxy- and all-*trans*-4-oxoretinoic acid are formed *in vivo* after administration of all-*trans*-[^{3}H]retinoic acid to hamsters maintained on a control diet. Subsequent work by Leo *et al.* (1984, 1989b) and Roberts *et al.* (1992) demonstrated that CYP isoforms in rat and in human liver preparations promote conversion of all-*trans*-retinoic acid to its 4-hydroxy and 4-oxo-forms. Hence, the CYP system plays a role in the physiological formation of these 4-oxygenated retinoids.

Barua *et al.* (1991) found that rats given large oral doses of all-*trans*-retinoic acid had significant amounts of both all-*trans*-4-hydroxy- and all-*trans*-4-oxoretinoic acid in their serum, small intestine, liver, kidney and stomach contents within 60 min. The intestinal mucosa of vitamin A-deficient rats given all-*trans*-[^{3}H]retinoic acid formed 5,6-epoxyretinoic acid, which was shown to be a metabolite of retinoic acid *in vivo* (McCormick *et al.*, 1978). Napoli *et al.* (1982) showed that all-*trans*-5,6-epoxyretinoyl-β-glucuronide was also formed in the small intestinal mucosa of vitamin A-deficient rats given intrajugular doses of all-*trans*-[^{3}H]5,6-epoxyretinoic acid. This compound was found in significant concentrations in the liver, small intestinal mucosa and intestinal contents, but not in the kidney, of vitamin A-deficient rats. It was synthesized in the kidney of vitamin A-sufficient rats given physiological doses of [^{3}H]retinol, suggesting that both retinoic acid and all-*trans*-5,6-epoxyretinoic acid are formed endogenously from retinol under normal physiological conditions (McCormick & Napoli, 1982). Barua *et al.* (1991) also reported that all-*trans*-5,6-epoxyretinoic acid was present in the serum, small intestine, liver and kidney of control rats given a large oral dose of all-*trans*-retinoic acid.

(ii) Enzymes and enzyme systems

CYP isoenzymes appear to be important in the formation of oxidized metabolites of all-*trans*-retinoic acid, and a novel isoform, CYP26, has been implicated as a catalyser in the oxidative metabolism of all-*trans*-retinoic acid. Formation of polar metabolites of retinoic acid is catalysed by rat intestine and liver microsomes, the activity being attributed to members of a class of mixed-function oxidases containing

CYPs (Roberts *et al.*, 1979). This activity requires NADPH and oxygen and is strongly inhibited by carbon monoxide. Leo *et al.* (1984) reported that rats fed a diet containing a 100-fold excess of retinyl acetate for two to three weeks had an increased hepatic microsomal CYP content. Purified cyto-chromes P450f and P450b catalysed the conversion of retinoic acid to polar metabolites, including 4-hydroxy-retinoic acid. The P450 isozyme P450IIC8 in human liver microsomes was shown to be responsible for oxidizing all-*trans*-retinoic acid to all-*trans*-4-hydroxyretinoic acid and all-*trans*-4-oxoretinoic acid (Leo *et al.*, 1989b).

Many rabbit liver CYP isoforms, including 2A4, 1A2, 2E1, 2E2, 2C3, 2G1 and 3A6, catalyse the 4-hydroxylation of retinoic acid (Roberts *et al.*, 1992). These cytochromes also catalysed the 4-hydroxylation of retinol and retinal but not the conversion of 4-hydroxyretinoids to the corresponding 4-oxoretinoids. Van Wauwe *et al.* (1992) showed that oral administration of a dose of 40 mg/kg body weight of liarazole, which inhibits CYP activity, enhanced the endogenous plasma concentrations of retinoic acid from less than 1.7 to 10–15 nmol/L.

CYP26, which can metabolize retinoic acid, was cloned independently by several groups (White *et al.*, 1996; Fujii *et al.*, 1997; Ray *et al.*, 1997; White *et al.*, 1997a). Fujii *et al.* (1997) reported that expressed murine P450RA cDNA catalysed the oxidation of all-*trans*-retinoic acid to all-*trans*-5,6-epoxyretinoic acid. Both 13-*cis*- and 9-*cis*-retinoic acid were found to serve as substrates for P450RA. This isoform was expressed in a stage- and region-specific fashion during mouse development, but expression did not appear to be inducible after exposure of mouse embryos to excess retinoic acid. In adult mice, P450RA was expressed only in liver.

White *et al.* (1996) reported the isolation and characterization of a cDNA for CYP26 from zebrafish, which they called P450RAI. This isoform was found to be expressed during gastrulation. When the cDNA for P450RAI was expressed in COS-1 cells, all-*trans*-retinoic acid was rapidly metabolized to more polar metabolites including all-*trans*-4-oxoretinoic acid and all-*trans*-4-hydroxyretinoic acid. Thus, P450RAI, which is induced after exposure to retinoic acid, catalyses the oxidative metabolism of retinoic acid. White *et al.* (1997a) cloned the human homologue of zebrafish P450RAI, the enzyme that catalyses hydroxylation of all-*trans*-retinoic acid to its 4-hydroxy, 4-oxo and 18-hydroxy forms. Moreover, *P450RAI* mRNA expression was highly induced by treatment of human LC-T, MCF-7, HB4 and HepG2 cells with retinoic acid.

Abu-Abed *et al.* (1998) reported the cloning of the mouse homologue for P450RAI which catalyses metabolism of retinoic acid into 4-hydroxyretinoic acid, 4-oxoretinoic acid and 18-hydroxyretinoic acid. They observed a direct relationship between the level of retinoic acid metabolic activity and retinoic acid-induced P450RAI mRNA in wild-type F9 cells and derivatives lacking RARs and/or RXRs, and suggested that RARγ and RXRα mediate the induction of the expression of the *P450RAI* gene by retinoic acid.

Simultaneously, Ray *et al.* (1997) reported the cloning of CYP26 (identical to P450RA and P450RAI) from a mouse embryonic stem cell library. Expression of this isoform was limited in the adult to liver and brain and was detectable in mouse embryos as early as day 8.5. Moreover, *CYP26* mRNA was upregulated during the retinoic acid-induced neural differentiation of mouse embryonic stem cells *in vitro*. These authors also cloned human CYP26, in which 195 of 200 amino acids were identical to those of mouse CYP26. In human adults, CYP26 is expressed in liver, brain and placenta.

The total retinol concentrations in liver from aryl hydrocarbon receptor-null ($AHR^{-/-}$) mice are approximately threefold higher than those in wild-type mice (Andreola *et al.*, 1997). In addition, $AHR^{-/-}$ mice showed a reduced capacity to oxidize retinoic acid and significantly lower hepatic levels of mRNA for retinaldehyde dehydrogenase types 1 and 2, although expression of CYP26 was similar in the AHR-deficient and wild-type mice, in keeping with earlier observations that mice and rats given 2,3,7,8-tetrachlorodibenzo-*para*-dioxin show a rapid decline in total hepatic retinol concentrations (Brouwer *et al.*, 1985; Chen *et al.*, 1992a).

A direct role for cellular retinoic acid-binding protein, type I (CRABP I) in the oxidative metabolism of retinoic acid was proposed by Fiorella and Napoli (1991). When all-*trans*-retinoic acid is bound to CRABP I, microsomal enzymes of rat testes catalyse the conversion of retinoic acid to 3,4-didehydro-, 4-hydroxy-, 4-oxo-, 16-hydroxy-4-oxo- and 18-hydroxy-retinoic acids. Ketoconazole inhibited oxidation by testis microsomes of both free and CRABP I-bound retinoic acid, suggesting that CYP isozymes are involved in this metabolism. CRABP I may well play a direct role in the oxidative metabolism of all-*trans*-retinoic acid.

2.2.2.5 *Other metabolism of all-*trans*-retinoic acid*

When all-*trans*-retinoic acid is given orally to rats, all-*trans*-retinoyl-β-glucuronide is secreted into the bile (Dunagin *et al.*, 1966; Swanson *et al.*, 1981; Skare & DeLuca, 1983; Frolik, 1984). Retinoyl-β-glucuronide can be synthesized from all-*trans*-retinoic acid and UDP-glucuronic acid in the liver, intestine, kidney and other tissues by several members of the microsomal glucuronyl transferase family of enzymes (Lippel & Olson, 1968; Frolik, 1984). The intestinal mucosa seems to be the most active tissue in synthesizing and retaining retinoyl-β-glucuronide (Zile *et al.*, 1982; McCormick *et al.*, 1983; Cullum & Zile, 1985; Barua *et al.*, 1991). After all-*trans*-retinoic acid was administered to rats, the all-*trans*-retinoyl-β-glucuronides were shown to consist of all-*trans*- and 13-*cis*-retinoyl-β-glucuronides in a ratio of 1.5 to 1.0 (Zile *et al.*, 1982), but when 13-*cis*-retinoic acid was administered all-*trans*-retinoyl-β-glucuronide was a major metabolite in rats *in vivo* (McCormick *et al.*, 1983; Meloche & Besner, 1986). Isomerization to all-*trans*-retinoic acid probably, but not necessarily, occurs before the conjugation reaction (McCormick *et al.*, 1983).

all-*trans*-Retinoyl-β-glucuronide can be synthesized from retinoic acid and UDP-glucuronic acid by extracts of rat liver, intestine, kidney and other tissues (Barua & Olson, 1986; Barua, 1997). Genchi *et al.* (1996) demonstrated that the relative rates of retinoid-β-glucuronide formation from uninduced rat liver microsomes were: 9-*cis*-retinoic acid > 13-*cis*-retinoic acid > all-*trans*-4-oxoretinoic acid > all-*trans*-retinoic acid > 13-*cis*-retinol > 9-*cis*-retinol > all-*trans*-retinol. They concluded that the rates of glucuronidation of retinoids depend on both the isomeric state and the chemical structure of the retinoids and that different UDP-glucuronosyl transferases might act on different geometric isomers of retinoic acid.

A specific UDP-glucuronosyl transferase with all-*trans*-[^{3}H]retinoic acid as a substrate was identified in rat liver microsomes by photoincorporation of both [^{32}P]5-azido-UDP-glucuronic acid and all-*trans*-[^{3}H]retinoic acid into the 52-kDa microsomal protein. This enzyme (and possibly similar ones) plays a physiologically relevant role in hepatic synthesis of retinoyl-β-glucuronides (Little & Radominska, 1997). Recombinant rat UDP-glucuronosyl transferase 1.1 catalyses the glucuronidation of all-*trans*-retinoic acid with an apparent K_m of 59.1 ± 5.4 mmol/L. Micro-somes from the livers of Gunn rats, which lack this enzyme, still have significant all-*trans*-retinoic acid glucuronidating activity, suggesting that other hepatic UDP-glucuronosyltransferase isoforms also contribute to retinoic acid glucuronidation (Radominska *et al.*, 1997).

Although retinoyl-β-glucuronides can be excreted into bile, they can also be hydrolysed to retinoic acid by enzymes present in rat liver, kidney and intestine (Kaul & Olson, 1998). The rates of hydrolysis were higher in tissue preparations from vitamin A-deficient rats than from controls, indicating that a regulatory mechanism may exist in vitamin A-deficiency that enhances the actions of tissue β-glucuronidases to increase the rate of retinoic acid formation from retinoyl-β-glucuronides.

About one-third of the retinoid-β-glucuronides excreted into the bile of rats is recycled back to the liver by enterohepatic circulation (Zachman *et al.*, 1966; Swanson *et al.*, 1981). Very little retinol, retinyl ester or retinoic acid is involved in the circulation, however, and the water-soluble retinyl-β-glucuronide and retinoyl-β-glucuronide are presumably reabsorbed by the portal rather than by the lymphatic route (Ribaya-Mercado *et al.*, 1988).

*2.2.2.6 Biosynthesis of 13-*cis*-retinoic acid*

Cullum and Zile (1985) reported that 13-*cis*-retinoic acid is endogenous in the intestinal mucosa, intestinal muscle and plasma of vitamin A-sufficient rats. When 20 mg all-*trans*-[^{3}H]retinyl acetate were administered to vitamin A-sufficient rats, 13-*cis*-retinoic acid appeared in the plasma and small intestine, and the authors concluded that 13-*cis*-retinoic acid is a naturally occurring metabolite of all-*trans*-retinyl acetate. Napoli *et al.* (1985) similarly demonstrated that 13-*cis*-retinoic acid is a naturally occurring form of retinoic acid in rat plasma, possibly arising from all-*trans*-retinoic acid.

Incubation of 13-*cis*-retinoic acid (0.83 mmol/L) with mouse liver microsomes in the presence of appropriate cofactors yielded all-*trans*-retinoic acid, 13-*cis*- and all-*trans*-4-hydroxyretinoic acid and 13-*cis*- and all-*trans*-4-oxoretinoic acid as the major metabolites. Metabolism of 13-*cis*-retinoic acid was not detectable in microsomal preparations from mouse skin, indicating that CYP isozymes in liver but not in skin are active towards 13-*cis*-retinoic acid (Oldfield, 1990).

Chen and Juchau (1997) reported that purified hepatic glutathione *S*-transferases from rats catalysed the isomerization of 13-*cis*-retinoic acid to all-*trans*-retinoic acid. The reaction was protein-dependent and independent of the presence of glutathione, indicating that the isomerization reaction is not linked to glutathione *S*-transferase activity. Chen and Juchau (1998) demonstrated that conversion of 13-*cis*- and 9-*cis*-retinoic acid to all-*trans*-retinoic acid can be catalysed by cell-free preparations from rat embryo tissue at day 12.5 of gestation. The isomerization was protein-dependent and showed substrate saturation kinetics.

When small doses of 13-*cis*-[^{3}H]retinoic acid were administered to hamsters on a control diet, the major metabolite formed was 13-*cis*-4-oxoretinoic acid (Frolik *et al.*, 1980). A 10 000 x *g* supernatant from hamster liver homogenate exposed to 13-*cis*-[^{3}H]retinoic acid similarly generated 13-*cis*-4-oxoretinoic acid as the major metabolite. 13-*cis*-4-Hydroxyretinoic acid, a putative metabolic precursor of 13-*cis*-4-oxoretinoic acid, was also identified in these experiments.

*2.2.2.7 Biosynthesis of 9-*cis*-retinoic acid*

As outlined in section 2.1.1.6., the 9-*cis*-isomer of retinoic acid may be formed directly from all-*trans*-retinoic acid or by an independent pathway. Membranes from bovine liver non-enzymatically catalyse the isomerization of all-*trans*-retinoic acid to 9-*cis*-retinoic acid due to the presence of free sulfhydryl groups (Urbach & Rando, 1994). Shih *et al.* (1997) also showed that interconversion of 9-*cis*-retinoic acid, all-*trans*-retinoic acid, 13-*cis*-retinoic acid and 9,13-di-*cis*-retinoic acid may be catalysed by sulfhydryl compounds of low relative molecular mass (including L-cysteine methyl ester, glutathione and *N*-acetyl-L-cysteine), and by proteins containing sulfhydryl groups (apoferritin, native microsomes and boiled microsomes). Sass *et al.* (1994) reported that both treated and untreated rat liver microsomes can catalyse the formation of 9-*cis*-retinoic acid and its glucuronide from all-*trans*- and 13-*cis*-retinoic acid.

Hébuterne *et al.* (1995) reported that 9-*cis*-β-carotene is a precursor for 9-*cis*-retinoic acid *in vivo* in perfused ferrets, but the significance of this pathway for the formation of 9-*cis*-retinoic acid has been questioned since the rate of cleavage of 9-*cis*-β-carotene in pigs is only 6–7% that of all-*trans*-β-carotene (Nagao & Olson, 1994). Furthermore, studies by You *et al.* (1996) in humans (see section 2.1.1) indicate that very little 9-*cis*-β-carotene is absorbed without undergoing isomerization to all-*trans*-β-carotene. Since rats and other animals maintained on carotenoid-free diets remain healthy, the conversion of 9-*cis*-β-carotene to 9-*cis*-retinoic acid cannot be essential for formation of this retinoic acid isomer. Labrecque *et al.* (1993, 1995) reported that the retinal dehydrogenase present in rat kidney can catalyse the oxidation of 9-*cis*-retinal to 9-*cis*-retinoic acid and suggested that a pathway starting with 9-*cis*-retinol may be important for 9-*cis*-retinoic acid formation. They demonstrated the presence of 9-*cis*-retinol in rat kidney at a concentration approximately 10% of that of all-*trans*-retinol.

An NAD^+-dependent retinol dehydrogenase which specifically oxidizes 9-*cis*-retinol but not all-*trans*-retinol has been cloned from a human

mammary tissue cDNA library (Mertz *et al.*, 1997; see section 2.1.1.6). Although it was suggested that the protein and mRNA of 11-*cis*-retinol dehydrogenase occur only in the retinal pigment epithelium (Simon *et al.*, 1995) and that the enzyme does not catalyse 9-*cis*-retinol oxidation (Suzuki *et al.*, 1993), this appears to be incorrect (Driessen *et al.*, 1998). The mouse homologue of 9-*cis*-retinol dehydrogenase is expressed in brain, liver, kidney and testis and at low concentrations in several other tissues and shows a marked substrate preference for 9-*cis*-retinol (Gamble *et al.*, 1999). 13-*cis*-Retinoic acid is a very potent inhibitor of 9-*cis*-retinol dehydrogenase activity. The enzyme is expressed in human and mouse kidney, tissues which have been reported to contain significant quantities of 9-*cis*-retinol and 9-*cis*-retinal dehydrogenase activity (Labrecque *et al.*, 1993, 1995).

A second *cis*-retinol dehydrogenase, also a member of the SCDR family, was described by Chai *et al.* (1997). The cDNA for this mouse liver enzyme, known as the *cis*-retinol/3α-hydroxy sterol short-chain dehydrogenase 1 (CRAD1), encodes an enzyme comprising 317 amino acids that recognizes 9-*cis*- and 11-*cis*-retinol, 5α-androstan-3α,17β-diol and 5α-androstan-3α-ol-17-one as substrates, although it has a greater affinity for sterol than for retinol substrates. The preferred cofactor of this mouse enzyme, which is closely homologous to mouse RoDH(I) (86%) and RoDH(II) isozymes (91%), is NAD^+, and it is expressed in liver, kidney, small intestine, heart, retinal pigment epithelium, brain, spleen, testis and lung.

Su *et al.* (1998) cloned a second *cis*-retinol/3α-hydroxysteroid short-chain dehydrogenase isozyme (CRAD2) from a mouse embryonic cDNA library, which has 87% amino acid identity with CRAD1, recognizes both androgens and retinols as substrates and has cooperative kinetics for 5α-androstan-3α,17β-diol (3α-adiol) and testosterone but Michaelis–Menten kinetics for androsterone, all-*trans*-, 11-*cis*- and 9-*cis* retinols. *CRAD2* mRNA was highly expressed in mouse liver and at much lower levels in lung, eye, kidney and brain.

*2.2.2.8 Subsequent metabolism of 9-*cis*-retinoic acid*

Horst *et al.* (1995) reported that 9,13-di-*cis*-retinoic acid is the major endogenous circulating retinoic acid isomer in bovine plasma. After intravenous administration of this retinoic acid to rats, circulating 9-*cis*-retinoic acid was found at a concentration that was approximately 3% of that of 9,13-di-*cis*-retinoic acid. When 9-*cis*-retinoic acid was injected intramuscularly into rats, the peak plasma concentration of 9,13-di-*cis*-retinoic acid followed that of 9-*cis*-retinoic acid by about 1.5 h, and detectable concentrations were seen for at least 24 h, whereas the concentration of 9-*cis*-retinoic decreased below the limit of detection (< 1.7 nmol/L) by 12 h after injection.

2.2.3 Plasma transport and kinetics

The plasma of rats contains low concentrations of all-*trans*- and 13-*cis*-retinoic acid (Cullum & Zile, 1985; Napoli *et al.*, 1985). Plasma all-*trans*-retinoic acid may be an important source of this retinoid for some but not all tissues (Kurlandsky *et al.*, 1995).

2.2.3.1 Plasma concentrations, transport and kinetics

Plasma all-*trans*-retinoic acid may be derived from dietary sources and presumably from endogenous metabolism of retinol within tissues. all-*trans*-Retinoic acid appears in the circulation after intraduodenal, oral or intraperitoneal administration of radiolabelled all-*trans*-retinoic acid to experimental animals (Zachman *et al.*, 1966; Geison & Johnson, 1970; Ito *et al.*, 1974; Skare & DeLuca, 1983). In rats, retinoic acid is absorbed in the intestine, and about two-thirds of the absorbed dose is distributed as retinoic acid throughout the body (Smith *et al.*, 1973). Lehman *et al.* (1972) and Smith *et al.* (1973) demonstrated that circulating retinoic acid is bound to serum albumin but not to retinol-binding protein. When radiolabelled all-*trans*-retinoic acid was administered in dimethyl sulfoxide by intrajugular injection to rats, it was rapidly (< 2 min) taken up by the intestines and other tissues; subsequently, 13-*cis*-retinoic acid and uncharacterized polar metabolites appeared in plasma (Cullum & Zile, 1985).

Two further extracellular proteins, epididymal retinoic acid-binding protein (Newcomer & Ong, 1990) and β-trace (Eguchi *et al.*, 1997; Tanaka *et al.*, 1997), are reported to bind all-*trans*-retinoic acid with high affinity. These proteins, like plasma retinol-binding protein, are members of the lipocalin family of extracellular lipid-binding proteins (Flower, 1996). Both epididymal retinoic acid-binding protein and β-trace have been proposed to play a role in the extracellular transport and economy of all-*trans*-retinoic acid in the body (Newcomer & Ong, 1990; Eguchi *et al.*, 1997). Targeted disruption of the mouse *β-trace* gene results in a lack of tactile pain (Eguchi *et al.*, 1999).

Using a steady-state tracer kinetic approach, Kurlandsky *et al.* (1995) explored the contribution of plasma all-*trans*-retinoic acid to tissue pools of all-*trans*-retinoic acid in chow-fed male rats. More than 75% of the all-*trans*-retinoic acid present in liver and brain was derived from the circulation, while the results were 23% for seminal vesicles, 9.6% for epididymis, 33% for kidney, 30% for epididymal fat, 24% for perirenal fat, 19% for spleen and 27% for lungs. Only 2.3% of the retinoic acid present in the pancreas and 4.8% of that in the eyes was contributed by the circulation. The testis did not take up any (< 1%) all-*trans*-retinoic acid from the circulation. The fractional catabolic rate for this compound in plasma was 30 plasma pools per hour and the absolute catabolic rate was 640 pmol/h. These rates are much greater than those of all-*trans*-retinol, the only other naturally occurring form of vitamin A studied under normal physiological conditions (Lewis *et al.*, 1990). Very little 9-*cis*- or 13-*cis*-retinoic acid was detected in any of these tissues.

Kurlandsky *et al.* (1995) also assessed the rate of plasma clearance of all-*trans*-3-[^{3}H]retinoic acid and [^{14}C]oleic acid bound to albumin and given simultaneously to rats maintained on a control or a totally retinoid-free diet. The rats on the retinoid-free diet had a significantly greater fractional catabolic rate for all-*trans*-retinoic acid (23 ± 5.5 versus 12 ± 4.6 pools/h) than those on control diet. Moreover, the fraction catabolic rates for oleic acid in these rats were not affected by dietary retinoid intake (43 ± 6.0 pools/h for controls and 38 ± 7.2 pools/h for the retinoid-free-group). Thus, dietary retinoid status influences all-*trans*-retinoic acid but not oleic acidturnover from the circulation The β-carotene content of the diet affected the serum concentrations of all-*trans*-retinoic acid in rabbits (Folman *et al.*, 1989).

2.2.3.2 Cellular uptake from plasma

Bovine serum albumin has three distinct binding sites for retinoic acid at mole ratios for retinoic acid:albumin of less than 1. Noy (1992a) reported that two of these binding sites correlated to two known binding sites for long-chain fatty acids, but one appeared to be a unique site for retinoic acid binding. Noy (1992b) found that the protonated (uncharged) form of retinoic acid, like other hydrophobic carboxylic acids (fatty acids and bile acids), was stabilized by incorporation into lipid bilayers. At physiological pH, uncharged retinoic acid crossed membranes rapidly and spontaneously. all-*trans*-Retinoic acid, although fully ionized in free solution at pH 7.4, is uncharged in a lipid environment (Noy, 1992a,b).

all-*trans*-Retinoic acid binds to the mannose-6-phosphate/insulin-like growth factor-II receptor on plasma membranes of rat cardiac myocytes (Kang *et al.*, 1998). The binding of all-*trans*-retinoic acid to this membrane receptor appears to be important in modulating the activity of cellular signal transduction pathways and cellular activity but not for facilitating its uptake from the circulation. Hodam and Creek (1996) showed that all-*trans*-[^{3}H]retinoic acid was taken up rapidly by a culture medium containing human foreskin keratinocytes and converted to polar compounds that were subsequently excreted. In contrast, retinoic acid bound to albumin was taken up and metabolized slowly.

2.2.4 Tissue distribution

Cullum and Zile (1985) reported the endogenous concentrations of all-*trans*- and 13-*cis*-retinoic acid and retinoyl-β-glucuronide and a polar metabolite fraction consisting of all-*trans*-5,6-epoxy-retinoic acid, all-*trans*-4-oxoretinoic acid and other polar metabolites in rat intestinal mucosa, intestinal muscle, plasma, erythro-

cytes and bile (Table 4). Heyman *et al.* (1992) estimated that the endogenous concentrations of 9-*cis*-retinoic acid in mouse liver and kidney were 13 and 100 pmol/g tissue, respectively. Kurlandsky *et al.* (1995) reported the concentrations of all-*trans*-retinoic acid in eight tissues of rats (Table 5). They did not detect 9-*cis*-retinoic acid in liver, spleen, kidney, brain, adipose tissue, testis or muscle even though the limit of detection for 9-*cis*-retinoic acid was only 4 pmol/g of tissue. The concentrations of all-*trans*- and 13-*cis*-retinoic acid have also been determined in rat conceptuses (Creech Kraft & Juchau, 1992).

2.2.5 *Intra- and inter-species variation*

The metabolism of all-*trans*-, 13-*cis*- and 9-*cis*-retinoic acid differs between species in a number of significant ways. The pharmacokinetics and metabolic profiles of all-*trans*- and 13-*cis*-retinoic acid in mice, monkeys and humans vary widely (Creech Kraft *et al.*, 1991a,b). The predominant metabolite of all-*trans*-retinoic acid is all-*trans*-4-oxoretinoic acid in mice and all-*trans*-retinoyl-β-glucuronide in monkeys. The predominant metabolite of 13-*cis*-retinoic acid is 13-*cis*-retinoyl-β-glucuronide in mice and 13-*cis*-4-oxoretinoic acid in monkeys and humans, although the metabolite is much more predominant in humans than in monkeys. all-*trans*-Retinoic acid was more rapidly cleared than 13-*cis*-retinoic acid in monkeys, while the reverse was observed in mice.

Marchetti *et al.* (1997) found that all-*trans*-, 13-*cis*- and 9-*cis*-retinoic acid each underwent substantial isomerization to the other two in hepatic microsome preparations from male and female Sprague-Dawley and Hairless rats. The isomerization was independent of NADPH and dependent on the presence of microsomal protein. The 4-oxo metabolites of these retinoic acid isomers were formed to a lesser extent. The authors concluded that the metabolism of retinoic acid isomers is influenced by sex but not strain.

3. Molecular mechanisms of retinoid action

Retinoids are signalling molecules that act through interaction with two families of retinoid receptors, RARs α, β and γ and RXRs α, β and γ. These receptors belong to the super-

Table 4. Endogenous vitamin A compounds in plasma, erythrocytes, small intestine and bile of vitamin A-sufficient rats

Endogenous vitamin A compound	Concentration (ng/g tissue)				
	Intestinal mucosa	Intestinal muscle	Plasma	Erythrocytes	Bile
Retinyl stearate	3.5	7.2	ND	ND	ND
Retinyl palmitate	30	22	0.8	1.1	ND
Retinyl linoleate	19	7.7	ND	ND	ND
Retinol	47	7.4	400	28	31
all-*trans*-Retinoic acid	2.3	1.1	2.7	1.1	7.7
13-*cis*-Retinoic acid	1.0	0.2	0.9	0.6	6.1
Retinoyl-β-glucuronide	1.3	ND	ND	ND	19
all-*trans*-5,6-Epoxyretinoic acid + all-*trans*-4-oxoretinoic acid + polar metabolites	7.5	13	5.5	2.8	390
Total retinoids	110	59	410	34	450

From Cullum & Zile (1985). Values represent the rounded average of two determinations for samples obtained from four rats 48 h after feeding of retinyl acetate and 12 h after fasting. Bile was collected 2 h before sacrifice; ND, not detected

Table 5. Concentrations of all-*trans*-retinoic acid in various tissues of rats

Tissue	Concentration (pmol/g tissue)
Liver	11 ± 4.7
Brain	6.8 ± 3.3
Testis	11 ± 2.7
Seminal vesicle	12 ± 7.0
Epididymis	4.2 ± 1.6
Kidney	8.3 ± 4.0
Pancreas	29 ± 16
Epididymal fat	16 ± 12
Perirenal fat	13 ± 8.7
Spleen	13 ± 12
Eye	120 ± 37
Plasma	1.8 ± 0.7 pmol/ml

From Kurlandsky *et al.* (1995). Each value is the mean and standard deviation for separate measurements in eight 400–450-g male Sprague-Dawley rats.

family of nuclear receptors, comprising such diverse receptors as those for steroids and thyroid hormones, retinoids and vitamin D3, present in vertebrates, arthropods and nematodes. The members of this superfamily act both as ligand-modulated transcriptional activators and suppressors, while no ligands have yet been found for a large group of so-called 'orphan' nuclear receptors. Nuclear receptors may have acquired ligand-binding ability during evolution, suggesting that the ancestral nuclear receptor was an orphan (Escriva *et al.*, 1997).

Retinoid receptors regulate complex physiological events that trigger key steps in development, control maintenance of homeostasis and induce or inhibit cellular proliferation, differentiation and death. Retinoid receptors have strong differentiation and anti-proliferative activity. Each of the subtypes of retinoic acid comprises three isotypes designated α, β and γ localized to chromosomes 17q21, 3p24 and 12q13, respectively. The RXR α, β and γ genes have been mapped to chromosomes 9q34.3, 6p21.3 and 1q22-23, respectively (Chambon, 1996). The RARs bind both all-*trans*-retinoic acid and 9-*cis*-retinoic acid, whereas the RXRs bind only 9-*cis*-retinoic acid. These receptors also bind a variety of synthetic retinoids, some of which show RAR or RXR selectivity or preferentially bind to specific RAR isotypes.

The genetic activities of retinoid receptors and other nuclear receptors result from both direct modulation of the activity of cognate gene programmes and mutual interference with the activity of other signalling pathways and regulatory events that occur at the post-transcriptional level (e.g. mRNA and protein stabilization or destabilization). More than 70 nuclear receptors have been identified. With the exception of some unusual nuclear receptors which appear to contain only regions homologous to the DNA- or ligand-binding domains, all have an identical structural organization, with an *N*-terminal region A/B followed by a DNA-binding domain comprised of two zinc fingers (region C), a linker region D and the ligand-binding domain. Some nuclear receptors contain a C-terminal region F of unknown function. Two autonomous *trans*-activation functions (AFs), a constitutively active AF-1 in region A/B and a ligand-dependent AF-2 in the ligand-binding domain, are responsible for the transcriptional activity of nuclear receptors (Gronemeyer & Laudet, 1995; Chambon, 1996). Figure 3 illustrates the canonical domain structure and associated functions of nuclear receptors.

For each RAR and RXR subtype, two to four isoforms (e.g. RARs β_1, β_2, β_3 and β_4) are generated by differential use of alternative promoters and alternative splicing; consequently, they differ in their A domain (Gronemeyer & Laudet, 1995; Chambon, 1996). Each receptor isoform may regulate a distinct subset of retinoid-responsive genes; these RARs have greater nucleotide sequence homology within single species (e.g. human RARα and human β), implying that RARs have distinct functions that have been conserved during evolution. Their expression is regulated spatio-temporally during embryonal development, and their expression patterns in certain adult tissues are distinct (Chambon, 1996). Studies with the embryonal carcinoma cell line F9, in which specific receptors have been disrupted by homologous

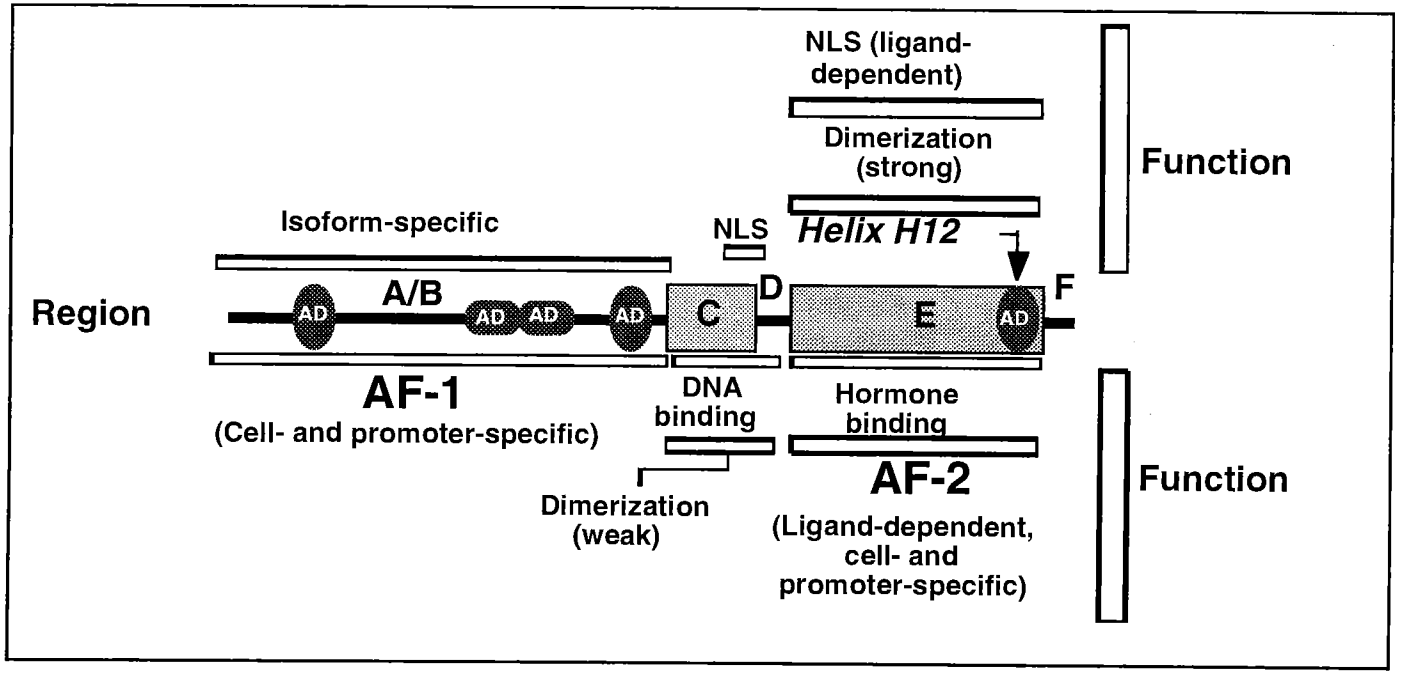

Figure 3. Schematic illustration of the structural and functional organization of nuclear receptors

The evolutionarily conserved regions C and E are indicated as boxes, and a black bar represents the divergent regions A/B, D and F. Note that region F may be absent in some receptors. Domain functions are depicted below and above the scheme; most were identified in structure–function studies of steroid, thyroid and retinoid receptors. Two transcription activation functions (AFs) have been described in several nuclear receptors: a constitutively active (only if taken out of the context of the full-length receptor) AF-1 in region A/B and a ligand-inducible AF-2 in region E. Within the AF-1s of some nuclear receptors, autonomous *trans*-activation domains (ADs) have been defined. At the C-terminal of the ligand-binding domain, the core of an activation domain, which is critical for AF-2 function, corresponds to the helix H12 in the three-dimensional structure of the ligand-binding domain. NLS, nuclear localization signal

recombination, have indicated that RARα regulates the expression of CRABP-II and the homeobox gene *Hoxb-1*, whereas RARγ mediates the expression of the *Hoxa-1*, *Hoxa-3*, *laminin B1*, *collage IV* (*alpha-1*), zinc finger transcription factor *GATA-4* and bone morphogenetic protein 2 (*BMP-2*) genes. Furthermore, RARα and RARγ were associated with opposite effects on the metabolism of all-*trans*-retinoic acid to more polar derivatives (Boylan *et al.*, 1995).

Nuclear receptors bind as homodimers (e.g. steroid receptors, RXR) and/or heterodimers (e.g. retinoic acid, thyroid and vitamin D receptors) with the promiscuous heterodimerization partner RXR to cognate response elements of target genes (Gronemeyer & Laudet, 1995; Chambon, 1996). Nuclear receptor activities are, however, not confined to cognate target genes; they can also 'cross-talk' in a ligand-dependent fashion with other signalling pathways, leading to mutual interference—positive or negative—with the *trans*-activation potentials of factors such as AP1 and NFκB (Pfahl, 1993; Göttlicher *et al.*, 1998). Other signalling pathways such as those operating through mitogen-activated protein kinase (Kato *et al.*, 1995; Hu *et al.*, 1996a) or cyclin-dependent kinase (CDK)7 (Rochette-Egly *et al.*, 1997) can target nuclear receptors directly and modify the activity of their AFs. For an illustration of the activities of nuclear receptors, see Figure 4.

Our understanding of nuclear receptor action at the molecular level has recently been enhanced dramatically due to progress in various domains, comprising:

- the identification and characterization of several novel classes of transcriptional mediators (transcription intermediary factors/co-regulators; co-activators and co-repressors) and the first steps towards the description of a plethora of interactions reported to occur between receptor, mediators, chromatin and the basal transcription machinery (Moras & Gronemeyer, 1998; Torchia *et al.*, 1998);
- the genetic analysis of receptor function (Beato *et al.*, 1995; Kastner *et al.*, 1995);
- the identification of novel (candidate) ligands for known and novel 'orphan' receptors (Mangelsdorf & Evans, 1995; Forman *et al.*, 1998; Kliewer *et al.*, 1998);
- determination of the three-dimensional structures of the apo-, holo- and antagonist-bound ligand-binding domains of several nuclear receptors (Brzozowski *et al.*, 1997; Moras & Gronemeyer, 1998; Nolte *et al.*, 1998; Shiau *et al.*, 1998) and prediction of a common fold of all nuclear receptor ligand-binding domains (Wurtz *et al.*, 1996);
- crystallization of complexes between agonist-bound nuclear receptor ligand-binding domains and peptides derived from the nuclear receptor-binding surface of co-activators which revealed the structural basis of (one type of) antagonism; and
- the synthesis, characterization and analysis of the three-dimensional structure of isotype-selective nuclear receptor ligands, particularly for RARs and RXRs.

3.1 DNA recognition by nuclear receptors

3.1.1 Response elements of nuclear receptors: The common principle

All nuclear receptors recognize derivatives of the same hexameric DNA core motif, 5'-PuGGTCA (Pu = A or G; this sequence corresponds to a so-called 'half-site' of the everted repeat (ER) recognition sequence in Figure 5). Nevertheless, mutation, extension, duplication and distinct relative orientations of repeats of this motif generate response elements that are selective for a given class of receptors (Figure 5). Apparently co-evolutionarily, nuclear receptors devised mechanisms to interact with these sequences optimally: they either modified residues which establish contacts to the nucleotides that specify a given response element or generated response element-adapted homo- or heterodimerization interfaces.

3.1.2 Synthetic ligand response elements and spacer 'rules'

A simple rule has been proposed to describe the preference of the various direct repeat (DR)-recognizing receptors for elements with a certain spacer length (Umesono & Evans, 1989; Kliewer *et al.*, 1992). According to this rule, DRn elements with *n* spacer nucleotides have the following specifications:

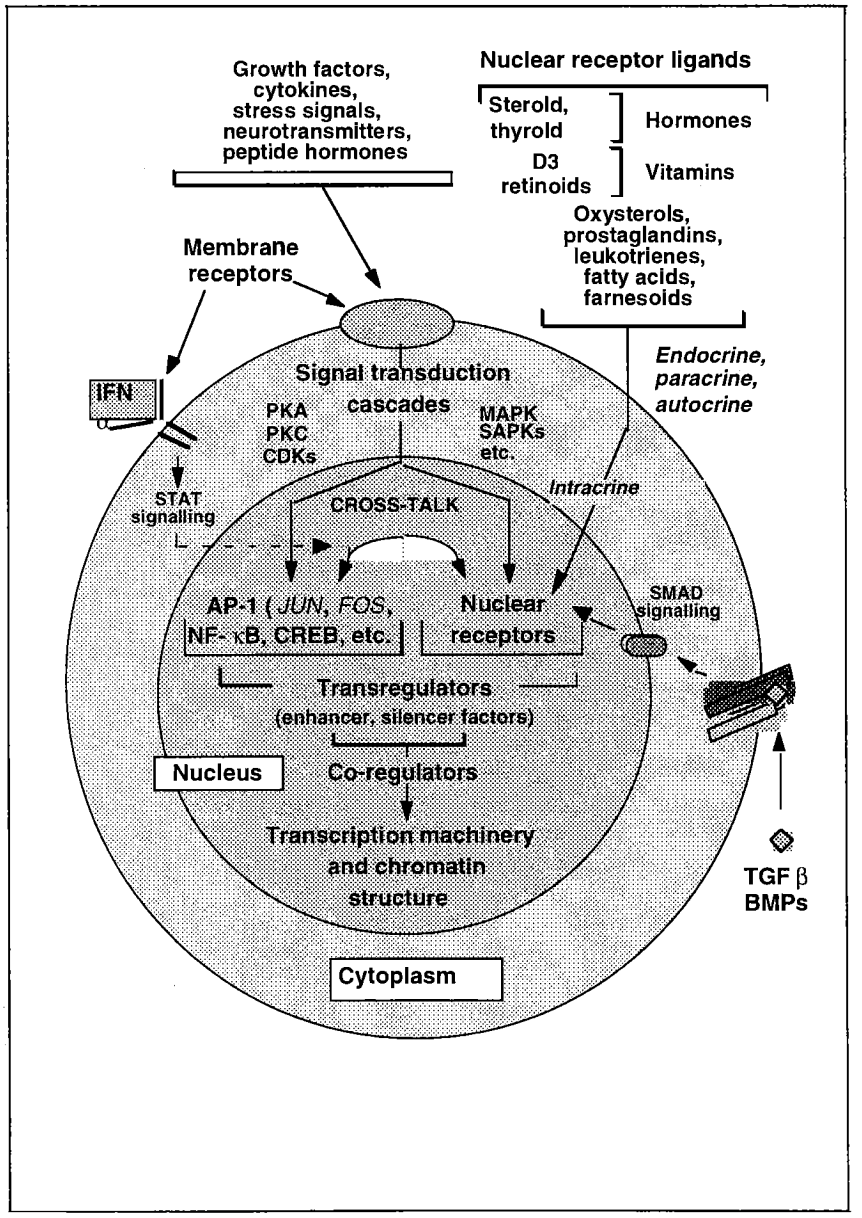

Figure 4. Schematic illustration of major signal transduction pathways involving membrane or nuclear receptors

Note the 'cross-talk' which gives rise to mutual interference between the various signalling cascades.

NR, nuclear receptor, IFN, interferon; PKA, protein kinase A; PKC, protein kinase C; CDK, cyclin-dependent kinase; MAPK, mitogen-activated protein kinase; SAPK, serum-activated protein kinase; STAT, signal transducer and activator of transcription; SMAD, mediator in transforming growth factor α (TGFα) signalling pathway; BMP, bone morphogenic protein

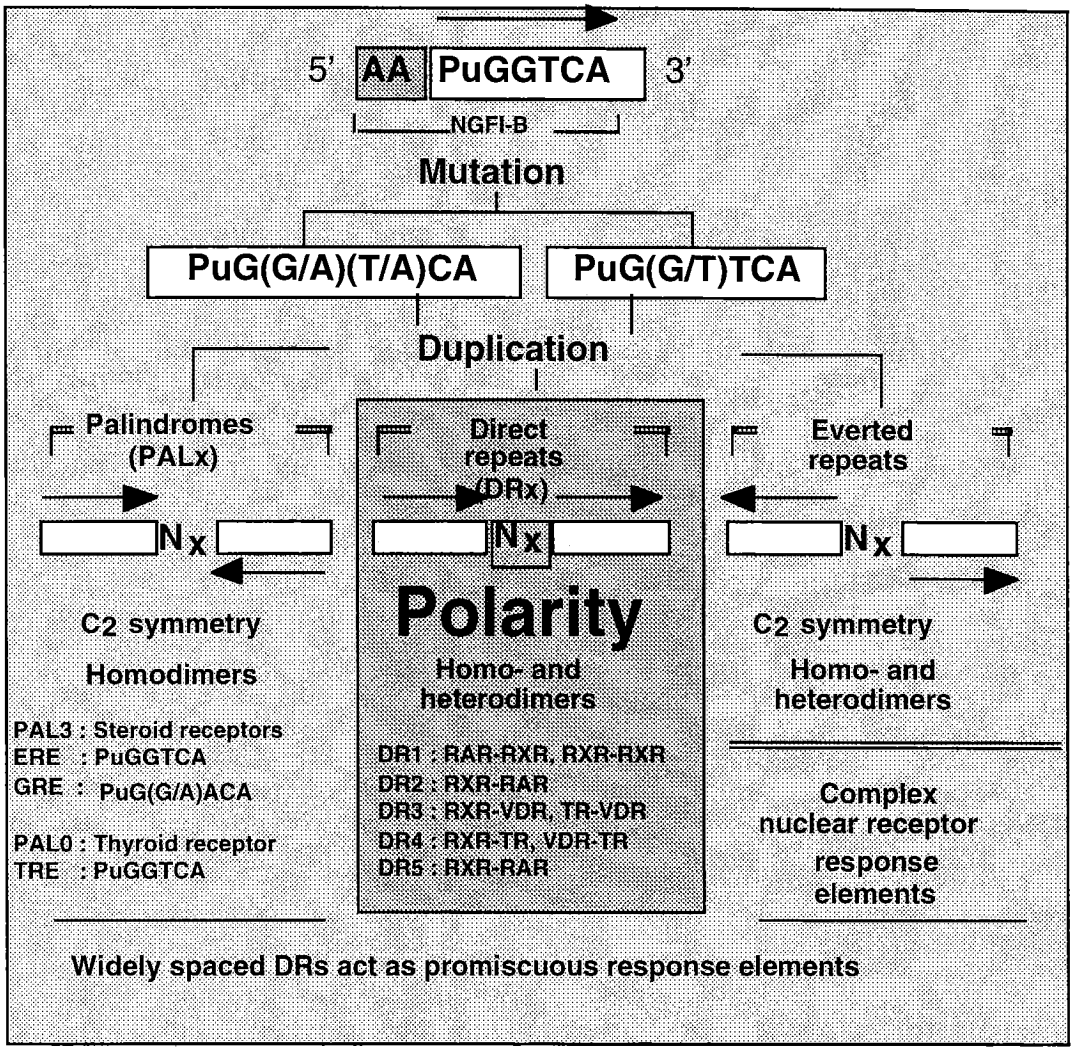

Figure 5. Response elements of nuclear receptors

The canonical core recognition sequence is 5′-PuGGTCA (arrows indicate the 5′ to 3′ direction) which, together with two 5′ As, is a response element of the orphan receptor NGFI-B. Duplication of the core sequence generates symmetrical palindromes (PALx) and everted repeats (ERx), and polar direct repeats (DRx), with x bp separating the two half-sites. PAL3 is an oestrogen-response element (ERE), while PAL0 corresponds to a thyroid hormone-response element (TRE). A single alteration at position 4 of the core sequence from T to A leads to PAL3 response elements (GREs) recognized by the glucocorticoid receptor (and also androgen, progesterone and mineralocorticoid receptors). Note that most response elements are far from ideal; often one of the half-sites contains one or more mutations. Whereas PALs bind homodimers, DRs can bind homo- or heterodimers with the specificities shown in the dark box (polarity of the receptors on their cognate DRs: left, 5′; right, 3′). Note that the 3′-positioned receptor makes minor groove DNA contacts in the spacer. Some everted repeats are response elements for homo- or heterodimers of the thyroid or retinoid receptors. Response elements are known which are comprised of complex arrangements of the core motif. TR, thyroid receptor; VDR vitamin D receptor

n	Systematic name	Acronym	Receptor complex
1	DR1	RXRE	RXR–RXR
2	DR2	RARE	RAR–RXR
3	DR3	VDRE	VDR–RXR
4	DR4	TRE	TR–RXR
5	DR5	RARE	RAR–RXR

where TR is thyroid receptor and VDR is vitamin D receptor.

This rule does not take into consideration a number of important aspects of receptor–DNA interaction:

- It is unclear whether DR1 RXREs exist in natural genes.
- DR1 elements have been shown to act as RAREs (Durand *et al.*, 1992).
- Several orphan receptors bind as homo- (HNF4) or heterodimers (COUP-TF/arp-1) with RXR to certain DR elements which 'belong' to other receptors according to the above spacer rule.
- The rule does not distinguish between homo- and heterodimers that may bind to distinct DR options (DR3 and DR6 VDREs).
- The rule does not take into consideration the polarity of the receptor–DNA complexes.

Note that, in addition to DR elements, several other types of (more complex) response elements exist (Figure 5).

3.1.3 Variability of the binding motif, spacer sequence and flanking nucleotides

There is considerable degeneration in the sequence of half-site motifs of a given type of natural retinoid response element (see Figure 6), and the various receptors have distinct preferences for certain motifs. For example, the preference for the half-site motif 5′-PuGGTCA over 5′-GTTCA follows the order TR > RXR > RAR (Mader *et al.*, 1993). A receptor-specific preference for certain nucleotides in the DR spacer is also seen. A DNA binding site selection with RAR/RXR, TR/RXR and VDR/RXR heterodimers to identify the optimal 3′-positioned motif and spacer sequence has been reported (Kurokawa *et al.*, 1993) and is easily rationalized in the light of the crystallographic data (Rastinejad *et al.*, 1995).

3.1.4 Response elements for retinoids

The classical retinoic acid response element (RARE), which was found in the P2 promoter of the *RARβ* gene and gives rise to *RARβ2* mRNA, is a 5 base pair-spaced direct repeat 4 (generally referred to as DR5) of the motif 5′-PuGTTCA (Figure 6). In addition, response elements with a DR5 containing the motif 5′-PuGGTCA (also termed DR5G to distinguish it from the DR5T of the RARβ2 promoter) act as perfect RAREs (de Thé *et al.*, 1990; Hoffmann *et al.*, 1990; Sucov *et al.*, 1990), as do direct 5′-PuGGTCA repeats spaced by one base pair (DR1) or two base pairs (DR2) (Figures 5 and 6). RAR–RXR heterodimers bind to and activate transcription from these three types of RARE, provided the target cells express both RARs and RXRs. DR1 elements bind not only RAR–RXR heterodimers but also RXR homodimers *in vitro*, and RXRs can *trans*-activate target genes containing DR1 elements in response to a RXR ligand. The notion that DR1 elements can act as functional retinoid X-receptor response elements (RXREs) *in vivo* is supported by their activity in yeast cells (Heery *et al.*, 1994), in which any contribution of endogenous RAR by heterodimerization with RXR can be excluded. The only reported natural RXRE is a DR1-related element found in the rat CRBPII promoter (Mangelsdorf *et al.*, 1991). RXR-specific induction of this CRBPII promoter *in vivo* has not yet been demonstrated, and the lack of conservation of the CRBPII RXRE in the mouse homologue (Nakshatri & Chambon, 1994) casts some doubt on its physiological role as an RXRE.

Further examples of genes that have a DR5 response element and are induced by retinoids include RARα2 (Leroy *et al.*, 1991), RARγ2 (Lehmann *et al.*, 1992), the rat cellular retinoid binding protein I (cCRBPI) (Husmann *et al.*, 1992), the medium chain acyl-coenzyme A dehydrogenase gene (Raisher *et al.*, 1992), rat α-fetoprotein gene (Liu *et al.*, 1994), *Hoxb-1* (Marshall *et al.*, 1994), the human tissue-type plasminogen activator gene (Bulens *et al.*, 1995), intercellular adhesion molecule-1 gene

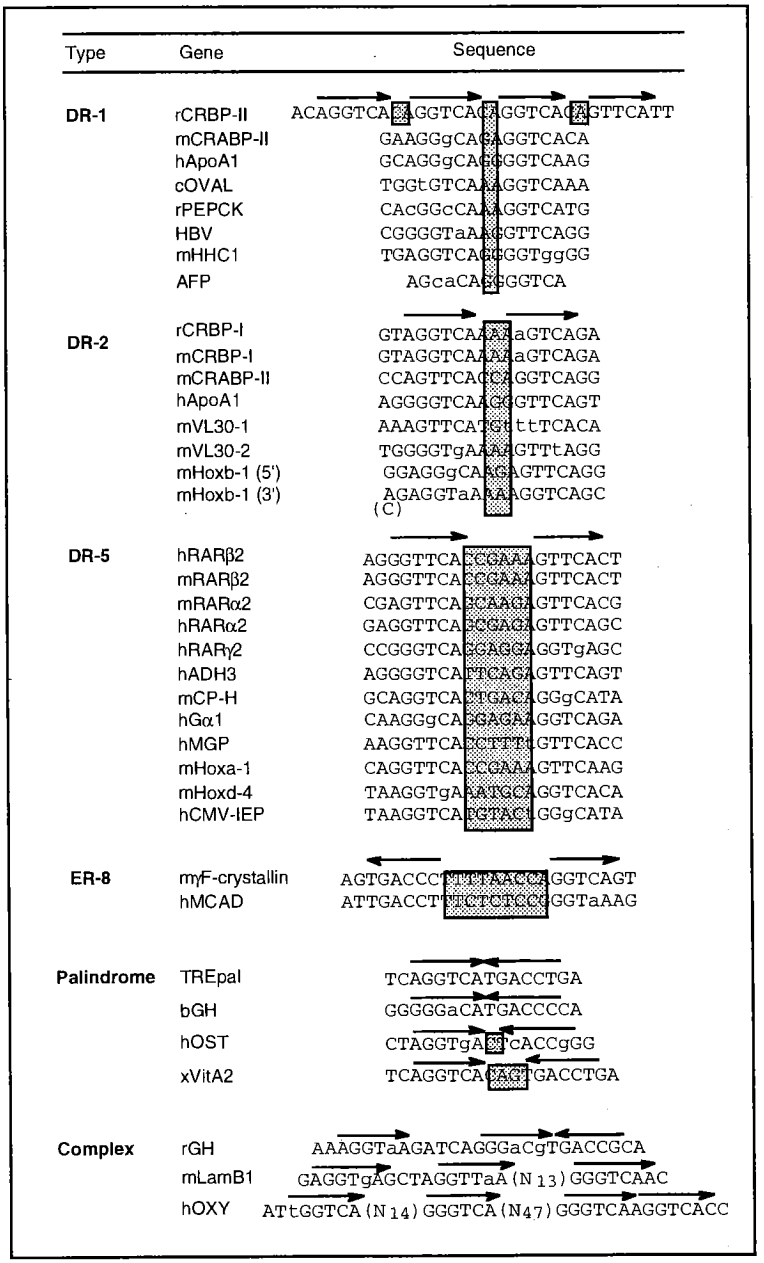

Figure 6. Natural retinoic acid response elements

Modified from Gronemeyer and Laudet (1995). Natural response elements that respond to retinoic acid are assembled into distinct direct-repeat (DR) groups and aligned according to their DR spacer (represented by a shaded box). The hexameric core motif and its orientation (compare with Figure 5) are indicated as an arrow. Non-consensus nucleotides are shown in small letters. ER, everted repeat

(Aoudjit *et al.*, 1995), the phosphoenolpyruvate carboxykinase gene (Scott *et al.*, 1996), the Pro alpha (I) collagen gene (Meisler *et al.*, 1997) and allelic human Pi class glutathione *S*-transferase gene (Lo & Ali-Osman, 1997).

In a few genes, RAREs have been found in the 3' flanking region rather than the 5' flanking region. These include the *Hox A* and *Hox B* homeobox gene cluster (Langston & Gudas, 1992; Langston *et al.*, 1997). A RARE was also found in the first intron of the human *CD38* gene (Kishimoto *et al.*, 1998).

In addition to RAR and RXR heterodimers, RXR homodimers may activate DR5 RARE such as the one in the RARβ promoter (Spanjaard *et al.*, 1995).

3.1.5 DNA binding and receptor dimerization

3.1.5.1 Homo- and heterodimerization

Nuclear receptors can bind their cognate response elements as monomers, homodimers or heterodimers with another family member (Glass, 1994). Dimerization is a general mechanism used to increase binding site affinity, specificity and diversity due to

- cooperative DNA binding (an extreme case of cooperative binding is the existence, in solution, of stable dimers),
- the lower frequency of two-hexamer rather than single-hexamer binding motifs separated by a defined spacer (statistically, a hexameric repeat like the oestrogen response element is 46 times less frequent than a single half-site motif) and
- the existence of recognition sites in heterodimers that are distinct from those of homodimers.

Steroid hormone receptors generally bind as homodimers to their response elements, while RAR, RXR, TR and VDR can homo- and heterodimerize. RXRs play a central role in these signal transduction pathways, since they can both homodimerize and act as promiscuous heterodimerization partners for RAR, TR, VDR and some orphan receptors. Heterodimerization has a threefold effect: it leads to a novel response element repertoire, increases the efficiency of DNA binding relative to the corresponding homodimers, and allows two signalling inputs, that of the ligands of RXR and its partner. A phenomenon called 'RXR subordination' maintains the identity of pathways for retinoic acid, thyroid and vitamin D signalling (see below). It is not clear whether some RXR complexes are permissive to RXR ligands in the absence of a ligand for the partner of RXR. Two dimerization interfaces can be distinguished in nuclear receptors, a weak one by the DNA-binding domains and a strong one by the ligand-binding domains. Ligand-binding domains dimerize in solution, while the interface of DNA-binding domains is apparently seen only when bound to DNA. The crystal structures of DNA-binding domain homo- and heterodimers and the RXR ligand-binding domain homodimer have defined the structures involved in dimerization (Gronemeyer & Moras, 1995). The response element repertoire described above for receptor homo- and heterodimers (Figure 5) is dictated by the DNA-binding domain, while the interface formed by the ligand-binding domains stabilizes the dimers but does not play any role in response element selection.

3.1.5.2 Specificity of DNA recognition

The specificity of the DNA response element (half-site sequence, spacing and orientation) is generated by recognition of the actual 'core' or 'half-site' motif and by the dimerization characteristics (mono-, homo- or heterodimerization; structure of the actual dimerization interface) of the receptor(s). The residues involved in distinguishing the hexameric half-site motives of oestrogen-response elements (5'-AGGTCA) and those recognized by glucocorticoid receptors (5'-AGAACA) were identified in a series of refined swapping experiments. Initially, DNA-binding domain swaps showed that specific half-site recognition depends on DNA-binding domain identity (Green & Chambon, 1987); subsequently, the N-terminal finger was found to differentiate between the two response elements (Green *et al.*, 1988). Three studies showed that two to three residues at the C-terminal 'knuckle' of the N-terminal finger, commonly referred to as the P-box (proximal box; see Figure 7), were responsible for the differentiation (Danielsen *et al.*, 1989; Mader *et al.*, 1989; Umesono & Evans, 1989).

A second region, the D-box (distal box; N-terminal 'knuckle' of the C-terminal finger; see Figure 7), was found to be involved in differentiating between binding to a three-base pair (characteristic of steroid receptor response elements) and a zero-base pair-spaced (one type of TRE) palindrome (Umesono & Evans, 1989). As was later confirmed by the crystal structures of GR and ER DNA-binding domains, this region does indeed contribute to the DNA-binding domain dimerization interface.

Two other boxes have been described within the DNA-binding domains of heterodimerizing receptors. The A-box was originally described as the sequence responsible for the recognition of two additional A nucleotides in the minor groove 5′ of the hexameric core motif, thus generating an NGFI-B response element (NBRE; 5′-AAAGGTCA) (Wilson *et al.*, 1992). This A-box was later found to play a similar role in heterodimers such as 5′-RXR-TR on DR4 elements, where it specifies to some extent the spacer 5′ of TR (Kurokawa *et al.*, 1993) and sets minimal spacing by steric hindrance phenomena (Zechel *et al.*, 1994). Interestingly, the A-box presents in the three-dimensional structure as a helix contacting the minor groove, and modelling indicates its role in setting a minimal distance between the half-sites (Rastinejad *et al.*, 1995).

The T-box (Figure 7) was originally defined in RXRβ (then H-2RIIBP) as a sequence required for dimerization on a DR1 element (Wilson *et al.*, 1992). Its role as a RXR homo- and heterodimerization surface has since been confirmed (Lee *et al.*, 1993; Zechel *et al.*, 1994).

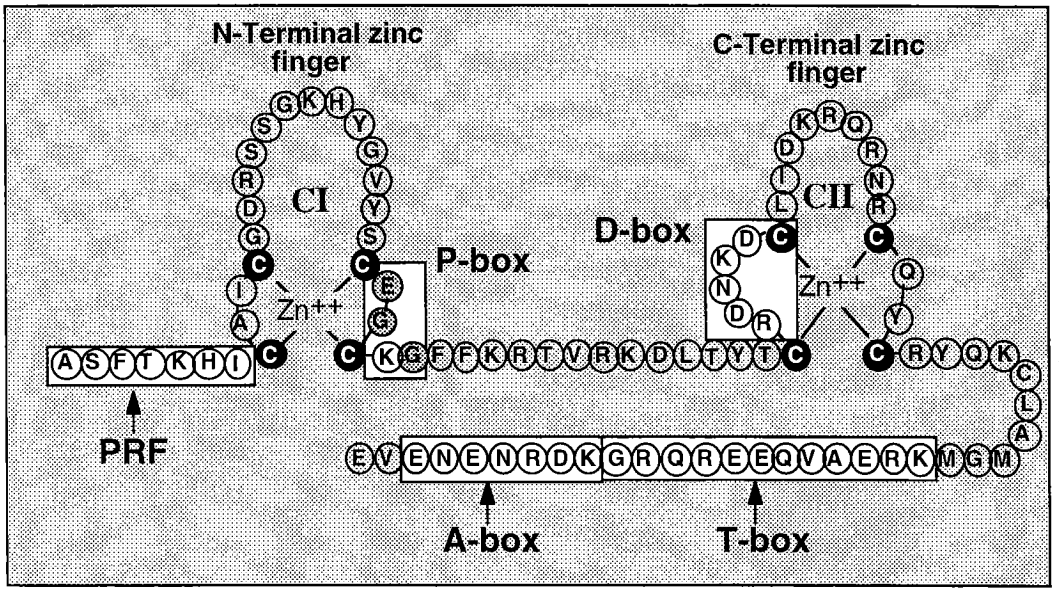

Figure 7. Schematic illustration of the retinoid X receptor DNA-binding domain

The boxes illustrate regions involved in response element selection. The P (proximal) box is part of the DNA recognition helix, and swapping of the EG. A residues (black circles, the sequence reads cEGckA) of the ER P-box with the corresponding GS..V residues of GR switches oestrogen-response element to that recognized by the glucocortocid receptor. The D (distal) box is responsible for PAL3/PAL0 selection by oestrogen or thyroid hormone receptors and contributes to the homodimerization interfaces of ER and GR DNA-binding domains. The T-box region forms a helix and corresponds to a dimerization surface in RXR homodimers. The TR A-box forms a helix which makes DNA backbone and minor groove contacts and precludes the formation of RXR–TR complexes on direct repeats spaced by less than 4 base pairs. CI and CII are the two zinc-finger motifs; PRF, pre-finger region.

3.2 Structure of nuclear receptor ligand-binding domains

3.2.1 Canonical fold

The crystal structures of six nuclear receptor ligand-binding domains have been described so far: the dimeric apo-RXRα (Figures 8 and 9; Bourguet *et al.*, 1995), monomeric holo-RARγ (Figures 8 and 9; Renaud *et al.*, 1995; Klaholz *et al.*, 1998), monomeric holo-TRα (Wagner *et al.*, 1995), dimeric holo (oestradiol, diethylstilboestrol)- and antagonist (raloxifene, tamoxifen)-bound ERα (Brzozowski *et al.*, 1997; Shiau *et al.*, 1998; Tanenbaum *et al.*, 1998), dimeric holo (progesterone)-bound PR (Williams & Sigler, 1998) and apo- and holo (thiazolidinedione)-peroxisome proliferator-activated receptor (PPAR)γ (Nolte *et al.*, 1998). These nuclear receptor ligand-binding domains display a common fold, as originally predicted (Wurtz *et al.*, 1996), with 12 α-helices (H1–H12) and one β-turn arranged as an antiparallel α-helical 'sandwich' in a three-layer structure (Figures 8 and 9). Note that some variability exists; for example, no helix H2 was found in RARγ (Renaud *et al.*, 1995), while an additional short helix H2' is present in PPARγ (Shiau *et al.*, 1998).

3.2.2 The mouse-trap model

Comparison of the various apo- and holo-ligand-binding domain structures (Figures 8 and 9) suggested a common mechanism by which AF-2 becomes transcriptionally competent: after ligand binding, H11 is repositioned in the continuity of H10, and the concomitant swinging of H12 unleashes the W-loop which flips over underneath H6, carrying along the N-terminal part of H3. In its final position, H12 seals the ligand-binding cavity like a 'lid' and further stabilizes ligand binding (in some but not all nuclear receptors) by contributing additional ligand–protein interactions. It is a general and essential feature of the ligand 'activation' of nuclear receptors that the transconformation of H12 and additional structural changes such as bending of helix H3 create distinct surfaces on the apo- and holo-ligand-binding domains. The novel surfaces generated after agonist binding allow bona fide co-activators, such as members of the SRC-1/transcription intermediary factor 2 family, to bind and recruit additional transcription factors (see below). Concomitantly, co-repressor proteins, which bind to presently unknown surface(s) of the apoligand-binding domain, dissociate after agonist but not necessarily antagonist binding (see below). Notably, certain antagonists 'force' H12 into a third position, distinct from the holo position whereby it impairs co-activator binding.

For a given receptor, the equilibrium between the apo and holo (or apo and antagonist) conformational states of a nuclear receptor ligand-binding domain can be affected through intramolecular interactions of H12, such as a salt bridge (holo-ligand-binding domain of RARγ, Figure 8; Renaud *et al.*, 1995) or hydrophobic contacts, as suggested for apo-ER (White *et al.*, 1997b). This implies that the apo conformation is not necessarily the default state, so that some nuclear receptors may be constitutive activators or repressors without possessing a cognate ligand. Moreover, an increase in co-activator concentration can generate a transcriptionally competent RAR under certain conditions (Voegel *et al.*, 1998), and the apo-ER conformation may be destabilized by phosphorylation (Weis *et al.*, 1996; White *et al.*, 1997b). Thus, overexpression of co-activators or receptor modification may generate ligand-independent receptors.

3.2.3 The dimer interface

Most studies of nuclear receptor co-regulator interactions consider only nuclear receptor monomers; however, heterodimers can have a very different gamut of co-regulator interactions. The roles of H1, H12 and (ligand-dependent) intradimeric allosteric effects must therefore be taken into account in order to have a comprehensive molecular view of nuclear receptor ligand-binding domain function.

As predicted from studies of mutagenesis, the homodimeric contacts involve helix H10. The three interfaces are very similar, as first seen in the crystal structure of apo-RXRα and now observed in ERα, PR and PPARγ. The key element that locks the dimer interface is H10, which can self-associate through hydrophobic

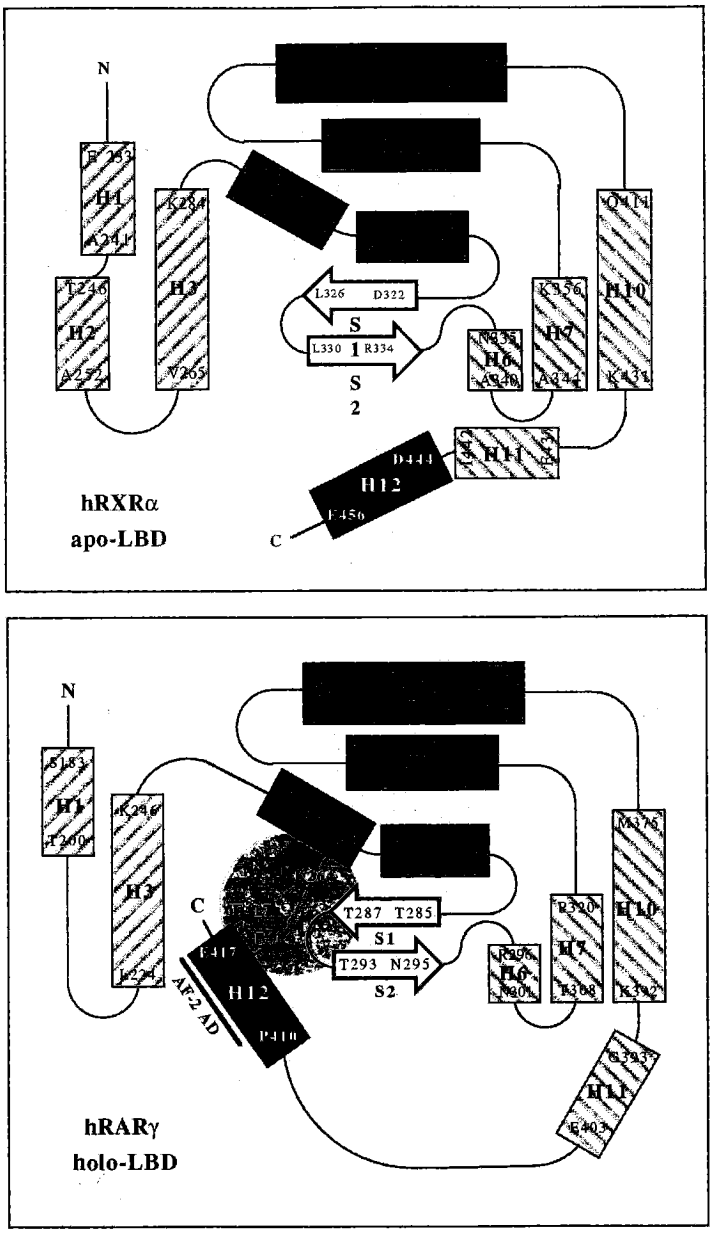

Figure 8. Topology of the apo-ligand-binding domain (LBD) of the retinoid X receptor (apo-RXRα) and the holo-LBD of the retinoic acid receptor (holo-RARγ).

Nuclear receptor ligand-binding domains form a β-helical sandwich with H4, H5, H8 and H9 (grey boxes) positioned between helices H1 to H3 and H6, H7, H10 (striped boxes). H12 (and to a lesser extent H11) undergoes major structural alteration ('*trans*-conformation') upon ligand binding. The N and C termini of the RXRα LBD are indicated, as well as the residues at the beginning and at the end of each α-helix or β-strand.

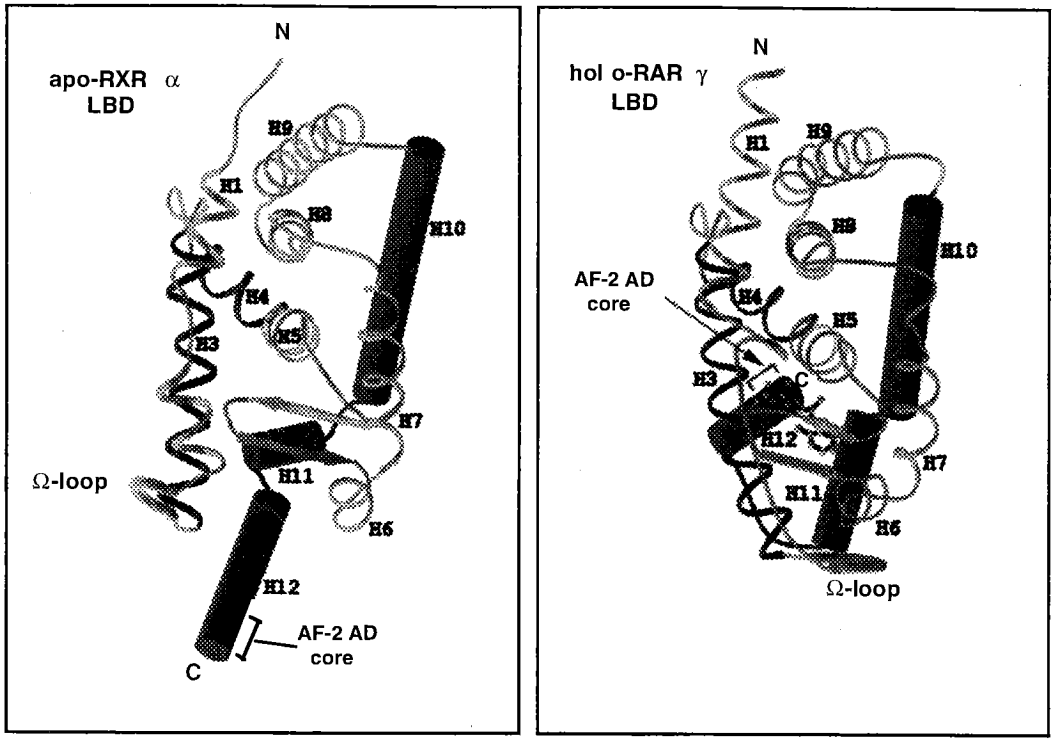

Figure 9. Three-dimensional structure of the apo-ligand-binding domain (LBD) of the retinoid X receptor (apo-RXRa) and the holo-LBD of the retinoic acid receptor (holo-RARγ)

The *trans*-conformation after ligand binding, which involves flipping of the W-loop, has been referred to as the 'mouse-trap mechanism' (see text and Renaud *et al.*, 1995). Note that these folds are prototypic folds which apply to the entire nuclear receptor superfamily (Wurtz *et al.*,1996). For illustration, helices H10, H11 and H12 are represented as black rods. The core of the activation func-

contacts, but helices H9 and H7 and the loops connecting H7 and H8 also contribute to dimer stability. The H12 of one subunit is proximal to H7 in the other, and this might provide the basis for allosteric cross-talk between subunits. If heterodimers were similarly associated, close proximity of H12 to the other subunit could play a significant role in the function of the nuclear receptor heterodimer, such as, for example, RXR subordination.

apo-RXR, in contrast to other apo-nuclear receptors, can form tetramers which dissociate after ligand binding (Kersten *et al.*, 1995, 1996; Chen *et al.*, 1998). These tetramers could be a storage form of this promiscuous heterodimerization partner.

3.2.4 The ligand-binding pocket

In all the crystal structures presently available, the ligand is embedded within the protein, with no clearly accessible entry or exit. PPARγ seems to be the only exception to that rule, since a potential access cleft to the ligand-binding protein was observed between H3 and the β-turn which could be of sufficient size to allow entry of small ligands without major adaptation. For all other receptors of known structure, significant conformational changes are necessary to generate potential entry sites. The mouse-trap model provides an easy solution to the problem: the mobility of H12 opens a channel by removing the 'lid' from the ligand pocket.

The ligand-binding pockets are lined with mostly hydrophobic residues. A few polar residues at the deep end of the pocket near the β-turn act as anchors for the ligand or play an essential role in the correct positioning and enforce the selectivity of the pocket. Most nuclear receptors contain a conserved arginine attached to H5 which points into this part of the cavity. These anchoring residues, conserved within a given subfamily, are indicative of the polar group characteristics of each family of ligands (i.e. carboxylate for retinoids and ketone for steroids).

3.2.5 Ligand selectivity

As shown in the cases of RARγ and TRβ, the shape of the ligand-binding pocket matches that of the ligand. The accordance of shape and volume maximize the number of mostly hydrophobic contacts, thus contributing to the stability of the complex and the selectivity of the pocket for the cognate ligand.

The RAR has a ligand-binding domain that can bind two chemically different ligands equally well: all-*trans*-retinoic acid and its 9-*cis* isomer. Crystallographic analysis of the two ligands in the RARγ ligand-binding domain showed that both adapt conformationally to the ligand-binding protein, which acts as a matrix (Klaholz *et al.*, 1998). Moreover, the conformation of a RARγ-selective agonist was shown to match that of the natural ligands in their bound state. The adaptation of ligands to the protein leads to an optimal number of interactions for binding and selectivity and justifies modelling approaches for ligand design.

For steroid receptors, the volume of ligand-binding protein is significantly larger than that of the corresponding ligands, and the rigidity of the ligand does not allow adaptability. Therefore, selectivity cannot be driven by multiple hydrophobic contacts, which could not suffice to discriminate between small structurally similar ligands. Very large volumes of ligand-binding protein allow binding of multiple ligands with different stereochemistry, as in the case of PPAR (Nolte *et al.*, 1998), often at the expense of lower binding affinities.

As in a structure-based sequence alignment, only three residues diverged in the ligand-binding proteins of RAR α, β and γ. It was predicted that these divergent residues were critically involved in differentiating between isotype-selective retinoids (Renaud *et al.*, 1995). Indeed, swapping of these residues confirmed the hypothesis (Gehin *et al.*, 1999) and also confirmed the agonistic/antagonistic response towards the ligand onto any other RAR isotype.

3.2.6 Molecular consequences of ligand binding

A model was proposed in which holo nuclear receptors transmitted their activity to the basal transcription machinery via transcription intermediary factors or co-regulators. This hypothesis was based on so-called 'squelching' experiments in which it was observed that overexpression of one receptor ('autosquelching'; Bocquel *et al.*, 1989) or of a different receptor ('heterosquelching'; Meyer *et al.*, 1989) inhibited agonist-induced *trans*-activation in a receptor dose- and ligand-dependent manner. These data were interpreted as the result of sequestration of transcription intermediary factors from the transcription initiation complex by either excess of the same or addition of another ligand-activated receptor.

3.3 Retinoid X receptor subordination

RXR cannot respond to RXR-selective agonists in the absence of an RAR ligand. This phenomenon is generally referred to as RXR 'subordination' or RXR 'silencing' by apoRAR. In the presence of an RAR agonist or certain RAR antagonists, RXR agonists further stimulate the transcriptional activity of RAR–RXR heterodimers synergistically (Figure 10; Chen *et al.*, 1996). RXR subordination solves a potential problem that arises from the ability of RXR to act as a promiscuous heterodimerization partner for numerous nuclear receptors, compromising signalling pathway identity, since in the absence of subordination RXR ligands could simultaneously activate multiple heterodimeric receptors, such as RAR–RXR, TR–RXR and VDR–RXR and confuse retinoic acid, thyroid hormone and vitamin D3 signalling, respectively. Retinoic acid-deprived animals do not, however, have abnormalities that could readily be related to impaired thyroid hormone

or vitamin D3 signalling. Moreover, RXR-selective ligands on their own could not trigger RXR–RAR heterodimer-mediated retinoic acid-induced events in various cell systems (Chen *et al.*, 1996; Clifford *et al.*, 1996; Taneja *et al.*, 1996). This is not due to an inability of the RXR partner to bind its cognate ligand in DNA-bound heterodimers, as was previously suggested (Kurokawa *et al.*, 1994), as RXR ligand binding has been demonstrated to occur in such complexes in several studies *in vitro*, and synergistic *trans*-activation induced by RAR- and RXR-selective ligands has been observed *in vivo* (Apfel *et al.*, 1995; Chen *et al.*, 1996, 1997; Clifford *et al.*, 1996; Kersten *et al.*, 1996; Taneja *et al.*, 1996; Li *et al.*, 1997; Minucci *et al.*, 1997). Thus, RAR apparently 'controls' the activity of RXR–RAR heterodimers in two ways: it induces transcription in response to its own ligand and silences RXR activity in the absence of an RAR ligand. Consequently, the only way for RXR to affect *trans*-activation in response to its ligand in RXR–RAR heterodimers is through synergy with RAR ligands (Figure 10). This concept of RXR silencing may not apply to all nuclear receptor partners, as the ligand-induced RXR activity was permissive in heterodimers with NGFI-B, leading even to a synergistic response (Forman *et al.*, 1995; Perlmann & Jansson, 1995). Neither the existence of an endogenous NGFI-B ligand nor weak constitutive activity of the NGFI-B AF-2 can be excluded; both could readily explain RXR activity and NGFI-B–RXR synergy due to the absence of RXR silencing.

3.4 Retinoid receptor cross-talk with other signalling pathways

Retinoid receptors can regulate gene expression by interacting directly, as homodimers and/or heterodimers, with cognate response elements in target gene promoters. At least three types of regulatory mechanism exist, by which several nuclear receptors, including retinoid receptors:

- can positively or negatively mutually interfere with gene activation in programmes of other signalling pathways (including so-called AP-1 *trans*-repression; Göttlicher *et al.*, 1998),
- act through the response elements of other transcription factors in a ligand-dependent but DNA binding-independent fashion (Gaub *et al.*, 1990; Paech *et al.*, 1997),
- be affected by the activity of other signalling pathways, resulting for example in receptor phosphorylation and concomitant modification of its activity (Kato *et al.*, 1995; Hu *et al.*, 1996a; Rochette-Egly *et al.*, 1997).

Transcriptional interference is not restricted to AP-1 but can also involve factors such as NFκB1, STAT5, C/EBP and Oct-2A. Examples of genes that have been shown to be suppressed by all-*trans*-retinoic acid through antagonism of AP-1 include stromelysin (Nicholson *et al.*, 1990), collagenase (matrix metalloproteinase-1) (Schüle *et al.*, 1991; Schroen & Brinckerhoff, 1996; Guerin *et al.*, 1997), involucrin (Monzon *et al.*, 1996) and relaxin (Bernacki *et al.*, 1998).

all-*trans*-Retinoic acid can increase AP-1 activity, and this may be associated with cell differentiation in melanoma cells (Desai & Niles, 1997) or cell growth enhancement in a lung carcinoma cell line (Wan *et al.*, 1997). Whether all-*trans*-retinoic acid suppresses or increases AP-1 activity appears to be cell type-specific and may depend on the differentiation programme elicited and the abundance of co-factors.

3.4.1 *Dissociation of* trans*-activation and AP-1* trans*-repression by synthetic retinoids*

Both RARs and RXRs (α, β and γ isotypes) can act as ligand-dependent *trans*-repressors of AP-1 (*c-Jun/c-Fos*) activity, and, reciprocally, AP-1 can inhibit *trans*-activation by RARs and RXRs. In the case of RAR, mutant analyses have shown that the integrity of both DNA and ligand-binding domains is required for efficient AP-1 repression. Notably, C-terminal truncation mutants lacking H12 fail to *trans*-repress, suggesting that H12 *trans*-conformation is involved in both *trans*-activation and *trans*-repression.

Several groups have generated ligands which are RAR isotype or RXR-selective agonists and antagonists and separate the *trans*-activation and *trans*-repression functions of

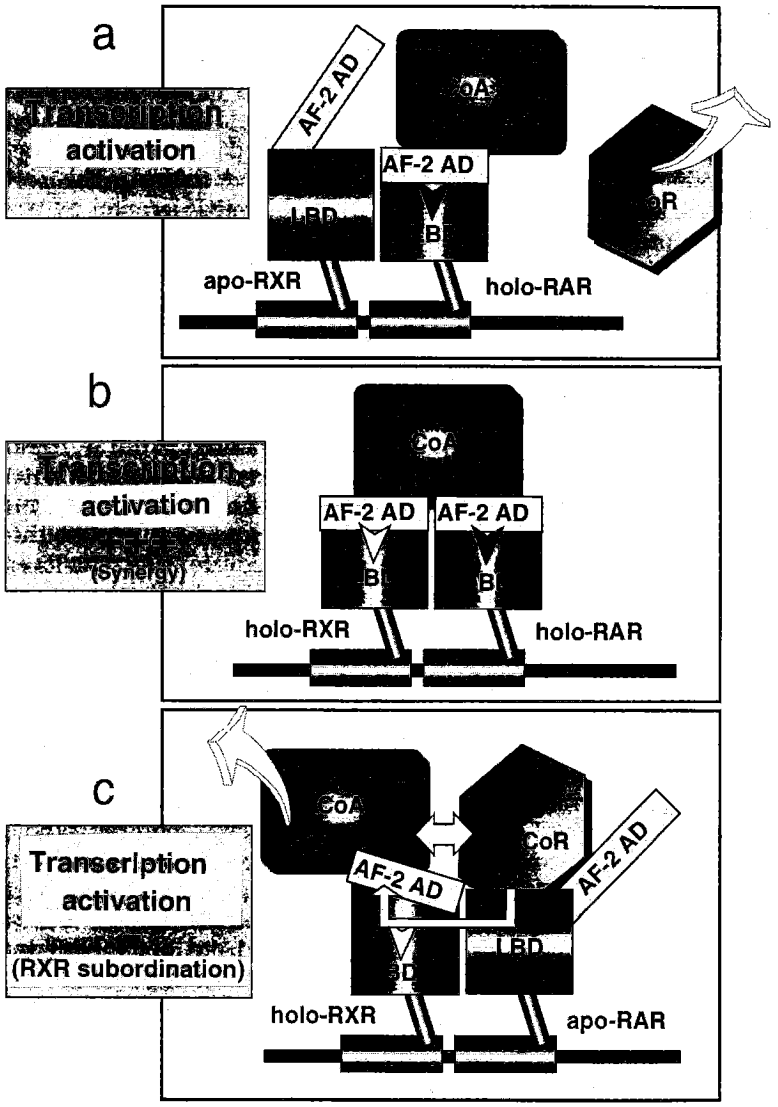

Figure 10. Model of retinoid X receptor (RXR) subordination by apo-retinoic acid receptor (RAR)

(a) In the presence of RAR agonists, co-repressor (CoR) dissociates from the RAR–RXR heterodimer and co-activator (CoA) is recruited and establishes a transcription activation-competent complex, probably through the RAR subunit. (b) Synergy between RAR and RXR agonists is due to cooperative binding of a co-activator through two of its LxxLL nuclear receptor boxes. (c) RXR ligand alone is unable to dissociate the co-repressor from the heterodimer but allows co-activator recruitment to the RXR subunit. Either allosteric or direct steric hindrance effects exerted by the co-repressor destabilize the co-activator complex, resulting in RXR subordination. LBD, ligand-binding protein

retinoid receptors. The aim is to find ligands which are largely devoid of the side-effects of all-*trans*-retinoic acid and function as inducers of either apoptosis and/or differentiation or inhibitors of cell proliferation, preferably in a cell-specific manner. Both subunits of the RAR–RXR heterodimer contribute synergistically to differentiation and apoptosis in the model NB4 and HL60 acute promyelocytic leukaemia cell lines (Chen *et al.*, 1996; Kizaki *et al.*, 1996). Retinoids that can *trans*-repress AP-1 but are largely devoid of the ability to *trans*-activate cognate target genes can inhibit the growth of tumour cells (Chen *et al.*, 1995). Huang *et al.* (1997) suggested that the antitumour effect of synthetic retinoids requires the AP-1 repressive but not the 'classical' *trans*-activation ability of the cognate receptor, but HL60 cells differentiated and ceased proliferation in response to RAR–RXR agonists and not to a retinoid with anti-AP-1 activity that did not *trans*-activate target genes (Kizaki *et al.*, 1996). Similar AP-1 antagonism-independent antitumour effects of retinoids have been reported for neuroblastoma (Giannini *et al.*, 1997) and prostate cancer (de Vos *et al.*, 1997) cells. It may be possible to target the antitumour effect of retinoids to specific cell types or tissues by designing synthetic retinoids with a defined pattern of RAR isotype and RXR selectivity and *trans*-activation or *trans*-repression ability.

There may be insufficient experimental evidence to establish that 9-*cis*-retinoic acid is 'the' or 'a' physiological ligand for the RXR family of receptors. There is a considerable body of literature on the formation of 9-*cis*-retinoic acid within cells, tissues and organisms and on the actions of 9-*cis*-retinoic acid in living systems. Heyman *et al.* (1992) reported that the concentrations of 9-*cis*-retinoic acid in adult mouse liver and kidney are 13 and 100 pmol/g tissue, respectively, but Kurlandsky *et al.* (1995), who reported a limit of detection of only 4 pmol/g of tissue, were unable to detect this compound in adult rat liver, spleen, kidney, brain, adipose tissue, testis or muscle. Since no further reports of 9-*cis*-retinoic acid concentrations in tissues have appeared, it can only be considered a putative physiological ligand for RXRs.

The mechanisms by which nuclear receptors cross-talk with other signalling pathways remain elusive. Three models are being discussed, none of which comprehensively accounts for the observed phenomena.

(i) The CBP (a 265-kDa CREB-binding protein) sequestration model (Kamei *et al.*, 1996) is based on the assumption that both nuclear receptors and AP-1 compete for CBP/p300 as a common cofactor. The problem with this model is that receptor mutants (Heck *et al.*, 1994) and antagonists (Chen *et al.*, 1995; Vayssière *et al.*, 1997) exist which do not induce *trans*-activation and do not recruit co-activators but nevertheless are potent *trans*-repressors of AP-1 activity.

(ii) Interference with the Jun N-terminal kinase (JNK) signalling pathway, based on the blockade by hormone-activated nuclear receptors of c-Jun phosphorylation on Ser-63/73, is required to recruit the transcriptional co-activator CBP (Caelles *et al.*, 1997). A similar mechanism was observed in human bronchial epithelial cells where all-*trans*-retinoic acid decreased the amount and activation of AP-1 components. It inhibited JNK and, to a lesser extent, extracellular signal-regulated kinase activity and also reduced c-fos mRNA (Lee *et al.*, 1998). Pretreatment of human skin with all-*trans*-retinoic acid was found to inhibit induction of c-Jun protein by ultraviolet radiation and, consequently, AP-1 via a post-transcriptional mechanism, since all-*trans*-retinoic acid did not inhibit induction of c-Jun mRNA (Fisher *et al.*, 1998). This mechanism does not account for the inhibition of nuclear receptor activity by AP-1.

(iii) Disruption of c-Jun/c-Fos dimerization by retinoid receptors (Zhou *et al.*, 1999), observed in a mammalian two-hybrid system, could account for the finding that agonist-bound RAR can inhibit DNA binding by AP-1 *in vitro*. The mechanism does not, however, apply for the glucocorticoid receptor, as inhibition of AP-1 DNA binding by glucocorticoid receptor has been shown to be an artefact (König *et al.*, 1992).

3.4.2 Post-transcriptional mechanisms

Although transcriptional control is recognized as a major mechanism of regulation of gene expression during differentiation and development, alterations in gene expression through a post-transcriptional process are also an acknowledged mechanism for modulating important growth regulatory gene products. Through this mechanism, all-*trans*-retinoic acid can enhance or suppress the expression of genes that are already being actively transcribed rather than only inducing the expression of silent genes. A number of genes have been reported to be regulated by retinoic acid at the post-transcriptional level, but the mechanism is not clear. It has been proposed that all-*trans*-retinoic acid modulates RNA processing or transport out of the nucleus (Rosenbaum & Niles, 1992). In most studies, it was found that all-*trans*-retinoic acid stabilizes mRNA, and the stable mRNA levels of the oncogenes *p53* and *c-myc* were found to decrease in F9 embryonal carcinoma cells induced to differentiate into parietal endoderm by treatment with all-*trans*-retinoic acid and dibutyryl cyclic AMP. The decrease in *c-myc* was rapid (< 24 h), whereas the decrease in *p53* was observed after two to three days, and both were due to post-transcriptional regulation, as indicated by nuclear transcription assay *in vitro* (Dony *et al.*, 1985). Thus, in F9 embryonal carcinoma cells, some genes are regulated by all-*trans*-retinoic acid at the transcriptional level and others at the post-transcriptional level. Likewise, nuclear run-on transcriptional analysis showed no changes in the transcription rate of the four homeobox genes (*HOX-1*, *HOX-2*, *HOX-3* and *HOX-5*) in human embryonal carcinoma cells treated with all-*trans*-retinoic acid and induced to differentiate into several cell types. These data suggest that the activation of homeobox gene expression in retinoic acid-induced embryonal carcinoma cells is controlled, at least in part, by post-transcriptional mechanisms (Simeone *et al.*, 1989). Other genes shown to be up-regulated by mRNA stabilization include protein kinase C in melanoma cells (Rosenbaum & Niles, 1992), alkaline phosphatase in pre-osteoblastic cells (Zhou *et al.*, 1994), keratin 19 in squamous carcinoma cells (Crowe, 1993), a secreted binding protein for fibroblast growth factors in squamous carcinoma cells (Liaudet-Coopman & Wellstein, 1996), connexin 43 in F9 embryonal carcinoma cells (Clairmont & Sies, 1997), Pbx protein (a heterodimerization partner of Hox proteins) in P19 embryonal carcinoma cells (Knoepfler & Kamps, 1997) and leukotriene C4 synthase in rat basophilic leukaemia cells (Hamasaki *et al.*, 1997). Although it is not clear how all-*trans*-retinoic acid stabilizes the mRNA of certain genes, it affects connexin 43 gene expression at the level of mRNA stability via elements located in the 3'-untranslated region (Clairmont & Sies, 1997). Another mechanism was suggested by studies on differentiation of the pre-osteoblastic cell line, UMR 201. In these cells, all-*trans*-retinoic acid caused a marked increase in the proficiency of post-transcriptional nuclear processing of alkaline phosphatase mRNA as a result of dephosphorylation of nuclear U1 70K spliceosome-component protein. A twofold decrease in mRNA expression of an isoform of alternative splicing factor that inhibits splicing was also observed (Manji *et al.*, 1999). Other mechanisms were described for the increased transcription of interleukin-1 β mRNA induced by all-*trans*-retinoic acid, although, unlike for other primary target genes, mRNA expression is regulated at pre-mRNA processing (Jarrous & Kaempfer, 1994).

A few studies have shown that all-*trans*-retinoic acid induces changes in protein stability. The half-life of bcl-2 protein was markedly shortened after treatment of blast stem cells of acute myeloblastic leukaemia with this compound (Hu *et al.*, 1996b). all-*trans*-Retinoic acid induced cyclin D1 proteolysis via proteasome in normal and immortalized human bronchial epithelial cells (Langenfeld *et al.*, 1997; Boyle *et al.*, 1999), and this effect may depend on retinoid receptors because retinoids that activated RARβ or RXR pathways preferentially led to a decrease in the amount of cyclin D1 protein (Boyle *et al.*, 1999). In contrast, all-*trans*-retinoic acid increased the stability of p53 protein in non-small cell lung cancer cells (Maxwell & Mukhopadhyay, 1994).

4. Cellular biology of retinoids

Normal tissue homeostasis depends on coordinated regulation of cell proliferation, differentiation and apoptosis. The putative cancer-preventive effects of retinoids are thought to be associated with their ability to modulate the growth, differentiation and apoptosis of normal, premalignant and malignant cells *in vitro* and *in vivo*. Although cancer prevention occurs by definition *in vivo*, many fundamental concepts of the mechanisms of action of chemopreventive agents can be explored in studies with cells in monolayer culture, semisolid media, three-dimensional raft cultures and organ cultures. Retinoids exert pleiotropic effects on transformation of cells and tissues in culture. They suppress the transforming effects of carcinogens, inhibit the growth of immortalized 'premalignant' cells and suppress the transformed phenotype of fully malignant cells.

4.1 Effects of retinoids on cell transformation

The effects of retinoids on transformation of normal cells *in vitro* were examined in mouse embryonal cell, rat tracheal epithelial cell and human keratinocyte model systems. Certain retinoids inhibited transformation induced by carcinogens whether they were chemicals (Bertram, 1983; Langenfeld *et al.*, 1996), physical agents (Harisiadis *et al.*, 1978), viruses (Talmage & Lackey, 1992; Agarwal *et al.*, 1991; Khan *et al.*, 1993; Pomponi *et al.*, 1996) or oncogenes (Roberts *et al.*, 1985; Cox *et al.*, 1991).

Retinoids suppressed the growth of immortalized keratinocytes that mimic a carcinoma *in situ* in raft culture (Shindoh *et al.*, 1995; Eicher & Lotan, 1996). They also reversed histological changes induced by vitamin A deficiency in hamster trachea (Newton *et al.*, 1980; Chopra, 1983) and by exposure of mouse prostate to carcinogens in organ culture (Lasnitzki, 1976). Some of these systems (e.g. the hamster trachea system) have been used as preclinical screens for chemopreventive effects of retinoids (Newton *et al.*, 1980).

4.2 Effects of retinoids on cell proliferation

Investigations have been conducted with cells derived from normal tissues and cells immortalized with simian virus 40 large T antigen (Reddel *et al.*, 1988; Langenfeld *et al.*, 1996; Sun *et al.*, 1999a), human papillomavirus (HPV) E6/E7 (Khan *et al.*, 1993; Shindoh *et al.*, 1995) or Epstein-Barr virus (Pomponi *et al.*, 1996). Some of the latter cells were further transformed by exposure to carcinogens *in vitro* (Langenfeld *et al.*, 1997; Boyle *et al.*, 1999) or *in vivo* (Klein-Szanto *et al.*, 1992). The immortal and transformed cells provided an isogenic system of carcinogenesis *in vitro* in which the activities of chemopreventive agents could be compared (Sun *et al.*, 1999a; Boyle *et al.*, 1999). Retinoids were found to suppress the growth of certain cells. Human keratinocytes immortalized by HPV 16 DNA became more sensitive than normal keratinocytes to growth control by retinoids (Pirisi *et al.*, 1992). Treatment decreased the level of HPV E2/E5/6/E7 transcripts (Pirisi *et al.*, 1992; Khan *et al.*, 1993). Whereas early-passage HKc/HPV16 cells were very sensitive to growth inhibition by all-*trans*-retinoic acid, as the cells progressed in culture they lost their sensitivity to the retinoid (Creek *et al.*, 1995).

Most studies on the effects of retinoids on cells *in vitro* have been performed with cell lines derived from carcinogen-induced rodent tumours and spontaneous human cancers (Amos & Lotan, 1990; Gudas *et al.*, 1994). In most cells that are sensitive to the growth-inhibitory effects of retinoids in monolayer or suspension cultures, there are changes in cell cycle, most often an accumulation of cells in the G1 phase and a concomitant decrease in the numbers of cells in S and G2/M (Zhu *et al.*, 1997; Matsuo & Thiele, 1998). Growth inhibition is usually observed within 24–48 h of treatment and requires the continuous presence of retinoids, as the effects are usually reversible (except when apoptosis is induced, as described below). The concentrations of retinoids required to induce 50% growth inhibition (IC_{50}) relative to control cultures can range from nanomolar (physiological) to micromolar (pharmacological) concentrations. They

depend on the solubility, hydro-phobicity and affinity for nuclear retinoid receptors of the retinoid and the rate of uptake, rate of catabolism of the retinoid, expression of cellular and nuclear retinoid-binding proteins and receptors and other factors related to the transcriptional machinery in the cell. The growth inhibitory effects of retinoids have been documented in a wide variety of tumour cell types, including melanoma, neuroblastoma, glioma, retinoblastoma, embryonal carcinoma, carcinomas of the lung, breast, prostate, bladder, colon, skin, head and neck and cervix and various types of sarcoma that grow as adherent monolayers on plastic tissue culture dishes, as well as in small-cell lung cancer cells isolated from pleural effusions and haematopoietic malignancies such as lymphomas, leukaemias, myelomas, premonocytic leukaemia and promyelocytic leukaemia which normally grow in suspension (Amos & Lotan, 1990; Gudas *et al.*, 1994).

Retinoids also suppress the ability of certain malignant cells to form colonies in semisolid media, an anchorage-independence property that is a hallmark of transformed cells (Lotan *et al.*, 1982; Amos & Lotan, 1990; Gudas *et al.*, 1994). Tumour cells examined for response to retinoids in this assay are often more sensitive than in monolayer cultures (Lotan *et al.*, 1982). Another advantage of the assay is that it has been adapted for analysis of the sensitivity to retinoids of tumour cells dissociated from fresh surgical specimens (Meyskens & Salmon, 1979; Meyskens *et al.*, 1983; Cowan *et al.*, 1983).

Natural and synthetic retinoids have been identified that are more effective in the various assays than all-*trans*-retinoic acid (Sun *et al.*, 1997; 1999a). Some of these retinoids may pave the way towards the next generation of chemopreventive retinoids if they are found to have greater cell specificity and fewer side-effects than those currently in use.

4.3 Effects of retinoids on cell differentiation

HPV-transformed ectocervical cells were sensitive to the ability of retinoids to suppress squamous differentiation in monolayer cultures, which is also observed in normal cells (Agarwal *et al.*, 1991). Many HPV-immortalized cell lines have shown greater sensitivity to suppression of squamous differentiation by all-*trans*-retinoic acid than normal cells (Choo *et al.*, 1995), but when HPV-immortalized keratinocytes derived in another laboratory were allowed to form three-dimensional organotypic raft cultures, they became less sensitive than normal cells to the suppressive effects of all-*trans*-retinoic acid on squamous differentiation (Merrick *et al.*, 1993). all-*trans*-Retinoic acid enhanced mucin secretion by SPOC1 cells (rat tracheal cells immortalized by growth on tracheal grafts *in vivo*) during the early plateau stage of culture (Randell *et al.*, 1996). Thus, it appears that all-*trans*-retinoic acid enhances the expression of the mucous phenotype while suppressing the squamous one.

Retinoids were found to modulate the differentiation of numerous types of cells derived from embryonal carcinoma, germ-cell tumour, choriocarcinoma, melanoma, neuroblastoma, astrocytoma, medulloblastoma, retinoblastoma, rhabdomyosarcoma, colon carcinoma, breast carcinoma, myeloid leukaemia, premonocytic leukaemia, erythroleukaemia and others (Lotan, 1995a). With the exception of normal and malignant keratinocytes, retinoids induced or enhanced the differentiation of these tumour cells (Gudas *et al.*, 1994; Lotan, 1995a). In cultured keratinocytes and squamous-cell carcinomas, retinoids inhibited keratinizing squamous differentiation (Jetten *et al.*, 1992; Lotan, 1993). Because in many tissues, such as buccal mucosa and bronchial epithelium, the keratinizing squamous differentiation of tumours derived from normally non-keratinizing epithelial cells is aberrant (i.e. it occurs during carcinogenesis or other pathological states), the inhibitory effect of retinoids on the expression of squamous differentiation markers can be viewed as restoration of the normal non-keratinizing phenotype (Lotan, 1993).

Although retinoids can induce many pathways of differentiation, extensive studies with cell lines derived from embryonal carcinoma, normal and malignant keratinocytes, premonocytic and myeloid leukaemias, neuroblastoma and melanoma indicate that they do not determine the pathway of differentiation but rather enhance predetermined programmes in cells that can undergo

differentiation along one or more specific pathways. Notable examples are the P19 embryonal carcinoma cells, which can be induced to undergo either myogenic differentiation or neuronal differentiation, depending on the concentration of all-*trans*-retinoic acid used (McBurney *et al.*, 1982). In F9 embryonal carcinoma cells, all-*trans*-retinoic acid can induce parietal or visceral endodermal differentiation depending on whether the cells are grown in monolayer culture or as three-dimensional aggregates (Strickland *et al.*, 1980; Gudas, 1992). Furthermore, all-*trans*-retinoic acid induced three distinct differentiation pathways: ectodermal, mesodermal and endodermal in a developmentally pluripotent germ-cell tumour (Damjanov *et al.*, 1993). The potential of all-*trans*-retinoic acid may sometimes be restricted to a specific pathway, as in HL-60 myeloid leukaemia cells which can undergo both myeloid and monocytoid differentiation, even though retinoids can induce only the granulocytic pathway (Breitman *et al.*, 1980). Presumably, the effects of retinoids on the differentiation pathway depend on various cellular factors that are either expressed constitutively (e.g. certain transcription factors) or are induced by retinoic acid and then turn on a specific differentiation pathway, such as *Hox* genes (Boncinelli *et al.*, 1991) and AP-2 (Luscher *et al.*, 1989).

The induction of differentiation of tumour cells is not restricted to established cell lines, since fresh leukaemic cells in short-term culture were also responsive to treatment with retinoids (Imaizumi & Breitman, 1987). Because retinoids can enhance the differentiation of malignant cells, they have been developed for clinical trials of 'differentiation therapy' aimed at inducing the differentiation of tumour cells *in vivo* (see section 6). So far, reports on induction of differentiation of solid tumour cells *in vivo* are restricted to murine embryonal carcinoma cells injected into syngeneic mice. A significant degree of differentiation was observed in teratocarcinomas when retinoic acid was injected directly into the tumour (Speers & Altmann, 1984) or when several retinoids were administered in the diet (McCue *et al.*, 1988).

4.4 Effects of retinoids on apoptosis

Activation of apoptosis in cells at risk of undergoing neoplastic transformation may constitute a physiological anti-neoplastic mechanism. Apoptosis of DNA-damaged cells can protect the organism from cancer development by eliminating cells that might otherwise replicate the damaged DNA, a process that may lead to mutations and eventually to cancer. Agents that can induce or restore the ability to undergo apoptosis in premalignant and malignant cells are expected, therefore, to be effective in cancer prevention and treatment (Thompson *et al.*, 1992). In this context, it is of special interest that retinoids have been found to induce apoptosis in mesenchymal, neuroectodermal, haematopoietic and epithelial cells during normal development and in cultured untransformed and tumour cells (Davies *et al.*, 1992; Lotan, 1995b).

Different retinoids were found to induce distinct types of apoptosis. One type involves initial differentiation followed by 'physiological' apoptosis of the differentiated cells. This pathway is exemplified by HL-60 myeloid leukaemia cells, which undergo differentiation into granulocytes and subsequently apoptosis by a process that requires six to eight days of treatment with all-*trans*-retinoic acid (Martin *et al.*, 1990). A second type of apoptosis does not require induction of differentiation and is usually more rapid; it can be accomplished in HL-60 cells by treatment with 9-*cis*-retinoic acid or RXR-selective retinoids (Nagy *et al.*, 1995). In HL-60 cells, activation of RARs by an RAR-specific ligand is sufficient to induce differentiation, whereas activation of RXRs by their ligand is essential for the induction of apoptosis (Nagy *et al.*, 1995). A third pattern of apoptosis is one in which cells that are resistant to induction of differentiation become responsive to induction of apoptosis, as described for a subclone of NB4 cells (a t15;17 human promyelocytic leukaemia cell line). When the parental cells were treated with all-*trans*-retinoic acid or 9-*cis*-retinoic acid, they differentiated into granulocytes and eventually underwent apoptosis as mature cells; however, the differentiation-resistant subclone underwent apoptosis after 72 h of treatment with

all-*trans*-retinoic acid (Bruel *et al.*, 1995). In these cells, apoptosis and differentiation may include events that cannot occur simultaneously. A fourth pattern of apoptosis was found in cultured PCC7-Mz1 embryonal carcinoma cells, which after exposure to all-*trans*-retinoic acid differentiated into neuronal, astroglial and fibroblast-like derivatives over several days. With the all-*trans*-retinoic acid concentrations that induce differentiation, apoptotic cells are detected as early as 10 h after exposure (Herget *et al.*, 1998).

Retinoids have also been found to protect cells from apoptosis. This phenomenon was described in the specific case of T cell receptor activation-induced T cell hybridoma death, which is mediated by the engagement of Fas by activation-up-regulated Fas ligand. Retinoic acids were found to suppress apoptosis by inhibiting the induction of Fas ligand expression (Yang *et al.*, 1993). Although 9-*cis*-retinoic acid was more effective than all-*trans*-retinoic acid, the effect required both nuclear retinoid receptors (RARs and RXRs) (Yang *et al.*, 1995).

An unusual induction of apoptosis was noted in SH SY 5Y human neuroblastoma cells which underwent apoptosis after treatment with 9-*cis*-retinoic acid for five days followed by incubation for four days without treatment. No apoptosis was observed when the cells were incubated under the same conditions with all-*trans*-retinoic acid or with LGD 1069, an RXR-selective retinoid or, surprisingly, when the cells were treated with 9-*cis*-retinoic acid for nine days continuously (Lovat *et al.*, 1997; Irving *et al.*, 1998). The mechanism of this effect remains to be explained.

Whereas all these studies were conducted with natural retinoic acids, several studies have demonstrated that some synthetic retinoids, such as *N*-(4-hydroxyphenyl)retinamide, are more potent than all-*trans*-retinoic acid or 9-*cis*-retinoic acid. *N*-(4-Hydroxyphenyl)retinamide induced apoptosis in all-*trans*-retinoic acid-resistant variant HL-60 cells and other leukaemia cells (Delia *et al.*, 1993), neuroblastoma cells (Ponzoni *et al.*, 1995), lung carcinoma (Kalemkerian *et al.*, 1995; Zou *et al.*, 1998), ovarian carcinoma (Supino *et al.*, 1996), head-and-neck squamous cell carcinomas (Oridate *et al.*, 1996), breast carcinoma (Wang & Phang, 1996) and prostate carcinoma cells (Roberson *et al.*, 1997; Sun *et al.*, 1999b). *N*-(4-Hydroxyphenyl)retinamide did not induce differentiation of HL-60 cells (Delia *et al.*, 1993) or neuroblastoma cells (Ponzoni *et al.*, 1995). Because this compound induced apoptosis in cells that are resistant to all-*trans*-retinoic acid and other retinoids, it may exert its effect by a distinct mechanism. Indeed, it was able to induce reactive oxygen species in leukaemia cells (Delia *et al.*, 1997) and cervical cancer cells (Oridate *et al.*, 1997); however, a survey of 13 cell lines indicated that only about 20% had increased production of reactive oxygen species after exposure to *N*-(4-hydroxyphenyl)retinamide, suggesting that other mechanisms should be considered (Sun *et al.*, 1999b,c).

5. The role of retinoids in embryogenesis

5.1 Involvement of retinoids in embryonic development

The unique features of retinoids in embryos is that they are endogenous and are required for normal embryonic development.

5.1.1 Endogenous retinoids in the embryo

Endogenous retinoids such as retinol, didehydroretinol, all-*trans*-retinoic acid, 4-oxoretinoic acid and didehydroretinoic acid have been identified by high-performance liquid chromatography in the embryos of all species examined to date. These include birds (Thaller & Eichele, 1987, 1990; Scott *et al.*, 1994; Dong & Zile, 1995), rodents (Satre *et al.*, 1989; Collins *et al.*, 1994; Tzimas *et al.*, 1995), rabbits (Tzimas *et al.*, 1996a), primates, including humans (Creech Kraft *et al.*, 1993; Hummler *et al.*, 1994; Sass, 1994; Tzimas *et al.*, 1996b), *Xenopus* (Durston *et al.*, 1989) and zebrafish (Costaridis *et al.*, 1996). Furthermore, retinoic acid is differentially distributed within single embryonic regions such as the chick limb bud where it forms a concentration gradient across the anterior–posterior axis, with the

highest concentrations posteriorly (Thaller & Eichele, 1987; Scott *et al.*, 1994). A similar gradient of all-*trans*-retinoic acid concentrations from the forebrain (lowest) to the spinal cord (highest) is found in the nervous system of early mouse embryos (Horton & Maden, 1995).

Transgenic mice carrying a reporter gene under the transcriptional control of a RARE (Rossant *et al.*, 1991; Mendelsohn *et al.*, 1991; Balkan *et al.*, 1992) and cell lines transfected with a retinoic acid-driven reporter gene (Chen *et al.*, 1992b; Wagner *et al.*, 1992; Chen *et al.*, 1994a) have been used to study the molecular basis of retinoid action. The response of the reporter system is assumed to reflect the presence of all-*trans*-retinoic acid and other active retinoids but cannot be considered to reflect the chemical identity of the retinoids conclusively. Despite this caveat, such methods demonstrate the existence of some 'hot spots' of embryonic retinoid concentrations which include the Hensen node of chicken embryos (Chen *et al.*, 1992b) and anterior–posterior gradients of retinoid concentrations in early neurula *Xenopus* embryos (Chen *et al.*, 1994a), the central nervous system tissue of rat embryos (Wagner *et al.*, 1992) and early chick embryos (Maden *et al.*, 1998).

5.1.2 Differential distribution of retinoid synthesizing enzymes

The enzymes that synthesize retinoic acid are distributed differentially in the embryo, a good example being the high level of activity of the enzyme retinaldehyde dehydrogenase(II) in mouse embryo spinal cord at the brachial and lumbar enlargements (McCaffery & Dräger, 1994). In each of these locations, there are more motor neurons than in the rest of the spinal cord. These neurons innervate the limbs which grow out at those two locations, and retinoic acid is required for motor neuron differentiation. Another example concerns the same enzyme, which is expressed in the mesenchyme of the early chick embryo (soon after gastrulation) in a domain that has an anterior border at the level of the second somite (Maden *et al.*, 1998).

5.1.3 Differential distribution of binding proteins and receptors

Retinoid cytoplasmic binding proteins and nuclear receptors are differently distributed in the embryo. The cytosolic binding proteins for retinoic acid and retinol (CRABPs and CRBPs, respectively) show very specific expression patterns within the embryo (Maden *et al.*, 1991; Gustafson *et al.*, 1993; Dekker *et al.*, 1994; Scott *et al.*, 1994). They may be involved in the metabolism of retinol to retinoic acid as well as in the control of local concentrations of 'free' all-*trans*-retinoic acid which is available for nuclear receptor binding. Indeed, CRBP occurs at relatively high concentrations in tissues sensitive to retinoid deficiency (such as embryonic heart), while CRABP occurs at high concentrations in tissues that are very sensitive to excess retinoic acid (such as the central nervous system). 'Knock-out' experiments have suggested, however, that these cytosolic binding proteins are 'dispensable' for embryonic development (Gorry *et al.*, 1994), as null mutant mice have no major developmental defects.

Studies of mouse embryos (Scott *et al.*, 1994) showed that the concentrations of all-*trans*-retinoic acid were much lower than those of its cellular binding protein CRABPI. In contrast, the whole-embryo concentration of retinol exceeded the concentration of its cellular binding protein CRBPI. Thus, it appears that all-*trans*-retinoic acid is predominantly bound to CRABPI in mouse embryos, while most of retinol remains unbound. CRABP can therefore function as a 'sink' for extensive accumulation of all-*trans*-retinoic acid in the embryo in the presence of low plasma concentrations (Tzimas *et al.*, 1996a).

The RARs and RXRs show very specific spatial and temporal distributions in the embryo (Dollé *et al.*, 1989, 1990; Ruberte *et al.*, 1990, 1991, 1993; Mangelsdorf *et al.*, 1994; Yamagata *et al.*, 1994). RARα is expressed ubiquitously throughout the embryo, while RARβ and RARγ show temporally and spatially restricted expression that is often mutually exclusive. For example, RARβ was found in the closed portion of the neural tube, while RARγ was present only in the open neural folds of the mouse embryo on

day 8.5 of gestation (Dollé *et al.*, 1990; Ruberte *et al.*, 1991, 1993), suggesting a role for RARβ and RARγ in the formation and closure of the neural tube. By day 13.5 of gestation, RARβ was no longer expressed in the cartilagenous condensations of the limb but was found in the interdigital mesenchyme, whereas RARγ was strongly expressed in the digits (Dollé *et al.*, 1990). These distribution patterns suggest that RARβ and RARγ are involved in the formation of the digits, and interference with retinoid signalling may cause interdigital webbing. Of the RXR family, RXRβ showed the widest distribution pattern within the embryo, while RXRα and RXRγ had more restricted patterns of expression (Mangelsdorf *et al.*, 1994).

5.1.4 Retinol deficiency and embryogenesis

When the concentrations of retinol and its metabolites in the embryo are reduced substantially, increased embryonic death and terata occur, as shown classically by removing vitamin A from the diet of the mother. Hale (1933) found that frank vitamin A deficiency in pigs induced anophthalmia in their offspring. Subsequent experiments by Warkany (1945), Wilson *et al.* (1953) and Kalter and Warkany (1959) showed that rodent embryos born to vitamin A-deficient mothers were afflicted with anophthalmia, microphthalmia, defects of the retina, hydrocephalus, cleft palate, malformed hind legs, cryptorchidism, cardiovascular malformations and urogenital tract malformations. Major defects in the developing central nervous system of the embryo could also be induced (Maden *et al.*, 1996).

Administration of various retinoids during specific developmental periods showed the efficiency of 'rescue' of embryonic structures by particular retinoids (Dickman *et al.*, 1997; Wellik *et al.*, 1997; Zile, 1998). In vitamin A-deficient rats, all-*trans*-retinoic acid can substitute for vitamin A in all instances except for reproduction (spermatogenesis) and vision. The embryos of vitamin A-deficient dams which were 'rescued' with all-*trans*-retinoic acid developed normally but were blind and sterile. Retinoic acid cannot be reduced to retinal, and no cofactor for rhodopsin can be produced from exogenous retinoic acid. Furthermore, retinoic acid cannot cross the blood–testis barrier (Kurlandsky *et al.*, 1995), and retinol is needed in the testis where it can be metabolized to retinoic acid.

It is clear, therefore, that any disturbance in the concentration of retinoids either by the provision of excess ligand or removal of ligand, disturbance of enzyme levels or removal of binding protein or receptors will result in terata. The terata are, as indicated above, remarkably uniform across all vertebrate species, although some differences between species are apparent. For example, at least in mammals, placental type may play a role. It is well established that rodents at different gestational ages have different types of placenta. In rats, the choriovitelline (yolk-sac) placenta is formed after angiogenesis of the chorionic placenta. An additional type of placenta, the chorioallantoic placenta, differentiates from day 11.5 of gestation and becomes functional from day 12 onwards (Beck, 1976; Jollie & Craig, 1979; Garbis-Berkvens & Peters, 1987). Most of the information on the transplacental distribution of retinoids in rodents is derived from studies performed on mid-organogenesis stages, such as day 11 in mice and day 12 in rats, thus prior to full development of the chorioallantoic placenta. In monkeys, development of the chorioallantoic placenta occurs much earlier during gestation and is already accomplished when embryonic retinoid concentrations can be measured (Beck, 1976).

Another factor is the presence of binding proteins in the embryo or the placenta. Harnish *et al.* (1992) found prolonged induction of CRABP II expression in mouse embryos after administration of teratogenic doses of retinoids. Increased CRABP II expression after exposure to all-*trans*-retinoic acid has also been observed in other systems and is compatible with the presence of a RARE and a RXRE in the promoter of the CRABP II gene (Durand *et al.*, 1992). In the presence of more CRABP II, more retinoic acid will be taken up by the embryo.

5.2 Molecular pathways for the teratogenicity of retinoids

During embryogenesis, the molecular code present in the genome is used to generate

mechanisms for the control of cell proliferation, differentiation and morphogenesis. Numerous processes must be strictly defined in developmental time and location to allow formation of the appropriate three-dimensional structure of the developing embryo during organogenesis and during all other developmental periods. Interference with such processes during organogenesis—when the most drastic structural defects can be induced—may be expected to be most efficient in altering gene expression. The complex retinoid signalling pathways, involving retinoid receptors, retinoid ligands and their strict spatio-temporal expression, are discussed in section 3. A major class of patterning genes which has been the subject of considerable research with regard to retinoids and teratogenesis are the *Hox* genes, many of which have retinoic acid response elements in their upstream sequences (Langston & Gudas, 1992; Pöpperl & Featherstone, 1993; Marshall *et al.*, 1994; Gould *et al.*, 1998; Packer *et al.*, 1998). Although the developing hindbrain of the central nervous system has been one focus of study, *Hox* genes are also expressed in other areas of the embryo, such as the limbs, the urogenital system, the genitalia and the gut, which helps to explain why retinoids have such pleiotropic effects.

5.3 New retinoids

New retinoids which can selectively activate individual RARs or RXRs may produce subsets of teratological defects in embryos. As the ability to bind the RARs decreases, so the teratogenicity of these retinoids decreases, such that RXR-selective compounds are not teratogenic *in vitro* or *in vivo* (Jiang *et al.*, 1994; Kochhar *et al.*, 1996). Thus, if RXR-selective compounds are found to be useful, they could eliminate the teratogenic side-effects of retinoid therapy.

6. Differentiation therapy of cancer

The fact that cancer may be envisaged as a disorder of differentiation provides at least a theoretical basis for therapy (Lynch, 1995). The possible therapeutic effect of retinoids has been recognized in this context for some years (Gudas, 1992; Smith *et al.*, 1992) and is considered to be strengthened by their ability to induce apoptosis (section 4.4). Uncontrolled proliferation of tumour cells may be due to the loss, altered expression or altered structure of particular gene products as a consequence of gene mutation or chromosomal translocation. The biology of leukaemia and a range of tumour types suggests that cancer may be understood as a disorder of differentiation. Thus, spontaneous differentiation of tumour cells has been described, specifically in teratocarcinoma (Pierce & Speers, 1988) and neuroblastoma.

The identification of *Patched*, a gene essentail for the embryonic development of *Drosophila melanogaster*, as a tumour suppressor gene provides direct support for the understanding that tumorigenesis involves abnormalities of development (Gailani *et al.*, 1996). Genes that play a critical role in cell cycle regulation may function as tumour suppressor genes. Loss of the cyclin-dependent kinase inhibitors p15 and p16 is evident in some tumours (Hunter & Pines, 1994). In turn, cellular proliferation is influenced by differentiation and DNA repair, the complex interrelationships being mediated in part by *p53* (Vogelstein & Kinzler, 1992). Thus, in the lymphoid and myeloid lineages there is evidence that wild-type *p53* is associated with growth regulation and stage-specific differentiation (Shaulsky *et al.*, 1991; Zhang *et al.*, 1992).

The *p53* tumour suppressor gene also exemplifies the close relationship between cell cycle regulation, DNA repair and apoptosis. Most currently used anticancer drugs inhibit DNA replication or formation of the mitotic spindle, thereby causing cell cycle arrest and in many cases initiating DNA repair processes. Depending on the cell type, prolonged cell cycle arrest or persistent DNA damage may result in cell death by either apoptosis or mitotic catastrophe (King & Cidlowski, 1995), although the same cytotoxic agents have been shown to induce differentiation of many cell lines at low concentrations (Darzynkiewicz, 1995). For example, adriamycin and daunomycin may mediate differentiation of HL-60 cells (Yung *et al.*, 1992)

Most, if not all cytotoxic drugs mediate cell death by apoptosis (Hickman *et al.*, 1994). In some cell populations and in response to particular stimuli, differentiation and apoptosis may be regulated independently (Delia *et al.*, 1993; Ponzoni *et al.*, 1995). In many instances, however, a close relationship between differentiation and apoptosis has been observed. For example, apoptosis is observed in human HL-60 cells induced to differentiate by phorbol esters and etoposide (Solary *et al.*, 1994).

Sachs (1978) proposed that an impairment of the proliferation–differentiation balance occurs in malignancy, raising the possibility of characterizing differentiating agents that can modify the biological response. In contradistinction to cytotoxic therapy, differentiation therapy is specifically targeted to the transformed malignant cell which is induced to differentiate terminally and to lose self-renewal potential. Hence, inducing malignant cells to differentiate is an established approach to cancer therapy. As differentiation of tumour cells to cells incapable of further division is not necessarily accompanied by depletion of the tumour stem-cell pool, differentiation therapy may have to be combined with other cytotoxic or apoptotic strategies.

6.1 Retinoid differentiation therapy in acute promyelocytic leukaemia

Studies on myeloid leukaemic cell lines have played a pivotal role. The demonstration of terminal differentiation of HL-60 cell line in response to retinoic acid (Breitman *et al.*, 1980) led to the finding that cells from patients with acute promyelocytic leukaemia are terminally differentiated by retinoic acid both *in vitro* (Chomienne *et al.*, 1990) and *in vivo* (Castaigne *et al.*, 1990), resulting in complete remission in 90% of cases. Despite a growing number of differentiating agents, however, few have been effective in clinical trials in improving the chances of long-term survival. Other models for differentiation therapy have been studied, such as the use of G-CSF for acute myeloid leukaemia associated with the t(8;21) translocation (Da Silva *et al.*, 1997).

6.1.1 Molecular basis of acute promyelocytic leukaemia

Acute promyelocytic leukaemia accounts for approximately 10% of all cases of acute myeloid leukaemia. It is characterized morphologically by blast cells (differentiation block at the promyelocytic stage; Bennett *et al.*, 1976), the t(15;17) chromosomal translocation (Larson *et al.*, 1984) and coagulopathy (Dombret *et al.*, 1992). The breakpoint has been cloned and has revealed a fusion between the *RARα* (located on chromosome 17) and *PML* (for promyelocytic leukaemia, located on chromosome 15) genes, giving rise to a *PML/RARα* chimaera (de Thé *et al.*, 1991). *PML* belongs to a gene family which encodes nuclear proteins with a zinc finger DNA-binding domain characteristic of many transcription factors that have a 'leucine zipper' motif, which functions as a dimerization interface in certain proteins (Kakizuka *et al.*, 1991). Each acute promyelocytic leukaemia patient is characterized by a specific *PML/RARα* fusion transcript termed *bcr1*, *bcr2* or *bcr3* (Grignani *et al.*, 1994). A variant translocation t(11;17) in an acute myeloid leukaemia patient has been cloned and another transcription factor has been identified, called PLZF, fused to RARα (Chen *et al.*, 1993). There is evidence that both fusion proteins alter the DNA binding and *trans*-activation properties of the wild-type RARα receptor (de Thé *et al.*, 1991; Chen *et al.*, 1994b) and lead to differentiation block (Rousselot *et al.*, 1994). Normal PML and PLZF proteins are located in large nuclear bodies which in the fusion protein have a microspeckled pattern (Koken *et al.*, 1994; Reid *et al.*, 1995). Two other rare fusions have been cloned from acute myeloid leukaemia patients with t(5;17) and t(11;17) translocations involving, respectively, the nucleophosmin (*NPM*) and nuclear mitotic apparatus (*NuMA*) genes with RARα (Redner *et al.*, 1996; Wells & Kamel-Reid, 1996).

6.1.2 Retinoic acid differentiation of acute promyelocytic leukaemic cells

Acute promyelocytic leukaemia cells are successfully induced to differentiate *in vitro* to polymorphonuclear cells in the presence of

retinoids, as demonstrated by morphology, nitroblue tetrazolium reduction assay and expression of differentiation antigens (CD11b, CD15). all-*trans*-Retinoic acid gave better results than the 13-*cis* isomer or 4-oxo metabolites, and a one-log difference in concentration for the same magnitude of effect was found (Chomienne *et al.*, 1989, 1990). The 9-*cis*-retinoic acid derivative gives results similar to those for all-*trans*-retinoic acid (Miller *et al.*, 1995). Cells that harbour chromosomal translocations resulting in rearrangements of the *RARα* gene but involving other fusion genes such as *PLZF* and *NPM* do not differentiate in the presence of all-*trans*-retinoic acid (Licht *et al.*, 1995).

6.1.3 all-trans-Retinoic acid differentiation therapy in acute promyelocytic leukaemia

The first clinical trial of all-*trans*-retinoic acid therapy in acute promyelocytic leukaemia was reported in 1988 from China (Huang *et al.*, 1988). About 90% of patients with newly diagnosed or first relapsed disease achieved complete remission with a dose of 45 mg/m^2 daily. When the data on 565 patients in studies conducted in China, France, Japan and the United States were combined, complete remission was found to have been achieved in 84%. Results for more than 1000 patients with acute promyelocytic leukaemia led to the conclusion that there was no resistance, the response was about 95% when the specific PML/RARα rearrangement was present, the differentiating effect was obtained without aplasia and coagulopathy rapidly improved (Warrell *et al.*, 1993). The major side-effect is the condition referred to as the 'all-*trans*-retinoic acid syndrome', sometimes associated with hyperleukocytosis and characterized by fever, respiratory distress, weight gain, pleural or pericardial effusion and sometimes renal failure (Frankel *et al.*, 1992). It occurs during the first month of treatment and can be prevented by giving chemotherapy when hyperleukocytosis occurs (Fenaux *et al.*, 1993). Steroids such as dexamethasone significantly reduce the severity and frequency of symptoms (Wiley & Firkin, 1995).

The shortness of the duration of complete remission after all-*trans*-retinoic acid therapy alone and the weaker activity of subsequent treatment led to the identification of acquired resistance due to autoinduced catabolism of all-*trans*-retinoic acid, by the induction of 4-hydroxylase of CYP involved in plasma catabolism (Lippman & Meyskens, 1987). A European multicentre randomized trial of chemotherapy alone and of all-*trans*-retinoic acid followed by the same chemotherapy in newly diagnosed cases of acute promyelocytic leukaemia was performed between 1991 and 1992. The trial was terminated when event-free actuarial survival at three years was 76% in the group receiving chemotherapy and all-*trans*-retinoic acid and 53% in the group given chemotherapy alone (Fenaux *et al.*, 1993). Subsequent clinical trials have established the superiority of combined treatment based on all-*trans*-retinoic acid and cytotoxic chemotherapy for acute promyelocytic leukaemia (Tallman *et al.*, 1997).

Patients at high risk for relapse can be identified by a positive reaction in a reverse transcriptase polymerase chain reaction by amplification of the different PML/RARα transcripts resulting from the t(15;17) translocation (Fenaux & Chomienne, 1996). Patients defined as poor responders, less than 50% of whose cells differentiate after three days of culture in the presence of all-*trans*-retinoic acid, have low intracellular concentrations of all-*trans*-retinoic acid and a greater risk for relapse (Agadir *et al.*, 1995).

In primary cultures of acute promyelocytic leukaemia cells *in vitro*, other agents have been shown to be more effective than all-*trans*-retinoic acid. These include RARα agonists (Cassinat *et al.*, 1998) and the aromatic retinoid AM80, which was effective in resistant patients (Takeshita *et al.*, 1996). 9-*cis*-Retinoic acid, which is as effective *in vitro* as all-*trans*-retinoic acid, also had equivalent activity in inducing complete remission *in vivo*. It has the added advantage of not decreasing 9-*cis*-retinoic acid plasma concentrations during treatment. Longer follow-up will be required to determine if 9-*cis*-retinoic acid is more effective in eradicating the leukaemia clone than all-*trans*-retinoic acid (Miller *et al.*, 1995).

6.2 Retinoid therapy in other haematological disorders

Myelodysplastic disorders are lethal, as they involve a stem-cell defect that affects the control of myeloid differentiation and apoptosis. In one-third of cases, the cause of death is acute myeloid leukaemia. 13-*cis*-Retinoic acid and all-*trans*-retinoic acid have been used in randomized trials alone or in association with other drugs with no significant effect (Greenberg *et al.*, 1985; Clark *et al.*, 1987; Koeffler *et al.*, 1988; Aul *et al.*, 1993; Kurzrock *et al.*, 1993). Subtypes of myelodysplastic disorders with a proliferative disease such as chronic myelomonocytic leukaemia or juvenile chronic myeloid leukaemia appear to benefit more from the antiproliferative effect of retinoids. Preliminary results in cases of juvenile chronic myeloid leukaemia have shown that 13-*cis*-retinoic acid can reduce lymphocyte counts and organomegaly in one-half of patients (Castleberry *et al.*, 1994). These results prompted Cambier *et al.* (1996) to test the efficacy of all-*trans*-retinoic acid in adult chronic myelomonocytic leukaemia, as this disorder has many features in common with the juvenile disease. Of 10 patients with advanced adult chronic myelomonocytic leukaemia treated with all-*trans*-retinoic acid (45 mg/m^2 per day), two developed 'all-*trans*-retinoic acid syndrome' and four had a significant response; two of the latter had reduced transfusion requirement and two showed increased platelet counts. all-*trans*-Retinoic acid alone was not associated with a reduction in hepatosplenomegaly or leukocytosis. The improvement in cytopenia observed by Cambier *et al.* (1996) in four patients was not reported in the cases of juvenile chronic myeloid leukaemia. Whether those differences in response to retinoids in adult and juvenile disease are due to intrinsic biological differences between the two disorders or to different actions of 13-*cis*-retinoic acid and all-*trans*-retinoic acid remains to be clarified.

6.3 Retinoid therapy in solid tumours

The efficacy of retinoids in inhibiting malignant cell growth *in vitro* and, in some cases, inducing differentiation and/or apoptosis have led to trials of their use for the therapy of invasive and preinvasive tumours of epithelial origin, including skin cancers such as basal-cell carcinomas and advanced squamous-cell carcinomas (Lippman *et al.*, 1987, 1995), cutaneous T-cell lymphoma, mycosis fungoides (Molin *et al.*, 1985) and Kaposi sarcoma (Bonhomme *et al.*, 1991). Pilot studies have been conducted with 13-*cis*-retinoic acid and etretinate for bladder cancer, prostate cancer and central nervous system cancers (Lippman & Meyskens, 1987; Grunberg & Itri, 1987; Reynolds *et al.*, 1990; Lippman *et al.*, 1992; Cobleigh *et al.*, 1993; Reynolds *et al.*, 1994; Defer, 1996). A phase II clinical trial of all-*trans*-retinoic acid showed no significant antitumour activity in patients with chemotherapy-refractory germ-cell tumours (Moasser *et al.*, 1995). Further, the combination of all-*trans*-retinoic acid and interferon α-2a was inactive in patients with advanced carcinoma of the cervix (Castaigne *et al.*, 1990).

On the basis of a small number of patients, it was suggested that 13-*cis*-retinoic acid might be effective in the treatment of Ki-1 anaplastic large-cell lymphoma (Chou *et al.*, 1996). A phaseII trial in children with recurrent neuroblastoma showed few responses, but the drug may be of more benefit in patients who have been treated by bone-marrow transplantation. It has been shown to be effective as a single agent in the treatment of squamous-cell carcinomas of the skin and mycosis fungoides (Villablanca *et al.*, 1995). In combination with interferon α-2a, 13-*cis*-retinoic acid was of benefit in the treatment of renal-cell carcinoma (Motzer *et al.*, 1995).

The lack of efficacy of retinoids against solid tumours is probably due to reduced accessibility, the heterogeneity of tumour cells and the lack of specific targeting of the retinoid to a tumour-specific retinoid receptor. In comparison with haematopoietic cells, which preferentially express the RARα receptor, a target for all-*trans*-retinoic acid, lung, nervous and thyroid cells express RARβ. In some of these tumours, RARβ expression is decreased or absent. *In vitro*, the response to retinoids has been shown to be linked to an increase in RARβ (Houle *et al.*, 1993; Lotan *et al.*, 1995; Carpentier *et al.*, 1997;

Schmutzler *et al.*, 1998). The general lack of efficacy, despite promising results in studies in cell lines, prompted trials with the more active receptor-targeted ligands such as targretin (RXR) (Miller *et al.*, 1997) and LGD 1550 (RAR).

7. Use of retinoids in the treatment of other conditions

7.1 Treatment of psoriasis

During the past decade, retinoids have been used increasingly for the treatment of psoriasis and other hyperkeratotic and parakeratotic skin diseases with or without dermal inflammation and as a standard treatment for severe acne and acne-related dermatoses (Peck *et al.*, 1982; Orfanos *et al.*, 1997). Vitamin A deficiency in experimental animals and humans is associated with xerosis, epithelial hyperkeratosis and squamous metaplasia of mucosal surfaces. Because these effects are reversible after intake of vitamin A and because of the similarity between certain disorders of keratinization and hypovitaminosis A, retinol and its esters were used in the treatment of psoriasis, ichthyoses and Darier disease in the 1940s. Topical all-*trans*-retinoic acid was later shown to have some activity in the treatment of psoriasis vulgaris. Later, etretinate was selected for clinical evaluation in psoriatic patients because of its favourable 'therapeutic index', which is 10 times better than that of all-*trans*-retinoic acid in animals.

Both etretinate and acitretin have been shown in extensive clinical trials to be effective in treating severe forms of psoriasis. The best responses are obtained in erythrodermic psoriasis and in localized or generalized pustular psoriasis, in which improvement has been reported in approximately 90% of all treated patients (Lowe *et al.*, 1988). Accordingly, retinoids have been used for topical and systemic treatment of psoriasis and other hyperkeratotic and parakeratotic skin disorders, keratotic genodermatoses and severe acne-related dermatoses (Bjerke & Geiger, 1989; Orfanos *et al.*, 1997). Etretinate was also used in the treatment of psoriatic arthritis, although its efficacy failed to achieve statistical significance in a meta-analysis (Jones *et al.*, 1997).

In psoriatic skin, etretinate has strong keratinolytic activity, leading to restoration of the normal epidermis by causing reappearance of the granular layer, disappearance of parakeratosis and reduction of acanthosis. Autoradiographic studies have demonstrated a decrease in the labelling index for [^{3}H]thymidine incorporation and prolonged DNA synthesis in the affected and uninvolved epidermis of psoriatic patients. Clinical benefit after initiation of etretinate therapy for psoriasis occurs one to two weeks after desquamation begins, and healing may be seen after three weeks (Gollnick *et al.*, 1990).

Acitretin is very similar in efficacy to etretinate for the treatment of psoriasis. In several 8–12-week randomized, double-blind clinical trials (Lauharanta & Geiger, 1989; Lassus & Geiger, 1988), acitretin was as effective as etretinate in patients with erythrodermic and pustular psoriasis. It has been suggested that etretinate may be better tolerated than acitretin when tested at equivalent doses (Cunningham & Geiger, 1992; Koo *et al.*, 1997). Acitretin was tested at administered doses of 10–75 mg/day in more than 1000 patients with psoriasis between 1985 and 1992 (Pilkington & Brogden, 1992) in non-comparative and placebo-controlled, double-blind clinical trials ranging in length from six weeks to 12 months. In the non-comparative studies, in which 8–52 evaluable patients per study received the drug orally for periods up to six months, body surface involvement was reduced by 33–96%. In the placebo-controlled trials, involving similar treatment protocols, a dose-dependent improvement of 6–85% was seen. In these and related studies, acitretin at 10–25 mg/day resulted in responses equivalent to those of the placebo, whereas doses of 50–75 mg/day significantly improved the symptoms of psoriasis (Koo *et al.*, 1997).

The mode of action of acitretin in psoriasis is unknown. Since retinoids have immunomodulatory and anti-inflammatory effects in human peripheral blood lymphocytes and polymorphonuclear leukocytes, an immune mechanism might be involved. During therapy

with acitretin and other agents, IgG-Fc receptor levels increase but do not reach those registered in normal controls, indicating an immunological defect that is not corrected by effective therapy (Bjerke *et al.*, 1994). Acitretin may mediate its effect on psoriasis through modification of cAMP-dependent protein kinases (Raynaud *et al.*, 1993).

Combination therapy involving acitretin and ultraviolet B radiation was of greater benefit to patients with plaque-type psoriasis than either treatment alone (Iest & Boer, 1989). Most of the trials lasted from 8 to 12 weeks and involved 34–88 patients taking 20–50 mg of acitretin per day alone or in combination with ultraviolet B radiation. With either monotherapy or combination therapy, 50% to over 90% of patients showed improvement (Koo *et al.*, 1997).

Acitretin is also effective in the treatment of psoriasis associated with HIV infection (Buccheri *et al.*, 1997). Etretinate has also been combined with other systemic treatments such as methotrexate, hydroxyurea and cyclosporine.

7.2 Other dermatological conditions

13-*cis*-Retinoic acid is regarded as an extremely effective drug when given systemically in the treatment of severe acne. Its efficacy is attributed to its ability to inhibit sebaceous gland activity. Orally administered retinoids such as etretinate and acitretin do not inhibit sebum production in humans and are ineffective against acne (Geiger, 1995).

Systemically administered retinoids such as etretinate and those given topically such as all-*trans*-retinoic acid are of benefit in the treatment of oral lichen planus (Lozada-Nur & Miranda, 1997). Acitretin has been used to treat skin complications in renal transplant recipients (Yuan *et al.*, 1995). Topical administration of all-*trans*-retinoic acid is useful for treating patients with dry eye disorders in which squamous metaplasia with keratinization of the ocular epithelium is present (Wright, 1985; Tseng, 1986; Murphy *et al.*, 1996). In addition, all-*trans*-retinoic acid can block the process of premature skin ageing induced by sunlight (Fisher *et al.*, 1997).

7.3 Other non-malignant conditions

The use of retinoids for a variety of conditions has been proposed on the basis of experimental observations. In a single case report, 13-*cis*-retinoic acid was of benefit in the treatment of thrombotic thrombocytopenic purpura (Raife *et al.*, 1998). Etretinate was proposed to be of benefit in the treatment of severe Reiter syndrome associated with HIV infection (Louthrenoo, 1993).

Studies of collagen production in human lung fibroblasts indicate that all-*trans*-retinoic acid inhibits the basal and transforming growth factor-β-stimulated production of types I and III collagen by these cells (Redlich *et al.*, 1995). In an isolated, spontaneously beating neonatal rat cardic myocyte preparation, all-*trans*-retinoic acid (10–20 μmol/L) prevented arrhythmia induced by isoproterenol or lysophosphatidylcholine (Kang & Leaf, 1995).

Retinoids like retinol and retinyl esters, which must be activated to retinoic acid, are beneficial in the treatment of bronchopulmonary dysplasia in premature infants (Chytil, 1996). In rats, all-*trans*-retinoic acid reversed the effects of elastase-induced pulmonary emphysema, and the efficacy of all-*trans*-retinoic acid in the treatment of emphysema is being assessed in clinical trials (Massaro & Massaro, 1996, 1997).

all-*trans*-Retinoic acid was also reported to be effective in reducing neointimal mass and eliciting favourable remodelling of vessel walls after balloon-withdrawal injury to the common carotid artery in rats (Miano *et al.*, 1998). Thus, all-*trans*-retinoic acid may prove useful for preventing or treating restenosis in patients with cardiovascular disease. Similarly, retinoids may prove effective for treating non-insulin-dependent diabetes mellitus, as studies of RXR-selective retinoids in mouse models for this disease indicate that RXR agonists function as insulin sensitizers and can decrease hyperglycaemia, hypertriglycidaemia and hyperinsulinaemia (Mukherjee *et al.*, 1997). The treatment of experimental mouse models of arthritis with any of several retinoids significantly inhibited the severity and development of arthritis (Takaoka *et al.*, 1997; Nagai *et al.*, 1999).

References

Anonymous (1990) Nomenclature policy: Generic descriptors and trivial names for vitamins and related compounds. *J. Nutr.*, **120**, 12–19

Abu-Abed, S.S., Beckett, B.R., Chiba, H., Chithalen, J.V., Jones, G., Metzger, D., Chambon, P. & Petkovich, M. (1998) Mouse *P450RAI* (CYP26) expression and retinoic acid-inducible retinoic acid metabolism in F9 cells are regulated by retinoic acid receptor γ and retinoid X receptor α. *J. Biol. Chem.*, **273**, 2409–2415

Agadir, A., Cornic, M., Lefebvre, P., Gourmel, B., Jerome, M., Degos, L., Fenaux, P. & Chomienne, C. (1995) All-*trans*-retinoic acid pharmacokinetics and bioavailability in acute promyelocytic leukemia: Intracellular concentrations and biologic response relationship. *J. Clin. Oncol.*, **13**, 2517–2523

Agarwal, C., Rorke, E.A., Irwin, J.C. & Eckert, R.L. (1991) Immortalization by human papillomavirus type 16 alters retinoid regulation of human ectocervical epithelial cell differentiation. *Cancer Res.*, **51**, 3982–3989

Allali-Hassani, A., Peralba, J.M., Martras, S., Farres, J. & Pares, X. (1998) Retinoids, omega-hydroxy-fatty acids and cytotoxic aldehydes as physiological substrates, and H2-receptor antagonists as pharmacological inhibitors, of human class IV alcohol dehydrogenase. *FEBS Lett.*, **426**, 362–366

Amos, B. & Lotan, R. (1990) Retinoid-sensitive cells and cell lines. In: Packer L., ed., *Methods in Enzymology: Retinoids*. Orlando, FL, Academic Press, pp. 217–225

Andreola, F., Fernandez Salguero, P.M., Chiantore, M.V., Petkovich, M.P., Gonzalez, F.J. & De Luca, L.M. (1997) Aryl hydrocarbon receptor knockout mice ($AHR^{-/-}$) exhibit liver retinoid accumulation and reduced retinoic acid metabolism. *Cancer Res.*, **57**, 2835–2838

Ang, H.L., Deltour, L., Zgombic, K.M., Wagner, M.A. & Duester, G. (1996a) Expression patterns of class I and class IV alcohol dehydrogenase genes in developing epithelia suggest a role for alcohol dehydrogenase in local retinoic acid synthesis. *Alcohol Clin. Exp. Res.*, **20**, 1050–1064

Ang, H.L., Deltour, L., Hayamizu, T.F., Zgombic-Knight, M. & Duester, G. (1996b) Retinoic acid synthesis in mouse embryos during gastrulation and craniofacial development linked to class IV alcohol dehydrogenase gene expression. *J. Biol. Chem.*, **271**, 9526–9534

Aoudjit, F., Brochu, N., Morin, N., Poulin, G., Stratowa, C. & Audette, M. (1995) Heterodimeric retinoic acid receptor-β and retinoid X receptor-α complexes stimulate expression of the intercellular adhesion molecule-1 gene. *Cell Growth Differ.*, **6**, 515–521

Apfel, C.M., Kamber, M., Klaus, M., Mohr, P., Keidel, S. & LeMotte, P.K. (1995) Enhancement of HL-60 differentiation by a new class of retinoids with selective activity on retinoid X receptor. *J. Biol. Chem.*, **270**, 30765–30772

Arnhold, T., Tzimas, G., Wittfoht, W., Plonait, S. & Nau, H. (1996) Identification of 9-*cis*-retinoic acid, 9,13-di-*cis*-retinoic acid, and 14-hydroxy-4,14-*retro*-retinol in human plasma after liver consumption. *Life Sci.*, **59**, L169–L177

Aul, C., Runde, V. & Gattermann, N. (1993) all-*trans*-Retinoic acid in patients with myelodysplastic syndromes: Results of a pilot study. *Blood*, **82**, 2967–2974

Baker, M.E. (1994) Sequence analysis of steroid- and prostaglandin-metabolizing enzymes: Application to understanding catalysis. *Steroids*, **59**, 248–258

Baker, M.E. (1998) Evolution of mammalian 11β- and 17β-hydroxysteroid dehydrogenases-type 2 and retinol dehydrogenases from ancestors in *Caenorhabditis elegans* and evidence for horizontal transfer of a eukaryote dehydrogenase to *E. coli*. *J. Steroid Biochem. Mol. Biol.*, **66**, 355–363

Balkan, W., Colbert, M., Bock, C. & Linney, E. (1992) Transgenic indicator mice for studying activated retinoic acid receptors during development. *Proc. Natl Acad. Sci. USA*, **89**, 3347–3351

Barua, A.B. (1997) Retinoyl β-glucuronide: A biologically active form of vitamin A. *Nutr. Rev.*, **55**, 259–267

Barua, A.B. & Olson, J.A. (1986) Retinoyl β-glucuronide: An endogenous compound of human blood. *Am. J. Clin. Nutr.*, **43**, 481–485

Barua, A.B., Gunning, D.B. & Olson, J.A. (1991) Metabolism *in vivo* of all-*trans*-[11-^{3}H]retinoic acid after an oral dose in rats. Characterization of retinoyl β-glucuronide in the blood and other tissues. *Biochem. J.*, **277**, 527–531

Beato, M., Herrlich, P. & Schütz, G. (1995) Steroid hormone receptors: Many actors in search of a plot. *Cell*, **83**, 851–857

Beck, F. (1976) Comparative placental morphology and function. *Environ. Health Perspect.*, **18**, 5–12

Ben-Amotz, A., Mokady, S. & Avron, M. (1988) The β-carotene-rich alga *Dunaliella bardawil* as a source of retinol in a rat diet. *Br. J. Nutr.*, **59**, 443–449

Bennett, J.M., Catovsky, D., Daniel, M.T., Flandrin, G., Galton, D.A., Gralnick, H.R. & Sultan, C. (1976) Proposals for the classification of the acute leukaemias. French– American–British (FAB) co-operative group. *Br. J. Haematol.*, **33**, 451–458

Bernacki, S.H., Medvedev, A., Holloway, G., Dawson, M., Lotan, R. & Jetten, A.M. (1998) Suppression of relaxin gene expression by retinoids in squamous differentiated rabbit tracheal epithelial cells. *Mol. Cell. Endocrinol.*, **138**, 115–125

Bertram, J.S. (1983) Inhibition of neoplastic transformation in vitro by retinoids. *Cancer Surv.*, **2**, 243–262

Bhat, P.V. (1998) Retinal dehydrogenase gene expression in stomach and small intestine of rats during postnatal development and in vitamin A deficiency. *FEBS Lett.*, **426**, 260–262

Bhat, P.V. & Samaha, H. (1999) Kinetic properties of the human liver cytosolic aldehyde dehydrogenase for retinal isomers. *Biochem. Pharmacol.*, **57**, 195–197

Bhat, P.V., Poissant, L., Falardeau, P. & Lacroix, A. (1988a) Enzymatic oxidation of all-*trans* retinal to retinoic acid in rat tissues. *Biochem. Cell. Biol.*, **66**, 735–740

Bhat, P.V., Poissant, L. & Lacroix, A. (1988b) Properties of retinal-oxidizing enzyme activity in rat kidney. *Biochim. Biophys. Acta*, **967**, 211–217

Bhat, P.V., Labrecque, J., Boutin, J.M., Lacroix, A. & Yoshida, A. (1995) Cloning of a cDNA encoding rat aldehyde dehydrogenase with high activity for retinal oxidation. *Gene*, **166**, 303–306

Bhat, P.V., Poissant, L. & Wang, X.L. (1996) Purification and partial characterization of bovine kidney aldehyde dehydrogenase able to oxidize retinal to retinoic acid. *Biochem. Cell. Biol.*, **74**, 695–700

Bhat, P.V., Marcinkiewicz, M., Li, Y. & Mader, S. (1998) Changing patterns of renal retinal dehydrogenase expression parallel nephron development in the rat. *J. Histochem. Cytochem.*, **46**, 1025–1032

Biswas, M.G. & Russell, D.W. (1997) Expression cloning and characterization of oxidative 17β- and 3α-hydroxysteroid dehydrogenases from rat and human prostate. *J. Biol. Chem.*, **272**, 15959–15966

Bjerke, J.R. & Geiger, J.M. (1989) Acitretin versus etretinate in severe psoriasis. A double-blind randomized Nordic multicenter study in 168 patients. *Acta Derm. Venereol. Suppl. Stockh.*, **146**, 206–207

Bjerke, J.R., Tigalonova, M., Jensen, T.S. & Matre, R. (1994) Fcγ-receptors in skin and serum from patients with psoriasis, before and after therapy. *Acta Derm. Venereol. Suppl. Stockh.*, **186**, 141–142

Blaner, W.S. & Churchich, J.E. (1980) The membrane bound retinol dehydrogenase from bovine rod outer segments. *Biochem. Biophys. Res. Commun.*, **94**, 820–826

Blaner, W.S. & Olson, J.A. (1994) Retinol and retinoic acid metabolism. In: Sporn, M.B., Roberts, A.B. & Goodman, D.S., eds, *The Retinoids: Biology, Chemistry, and Medicine*, 2nd ed. New York, Raven Press, pp. 229–255

Bocquel, M.T., Kumar, V., Stricker, C., Chambon, P. & Gronemeyer, H. (1989) The contribution of the N- and C-terminal regions of steroid receptors to activation of transcription is both receptor and cell-specific. *Nucleic Acids Res.*, **17**, 2581–2595

Boerman, M.H. & Napoli, J.L. (1995) Characterization of a microsomal retinol dehydrogenase: A short-chain alcohol dehydrogenase with integral and peripheral membrane forms that interacts with holo-CRBP (type I). *Biochemistry*, **34**, 7027–7037

Boleda, M.D., Saubi, N., Farres, J. & Pares, X. (1993) Physiological substrates for rat alcohol dehydrogenase classes: Aldehydes of lipid peroxidation, omega-hydroxyfatty acids, and retinoids. *Arch. Biochem. Biophys.*, **307**, 85–90

Bollag, W. & Holdener, E.E. (1992) Retinoids in cancer prevention and therapy. *Ann. Oncol.*, **3**, 513–526

Boncinelli, E., Simeone, A., Acampora, D. & Mavilio, F. (1991) *HOX* gene activation by retinoic acid. *Trends Genet.*, **7**, 329–334

Bonhomme, L., Fredj, G., Averous, S., Szekely, A.M., Ecstein, E., Trumbic, B., Meyer, P., Lang, J.M., Misset, J.L. & Jasmin, C. (1991) Topical treatment of epidemic Kaposi's sarcoma with all-*trans*-retinoic acid. *Ann. Oncol.*, **2**, 234–235

Bourguet, W., Ruff, M., Chambon, P., Gronemeyer, H. & Moras, D. (1995) Crystal structure of the ligand-binding domain of the human nuclear receptor RXR-α. *Nature*, **375**, 377–382

Boylan, J.F., Lufkin, T., Achkar, C.C., Taneja, R., Chambon, P. & Gudas, L.J. (1995) Targeted disruption of retinoic acid receptor α (RARα) and RARγ results in receptor-specific alterations in retinoic acid-mediated differentiation and retinoic acid metabolism. *Mol. Cell. Biol.*, **15**, 843–851

Boyle, J.O., Langenfeld, J., Lonardo, F., Sekula, D., Reczek, P., Rusch, V., Dawson, M.I. & Dmitrovsky, E. (1999) Cyclin D1 proteolysis: A retinoid chemoprevention signal in normal, immortalized, and transformed human bronchial epithelial cells. *J. Natl Cancer Inst.*, **91**, 373–379

Breitman, T.R., Selonick, S.E. & Collins, S.J. (1980) Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. *Proc. Natl Acad. Sci. USA*, 77, 2936–2940

Brouwer, A., van den Berg, K.J. & Kukler, A. (1985) Time and dose responses of the reduction in retinoid concentrations in C57BL/Rij and DBA/2 mice induced by 3,4,3',4'-tetrachlorobiphenyl. *Toxicol. Appl. Pharmacol.*, **78**, 180–189

Bruel, A., Benoit, G., De-Nay, D., Brown, S. & Lanotte, M. (1995) Distinct apoptotic responses in maturation sensitive and resistant t(15;17) acute promyelocytic leukemia NB4 cells. 9-cis Retinoic acid induces apoptosis independent of maturation and Bcl-2 expression. *Leukemia*, **9**, 1173–1184

Brzozowski, A.M., Pike, A.C., Dauter, Z., Hubbard, R.E., Bonn, T., Engstrom, O., Ohman, L., Greene, G.L., Gustafsson, J.A. & Carlquist, M. (1997) Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature*, **389**, 753–758

Buccheri, L., Katchen, B.R., Karter, A.J. & Cohen, S.R. (1997) Acitretin therapy is effective for psoriasis associated with human immunodeficiency virus infection. *Arch. Dermatol.*, **133**, 711–715

Bulens, F., Ibanez-Tallon, I., Van Acker, P., De Vriese, A., Nelles, L., Belayew, A. & Collen, D. (1995) Retinoic acid induction of human tissue-type plasminogen activator gene expression via a direct repeat element (DR5) located at –7 kilobases. *J. Biol. Chem.*, **270**, 7167–7175

Caelles, C., González-Sancho, J.M. & Muñoz, A. (1997) Nuclear hormone receptor antagonism with AP-1 by inhibition of the JNK pathway. *Genes Dev.*, **11**, 3351–3364

Cambier, N., Wattel, E., Menot, M.L., Guerci, A., Chomienne, C. & Fenaux, P. (1996) All-*trans* retinoic acid in adult chronic myelomonocytic leukemia: Results of a pilot study. *Leukemia*, **10**, 1164–1167

Carpentier, A., Balitrand, N., Rochette-Egly, C., Shroot, B., Degos, L. & Chomienne, C. (1997) Distinct sensitivity of neuroblastoma cells for retinoid receptor agonists: Evidence for functional receptor heterodimers. *Oncogene*, **15**, 1805–1813

Cassinat, B., Balitrand, N., Zassadowski, F., Barbey, C., Delva, L., Bastie, J.N., Rain, J.D., Degos, L., Fenaux, P. & Chomienne, C. (1998) Combination of ATRA with G-CSF or RARα agonists enhances differentiation induction of fresh APL cells. *Blood*, **92** (Suppl. 1), 596a

Castaigne, S., Chomienne, C., Daniel, M.T., Ballerini, P., Berger, R., Fenaux, P. & Degos, L. (1990) All-*trans*-retinoic acid as a differentiation therapy for acute promyelocytic leukemia. I. Clinical results. *Blood*, **76**, 1704–1709

Castleberry, R.P., Emanuel, P.D., Zuckerman, K.S., Cohn, S., Strauss, L., Byrd, R.L., Homans, A., Chaffee, S., Nitschke, R. & Gualtieri, R.J. (1994) A pilot study of isotretinoin in the treatment of juvenile chronic myelogenous leukemia. *N. Engl. J. Med.*, **331**, 1680–1684

Chai, X., Zhai, Y., Popescu, G. & Napoli, J.L. (1995) Cloning of a cDNA for a second retinol dehydrogenase type II. Expression of its mRNA relative to type I. *J. Biol. Chem.*, **270**, 28408–28412

Chai, X., Zhai, Y. & Napoli, J.L. (1996) Cloning of a rat cDNA encoding retinol dehydrogenase isozyme type III. *Gene*, **169**, 219–222

Chai, X., Zhai, Y. & Napoli, J.L. (1997) cDNA cloning and characterization of a *cis*-retinol/3α-hydroxysterol short-chain dehydrogenase. *J. Biol. Chem.*, **272**, 33125–33131

Chambon, P. (1996) A decade of molecular biology of retinoic acid receptors. *FASEB J.*, **10**, 940–954

Chen, H. & Juchau, M.R. (1997) Glutathione S-transferases act as isomerases in isomerization of 13-*cis*-retinoic acid to all-*trans*-retinoic acid in vitro. *Biochem.J.*, **327**, 721–726

Chen, H. & Juchau, M.R. (1998) Biotransformation of 13-*cis*- and 9-*cis*-retinoic acid to all-*trans*-retinoic acid in rat conceptal homogenates. Evidence for catalysis by a conceptal isomerase. *Drug Metab. Dispos.*, **26**, 222–228

Chen, L.C., Berberian, I., Koch, B., Mercier, M., Azais, B., V, Glauert, H.P., Chow, C.K. & Robertson, L.W. (1992a) Polychlorinated and polybrominated biphenyl congeners and retinoid levels in rat tissues: Structure–activity relationships. *Toxicol. Appl. Pharmacol.*, **114**, 47–55

Chen, Y., Huang, L., Russo, A.F. & Solursh, M. (1992b) Retinoic acid is enriched in Hensen's node and is developmentally regulated in the early chicken embryo. *Proc. Natl Acad. Sci. USA*, **89**, 10056–10059

Chen, Z., Brand, N.J., Chen, A., Chen, S.J., Tong, J.H., Wang, Z.Y., Waxman, S. & Zelent, A. (1993) Fusion between a novel *Krüppel*-like zinc finger gene and the retinoic acid receptor-α locus due to a variant t(11;17) translocation associated with acute promyelocytic leukaemia. *EMBO J.*, **12**, 1161–1167

Chen, Y., Huang, L. & Solursh, M. (1994a) A concentration gradient of retinoids in the early *Xenopus laevis* embryo. *Dev. Biol.*, **161**, 70–76

Chen, Z., Guidez, F., Rousselot, P., Agadir, A., Chen, S.J., Wang, Z.Y., Degos, L., Zelent, A., Waxman, S. & Chomienne, C. (1994b) PLZF-RARα fusion proteins generated from the variant t(11;17)(q23;q21) translocation in acute promyelocytic leukemia inhibit ligand-dependent transactivation of wild-type retinoic acid receptors. *Proc. Natl Acad. Sci. USA*, **91**, 1178–1182

Chen, J.Y., Penco, S., Ostrowski, J., Balaguer, P., Pons, M., Starrett, J.E., Reczek, P., Chambon, P. & Gronemeyer, H. (1995) RAR-specific agonist/antagonists which dissociate transactivation and AP1 transrepression inhibit anchorage-independent cell proliferation. *EMBO J.*, **14**, 1187–1197

Chen, J.Y., Clifford, J., Zusi, C., Starrett, J., Tortolani, D., Ostrowski, J., Reczek, P.R., Chambon, P. & Gronemeyer, H. (1996) Two distinct actions of retinoid-receptor ligands. *Nature*, **382**, 819–822

Chen, H., Lin, R.J., Schiltz, R.L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M.L., Nakatani, Y. & Evans, R.M. (1997) Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell*, **90**, 569–580

Chen, Z.P., Iyer, J., Bourguet, W., Held, P., Mioskowski, C., Lebeau, L., Noy, N., Chambon, P. & Gronemeyer, H. (1998) Ligand- and DNA-induced dissociation of RXR tetramers. *J. Mol. Biol.*, **275**, 55–65

Chomienne, C., Ballerini, P., Balitrand, N., Amar, M., Bernard, J.F., Boivin, P., Daniel, M.T., Berger, R., Castaigne, S. & Degos, L. (1989) Retinoic acid therapy for promyelocytic leukaemia. *Lancet*, **2**, 746–747

Chomienne, C., Ballerini, P., Balitrand, N., Daniel, M.T., Fenaux, P., Castaigne, S. & Degos, L. (1990) All-*trans* retinoic acid in acute promyelocytic leukemias. II. In vitro studies: Structure–function relationship. *Blood*, **76**, 1710–1717

Choo, C.K., Rorke, E.A. & Eckert, R.L. (1995) Retinoid regulation of cell differentiation in a series of human papillomavirus type 16-immortalized human cervical epithelial cell lines. *Carcinogenesis*, **16**, 375–381

Chopra, D.P. (1983) Retinoid reversal of squamous metaplasia in organ cultures of tracheas derived from hamsters fed on vitamin A-deficient diet. *Eur. J. Cancer Clin. Oncol.*, **19**, 847–857

Chou, W.C., Su, I.J., Tien, H.F., Liang, D.C., Wang, C.H., Chang, Y.C. & Cheng, A.L. (1996) Clinicopathologic, cytogenetic, and molecular studies of 13 Chinese patients with Ki-1 anaplastic large cell lymphoma. Special emphasis on the tumor response to 13-*cis*-retinoic acid. *Cancer*, **78**, 1805–1812

Chytil, F. (1996) Retinoids in lung development. *FASEB J.*, **10**, 986–992

Clairmont, A. & Sies, H. (1997) Evidence for a posttranscriptional effect of retinoic acid on connexin43 gene expression via the 3'-untranslated region. *FEBS Lett.*, **419**, 268–270

Clark, R.E., Jacobs, A., Lush, C.J. & Smith, S.A. (1987) Effect of 13-*cis*-retinoic acid on survival of patients with myelodysplastic syndrome. *Lancet*, **i**, 763–765

Clifford, J., Chiba, H., Sobieszczuk, D., Metzger, D. & Chambon, P. (1996) RXRα-null F9 embryonal carcinoma cells are resistant to the differentiation, anti-proliferative and apoptotic effects of retinoids. *EMBO J.*, **15**, 4142–4155

Cobleigh, M.A., Dowlatshahi, K., Deutsch, T.A., Mehta, R.G., Moon, R.C., Minn, F., Benson, A.B., Rademaker, A.W., Ashenhurst, J.B., Wade, J.L. & Wolter, J. (1993) Phase I/II trial of tamoxifen with or without fenretinide, an analog of vitamin A, in women with metastatic breast cancer. *J. Clin. Oncol.*, **11**, 474–477

Collins, M.D., Tzimas, G., Hummler, H., Burgin, H. & Nau, H. (1994) Comparative teratology and transplacental pharmacokinetics of all-*trans*-retinoic acid, 13-*cis*-retinoic acid, and retinyl palmitate following daily administrations in rats. *Toxicol. Appl. Pharmacol.*, **127**, 132–144

Connor, M.J. & Smit, M.H. (1987) Terminal-group oxidation of retinol by mouse epidermis. Inhibition in vitro and in vivo. *Biochem.J.*, **244**, 489–492

Costaridis, P., Horton, C., Zeitlinger, J., Holder, N. & Maden, M. (1996) Endogenous retinoids in the zebrafish embryo and adult. *Dev. Dyn.*, **205**, 41–51

Cowan, J.D., Von Hoff, D.D., Dinesman, A. & Clark, G. (1983) Use of a human tumor cloning system to screen retinoids for antineoplastic activity. *Cancer*, **51**, 92–96

Cox, L.R., Motz, J., Troll, W. & Garte, S.J. (1991) Effects of retinoic acid on NIH3T3 cell transformation by the H-*ras* oncogene. *J. Cancer Res. Clin. Oncol.*, **117**, 102–108

Creech Kraft, J. & Juchau, M.R. (1992) Conceptual biotransformation of 4-oxo-all-*trans*-retinoic acid, 4-oxo-13-*cis*-retinoic acid and all-*trans*-retinoyl-β-glucuronide in rat whole embryo culture. *Biochem.Pharmacol.*, **43**, 2289–2292

Creech Kraft, J., Slikker, W., Bailey, J.R., Roberts, L.G., Fischer, B., Wittfoht, W. & Nau, H. (1991a) Plasma pharmacokinetics and metabolism of 13-*cis*- and all-*trans*-retinoic acid in the cynomolgus monkey and the identification of 13-*cis*- and all-*trans*-retinoyl-β-glucuronides. A comparison to one human case study with isotretinoin. *Drug Metab. Dispos.*, **19**, 317–324

Creech Kraft, J., Eckhoff, C., Kochhar, D.M., Bochert, G., Chahoud, I. & Nau, H. (1991b) Isotretinoin (13-*cis*-retinoic acid) metabolism, *cis-trans* isomerization, glucuronidation, and transfer to the mouse embryo: Consequences for teratogenicity. *Teratog. Carcinog. Mutag.*, **11**, 21–30

Creech Kraft, J., Shepard, T. & Juchau, M.R. (1993) Tissue levels of retinoids in human embryos/fetuses. *Reprod. Toxicol.*, **7**, 11–15

Creek, K.E., Geslani, G., Batova, A. & Pirisi, L. (1995) Progressive loss of sensitivity to growth control by retinoic acid and transforming growth factor-beta at late stages of human papillomavirus type 16-initiated transformation of human keratinocytes. *Adv. Exp. Med. Biol.*, **375**, 117–135

Crowe, D.L. (1993) Retinoic acid mediates post-transcriptional regulation of keratin 19 mRNA levels. *J. Cell Sci.*, **106**, 183–188

Cullum, M.E. & Zile, M.H. (1985) Metabolism of all-*trans*-retinoic acid and all-*trans*-retinyl acetate. Demonstration of common physiological metabolites in rat small intestinal mucosa and circulation. *J. Biol. Chem.*, **260**, 10590–10596

Cunningham, W.J. & Geiger, J.M. (1992) Practical use of retinoids in psoriasis. *Semin. Dermatol.*, **11**, 291–301

Damjanov, I., Horvat, B. & Gibas, Z. (1993) Retinoic acid-induced differentiation of the developmentally pluripotent human germ cell tumor-derived cell line, NCCIT. *Lab. Invest.*, **68**, 220–232

Danielsen, M., Hinck, L. & Ringold, G.M. (1989) Two amino acids within the knuckle of the first zinc finger specify DNA response element activation by the glucocorticoid receptor. *Cell*, **57**, 1131–1138

Darzynkiewicz, Z. (1995) Apoptosis in antitumor strategies: Modulation of cell cycle or differentiation. *J. Cell Biochem.*, **58**, 151–159

Da Silva, N., Meyer-Monard, S., Menot, M.L., Degos, L., Baruchel, A., Dombret, H. & Chomienne, C. (1997) G-CSF activates STAT pathways in Kasumi-1 myeloid leukemic cells with the t(8;21) translocation: Basis for potential therapeutic efficacy. *Cytokines Cell. Mol. Ther.*, **3**, 75–80

Davies, P.J., Stein, J.P., Chiocca, E.A., Basilon, I.P., Gentile, V., Thomazy, V. & Fesus, L. (1992) Retinoid-regulated expression of transglutaminases: Links to the biochemistry of pro-

grammed cell death. In: Morriss-Kay, G., ed., *Retinoids in Normal Development and Teratogenesis*. Oxford, Oxford University Press, pp. 249–263

Defer, G. (1996) Retinoids and glial tumors. A new therapeutic approach? *Rev. Neurol. Paris,* **152**, 653–657

Dekker, E.J., Vaessen, M.J., van den Berg, C., Timmermans, A., Godsave, S., Holling, T., Nieuwkoop, P., Geurts-van, K.A. & Durston, A. (1994) Overexpression of a cellular retinoic acid binding protein (xCRABP) causes anteroposterior defects in developing Xenopus embryos. *Development,* **120**, 973–985

De Leenheer, A.P., Lambert, W.E. & Claeys, I. (1982) All-*trans*-retinoic acid: Measurement of reference values in human serum by high performance liquid chromatography. *J. Lipid Res.*, **23**, 1362–1367

Delia, D., Aiello, A., Lombardi, L., Pelicci, P.G., Grignani, F., Formelli, F., Ménard, S., Costa, A., Veronesi, U. & Pierotti, M.A. (1993) *N*-(4-Hydroxyphenyl)retinamide induces apoptosis of malignant hemopoietic cell lines including those unresponsive to retinoic acid. *Cancer Res.*, **53**, 6036–6041

Delia, D., Aiello, A., Meroni, L., Nicolini, M., Reed, J.C. & Pierotti, M.A. (1997) Role of antioxidants and intracellular free radicals in retinamide-induced cell death. *Carcinogenesis,* **18**, 943–948

Desai, S.H. & Niles, R.M. (1997) Characterization of retinoic acid-induced AP-1 activity in B16 mouse melanoma cells. *J. Biol. Chem.*, **272**, 12809–12815

Dickman, E.D., Thaller, C. & Smith, S.M. (1997) Temporally-regulated retinoic acid depletion produces specific neural crest, ocular and nervous system defects. *Development,* **124**, 3111–3121

Dollé, P., Ruberte, E., Kastner, P., Petkovich, M., Stoner, C.M., Gudas, L.J. & Chambon, P. (1989) Differential expression of genes encoding α, β and γ retinoic acid receptors and CRABP in the developing limbs of the mouse. *Nature,* **342**, 702–705

Dollé, P., Ruberte, E., Leroy, P., Morriss-Kay, G. & Chambon, P. (1990) Retinoic acid receptors and cellular retinoid binding proteins. I. A systematic study of their differential pattern of transcription during mouse organogenesis. *Development,* **110**, 1133–1151

Dombret, H., Sutton, L., Duarte, M., Daniel, M.T., Leblond, V., Castaigne, S. & Degos, L. (1992) Combined therapy with all-*trans*-retinoic acid and high-dose chemotherapy in patients with hyperleukocytic acute promyelocytic leukemia and severe visceral hemorrhage. *Leukemia,* **6**, 1237–1242

Dong, D. & Zile, M.H. (1995) Endogenous retinoids in the early avian embryo. *Biochem. Biophys. Res. Commun.*, **217**, 1026–1031

Dony, C., Kessel, M. & Gruss, P. (1985) Post-transcriptional control of myc and p53 expression during differentiation of the embryonal carcinoma cell line F9. *Nature,* **317**, 636–639

Driessen, C.A., Winkens, H.J., Kuhlmann, E.D., Janssen, A.P., van-Vugt, A.H., Deutman, A.F. & Janssen, J.J. (1998) The visual cycle retinol dehydrogenase: Possible involvement in the 9-*cis*-retinoic acid biosynthetic pathway. *FEBS Lett.*, **428**, 135–140

Duell, E.A., Kang, S. & Voorhees, J.J. (1996) Retinoic acid isomers applied to human skin *in vivo* each induce a 4-hydroxylase that inactivates only *trans*-retinoic acid. *J. Invest. Dermatol.*, **106**, 316–320

Duester, G. (1996) Involvement of alcohol dehydrogenase, short-chain dehydrogenase/reductase, aldehyde dehydrogenase, and cytochrome P450 in the control of retinoid signaling by activation of retinoic acid synthesis. *Biochemistry,* **35**, 12221–12227

Dunagin, P.E.Jr., Zachman, R.D. & Olson, J.A. (1966) The identification of metabolites of retinal and retinoic acid in rat bile. *Biochim. Biophys. Acta,* **124**, 71–85

Durand, B., Saunders, M., Leroy, P., Leid, M. & Chambon, P. (1992) All-*trans*- and 9-*cis*-retinoic acid induction of CRABPII transcription is mediated by RAR–RXR heterodimers bound to DR1 and DR2 repeated motifs. *Cell,* **71**, 73–85

Durston, A.J., Timmermans, J.P., Hage, W.J., Hendriks, H.F., de Vries, N.J., Heideveld, M. & Nieuwkoop, P.D. (1989) Retinoic acid causes an anteroposterior transformation in the developing central nervous system. *Nature,* **340**, 140–144

Eckhoff, C. & Nau, H. (1990) Identification and quantitation of all-*trans*- and 13-*cis*-retinoic acid and 13-*cis*-4-oxoretinoic acid in human plasma. *J. Lipid Res.*, **31**, 1445–1454

Eckhoff, C., Collins, M.D. & Nau, H. (1991) Human plasma all-*trans*-, 13-*cis*- and 13-*cis*-4-oxoretinoic acid profiles during subchronic vitamin A supplementation: Comparison to retinol and retinyl ester plasma levels. *J. Nutr.*, **121**, 1016–1025

Edwards, R.B., Adler, A.J., Dev, S. & Claycomb, R.C. (1992) Synthesis of retinoic acid from retinol by cultured rabbit Müller cells. *Exp. Eye Res.*, **54**, 481–490

Eguchi, Y., Eguchi, N., Oda, H., Seiki, K., Kijima, Y., Matsu, U.Y., Urade, Y. & Hayaishi, O. (1997) Expression of lipocalin-type prostaglandin D synthase (beta-trace) in human heart and its accumulation in the coronary circulation of angina patients. *Proc. Natl Acad. Sci. USA*, **94**, 14689–14694

Eguchi, N., Minami, T., Shirafuji, N., Kanaoka, Y., Tanaka, T., Nagata, A., Yoshida, N., Urade, Y., Ito, S. & Hayaishi, O. (1999) Lack of tactile pain (allodynia) in lipocalin-type prostaglandin D synthase-deficient mice. *Proc. Natl Acad. Sci. USA*, **96**, 726–730

Eicher, S.A. & Lotan, R. (1996) Differential effects of retinoic acid and N-(4-hydroxyphenyl)retinamide on head and neck squamous cell carcinoma cells. *Laryngoscope*, **106**, 1471–1475

El Akawi, Z. & Napoli, J.L. (1994) Rat liver cytosolic retinal dehydrogenase: Comparison of 13-*cis*-, 9-*cis*-, and all-*trans*-retinal as substrates and effects of cellular retinoid-binding proteins and retinoic acid on activity. *Biochemistry*, **33**, 1938–1943

Escriva, H., Safi, R., Hänni, C., Langlois, M.C., Saumitou-Laprade, P., Stehelin, D., Capron, A., Pierce, R. & Laudet, V. (1997) Ligand binding was acquired during evolution of nuclear receptors. *Proc. Natl Acad. Sci. USA*, **94**, 6803–6808

Fenaux, P. & Chomienne, C. (1996) Biology and treatment of acute promyelocytic leukemia. *Curr. Opin. Oncol.*, 8, 3–12

Fenaux, P., Le Deley, M.C., Castaigne, S., Archimbaud, E., Chomienne, C., Link, H., Guerci, A., Duarte, M., Daniel, M.T., Bowen, D., Huebner, G., Bauters, F., Fegueux, N., Fey, M., Sanz, M., Lowenberg, B., Maloisel, F., Auzanneau, G., Sadoun, A., Gardin, C., Bastion, Y., Ganser, A., Jacky, E., Dombret, H., Chastang, C. & Degos, L. (1993) Effect of all-*trans*-retinoic acid in newly diagnosed acute promyelocytic leukemia. Results of a multicenter randomized trial. European APL 91 Group. *Blood*, 82, 3241–3249

Fiorella, P.D. & Napoli, J.L. (1991) Expression of cellular retinoic acid binding protein (CRABP) in *Escherichia coli*. Characterization and evidence that holo-CRABP is a substrate in retinoic acid metabolism. *J. Biol. Chem.*, 266, 16572–16579

Fisher, G.J., Wang, Z.Q., Datta, S.C., Varani, J., Kang, S. & Voorhees, J.J. (1997) Patho-physiology of premature skin aging induced by ultraviolet light. *N. Engl. J. Med.*, **337**, 1419–1428

Fisher, G.J., Talwar, H.S., Lin, J., Lin, P., McPhillips, F., Wang, Z., Li, X., Wan, Y., Kang, S. & Voorhees, J.J. (1998) Retinoic acid inhibits induction of c-Jun protein by ultraviolet radiation that occurs subsequent to activation of mitogen-activated protein kinase pathways in human skin in vivo. *J. Clin. Invest.*, **101**, 1432–1440

Flower, D.R. (1996) The lipocalin protein family: Structure and function. *Biochem. J.*, **318**, 1–14

Folman, Y., Russell, R.M., Tang, G.W. & Wolf, D.G. (1989) Rabbits fed on beta-carotene have higher serum levels of all-*trans*-retinoic acid than those receiving no beta-carotene. *Br. J. Nutr.*, **62**, 195–201

Forman, B.M., Umesono, K., Chen, J. & Evans, R.M. (1995) Unique response pathways are established by allosteric interactions among nuclear hormone receptors. *Cell*, 81, 541–550

Forman, B.M., Tzameli, I., Choi, H.S., Chen, J., Simha, D., Seol, W., Evans, R.M. & Moore, D.D. (1998) Androstane metabolites bind to and deactivate the nuclear receptor CAR-β. *Nature*, **395**, 612–615

Frankel, S.R., Eardley, A., Lauwers, G., Weiss, M. & Warrell, R.P.J. (1992) The 'retinoic acid syndrome' in acute promyelocytic leukemia. *Ann. Intern. Med*, **117**, 292–296

Frolik, C.A. (1984) Metabolism of retinoids. In: Sporn, M.B., Roberts, A.B., & Goodman, D.S., eds, *The Retinoids*. Orlando, FL, Academic Press, pp. 177-208

Frolik, C.A., Roller, P.P., Roberts, A.B. & Sporn, M.B. (1980) *In vitro* and *in vivo* metabolism of all-*trans*- and 13-*cis*-retinoic acid in hamsters. Identification of 13-*cis*-4-oxoretinoic acid. *J. Biol. Chem.*, **255**, 8057–8062

Fujii, H., Sato, T., Kaneko, S., Gotoh, O., Fujii-Kuriyama, Y., Osawa, K., Kato, S. & Hamada, H. (1997) Metabolic inactivation of retinoic acid by a novel P450 differentially expressed in developing mouse embryos. *EMBO J.*, **16**, 4163–4173

Gailani, M.R., Stahle-Backdahl, M., Leffell, D.J., Glynn, M., Zaphiropoulos, P.G., Pressman, C., Unden, A.B., Dean, M., Brash, D.E., Bale, A.E. & Toftgard, R. (1996) The role of the human homologue of *Drosophila patched* in sporadic basal cell carcinomas. *Nat. Genet.*, **14**, 78–81

Gallup, J.M., Barua, A.B., Furr, H.C. & Olson, J.A. (1987) Effects of retinoid beta-glucuronides and N-retinoyl amines on the differentiation of HL-60 cells in vitro. *Proc. Soc. Exp. Biol. Med.*, **186**, 269–274

Gamble, M.V., Shang, E., Piantedosi, R., Mertz, J.R., Wolgemuth, D.J. & Blaner, W.S. (1999) Biochemical properties, tissue expression, and gene structure of a short chain dehydrogenase/reductase able to catalyze *cis*-retinol oxidation. *J. Lipid Res.*, **40**, pp. 2279–2292

Garbis-Berkvens, J.M. & Peters, P.W.J. (1987) Comparative morphology and physiology of embryonic and fetal membranes. In: Nau H. & Scott W.J.J., eds, *Pharmacokinetics in Teratogenesis*. Boca Raton, FL, CRC Press, pp. 13–44

Gaub, M.P., Bellard, M., Scheuer, I., Chambon, P. & Sassone-Corsi, P. (1990) Activation of the ovalbumin gene by the estrogen receptor involves the fos-jun complex. *Cell*, **63**, 1267–1276

Gehin, M., Vivat, V., Wurtz, J.M., Losson, R., Chambon, P., Moras, D. & Gronemeyer, H. (1999) Structural basis for engineering of retinoic acid receptor isotype-selective agonists and antagonists. *Chem. Biol.*, **6**, 519–529

Geiger, J.M. (1995) Retinoids and sebaceous gland activity. *Dermatology*, **191**, 305–310

Geison, R.L. & Johnson, B.C. (1970) Studies on the in vivo metabolism of retinoic acid in the rat. *Lipids*, **5**, 371–378

Genchi, G., Wang, W., Barua, A., Bidlack, W.R. & Olson, J.A. (1996) Formation of β-glucuronides and of β-galacturonides of various retinoids catalyzed by induced and noninduced microsomal UDP-glucuronosyltransferases of rat liver. *Biochim. Biophys. Acta*, **1289**, 284–290

Giannini, G., Dawson, M.I., Zhang, X. & Thiele, C.J. (1997) Activation of three distinct RXR/RAR heterodimers induces growth arrest and differentiation of neuroblastoma cells. *J. Biol. Chem.*, **272**, 26693–26701

Glass, C.K. (1994) Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers. *Endocr. Rev.*, **15**, 391–407

Gollnick, H., Ehlert, R., Rinck, G. & Orfanos, C.E. (1990) Retinoids: An overview of pharmacokinetics and therapeutic value. *Methods Enzymol.*, **190**, 291–304

Goodman, D.S. & Blaner, W.S.(1984) Biosynthesis, absorption, and hepatic metabolism of retinol. In: Sporn, M.B., Roberts, A.B. & Goodman, D.S., eds, *The Retinoids*. New York, Academic Press, pp. 1–39

Gorry, P., Lufkin, T., Dierich, A., Rochette-Egly, C., Decimo, D., Dollé, P., Mark, M., Durand, B. & Chambon, P. (1994) The cellular retinoic acid binding protein I is dispensable. *Proc. Natl Acad. Sci. USA*, **91**, 9032–9036

Göttlicher, M., Heck, S. & Herrlich, P. (1998) Transcriptional cross-talk, the second mode of steroid hormone receptor action. *J. Mol. Med.*, **76**, 480–489

Gough, W.H., VanOoteghem, S., Sint, T. & Kedishvili, N.Y. (1998) cDNA cloning and characterization of a new human microsomal NAD^+-dependent dehydrogenase that oxidizes all-*trans*-retinol and 3α-hydroxysteroids. *J. Biol. Chem.*, **273**, 19778–19785

Gould, A., Itasaki, N. & Krumlauf, R. (1998) Initiation of rhombomeric Hoxb4 expression requires induction by somites and a retinoid pathway. *Neuron*, **21**, 39–51

Green, S. & Chambon, P. (1987) Oestradiol induction of a glucocorticoid-responsive gene by a chimaeric receptor. *Nature*, **325**, 75–78

Green, S., Kumar, V., Theulaz, I., Wahli, W. & Chambon, P. (1988) The N-terminal DNA-binding 'zinc finger' of the oestrogen and glucocorticoid receptors determines target gene specificity. *EMBO J.*, **7**, 3037–3044

Greenberg, B.R., Durie, B.G., Barnett, T.C. & Meyskens, F.L.J. (1985) Phase I–II study of 13-*cis*-retinoic acid in myelodysplastic syndrome. *Cancer Treat. Rep.*, **69**, 1369–1374

Grignani, F., Fagioli, M., Alcalay, M., Longo, L., Pandolfi, P.P., Donti, E., Biondi, A., Lo Coco, F. & Pelicci, P.G. (1994) Acute promyelocytic leukemia: From genetics to treatment. *Blood*, **83**, 10–25

Gronemeyer, H. & Laudet, V. (1995) Transcription factors 3: Nuclear receptors. *Protein Profile*, **2**, 1173–1308

Gronemeyer, H. & Moras, D. (1995) Nuclear receptors. How to finger DNA. *Nature*, **375**, 190–191

Grunberg, S.M. & Itri, L.M. (1987) Phase II study of isotretinoin in the treatment of advanced non-small cell lung cancer. *Cancer Treat. Rep.*, **71**, 1097–1098

Gudas, L.J. (1992) Retinoids, retinoid-responsive genes, cell differentiation, and cancer. *Cell Growth Differ.*, **3**, 655–662

Gudas, L.J., Sporn, M.B. & Roberts, A.B. (1994) Cellular biology and biochemistry of the retinoids. In: Sporn M.B., Roberts A.B. & Goodman D.S., eds, *The Retinoids. Biology, Chemistry and Medicine*, 2nd Ed. New York, Raven Press, pp. 443–520

Guerin, E., Ludwig, M.G., Basset, P. & Anglard, P. (1997) Stromelysin-3 induction and interstitial collagenase repression by retinoic acid. Therapeutical implication of receptor-selective retinoids dissociating transactivation and AP-1-mediated transrepression. *J. Biol. Chem.*, **272**, 11088–11095

Gunning, D.B., Barua, A.B. & Olson, J.A. (1993) Comparative teratogenicity and metabolism of all-*trans*-retinoic acid, all-*trans*-retinoyl beta-glucose, and all-*trans*-retinoyl beta-glucuronide in pregnant Sprague-Dawley rats. *Teratology*, **47**, 29–36

Gustafson, A.L., Dencker, L. & Eriksson, U. (1993) Non-overlapping expression of CRBP I and CRABP I during pattern formation of limbs and craniofacial structures in the early mouse embryo. *Development*, **117**, 451–460

Haeseleer, F., Huang, J., Lebioda, L., Saari, J.C. & Palczewski, K. (1998) Molecular characterization of a novel short-chain dehydrogenase/reductase that reduces all-*trans*-retinal. *J. Biol. Chem.*, **273**, 21790–21799

Hale, F. (1933) Pigs born without eye balls. *J. Hered.*, **24**, 105–106

Hamasaki, Y., Murakami, M., Kudo, I. & Miyazaki, S. (1997) Post-transcriptional regulation of LTC4 synthase activity by retinoic acid in rat basophilic leukemia cells. *Biochim. Biophys. Acta*, **1347**, 205–211

Han, I.S. & Choi, J.H. (1996) Highly specific cytochrome P450-like enzymes for all-*trans*-retinoic acid in T47D human breast cancer cells. *J. Clin. Endocrinol. Metab.*, **81**, 2069–2075

Harisiadis, L., Miller, R.C., Hall, E.J. & Borek, C. (1978) A vitamin A analogue inhibits radiation-induced oncogenic transformation. *Nature*, **274**, 486–487

Harnish, D.C., Soprano, K.J. & Soprano, D.R. (1992) Mouse conceptuses have a limited capacity to elevate the mRNA level of cellular retinoid binding proteins in response to teratogenic doses of retinoic acid. *Teratology*, **46**, 137–146

Haselbeck, R.J. & Duester, G. (1998a) ADH1 and ADH4 alcohol/retinol dehydrogenases in the developing adrenal blastema provide evidence for embryonic retinoid endocrine function. *Dev. Dyn.*, **213**, 114–120

Haselbeck, R.J. & Duester, G. (1998b) ADH4-lacZ transgenic mouse reveals alcohol dehydrogenase localization in embryonic midbrain/hindbrain, otic vesicles, and mesencephalic, trigeminal, facial, and olfactory neural crest. *Alcohol Clin. Exp. Res.*, **22**, 1607–1613

Haselbeck, R.J., Ang, H.L. & Duester, G. (1997) Class IV alcohol/retinol dehydrogenase localization in epidermal basal layer: Potential site of retinoic acid synthesis during skin development. *Dev. Dyn.*, **208**, 447–453

Hébuterne, X., Wang, X.D., Johnson, E.J., Krinsky, N.I. & Russell, R.M. (1995) Intestinal absorption and metabolism of 9-*cis*-β-carotene in vivo: Biosynthesis of 9-*cis*-retinoic acid. *J. Lipid Res.*, **36**, 1264–1273

Heck, S., Kullmann, M., Gast, A., Ponta, H., Rahmsdorf, H.J., Herrlich, P. & Cato, A.C. (1994) A distinct modulating domain in glucocorticoid receptor monomers in the repression of activity of the transcription factor AP-1. *EMBO J.*, **13**, 4087–4095

Heery, D.M., Pierrat, B., Gronemeyer, H., Chambon, P. & Losson, R. (1994) Homo- and heterodimers of the retinoid X receptor (RXR) activated transcription in yeast. *Nucleic Acids Res.*, **22**, 726–731

Herget, T., Specht, H., Esdar, C., Oehrlein, S.A. & Maelicke, A. (1998) Retinoic acid induces apoptosis-associated neural differentiation of a murine teratocarcinoma cell line. *J. Neurochem.*, **70**, 47–58

Heyman, R.A., Mangelsdorf, D.J., Dyck, J.A., Stein, R.B., Eichele, G., Evans, R.M. & Thaller, C. (1992) 9-*cis*-Retinoic acid is a high affinity ligand for the retinoid X receptor. *Cell*, **68**, 397–406

Hickman, J.A., Potten, C.S., Merritt, A.J. & Fisher, T.C. (1994) Apoptosis and cancer chemotherapy. *Phil. Trans. R. Soc. Lond. B Biol. Sci.*, **345**, 319–325

Hodam, J.R. & Creek, K.E. (1996) Uptake and metabolism of [^{3}H]retinoic acid delivered to human foreskin keratinocytes either bound to serum albumin or added directly to the culture medium. *Biochim. Biophys. Acta*, **1311**, 102–110

Hoffmann, B., Lehmann, J.M., Zhang, X.K., Hermann, T., Husmann, M., Graupner, G. & Pfahl, M. (1990) A retinoic acid receptor-specific element controls the retinoic acid receptor-β promoter. *Mol. Endocrinol.*, **4**, 1727–1736

Hofmann, C. & Eichele, G.(1994) Retinoids in development. In: Sporn, M.B., Roberts, A.B., & Goodman, D.S., eds, *The Retinoids. Biology, Chemistry and Medicine*, 2nd Ed. New York, Raven Press, pp. 387-441

Horst, R.L., Reinhardt, T.A., Goff, J.P., Nonnecke, B.J., Gambhir, V.K., Fiorella, P.D. & Napoli, J.L. (1995) Identification of 9-*cis*,13-*cis*-retinoic acid as a major circulating retinoid in plasma. *Biochemistry*, **34**, 1203–1209

Horton, C. & Maden, M. (1995) Endogenous distribution of retinoids during normal development and teratogenesis in the mouse embryo. *Dev. Dyn.* , **202**, 312–323

Houle, B., Rochette-Egly, C. & Bradley, W.E. (1993) Tumor-suppressive effect of the retinoic acid receptor β in human epidermoid lung cancer cells. *Proc. Natl Acad. Sci. USA*, **90**, 985–989

Hu, E., Kim, J.B., Sarraf, P. & Spiegelman, B.M. (1996a) Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARγ. *Science*, **274**, 2100–2103

Hu, Z.B., Minden, M.D. & McCulloch, E.A. (1996b) Post-transcriptional regulation of bcl-2 in acute myeloblastic leukemia: Significance for response to chemotherapy. *Leukemia*, **10**, 410–416

Huang, C., Ma, W.Y., Dawson, M.I., Rincon, M., Flavell, R.A. & Dong, Z. (1997) Blocking activator protein-1 activity, but not activating retinoic acid response element, is required for the antitumor promotion effect of retinoic acid. *Proc. Natl Acad. Sci. USA*, **94**, 5826–5830

Huang, M.E., Ye, Y.C., Chen, S.R., Chai, J.R., Lu, J.X., Zhoa, L., Gu, L.J. & Wang, Z.Y. (1988) Use of all-*trans*-retinoic acid in the treatment of acute promyelocytic leukemia. *Blood*, **72**, 567–572

Hummler, H., Hendrickx, A.G. & Nau, H. (1994) Maternal toxicokinetics, metabolism, and embryo exposure following a teratogenic dosing regimen with 13-*cis*-retinoic acid (isotretinoin) in the cynomolgus monkey. *Teratology*, **50**, 184–193

Hunter, T. & Pines, J. (1994) Cyclins and cancer. II: Cyclin D and CDK inhibitors come of age. *Cell*, **79**, 573–582

Hupert, J., Mobarhan, S., Layden, T.J., Papa, V.M. & Lucchesi, D.J. (1991) In vitro formation of retinoic acid from retinal in rat liver. *Biochem. Cell. Biol.*, **69**, 509–514

Husmann, M., Hoffmann, B., Stump, D.G., Chytil, F. & Pfahl, M. (1992) A retinoic acid response element from the rat CRBPI promoter is activated by an RAR/RXR heterodimer. *Biochem. Biophys. Res. Commun.*, **187**, 1558–1564

IARC (1997) *IARC Handbooks of Cancer Prevention*, Vol. 1, *Non-steroidal Anti-inflammatory Drugs*, Lyon, International Agency for Research on Cancer, 202 pp.

IARC (1998a) *IARC Handbooks of Cancer Prevention*, Vol. 2, *Carotenoids*, Lyon, International Agency for Research on Cancer, 326 pp.

IARC (1998b) *IARC Handbooks of Cancer Prevention*, Vol. 3, *Vitamin A*, Lyon, International Agency for Research on Cancer, 261 pp.

Iest, J. & Boer, J. (1989) Combined treatment of psoriasis with acitretin and UVB phototherapy compared with acitretin alone and UVB alone. *Br. J. Dermatol.*, **120**, 665–670

Imaizumi, M. & Breitman, T.R. (1987) Retinoic acid-induced differentiation of the human promyelocytic leukemia cell line, HL-60, and fresh human leukemia cells in primary culture: A model for differentiation inducing therapy of leukemia. *Eur. J. Haematol.*, **38**, 289–302

Imaoka, S., Wan, J., Chow, T., Hiroi, T., Eyanagi, R., Shigematsu, H. & Funae, Y. (1998) Cloning

and characterization of the CYP2D1-binding protein, retinol dehydrogenase. *Arch. Biochem. Biophys.*, **353**, 331–336

Irving, H., Lovat, P.E., Campbell Hewson, Q., Malcolm, A.J., Pearson, A.D. & Redfern, C.P. (1998) Retinoid-induced differentiation of neuroblastoma: Comparison between LG69, an RXR-selective analogue and 9-*cis*-retinoic acid. *Eur. J. Cancer*, **34**, 111–117

Ito, Y., Zile, M., DeLuca, H.F. & Ahrens, H.M. (1974) Metabolism of retinoic acid in vitamin A-deficient rats. *Biochim. Biophys. Acta*, **369**, 338–350

IUPAC Commission on the Nomenclature of Biological Chemistry (1960) Definitive rules for the nomenclature of amino acids, steroids, vitamins, and carotenoids. *J. Am. Chem. Soc.*, **82**, 5575–5584

IUPAC–IUB Commission on Biochemical Nomenclature (1966) IUPAC–IUB Commission on Biochemical Nomenclature. Trivial names of miscellaneous compounds of importance in biochemistry. *J. Biol. Chem.*, **241**, 2987–2988

IUPAC–IUB Joint Commission on Biochemical Nomenclature (1983) IUPAC–IUB Joint Commission on Biochemical Nomenclature (JCBN). Nomenclature of Retinoids. Recom-mendations 1981. *J. Biol. Chem.*, **258**, 5329–5333

IUPAC–IUB Joint Commission on Biochemical Nomenclature (1998) IUPAC–IUBMB Joint Commission on Biochemical Nomenclature (JCBN) and Nomenclature Committee of IUBMB (NC–IUBMB). Newsletter 1996. *J. Mol. Biol.*, **275**, 527–537

Janick-Buckner, D., Barua, A.B. & Olson, J.A. (1991) Induction of HL-60 cell differentiation by water-soluble and nitrogen-containing conjugates of retinoic acid and retinol. *FASEB J.*, **5**, 320–325

Jarrous, N. & Kaempfer, R. (1994) Induction of human interleukin-1 gene expression by retinoic acid and its regulation at processing of precursor transcripts. *J. Biol. Chem.*, **269**, 23141–23149

Jetten, A.M., Nervi, C. & Vollberg, T.M. (1992) Control of squamous differentiation in tracheobronchial and epidermal epithelial cells: Role of retinoids. *Natl Cancer Inst. Monogr.*, 93–100

Jiang, H., Gyda, M., Harnish, D.C., Chandraratna, R.A., Soprano, K.J., Kochhar, D.M. & Soprano, D.R. (1994) Teratogenesis by retinoic acid analogs positively correlates with elevation of retinoic acid receptor-beta 2 mRNA levels in treated embryos. *Teratology*, **50**, 38–43

Jollie, W.P. & Craig, S.S. (1979) The fine structure of placental junctional zone cells during prolonged pregnancy in rats. *Acta Anat. Basel*, **105**, 386–400

Jones, G., Crotty, M. & Brooks, P. (1997) Psoriatic arthritis: A quantitative overview of therapeutic options. The Psoriatic Arthritis Meta-analysis Study Group. *Br. J. Rheumatol.*, **36**, 95–99

Jörnvall, H. & Höög, J.O. (1995) Nomenclature of alcohol dehydrogenases. *Alcohol Alcohol.*, **30**, 153–161

Jörnvall, H., Persson, B., Krook, M., Atrian, S., Gonzalez, D.R., Jeffery, J. & Ghosh, D. (1995) Short-chain dehydrogenases/reductases (SDR). *Biochemistry*, **34**, 6003–6013

Julia, P., Farres, J. & Pares, X. (1986) Ocular alcohol dehydrogenase in the rat: Regional distribution and kinetics of the ADH-1 isoenzyme with retinol and retinal. *Exp. Eye Res.*, **42**, 305–314

Kakizuka, A., Miller, W.H.J., Umesono, K., Warrell, R.P.J., Frankel, S.R., Murty, V.V., Dmitrovsky, E. & Evans, R.M. (1991) Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RARα with a novel putative transcription factor, PML. *Cell*, **66**, 663–674

Kalemkerian, G.P., Slusher, R., Ramalingam, S., Gadgeel, S. & Mabry, M. (1995) Growth inhibition and induction of apoptosis by fenretinide in small-cell lung cancer cell lines. *J. Natl Cancer Inst.*, **87**, 1674–1680

Kalter, H. & Warkany, J. (1959) Experimental production of congenital malformations in mammals by metabolic procedure. *Physiol. Rev.*, **39**, 69–115

Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S.C., Heyman, R.A., Rose, D.W., Glass, C.K. & Rosenfeld, M.G. (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell*, **85**, 403–414

Kang, J.X. & Leaf, A. (1995) Protective effects of all-*trans*-retinoic acid against cardiac arrhythmias induced by isoproterenol, lysophos-

phatidylcholine or ischemia and reperfusion. *J. Cardiovasc. Pharmacol.*, **26**, 943–948

Kang, J.X., Li, Y. & Leaf, A. (1998) Mannose-6-phosphate/insulin-like growth factor-II receptor is a receptor for retinoic acid. *Proc. Natl Acad. Sci. USA*, **95**, 13671–13676

Kastner, P., Mark, M. & Chambon, P. (1995) Nonsteroid nuclear receptors: What are genetic studies telling us about their role in real life? *Cell*, **83**, 859–869

Kato, S., Endoh, H., Masuhiro, Y., Kitamoto, T., Uchiyama, S., Sasaki, H., Masushige, S., Gotoh, Y., Nishida, E., Kawashima, H., Metzger, D. & Chambon, P. (1995) Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. *Science*, **270**, 1491–1494

Kaul, S. & Olson, J.A. (1998) Effect of vitamin A deficiency on the hydrolysis of retinoyl beta-glucuronide to retinoic acid by rat tissue organelles in vitro. *Int. J. Vitam. Nutr. Res.*, **68**, 232–236

Kedishvili, N.Y., Gough, W.H., Chernoff, E.A., Hurley, T.D., Stone, C.L., Bowman, K.D., Popov, K.M., Bosron, W.F. & Li, T.K. (1997) cDNA sequence and catalytic properties of a chick embryo alcohol dehydrogenase that oxidizes retinol and 3β,5α-hydroxysteroids. *J. Biol. Chem.*, **272**, 7494–7500

Kedishvili, N.Y., Gough, W.H., Davis, W.I., Parsons, S., Li, T.K. & Bosron, W.F. (1998) Effect of cellular retinol-binding protein on retinol oxidation by human class IV retinol/alcohol dehydrogenase and inhibition by ethanol. *Biochem. Biophys. Res. Commun.*, **249**, 191–196

Kersten, S., Kelleher, D., Chambon, P., Gronemeyer, H. & Noy, N. (1995) Retinoid X receptor α forms tetramers in solution. *Proc. Natl Acad. Sci. USA*, **92**, 8645–8649

Kersten, S., Dawson, M.I., Lewis, B.A. & Noy, N. (1996) Individual subunits of heterodimers comprised of retinoic acid and retinoid X receptors interact with their ligands independently. *Biochemistry*, **35**, 3816–3824

Khan, M.A., Jenkins, G.R., Tolleson, W.H., Creek, K.E. & Pirisi, L. (1993) Retinoic acid inhibition of human papillomavirus type 16-mediated transformation of human keratinocytes. *Cancer Res.*, **53**, 905–909

Kim, C.I., Leo, M.A. & Lieber, C.S. (1992) Retinol forms retinoic acid via retinal. *Arch. Biochem. Biophys.*, **294**, 388–393

Kim, S.Y., Han, I.S., Yu, H.K., Lee, H.R., Chung, J.W., Choi, J.H., Kim, S.H., Byun, Y., Carey, T.E. & Lee, K.S. (1998) The induction of P450-mediated oxidation of all-*trans*-retinoic acid by retinoids in head and neck squamous cell carcinoma cell lines. *Metabolism*, **47**, 955–958

King, K.L. & Cidlowski, J.A. (1995) Cell cycle and apoptosis: Common pathways to life and death. *J. Cell Biochem.*, **58**, 175–180

Kishimoto, H., Hoshino, S., Ohori, M., Kontani, K., Nishina, H., Suzawa, M., Kato, S. & Katada, T. (1998) Molecular mechanism of human CD38 gene expression by retinoic acid. Identification of retinoic acid response element in the first intron. *J. Biol. Chem.*, **273**, 15429–15434

Kizaki, M., Dawson, M.I., Heyman, R., Elster, E., Morosetti, R., Pakkala, S., Chen, D.L., Ueno, H., Chao, W., Morikawa, M., Ikeda, Y., Heber, D., Pfahl, M. & Koeffler, H.P. (1996) Effects of novel retinoid X receptor-selective ligands on myeloid leukemia differentiation and proliferation in vitro. *Blood*, **87**, 1977–1984

Klaholz, B.P., Renaud, J.P., Mitschler, A., Zusi, C., Chambon, P., Gronemeyer, H. & Moras, D. (1998) Conformational adaptation of agonists to the human nuclear receptor RARγ. *Nat. Struct. Biol.*, **5**, 199–202

Klein-Szanto, A.J., Iizasa, T., Momiki, S., Garcia, P., I, Caamano, J., Metcalf, R., Welsh, J. & Harris, C.C. (1992) A tobacco-specific *N*-nitrosamine or cigarette smoke condensate causes neoplastic transformation of xenotransplanted human bronchial epithelial cells. *Proc. Natl Acad. Sci. USA*, **89**, 6693–6697

Kliewer, S.A., Umesono, K., Heyman, R.A., Mangelsdorf, D.J., Dyck, J.A. & Evans, R.M. (1992) Retinoid X receptor-COUP-TF interactions modulate retinoic acid signaling. *Proc. Natl Acad. Sci. USA*, **89**, 1448–1452

Kliewer, S.A., Moore, J.T., Wade, L., Staudinger, J.L., Watson, M.A., Jones, S.A., McKee, D.D., Oliver, B.B., Willson, T.M., Zetterström, R.H., Perlmann, T. & Lehmann, J.M. (1998) An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell*, **92**, 73–82

Knoepfler, P.S. & Kamps, M.P. (1997) The Pbx family of proteins is strongly upregulated by a post- transcriptional mechanism during retinoic acid-induced differentiation of P19 embryonal carcinoma cells. *Mech. Dev.*, **63**, 5–14

Kochhar, D.M., Jiang, H., Penner, J.D., Beard, R.L. & Chandraratna, R.A. (1996) Differential teratogenic response of mouse embryos to receptor selective analogs of retinoic acid. *Chem. Biol. Interact.*, **100**, 1–12

Koeffler, H.P., Heitjan, D., Mertelsmann, R., Kolitz, J.E., Schulman, P., Itri, L., Gunter, P. & Besa, E. (1988) Randomized study of 13-*cis* retinoic acid *v* placebo in the myelodysplastic disorders. *Blood*, **71**, 703–708

Koken, M.H., Puvion-Dutilleul, F., Guillemin, M.C., Viron, A., Linares-Cruz, G., Stuurman, N., de Jong, L., Szostecki, C., Calvo, F., Chomienne, C., Degos, L., Puvion, E. & de Thé, H. (1994) The t(15;17) translocation alters a nuclear body in a retinoic acid-reversible fashion. *EMBO J.*, **13**, 1073–1083

König, H., Ponta, H., Rahmsdorf, H.J. & Herrlich, P. (1992) Interference between pathway-specific transcription factors: Glucocorticoids antagonize phorbol ester-induced AP-1 activity without altering AP-1 site occupation *in vivo*. *EMBO J.*, **11**, 2241–2246

Koo, J., Nguyen, Q. & Gambla, C. (1997) Advances in psoriasis therapy. *Adv. Dermatol.*, **12**, 47–73

Krekels, M.D., Verhoeven, A., van Dun, J., Cools, W., Van Hove, C., Dillen, L., Coene, M.C. & Wouters, W. (1997) Induction of the oxidative catabolism of retinoid acid in MCF-7 cells. *Br. J. Cancer*, **75**, 1098–1104

Kurlandsky, S.B., Gamble, M.V., Ramakrishnan, R. & Blaner, W.S. (1995) Plasma delivery of retinoic acid to tissues in the rat. *J. Biol. Chem.*, **270**, 17850–17857

Kurokawa, R., Yu, V.C., Näär, A., Kyakumoto, S., Han, Z., Silverman, S., Rosenfeld, M.G. & Glass, C.K. (1993) Differential orientations of the DNA-binding domain and carboxy-terminal dimerization interface regulate binding site selection by nuclear receptor heterodimers. *Genes Dev.*, **7**, 1423–1435

Kurokawa, R., DiRenzo, J., Boehm, M., Sugarman, J., Gloss, B., Rosenfeld, M.G., Heyman, R.A. & Glass, C.K. (1994) Regulation of retinoid signalling by receptor polarity and allosteric control of ligand binding. *Nature*, **371**, 528–531

Kurzrock, R., Estey, E. & Talpaz, M. (1993) All-*trans*-retinoic acid: Tolerance and biologic effects in myelodysplastic syndrome. *J. Clin. Oncol.*, **11**, 1489–1495

Labrecque, J., Bhat, P.V. & Lacroix, A. (1993) Purification and partial characterization of a rat kidney aldehyde dehydrogenase that oxidizes retinal to retinoic acid. *Biochem. Cell. Biol.*, **71**, 85–89

Labrecque, J., Dumas, F., Lacroix, A. & Bhat, P.V. (1995) A novel isoenzyme of aldehyde dehydrogenase specifically involved in the biosynthesis of 9-*cis* and all-*trans* retinoic acid. *Biochem. J.*, **305**, 681–684

Langenfeld, J., Lonardo, F., Kiyokawa, H., Passalaris, T., Ahn, M.J., Rusch, V. & Dmitrovsky, E. (1996) Inhibited transformation of immortalized human bronchial epithelial cells by retinoic acid is linked to cyclin E downregulation. *Oncogene*, **13**, 1983–1990

Langenfeld, J., Kiyokawa, H., Sekula, D., Boyle, J. & Dmitrovsky, E. (1997) Posttranslational regulation of cyclin D1 by retinoic acid: A chemoprevention mechanism. *Proc. Natl Acad. Sci. USA*, **94**, 12070–12074

Langston, A.W. & Gudas, L.J. (1992) Identification of a retinoic acid responsive enhancer 3′ of the murine homeobox gene *Hox-1.6*. *Mech. Dev.*, **38**, 217–227

Langston, A.W., Thompson, J.R. & Gudas, L.J. (1997) Retinoic acid-responsive enhancers located 3′ of the *Hox A* and *Hox B* homeobox gene clusters. Functional analysis. *J. Biol. Chem.*, **272**, 2167–2175

Lansink, M., Van Bennekum, A.M., Blaner, W.S. & Kooistra, T. (1997) Differences in metabolism and isomerization of all-*trans*-retinoic acid and 9-*cis*-retinoic acid between human endothelial cells and hepatocytes. *Eur. J. Biochem.*, **247**, 596–604

Larson, R.A., Kondo, K., Vardiman, J.W., Butler, A.E., Golomb, H.M. & Rowley, J.D. (1984) Evidence for a 15;17 translocation in every patient with acute promyelocytic leukemia. *Am. J. Med.*, **76**, 827–841

Lasnitzki, I. (1976) Reversal of methylcholanthrene-induced changes in mouse prostates *in vitro* by retinoic acid and its analogues. *Br. J. Cancer*, **34**, 239–248

Lassus, A. & Geiger, J.M. (1988) Acitretin and etretinate in the treatment of palmoplantar pustulosis: A double-blind comparative trial. *Br. J. Dermatol.*, **119**, 755–759

Lauharanta, J. & Geiger, J.M. (1989) A double-blind comparison of acitretin and etretinate in combination with bath PUVA in the treatment of extensive psoriasis. *Br. J. Dermatol.*, **121**, 107–112

Lee, M.O., Manthey, C.L. & Sladek, N.E. (1991) Identification of mouse liver aldehyde dehydrogenases that catalyze the oxidation of retinaldehyde to retinoic acid. *Biochem. Pharmacol.*, **42**, 1279–1285

Lee, M.S., Kliewer, S.A., Provencal, J., Wright, P.E. & Evans, R.M. (1993) Structure of the retinoid X receptor α DNA binding domain: A helix required for homodimeric DNA binding. *Science*, **260**, 1117–1121

Lee, H.Y., Walsh, G.L., Dawson, M.I., Hong, W.K. & Kurie, J.M. (1998) All-*trans*-retinoic acid inhibits Jun N-terminal kinase-dependent signaling pathways. *J. Biol. Chem.*, **273**, 7066–7071

Lehman, E.D., Spivey, H.O., Thayer, R.H. & Nelson, E.C. (1972) The binding of retinoic acid to serum albumin in plasma. *Fed. Proc. Am. Soc. Exp. Biol.*, **31**, A672–A672

Lehmann, J.M., Zhang, X.K. & Pfahl, M. (1992) RARγ2 expression is regulated through a retinoic acid response element embedded in Sp1 sites. *Mol. Cell. Biol.*, **12**, 2976–2985

Leo, M.A. & Lieber, C.S. (1984) Normal testicular structure and reproductive function in deer-mice lacking retinol and alcohol dehydrogenase activity. *J. Clin. Invest.*, **73**, 593–596

Leo, M.A., Iida, S. & Lieber, C.S. (1984) Retinoic acid metabolism by a system reconstituted with cytochrome P-450. *Arch. Biochem. Biophys.*, **234**, 305–312

Leo, M.A., Kim, C.I. & Lieber, C.S. (1987) NAD^+-dependent retinol dehydrogenase in liver microsomes. *Arch. Biochem. Biophys.*, **259**, 241–249

Leo, M.A., Kim, C.I., Lowe, N. & Lieber, C.S. (1989a) Increased hepatic retinal dehydrogenase activity after phenobarbital and ethanol administration. *Biochem. Pharmacol.*, **38**, 97–103

Leo, M.A., Lasker, J.M., Raucy, J.L., Kim, C.I., Black, M. & Lieber, C.S. (1989b) Metabolism of retinol and retinoic acid by human liver cytochrome P450IIC8. *Arch. Biochem. Biophys.*, **269**, 305-312

Leroy, P., Nakshatri, H. & Chambon, P. (1991) Mouse retinoic acid receptor alpha 2 isoform is transcribed from a promoter that contains a retinoic acid response element. *Proc. Natl Acad. Sci. USA*, **88**, 10138–10142

Levin, G. & Mokady, S. (1994) 9-*cis*-β-Carotene as a precursor of retinol isomers in chicks. *Int. J. Vitam. Nutr. Res.*, **64**, 165–169

Lewis, K.C., Green, M.H., Green, J.B. & Zech, L.A. (1990) Retinol metabolism in rats with low vitamin A status: A compartmental model. *J. Lipid Res.*, **31**, 1535–1548

Li, C., Schwabe, J.W., Banayo, E. & Evans, R.M. (1997) Coexpression of nuclear receptor partners increases their solubility and biological activities. *Proc. Natl Acad. Sci. USA*, **94**, 2278–2283

Liaudet-Coopman, E.D.E. & Wellstein, A. (1996) Regulation of gene expression of a binding protein for fibroblast growth factors by retinoic acid. *J. Biol. Chem.*, **271**, 21303–21308

Licht, J.D., Chomienne, C., Goy, A., Chen, A., Scott, A.A., Head, D.R., Michaux, J.L., Wu, Y., DeBlasio, A., Miller, W.H.J., Zelenetz, A.D., Willman, C.L., Chen, Z., Chen, S.J., Zelent, A., Macintyre, E., Veil, A., Cortes, J., Kantarjian, H. & Waxman, S. (1995) Clinical and molecular characterization of a rare syndrome of acute promyelocytic leukemia associated with translocation (11;17). *Blood*, **85**, 1083–1094

Lion, F., Rotmans, J.P., Daemen, F.J. & Bonting, S.L. (1975) Biochemical aspects of the visual process. XXVII. Stereospecificity of ocular retinol dehydrogenases and the visual cycle. *Biochim. Biophys. Acta* , **384**, 283–292

Lippel, K. & Olson, J.A. (1968) Biosynthesis of beta-glucuronides of retinol and of retinoic acid in vivo and in vitro. *J. Lipid Res.*, **9**, 168–175

Lippman, S.M. & Meyskens, F.L.Jr. (1987) Treatment of advanced squamous cell carcinoma of the skin with isotretinoin. *Ann. Intern. Med*, **107**, 499–502

Lippman, S.M., Kessler, J.F. & Meyskens, F.L.J. (1987) Retinoids as preventive and therapeutic anticancer agents (Part II). *Cancer Treat. Rep.*, **71**, 493–515

Lippman, S.M., Kavanagh, J.J., Paredes-Espinoza, M., Delgadillo-Madrueno, F., Paredes-Casillas,

P., Hong, W.K., Holdener, E. & Krakoff, I.H. (1992) 13-*cis*-Retinoic acid plus interferon α-2a: Highly active systemic therapy for squamous cell carcinoma of the cervix. *J. Natl Cancer Inst.*, **84**, 241–245

Lippman, S.M., Heyman, R.A., Kurie, J.M., Benner, S.E. & Hong, W.K. (1995) Retinoids and chemoprevention: Clinical and basic studies. *J. Cell Biochem. Suppl.*, **22**, 1–10

Little, J.M. & Radominska, A. (1997) Application of photoaffinity labeling with [11,12-^{3}H]all-*trans*-retinoic acid to characterization of rat liver microsomal UDP-glucuronosyltransferase(s) with activity toward retinoic acid. *Biochem. Biophys. Res. Commun.*, **230**, 497–500

Liu, Y., Chen, H. & Chiu, J.F. (1994) Identification of a retinoic acid response element upstream of the rat alpha-fetoprotein gene. *Mol. Cell. Endocrinol.*, **103**, 149–156

Lo, H.W. & Ali-Osman, F. (1997) Genomic cloning of hGSTP1*C, an allelic human Pi class glutathione S-transferase gene variant and functional characterization of its retinoic acid response elements. *J. Biol. Chem.*, **272**, 32743–32749

Lotan, R.(1993) Retinoids and squamous cell differentiation. In: Hong, W.K. & Lotan, R., eds, *Retinoids in Oncolog.*, New York, Marcel Dekker, pp. 43–72

Lotan, R. (1995a) Cellular biology of the retinoids. In: Degos L. & Parkinson D.R., eds, *Retinoids in Oncology.* Berlin, Springer Verlag, pp. 27–42

Lotan, R. (1995b) Retinoids and apoptosis: Implications for cancer chemoprevention and therapy. *J. Natl Cancer Inst.*, **87**, 1655–1657

Lotan, R., Lotan, D. & Kadouri, A. (1982) Comparison of retinoic acid effects on anchorage-dependent growth, anchorage-independent growth and fibrinolytic activity of neoplastic cells. *Exp. Cell Res.*, **141**, 79–86

Lotan, R., Dawson, M.I., Zou, C.C., Jong, L., Lotan, D. & Zou, C.P. (1995) Enhanced efficacy of combinations of retinoic acid- and retinoid X receptor-selective retinoids and α-interferon in inhibition of cervical carcinoma cell proliferation. *Cancer Res.*, **55**, 232–236

Louthrenoo, W. (1993) Successful treatment of severe Reiter's syndrome associated with human immunodeficiency virus infection with etretinate. Report of 2 cases. *J. Rheumatol.*, **20**, 1243–1246

Lovat, P.E., Irving, H., Annicchiarico-Petruzzelli, M., Bernassola, F., Malcolm, A.J., Pearson, A.D., Melino, G. & Redfern, C.P. (1997) Retinoids in neuroblastoma therapy: Distinct biological properties of 9-*cis*- and all-*trans*-retinoic acid. *Eur. J. Cancer*, **33**, 2075–2080

Lowe, N.J., Lazarus, V. & Matt, L. (1988) Systemic retinoid therapy for psoriasis. *J. Am. Acad. Dermatol.*, **19**, 186–191

Lozada-Nur, F. & Miranda, C. (1997) Oral lichen planus: Topical and systemic therapy. *Semin. Cutan. Med. Surg.*, **16**, 295–300

Luscher, B., Mitchell, P.J., Williams, T. & Tjian, R. (1989) Regulation of transcription factor AP-2 by the morphogen retinoic acid and by second messengers. *Genes Dev.*, **3**, 1507–1517

Lynch, R.G. (1995) Differentiation and cancer: The conditional autonomy of phenotype. *Proc. Natl Acad. Sci. USA*, **92**, 647–648

Maden, M., Hunt, P., Eriksson, U., Kuroiwa, A., Krumlauf, R. & Summerbell, D. (1991) Retinoic acid-binding protein, rhombomeres and the neural crest. *Development*, **111**, 35–43

Maden, M., Gale, E., Kostetskii, I. & Zile, M. (1996) Vitamin A-deficient quail embryos have half a hindbrain and other neural defects. *Curr. Biol.*, **6**, 417–426

Maden, M., Sonneveld, E., van der Saag, P.T. & Gale, E. (1998) The distribution of endogenous retinoic acid in the chick embryo: Implications for developmental mechanisms. *Development*, **125**, 4133–4144

Mader, S., Kumar, V., de Verneuil, H. & Chambon, P. (1989) Three amino acids of the oestrogen receptor are essential to its ability to distinguish an oestrogen from a glucocorticoid-responsive element. *Nature*, **338**, 271–274

Mader, S., Chen, J.Y., Chen, Z., White, J., Chambon, P. & Gronemeyer, H. (1993) The patterns of binding of RAR, RXR and TR homo- and heterodimers to direct repeats are dictated by the binding specificites of the DNA binding domains. *EMBO J.*, **12**, 5029–5041

Mangelsdorf, D.J. & Evans, R.M. (1995) The RXR heterodimers and orphan receptors. *Cell*, **83**, 841–850

Mangelsdorf, D.J., Umesono, K., Kliewer, S.A., Borgmeyer, U., Ong, E.S. & Evans, R.M. (1991)

A direct repeat in the cellular retinol-binding protein type II gene confers differential regulation by RXR and RAR. *Cell*, **66**, 555–561

Mangelsdorf, D.J., Umesono, K. & Evans, R.M. (1994) The retinoid receptors. In: Sporn M.B., Roberts A.B. & Goodman D.S., eds, *The Retinoids: Biology, Chemistry, and Medicine*, 2nd Ed. New York, Raven Press, pp. 319–350

Manji, S.S., Pearson, R.B., Pardee, M., Paspaliaris, V., d'Apice, A., Martin, T.J. & Ng, K.W. (1999) Dual posttranscriptional targets of retinoic acid-induced gene expression. *J. Cell Biochem.*, **72**, 411–422

Marchetti, M.N., Sampol, E., Bun, H., Scoma, H., Lacarelle, B. & Durand, A. (1997) *In vitro* metabolism of three major isomers of retinoic acid in rats. Intersex and interstrain comparison. *Drug Metab. Dispos.*, **25**, 637–646

Marshall, H., Studer, M., Pöpperl, H., Aparicio, S., Kuroiwa, A., Brenner, S. & Krumlauf, R. (1994) A conserved retinoic acid response element required for early expression of the homeobox gene *Hoxb-1*. *Nature*, **370**, 567–571

Martin, S.J., Bradley, J.G. & Cotter, T.G. (1990) HL-60 cells induced to differentiate towards neutrophils subsequently die via apoptosis. *Clin. Exp. Immunol.*, **79**, 448–453

Massaro, G.D. & Massaro, D. (1996) Postnatal treatment with retinoic acid increases the number of pulmonary alveoli in rats. *Am. J. Physiol.*, **270**, L305–L310

Massaro, G.D. & Massaro, D. (1997) Retinoic acid treatment abrogates elastase-induced pulmonary emphysema in rats. *Nat. Med.*, **3**, 675–677

Matsuo, T. & Thiele, C.J. (1998) p27Kip1: A key mediator of retinoic acid induced growth arrest in the SMS-KCNR human neuroblastoma cell line. *Oncogene*, **16**, 3337–3343

Maxwell, S.A. & Mukhopadhyay, T. (1994) Transient stabilization of p53 in non-small cell lung carcinoma cultures arrested for growth by retinoic acid. *Exp. Cell Res.*, **214**, 67–74

McBurney, M.W., Jones-Villeneuve, E.M., Edwards, M.K. & Anderson, P.J. (1982) Control of muscle and neuronal differentiation in a cultured embryonal carcinoma cell line. *Nature*, **299**, 165–167

McCaffery, P. & Dräger, U.C. (1994) Hot spots of retinoic acid synthesis in the developing spinal cord. *Proc. Natl Acad. Sci. USA*, **91**, 7194–7197

McCaffery, P. & Dräger, U.C. (1995) Retinoic acid synthesizing enzymes in the embryonic and adult vertebrate. *Adv. Exp. Med. Biol.*, **372**, 173–183

McCormick, A.M. & Napoli, J.L. (1982) Identification of 5,6-epoxyretinoic acid as an endogenous retinol metabolite. *J. Biol. Chem.*, **257**, 1730–1735

McCormick, A.M., Napoli, J.L., Schnoes, H.K. & DeLuca, H.F. (1978) Isolation and identification of 5,6-epoxyretinoic acid: A biologically active metabolite of retinoic acid. *Biochemistry*, **17**, 4085–4090

McCormick, A.M., Kroll, K.D. & Napoli, J.L. (1983) 13-*cis*-Retinoic acid metabolism *in vivo*. The major tissue metabolites in the rat have the all-*trans* configuration. *Biochemistry*, **22**, 3933–3940

McCue, P.A., Thomas, R.S., Schroeder, D., Gubler, M.L. & Sherman, M.I. (1988) Effects of dietary retinoids upon growth and differentiation of tumors derived from several murine embryonal carcinoma cell lines. *Cancer Res.*, **48**, 3772–3779

Mehta, R.G., Barua, A.B., Moon, R.C. & Olson, J.A. (1992) Interactions between retinoid-β-glucuronides and cellular retinol- and retinoic acid-binding proteins. *Int. J. Vitam. Nutr. Res.*, **62**, 143–147

Meisler, N.T., Parrelli, J., Gendimenico, G.J., Mezick, J.A. & Cutroneo, K.R. (1997) All-*trans*-retinoic acid inhibition of Pro alpha1(I) collagen gene expression in fetal rat skin fibroblasts: Identification of a retinoic acid response element in the Pro alpha1(I) collagen gene. *J. Invest. Dermatol.*, **108**, 476–481

Meloche, S. & Besner, J.G. (1986) Metabolism of isotretinoin. Biliary excretion of isotretinoin glucuronide in the rat. *Drug Metab. Dispos.*, **14**, 246–249

Mendelsohn, C., Ruberte, E., LeMeur, M., Morriss Kay, G. & Chambon, P. (1991) Developmental analysis of the retinoic acid-inducible RAR-β2 promoter in transgenic animals. *Development*, **113**, 723–734

Merrick, D.T., Gown, A.M., Halbert, C.L., Blanton, R.A. & McDougall, J.K. (1993) Human papillomavirus-immortalized keratinocytes are resistant to the effects of retinoic acid on terminal differentiation. *Cell Growth Differ.*, **4**, 831–840

Mertz, J.R., Shang, E., Piantedosi, R., Wei, S., Wolgemuth, D.J. & Blaner, W.S. (1997) Identification and characterization of a stereospecific human enzyme that catalyzes 9-*cis*-retinol oxidation. A possible role in 9-*cis*-retinoic acid formation. *J. Biol. Chem.*, **272**, 11744–11749

Meyer, M.E., Gronemeyer, H., Turcotte, B., Bocquel, M.T., Tasset, D. & Chambon, P. (1989) Steroid hormone receptors compete for factors that mediate their enhancer function. *Cell*, **57**, 433–442

Meyskens, F.L., Jr & Salmon, S.E. (1979) Inhibition of human melanoma colony formation by retinoids. *Cancer Res.*, **39**, 4055–4057

Meyskens, F.L., Jr, Alberts, D.S. & Salmon, S.E. (1983) Effect of 13-*cis*-retinoic acid and 4-hydroxyphenyl-all-*trans*-retinamide on human tumor colony formation in soft agar. *Int. J. Cancer*, **32**, 295–299

Mezey, E. & Holt, P.R. (1971) The inhibitory effect of ethanol on retinol oxidation by human liver and cattle retina. *Exp. Mol. Pathol.*, **15**, 148–156

Miano, J.M., Kelly, L.A., Artacho, C.A., Nuckolls, T.A., Piantedosi, R. & Blaner, W.S. (1998) all-*trans*-Retinoic acid reduces neointimal formation and promotes favorable geometric remodeling of the rat carotid artery after balloon withdrawal injury. *Circulation*, **98**, 1219–1227

Miller, W.H.J., Jakubowski, A., Tong, W.P., Miller, V.A., Rigas, J.R., Benedetti, F., Gill, G.M., Truglia, J.A., Ulm, E., Shirley, M. & Warrell, R.P.J. (1995) 9-*cis*-Retinoic acid induces complete remission but does not reverse clinically acquired retinoid resistance in acute promyelocytic leukemia. *Blood*, **85**, 3021–3027

Miller, V.A., Benedetti, F.M., Rigas, J.R., Verret, A.L., Pfister, D.G., Straus, D., Kris, M.G., Crisp, M., Heyman, R., Loewen, G.R., Truglia, J.A. & Warrell, R.P.J. (1997) Initial clinical trial of a selective retinoid X receptor ligand, LGD1069. *J. Clin. Oncol.*, **15**, 790–795

Minucci, S., Leid, M., Toyama, R., Saint Jeannet, J.P., Peterson, V.J., Horn, V., Ishmael, J.E., Bhattacharyya, N., Dey, A., Dawid, I.B. & Ozato, K. (1997) Retinoid X receptor (RXR) within the RXR-retinoic acid receptor heterodimer binds its ligand and enhances retinoid-dependent gene expression. *Mol. Cell. Biol.*, **17**, 644–655

Moasser, M.M., Motzer, R.J., Khoo, K.S., Lyn, P., Murphy, B.A., Bosl, G.J. & Dmitrovsky, E. (1995) all-*trans*-Retinoic acid for treating germ cell tumors. In vitro activity and results of a phase II trial. *Cancer*, **76**, 680–686

Moffa, D.J., Lotspeich, F.J. & Krause, R.F. (1970) Preparation and properties of retinal-oxidizing enzyme from rat intestinal mucosa. *J. Biol. Chem.*, **245**, 439–447

Molin, L., Thomsen, K., Volden, G. & Lange Wantzin, G. (1985) Retinoid dermatitis mimicking progression in mycosis fungoides: A report from the Scandinavian Mycosis Fungoides Group. *Acta Derm. Venereol.*, **65**, 69–71

Monzon, R.I., LaPres, J.J. & Hudson, L.G. (1996) Regulation of involucrin gene expression by retinoic acid and glucocorticoids. *Cell Growth Differ.*, **7**, 1751–1759

Moras, D. & Gronemeyer, H. (1998) The nuclear receptor ligand-binding domain: Structure and function. *Curr. Opin. Cell Biol.*, **10**, 384–391

Motzer, R.J., Schwartz, L., Law, T.M., Murphy, B.A., Hoffman, A.D., Albino, A.P., Vlamis, V. & Nanus, D.M. (1995) Interferon alfa-2a and 13-*cis*-retinoic acid in renal cell carcinoma: Antitumor activity in a phase II trial and interactions in vitro. *J. Clin. Oncol.*, **13** , 1950–1957

Muindi, J.R., Frankel, S.R., Huselton, C., DeGrazia, F., Garland, W.A., Young, C.W. & Warrell,R.P., Jr (1992) Clinical pharmacology of oral all-*trans*-retinoic acid in patients with acute promyelocytic leukemia. *Cancer Res.*, **52**, 2138–2142

Mukherjee, R., Davies, P.J., Crombie, D.L., Bischoff, E.D., Cesario, R.M., Jow, L., Hamann, L.G., Boehm, M.F., Mondon, C.E., Nadzan, A.M., Paterniti, J.R., Jr & Heyman, R.A. (1997) Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists. *Nature*, **386**, 407–410

Murphy, P.T., Sivakumaran, M., Fahy, G. & Hutchinson, R.M. (1996) Successful use of topical retinoic acid in severe dry eye due to chronic graft-versus-host disease. *Bone Marrow Transplant.*, **18**, 641–642

Nagai, H., Matsuura, S., Bouda, K., Takaoka, Y., Wang, T., Niwa, S. & Shudo, K. (1999) Effect of Am-80, a synthetic derivative of retinoid, on

experimental arthritis in mice. *Pharmacology*, **58**, 101–112

Nagao, A. & Olson, J.A. (1994) Enzymatic formation of 9-*cis*, 13-*cis*, and all-*trans*-retinals from isomers of β-carotene. *FASEB J.*, **8**, 968–973

Nagy, L., Thomazy, V.A., Shipley, G.L., Fesus, L., Lamph, W., Heyman, R.A., Chandraratna, R.A. & Davies, P.J. (1995) Activation of retinoid X receptors induces apoptosis in HL-60 cell lines. *Mol. Cell. Biol.*, **15**, 3540–3551

Nakshatri, H. & Chambon, P. (1994) The directly repeated RG(G/T)TCA motifs of the rat and mouse cellular retinol-binding protein II genes are promiscuous binding sites for RAR, RXR, HNF-4, and ARP-1 homo- and heterodimers. *J. Biol. Chem.*, **269**, 890–902

Napoli, J.L. (1986) Retinol metabolism in LLC-PK1 Cells. Characterization of retinoic acid synthesis by an established mammalian cell line. *J. Biol. Chem.*, **261**, 13592–13597

Napoli, J.L. (1996) Retinoic acid biosynthesis and metabolism. *FASEB J.*, **10**, 993–1001

Napoli, J.L. & Race, K.R. (1988) Biogenesis of retinoic acid from β-carotene. Differences between the metabolism of β-carotene and retinal. *J. Biol. Chem.*, **263**, 17372–17377

Napoli, J.L., Khalil, H. & McCormick, A.M. (1982) Metabolism of 5,6-epoxyretinoic acid in vivo: Isolation of a major intestinal metabolite. *Biochemistry*, **21**, 1942–1949

Napoli, J.L., Pramanik, B.C., Williams, J.B., Dawson, M.I. & Hobbs, P.D. (1985) Quantification of retinoic acid by gas–liquid chromatography–mass spectrometry: total versus all-*trans*-retinoic acid in human plasma. *J. Lipid Res.*, **26**, 387–392

Newcomer, M.E. & Ong, D.E. (1990) Purification and crystallization of a retinoic acid-binding protein from rat epididymis. Identity with the major androgen-dependent epididymal proteins. *J. Biol. Chem.*, **265**, 12876–12879

Newton, D.L., Henderson, W.R. & Sporn, M.B. (1980) Structure–activity relationships of retinoids in hamster tracheal organ culture. *Cancer Res.*, **40**, 3413–3425

Nicholson, R.C., Mader, S., Nagpal, S., Leid, M., Rochette-Egly, C. & Chambon, P. (1990) Negative regulation of the rat stromelysin gene promoter by retinoic acid is mediated by an AP1 binding site. *EMBO J.*, **9**, 4443–4454

Nicotra, C. & Livrea, M.A. (1976) Alcohol dehydrogenase and retinol dehydrogenase in bovine retinal pigment epithelium. *Exp. Eye Res.*, **22**, 367–376

Nicotra, C. & Livrea, M.A. (1982) Retinol dehydrogenase from bovine retinal rod outer segments. Kinetic mechanism of the solubilized enzyme. *J. Biol. Chem.*, **257**, 11836–11841

Niederreither, K., McCaffery, P., Dräger, U.C., Chambon, P. & Dollé, P. (1997) Restricted expression and retinoic acid-induced downregulation of the retinaldehyde dehydrogenase type 2 (RALDH-2) gene during mouse development. *Mech. Dev.*, **62**, 67–78

Nierenberg, D.W. (1984) Determination of serum and plasma concentrations of retinol using high-performance liquid chromatography. *J. Chromatogr.*, **311**, 239–248

Nolte, R.T., Wisely, G.B., Westin, S., Cobb, J.E., Lambert, M.H., Kurokawa, R., Rosenfeld, M.G., Willson, T.M., Glass, C.K. & Milburn, M.V. (1998) Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-γ. *Nature*, **395**, 137–143

Noy, N. (1992a) The ionization behavior of retinoic acid in aqueous environments and bound to serum albumin. *Biochim. Biophys. Acta*, **1106**, 151–158

Noy, N. (1992b) The ionization behavior of retinoic acid in lipid bilayers and in membranes. *Biochim. Biophys. Acta*, **1106**, 159–164

Oldfield, N. (1990) The metabolic fate of 13-*cis*-retinoic acid in mouse skin microsomal preparations. *Drug Metab. Dispos.*, **18**, 1105–1107

Olson, J.A.(1990) Vitamin A. In: Machlin, L.J., ed., *Handbook of Vitamins*. New York, Marcel Dekker, pp. 1–57

Olson, J.A., Moon, R.C., Anders, M.W., Fenselau, C. & Shane, B. (1992) Enhancement of biological activity by conjugation reactions. *J. Nutr.*, **122**, 615–624

Orfanos, C.E., Zouboulis, C.C., Almond-Roesler, B. & Geilen, C.C. (1997) Current use and future potential role of retinoids in dermatology. *Drugs*, **53**, 358–388

Oridate, N., Lotan, D., Xu, X.C., Hong, W.K. & Lotan, R. (1996) Differential induction of apoptosis by all-*trans*-retinoic acid and *N*-(4-hydroxyphenyl)retinamide in human head and neck squamous cell carcinoma cell lines. *Clin. Cancer Res.*, **2**, 855–863

Oridate, N., Suzuki, S., Higuchi, M., Mitchell, M.F., Hong, W.K. & Lotan, R. (1997) Involvement of reactive oxygen species in *N*-(4-hydroxyphenyl)-retinamide-induced apoptosis in cervical carcinoma cells. *J. Natl Cancer Inst.*, **89**, 1191–1198

Packer, A.I., Crotty, D.A., Elwell, V.A. & Wolgemuth, D.J. (1998) Expression of the murine Hoxa4 gene requires both autoregulation and a conserved retinoic acid response element. *Development*, **125**, 1991–1998

Paech, K., Webb, P., Kuiper, G.G., Nilsson, S., Gustafsson, J., Kushner, P.J. & Scanlan, T.S. (1997) Differential ligand activation of estrogen receptors ERα and ERβ at AP1 sites. *Science*, **277**, 1508–1510

Peck, G.L., Gross, E.G., Butkus, D. & DiGiovanna, J.J. (1982) Chemoprevention of basal cell carcinoma with isotretinoin. *J. Am. Acad. Dermatol.*, **6**, 815–823

Perlmann, T. & Jansson, L. (1995) A novel pathway for vitamin A signaling mediated by RXR heterodimerization with NGFI-B and NURR1. *Genes Dev.*, **9**, 769–782

Pfahl, M. (1993) Nuclear receptor/AP-1 interaction. *Endocr. Rev.*, **14**, 651–658

Pierce, G.B. & Speers, W.C. (1988) Tumors as caricatures of the process of tissue renewal: Prospects for therapy by directing differentiation. *Cancer Res.*, **48**, 1996–2004

Pilkington, T. & Brogden, R.N. (1992) Acitretin. A review of its pharmacology and therapeutic use. *Drugs*, **43**, 597–627

Pirisi, L., Batova, A., Jenkins, G.R., Hodam, J.R. & Creek, K.E. (1992) Increased sensitivity of human keratinocytes immortalized by human papillomavirus type 16 DNA to growth control by retinoids. *Cancer Res.*, **52**, 187–193

Pomponi, F., Cariati, R., Zancai, P., De Paoli, P., Rizzo, S., Tedeschi, R.M., Pivetta, B., De Vita, S., Boiocchi, M. & Dolcetti, R. (1996) Retinoids irreversibly inhibit in vitro growth of Epstein-Barr virus-immortalized B lymphocytes. *Blood*, **88**, 3147–3159

Ponzoni, M., Bocca, P., Chiesa, V., Decensi, A., Pistoia, V., Raffaghello, L., Rozzo, C. & Montaldo, P.G. (1995) Differential effects of *N*-(4-hydroxyphenyl)retinamide and retinoic acid on neuroblastoma cells: Apoptosis *versus* differentiation. *Cancer Res.*, **55**, 853–861

Pöpperl, H. & Featherstone, M.S. (1993) Identification of a retinoic acid response element upstream of the murine *Hox-4.2* gene. *Mol. Cell. Biol.*, **13**, 257–265

Posch, K.C. & Napoli, J.L. (1992) Multiple retinoid dehydrogenases in testes cytosol from alcohol dehydrogenase negative or positive deermice. *Biochem.Pharmacol.*, **43**, 2296–2298

Posch, K.C., Boerman, M.H., Burns, R.D. & Napoli, J.L. (1991) Holocellular retinol binding protein as a substrate for microsomal retinal synthesis. *Biochemistry*, **30**, 6224–6230

Posch, K.C., Burns, R.D. & Napoli, J.L. (1992) Biosynthesis of all-trans-retinoic acid from retinal. Recognition of retinal bound to cellular retinol binding protein (type I) as substrate by a purified cytosolic dehydrogenase. *J. Biol. Chem.*, **267**, 19676–19682

Radominska, A., Little, J.M., Lehman, P.A., Samokyszyn, V., Rios, G.R., King, C.D., Green, M.D. & Tephly, T.R. (1997) Glucuronidation of retinoids by rat recombinant UDP: Glucuronosyltransferase 1.1 (bilirubin UGT). *Drug Metab. Dispos.*, **25**, 889–892

Raife, T.J., McArthur, J., Peters, C., Kisker, C.T. & Lentz, S.R. (1998) Remission after 13-*cis*-retinoic acid in thrombotic thrombocytopenic purpura. *Lancet*, **352**, 454–455

Raisher, B.D., Gulick, T., Zhang, Z., Strauss, A.W., Moore, D.D. & Kelly, D.P. (1992) Identification of a novel retinoid-responsive element in the promoter region of the medium chain acyl-coenzyme A dehydrogenase gene. *J. Biol. Chem.*, **267**, 20264–20269

Randell, S.H., Liu, J.Y., Ferriola, P.C., Kaartinen, L., Doherty, M.M., Davis, C.W. & Nettesheim, P. (1996) Mucin production by SPOC1 cells—An immortalized rat tracheal epithelial cell line. *Am. J. Respir. Cell. Mol. Biol.*, **14**, 146–154

Rastinejad, F., Perlmann, T., Evans, R.M. & Sigler, P.B. (1995) Structural determinants of nuclear receptor assembly on DNA direct repeats. *Nature*, **375**, 203–211

Ray, W.J., Bain, G., Yao, M. & Gottlieb, D.I. (1997) CYP26, a novel mammalian cytochrome P450, is induced by retinoic acid and defines a new family. *J. Biol. Chem.*, **272**, 18702–18708

Raynaud, F., Gerbaud, P., Bouloc, A., Gorin, I., Anderson, W.B. & Evain, B.D. (1993) Rapid

effect of treatment of psoriatic erythrocytes with the synthetic retinoid acitretin to increase 8-azido cyclic AMP binding to the RI regulatory subunit. *J. Invest. Dermatol.*, **100**, 77–81

Reddel, R.R., Ke, Y., Gerwin, B.I., McMenamin, M.G., Lechner, J.F., Su, R.T., Brash, D.E., Park, J.B., Rhim, J.S. & Harris, C.C. (1988) Transformation of human bronchial epithelial cells by infection with SV40 or adenovirus-12 SV40 hybrid virus, or transfection via strontium phosphate coprecipitation with a plasmid containing SV40 early region genes. *Cancer Res.*, **48**, 1904–1909

Redlich, C.A., Delisser, H.M. & Elias, J.A. (1995) Retinoic acid inhibition of transforming growth factor-β-induced collagen production by human lung fibroblasts. *Am. J. Respir. Cell. Mol. Biol.*, **12**, 287–295

Redner, R.L., Rush, E.A., Faas, S., Rudert, W.A. & Corey, S.J. (1996) The t(5;17) variant of acute promyelocytic leukemia expresses a nucleophosmin-retinoic acid receptor fusion. *Blood*, **87**, 882–886

Reid, A., Gould, A., Brand, N., Cook, M., Strutt, P., Li, J., Licht, J., Waxman, S., Krumlauf, R. & Zelent, A. (1995) Leukemia translocation gene, *PLZF*, is expressed with a speckled nuclear pattern in early hematopoietic progenitors. *Blood*, **86**, 4544–4552

Renaud, J.P., Rochel, N., Ruff, M., Vivat, V., Chambon, P., Gronemeyer, H. & Moras, D. (1995) Crystal structure of the RAR-γ ligand-binding domain bound to all-*trans*-retinoic acid. *Nature*, **378**, 681–689

Reynolds, C.P., Matthay, K. & Crouse, K. (1990) Response of neuroblastoma bone marrow metastases to 13-*cis*-retinoic acid. *Proc. Am. Soc. Clin. Oncol.*, **9**, 54–54

Reynolds, C.P., Schindler, P.F., Jones, D.M., Gentile, J.L., Proffitt, R.T. & Einhorn, P.A. (1994) Comparison of 13-*cis*-retinoic acid to *trans*-retinoic acid using human neuroblastoma cell lines. *Prog. Clin. Biol. Res.*, **385**, 237–244

Ribaya-Mercado, J.D., Kassarjian, Z. & Russell, R.M. (1988) Quantitation of the enterohepatic circulation of retinol in the rat. *J. Nutr.*, **118**, 33–38

Rigas, J.R., Francis, P.A., Muindi, J.R., Kris, M.G., Huselton, C., DeGrazia, F., Orazem, J.P., Young, C.W. & Warrell-RP, J. (1993) Constitutive variability in the pharmacokinetics of the natural retinoid, all-*trans*-retinoic acid, and its modulation by ketoconazole. *J. Natl Cancer Inst.*, **85**, 1921–1926

Rigas, J.R., Miller, V.A., Zhang, Z.F., Klimstra, D.S., Tong, W.P., Kris, M.G. & Warrell, R.P. (1996) Metabolic phenotypes of retinoic acid and the risk of lung cancer. *Cancer Res.*, **56**, 2692–2696

Roberson, K.M., Penland, S.N., Padilla, G.M., Selvan, R.S., Kim, C.S., Fine, R.L. & Robertson, C.N. (1997) Fenretinide: Induction of apoptosis and endogenous transforming growth factor beta in PC-3 prostate cancer cells. *Cell Growth Differ.*, **8**, 101–111

Roberts, A.B., Nichols, M.D., Newton, D.L. & Sporn, M.B. (1979) *In vitro* metabolism of retinoic acid in hamster intestine and liver. *J. Biol. Chem.*, **254**, 6296–6302

Roberts, A.B., Lamb, L.C. & Sporn, M.B. (1980) Metabolism of all-*trans*-retinoic acid in hamster liver microsomes: Oxidation of 4-hydroxy- to 4-keto-retinoic acid. *Arch. Biochem. Biophys.*, **199**, 374–383

Roberts, A.B., Roche, N.S. & Sporn, M.B. (1985) Selective inhibition of the anchorage-independent growth of *myc*-transfected fibroblasts by retinoic acid. *Nature*, **315**, 237–239

Roberts, E.S., Vaz, A.D. & Coon, M.J. (1992) Role of isozymes of rabbit microsomal cytochrome P-450 in the metabolism of retinoic acid, retinol, and retinal. *Mol. Pharmacol.*, **41**, 427–433

Rochette-Egly, C., Adam, S., Rossignol, M., Egly, J.M. & Chambon, P. (1997) Stimulation of RARα activation function AF-1 through binding to the general transcription factor TFIIH and phosphorylation by CDK7. *Cell*, **90**, 97–107

Rosenbaum, S.E. & Niles, R.M. (1992) Regulation of protein kinase C gene expression by retinoic acid in B16 mouse melanoma cells. *Arch. Biochem. Biophys.*, **294**, 123–129

Rossant, J., Zirngibl, R., Cado, D., Shago, M. & Giguère, V. (1991) Expression of a retinoic acid response element-*hsplacZ* transgene defines specific domains of transcriptional activity

during mouse embryogenesis. *Genes Dev.*, **5**, 1333–1344

Rousselot, P., Hardas, B., Patel, A., Guidez, F., Gaken, J., Castaigne, S., Dejean, A., de Thé, H., Degos, L., Farzaneh, F. & Chomienne, C. (1994) The PML-RARα gene product of the t(15;17) translocation inhibits retinoic acid-induced granulocytic differentiation and mediated transactivation in human myeloid cells. *Oncogene*, **9**, 545–551

Ruberte, E., Dollé, P., Krust, A., Zelent, A., Morriss, K.G. & Chambon, P. (1990) Specific spatial and temporal distribution of retinoic acid receptor gamma transcripts during mouse embryogenesis. *Development*, **108**, 213–222

Ruberte, E., Dollé, P., Chambon, P. & Morriss, K.G. (1991) Retinoic acid receptors and cellular retinoid binding proteins. II. Their differential pattern of transcription during early morphogenesis in mouse embryos. *Development*, **111**, 45–60

Ruberte, E., Friederich, V., Chambon, P. & Morriss, K.G. (1993) Retinoic acid receptors and cellular retinoid binding proteins. III. Their differential transcript distribution during mouse nervous system development. *Development*, **118**, 267–282

Saari, J.C. (1994) Retinoids in photosensitive systems. In: Sporn, M.B., Roberts, A.B., & Goodman, D.S., eds, *The Retinoids: Biology, Chemistry, and Medicine*, 2nd Ed. New York, Raven Press, pp. 351–385

Saari, J.C. & Bredberg, L. (1982) Enzymatic reduction of 11-*cis*-retinal bound to cellular retinal-binding protein. *Biochim. Biophys. Acta*, **716**, 266–272

Sachs, L. (1978) Control of normal cell differentiation and the phenotypic reversion of malignancy in myeloid leukaemia. *Nature*, **274**, 535–539

Sani, B.P., Barua, A.B., Hill, D.L., Shih, T.W. & Olson, J.A. (1992) Retinoyl beta-glucuronide: Lack of binding to receptor proteins of retinoic acid as related to biological activity. *Biochem. Pharmacol.*, **43**, 919–922

Sass, J.O. (1994) 3,4-Didehydroretinol may be present in human embryos/fetuses. *Reprod. Toxicol.*, **8**, 191–191

Sass, J.O., Forster, A., Bock, K.W. & Nau, H. (1994) Glucuronidation and isomerization of all-*trans*- and 13-*cis*-retinoic acid by liver microsomes of phenobarbital- or 3-methylcholanthrene-treated rats. *Biochem. Pharmacol.*, **47**, 485–492

Satre, M.A., Penner, J.D. & Kochhar, D.M. (1989) Pharmacokinetic assessment of teratologically effective concentrations of an endogenous retinoic acid metabolite. *Teratology*, **39**, 341–348

Schmutzler, C., Brtko, J., Winzer, R., Jakobs, T.C., Meissner-Weigl, J., Simon, D., Goretzki, P.E. & Kohrle, J. (1998) Functional retinoid and thyroid hormone receptors in human thyroid-carcinoma cell lines and tissues. *Int. J. Cancer*, **76**, 368–376

Schroen, D.J. & Brinckerhoff, C.E. (1996) Inhibition of rabbit collagenase (matrix metalloproteinase-1; MMP-1) transcription by retinoid receptors: Evidence for binding of RARs/RXRs to the –77 AP-1 site through interactions with c-Jun. *J. Cell Physiol.*, **169**, 320–332

Schüle, R., Rangarajan, P., Yang, N., Kleiwer, S., Ransone, L.J., Bolado, J., Verma, I.M. & Evans, R.M. (1991) Retinoic acid is a negative regulator of AP-1-responsive genes. *Proc. Natl Acad. Sci. USA*, **88**, 6092–6096

Scott, W.J., Jr, Walter, R., Tzimas, G., Sass, J.O., Nau, H. & Collins, M.D. (1994) Endogenous status of retinoids and their cytosolic binding proteins in limb buds of chick vs mouse embryos. *Dev. Biol.*, **165**, 397–409

Scott, D.K., Mitchell, J.A. & Granner, D.K. (1996) Identification and characterization of the second retinoic acid response element in the phosphoenolpyruvate carboxykinase gene promoter. *J. Biol. Chem.*, **271**, 6260–6264

Shaulsky, G., Goldfinger, N., Peled, A. & Rotter, V. (1991) Involvement of wild-type p53 in pre-B-cell differentiation *in vitro*. *Proc. Natl Acad. Sci. USA*, **88**, 8982–8986

Shiau, A.K., Barstad, D., Loria, P.M., Cheng, L., Kushner, P.J., Agard, D.A. & Greene, G.L. (1998) The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell*, **95**, 927–937

Shih, T.W. & Hill, D.L. (1991) Conversion of retinol to retinal by rat liver microsomes. *Drug Metab. Dispos.*, **19**, 332–335

Shih, T.W., Lin, T.H., Shealy, Y.F. & Hill, D.L. (1997) Nonenzymatic isomerization of 9-*cis*-retinoic acid catalyzed by sulfhydryl compounds. *Drug Metab. Dispos.*, **25**, 27–32

Shindoh, M., Sun, Q., Pater, A. & Pater, M.M. (1995) Prevention of carcinoma in situ of human papillomavirus type 16-immortalized human endocervical cells by retinoic acid in organotypic raft culture. *Obstet. Gynecol.*, **85**, 721–728

Sietsema, W.K. & DeLuca, H.F. (1982) A new vaginal smear assay for vitamin A in rats. *J. Nutr.*, **112**, 1481–1489

Simeone, A., Acampora, D., D'Esposito, M., Faiella, A., Pannese, M., Scotto, L., Montanucci, M., D'Alessandro, G., Mavilio, F. & Boncinelli, E. (1989) Posttranscriptional control of human homeobox gene expression in induced NTERA-2 embryonal carcinoma cells. *Mol. Reprod. Dev.*, **1**, 107–115

Simon, A., Hellman, U., Wernstedt, C. & Eriksson, U. (1995) The retinal pigment epithelial-specific 11-*cis* retinol dehydrogenase belongs to the family of short chain alcohol dehydrogenases. *J. Biol. Chem.*, **270**, 1107–1112

Skare, K.L. & DeLuca, H.F. (1983) Biliary metabolites of all-*trans*-retinoic acid in the rat. *Arch. Biochem. Biophys.*, **224**, 13–18

Smith, J.E., Milch, P.O., Muto, Y. & Goodman, D.S. (1973) The plasma transport and metabolism of retinoic acid in the rat. *Biochem. J.*, **132**, 821–827

Smith, M.A., Parkinson, D.R., Cheson, B.D. & Friedman, M.A. (1992) Retinoids in cancer therapy. *J. Clin. Oncol.*, **10**, 839–864

Solary, E., Bertrand, R. & Pommier, Y. (1994) Apoptosis of human leukemic HL-60 cells induced to differentiate by phorbol ester treatment. *Leukemia*, **8**, 792–797

Sonneveld, E., van den Brink, C.E., van der Leede, B.M., Schulkes, R.K., Petkovich, M., van der Burg, B. & van der Saag, P.T. (1998) Human retinoic acid (RA) 4-hydroxylase (CYP26) is highly specific for all-*trans*-RA and can be induced through RA receptors in human breast and colon carcinoma cells. *Cell Growth Differ.*, 9, 629–637

Spanjaard, R.A., Sugawara, A., Ikeda, M. & Chin, W.W. (1995) Evidence that retinoid X receptors mediate retinoid-dependent transcriptional activation of the retinoic acid receptor beta gene in S91 melanoma cells. *J. Biol. Chem.*, **270**, 17429–17436

Speers, W.C. & Altmann, M. (1984) Chemically induced differentiation of murine embryonal carcinoma *in vivo*: Transplantation of differentiated tumors. *Cancer Res.*, **44**, 2129–2135

Strickland, S., Smith, K.K. & Marotti, K.R. (1980) Hormonal induction of differentiation in teratocarcinoma stem cells: Generation of parietal endoderm by retinoic acid and dibutyryl cAMP. *Cell*, **21**, 347–355

Su, J., Chai, X., Kahn, B. & Napoli, J.L. (1998) cDNA cloning, tissue distribution, and substrate characteristics of a *cis*-retinol/3α-hydroxysterol short-chain dehydrogenase isozyme. *J. Biol. Chem.*, **273**, 17910–17916

Sucov, H.M., Murakami, K.K. & Evans, R.M. (1990) Characterization of an autoregulated response element in the mouse retinoic acid receptor type β gene. *Proc. Natl Acad. Sci. USA*, **87**, 5392–5396

Sun, S.Y., Yue, P., Dawson, M.I., Shroot, B., Michel, S., Lamph, W.W., Heyman, R.A., Teng, M., Chandraratna, R.A., Shudo, K., Hong, W.K. & Lotan, R. (1997) Differential effects of synthetic nuclear retinoid receptor-selective retinoids on the growth of human non-small cell lung carcinoma cells. *Cancer Res.*, **57**, 4931–4939

Sun, S.Y., Kurie, J.M., Yue, P., Dawson, M.I., Shroot, B., Chandraratna, R.A., Hong, W.K. & Lotan, R. (1999a) Differential responses of normal, premalignant, and malignant human bronchial epithelial cells to receptor-selective retinoids. *Clin. Cancer Res.*, **5**, 431–437

Sun, S.Y., Yue, P. & Lotan, R. (1999b) Induction of apoptosis by *N*-(4-hydroxyphenyl)retinamide and its association with reactive oxygen species, nuclear retinoic acid receptors, and apoptosis-related genes in human prostate carcinoma cells. *Mol. Pharmacol.*, **55**, 403–410

Sun, S.Y., Li, W., Yue, P., Lippman, S.M., Hong, W.K. & Lotan, R. (1999c) Mediation of N-(4-hydroxyphenyl) retinamide-induced apoptosis in human cancer cells by different mechanisms. *Cancer Res.*, **59**, 2493–2498

Supino, R., Crosti, M., Clerici, M., Warlters, A., Cleris, L., Zunino, F. & Formelli, F. (1996) Induction of apoptosis by fenretinide (4HPR) in human ovarian carcinoma cells and its association with retinoic acid receptor expression. *Int. J. Cancer*, **65**, 491–497

Suzuki, Y., Ishiguro, S. & Tamai, M. (1993) Identification and immunohistochemistry of retinol dehydrogenase from bovine retinal pigment epithelium. *Biochim. Biophys. Acta*, **1163**, 201–208

Swanson, B.N., Frolik, C.A., Zaharevitz, D.W., Roller, P.P. & Sporn, M.B. (1981) Dose-dependent kinetics of all-*trans*-retinoic acid in rats. Plasma levels and excretion into bile, urine, and faeces. *Biochem. Pharmacol.*, **30**, 107–113

Takaoka, Y., Nagai, H., Mori, H. & Tanahashi, M. (1997) The effect of TRK-530 on experimental arthritis in mice. *Biol. Pharm. Bull.*, **20**, 1147–1150

Takeshita, A., Shibata, Y., Shinjo, K., Yanagi, M., Tobita, T., Ohnishi, K., Miyawaki, S., Shudo, K. & Ohno, R. (1996) Successful treatment of relapse of acute promyelocytic leukemia with a new synthetic retinoid, Am80. *Ann. Intern. Med*, **124**, 893–896

Tallman, M.S., Andersen, J.W., Schiffer, C.A., Appelbaum, F.R., Feusner, J.H., Ogden, A., Shepherd, L., Willman, C., Bloomfield, C.D., Rowe, J.M. & Wiernik, P.H. (1997) All-*trans*-retinoic acid in acute promyelocytic leukemia. *N. Engl. J. Med.*, **337**, 1021–1028

Talmage, D.A. & Lackey, R.S. (1992) Retinoic acid receptor α suppresses polyomavirus transformation and c-*fos* expression in rat fibroblasts. *Oncogene*, 7, 1837–1845

Tanaka, T., Urade, Y., Kimura, H., Eguchi, N., Nishikawa, A. & Hayaishi, O. (1997) Lipocalin-type prostaglandin D synthase (β-trace) is a newly recognized type of retinoid transporter. *J. Biol. Chem.*, **272**, 15789–15795

Taneja, R., Roy, B., Plassat, J.L., Zusi, C.F., Ostrowski, J., Reczek, P.R. & Chambon, P. (1996) Cell-type and promoter-context dependent retinoic acid receptor (RAR) redundancies for *RARβ 2* and *Hoxa-1* activation in F9 and P19 cells can be artefactually generated by gene knockouts. *Proc. Natl Acad. Sci. USA*, **93**, 6197–6202

Tanenbaum, D.M., Wang, Y., Williams, S.P. & Sigler, P.B. (1998) Crystallographic comparison of the estrogen and progesterone receptor's ligand binding domains. *Proc. Natl Acad. Sci. USA*, **95**, 5998–6003

Tang, G.W. & Russell, R.M. (1990) 13-*cis*-Retinoic acid is an endogenous compound in human serum. *J. Lipid Res.*, **31**, 175–182

Tang, G. & Russell, R.M. (1991) Formation of all-*trans*-retinoic acid and 13-*cis*-retinoic acid from all-*trans*-retinyl palmitate in humans. *J. Nutr. Biochem.*, **2**, 210–213

Tang, G., Shiau, A., Russell, R.M. & Mobarhan, S. (1995) Serum retinoic acid levels in patients with resected benign and malignant colonic neoplasias on β-carotene supplementation. *Nutr. Cancer*, **23**, 291–298

Thaller, C. & Eichele, G. (1987) Identification and spatial distribution of retinoids in the developing chick limb bud. *Nature*, **327**, 625–628

Thaller, C. & Eichele, G. (1990) Isolation of 3,4-didehydroretinoic acid, a novel morphogenetic signal in the chick wing bud. *Nature*, **345**, 815–819

de Thé, H., Vivanco-Ruiz, M.M., Tiollais, P., Stunnenberg, H. & Dejean, A. (1990) Identification of a retinoic acid responsive element in the retinoic acid receptor β gene. *Nature*, **343**, 177–180

de Thé, H., Lavau, C., Marchio, A., Chomienne, C., Degos, L. & Dejean, A. (1991) The PML-RARα fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. *Cell*, **66**, 675–684

Thompson, H.J., Strange, R. & Schedin, P.J. (1992) Apoptosis in the genesis and prevention of cancer. *Cancer Epidemiol. Biomarkers. Prev.*, **1**, 597–602

Torchia, J., Glass, C. & Rosenfeld, M.G. (1998) Co-activators and co-repressors in the integration of transcriptional responses. *Curr. Opin. Cell Biol.*, **10**, 373–383

Trofimova-Griffin, M.E. & Juchau, M.R. (1998) Expression of cytochrome P450RAI (CYP26) in human fetal hepatic and cephalic tissues. *Biochem. Biophys. Res. Commun.*, **252**, 487–491

Tseng, S.C. (1986) Topical tretinoin treatment for severe dry-eye disorders. *J. Am. Acad. Dermatol.*, **15**, 860–866

Tzimas, G., Collins, M.D. & Nau, H. (1995) Developmental stage-associated differences in the transplacental distribution of 13-*cis*- and all-*trans*-retinoic acid as well as their glucuronides in rats and mice. *Toxicol. Appl. Pharmacol.*, **133**, 91–101

Tzimas, G., Collins, M.D., Bürgin, H., Hummler, H. & Nau, H. (1996a) Embryotoxic doses of vitamin A to rabbits result in low plasma but high embryonic concentrations of all-*trans*-retinoic acid: Risk of vitamin A exposure in humans. *J. Nutr.*, **126**, 2159–2171

Tzimas, G., Nau, H., Hendrickx, A.G., Peterson, P.E. & Hummler, H. (1996b) Retinoid metabo-

lism and transplacental pharmacokinetics in the cynomolgus monkey following a nonteratogenic dosing regimen with all-*trans*-retinoic acid. *Teratology,* **54**, 255–265

Umesono, K. & Evans, R.M. (1989) Determinants of target gene specificity for steroid/thyroid hormone receptors. *Cell,* **57**, 1139–1146

Urbach, J. & Rando, R.R. (1994) Isomerization of all-*trans*-retinoic acid to 9-*cis*-retinoic acid. *Biochem. J.*, **299**, 459–465

Van Wauwe, J., Van-Nyen, G., Coene, M.C., Stoppie, P., Cools, W., Goossens, J., Borghgraef, P. & Janssen, P.A. (1992) Liarozole, an inhibitor of retinoic acid metabolism, exerts retinoid-mimetic effects in vivo. *J. Pharmacol. Exp. Ther.*, **261**, 773–779

Vayssière, B.M., Dupont, S., Choquart, A., Petit, F., Garcia, T., Marchandeau, C., Gronemeyer, H. & Resche-Rigon, M. (1997) Synthetic glucocorticoids that dissociate transactivation and AP-1 transrepression exhibit antiinflammatory activity in vivo. *Mol. Endocrinol.*, **11**, 1245–1255

Villablanca, J.G., Khan, A.A., Avramis, V.I., Seeger, R.C., Matthay, K.K., Ramsay, N.K. & Reynolds, C.P. (1995) Phase I trial of 13-*cis*-retinoic acid in children with neuroblastoma following bone marrow transplantation. *J. Clin. Oncol.*, **13**, 894–901

Voegel, J.J., Heine, M.J., Tini, M., Vivat, V., Chambon, P. & Gronemeyer, H. (1998) The coactivator TIF2 contains three nuclear receptor-binding motifs and mediates transactivation through CBP binding-dependent and -independent pathways. *EMBO J.*, **17**, 507–519

Vogelstein, B. & Kinzler, K.W. (1992) p53 Function and dysfunction. *Cell,* **70**, 523–526

de Vos, S., Dawson, M.I., Holden, S., Le, T., Wang, A., Cho, S.K., Chen, D.L. & Koeffler, H.P. (1997) Effects of retinoid X receptor-selective ligands on proliferation of prostate cancer cells. *Prostate,* **32**, 115–121

Wagner, M., Han, B. & Jessell, T.M. (1992) Regional differences in retinoid release from embryonic neural tissue detected by an in vitro reporter assay. *Development,* **116**, 55–66

Wagner, R.L., Apriletti, J.W., McGrath, M.E., West, B.L., Baxter, J.D. & Fletterick, R.J. (1995) A structural role for hormone in the thyroid hormone receptor. *Nature,* **378**, 690–697

Wan, H., Dawson, M.I., Hong, W.K. & Lotan, R. (1997) Enhancement of Calu-1 human lung carcinoma cell growth in serum-free medium by retinoids: Dependence on AP-1 activation, but not on retinoid response element activation. *Oncogene,* **15**, 2109–2118

Wang, T.T. & Phang, J.M. (1996) Effect of *N*-(4-hydroxyphenyl)retinamide on apoptosis in human breast cancer cells. *Cancer Lett.,* **107**, 65–71

Wang, X.D., Tang, G.W., Fox, J.G., Krinsky, N.I. & Russell, R.M. (1991) Enzymatic conversion of beta-carotene into beta-apo-carotenals and retinoids by human, monkey, ferret, and rat tissues. *Arch. Biochem. Biophys.*, **285**, 8–16

Wang, X.D., Krinsky, N.I., Tang, G.W. & Russell, R.M. (1992) Retinoic acid can be produced from excentric cleavage of beta-carotene in human intestinal mucosa. *Arch. Biochem. Biophys.*, **293**, 298–304

Wang, X., Penzes, P. & Napoli, J.L. (1996a) Cloning of a cDNA encoding an aldehyde dehydrogenase and its expression in *Escherichia coli*. Recognition of retinal as substrate. *J. Biol. Chem.*, **271**, 16288–16293

Wang, X.D., Russell, R.M., Liu, C., Stickel, F., Smith, D.E. & Krinsky, N.I. (1996b) Beta-oxidation in rabbit liver in vitro and in the perfused ferret liver contributes to retinoic acid biosynthesis from beta-apocarotenoic acids. *J. Biol. Chem.*, **271**, 26490–26498

Warkany, J. (1945) Manifestations of prenatal nutritional deficiency. *Vitam. Hormones,* **3**, 73–103

Warrell, R.P., Jr, de Thé, H., Wang, Z.Y. & Degos, L. (1993) Acute promyelocytic leukemia. *N. Engl. J. Med.,* **329**, 177–189

Weis, K.E., Ekena, K., Thomas, J.A., Lazennec, G. & Katzenellenbogen, B.S. (1996) Constitutively active human estrogen receptors containing amino acid substitutions for tyrosine 537 in the receptor protein. *Mol. Endocrinol.,* **10**, 1388–1398

Wellik, D.M., Norback, D.H. & DeLuca, H.F. (1997) Retinol is specifically required during midgestation for neonatal survival. *Am. J. Physiol.,* **272**, E25–E29

Wells, R.A. & Kamel-Reid, S. (1996) NuMA-RARα, a new gene fusion in acute promyelocytic leukaemia. *Blood,* **88** (Suppl. 1), 365a

White, J.A., Guo, Y.D., Baetz, K., Beckett-Jones, B., Bonasoro, J., Hsu, K.E., Dilworth, F.J., Jones, G. & Petkovich, M. (1996) Identification of the

retinoic acid-inducible all-*trans*-retinoic acid 4-hydroxylase. *J. Biol. Chem.*, **271**, 29922–29927

White, J.A., Beckett-Jones, B., Guo, Y.D., Dilworth, F.J., Bonasoro, J., Jones, G. & Petkovich, M. (1997a) cDNA cloning of human retinoic acid-metabolizing enzyme (hP450RAI) identifies a novel family of cytochromes P450. *J. Biol. Chem.*, **272**, 18538–18541

White, R., Sjöberg, M., Kalkhoven, E. & Parker, M.G. (1997b) Ligand-independent activation of the oestrogen receptor by mutation of a conserved tyrosine. *EMBO J.*, **16**, 1427–1435

Wiley, J.S. & Firkin, F.C. (1995) Reduction of pulmonary toxicity by prednisolone prophylaxis during all-*trans*-retinoic acid treatment of acute promyelocytic leukemia. Australian Leukaemia Study Group. *Leukemia*, **9**, 774–778

Williams, S.P. & Sigler, P.B. (1998) Atomic structure of progesterone complexed with its receptor. *Nature*, **393**, 392–396

Williams, J.B., Pramanik, B.C. & Napoli, J.L. (1984) Vitamin A metabolism: Analysis of steady-state neutral metabolites in rat tissues. *J. Lipid Res.*, **25**, 638–645Wilson, J.G., Roth, B. & Warkany, J. (1953) An analysis of the syndrome of malformations induced by maternal vitamin A deficiency. Effects of restoration of vitamin A at various times during gestation. *Am. J. Anat.*, **92**, 189–217

Wilson, T.E., Paulsen, R.E., Padgett, K.A. & Milbrandt, J. (1992) Participation of non-zinc finger residues in DNA binding by two nuclear orphan receptors. *Science*, **256**, 107–110

Wright, P. (1985) Topical retinoic acid therapy for disorders of the outer eye. *Trans. Ophthalmol. Soc. UK*, **104**, 869–874

Wurtz, J.M., Bourguet, W., Renaud, J.P., Vivat, V., Chambon, P., Moras, D. & Gronemeyer, H. (1996) A canonical structure for the ligand-binding domain of nuclear receptors. *Nat. Struct. Biol.*, **3**, 87–94

Yamagata, T., Momoi, M.Y., Yanagisawa, M., Kumagai, H., Yamakado, M. & Momoi, T. (1994) Changes of the expression and distribution of retinoic acid receptors during neurogenesis in mouse embryos. *Brain Res. Dev. Brain Res.*, **77**, 163–176

Yang, Y., Vacchio, M.S. & Ashwell, J.D. (1993) 9-*cis*-Retinoic acid inhibits activation-driven T-cell apoptosis: Implications for retinoid X receptor involvement in thymocyte development. *Proc. Natl Acad. Sci. USA*, **90**, 6170–6174

Yang, Z.N., Davis, G.J., Hurley, T.D., Stone, C.L., Li, T.K. & Bosron, W.F. (1994) Catalytic efficiency of human alcohol dehydrogenases for retinol oxidation and retinal reduction. *Alcohol Clin. Exp. Res.*, **18**, 587–591

Yang, Y., Minucci, S., Ozato, K., Heyman, R.A. & Ashwell, J.D. (1995) Efficient inhibition of activation-induced Fas ligand up-regulation and T cell apoptosis by retinoids requires occupancy of both retinoid X receptors and retinoic acid receptors. *J. Biol. Chem.*, **270**, 18672–18677

You, C.S., Parker, R.S., Goodman, K.J., Swanson, J.E. & Corso, T.N. (1996) Evidence of *cis–trans* isomerization of 9-*cis*-β-carotene during absorption in humans. *Am. J. Clin. Nutr.*, **64**, 177–183

Yuan, Z.F., Davis, A., Macdonald, K. & Bailey, R.R. (1995) Use of acitretin for the skin complications in renal transplant recipients. *N. Z. Med. J.*, **108**, 255–256

Yung, B.Y., Luo, K.J. & Hui, E.K. (1992) Interaction of antileukemia agents adriamycin and daunomycin with sphinganine on the differentiation of human leukemia cell line HL-60. *Cancer Res.*, **52**, 3593–3597

Zachman, R.D. & Olson, J.A. (1961) A comparison of retinene reductase and alcohol dehydrogenase of rat liver. *J. Biol. Chem.*, **236**, 2309–2313

Zachman, R.D., Dunagin, P.E., Jr & Olson, J.A. (1966) Formation and enterohepatic circulation of metabolites of retinol and retinoic acid in bile duct-cannulated rats. *J. Lipid Res.*, **7**, 3–9

Zechel, C., Shen, X.Q., Chen, J.Y., Chen, Z.P., Chambon, P. & Gronemeyer, H. (1994) The dimerization interfaces formed between the DNA binding domains of RXR, RAR and TR determine the binding specificity and polarity of the full-length receptors to direct repeats. *EMBO J.*, **13**, 1425–1433

Zhai, Y., Higgins, D. & Napoli, J.L. (1997) Coexpression of the mRNAs encoding retinol dehydrogenase isozymes and cellular retinol-binding protein. *J. Cell Physiol.*, **173**, 36–43

Zhang, W., Hu, G., Estey, E., Hester, J. & Deisseroth, A. (1992) Altered conformation of

the p53 protein in myeloid leukemia cells and mitogen-stimulated normal blood cells. *Oncogene*, **7**, 1645–1647

Zhao, D., McCaffery, P., Ivins, K.J., Neve, R.L., Hogan, P., Chin, W.W. & Dräger, U.C. (1996) Molecular identification of a major retinoic-acid-synthesizing enzyme, a retinaldehyde-specific dehydrogenase. *Eur. J. Biochem.*, **240**, 15–22

Zhou, H., Manji, S.S., Findlay, D.M., Martin, T.J., Heath, J.K. & Ng, K.W. (1994) Novel action of retinoic acid. Stabilization of newly synthesized alkaline phosphatase transcripts. *J. Biol. Chem.*, **269**, 22433–22439

Zhou, X.F., Shen, X.Q. & Shemshedini, L. (1999) Ligand-activated retinoic acid receptor inhibits AP-1 transactivation by disrupting c-Jun/c-Fos dimerization. *Mol. Endocrinol.*, **13**, 276–285

Zhu, W.Y., Jones, C.S., Kiss, A., Matsukuma, K., Amin, S. & De-Luca, L.M. (1997) Retinoic acid inhibition of cell cycle progression in MCF-7 human breast cancer cells. *Exp. Cell Res.*, **234**, 293–299

Zile, M.H. (1998) Vitamin A and embryonic development: aAn overview. *J. Nutr.*, **128**, 455S–458S

Zile, M.H., Inhorn, R.C. & DeLuca, H.F. (1982) Metabolism in vivo of all-trans-retinoic acid. Biosynthesis of 13-*cis*-retinoic acid and all-*trans*- and 13-*cis*-retinoyl glucuronides in the intestinal mucosa of the rat. *J. Biol. Chem.*, **257**, 3544–3550

Zile, M.H., Cullum, M.E., Simpson, R.U., Barua, A.B. & Swartz, D.A. (1987) Induction of differentiation of human promyelocytic leukemia cell line HL-60 by retinoyl glucuronide, a biologically active metabolite of vitamin A. *Proc. Natl Acad. Sci. USA*, **84**, 2208–2212

Zou, C.P., Kurie, J.M., Lotan, D., Zou, C.C., Hong, W.K. & Lotan, R. (1998) Higher potency of *N*-(4-hydroxyphenyl)retinamide than all-*trans*-retinoic acid in induction of apoptosis in non-small cell lung cancer cell lines. *Clin. Cancer Res.*, **4**, 1345–1355