ELSEVIER

Review

The role of vitamin A in differentiation and skin carcinogenesis

Luigi M. De Luca, Karolina Kosa, and Fausto Andreola

Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-4255

The field of vitamin A research has witnessed a remarkable surge in interest since the late 1980's, when the retinoid receptors were discovered and their genes cloned. Heterodimeric interactions between the retinoid X receptors (RXRs α, β, and γ), which bind 9-cis-retinoic acid, and other hormone receptors, including the retinoic acid receptors (RARs α, β, and γ), the thyroid hormone receptor (TR), the vitamin D receptor (VDR), the peroxisomal proliferator activated receptor (PPAR), and others make hormone action dependent on retinoid homeostasis. Retinoid response elements (RAREs) are present in the promoter and/or enhancer regions of several genes, including some of the homeobox genes, which control development and differentiation. The interaction between hormones and retinoids is added additional orders of complexity by the diversity of the RAREs including the spacer length, their 5' or 3' position, and their coexistence in composite sequences with other hormone response elements (e.g., an estrogen response element in the lactalbumin gene promoter, see Table 2). Control of normal epithelial differentiation is a fundamental function of retinoids. The histogenesis of squamous metaplasia caused by vitamin A deficiency is a stepwise process, which permits the gradual transition of phenotypes from simple-columnar, typical of the endocervical epithelium, to pseudostratified, to stratified-squamous and, eventually, to keratinizing. Conversely, the maintenance of the squamous keratinizing differentiation in the ectocervix and vagina requires estrogen. In the absence of this hormone, the squamous stratified ectocervical epithelium retrogrades to a simpler, two or three cell layer morphology, with the topmost layer expressing keratin K8, typical of the endocervical epithelium and mucous cells. Retinoid and estrogen receptor transcript expression is governed by dietary retinoid status and by estrogen availability, with squamous cells mostly expressing RARγ and estrogen receptor transcripts, and columnar cells mostly expressing RARβ. RXR transcripts appear mostly expressed in proliferating cells. The relevance of the retinoid receptors to carcinogenesis is highlighted in the work on acute promyelocytic leukemia. This work has demonstrated that the fusion gene PML-RARα, resulting from the t(15;17) chromosomal translocation, is etiologically connected with the disease and with complete remission after oral retinoid administration. Developments in retinoid metabolism, including the cloning of the cytochrome P450RAI and the connection between RA metabolism and cell growth inhibition, have recently taken place. Recent work has also shown that pharmacological dietary retinoic acid specifically inhibits malignant conversion in the mouse two-stage carcinogenesis system. Because RA upregulates retinoid receptor expression, it seems that retinoid receptors function as tumor suppressors.

This field should serve as a paradigm for things to come for other essential nutrients, and spells out the notion that nutritional sciences are indeed fundamentally important, because they can contribute significantly to our understanding of different diseases and provide effective therapeutic approaches. (J. Nutr. Biochem. 8: 426–437, 1997) © *Elsevier Science Inc. 1997*

Keywords: retinoic acid; estrogen; nuclear receptors; differentiation; carcinogenesis; epithelium

Introduction

I (LDL) have spent some 30 years of my professional life working on vitamin A, and I am more excited than ever about this vitamin. Though undoubtedly this attachment is a sign of my own limitations, the majority of the credit for the

Address correspondence to Dr. Luigi M. De Luca at Building 37, Room 3A-17, 37 Convent Drive, NIH, Bethesda, MD 20892-4255.
Received January 30, 1997; accepted April 1, 1997.

655 Avenue of the Americas, New York, NY 10010

0955-2863/97/$17.00
PII S0955-2863(97)00063-6

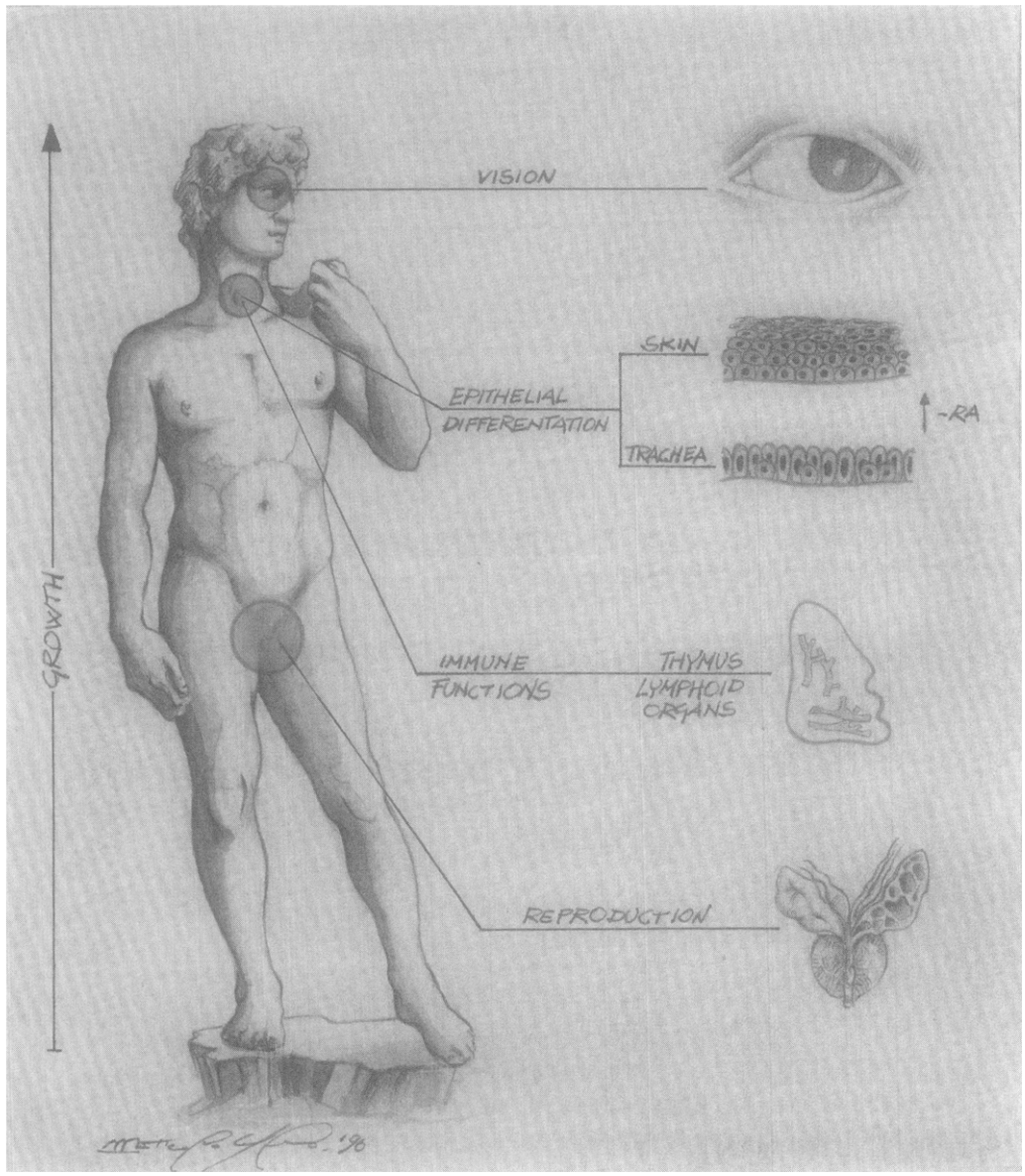

Figure 1 Artistic rendition to highlight the various functions of vitamin A.

enthusiasm goes to the nature of the substance itself. The wide scope of its involvement in fundamental biological processes is evident from *Figure 1*, which highlights its requirement for growth, reproduction, vision, epithelial differentiation, and immune functions. The field of vitamin A research has continued to grow since the 1960's, when it was mostly the realm of nutritional biochemists; through the 1970's, with the realization that retinoids might be the golden key to cancer chemoprevention; and the 1980's, when some, apparently unrelated findings in molecular biology, permitted the cloning of the retinoid receptors. Retinoids are now of fundamental interest to researchers and clinicians in fields as diverse, and yet interrelated, as dermatology, oncology, embryogenesis, etc.

This review considers some aspects of the field and falls short of being comprehensive, both because of space limitations and those of the senior reviewer himself.

Retinoid metabolism

Excellent reviews on retinol and retinoic acid metabolism[1,2] and transport[3,4] have been published. *Figure 2* shows the chemical structures of the most common retinoids. *Figure 3* shows the salient points in the oxidative pathway from retinol to retinoic acid and its derivative 4-hydroxy and 4-oxo-RA. Here, we focus our attention on how RA itself can actively contribute to control its own levels within the cell by regulating both its biosynthesis and metabolism. Once taken up, retinol binds the cellular retinol binding protein (CRBP). The resulting holo-CRBP then functions as a substrate for two different families of enzymes, which compete for its availability: a) the microsomal lecithin: retinol acyl transferase (LRAT),[5] which synthesizes retinyl esters, the storage form of retinol in many tissues and cells[3] and b) the microsomal retinol dehydrogenases,[6,7] which

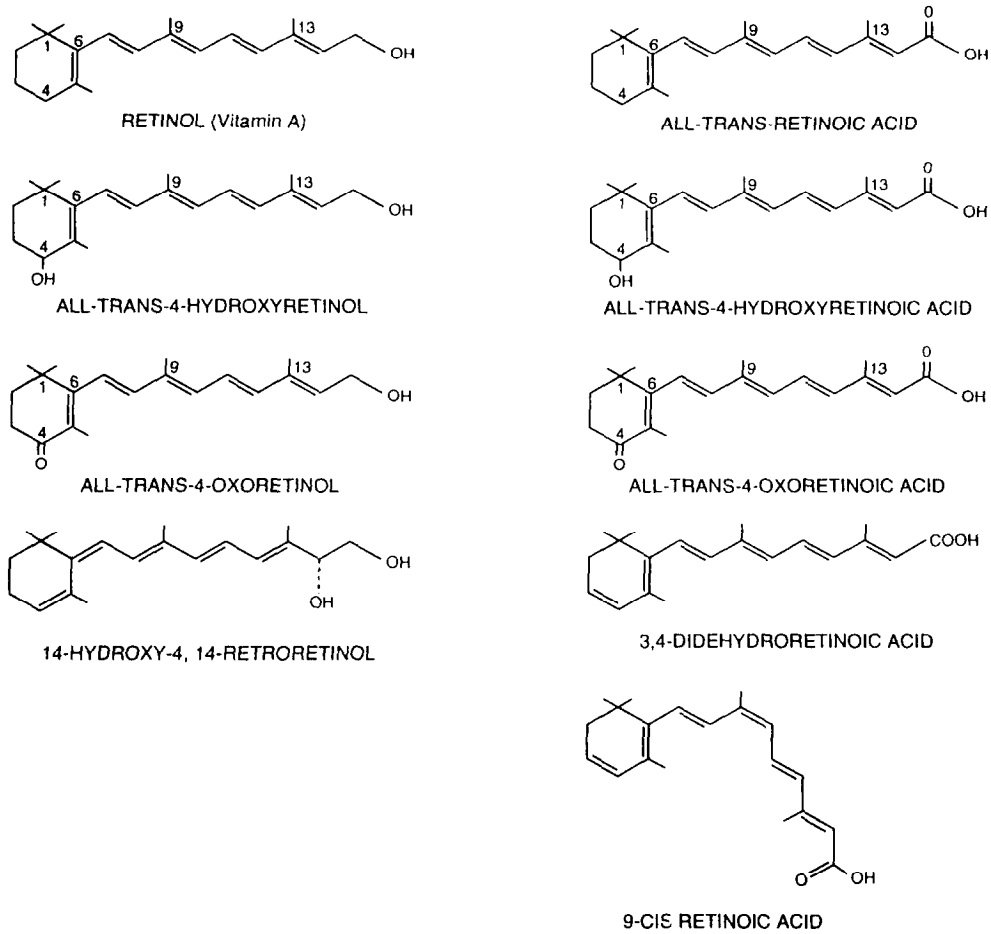

Figure 2 The structures of the most common natural retinoids are shown.

catalyze the oxidation of retinol to retinal, the rate-limiting step in the oxidative formation of RA.[2]

Recently, a new interesting autoregulative system has been described in human keratinocytes. In these cells RA-treatment causes both a strong induction of LRAT activity and a 50% reduction in the conversion of retinol to RA. It has been suggested that RA, through the activation of LRAT, sequesters retinol from the oxidative pathway leading to RA; in fact, when LRAT is inhibited by other agents such as the serine protease inhibitor PMSF or apo-CRBP,

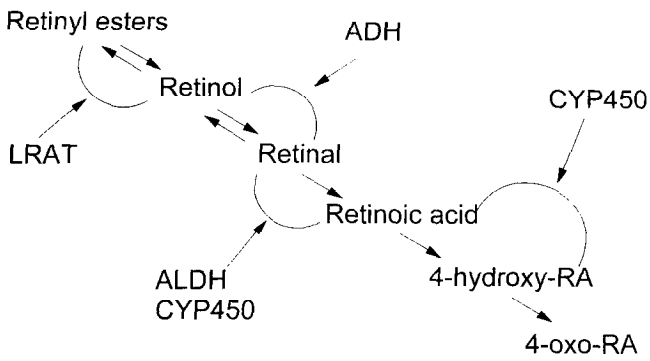

Figure 3 The metabolic flow from retinyl palmitate to retinoic acid and its oxidation products is shown. Note that the oxidation of retinal to retinoic acid and further oxidation reactions are irreversible.

RA synthesis rises to control levels, i.e., levels of RA in solvent-treated cells.[8]

As shown in *Figure 3*, RA can be further oxidized to 4-hydroxy and eventually 4-oxo-RA. These metabolites have been considered inactive forms of RA, but some recent evidence credits them with a pivotal role in development, growth and differentiation.[9,10] It is thought that they might act by binding to and activating RARs. In particular, a receptor specificity has been postulated for 4-oxo-RA, as it binds avidly to RARβ, but poorly to RARγ.[10,11]

Cytochrome P-450s are the enzymes responsible for the biosynthesis of these RA metabolites; several of these enzymes have been found to be able to oxidize at the carbon 4 position, not only RA, but also retinal and retinol.[12]

A new cytochrome (P450RAI) has been cloned recently[13] from regenerating zebrafish fins and shown to generate 4-hydroxy and 4-oxo-RA. P450RAI is not normally expressed, but it becomes expressed after wounding, in the process of regeneration, and the transcripts are specifically expressed deep in the epithelial layer at the distal tip of the blastemal mesenchyme.[13] The cloning of CYP-RAI is obviously an important development in our understanding of retinoid metabolism and function.

We have studied RA metabolism in 15 different cell lines from diverse sources.[14] Nine cell lines were growth inhibited by RA and quickly metabolized it to oxidation products. In sharp contrast, the 6 RA-resistant cell lines accu-

mulated as much as 20 fold more RA because of their inability to metabolize and/or use it.[14] These data would suggest that the RA-sensitive cells synthesize a metabolite that is responsible for the inhibition of cell growth. This possibility is presently being tested in our laboratory.

Recent studies have shown that 4-oxo-retinol (*Figure 2*), is a good activator of RARs and is able to induce differentiation of the embryonal carcinoma cells F-9.[15] Of interest are also the 4, 14 dihydroxy retinol compounds (*Figure 2*), which have been shown by Buck, Hammerling, and collaborators, to be essential for lymphocyte cell growth.[16]

Retinoids and epithelial differentiation

It has been known since 1925 that vitamin A deficiency leads to metaplastic changes in epithelial tissues,[17] similar to those caused by chemical carcinogens. Sporn and colleagues have used the hamster trachea organ culture system to grade the potency of retinoids in differentiation, using H and E staining to monitor epithelial changes.[9] Our laboratory has studied the dynamics of epithelial cell replacement and differentiation in hamster tracheal epithelium and in mouse endocervical epithelia undergoing squamous metaplasia as the result of nutritional vitamin A deficiency. We have defined stages during the formation of the preneoplastic lesion squamous metaplasia.[18,19] These stages are best observed in the simplest system, the endocervical epithelium of the mouse. This epithelium is of the simple-columnar type, so called because it is made up of one layer of columnar cells (*Figure 4*). These cells express keratin K8, cease to divide and are replaced by squamous K8-negative, but K5-positive cells, under conditions of vitamin A deficiency (*Figure 4*). Our work has shown that this replacement is gradual and proceeds through the following stages: formation of pseudostratified, stratified-squamous, and keratinized metaplastic lesions.[18,19] The characterization of these stages has been achieved through the use of specific keratin antibodies, which have permitted monitoring for the appearance of K5-positive basal cells in a loan of negative columnar cells (simple to pseudostratified transition); the formation of multiple K5-positive squamous cell layers (pseudostratified to squamous-stratified transition); and finally the formation of a keratinizing epithelium.[19,20] *Figure 4* indicates that vitamin A deficiency causes a simple-columnar epithelium to gradually become more complex through the acquisition of phenotypes normally present in other, more stratified epithelia: i.e., the simple epithelium in succession becomes pseudostratified (i.e., similar to the normal phenotype of trachea), squamous stratified (similar to buccal mucosa) and eventually keratinizing (similar to the epidermis).[19] These data suggest that homeostatic mechanisms dependent on the cellular millieu control the phenotypic expression of epithelial cells under normal conditions, and that vitamin A concentration may be an important factor in maintaining a simple or more stratified epithelial morphology. The concentration of retinoids and their second messengers in different epithelia, therefore, seem to be important determinants of differentiation.

Epithelial, cell type specific expression of retinoid receptor transcripts

We have reported a cell type-specific expression of the retinoid receptor transcripts.[21] Retinoic acid receptor RARβ transcripts were found specifically expressed in the columnar cells of the endocervix (*Figure 5*) and in the proliferating cells of the squamous metaplastic lesions (*Figure 4*); RARγ transcripts were absent from the normal columnar endocervical epithelium and is normally present in squamous-stratified epithelium of the ectocervix (*Figure 5*). It also is expressed in squamous cells of the metaplastic lesions of the endocervix (*Figure 4*); RARα transcripts seem ubiquitous, as expected of a house-keeping gene; retinoid-X-receptor RXRα and β transcript expression was very high in proliferating cells of the endocervix and squamous lesions (*Figure 4*).[21]

Retinoid-steroid interactions in cervical epithelial differentiation

Estrogen is involved in the differentiation of vaginal epithelial phenotypes and usually counteracts the action of retinoids, in that it causes the normally squamous, nonkeratinizing epithelium of the cervix and vagina to become keratinizing.[22] The interplay among steroid hormones, the thyroid hormone and the retinoids, and possibly other members of this superfamily, has been demonstrated with the discovery that heterodimers of their receptors are formed and can either activate or repress gene transcription. In particular, the 9-cis-retinoic acid receptor, RXR, has been shown to form heterodimers with a variety of other transcriptional activators, which bind to their specific response elements.[21,23]

The availability or deficiency of estrogen profoundly alters the phenotype of the cervical and vaginal epithelia. Ovariectomy caused the normally squamous stratified ectocervical epithelium to change into an epithelium with, at the most, three cell layers. The topmost cells were columnar and keratin K8-positive. The epithelium lacked keratin K1, normally occurring at the suprabasal level in squamous stratified ectocervix. After estrogen administration these K8-positive cells disappeared and the stratified morphology resumed with the appearance of keratin K1-positive cells in suprabasal position. Stimulation of RARγ, estrogen receptor and of the oncogene *fos* occurred 1 to 2 hr after estrogen.[24] Our most recent data (Kirkhof et al., in preparation) show that induction of vitamin A deficiency in ovariectomized mice takes longer than normal, suggesting an interaction between the steroid and the retinoid pathways in the maintenance of normal differentiation.

The two families of retinoid receptors

The cloning and characterization of the retinoid receptors represents a landmark in nutritional biochemistry and molecular biology. The finding was a direct consequence of basic work in the field of steroid and thyroid hormone receptors. All these receptors contain six different domains (A to F) with different functions as reviewed previously.[21] The C-domain is also known as the DNA-binding domain or

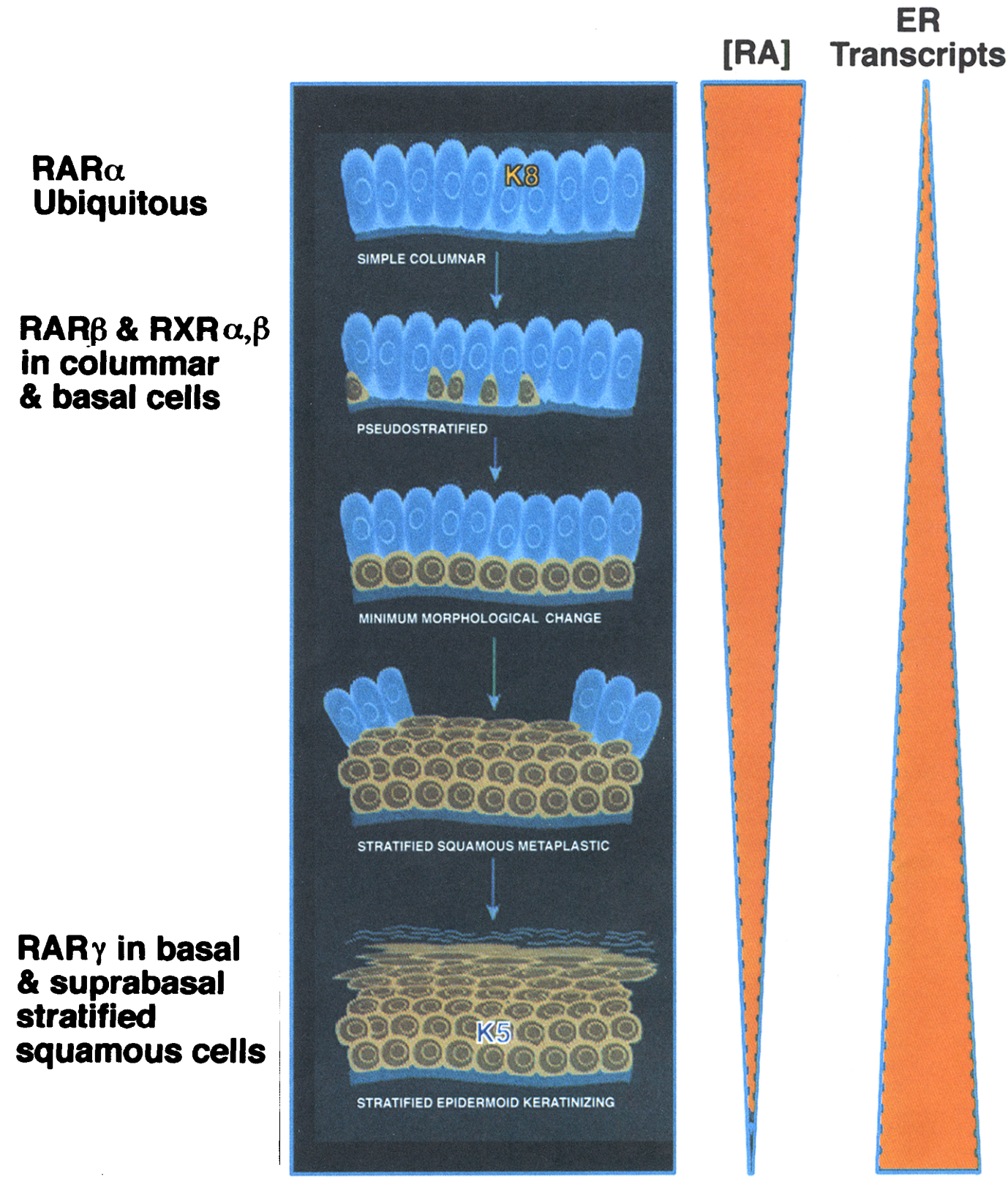

Figure 4 The figure shows the stepwise pathological process of squamous metaplasia formation in the simple-columnar epithelium of the endocervix. A single row of K8 positive columnar cells characterizes the simple-columnar epithelium of the endocervix, as it is under normal conditions of vitamin A nutriture. Under conditions of vitamin A deficiency, K5-positive basal cells appear in subcolumnar positions. Eventually a continuous layer of subcolumnar basal cells lifts the columnar cells, which cease to divide and are shed off, whereas the K5-positive cells proliferate and form a stratified squamous-metaplastic focus. Different foci merge into a stratified squamous keratinizing epithelium. These changes are reversible upon administration of vitamin A. Conversely, the stratified-squamous keratinizing epithelium of the ectocervix and vagina acquire characteristics of a simpler epithelium with K8-positive cells in ovariectomized mice, resembling the minimal morphological stage attributable to vitamin A deficiency in the endocervical epithelium. The figure also indicates changes in the expression of retinoid and estrogen receptor transcripts during the establishment of squamous metaplasia.

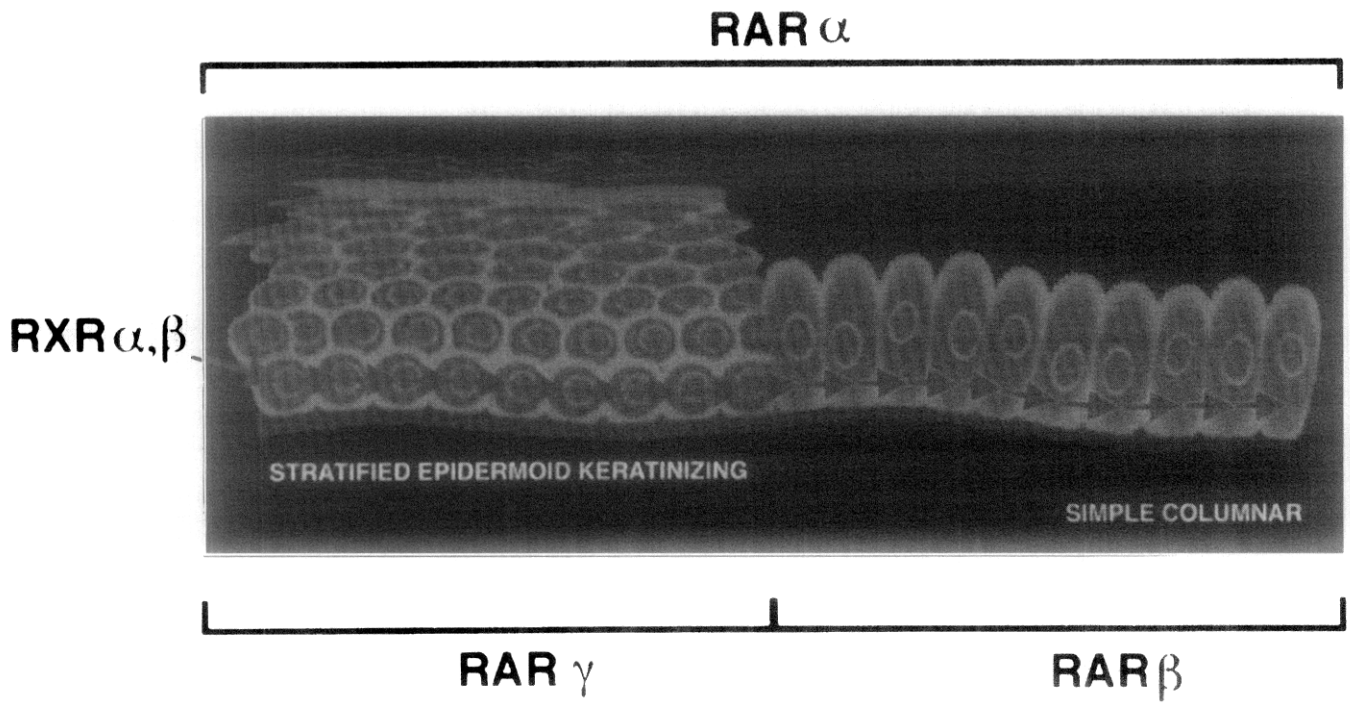

Figure 5 The figure shows the expression of retinoid receptor transcripts as studied by in situ hybridization. Schematics of the normal epithelial junction (squamo-columnar junction) of the cervix showing the cell type specific expression of retinoid receptor transcripts.

DBD and is very highly conserved between these different receptors so that a 24-nucleotide sequence shared by all the receptors can be used as a common probe to detect all the various receptors, including those whose ligands are unknown (orphan receptors). Retinoid receptor cloning was in fact possible because of the common 24-nucleotide probe used to identify new orphan receptors.[25,26]

The approach to demonstrating that the new orphan receptors were RA-dependent transcriptional activators required the molecular engineering of a chimeric receptor with the DNA binding domain of the estrogen receptor, because no RA-dependent genes were known.[25,26] Activation of ER-dependent reporter constructs was thus induced by RA binding to the RAR, which then bound to the ERE of the estrogen receptor. Soon, this and similar approaches permitted the characterization of three genes (α, β, and γ) for RARs and for RXRs.[27] The latter class of receptors specifically bind 9-cis-RA[27,28] and RARs, on the other hand, bind both RA and 9-cis-RA.[23]

Genes containing RAREs in their promoters are known to be involved in diverse and yet interconnected biological processes, such as embryogenesis, growth and differentiation.[23,29,30] The complexity of interactions becomes greater, if one considers that RXRs, in addition to forming homodimers,[27] are also able to form heterodimers with other receptors,[27,31–33] including RARs, the thyroid hormone receptor, TR, the 1,25-dihydroxy-vitamin D3 receptor VDR,[31] the peroxisomal proliferator activated receptor, PPAR,[34–36] and other members. Complexity increases if one also considers that there are some 18 isoforms of the RARs and probably just as many RXRs through the use of different promoters or alternative splicing.[37]

In addition to the activation function AF-1 in the amino terminal A/B regions of the receptors, the use of various reporter gene assays has made it possible to identify a transcriptional activation function (AF-2), which overlaps the ligand binding domain (LBD) in the E-region of the RARs and RXRs. RAR AF-2s are activated similarly by all-*trans* and 9-cis-RA and their 3,4-didehydroderivatives (*Figure 1*), whereas RXR AF-2s are only efficiently activated by 9-*cis* RA and by 9-*cis*-3,4-didehydroRA (Ref. 37 and references therein).

A third level of complexity accrues from interactive elements on different promoters. These may differ for the same receptor heterodimer and for different heterodimers. For instance, response elements containing direct repeats (DR) of the canonical sequence AGGTCA with a spacer of two nucleotides (DR2) or five (DR5) are known to occur in the gene for the homeobox b-1[38,39] and in the RARβ2 gene. In addition, the actual sequence of the direct repeats and the types of flanking bases seem to be important determinants for RAR- and RXR-binding efficiencies.[40] Moreover, interactions with other proteins, which may either function as transcriptional repressors[41–44] or ligand-dependent activators,[45–47] such as the cyclic AMP response element binding protein (CBP), have also been reported and may add another level of complexity and permit a variety of interactive pathway to specific gene expression regulation.

Speculations on orders of receptor interactions

These considerations suggest different orders of interactions that may lead to diverse biological results. We suggest that effects of interactions between RXR-RAR heterodimers and

Table 1 The table shows natural retinoic acid response elements (RAREs) from mouse and human RARα2[82,83] and RARβ2[84-86] promoters, human RARγ2 promoter,[83] the human alcohol dehydrogenase 3 promoter (ADH3),[87] the mouse cellular retinol binding protein I (mCRBPI),[88] laminin BI (mLB1),[89,90] the human apolipoprotein AI promoter,[91] the complement factor H (mCP-H)[92] promoter, the rat cellular retinol binding protein II (rCRBPII) promoter,[93] the putative RARE from the promoter of the human medium chain acyl-coenzyme A dehydrogenase (hMCAD),[94] composite element containing an RARE and ERE from the human lactoferrin promoter,[95] two RAREs from the mouse cellular retinoic acid binding protein II (mCRABPII)[96] promoter and RARE from the rat phosphoenol pyruvate carboxykinase (rPEPCK)[97] and the mouse transglutaminase type II promoter (mTGRRE1),[98] which contains three hexad repeats (DR5 and DR7 motif) capable to bing both RXR homodimers as well as RXR-RAR heterodimers, are shown. Repeated hexad motifs in the 3′ enhancer of the mouse Hox1.6 (mHox1.6),[99] the mouse Hoxb-1[38,39] and the mouse Hoxb-1 5′ repressor[38,39] are also shown. The repeated hexad motifs, identified as the specific response elements[40] are highlighted.

Natural retinoic acid response elements (RAREs)	
mRARα2	5′-(−59)GGCG**AGTTCA**GCAAG**AGTTCA**GCCGA(−34)-3′ (82)
hRARα2	5′-(−58)GGCG**AGTTCA**GCGAG**AGTTCA**GCCGC(−33)-3′ (83)
mRARβ2	5′-(−57)GAAG**GGTTCA**CCGAA**AGTTCA**CTCGC(−32)-3′ (84)
hRARβ2	5′-(−57)GTAG**GGTTCA**CCGAA**AGTTCA**CTCGC(−32)-3′ (85,86)
hRARγ2	5′-(401)GGCC**GGGTCA**GGAGG**AGGTGA**GCGCGC(−375)-3′ (83)
hADH3	5′-(−280)ACAG**GGGTCA**TTCAG**AGTTCA**GTTTT(−305)-3′ (87)
mCRBPI	5′-(−1015)TAGT**AGGTCA**═AA**AGGTCA**GACAC(−993)-3′ (88)
mLamBI	5′-(−432)═G**AGGTGA**GCT═A**GGTTAA**GCCCTTAGAA
	AAA**GGGTCAA**(−468)-3′ (89,90)
hapoAI	5′-(−192)**AGGGCAGGGGTCA**═AG**GGTTCA**GTGGG(−217)-3′ (91)
mCP-H	5′-(−147)CAGC**AGGTCA**CTGAC**AGGGCA**TAGTA(−122)-3′ (92)
rCRBPII	5′-(−639)GCT**GTCACAGGTCAC**═**AGGTCAC**
	AGGTCAC═**AGTTCA**(−605)-3′ (93)
hMCAD	5′-(−341)GGGTTTGACCTTTCTCTCC**GGGTAAAGGTGA**AGG(−308)-3′
	3′-CCCAA**ACTGGA**AAGAGAGGCCCATTTCCACTTCC-5′ (94)
hLactoferrin	5′-(−351)AA**GTGTCACAGGTCA**(RXRE)AGGTAACCCAC(−326) (95)
Composite	TTCACAGTGTCC(ERE)**AGTTCCATTGGG**TG
RARE/ERE	
mCRABPII	
RARE1:	5′-(−1162)CCCC**AGTTCA**CC═**AGGTCA**GGGCT(−1140)-3′ (96)
RARE2:	5′-(−657)CTG**TGACCT**C═**TGCCCT**TCT(−639)-3′ (96)
rPEPCK	5′-(−451)**TGACCT**TTGGCCGTGGGA(−434)-3′ (97)
mTGase (mTGRRE1)	5′-(−1703)CATGG**GGGTCA**CTGTGAG**AGGTCC**CAG
	TG**GGGTCA**GGATTA(−1751)-3′ antisense (98)
mHox1.6	5′-(Sac-74)CA**GGTTCA**CCGAA**AGTTCA**AG(Sac-55)-3′ (99)
3′ enhancer	
mHoxb-1	5′-CTTAG**AGGTAA**AA**AGGTCA**GCCCAG-3′ (38,39)
3′ enhancer	
RARE	
mHoxb-1	5′-**AGGGCA**AG**AGTTCA**-3′ (38,39)
5′ repressor	
RARE	
Consensus	G T A T 5′- G TCA (X) G TCA-3′ A G G G 1 2 3 4 5 6 1 2 3 4 5 6

target gene promoters are the most immediate and would be the first to respond to retinoid depletion or excess. These reactions would acquire the characteristic acute response, if the RARE is located in the promoter of the receptor gene itself. This is the case for the RARβ2 gene, which is therefore a "first order dependence gene." Other first-order dependence genes would be the RARγ2 and α2 and all the genes that contain a RARE in their promoters. Some of these are listed in *Table 1*. First order dependence would also be observed with ligands activating RXR-RXR homodimers, such as for 9-cis-RA in the activation of the CRBPII gene (*Table 1*).

RXR cognate receptors other than RARs, such as VDR, TR,[33,48-50] COUP-TF,[51] and PPAR[36] would mediate second-order types of retinoid responses, in that their responses, though possibly dependent on 9-*cis*-RA, are also dependent on other hormonal ligands such as vitamin D3, thyroxin,[31] as well as xenobiotic agents.

We suggest that genes belonging to the second-order dependence are probably much more numerous than those of first-order dependence. These genes control most gene activation processes dependent on thyroid hormone, vitamin D, and other important hormonal and xenobiotic ligands.

We call third-order dependence genes all those genes that would be activated as the result of secondary transcriptional events. An example of this type of dependence is genes that depend on AP-1 complexes. It is well known that transcriptional transrepression occurs at the AP-1 site concomitant with RA mediated gene activation on various RAREs, especially when RA is in excess. Genes containing TPA-responsive elements (TREs), such as the stromelysin or the collagenase genes, are negatively regulated by RA. This negative regulation was thought to be attributable to binding of the AP-1 Jun-Fos protein to the RARs, thus removing them from interactions with the TRE. More recent results indicate that *trans*-repression is the result of limiting concentrations of the (cyclic-AMP response element binding protein) CREB-binding protein (CBP). This protein

apparently binds with high affinity to CREB as well as to RARs, ERs, and possibly a variety of other receptors.[45] Whereas the binding and transcriptional activation by the CREB homodimer is independent of ligands, CBP binding to RARs and other hormone receptors and consequent transcriptional activation is ligand dependent.[45] Because CBP is present in limiting amounts, AP-1 transrepression normally occurs when RAR or ER concentrations are increased. When CBP is nonlimiting, transrepression is not observed. Therefore, the concentration of CBP and of other ligand-dependent coactivators, as well as that of corepressors, add another level of control to transcription of the various genes. Recent reviews on the important aspects of structural configuration and space filling models[27] of the retinoid receptors and on the genetic knockout[52] of their genes have recently been published and the reader is referred to those reviews for these subjects.

Mutants of RARs and their biological consequences

Acute promyelocytic leukemia (APL) is a rare leukemia that is characterized by accumulation of immature cells, the promyelocytes, in the blood of the patient. Normally these cells do not accumulate because the normal process of differentiation would make them become granulocytes, the end cell differentiated phenotype in this lineage. Approaches to reestablish normal differentiation by inducing the promyelocyte to granulocyte pathway would result in remission from the disease through what is known as "differentiation therapy."

Blood cells from patients with APL contain the APL-RARα transcript and protein.[53] This transcript comes from a gene that is the fusion product resulting from the balanced translocation of the long arm of chromosome 17 and chromosome 15, t(15;17).[54] Other translocations involving chromosome 17 are also known to occur, however, by far the most common and studied translocation is the t15;17 translocation.[55,56] The presence of PML-RARα is a reliable marker of the disorder.[54,57–59] Even though RAR is fused to PML in PML-RAR, APL is highly responsive to RA differentiation therapy.[58–60] This is probably because RA may use other receptors for its function. The other possibility is that RA may still work through PML-RAR.[61] This fusion protein is in fact transcriptionally active, albeit under in vitro conditions in assays based on reporter gene activation.[62] Transcriptional activation assays are highly dependent on newly discovered factors such as CBP, therefore investigations to determine whether PML-RAR is equally active as RAR under optimal conditions, i.e., with CBP also present are very important.

Even though PML-RAR transcripts are used as a diagnostic tool for APL, their presence and the presence of the t(15;17) translocation does not establish that PML-RAR is the causative agent of APL. Recent work by Pandolfi and collaborators (personal communication) has demonstrated that transgenic overexpression of PML-RAR causes the development of APL in mice. Moreover, mouse genetic knockout of the normal PML gene is associated with splenomegaly and other lymphoid organ disorders (Pandolfi, personal communication).

Chemoprevention of skin carcinogenesis by dietary retinoic acid

The skin carcinogenesis system[63] has been used in studies of chemoprevention by retinoids, because it offers the advantage of observing the neoplastic lesions without having to kill the animal. The process of skin carcinogenesis has been divided in three stages:[64] initiatiation, promotion (which gives rise to benign tumors, papillomas), and malignant conversion (which generates carcinomas from the benign tumors). In the relatively large literature on retinoids and skin carcinogenesis,[63] our studies are among the very few to use dietary, rather than topical retinoids, and a semipurified diet supplemented with different levels of retinoic acid.

Dietary retinoic acid inhibits malignant conversion

We conducted a series of experiments using a purified vitamin A-free diet supplemented with either low (0.3 μg/gm diet; 0.3RA diet); intermediate (3 μg/gm diet; 3RA diet) or pharmacological (30 μg/gm diet; 30RA diet) doses of RA. Dietary retinoic acid at pharmacological concentrations (30RA diet) has little effect on the formation of papillomas (low-risk tumors), but specifically inhibits carcinoma incidence in the two-stage (DMBA, TPA) system of skin carcinogenesis in female SENCAR mice,[63,65–67] independent of age at initiation.[68] The 30RA diet protected against mezerein-induced high-risk papillomas and against carcinomas,[69] but had no effect on papilloma and carcinoma formation by the complete carcinogenesis protocol, which uses weekly dorsal applications of DMBA, as both the initiator and the tumor promoter.[63,66,70] The 30RA diet retains the inhibitory activity on malignant conversion in the DMBA-TPA system, even if given from week 20, i.e., the time of maximum papilloma formation. This experiment excludes the possibility that RA acts via a direct effect on initiation in this system, because the DMBA dose was given 17 weeks before switching the diet from 3 to 30RA.[63]

Downregulation of RARs during skin carcinogenesis

We have defined the cellular localization of several of RAR (α1 and γ1) and RXR (α, β) transcripts in normal skin and in chemically-induced mouse skin tumors at several stages of skin tumor progression. A key finding is that RXRα transcripts, unlike RARs, are mainly compartmentalized in the basal cell layers and concentrated in hair follicles in normal skin and are abundant in skin malignancies.[71] RXRβ transcripts are more widespread in skin malignancies, whereas RARα1 and RARγ1 are decreased relative to normal skin. In normal skin, RXRα transcripts are mainly localized in the rapidly proliferating basal cells and are concentrated in the outer root sheath and matrix cells of hair follicles. Skin tumor progression results in increased levels of RXR and reduced levels of RAR transcripts, which could result in lower levels of RXR/RAR heterodimers. Spindle cell carcinomas, which have lost the epithelial phenotype

and most epithelial markers, namely keratins as well as the RARs, still retain the RXRs.[71] Therefore, malignant progression in the two-stage system reduced RARα and γ expression, whereas RXRα and β expression remained high in proliferating cells.[71]

Retinoic acid receptors are altered during premalignant progression and malignant conversion

Current studies indicate that RARα protein is reduced in high-risk papillomas, relative to low-risk papillomas, and the promoting agent mezerein, that induces high-risk papillomas, causes a substantial reduction in RARα in nontumorous skin. Transducing cultured normal keratinocytes with v-*ras*^{Ha} reduces RARα and γ proteins in nuclear extracts and decreases RAR mediated transcriptional activity.[72] Introduction of a recombinant RARα expression vector into papilloma cells inhibits growth in response to retinoic acid.[72] Modulation of RARs could contribute to the neoplastic phenotype directly or indirectly through interaction with AP-1 and may be involved in the differential promoting activity of mezerein and TPA, influencing the selection of tumors at high risk for malignant conversion.

Conclusive remarks

Progress in the field of vitamin A research has been remarkable. Who could have foreseen in the 1960s that retinoic acid would be used as a routine form of treatment for acute promyelocytic leukemia patients? And who could have imagined the complexity of receptor interactions connecting thyroid hormone, vitamin D, and xenobiotics with retinoid homeostasis? It is these interactions that make vitamin A essential for many biological processes, including embryogenesis and the maintenance of epithelial differentiation in the adult organism. Recently Tontonoz et al.[73] have shown that PPARγ plays a central role in adipogenesis and Lehmann et al.[74] have shown that the antidiabetic agents thiazolidinediones are high-affinity ligands for this receptor. This latter observation led to the discovery of 15-dioxy-$\delta^{12,14}$-prostaglandin J_2 as a natural PPARγ ligand.[75,76] These data illustrate yet another unexpected connection, that between prostanoids, adipogenesis, glucose homeostasis,[27] and their dependence on an RXR pathway of gene activation.

For those of us who have been in this field long enough, it is to be expected that additional and perhaps even more exciting developments will follow. In particular, it is conceivable that rearrangements similar to the PML-RARα, involving a retinoid receptor, might explain additional diseases resulting in loss of growth control and altered differentiation, consistent with the suggestion that retinoid receptors may function as tumor suppressor genes.[77]

A related area of interest is the connection between metabolism and control of cell growth. This connection was made in our laboratory, with the realization that only cell lines able to metabolize RA at a fast rate were also growth-inhibited.[14] This would suggest the synthesis of a RA metabolite responsible for growth inhibition. Metabolic activation may also occur through the formation of covalent derivatives of RA or its metabolites with various macromolecules. This area of research is also growing and may explain some of the less well understood effects of retinoids.[78]

Another area of interest is the use of retinoids as chemopreventive agents, particularly in populations at high risk for cancer development. Retinoids have been shown to be very effective inhibitors of skin,[79] head and neck,[80] and liver[81] carcinogenesis in humans and the list of applications of these compounds as chemopreventive agents will probably grow in the near future.

We conclude this review with the hope that it will provide incentive to those students who are considering nutritional biochemistry as a field for their professional life. We are optimistic that nutritional biochemistry will grow considerably in the next decades and that similar interest will continue to develop for other essential nutrients and dietary factors as it has for vitamin A.

References

1 Blaner, W.S. and Olson, J.A. (1994). Retinol and retinoic acid metabolism. In *The Retinoids* (M.B. Sporn, A.B. Roberts, and D.S. Goodman, eds.), pp. 229–255, Raven Press, New York, NY USA

2 Napoli, J.L. (1996). Retinoic acid biosynthesis and metabolism. *FASEB J.* **10,** 993–1001

3 Blomhoff, R., Green, M.H., Berg, T., and Norum, K.R. (1990). Transport and storage of vitamin A. *Science* **250,** 399–404

4 Blomhoff, R., Green, M.H., Green, J.B., Berg, T., and Norum, K.R. (1991). Vitamin A metabolism: new perspectives on absorption, transport, and storage. *Physiol. Rev.* **71,** 951–990

5 MacDonald, P.N. and Ong, D.E. (1988). Evidence for a lecithin-retinol acyltransferase activity in the rat small intestine. *J. Biol. Chem.* **263,** 12478–12482

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

7 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

8 Kurlandsky, S.B., Duell, E.A., Kang, S., Voorhees, J.J., and Fisher, G.J. (1996). Auto-regulation of retinoic acid biosynthesis through regulation of retinol esterification in human keratinocytes. *J. Biol. Chem.* **271,** 15346–15352

9 Sporn, M.B. and Newton, D.L. (1979). Chemoprevention of cancer with retinoids. *Fed. Proc.* **38,** 2528–2534

10 Pijnappel, W.W., Hendriks, H.F., Folkers, G.E., van den Brink, C.E., Dekker, E.J., Edelenbosch, C., van der Saag, P.T., and Durston, A.J. (1993). The retinoid ligand 4-oxo-retinoic acid is a highly active modulator of positional specification. *Nature* **366,** 340–344

11 Reddy, A.P., Chen, J.Y., Zacharewski, T., Gronemeyer, H., Voorhees, J.J., and Fisher, G.J. (1992). Characterization and purification of human retinoic acid receptor-gamma I overexpressed in the baculovirus-insect cell system. *Biochem. J.* **287,** 833–840

12 Raner, G.M., Vaz, A.D., and Coon, M.J. (1996). Metabolism of *all-trans*, 9-*cis*, and 13-*cis* isomers of retinal by purified isozymes of microsomal cytochrome P450 and mechanism-based inhibition of retinoid oxidation by citral. *Mol. Pharmacol.* **49,** 515–522

13 White, J.A., Guo, Y.D., Baetz, K., Beckett-Jones, B., Bonasoro, J., Hsu, K.E., Dilworth, F.J., Jones, G., and Petkovich, M. (1996). Identification of the retinoic acid-inducible all-*trans*-retinoic acid 4-hydroxylase. *J. Biol. Chem.* **271,** 29922–29927

14 Takatsuka, J., Takahashi, N., and De Luca, L.M. (1996). Retinoic acid metabolism and inhibition of cell proliferation: an unexpected liaison. *Cancer Res.* **56,** 675–678

15 Achkar, C.C., Derguini, F., Blumberg, B., Langston, A., Levin, A.A., Speck, J., Evans, R.M., Bolado, J., Jr., Nakanishi, K., and Buck, J. (1996). 4-Oxoretinol, a new natural ligand and transactivator of the retinoic acid receptors. *Proc. Natl. Acad. Sci. USA* **93,** 4879–4884

16 Buck, J., Derguini, F., Levi, E., Nakanishi, K., and Hammerling, U.

(1991). Intracellular signaling by 14-hydroxy-4,14-retro retinol. *Science* **254,** 1654–1656
17 Wolbach, S.B. and Howe, P.R. (1925). Tissue changes following deprivation of fat-soluble A-vitamin. *J. Exp. Med.* **42,** 753–778
18 Rosenthal, D., Lancillotti, F., Darwiche, N., Sinha, R., and De Luca, L.M. (1994). Retinoids in epithelial differentiation and tumorigenesis. In *Vitamin A in Health and Disease* (R. Blomhoff, ed.), pp. 425–450, Marcel Dekker, New York, NY USA
19 De Luca, L.M., Darwiche, N., Jones, C.S., and Scita, G. (1995). Retinoids in differentiation and neoplasia. *Scient. Am. Sci. Med.* **2,** 28–37
20 Darwiche, N. and De Luca, L.M. (1995). Retinoids in differentiation and prevention of malignant transformation. In *Intraepithelial Neoplasia of the Female Lower Genital Tract* (J. Jordan, D. Luesley, and R. Richart, eds.), pp. 309–324, Churchill Livingstone, London, UK
21 Darwiche, N., Celli, G., and De Luca, L.M. (1994). Specificity of retinoid receptor gene expression in mouse cervical epithelia. *Endocrinology* **134,** 2018–2025
22 Jetten, A.M., De Luca, L.M., Nelson, K., Schroeder, W., Burlingame, S., and Fujimoto, W. (1996). Regulation of cornifin α expression in the vaginal and uterine epithelium by estrogen and retinoic acid. *Mol. Cell Endocrinol.* **123,** 7–15
23 De Luca, L.M. (1991). Retinoids and their receptors in differentiation, embryogenesis and neoplasia. *FASEB J.* **5,** 2924–2933
24 Celli, G., Darwiche, N., and De Luca, L.M. (1996). Estrogen induces retinoid receptor expression in mouse cervical epithelia. *Exp. Cell Res.* **226,** 273–282
25 Petkovich, M., Brand, N.J., Krust, A., and Chambon, P. (1987). A human retinoic acid receptor which belongs to the family of nuclear receptors. *Nature* **330,** 444–450
26 Giguere, V., Ong, E.S., Segui, P., and Evans, R.M. (1987). Identification of a receptor for the morphogen retinoic acid. *Nature* **330,** 624–629
27 Mangelsdorf, D.J. and Evans, R.M. (1995). The RXR heterodimers and orphan receptors. *Cell* **83,** 841–850
28 Levin, A.A., Sturzenbecker, L.J., Kazmer, S., Bosakowski, T., Huselton, C., Allenby, G., Speck, J., Kratzeisen, G., Rosenberger, M., Lovey, A., and Grippo, J.F. (1992). 9-cis Retinoic acid stereoisomer binds and activates the nuclear receptor RXRα. *Nature* **355,** 359–361
29 Mangelsdorf, D.J., Umesono, K., and Evans, R.M. (1996). In *The Retinoids: Biology, Chemistry* (M.B. Sporn, A.B. Robert, and D.S. Goodman, eds.), pp. 319–349, Raven Press, New York, NY USA
30 Mangelsdorf, D.J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., and Chambon, P. (1995). The nuclear receptor superfamily: the second decade. *Cell* **83,** 835–839
31 Yu, V.C., Delsert, C., Andersen, B., Holloway, J.M., Devary, O.V., Naar, A., Kim, M., Boutin, S.Y., Glass, C.K., and Rosenfeld, M.G. (1991). RXRβ: a coregulator that enhances binding of retinoic acid, thyroid hormone, and vitamin D receptors to their cognate response elements. *Cell* **67,** 1251–1266
32 Leid, M., Kastner, P., Lyons, R., Nakshatri, H., Saunders, M., Zacharewski, T., Chen, J., Staub, A., Garnier, J., Mader, S., and Chambon, P. (1992). Purification, cloning, and RXR identity of the HeLa cell factor with which RAR or TR heterodimerizes to bind target sequences efficiently. *Cell* **68,** 377–395
33 Marks, M.S., Hallenbeck, P.L., Nagata, T., Segars, J.H., Appella, E., Nikodem, V.M., and Ozato, K. (1992). H-2RIIBP (RXRβ) heterodimerization provides a mechanism for combinatorial diversity in the regulation of retinoic acid and thyroid hormone responsive genes. *EMBO J.* **11,** 1419–1435
34 Kliewer, S.A., Umesono, K., Evans, R., and Mangelsdorf, D.J. (1994). The retinoid X receptors: modulators of multiple hormonal signaling pathways. In *Vitamin A in Health and Disease* (R. Blomhoff, ed.), pp. 239–255, Marcel Dekker, New York, NY USA
35 Kliewer, S.A., Umesono, K., Noonan, D.J., Heyman, R.A., and Evans, R.M. (1992). Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. *Nature* **358,** 771–774
36 Tugwood, J.D., Issemann, I., Anderson, R.G., Bundell, K.R., McPheat, W.L., and Green, S. (1992). The mouse peroxisome proliferator activated receptor recognizes a response element in the 5′ flanking sequence of the rat acyl CoA oxidase gene. *EMBO J.* **11,** 433–439
37 Leid, M., Kastner, P., and Chambon, P. (1992). Multiplicity generates diversity in the retinoic acid signalling pathways. *TIBS* **17,** 427–433
38 Marshall, H., Studer, M., Popperl, H., Aparicio, S., Kuroiwa, A., Brenner, S., and Krumlauf, R. (1994). A conserved retinoic acid response element required for early expression of the homeobox gene Hoxb-1. *Nature* **370,** 567–571
39 Studer, M., Popperl, H., Marshall, H., Kuroiwa, A., and Krumlauf, R. (1994). Role of a conserved retinoic acid response element in rhombomere restriction of *Hoxb-1*. *Science* **265,** 1728–1732
40 Mader, S., Leroy, P., Chen, J.Y., and Chambon, P. (1993). Multiple parameters control the selectivity of nuclear receptors for their response elements. Selectivity and promiscuity in response element recognition by retinoic acid receptors and retinoid X receptors. *J. Biol. Chem.* **268,** 591–600
41 Glass, C.K. (1996). Some new twists in the regulation of gene expression by thyroid hormone and retinoic acid receptors. *J. Endocrinol.* **150,** 349–357
42 Zamir, I., Harding, H.P., Atkins, G.B., Horlein, A., Glass, C.K., Rosenfeld, M.G., and Lazar, M.A. (1996). A nuclear hormone receptor corepressor mediates transcriptional silencing by receptors with distinct repression domains. *Mol. Cell Biol.* **16,** 5458–5465
43 Kurokawa, R., Soderstrom, M., Horlein, A., Halachmi, S., Brown, M., Rosenfeld, M.G., and Glass, C.K. (1995). Polarity-specific activities of retinoic acid receptors determined by a co-repressor. *Nature* **377,** 451–454
44 Horlein, A.J., Naar, A.M., Heinzel, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamei, Y., Soderstrom, M., and Glass, C.K. (1995). Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* **377,** 397–404
45 Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa,R., Gloss, B., Lin, S.C., Heyman, R.A., Rose, D.W., Glass, C.K., and Rosenfeld, M.G. (1996). A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell* **85,** 403–414
46 Chakravarti, D., LaMorte, V.J., Nelson, M.C., Nakajima, T., Schulman, I.G., Juguilon, H., Montminy, M., and Evans, R.M. (1996). Role of CBP/P300 in nuclear receptor signalling. *Nature* **383,** 99–103
47 Chen, J.D., Umesono, K., and Evans, R.M. (1996). SMRT isoforms mediate repression and anti-repression of nuclear receptor heterodimers. *Proc. Natl. Acad. Sci. USA* **93,** 7567–7571
48 Kliewer, S.A., Umesono, K., Mangelsdorf, D.J., and Evans, R.M. (1992). Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D_3 signalling. *Nature* **355,** 446–449
49 Zhang, X., Hoffmann, B., Tran, P.B.V., Graupner, G., and Pfahl, M. (1992). Retinoid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors. *Nature* **355,** 441–446
50 Bugge, T.H., Pohl, J., Lonnoy, O., and Stunnenberg, H.G. (1992). RXRα, a promiscuous partner of retinoic acid and thyroid hormone receptors. *EMBO J.* **11,** 1409–1418
51 Kliewer, S.A., Umesono, K., Heyman, R.A., Mangelsdorf, D.J., Dyck, J.A., and Evans, R.M. (1992). Retinoid X receptor-COUP-TF interactions modulate retinoic acid signaling. *Proc. Natl. Acad. Sci. USA* **89,** 1448–1452
52 Kastner, P., Mark, M., and Chambon, P. (1995). Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life? *Cell* **83,** 859–869
53 Dyck, J.A., Warrell, R.P., Jr., Evans, R.M., and Miller, W.H., Jr. (1995). Rapid diagnosis of acute promyelocytic leukemia by immunohistochemical localization of PML/RAR-alpha protein. *Blood* **86,** 862–867
54 Warrell, R.P., Jr. (1996). Pathogenesis and management of acute promyelocytic leukemia. *Annu. Rev. Med.* **47,** 555–565
55 Kakizuka, A., Miller, W.H., Jr., Umesono, K., Warrell, R.P., Jr., Frankel, S.R., Murty, V.V., Dmitrovsky, E., and Evans, R.M. (1991). Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RAR alpha with a novel putative transcription factor, PML. *Cell* **66,** 663–674
56 de The, H., Chomienne, C., Lanotte, M., Degos, L., and Dejean, A.

(1990). The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor alpha gene to a novel transcribed locus. *Nature* **347,** 558–561

57 Cornic, M., Delva, L., Castaigne, S., Lefebvre, P., Balitrand, N., Degos, L., and Chomienne, C. (1994). *In vitro* all-*trans* retinoic acid (ATRA) sensitivity and cellular retinoic acid binding protein (CRABP) levels in relapse leukemic cells after remission induction by ATRA in acute promyelocytic leukemia. *Leukemia* **8,** 914–917

58 Degos, L., Chomienne, C., Ballerini, P., Huang, M.E., Daniel, M.T., Berger, R., and Castaigne, S. (1990). All-trans retinoic acid: a novel differentiation therapy for acute promyelocytic leukemia. *Proc. Am. Soc. Clin. Oncol.* **9,** 207

59 Alcalay, M., Zangrilli, D., Fagioli, M., Pandolfi, P.P., Mencarelli, A., Lo Coco, F., Biondi, A., Grignani, F., and Pelicci, P.G. (1992). Expression pattern of the RARα-PML fusion gene in acute promyelocytic leukemia. *Proc. Natl. Acad. Sci. USA* **89,** 4840–4844

60 Vyas, R.C., Frankel, S.R., Agbor, P., Miller, W.H., Jr., Warrell, R.P., Jr., and Hittelman, W.N. (1996). Probing the pathobiology of response to all-trans retinoic acid in acute promyelocytic leukemia: premature chromosome condensation/fluorescence in situ hybridization analysis. *Blood* **87,** 218–226

61 Raelson, J.V., Nervi, C., Rosenauer, A., Benedetti, L., Monczak, Y., Pearson, M., Pelicci, P.G., and Miller, W.H., Jr. (1996). The PML/RAR alpha oncoprotein is a direct molecular target of retinoic acid in acute promyelocytic leukemia cells. *Blood* **88,** 2826–2832

62 Fagioli, M., Grignani, F., Ferrucci, P.F., Alcalay, M., Mencarelli, A., Nicoletti, I., and Pelicci, P.G. (1994). Effect of the acute promyelocytic leukemia PML/RAR alpha protein on differentiation and survival of myeloid precursors. *Leukemia* **8(Suppl 1),** S7–11

63 Chen, L.C. and De Luca, L.M. (1995). Retinoids and skin cancer. In *Skin Cancer: Mechanisms and Human Relevance* (H. Mukhtar, ed.), pp. 401–424, CRC Press Inc, Boca Raton, FL USA

64 Yuspa, S.H., Dlugosz, A.A., Denning, M.F., and Glick, A.B. (1996). Multistage carcinogenesis in the skin. *J. Invest. Dermatol.* **1,** 147–150

65 De Luca, L.M., Sly, L., Jones, C.S., and Chen, L.C. (1993). Effects of dietary retinoic acid on skin papilloma and carcinoma formation in female SENCAR mice. *Carcinogenesis* **14,** 539–542

66 Chen, L.C., Kirchhof, S., and De Luca, L.M. (1994). Effect of excess dietary retinoic acid on skin papilloma and carcinoma formation induced by a complete carcinogenesis protocol in SENCAR mice. *Cancer Lett.* **78,** 63–67

67 Chen, L.C., Sly, L., and De Luca, L.M. (1994). High dietary retinoic acid prevents malignant conversion of skin papillomas induced by a two-stage carcinogenesis protocol in female SENCAR mice. *Carcinogenesis* **15,** 2383–2386

68 De Luca, L.M., Tarone, R., Huynh, M., Jones, C.S., and Chen, L.C. (1996). Dietary retinoic acid inhibits mouse skin carcinogenesis irrespective of age at initiation. *Nutr. Cancer* **25,** 249–257

69 Chen, L.C., Tarone, R., Huynh, M., and De Luca, L.M. (1995). High dietary retinoic acid inhibits tumor promotion and malignant conversion in a two-stage skin carcinogenesis protocol using 7,12-dimethylbenz(a)anthracene as the initiator and mezerein as the tumor promoter in female SENCAR mice. *Cancer Lett.* **95,** 113–118

70 Chen, L.C., Sly, L., Jones, C.S., and De Luca, L.M. (1993). Differential effects of dietary β-carotene on papilloma and carcinoma formation induced by an initiation-promotion protocol in SENCAR mouse skin. *Carcinogenesis* **14,** 713–717

71 Darwiche, N., Celli, G., Tennenbaum, T., Glick, A.B., Yuspa, S.H., and De Luca, L.M. (1995). Mouse skin tumor progression results in differential expression of retinoic acid and retinoid X receptors. *Cancer Res.* **55,** 2774–2782

72 Darwiche, N., Scita, G., Jones, C., Rutberg, S., Greenwald, E., Tennenbaum, T., Collins, S.J., De Luca, L.M., and Yuspa, S.H. (1996). Loss of retinoic acid receptors in mouse skin and skin tumors is associated with activation of the ras^{Ha} oncogene and high risk for premalignant progression. *Cancer Res.* **56,** 4942–4959

73 Tontonoz, P., Hu, E., and Spiegelman, B.M. (1994). Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell* **79,** 1147–1156

74 Lehmann, J.M., Moore, L.B., Smith-Oliver, T.A., Wilkison, W.O., Willson, T.M., and Kliewer, S.A. (1995). An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J. Biol. Chem.* **270,** 12953–12956

75 Forman, B.M., Tontonoz, P., Chen, J., Brun, R.P., Spiegelman, B.M., and Evans, R.M. (1995). 15-Deoxy-delta^{12,14}-prostaglandin J_2 is a ligand for the adipocyte determination factor PPAR gamma. *Cell* **83,** 803–812

76 Kliewer, S.A., Lenhard, J.M., Willson, T.M., Patel, I., Morris, D.C., and Lehmann, J.M. (1995). A prostaglandin J_2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. *Cell* **83,** 813–819

77 Xu, X.C., Ro, J.Y., Lee, J.S., Shin, D.M., Hong, W.K., and Lotan, R. (1994). Differential expression of nuclear retinoid receptors in normal, premalignant, and malignant head and neck tissues. *Cancer Res.* **54,** 3580–3587

78 Takahashi, N. and Breitman, T.R. (1989). Retinoic acid acylation (retinoylation) of a nuclear protein in the human acute myeloid leukemia cell line HL60. *J. Biol. Chem.* **264,** 5159–5163

79 Kraemer, K.H., DiGiovanna, J.J., Moshell, A.N., Tarone, R.E., and Peck, G.L. (1988). Prevention of skin cancer in xeroderma pigmentosum with the use of oral isotretinoin. *N. Engl. J. Med.* **318,** 1633–1637

80 Hong, W.K., Lippman, S.M., Itri, L.M., Karp, D.D., Lee, J.S., Byers, R.M., Schantz, S.P., Kramer, A.M., Lotan, R., Peters, L.J., Dimery, I.W., Brown, B.W., and Goepfert, H. (1990). Prevention of second primary tumors with isotretinoin in squamous-cell carcinoma of the head and neck. *N. Engl. J. Med.* **323,** 795–801

81 Muto, Y., Moriwaki, H., Ninomiya, M., Adachi, S., Saito, A., Takasaki, K.T., Tanaka, T., Tsurumi, K., Okuno, M., Tomita, E., Nakamura, T., and Kojima, T. (1996). Prevention of second primary tumors by an acyclic retinoid, polyprenoic acid, in patients with hepatocellular carcinoma. Hepatoma Prevention Study Group. *N. Engl. J. Med.* **334,** 1561–1567

82 Leroy, P., Nakshatri, H., and Chambon, P. (1991). Mouse retinoic acid receptor α2 isoform is transcribed from a promoter that contains a retinoic acid response element. *Proc. Natl. Acad. Sci. USA* **88,** 10138–10142

83 Mattei, M.G., Petkovich, M., Mattei, J.F., Brand, N., and Chambon, P. (1988). Mapping of the human retinoic acid receptor to the q21 band of chromosome 17. *Hum. Genet.* **80,** 186–188

84 Sucov, H.M., Murakami, K.K., and Evans, R.M. (1990). Characterization of an autoregulated response element in the mouse retinoic acid receptor type beta gene. *Proc. Natl. Acad. Sci. USA* **87,** 5392–5396

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

86 de The, H., Vivanco-Ruiz, M., Tiollais, P., Stunnenberg, H., and Dejean, A. (1990). Identification of a retinoic acid responsive element in the retinoic acid receptor β gene. *Nature* **343,** 177–180

87 Duester, G., Shean, M.L., McBride, M.S., and Stewart, M.J. (1991). Retinoic acid response element in the human alcohol dehydrogenase gene ADH3: implications for regulation of retinoic acid synthesis. *Mol. Cell Biol.* **11,** 1638–1646

88 Smith, W.C., Nakshatri, H., Leroy, P., Rees, J., and Chambon, P. (1991). A retinoic acid response element is present in the mouse cellular retinol binding protein I (mCRBPI) promoter. *EMBO J.* **10,** 2223–2230

89 Vasios, G.W., Gold, D.G., Petkovich, M., Chambon, P., and Gudas, L.J. (1989). A retinoic acid-responsive element is present in the 5′ flanking region of the laminin B1 gene. *Proc. Natl. Acad. Sci. USA* **86,** 9099–9103

90 Vasios, G., Mader, S., Gold, J.D., Leid, M., Lutz, Y., Gaub, M.P., Chambon, P., and Gudas, L. (1991). The late retinoic acid induction of laminin B1 gene transcription involves RAR binding to the responsive element. *EMBO J.* **10,** 1149–1158

91 Rottman, J.N., Widom, R.L., Nadal-Ginard, B., Mahdavi, V., and Karathanasis, S.K. (1991). A retinoic acid-responsive element in the apolipoprotein AI gene distinguishes between two different retinoic acid response pathways. *Mol. Cell Biol.* **11,** 3814–3820

92 Munos-Canores, Vik, D.P., and Tack, B.F. (1990). Mapping of a retinoic acid-responsive element in the promoter region of the complement factor H gene. *J. Biol. Chem.* **265,** 20065–20068

93 Mangelsdorf, D.J., Umesono, K., Kliewer, S.A., Borgmeyer, U.,

Ong, E.S., and 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

94 Raisher, B.D., Gulick, T., Zhang, Z., Strauss, A.W., Moore, D.D., and 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

95 Lee, M.O., Liu, Y., and Zhang, X.K. (1995). A retinoic acid response element that overlaps an estrogen response element mediates multihormonal sensitivity in transcriptional activation of the lactoferrin gene. *Mol. Cell Biol.* **15,** 4194–4207

96 Durand, B., Saunders, M., Leroy, P., Leid, M., and Chambon, P. (1992). All-trans and 9-cis retinoic acid induction of mouse CRABPII gene transcription is mediated by RAR-RXR heterodimers. *Cell* **71,** 73–85

97 Lucas, P.C., O'Brien, R.M., Mitchell, J.A., Davis, C.M., Imai, E., Forman, B.M., Samuels, H.H., and Granner, D.K. (1991). A retinoic acid response element is part of a pleiotropic domain in the phosphoenolpyruvate carboxykinase gene. *Proc. Natl. Acad. Sci. USA* **88,** 2184–2188

98 Nagy, L., Saydak, M., Shipley, N., Lu, S., Basilion, J.P., Yan, Z.H., Syka, P., Chandraratna, R.A., Stein, J.P., Heyman, R.A., and Davies, P.J. (1996). Identification and characterization of a versatile retinoid response element (retinoic acid receptor response element-retinoid X receptor response element) in the mouse tissue transglutaminase gene promoter. *J. Biol. Chem.* **271,** 4355–4365

99 Langston, A.W. and 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