The connection between vitamin A and development of cancer was made rather soon after the discovery of this vitamin and its chemical structure. Wolbach and Howe’s pioneering studies in 1925 showed that vitamin-A deficiency inhibited proliferation and differentiation of epithelial cells. Throughout the next several decades, emphasis was placed on elucidating the chemical structure of vitamin A and how vitamin A was processed and metabolized in the organism. In the mid-1960s, a number of reports demonstrated that vitamin-A deficiency induced an increase in the number of spontaneous and chemically induced tumors in animals. During this period, much effort was made to determine the physiological metabolites of vitamin A and how vitamin A was processed and circulated through the circulatory system. Evidence from these studies suggested that vitamin-A acid (retinoic acid) is the major physiologically active retinoid. Retinol circulates as a complex with plasma retinol-binding protein and transthyretin (prealbumin). It is taken up by cells and bound within these cells by cellular retinol-binding protein. The cellular retinol-binding protein then delivers retinol to intracellular sites, where it is converted to retinoic acid.

A key observation was made by Strickland and Mahdavi who demonstrated that retinoic acid could induce the differentiation of teratocarcinoma cells in vitro and in vivo. Also during this period, Lotan and Nicholson established that retinoic acid inhibited the growth of many “normal” transformed and tumor cells in culture. The initial discovery that retinoic acid could induce the differentiation of malignant leukemia cells was reported by Breitman et al. In addition to these findings, the same group found that retinoic acid could induce the differentiation of fresh leukemic cells from patients with promyelocytic leukemia into mature granulocytes. Despite the extraordinary promise of these findings by a research team at the National Institutes of Health, this basic research was not translated into clinical trials until 1988, when Huang et al. reported a series of successful clinical trials using retinoic acid to treat patients with promyelocytic leukemia. After replication of Huang et al.’s results by investigators in France, clinical trials were finally conducted in the United States that verified the findings reported in the other studies.
A major advance in the understanding of how retinoic acid might inhibit cancer development and progression occurred in 1987 when Giguere et al. and Petkovitch et al. reported the discovery of nuclear receptors for retinoic acid. These receptors were found to contain the same structural modules as the family of steroid hormone receptors. Additional studies showed that there is a family of retinoic-acid nuclear receptors. One class of receptors (RARα, β, and γ) bind all-trans and 9-cis-retinoic acid, whereas the other class of receptors (RXR α, β, and γ) bind only 9-cis-retinoic acid with high affinity. It was also determined that the RXR formed heterodimers with a number of other nuclear receptors such as RAR, vitamin-D3 receptor, thyroid-hormone receptor, and peroxisomal-proliferator–activator receptor. Under physiologic conditions, only the RXR:RAR heterodimer leads to productive DNA binding.

Between 1992 and 1995, it was discovered that coactivator and corepressor proteins also regulated the activity of the RAR and RXR. The corepressors bind to the receptors in the absence of ligand and prevent it from activating transcription of the target genes. In contrast, coactivators bind to the receptors only when ligand is bound to the receptor, and they enhance the activation of transcription. One of the coactivators, CBP/p300, acts to enhance the activity of a plethora of transcription factors. CBP and a companion protein, pCAF, contain histone acetyltransferase activity. It was shown that some of these cofactors serve to remodel chromatin and thus alter the accessibility of the receptor to DNA. Our current model of how the complex of RXR:RAR and cofactors regulate retinoic-acid–induced gene expression is shown in Figure 1.

### TABLE I.

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1925</td>
<td>Wolbach and Howe</td>
<td>First described the effect of vitamin-A deficiency on the proliferation and differentiation of epithelial cells</td>
</tr>
<tr>
<td>1950–1960</td>
<td>Various investigators</td>
<td>Demonstrated that vitamin-A deficiency induces an increase in the number of spontaneous and chemically induced tumors in animals</td>
</tr>
<tr>
<td>1960–1970</td>
<td>A variety of studies</td>
<td>Report that exogenously added retinoids can suppress the incidence of chemically induced tumors in animals</td>
</tr>
<tr>
<td>1977</td>
<td>Lotan and Nicholson</td>
<td>Report that retinoids inhibit the growth of many “normal” transformed and tumor cells in culture</td>
</tr>
<tr>
<td>1978</td>
<td>Strickland and Mahdavi</td>
<td>Report that retinoic acid induces differentiation of teratocarcinoma cells grown in tissue culture</td>
</tr>
<tr>
<td>1980–1981</td>
<td>Breitman et al.</td>
<td>Find that retinoid acid induces the differentiation of the HL-60 human leukemic cells and also cells from patients with promyelocytic leukemia; these basic research findings form the basis of future clinical treatment of this disease with retinoids</td>
</tr>
<tr>
<td>1987</td>
<td>The discovery of nuclear retinoic-acid receptors</td>
<td>Is reported independently by Giguere et al. and by Petkovitch et al.</td>
</tr>
<tr>
<td>1988</td>
<td>Huang et al.</td>
<td>Report the successful use of retinoic acid to treat patients with acute promyelocytic leukemia</td>
</tr>
<tr>
<td>1993</td>
<td>Lippman et al.</td>
<td>Report that retinoids significantly decrease the incidence of second primary tumors in patients with head-and-neck cancer</td>
</tr>
<tr>
<td>1992</td>
<td>Coactivators and corepressors for RAR are reported by different investigators</td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>Bischoff et al.</td>
<td>Report that targetretin, an RXR-selective retinoid, causes complete remission of manary cancer in rats</td>
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</tbody>
</table>

FIG. 1. Schematic diagram of the assembly of a transcriptionally active RXR:RAR complex on target genes. (A) Inactive RXR:RAR complex bound to corepressor. The RXR ligand-binding site is sterically blocked by RAR. Corepressor has histone deacetylase activity, which keeps the chromatin in the “condensed” state. (B) Assembly of active complex. Addition of the ligand all-trans-retinoic acid, delivered to the nucleus by CRABP II, induces a conformational change in RAR. As a result of the change, the co-repressor is released, the RXR is free to bind its ligand 9-cis-retinoic acid, and coactivators such as CBP/p300, pCAF, and others (e.g., steroid coactivator-1 [SRC-1]) bind to the RXR and RAR. CBP/p300 and pCAF have histone-acetyltransferase activity that induces a “loose” state of chromatin structure required for active gene transcription.
A significant amount of data suggest that RARβ may be a tumor-suppressor gene because its expression is lost in a variety of tumors. Further, induction of RARβ expression invariably accompanies the growth inhibition and stimulation of differentiation of tumor cells by retinoic acid. However, mutations in some of the cofactors required for RAR:RXR activity could also lead to loss of retinoic-acid control of gene expression. Because some of the coactivators needed for optimal RAR activity are also required by other nuclear receptors and transcription factors, the stoichiometry of all these different molecules will determine the intensity of the response to retinoic acid. In other words, the relative amounts of the RARs and RXRs and their ability to compete for coactivators will influence the ability of retinoic acid to control the physiologic functions of cells.

One of the problems with using retinoic acid as a therapeutic agent is that at pharmacologic concentrations it has a number of potential side effects such as liver toxicity, central nervous system toxicity, and elevation of serum triacylglycerols. These side effects are thought to be due to the ability of all-trans-retinoic acid to bind and activate all of the different RARs. There has been a concerted effort in recent years to develop receptor-selective retinoids. Currently, there are a variety of retinoid analogs that have relatively good RAR-selective agonist or antagonist activity. It should be emphasized that there are no absolutely specific RAR-subtype retinoids. When used at high concentrations, these compounds will bind and activate other retinoid receptors. This could present a problem in clinical settings, where there may be a need to use higher concentrations of the retinoid. Therefore, it was encouraging to learn of the recent work of Bischoff et al. who found that targetretin, an RXR-selective retinoid, caused complete remission of mammary cancer in rats. There are also reports that RAR-selective retinoids can overcome the retinoid resistance found in cases of promyelocytic leukemia that have become refractory to treatment with retinoic acid. These studies suggest retinoids with receptor-subtype selectivity may have therapeutic usefulness in the prevention and treatment of certain types of cancer.

**FUTURE PREDICTIONS AND AREAS IN NEED OF EXPLORATION (TABLE II)**

Substantial progress has been made during the last 20 y in advancing our knowledge of how retinoids achieve their biologic effects and in turn how they may be involved in the prevention and treatment of cancer. Although some epidemiologic studies have suggested that there may be a correlation between low dietary vitamin-A intake and development of certain cancers, molecular studies have indicated that defects in the retinoid-signaling pathway likely contribute to the development of many types of cancers. In particular, a significant amount of experimental data supports the hypothesis that RARβ may function as a tumor-suppressor gene. Expression of RARβ in tumor cells not expressing this gene restores retinoid-induced growth inhibition and apoptosis. Transgenic mice in which RARβ gene expression is downregulated have an increased incidence of lung cancer. Decreased RARβ expression also appears to be responsible for a lack of antitumor activity of retinoids in animals. Deletion of the short arm of chromosome 3p24, a region containing the RARβ gene, occurs with high frequency in human tumors. Also, a variety of tumors have a high frequency of abnormal expression of the RARβ gene but not of the genes encoding the other RAR subtypes. In light of the data already accumulated, it is likely that within the next decade the tumor-suppressor hypothesis will be proven correct and that RARβ will become the target of therapeutic strategies.

To develop these therapeutics, we need a better understanding of retinoid-receptor biology. A variety of proteins that interact with these nuclear retinoid receptors have been discovered in the past few years. It is not clear how all of the proteins assemble into a complex to activate transcription of target genes. Some of these coactivators and corepressors appear to be ubiquitously expressed, whereas others show tissue-specific expression. Tissue-specific expression of coactivators is intriguing because it suggests that they may control tissue-specific retinoid-regulated gene expression. In addition to proteins that bind and modulate the activity of these receptors, the nuclear retinoid receptors can be regulated at the level of synthesis (cyclic AMP can either induce or repress RAR expression, depending on the cell type) and through phosphorylation. Quite recently, it was discovered that the RARs are degraded through the ubiquitination–proteasome pathway and that their degradation can be accelerated by ligand binding. Because the affinity of the RAR for retinoic acid is so strong, accelerated degradation of the ligand-bound receptor provides a means for turning off the induction of target gene transcription that is independent of ligand dissociation. Because there are so many ways by which the activity of the nuclear retinoid receptors can be regulated, it will take a considerable amount of time before we fully understand how nuclear retinoid receptors function in vivo under different physiologic scenarios.

A fundamental question, still unanswered, is the exact pathway (enzymes or subcellular localization) by which retinol is taken up by cells, converted to all-trans-retinoic acid or 9-cis–retinoic acid and then delivered to the nuclear retinoid receptors. Indeed, in light of the very-low cellular concentration of 9-cis–retinoic acid, there is some lingering doubt that this retinoid is the natural ligand for RXR. The recent, unexpected, finding that CRABP II is found in the nucleus, binds to RAR, and stimulates its transcriptional activity provides a potential pathway for nuclear delivery of this retinoid. It is likely that these pathways for regulating ligand availability and for the regulation of nuclear retinoid-receptor function will also be disrupted in some cancers.

The exact pathway(s) by which retinoids control proliferation, apoptosis, and differentiation remains a mystery. It is likely that different pathways, i.e., different sets of genes, will be used by different cell types. The use of DNA microarrays, by which one can examine changes in the expression of thousands of genes in one experiment, will provide abundant data regarding which genes are directly regulated by retinoids, whose activation in turn regulates downstream target genes, ultimately leading to the biologic response. It is likely that in the next 20 y these retinoid-regulated pathways will be mapped in a variety of cell types. This information will provide additional insight into how alterations in the retinoid-signaling pathway might contribute to carcinogenesis and yield target identification for the development of new molecular-based therapies for the treatment of cancer.

**TABLE II.**  
**IMPORTANT QUESTIONS FOR RETINOIDS AND CANCER BIOLOGY IN THE 21ST CENTURY**  
<table>
<thead>
<tr>
<th>Question</th>
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<tr>
<td>Does RARβ function as a tumor suppressor gene?</td>
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<tr>
<td>How does the complex of nuclear retinoid receptors and coactivators result in stimulation of target gene expression?</td>
</tr>
<tr>
<td>What is the role of phosphorylation and protein stability in the regulation of nuclear retinoid-receptor function?</td>
</tr>
<tr>
<td>How is the production of intracellular retinoic acid regulated and how is it delivered to the nuclear receptors? Is this process altered in cancer cells?</td>
</tr>
<tr>
<td>What are the pathways by which retinoids regulate proliferation, apoptosis, and differentiation? What is the identity of the primary and downstream target genes? Are these retinoid-regulated pathways deranged in cancer cells?</td>
</tr>
</tbody>
</table>
REFERENCES


32. Xiao Y-H, Desai D, Quick TC, Niles RM. Control of retinoic acid receptor expression in mouse melanoma cells by cyclic AMP. J Cell Physiol 1996;176:413