Carotenoids and Retinoids
Molecular Aspects and Health Issues

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Preface

Carotenoids synthesized in plants are essential for the assembly, function, and stability of photosynthetic pigment–protein complexes. A light-harvesting function of carotenoids allows blue and green sunlight to be used for energy conservation, a process that involves energy transfer from carotenoid excited states to nearby chlorophylls at the active center of oxygenic photosynthetic pigment complexes. Carotenoids also protect against oxidative and photooxidative damage by quenching free radicals that are produced during photosynthesis; this function gains further significance when considering that free radicals or reactive oxygen species are by-products of metabolism in humans.

Indeed, the presence of carotenoids in the diet and their role in human health has become a subject of unprecedented interest. Some carotenoids are called provitamin A compounds because they are precursors of retinol and retinoic acid. The type of carotenoids found in human plasma depends on the extent to which people consume diets rich in green, yellow/red, or yellow/orange vegetables. Fifty to sixty different carotenoid compounds are typically present in the human diet, including the most abundant forms in plasma: β-carotene, lycopene, lutein, cryptoxanthin, α-carotene, and zeaxanthin. Carotenoids are potent antioxidants known to affect different cellular pathways. For example, lutein and zeaxanthin accumulate in the fovea (macular region) of the human eye and are thought to prevent blue light damage to the eye. A low dietary supply of these carotenoids (xanthophylls) is thought to be associated with age-related macular degeneration, one of the most common causes of irreversible blindness in the Western world. Numerous epidemiological, interventional, and prospective human studies, as well as an incredible array of fundamental research, are currently underway to elucidate the role of carotenoids, vitamin A (retinol), retinoids, and their stereoisomers and metabolites in biological processes and health and disease prevention.

A roundtable discussion on the Safety of β-Carotene and a workshop on Carotenoids and Retinoids: Molecular Aspects and Health Issues were held at the annual Oxygen Club of California (OCC) conference in Santa Barbara, California, on March 10–13, 2004. These events were co-organized by the editors of this volume and sponsored by the Scientific Affairs, Strategic Marketing Human Nutrition unit of BASF, Ludwigshafen, Germany. The chapters in this book represent an account of the information presented at the workshop together with several additional invited contributions to cover topics more completely that are currently at the cutting edge of research. The editors have sought the timely publication of this book in cooperation with AOCS Press.

Some of the highlights of this book on Carotenoids and Retinoids: Molecular Aspects and Health Issues are summarized below.

The book commences with comprehensive overview chapters on vitamin A, retinoids, and carotenoids, including different aspects of their uptake, molecular
structure, transport, storage, metabolism, transcriptional activity, and roles in human health. There is also a thorough review of the special role that vitamin A intake plays in the health status of developing countries.

Another chapter addresses the essential role of vitamin A in cell signaling and covers historical aspects followed by receptor action, molecular aspects of action with kinases, redox regulation, and the significance of vitamin A in oxygen biology. A chapter on the role of carotenoids and retinoids in cellular/tissue gap junctional communication focuses on structure/activity relationships and interactions with other micronutrients. The molecular structure of carotenoid metabolites and their intracellular distribution are thoroughly reviewed, with an emphasis on human data.

Owing to the unique molecular structure of the hydrocarbon chain in carotenoids, resonance Raman spectroscopy is a useful tool for investigating carotenoids in photosynthetic processes, noninvasively measuring carotenoid content in the macular region of the human eye and skin. With this technology, the age-related loss of carotenoids in the human macula has been demonstrated. Recent studies have also provided evidence of a close correlation between serum (high-performance liquid chromatography) and stratum corneum (laser Raman scattering intensity) levels of carotenoids in the skin. This discovery broadens the application of this technology to human studies.

The oxidation of carotenoids and their cleavage reactions result in the formation of metabolites whose biological function requires elucidation. Two chapters describe studies on the actions of these metabolites in cell and mitochondrial systems and the formation of oxidative metabolites in inflamed lung tissue.

Epidemiological studies in two human trials revealed that the presence of β-carotene increased the incidence of lung cancer in individuals exposed to cigarette smoke and asbestos, thus stimulating interest in the scientific community to elucidate the relation to cancer. The molecular targets involved in carotenoid action in smoke-induced lung pathology are described. Another chapter deals with the up-regulation of gap junctional proteins by carotenoids and retinoids and, hence, their cancer-preventive actions. One of the best-studied roles of carotenoids in cancer prevention is the inverse association between consumption of tomato-based foods and lycopene and the incidence of prostate cancer.

A role of phytochemicals in cancer prevention is their induction of Phase II enzymes, which are important in detoxification reactions and antioxidant defense. Recent studies demonstrate that carotenoids and their oxidation products regulate transcription factors that control the induction of key Phase II enzymes in cell culture models. These findings may also serve to explain the antiproliferative effects of carotenoids.

Carotenoids also have been reported to be beneficial in cardiovascular health from studies involving the consumption of fruits and vegetables rich in carotenoids. One of the chapters reports on the evidence of a reduced risk associated with dietary lycopene. In another study, the relationship between consumption of carotenoid-rich fruits and vegetables, their uptake, and the oxidizability of serum carotenoids is reported. Relevant to this topic is the metabolic mechanism of carotenoid oxidizability: The discovery and elucidation of carotenoid oxygenases and dioxygenases.
has led to remarkable new insights into the role of these cleavage enzymes in vitamin A and retinoid metabolism, as well as their action in developmental processes.

In view of the enormous interest in the health aspects of carotenoids, it is important to summarize and critically evaluate the human epidemiological evidence for the association of carotenoids and human health. A synopsis of the most important and ongoing investigations is given in one of the final chapters. Also, the final chapter presents a brief synopsis of the Round Table Discussion on Safety of β-Carotene attended by many of the leading investigators in the field. Topics discussed were mainly related to the human safety of β-carotene, especially with regard to smokers and individuals exposed to hazardous environments. The author of this insightful account presents the reader with future perspectives and research directions. A feature on Future Horizons of Research on Carotenoids and Retinoids is presented by one of the volume co-editors.

Lester Packer
Ute Obermüller-Jevic
Klaus Kraemer
Helmut Sies

September 10, 2004
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Chapter 1

Introduction to Retinoids

Sheila M. O’Byrne\textsuperscript{a} and William S. Blaner\textsuperscript{a,b}

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Introduction

What are retinoids? The term retinoid refers to both naturally occurring and synthetic compounds that bear a structural resemblance to vitamin A (all-trans-retinol) with or without the biological activity of vitamin A (1). Figure 1.1 shows the chemical structures of some naturally occurring retinoids. The term vitamin A is often used as a general term for all compounds that exhibit the biological activity of retinol. There are many natural and synthetic retinoids. All of the synthetic retinoids were developed as potential pharmacologic agents for use in treating ailments ranging from cancer to acne. For the remainder of this chapter, we will focus only on the metabolism, storage, and transport of natural retinoid forms (vitamin A and its metabolites) that are found in the diet and in the body.

Many of the chapters in this book focus on carotenoids. Some carotenoids may be converted by higher animals to retinoids. These carotenoids are collectively known as provitamin A carotenoids. The best-studied of the provitamin A carotenoids is \(\beta\)-carotene. Within the body, \(\beta\)-carotene can (but does not necessarily have to) undergo cleavage to retinal, which is then reduced to give rise to two molecules of retinol. We will briefly describe the carotene cleavage process as it occurs in the intestine, but we will not deal directly with the metabolism or transport of other members of the large carotenoid family in this chapter.

To understand retinoid metabolism, storage, and transport, we believe that it is necessary to understand the proteins that bind these hydrophobic molecules within the aqueous environment of cells and the extracellular fluids, the enzymes that act upon them to render them biologically active or inactive, and the proteins that are required to facilitate their actions within the body. Many of these proteins will be discussed throughout the course of this chapter to paint a picture of the complexity of the metabolic trafficking that retinoids undergo. The different retinoid forms present in the body relate to and are a result of the actions of these proteins. Most of the enzymatic reactions central to retinoid metabolism, with the notable exception of retinal oxidation to retinoic acid, are reversible. This allows the retinoid metabolism to be finely regulated in response to the body’s needs. A listing of binding proteins that shuttle and sequester retinoids, of enzymes that maintain retinoids as inactive forms, and enzymes that activate retinoids, and of nuclear pro-

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Proteins that bring about the transcriptional activator properties of retinoids will be discussed in the text and summarized in tables. Many of the proteins will be discussed in the text and the reader is referred to these tables to facilitate ease of understanding.

Finally, this chapter will not be exhaustive. It is aimed primarily at giving the reader a brief but detailed overview of our current scientific knowledge of mammalian storage and metabolism of natural retinoids.

**Dietary Sources of Retinoid**

Retinoids are essential micronutrients (i.e., our bodies cannot synthesize them *de novo* and they are required in microamounts) and must therefore be obtained from the diet. There are two sources of retinoid in the diet; the first is as provitamin A carotenoids obtained from dark green and colorful vegetables and the second is as preformed vitamin A from animal products such as meat and dairy (where the retinoid has been preformed from fruits and vegetables consumed by animals).
Provitamin A carotenoids such as β-carotene can be cleaved to form two molecules of retinal, which are then reduced to form two molecules of retinol. The retinoid forms found in animal food products are mainly retinol and retinyl esters. Higher animals are capable of storing retinoid in the liver and to a lesser extent in other tissues, thus alleviating the obligate need for daily dietary intake because the stored retinoid can be mobilized in times of insufficient dietary retinoid intake (2,3).

**Major Retinoid Forms in the Body**

The different retinoid forms present within the body are generated primarily through modifications to the terminal polar end of the molecule (see Fig. 1.1). Retinol and retinyl esters are the most abundant retinoid forms found in the body. All-trans-retinol is by definition vitamin A. When a fatty acyl group is esterified to the hydroxyl terminus of all-trans-retinol, a storage form of retinol, the retinyl ester, is formed. The most abundant retinyl esters are those of palmitic, oleic, steric, and linoleic acids (2,3). Retinyl acetate is often used as a dietary supplement, but this short-chain retinyl ester does not occur naturally (2,3). In times of retinoid need or in the intestine upon retinyl ester intake from the diet, the ester bond undergoes hydrolysis to retinol. The formation of retinyl esters makes the retinoid less toxic and allows for its storage within intracellular lipid droplets.

Retinol itself has no known biological activity. However, it can be reversibly oxidized to retinal, which as the 11-cis isomer is essential for the visual cycle (4,5). Rhodopsin, the visual pigment responsible for photoperception, consists of 11-cis-retinal covalently bound to a lysine residue present in the protein opsin (4,5). As the primal event in vision, a photon of light strikes the rhodopsin molecule, resulting in the photoisomerization of 11-cis-retinal to all-trans-retinal (4,5). In all tissues aside from the eye, retinal has no other known function beyond serving as an intermediate in the synthesis of retinoic acid (6,7).

The all-trans and 9-cis-isomers of retinoic acid are transcriptionally active retinoids and are thought to account for the gene regulatory properties of retinoids within cells and tissues (6,7). The concentration of retinoic acid within tissues is generally very low and is usually 100–1000 times less than that of retinol (2,3). All-trans-retinoic acid is formed through the irreversible oxidation of all-trans-retinal. This is one of the few irreversible steps in retinoid metabolism and must therefore be finely regulated. All-trans-retinoic acid can be isomerized through a nonenzymatic process to form the 9-cis- or 13-cis-isomers (3). It is possible that some 9-cis-retinoic acid may be formed from 9-cis-retinol through a two-step oxidation process similar to that described above for all-trans-retinoic acid (8,9). Retinoic acid formed outside of the nucleus can move to the nucleus where it binds and activates one of its nuclear hormone receptors (6,7). This binding leads to the transcription of retinoid-responsive target genes that give rise to the biological activities of vitamin A (6,7). This transcriptional activity is described in more detail in other chapters. Although 13-cis-retinoic acid is a naturally occurring
retinoid that is present in blood and tissues (2,3), it possesses much less transcriptional activity than either the all-trans- or the 9-cis-isomer (6).

Various oxo- and hydroxy-forms of retinol and retinoic acid as well as glucuronides of retinol and retinoic acid are present in the body, albeit at very low concentrations relative to retinol and retinyl esters (2,3). Although some of these oxidized and conjugated retinoid forms may have biological/transcriptional activity, it appears likely that most of these forms are catabolic in nature and destined for elimination from the body. Because there are no known enzymes that can reduce retinoic acid to retinal, excessive or unneeded retinoic acid is not recycled and must be catabolized. As described below in more detail, this catabolism is thought to be catalyzed by one of several cytochromes (CYP) (2,3,10–14), giving rise to the more water-soluble retinoid forms that can be easily excreted.

Finally, retro- and anhydro-retinoids are also naturally occurring retinoid forms that can be synthesized by cells and tissues and that are present within the body (15). Enzymes able to catalyze the formation of retro- and anhydro-retinoids were identified (15). It was proposed that the retro- and anhydro-retinoids may have actions in regulating immune function, but the mechanisms responsible for these actions have not yet been elucidated (15,16).

**Retinoid-Binding Proteins**

To solubilize, protect, and detoxify retinoids in the intracellular and extracellular environment, retinol, retinal, and retinoic acid are usually found bound to specific retinoid-binding proteins. The known retinoid-binding proteins are summarized in Table 1.1. These can be classified using several different criteria. Some of these proteins, specifically retinol-binding protein (RBP), interphotoreceptor matrix retinoid-binding protein (IRBP), epididymal retinoic acid-binding protein (ERABP), and \(\beta\)-trace are found only in extracellular fluids, whereas the remainder are found only intracellularly. Of the intracellular binding proteins, some bind only retinoic acid [cellular retinoic acid-binding protein, type I (CRABP I) and cellular retinoic acid-binding protein, type II (CRABP II)]; some preferentially bind both retinol and retinal [cellular retinol-binding protein, type I (CRBP I) and cellular retinol-binding protein, type II (CRBP II)]; some preferentially bind retinol [cellular retinol-binding protein, type III (CRBP III) and cellular retinol-binding protein, type IV (CRBP IV)]; and one preferentially binds retinal [cellular retinal-binding protein (CRALBP)]. These proteins can also be grouped by the protein families to which they belong. RBP, ERABP, and \(\beta\)-trace are all members of the lipocalin protein family (17–19). CRBP I, II, III, and IV as well as CRABP I and CRABP II are members of the fatty acid-binding protein family of proteins (20–24). CRALBP is a member of the CRAL-TRIO protein family, which also contains a vitamin E–binding protein (25,26).

Each of the known retinoid-binding proteins was proposed to have a role in facilitating retinoid transport and/or metabolism (see Table 1.1). However, none of
<table>
<thead>
<tr>
<th>Protein</th>
<th>Ligand(s)</th>
<th>Proposed functions</th>
<th>Tissue site(s) of synthesis</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Retinoic acid-binding protein (RBP)</td>
<td>Retinol</td>
<td>Mobilization of liver stores and transport in blood</td>
<td>Liver, adipose, others</td>
<td>17, 21</td>
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<tr>
<td>Epididymal retinoic acid-binding protein (ERABP)</td>
<td>Retinoic acid</td>
<td>Epididymal lumen transport of retinoic acid</td>
<td>Epididymis, others</td>
<td>18</td>
</tr>
<tr>
<td>β-Thoc (Lipocalin-type prostaglandin D synthase)</td>
<td>Retinoic acid</td>
<td>Binds retinoic acid in the cerebral spinal fluid (CSF)</td>
<td>Brain (CSF)</td>
<td>19</td>
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<tr>
<td>Interphotoreceptor retinoic acid-binding protein (IRBP)</td>
<td>Retinol and 11-cis-retinal</td>
<td>Specialized shuttling protein involved in the visual cycle</td>
<td>Eye (retina)</td>
<td>5</td>
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<tr>
<td>Intracellular</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellular retinoic acid-binding protein (CRAIBP)</td>
<td>Retinol and 11-cis-retinal</td>
<td>Specialized binding protein involved in the visual cycle</td>
<td>Eye (retina)</td>
<td>5, 21, 25, 26</td>
</tr>
<tr>
<td>Cellular retinoic acid-binding protein, type II (CRBP II)</td>
<td>Retinol and retinal</td>
<td>Dietary retinoic uptake and metabolism in the intestinal mucosa</td>
<td>Small intestine</td>
<td>20-22</td>
</tr>
<tr>
<td>Cellular retinoic acid-binding protein, type III (CRBP III)</td>
<td>Retinol</td>
<td>Cellular retinoic uptake, transport and metabolism</td>
<td>Heart, mammary, fat</td>
<td>23</td>
</tr>
<tr>
<td>Cellular retinoic acid-binding protein, type IV (CRBP IV)</td>
<td>Retinol</td>
<td>Cellular retinoic uptake, transport and metabolism</td>
<td>Liver, kidney, heart, others</td>
<td>24</td>
</tr>
<tr>
<td>Cellular retinoic acid-binding protein, type I (CRABP I)</td>
<td>Retinoic acid and metabolites</td>
<td>Transport and metabolism of retinoic acid within the cell</td>
<td>Liver, kidney, liver, others</td>
<td>20, 21</td>
</tr>
<tr>
<td>Cellular retinoic acid-binding protein, type II (CRABP II)</td>
<td>Retinoic acid and metabolites</td>
<td>Retinoic acid metabolism shuttling of retinoic acid to the nucleus and involvement in transcription</td>
<td>Skin, uterus, ovaries</td>
<td>20, 21</td>
</tr>
</tbody>
</table>
these proteins can have an essential role in facilitating these processes. The genes for nearly all of these retinoid-binding proteins were ablated in mouse models, and none of the gene disruptions were lethal or even associated with severe phenotypes (27–33). It seems likely that these proteins are used to facilitate optimal retinoid retention, transport, and metabolism. When dietary retinoid availability is not impaired, the actions of the binding proteins are likely not essential or possibly even critical for maintaining retinoid status of the body or the health of the organism. However, in times of dietary retinoid deficiency, the binding proteins and the enhanced metabolic efficiency and retention that they afford convey an advantage to the organism. We note that throughout evolution, dietary retinoid deficiency was the norm rather than the exception it is today.

**Transcriptional Activity of Retinoids**

Retinoids are required for maintaining reproduction including spermatogenesis, conception, placenta formation, and embryogenesis and processes that are dependent on cell differentiation such as bone remodeling, epithelial and skin differentiation, and immune system function (34). These retinoid actions are mediated by all-trans-retinoic acid and 9-cis-retinoic acid through effects on retinoid-responsive gene expression. Retinoic acid can bind three retinoic acid receptors (RARα, RARβ, and RARγ) and three retinoid X receptors (RXRα, RXRβ, and RXRγ), which are then activated and can regulate gene expression in the nucleus of cells (6,7,35,36). All-trans-retinoic acid binds well and readily transactivates the three RAR and the three RXR, whereas 9-cis-retinoic acid binds and transactivates well only the RXR. Thus, all-trans-retinoic acid is usually thought of as the natural ligand for the RAR, and 9-cis-retinoic acid is considered to be the natural ligand for the RXR (6,7,35,36). Because the RXR can interact with nuclear vitamin D receptors (VDR), thyroid hormone receptors (TR), and peroxisomal proliferator activator receptors (PPAR), retinoic acid helps to regulate a broad spectrum of hormonally responsive genes (6,7,35–37). Well over 500 genes may be regulated by retinoic acid (37). Because these receptors will be discussed in detail in other chapters, we will not discuss them further in this chapter aside from summarizing them in Table 1.2.

**Intestinal Absorption and Processing of Retinoids**

As mentioned earlier, in most of the Western and developed world, much of the retinoid obtained in the diet arises from animal food sources and therefore consists of retinol and retinyl ester. Because retinol but not retinyl esters can enter the intestinal mucosa, dietary retinyl ester must first be acted upon by a hydrolase to yield free retinol. Retinyl esters can be hydrolyzed within the intestinal lumen by nonspecific pancreatic enzymes such as pancreatic triglyceride lipase and cholesteryl ester hydrolase or at the mucosal cell surface where a retinyl ester hydrolase is associated with the intestinal brush border (2,3). The free retinol formed upon hydrolysis of the retinyl ester or retinol arriving as such from the diet is taken up
into the intestinal cells (2,3). In contrast to dietary preformed vitamin A, dietary provitamin A carotenoids are absorbed unmodified by the same intestinal cells. These carotenoids can be cleaved within the intestinal cells by carotene-15,15′-monooxygenase (also called carotene cleavage enzyme)\(^1\) to retinal (38–42), which is then reduced to retinol by the enzyme retinal reductase (2,3). After carotene cleavage and retinal reduction, the retinol arriving from the diet as preformed vitamin A and as provitamin A carotenoids cannot be distinguished metabolically.

CRBP II is present in the small intestine and binds both retinal and retinol (20–22). Retinal bound to CRBP II is the preferred substrate for reduction to retinol by the intestinal retinal reductase. Retinol bound to CRBP II is then reesterified with long-chain fatty acids through the action of the enzyme lecithin:retinol acyltransferase (LRAT), which utilizes preferentially retinol bound to CRBP II as a substrate for esterification (20,21,43). The resulting retinyl esters are then packaged along with the rest of the dietary lipids into nascent chylomicrons and secreted into the lymphatic system for uptake into the general circulation (2,3). Figure 1.2 gives a schematic representation of this process. A partial listing of enzymes and enzyme families that are thought to have roles in catalyzing intestinal metabolism and the metabolic processes discussed later in the chapter is provided in Table 1.3.

\(^1\)The older literature refers to carotene-15,15′-monooxygenase as carotene-15,15′-dioxygenase, but recent mechanistic studies indicate that this enzyme acts through a monooxygenase mechanism rather than that of a dioxygenase (Leuenberger, M.G., Engeloch-Jarret, C., and Woggon, W.-D. (2001) The Reaction Mechanism of the Enzyme-Catalyzed Central Cleavage of β-Carotene to Retinal, Angew. Chem. Int. Ed. 40, 2613–2617). As indicated in the text, this enzyme is also referred to in the literature as carotene cleavage enzyme.

### TABLE 1.2

<table>
<thead>
<tr>
<th>Nuclear receptor</th>
<th>Retinoic acid ligand(s)</th>
<th>Function(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinoic acid receptor α (RARα)</td>
<td>All-trans and 9-cis</td>
<td>Transcriptional mediator; heterodimerizes with RXR</td>
</tr>
<tr>
<td>Retinoic acid receptor β (RARβ)</td>
<td>All-trans and 9-cis</td>
<td>Transcriptional mediator; heterodimerizes with RXR</td>
</tr>
<tr>
<td>Retinoic acid receptor γ (RARγ)</td>
<td>All-trans and 9-cis</td>
<td>Transcriptional mediator; heterodimerizes with RXR</td>
</tr>
<tr>
<td>Retinoid X receptor α (RXRα)</td>
<td>9-cis</td>
<td>Transcriptional mediator; homodimerizes; heterodimerizes with RAR, TR, VDR, PPAR and others</td>
</tr>
<tr>
<td>Retinoid X receptor β (RXRβ)</td>
<td>9-cis</td>
<td>Transcriptional mediator; homodimerizes; heterodimerizes with RAR, TR, VDR, PPAR and others</td>
</tr>
<tr>
<td>Retinoid X receptor γ (RXRγ)</td>
<td>9-cis</td>
<td>Transcriptional mediator; homodimerizes; heterodimerizes with RAR, TR, VDR, PPAR and others</td>
</tr>
</tbody>
</table>

\(^a\)Abbreviations: TR, thyroid hormone receptors; VDR, vitamin D receptors; PPAR, peroxisome proliferator activator receptors.
Fig. 1.2. Schematic representation of intestinal absorption and processing of dietary preformed vitamin A and provitamin A carotenoids. Retinoids are present in the diet as preformed vitamin A (primarily as retinol and retinyl esters) or as provitamin A carotenoids (e.g., β-carotene, α-carotene, or β-cryptoxanthin). The dietary carotenoids can enter the intestinal mucosal cell and either be packaged directly into the nascent chylomicrons or be cleaved by carotene 15,15'-monooxygenase to yield two molecules of retinal. The retinal can then be transformed enzymatically to retinol by the action of an intestinal retinal reductase(s). Dietary retinyl esters are unable to pass directly through the intestinal brush border and must first be hydrolyzed to free retinol by either pancreatic retinyl ester hydrolases or a brush border retinyl ester hydrolase. This retinol, like free retinol arriving as such in the diet, readily traverses the intestinal brush border and binds within the enterocyte cellular retinol-binding protein, type II (CRBP II). This protein can also bind the free retinal from the previous step (not shown in this schematic representation). Retinol bound to CRBP II is then esterified to form retinyl esters through the actions of lecithin:retinol acyltransferase (LRAT). These retinyl esters (RE) are incorporated through some undefined mechanism into the nascent chylomicrons along with other dietary lipids; these are secreted into the lymphatic system and subsequently enter the general circulation. Most chylomicron retinyl ester is taken up by the liver, but a substantial percentage is also taken up by peripheral tissues.
<table>
<thead>
<tr>
<th>Name(s)</th>
<th>Substrate(s)</th>
<th>Proposed physiological function(s)</th>
<th>Primary location</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecithin retinol acyltransferase (LRAT)</td>
<td>Retinol</td>
<td>Transfer fatty acid moiety from retinol to form retinyl ester</td>
<td>Most tissues, particularly eye, intestine, liver</td>
<td>3, 5, 20, 21, 43, 51-53</td>
</tr>
<tr>
<td>Acyl CoA:retinol acyltransferase (ARAT)</td>
<td>Retinol</td>
<td>Retinyl ester formation from fatty acyl CoA</td>
<td>Mammary tissue, possibly others</td>
<td>2, 3, 55, 56</td>
</tr>
<tr>
<td>Carotene-15,15' monooxygenase or carotene cleavage enzyme (CCE)</td>
<td>ß-Carotene and other provitamin A carotenoids</td>
<td>Central cleavage of carotenoids to form retinol</td>
<td>Intestine, liver, tissues, others</td>
<td>28-12</td>
</tr>
<tr>
<td>Retinyl ester hydrolases (REH)</td>
<td>Retinyl esters</td>
<td>Mobilization of retinyl ester from stores</td>
<td>Intestinal cells, liver, many others</td>
<td>2, 3, 57-69</td>
</tr>
<tr>
<td>Bile salt sensitive retinylester hydrolase (BS-RH) or carboxyl ester lipase (CEL)</td>
<td>Retinyl esters, cholesteryl esters</td>
<td>Hydrolases of dietary retinyl ester, in intestinal lumen and hepatic retinyl ester</td>
<td>Lumen of the intestine, liver</td>
<td>2, 3, 60</td>
</tr>
<tr>
<td>Medium chain alcohol dehydrogenases (ADH)</td>
<td>Retinol, other alcohols</td>
<td>Oxidation of retinol to retinal; for retinoid formation</td>
<td>Most tissues, liver, eye, others</td>
<td>61-65, 69-71</td>
</tr>
<tr>
<td>Short chain dehydrogenases/ reductases (SDR)</td>
<td>Retinol, retinal, other alcohols and aldehydes</td>
<td>Oxidation of retinol to retinal; or retinal reduction to retinol involved in retinoid acid formation</td>
<td>Most tissues, liver, skin, eye, tests, kidney and lung</td>
<td>2, 3, 61-65</td>
</tr>
<tr>
<td>Aldehyde dehydrogenases (ALDH)</td>
<td>Retinol, other aldehydes</td>
<td>Irreversible oxidation of retinol to retinoid</td>
<td>Most tissues</td>
<td>73-77</td>
</tr>
<tr>
<td>Cytochrome P450, CYP36 isoforms</td>
<td>Retinoic acid</td>
<td>Oxidative metabolism of retinoic acid to retinoid and cellular retinoid</td>
<td>Most tissues, well studied in embryonic tissues</td>
<td>2, 3, 10-14, 78-81</td>
</tr>
<tr>
<td>UDP glucuronosyltransferases</td>
<td>Retinoic acid and retinal</td>
<td>Conjugation to form water soluble glucuronides for elimination from the body</td>
<td>Liver, other tissues</td>
<td>2, 3, 82, 83</td>
</tr>
</tbody>
</table>
Postprandial Retinoid Transport and Hepatic Storage

Retinyl esters in chylomicrons enter the circulation and are taken up by tissues; ~70% of chylomicron retinyl ester is taken up by the liver, and the remainder is cleared by peripheral tissues (44). Before uptake by tissues, chylomicron retinyl ester must undergo hydrolysis. In the liver, the process of retinyl ester hydrolysis occurs as the chylomicron remnant particle is being cleared by hepatocytes (liver parenchymal cells) (2,3) but it is not established what enzyme(s) is responsible for hydrolysis. It was proposed that the enzyme lipoprotein lipase (LPL) performs this function in peripheral tissues, facilitating retinol uptake (45,46). Free postprandial retinol taken up by cells is thought to bind immediately to CRBP I (see Table 1.1) that are present in tissues (20,21,47). It was suggested that CRBP I facilitate/optimize the retinol uptake process (21,47). It is well established that hepatocytes are responsible for the uptake of postprandial retinoid into the liver (3,48).

Because retinoid action is very important for maintaining good health, higher animals have developed the capacity to store retinoid as retinyl esters in the liver (2,3). After postprandial retinoid is taken up by the liver, this retinoid is either secreted back into the circulation bound to RBP (see below) or transferred to the hepatic stellate cells for storage (2,3,48). Within the stellate cells, retinoid is stored as retinyl esters in the large lipid droplets that are characteristic of these cells. Thus, after the postprandial retinoid is taken up by hepatocytes and hydrolyzed to retinol, this retinol must again undergo esterification via the actions of LRAT before storage in stellate cell lipid droplets.

The major tissue storage site for retinoid is the liver, although other tissues including the eye, lung, adipose tissue, and skin have the ability to store retinoid, albeit to a much lesser degree than liver. It was estimated that for healthy well-nourished individuals, ~60–80% of the retinoid present in the body will be stored in the liver and ~70% of that is present in the hepatic stellate cells (2,3,48,49). When the body senses a need for retinoid, these esters are hydrolyzed by retinyl ester hydrolase (REH; see below for more detail) to free retinol, which through some poorly characterized process is mobilized from the liver bound to its plasma transport protein, RBP. It remains to be established how a signal is conveyed by the peripheral tissues to the liver in terms of retinoid having to allow for retinoid mobilization from the liver.

Retinol-RBP is secreted from the liver into the circulation as a means of delivering retinol to peripheral tissues (1,17). In the fasted state, for a well-nourished healthy person, >95% of the retinoid in the circulation will be present as retinol-RBP. The liver is the major site of synthesis of RBP in the body, and within the liver, the hepatocyte is the sole cellular site of RBP synthesis (17). Other tissues including adipose tissue, kidney, lung, heart, skeletal muscle, spleen, eye, and testis also express RBP, and this may be important for recycling retinoid from peripheral tissues back to the liver (17). The retinol-RBP complex binds another plasma protein, transthyretin (TTR), and this stabilizes the complex and reduces renal filtering of the retinoid (1,17,50). Once retinol is delivered as retinol-RBP-TTR, it is taken up by cells and either stored
within the cell as retinyl ester or oxidized to retinoic acid for use in regulating gene expression. These processes are summarized in Figure 1.3 and discussed in more detail in the text below.

**Fig. 1.3.** Cellular uptake and processing of retinoids. Retinol is delivered to cells by the circulation after its secretion from the liver of retinol bound to retinol-binding protein (RBP). In the circulation, the retinol-RBP complex undergoes protein-protein interaction with transthyretin (TTR) and circulates as the ternary retinol-RBP-TTR complex (A). Retinol arriving at the cell is then taken into the cell where it is immediately bound by one of the cellular retinol-binding protein family members. In most tissues, this will involve the actions of cellular retinol-binding protein, type I (CRBP I) because CRBP I is widely expressed throughout the body. However, the other three CRBP forms may have roles in this process in tissues where they are expressed (B). Upon cell uptake, there are two possible fates for the retinol. It may be esterified by lecithin:retinol acyltransferase (LRAT) (C) and stored as retinyl esters (RE) in lipid droplets present in the cell. In times of cellular need, a retinyl ester hydrolase(s) can liberate the retinol from the retinyl ester stores (D). Alternatively, retinol can be acted on by one of a number of the retinol dehydrogenases (Retinol DH), which are able to oxidize it to retinal (E). This step is reversible, and retinal can be converted back to retinol by cellular retinal reductases (F). Upon its formation, retinol is usually quickly acted upon by one of several retinal dehydrogenases (Retinal DH) and is irreversibly converted to retinoic acid (G). Retinoic acid then enters the nucleus, binds, and activates one of the retinoid nuclear receptors (H) that regulate transcription of a wide range of target genes (I).
**Retinol Esterification and Retinyl Ester Hydrolysis**

Although the liver and intestine are the major tissue sites of retinol esterification in the body, many tissues are able to esterify retinol and to accumulate some retinyl ester stores. It appears that the major, if not sole, enzyme responsible for catalyzing retinyl ester formation is LRAT. This enzyme catalyzes retinol esterification with long-chain fatty acids (primarily palmitic, stearic, oleic, and linoleic acids) present in membrane phospholipids (2,3,43,51,52). LRAT in the liver is thought to be identical to intestinal LRAT, which synthesizes retinyl esters from dietary retinol for incorporation into nascent chylomicrons. LRAT is also found in the eye and has an important role in the visual cycle (52,53). This role in vision was verified recently by studies in mice that lack the LRAT protein and whose vision is severely attenuated starting at a young age (53). Interestingly, hepatic but not intestinal LRAT activity is regulated by retinoid nutritional status. This regulation seems to involve the presence of a retinoic acid response element that is present in the LRAT gene and probably the actions of RAR and/or RXR (54). This regulation is suggested to give rise to a positive feedback loop when cellular retinoic acid levels are high.

There is also one other candidate enzyme that may be physiologically relevant for catalyzing retinyl ester formation, i.e., acyl-CoA:retinol acyltransferase (ARAT). ARAT esterifies retinol using fatty acids present in the acyl-CoA pool (55). ARAT also differs in the form of retinol it utilizes as a substrate compared with LRAT because ARAT is incapable of esterifying retinol when it is bound to CRBP I or CRBP II (56).

Because retinyl esters represent a storage form of retinoids, they must first be hydrolyzed to retinol before activation to retinoic acid. Unlike LRAT, which is accepted to be the major enzyme responsible for retinyl ester formation, there are many REH that may be responsible for the generation of free retinol from retinyl ester stores (57–59). One is a bile salt–dependent REH (BS-REH). Most or all of the BS-REH activity in liver probably arises from the actions of bile salt–activated carboxylester lipase (CEL) (60). However, because mice lacking CEL display no alterations in retinoid storage, metabolism, or actions, this enzyme cannot be the sole physiologically relevant REHs (60). Another group of enzymes, collectively known as bile salt–independent REHs were described. There are two groups of REHs based on their pH optima, neutral REHs and acidic REHs. It was reported that the activities of the neutral and acidic REHs are unaffected by retinoid nutritional status (58). There also is evidence demonstrating that three known hepatic carboxylesterases (also known in the literature as ES-2, ES-4, and ES-10) act as REHs in vitro (57,59). However, it is not yet established whether any or all of these REHs are physiologically important in retinoid metabolism.

**Retinol Oxidation**

Members of two enzyme families are proposed to have important roles in catalyzing retinol oxidation to retinal, the first of two oxidative steps required for retinoic
acid formation (61–65). The first of these enzyme families is the short-chain dehydrogenase/reductase (SDR) family. The second family is the medium-chain alcohol dehydrogenase (ADH) family. These two enzyme families will be considered separately below.

The SDR Family. At the time this chapter was written, at least a dozen distinct SDRs able to catalyze retinol oxidation were proposed as being important for catalyzing retinol oxidation. The members of the SDR family all range in size between 26 and 34 kDa and are usually associated with cell membrane fractions (61–65). SDRs able to catalyze the oxidation of only all-trans-retinol as well as others that can also catalyze oxidation of 9/13-cis-retinol have been identified (8,9,61–65). It was proposed that this latter group may play an important role in the synthesis of 9-cis-retinoic acid (8,9). These are distributed throughout the tissues of the body, and one or more SDR with retinol dehydrogenase activity are present in tissues such as liver, skin, eye, testis, kidney, and lung, which are known to have a high capacity for retinoid metabolism and/or a great need for retinoic acid. Most SDRs proposed as being physiologically relevant for catalyzing retinol oxidation also possess steroid dehydrogenase activity toward androgens and/or estrogens (62,64–66). It was suggested that the retinoid and steroid metabolisms are connected through these enzymes, which have dual responsibility for the metabolism of each member of each family of very bioactive lipids. However, no data derived from in vivo studies are currently available concerning how the metabolism of either retinoids or steroids influences the metabolism of the other. Nevertheless, the possibility of such interactions is very intriguing and one worthy of much future research attention.

Some SDRs are able to utilize retinol bound to CRBP I as a substrate, but usually these enzymes also will catalyze in vitro oxidation of retinol that was dispersed in a detergent or solubilized in some other manner. Because most of the retinol present in CRBP I-expressing cells is bound to CRBP I, the ability of SDR to recognize retinol bound to CRBP I as substrate was taken as an indication that SDRs with retinol dehydrogenases are physiologically important for retinoic acid formation. Although the biochemical properties of SDRs that have retinol dehydrogenase activity were thoroughly investigated and characterized, the actions of these enzymes are less well studied in living cells or animals. Thus, it remains difficult to assign definitive physiologic roles for these enzymes as mediators of retinol oxidation in the body.

Interestingly, two members of the SDR family, Ret/SDR and RalR1, were identified as being retinal reductases and proposed as physiologically relevant enzymes for reducing retinal formed upon the cleavage of provitamin A carotenoids (67,68). Although both of these enzymes are expressed in the intestine as well as other tissues that express carotene-15,15′-monooxygenase (38–42), the proposed roles of these SDRs in the formation of retinoid from provitamin A carotenoids have not been investigated in a physiologically meaningful context.
However, no studies convincingly confirmed this physiological role for either Ret/SDR or RalR1.

The ADH Family. Retinol oxidation also can be catalyzed by several members of the ADH family. This enzyme family is composed of cytosolic proteins of ~40 kDa and comprises the “classic” alcohol dehydrogenases that were first identified >70 years ago. There are three major ADH isotypes, ADH1, ADH3, and ADH4, proposed to be involved in retinal formation (62–65,69–71). Each of these ADH isotypes is able to use free or detergent-solubilized retinol as a substrate but not retinol bound to CRBP I.

Both the biochemistry and physiology of the ADH enzymes are well studied. Although the ADH have a very broad substrate specificity for alcohols, comparisons of catalytic efficiencies ($k_{cat}/K_m$) for these enzymes indicate that retinol is one of the best naturally occurring substrates for the enzymes (63,69–71). One of the important arguments in favor of the physiologic relevance of the ADH in retinoic acid synthesis is that the different isotypes are strongly expressed in embryonic tissues that synthesize and require high amounts of retinoic acid. This provides circumstantial evidence for an involvement of the ADH in retinoic acid synthesis. Targeted disruptions of ADH1, ADH3, and ADH4 were reported and mice lacking one or more of these ADH isotypes are viable (72). However, when ADH4-deficient mice are stressed through administration of a retinoid-deficient diet, they more quickly succumb to retinoid deficiency than do wild-type mice (72). ADH1-deficient mice are no more susceptible to retinoid deficiency than wild-type mice. Interestingly, however, ADH1/4-double knockout mice are more resistant to retinoid deficiency than the single ADH4-knockout mice. This suggests that ADH1 absence in some undefined manner partially rescues ADH4-deficient mice from their increased sensitivity to retinoid deficiency.

Retinal Oxidation

The second and final step needed for retinoic acid synthesis is the oxidation of retinal to retinoic acid. Several distinct cytosolic aldehyde dehydrogenases (ALDH), which are referred to as retinal dehydrogenases (RALDH) when discussed in the context of retinoid physiology, catalyze the irreversible oxidation of retinal to retinoic acid. These RALDH, RALDH1 (ALDH1a1), RALDH2 (ALDH1a2), RALDH3 (ALDH1a3), and RALDH4 (ALDH8a1) are all distinct members of the ALDH protein family (63–65,73,74). The members of the ALDH family catalyze the irreversible oxidation of aldehydes to acids (63–65,73). This irreversible reaction, as the final step of retinoic acid synthesis, must be tightly regulated to control retinoic acid--induced signaling (63–65,73–75). RALDH1, RALDH2, and RALDH3 were studied in the context of all-trans-retinoic acid formation (63–65,71,75). RALDH4 was suggested to be involved in catalyzing 9-cis-retinal oxidation and consequently might have an important role in the biosynthesis of 9-cis-retinoic acid (74). Unlike the situ-
ation for retinol oxidation, the physiologic roles of these RALDH are generally well defined.

Of the RALDH, RALDH2 is the best studied. The targeted disruption of the gene for RALDH2 gives rise to embryonic lethality at E11.5 due to severe trunk, hindbrain, and heart defects (75). Growth defects were first observed from E8.5 to E10.5, but these could be reversed upon all-trans-retinoic acid administration (75). Using a LacZ reporter construct driven by a retinoic acid sensitive promoter, it was possible to demonstrate that the absence of RALDH2 diminished retinoic acid synthesis; consequently, RALDH2 must be viewed as an essential enzyme responsible for retinoic acid synthesis (75). Disruption of the gene encoding RALDH1 does not result in embryonic lethality but does give rise to lessened synthesis of retinoic acid in the developing eye and to microphthalmia in the adult (76). Thus, RALDH1 appears to have an important role in the synthesis of retinoic acid in the developing eye. Like RALDH2, RALDH3 appears to have an essential function during embryogenesis and/or early in the postnatal period because its absence results in lethality shortly after birth due to choanal atresia giving rise to respiratory distress and death (77). Lethality is reversible upon maternal retinoic acid treatment (77). Clearly RALDH1, RALDH2, and RALDH3 each have an important role in catalyzing retinoic acid formation from retinal. Why distinct RALDH forms are needed by the body to catalyze this reaction is not well understood at present.

Oxidative and Conjugative Metabolism of Retinoids

Once retinoic acid has activated its receptor, it is important for the cell's health that the signal be terminated by the removal of the retinoic acid. This is accomplished through the generation of hydroxyl- and oxo-retinoic acid species and glucuronides of retinoic acid (2,3). In the late 1990s, several groups reported the identification of a specific retinoic acid–inducible cytochrome P450-related retinoic acid hydroxylase, CYP26 (10–14). This enzyme shares structural motifs with other cytochrome P450 species. Expression of CYP26 in cultured naïve cells conferred on these cells the ability to oxidize all-trans-retinoic acid to products identified as its 4-hydroxy- and 4-oxo-metabolites (10–14). Several different isotypes of CYP26 were identified, and each of these seems to have an important role in catalyzing the oxidative breakdown of retinoic acid. Inactivation of CYP26 isotypes through targeted gene disruptions results in impaired embryologic development (10–14,78–81). The lack of CYP26 expression was shown to result in excessive retinoic acid accumulation in embryonic structures (79,81). It is clear from these elegant studies that the oxidative metabolism of retinoic acid catalyzed by CYP26 is an essential mechanism through which the body prevents excessive or unneeded retinoic acid accumulation.

Cytochrome P450 species, other than the CYP26 isotypes, are also reported to catalyze in vitro oxidation of retinoic acid (2,3). Most of these are present in adult tissues and each may have a role in mediating some aspect of retinoid physiology.
in adults. However, the precise role(s) of these enzymes in normal retinoid physiology remains to be established.

Both retinol and retinoic acid undergo conjugation with glucuronic acid, giving rise to retinol- and retinoic acid-β-glucuronides (2,3). The retinoid-β-glucuronides are very water soluble, and their formation likely reflects a second pathway through which excess or unneeded retinoids are eliminated from the body. Several UDP-glucuronosyltransferases were identified; they can catalyze the formation of retinoid-β-glucuronides and have broad substrate specificity for compounds other than retinoids (82,83). There is no information available concerning whether any of these enzymes is specifically responsive to retinoic acid.

Concluding Remarks

In the last 20 years, there has been tremendous growth in our understanding of retinoid metabolism and actions. With the identification and characterization of the retinoic acid nuclear receptors, it became clear how retinoids act physiologically to mediate an apparently diverse array of physiologically essential processes. Many of the enzymes and binding proteins responsible for maintaining normal retinoid physiology also were identified and characterized in this period. We now have a very clear understanding of the enzymatic processes responsible for the storage of retinoids and for the catabolism of excess or unneeded retinoid. We also now have a good understanding of how retinoids are delivered to tissues and how tissues and cells acquire and maintain retinoid pools. However, there are still many areas of retinoid physiology that require elucidation. The true physiologic relevance of many enzymes that are proposed to catalyze retinol oxidation remains to be established. Possible metabolic linkages between steroid and retinoid metabolism require examination. Understanding of how different tissues communicate with each other to facilitate retinoid economy within the body is still lacking. Ultimately, we have a better understanding of the actions of retinoids for maintaining optimal health and preventing disease, but much has yet to be learned if we are to appreciate fully the actions of retinoids within the body.

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