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Natural retinoids and β-carotene: From food to their actions on gene expression

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*Vitamin A (retinol) is found in foods of animal origin in the form of retinyl ester, or in fruits and vegetables as carotenoids with provitamin A activity, especially β-carotene. Inside the enterocytes, retinol binds to cellular retinol-binding protein, type II (CRBPII), which directs its esterification by the enzyme lecithin:retinol acyltransferase (LRAT). β-Carotene may undergo central or eccentric cleavage with the formation of retinoids, or may be released into the circulation unchanged. The retinyl esters synthesized, as well as β-carotene that has not undergone cleavage, are incorporated into chylomicrons and taken up mainly by hepatocytes. In the liver, retinol may be stored in stellate cells as retinyl esters, be oxidized to retinoic acid, or be liberated toward target cells bound to retinol-binding protein (RBP). All-*trans *retinoic acid and its 9-*cis *isomer have binding affinity for nuclear receptors that activate transcription and that bind as dimers to specific nucleotide sequences present in promoters of target genes. Retinoids also modulate gene expression acting at the posttranscriptional level. The existence of nuclear receptors of β-carotene has not been reported thus far. However, it has been demonstrated that β-carotene can also modulate gene expression. Information about the metabolism of retinoids and of β-carotene has also had important repercussions from a clinical viewpoint, such as the administration of all-*trans *retinoic acid to patients with acute promyelocytic leukemia.* (J. Nutr. Biochem. 9:446–456, 1998) © *Elsevier Science Inc. 1998*

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Introduction

It is important to emphasize the recent advances in our understanding of the metabolism of retinoids and carotenoids because of the clinical repercussions of these compounds. This paper will focus on the metabolism of natural retinoids and on β-carotene, because little information is available in the literature about the metabolism of other carotenoids. The term *retinoid* refers to substances including vitamin A (retinol) and its natural metabolites retinaldehyde and retinoic acid, in addition to its synthetic derivatives.[1] This liposoluble vitamin has various actions in many biological processes, participating, for example, in fetal development and the regulation of several aspects of cell metabolism.[2]

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Vitamin A mainly occurs in foods of animal origin in the form of preformed vitamin, and in orange and dark green vegetables and fruits in the form of carotenoids.[3] Approximately 600 carotenoids have been identified thus far in nature. However, less than 10% of them are vitamin A precursors, being denoted as pro-vitamins A. Among these, β-carotene is the carotenoid presenting the highest biological activity.[4] This carotenoid is found in different organs and tissues including blood,[5] both in humans and in animals, where it performs its antioxidant action. There is also evidence that it has anticarcinogenic activity.[6–9] *Figure 1* presents a general view of the metabolism of natural retinoids and of β-carotene, from their presence in the intestinal lumen to their actions at the target cell level.

From foods to the release from enterocytes

Soon after ingestion, foods containing preformed vitamin A in the form of retinyl ester and the carotenoids undergo enzymatic hydrolysis. Afterwards, the subsequent release and aggregation to lipid globules takes place (*Figure 2*).

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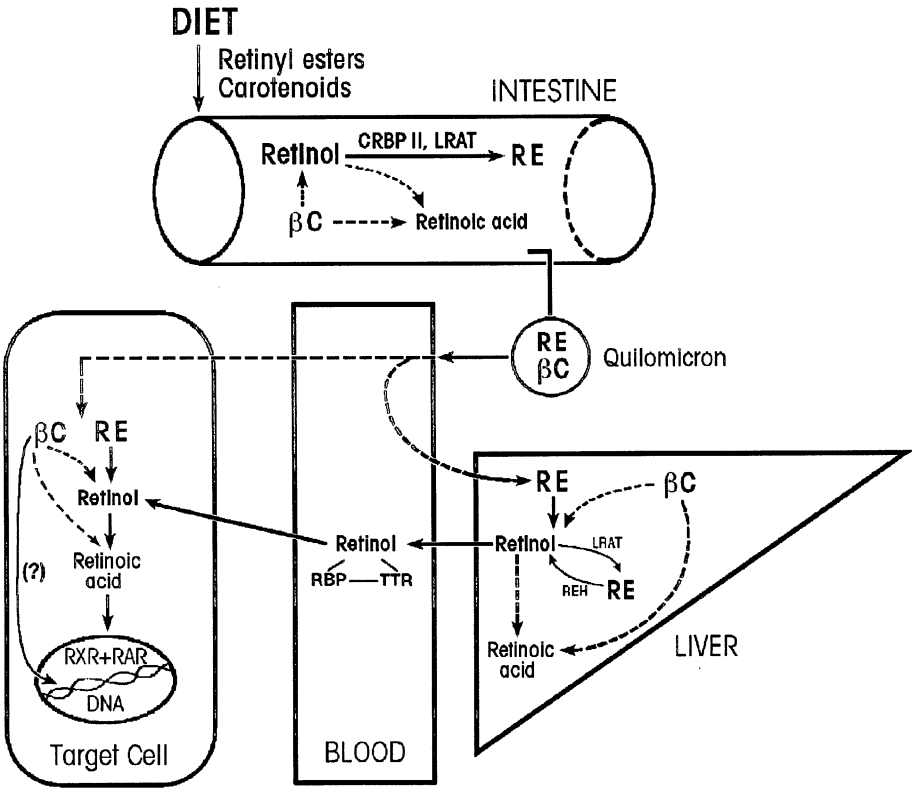

Figure 1 A general view of the metabolism of natural retinoids and of β-carotene, from their presence in the intestinal lumen to their actions at the target cell level. (β-Carotene, βC; retinyl ester, RE; lecithin:retinol acyltransferase, LRAT; cellular retinol-binding protein typeII, CRBPII; retinyl ester hydrolase, REH; retinol binding protein, RBP; transthyretin, TTR; retinoic acid receptor, RAR; retinoid X receptor, RXR).

β-*Carotene*

The absorption of carotenoids basically occurs by passive diffusion and depends on adequate amounts of lipids, proteins, calories, and food fibers in the intestinal lumen.[10,11] High β-carotene concentrations may inhibit diffusion.[12] In the enterocytes, after being converted to retinoids or even after being absorbed unchanged, β-carotene may be incorporated into chylomicrons for transport to other cells of the organism.[13]

Two hypotheses have been suggested for the conversion of this carotenoid to retinoids, i.e., that hydrolysis of β-carotene molecules occurs by central cleavage and/or by eccentric cleavage.[13] In the decade of the 1960s and 1980s, it was demonstrated that β-carotene can be converted to vitamin A through the action of the enzyme 15.15′-dioxygenase (involved in central cleavage), which is present in the cytosol of enterocytes, in the liver, and in the corpus luteum.[14–16] In this case, molecular oxygen may first react with the central two carbon atoms of β-carotene, producing two retinal molecules. These are subsequently reduced to retinol (vitamin A) by the enzyme retinal reductase, or even oxidized to retinoic acid. A later study on the intestinal mucosa of rabbits and rats confirmed the formation of retinal molecules from β-carotene.[17]

The occurrence of eccentric cleavage of β-carotene was first detected in plants and microorganisms,[18] and later in the intestine of rats, ferrets, monkeys, and human beings.[19,20] The reaction gives origin to different chains of β-carotenals 12′-, 10′-, 8′-, which may be converted into retinal or even be oxidized to β-apo-carotenoic acids. The latter are also possibly precursors of retinoic acid (*Figure 2*).[21] However, there is no consensus in the literature about the importance of central or eccentric cleavage in the conversion of β-carotene to retinoids, although both are known to occur.[22]

It is assumed that in humans 1/6 of the β-carotene present in food is converted to vitamin A in the organism.[23] This ratio also depends on factors such as thyroid activity, the presence or absence of stress, and on the presence of dietary interferences such as higher or lower concentrations of nitrates, proteins or lipids, and especially on the concentrations of the carotenoid itself in food.[24]

In humans, most of the absorbed β-carotene is converted to retinol in the enterocytes. However, approximately 15% of this carotenoid is found in the lymphatic system in the intact form.[25] Thus, human beings can accumulate the intact β-carotene molecule in the blood and tissues, the same occurring with other mammals such as horses and ferrets.[26] In other animals, such as rats and chickens, it has been reported that large amounts of the carotenoid present in food are converted to vitamin A. Thus, the rat may not be indicated as an in vivo model for the study of β-carotene metabolism.[27] However, recent studies have demonstrated that this species can accumulate large amounts of β-carotene, especially in the liver, when the substance is added to the diet or administered by gavage.[7–9,28] The presence of a protein-carotenoid complex has also been demonstrated in the liver of rats fed β-carotene, and it has been suggested that this complex may be involved in the storage and transport of the intact molecule of this carotenoid.[29]

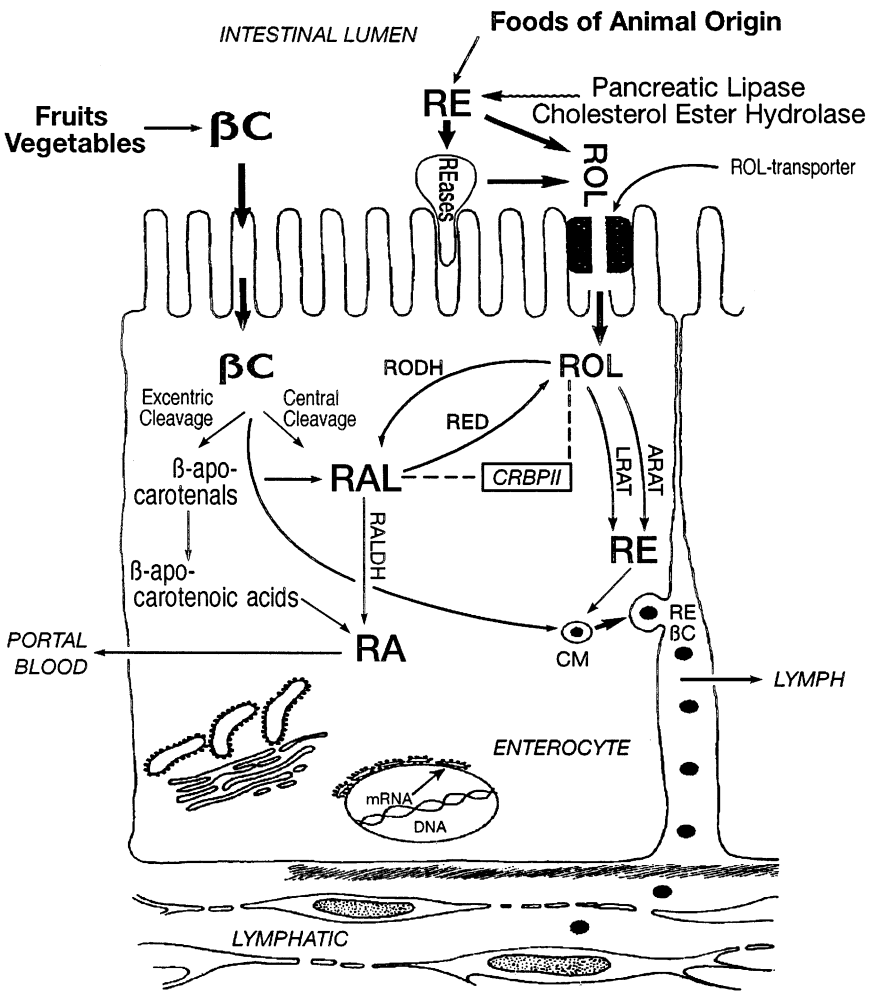

Figure 2 Aspects of the metabolism of natural retinoids and β-carotene, from their presence in the intestinal lumen to their release from enterocytes. (β-Carotene, βC; retinyl ester, RE; retinol, ROL; retinal, RAL; retinoic acid, RA; brush border retinyl ester hydrolases, REases; retinol dehydrogenase, RODH; retinal dehydrogenase, RALDH; retinal reductase, RED; lecithin:retinol acyltransferase, LRAT; acyl:CoA retinol acyltransferase, ARAT; cellular retinol-binding protein typeII, CRBPII; chylomicron, CM).

Retinoids

Two pancreatic enzymes have been identified in the lumen of the small intestine as being responsible for the hydrolysis of retinyl esters. One of them, cholesterol ester hydrolase, is stimulated by bile salts, acting on a wide variety of esterified substrates. In contrast, the other, pancreatic lipase, is inhibited by bile salts.[30] In this respect, little attention has been paid to the activity of pancreatic lipase, and it is believed that cholesterol ester hydrolase is mainly responsible for the hydrolysis of retinyl esters in the intestinal lumen.

Two enzymes located on the apical plasma membrane of the brush borders of enterocytes have also been reported to have hydrolytic activity on retinyl esters. These enzymes were first detected in rats and, more recently, in human beings.[31] One of them has properties similar to those of pancreatic cholesterol ester hydrolase, i.e., it is stimulated by bile salts, especially taurocholate, and acts more specifically in the hydrolysis of short-chain retinyl esters. As to its origin, it is still being debated whether it is in the pancreas itself with later binding to the membrane of the brush border of enterocytes. The other enzyme, intrinsically present in this membrane, is stimulated by bile salts, especially deoxycholate, and preferentially acts in the hydrolysis of long-chain retinyl esters.[31]

Free retinol is obtained as a result of the activity of these enzymes and is absorbed by the enterocytes by facilitated diffusion at physiological concentrations (*Figure 2*), or by passive diffusion at pharmacological concentrations.[32] After being absorbed, vitamin A (retinol) undergoes esterification with long-chain fatty acids, especially palmitic or stearic acid. The enzymes involved are lecithin: retinol acyltransferase (LRAT), which utilizes the acyl group at the *sn*-1 position of phosphatidylcholine as a source of fatty acids, and acyl:CoA retinol acyltransferase (ARAT), which utilizes free acyl groups.[33,34]

Retinol in its free form may cause deleterious effects on cells.[35] However, this vitamin has a high binding affinity for two specific cytoplasmic proteins, the cellular retinol-binding proteins CRBP and CRBPII.[35] These, although sharing 56% of identical amino-acid sequences, differ in their physiological actions.[36] CRBPII is located exclusively in enterocytes, where it represents about 1% of cytosol protein, whereas CRBP is present in different tissues such as liver, kidneys, testicles, lungs, and small intestine muscles.[37,38] CRBPII and CRBP bind to retinaldehyde, the first

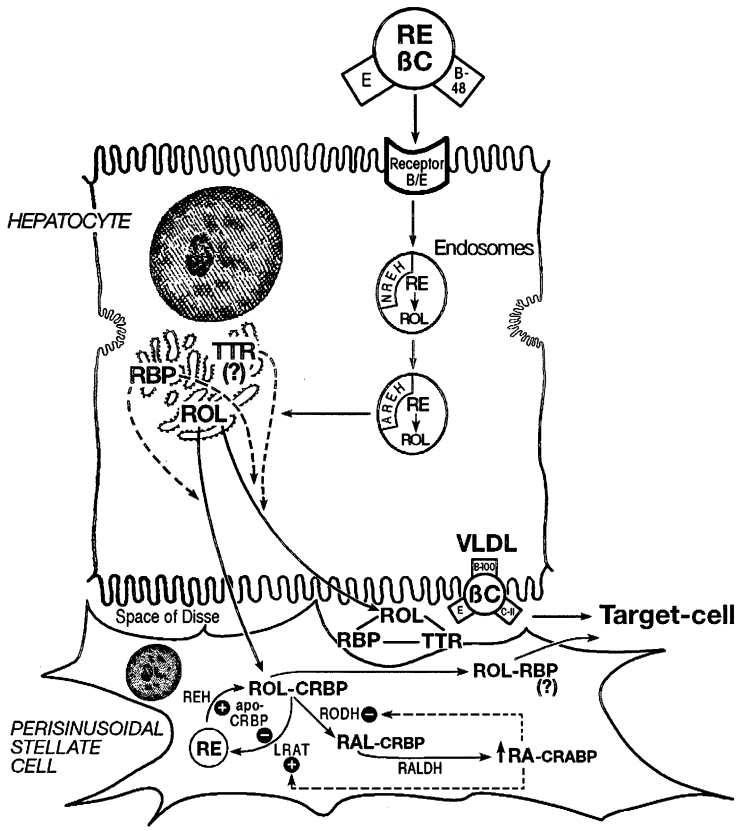

Figure 3 Aspects of the hepatic metabolism of natural retinoids and of β-carotene, from their uptake by the hepatocyte to their release toward the respective target cells. (β-carotene, βC; retinyl ester, RE; retinol, ROL; retinal, RAL; retinoic acid, RA; neutral and acid retinyl ester hydrolases, NREH and AREH; retinol dehydrogenase, RODH; retinal dehydrogenase, RALDH; retinyl ester hydrolase, REH; lecithin:retinol acyltransferase, LRAT; retinol binding protein, RBP; transthyretin, TTR; cellular retinoic acid binding protein, CRABP; cellular retinol binding protein, CRBP; very low density lipoprotein, VLDL).

with the same affinity it shows for retinol. Retinaldehyde, however, can dislocate retinol only from CRBPII.[39] Furthermore, CRBPII has the ability to bind to the 13-*cis* isomer of retinol and to 3-dehydroretinol.

Several lines of evidence suggest that CRBPII plays fundamental roles in the regulation of retinol absorption into enterocytes and its intracellular metabolism, also acting on the reaction of retinaldehyde (a product of β-carotene cleavage) reduction to retinol.[38,40,41] CRBPII bound to retinol directs the aldehyde more specifically toward microsomal reductase, thus preventing its free access to cytosol reductases.[41] Inside the enterocytes, CRBPII-bound retinol undergoes esterification specifically by the enzyme LRAT (*Figure 2*). However, when present in high intracellular concentrations, vitamin A will be esterified by the enzyme ARAT.[42]

From the enterocyte to the hepatocyte and to the stellate perisinusoidal cell

Synthesized retinyl esters, as well as carotenoids that did not undergo cleavage, are incorporated into chylomicrons, which are transferred to the lymphatic system by exocytosis (*Figure 2*). In the systemic circulation, this lipoprotein is submitted to hydrolysis of its triacylglycerols and also to changes in the respective apoproteins, resulting in the formation of chylomicron remnants.[43] These remnants, in turn, play an important role in the distribution of retinoids and carotenoids to the liver, to adipose and skeletal muscle tissue, and to bone marrow.[42] *Figure 3* illustrates aspects of the hepatic metabolism of natural retinoids and of β-carotene, from their uptake by the hepatocytes to their release toward the respective target cells.

β-*Carotene*

The carotenoids present in the chylomicron remnants may be converted to retinoids in the liver or be incorporated into very low density lipoproteins (VLDL) and thus be again transported to peripheral cells.[44] Low density lipoprotein (LDL) represents the major carrier of β-carotene in plasma.[45]

It has been observed that the liver is the major site of β-carotene accumulation in the organism when diets supplemented with this carotenoid are administered to various species such as ferrets, rats, and chickens.[27,28] With respect to its subcellular localization, studies in chicken and rat liver have demonstrated that β-carotene is mainly present in the mitochondrial fraction, followed by the lysosomes.[29,46]

Retinoids

Most of the retinyl esters present in chylomicron remnants, about 70 to 80%, are taken up by the liver.[47] The presence of two different cell types responsible for vitamin A metabolism has been reported in this organ. One of them, represented by hepatocytes, is directly involved in the uptake of chylomicron remnants as well as with the synthesis and secretion of retinol-binding protein (RBP). The

other, consisting of the stellate, or Ito or perisinusoidal cells, is responsible for the storage of vitamin A in the form of retinyl esters, especially in the periportal region.[42,48,49]

Thus, the chylomicron remnants are taken up by hepatocytes, which present B/E receptors on their surface,[50] with subsequent endocytosis. The retinyl esters constituting these remnants are then rapidly hydrolyzed. This is possibly brought about by acid and neutral hydrolases, with activities independent of bile salts, and which are present on the plasma membrane and/or in the endosome of the hepatocyte.[51,52] The retinol formed, instead of being transferred to the lysosomes, goes to the endoplasmic reticulum, where RBP is also synthesized and is present in high concentrations (*Figure 3*). After retinol binding to RBP, the holoprotein migrates to the Golgi complex, with subsequent secretion outside the cell.[53] Each retinol molecule circulates several times between the liver and extrahepatic tissues before being irreversibly degraded. As the half-life of RBP in plasma is shorter than the half-life of retinol, this implies the need for additional RBP synthesis for vitamin A recirculation to occur.[54]

In this way, the retinol-RBP complex liberated by the hepatocytes has the basic function of satisfying the vitamin A requirements of the organism, with the remainder being stored in stellate cells.[48] The mechanisms by which retinol is transferred from hepatocytes to stellate cells are not fully understood. It has been suggested that the holo-RBP molecule may be responsible for intercellular transfer. The possible occurrence of incorporation of this complex into stellate cells has been discussed.[55–57] Another possibility may be the existence of a carrier protein identified thus far only in the retina,[58] but not in the liver. This protein may mediate retinol transfer between hepatocytes and stellate cells. Furthermore, it has been suggested that retinol may be directly transferred by intracellular communications of the desmosome type.[57,59]

Stellate cells represent the major site of vitamin A storage in the liver, containing 80 to 90% of total hepatic retinol, which is present in the form of retinyl esters in 98% of cases.[55] Stellate cells also have large quantities of cellular retinol binding protein (CRBP) and cellular retinoic acid binding protein (CRABP), as well as enzymes capable of synthesizing (LRAT and ARAT) and hydrolyzing retinyl ester.[49,60] As also observed in the small intestine, under physiological conditions, retinol bound to CRBP is esterified in the liver preferentially by the enzyme LRAT (*Figure 3*). When present in high concentrations, retinol can also be esterified by the enzyme ARAT. Thus, the two enzymes may be involved in the esterification of hepatic retinol depending on the concentrations of the latter and whether or not it is bound to CRBP.[42,61] However, the low level of saturation of the CRBP molecule, i.e., the presence of high apo-CRBP concentrations, blocks retinol esterification in addition to stimulating the mobilization of stored retinyl esters. This basically occurs through two major mechanisms, i.e., LRAT inhibition, and stimulation of retinyl ester hydrolysis independent of bile salts (*Figure 3*).[62,63]

Furthermore, it has been reported that CRBP is also involved in the conversion of hepatic retinol to retinoic acid via retinaldehyde.[64] Retinoic acid, in turn, also plays an important role in the regulation of hepatic retinol metabolism. An in vitro study demonstrated that retinoic acid at high concentrations inhibits the conversion of retinol to retinal in addition to stimulating retinyl ester synthesis (*Figure 3*).[65]

For retinol to be released from stellate cells into the circulation, the stored retinyl esters must first be hydrolyzed. After retinol formation occurs, retinol associates with the RBP molecule. However, the ability of stellate cells to synthesize and secrete RBP is debated (*Figure 3*).[66,67]

Excessive retinol ingestion over long periods of time (hypervitaminosis A) may cause liver damage and later cirrhosis. It has been suggested that the toxicity of vitamin A may be caused by the high retinol concentration that may exceed the binding ability of the RBP molecule, resulting into retinol precipitation and therefore damage to the cell membranes.[68] Another possible mechanism responsible for toxicity may be through the interaction of retinoids with their respective nuclear receptors.

Transport to the target cell and subsequent uptake

The retinol-RBP complex of hepatic cells associates in the systemic circulation, or even while still inside the cell in the lumen of the endoplasmic reticulum (*Figure 3*), with the protein transthyretin (TTR).[42] This protein also presents a binding site with a high affinity for thyroxine, and its function is to reduce glomerular filtration of the retinol-RBP complex. However, a recent study on knockout TTR^- mice demonstrated that, even though these animals have low plasma concentrations of the retinol-RBP complex, they do not present vitamin A deficiency. This may be possibly justified by the fact that mice also show increased plasma concentrations of all-*trans* retinoic acid, suggesting the existence of a possible compensatory mechanism.[69] Furthermore, no increase in RBP concentrations was observed in the kidney and urine in the same study, suggesting that transthyretin probably controls the hepatic release of the retinol-RBP complex.[69,70] *Figure 4* illustrates aspects of the metabolism of natural retinoids and of β-carotene from their uptake by target cells to their eventual actions at the gene expression level.

The mechanisms by which the cells of the organism take up retinol present in the retinol-RBP-transthyretin complex are still quite debatable. Some studies have suggested the existence of a specific RBP receptor present on the cell membranes.[71–73] This was first identified in pigmented retinal cells and later also in the placenta, kidneys, small intestine, spleen, liver, bone marrow, choroid plexus, and the pineal gland.[73] In 1991, the RBP receptor present on the membrane of the retinal epithelium was isolated and found to be a 63-kDa glycosylated protein.[74]

There is also evidence that cells can take up retinol from the retinol-RBP-transthyretin complex without the need for specific receptors present on the plasma membrane.[56] Thus, it has also been demonstrated that retinol crosses the plasma membrane simply by a "flip-flop" mechanism and that this occurs in the presence of high intracellular apo-CRBP concentrations.[75]

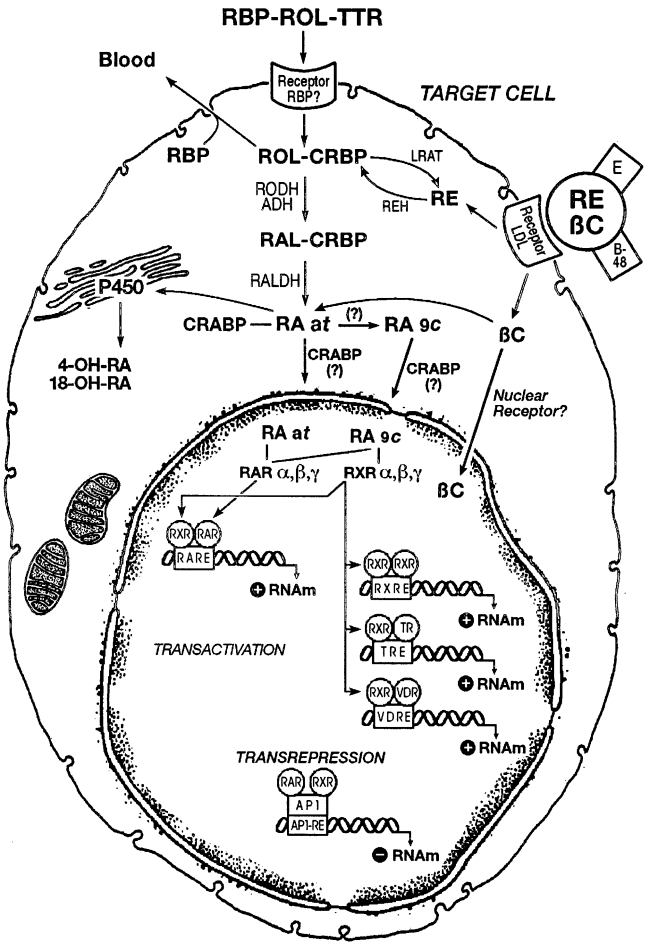

Figure 4 Aspects of the metabolism of natural retinoids and of β-carotene, from their uptake by target cells to their eventual actions at the gene expression level. (β-carotene, βC; retinyl ester, RE; retinol, ROL; retinal, RAL; all-*trans* retinoic acid, RA *at*; 9-*cis* retinoic acid, RA9*c*; retinol binding protein, RBP; transthyretin, TTR; cellular retinol-binding protein, CRBP; cellular retinoic acid binding protein, CRABP; retinol dehydrogenase, RODH; retinal dehydrogenase, RALDH; retinyl ester hydrolase, REH; lecithin:retinol acyltransferase, LRAT; alcohol dehydrogenase, ADH; retinoic acid receptor, RAR; retinoid X receptor, RXR; retinoic acid response element, RARE; retinoid X response element, RXRE; vitamin D response element, VDRE; thyroid hormone response element, TRE; activator protein 1, AP1; AP1 response element, AP1-RE).

Metabolism of retinol to retinoic acid

Inside the cells, retinol may follow different metabolic pathways (*Figure 4*). It may be stored in the form of retinyl esters, it may be metabolized to retinoic acid, or it may be again recycled into the systemic circulation, because several tissues such as the kidney, placenta, Sertoli cells, adipose tissue, and the liver can synthesize RBP.[36,44] Retinoic acid is also present in plasma in low concentrations and is taken up by the cells, presumably by diffusion.[36]

After 1987, with the important discovery of the existence of nuclear retinoic acid receptors, researchers started to devote more time to studies related to the mechanisms involved in the regulation of the intracellular concentrations of this retinoid.[76,77] Conversion of retinol to retinoic acid initially involves the oxidation of vitamin A to retinal and later the oxidation of the latter to retinoic acid. In this respect, the enzymes alcohol dehydrogenase and retinol dehydrogenase have been reported to act on the formation of retinal, the limiting step in the pathway of retinoic acid formation.[78] Alcohol dehydrogenase is present in the cell cytosol where it catalyzes the oxidation not only of ethyl alcohol and other substrates, but also of all-*trans* retinol and its 9-*cis* and 13-*cis* isomers.[79] In contrast, retinol dehydrogenase is present both in the cytosol and in the microsome fraction, where it catalyzes the oxidation of retinol to retinal.[80] The retinol dehydrogenase isoenzyme present in the cytosol has little affinity for the substrate and is inhibited by apo-CRBP. In contrast, the microsomal isoenzyme has high affinity for retinol in its free or CRBP-bound forms, and is not inhibited by the latter. Neither enzyme can oxidize the 9-*cis* or 13-*cis* isomers of retinol.[41,81–83]

The enzyme retinal dehydrogenase and members of the cytochrome P450 family, its 1A1 and 1A2 forms, have been described as participating in the oxidation reactions of retinal and of its 9-*cis* isomer to their respective retinoic acids. Four retinal dehydrogenase isoenzymes have been identified thus far. They recognize as a substrate the retinal generated by the enzyme retinol dehydrogenase, synthesizing in this way retinoic acid. Several other isoenzymes of the cytochrome P450 system can also participate in the metabolism of retinoid acid, producing polar compounds such as 4-OH and 18-OH-retinoic acids (*Figure 4*), which will later be excreted by the cell.[82,84,85]

Thus, in view of the observation that the microsomal enzyme retinol dehydrogenase recognizes the retinol-CRBP complex as a substrate, it has been suggested that retinol is transferred from this complex through a protein-protein interaction to the enzyme retinol dehydrogenase, which oxidizes vitamin A, producing retinaldehyde.[86] The latter, in the form of a retinal-CRBP complex, then migrates to the cytosol, where the enzyme retinal dehydrogenase is also present and can oxidize it to retinoic acid. It has been reported that the derivation of retinoic acid from β-carotene (*Figure 4*) may preferentially occur from the eccentric pathway of cleavage of the carotenoid molecule.[22] This pathway results in β-apo-carotenals that may give origin to retinoic acid directly, and not retinaldehyde, as the latter, when bound to CRBPII, is not so actively directed toward the enzyme retinal dehydrogenase.

In situations of relative vitamin A deficiency, there is an increase in apo-CRBP concentration.[41] In turn, this can stimulate the hydrolysis of stored retinyl esters and inhibit LRAT, but not microsomal retinol dehydrogenase, thus permitting the continuous production of retinoic acid under these conditions.[87]

Cells of different tissues such as brain, ovary, testicle, uterus, kidney, and liver present a cytosol protein capable of binding to retinoic acid (CRABP) whose real function is still unclear (*Figure 4*).[36] This protein mainly has binding affinity for all-*trans* retinoic acid but also binds to its 9-*cis* and 13-*cis* isomers and to its polar metabolite, 4-OH-retinoic acid.[88,89] Furthermore, a protein with affinity for retinoic acid has been reported, CRABPII, which has properties similar to those of CRABP. This protein is

detected during embryogenesis, being restricted to the skin in adults.[90]

The CRABP molecule has been reported to be possibly involved in the transport of retinoic acid from the cytosol to the nucleus (*Figure 4*). However, CRABP can also direct this retinoid to the cytochrome P450 system. On this basis, it has been suggested that this protein participates in the homeostasis of retinoic acid inside the cell, sequestering it and limiting its distribution and biological effects.[36,83]

Formation of 9-*cis* retinoic acid

The observation that 9-*cis* retinoic acid has a high binding affinity for nuclear receptors has led to questions about its origin in the organism. However, its exact mechanism of synthesis is unknown. A possibility is the isomerization of all-*trans* retinoic acid to 9-*cis*, with questions about the participation of enzymes in this process.[91] Another possibility is that 9-*cis* retinoic acid is formed in the organism from 9-*cis* β-carotene and from 9-*cis* retinol, which are present in the diet.[92] These compounds, after cleavage (9-*cis* β-carotene) or oxidation (9-*cis* retinol) of their molecules may give origin to 9-*cis* retinal. This, in turn, after being oxidized by the enzyme retinal dehydrogenase, may give origin to 9-*cis* retinoic acid.[93,94] A recent study on humans has reported that after the administration of 9-*cis* β-carotene, *cis-trans* isomerization of the carotenoid occurred before its secretion into the bloodstream.[95] This mechanism may be responsible for the reduced concentrations of this isomer in chylomicrons, a fact that might actually limit the synthesis of 9-*cis* retinoids at the tissue level.[96] Nevertheless, 9-*cis* β-carotene is found in tissues, representing 25% of the total β-carotene in the liver.[97]

Nuclear receptors for retinoids

Many of the actions of retinoids are mediated by nuclear receptors belonging to the family of receptors for steroid and thyroid hormones, as well as receptors for vitamin D, which act as transcription factors depending on their ligands.[98] These nuclear receptors are structurally similar, presenting an A/B region close to the amino-terminal end of the protein, which is important in the activation of gene transcription. They also have a C region containing two zinc-binding motifs involved in receptor binding to specific DNA sequences. Finally, an E region close to the carboxyl-terminal region is responsible for the receptor binding to its ligand and for the formation of dimers. The functions of the D and F regions of these receptors have not been fully clarified.[99]

Two different types of nuclear receptors for retinoids have been described, i.e., RAR, and RXR receptors, with their respective α, β, and γ subtypes (*Figure 4*), coded by separate genes. RAR receptors can bind both to all-*trans* retinoic acid and to its isomer, 9-*cis* retinoic acid, and to 4-oxo-retinol. However, they have no affinity for the 13-*cis* isomer. In contrast, RXR receptors have affinity only for 9-*cis* retinoic acid.[100]

The RAR and RXR receptors vary in terms of distribution in the various tissues both during development and in adult life. Thus, it has been suggested that they have distinct functions in the regulation of gene transcription. For example, expression of the genes that code for RARα and RXRβ receptors has been observed in several tissues of both embryos and adult animals, whereas expression of the gene that codes for the RARβ receptor has been observed only in the kidneys, muscle, and prostate of adult animals.[99,100] In addition, expression of the gene that codes for the RARγ receptor has been observed in the skin, suggesting its involvement with morphogenesis and epithelial differentiation.[101] RXRα and RXRγ receptors are expressed in only some tissues, with the former being present in the liver, skin, and kidneys, and the latter only in muscle and in the heart.[100]

Studies have been conducted on knockout mice to identify the functions of these receptors in vivo. Thus, RARγ knockout mice have been reported to present congenital alterations, growth deficiency, and premature death.[102] The same features have been observed in RARα knockout mice, which also demonstrate testicular degeneration.[103,104] Furthermore, in a recent study on F9 cells, in which mutation of the gene for the RARγ receptor was induced, there was a loss of expression of some genes that respond to retinoids, such as Hoxa-1, Hoxa-3, laminin B1, and collagenase IV, as well as reduced metabolism of all-*trans* retinoic acid to its polar derivatives.[105] In this same study, the induction of mutation in the RARα gene resulted in increased metabolism of the retinoid and reduction of the expression of the genes for CRBPII and Hoxb-1. On this basis, it can be concluded that each receptor subtype presents a specific function, modulating the expression of certain genes.

Actions of retinoids on gene expression

Nuclear retinoid receptors are believed to act as activators of gene transcription, binding as dimers to specific nucleotide sequences present in the response elements of their target genes.[100] This process is called *transactivation*. RXR receptors are the only ones capable of forming homodimers (RXR-RXR), acting in this way on genes such as CRBPII and apo-A1. On the other hand, they also form heterodimers with receptors for retinoic acid (RXR-RAR), for vitamin D (RXR-DR), for thyroid hormone (RXR-TR) (*Figure 4*), and with the peroxisome proliferator activated receptor (PPAR). Heterodimers are more stable than homodimers and have greater affinity for the promoting region of DNA.[100,106]

The regulation of transcription by the RXR-RAR heterodimer has been reported to require only the presence of the RAR receptor ligand, i.e., the presence of all-*trans* retinoic acid.[107] However, recent studies have reported the presence of the two ligands in the RXR-RAR heterodimer (*Figure 4*), suggesting an active role of RXR also in gene transcription. RXR may stabilize the binding of the heterodimer to specific DNA sequences. It has also been demonstrated that only the ligand of the RAR receptor stimulates the binding of the heterodimer to the DNA promoting region, but the presence of the RXR receptor ligand enhances gene expression.[108]

The RXR-RAR heterodimer and the RXR-RXR homodimer recognize specific sequences of nucleotides present in retinoid-responsive genes (*Figure 4*). These

elements consist of three or more repetitions of the AGG TCA sequence. The sequences of the retinoic acid response element (RARE), i.e., for the RXR-RAR heterodimer, are separated by two or five nucleotides (DR2 and DR5), with the DR5 spacing being actually the most frequent. In contrast, the RXR-RXR homodimer response element (RXRE) recognizes these same sequences when they are separated by only one nucleotide (DR1). RAREs and RXREs are also able to bind homo- or heterodimer complexes of orphan nuclear receptors such as the apo-A1 regulatory protein (ARP-1), nuclear hepatocyte receptors (HNF-4), and the v-*erb*A-related protein 3 (EAR 3).[106,109,110]

Retinoids can stimulate or even inhibit gene transcription. Examples of transactivation are the genes for growth hormone and oxytocin, some cell growth factors, enzymes such as phosphoenolpyruvate and alcohol dehydrogenase, genes for the CRBPI and CRABPII proteins, and some proteins of the extracellular matrix, such as certain collagenases and laminin B.[111] In this case, retinoids may act in a direct manner by synthesizing a protein after the binding of their nuclear receptors (RAR or RXR) to the response element of a given gene in DNA, or even in an indirect manner, producing a transcription factor that will act on target genes. Examples of genes whose transcription is inhibited by retinoids are the genes for certain growth factors such as IGF-I and TGF-α,[111] as well as the gene for protein Gla of the extracellular matrix.[112]

The actions of retinoids on gene transcription depend on the amounts and types of receptors present, as well as on the concentrations of their ligands. Thus, in the presence of a low 9-*cis* retinoic acid concentration and/or large amounts of RAR receptors, there is a preferential formation of the RXR-RAR heterodimer. Under these conditions, the RXR-RAR heterodimer binds to the RXRE element, thus inhibiting gene transcription, as in the case of the gene for protein CRBPII.[113]

Retinoids can also act at the posttranscriptional level, increasing the stability[114] or half-life of messenger RNA,[115] and regulating the processing of transcript precursors.[116] Furthermore, retinoids can inhibit the actions of some oncogenes, of certain collagenases, and of TGF-β1 by the interaction of RAR and RXR receptors with the AP-1 protein complex (a dimer of the fos and jun proteins). This process is denoted *transrepression* and results in the loss of the ability of this transcription factor to bind to the response elements of the respective genes (*Figure 4*).[111,117] AP-1 deregulation is associated with inflammatory states and malignant transformation.[118] Thus, AP-1 transrepression seems to represent an important mechanism responsible for the anti-inflammatory and antiproliferative activities of retinoids. Retinoids that preferentially transrepress AP-1 may represent valuable therapeutic agents.[118]

Actions of β-carotene on gene expression

There are no reports thus far of the existence of nuclear carotenoid receptors or of data demonstrating their affinity for the nuclear retinoid receptors themselves. However, the possible existence of a new class of nuclear receptors for certain carotenoids has been suggested (*Figure 4*) as it has been observed that β-carotene can also act at the molecular level. This carotenoid has been reported to regulate the expression of the connexin 43 gene in an intrinsic manner independent of its activity as pro-vitamin A.[119] β-Carotene can also act at the posttranscriptional level. Thus, it has been described that this carotenoid inhibits HMGCoA reductase gene expression, an enzyme considered to be the rate-limiting step in the metabolism of cholesterol, in rat treated with the carotenoid and submitted to partial hepatectomy.[120]

Retinoids and acute promyelocytic leukemia

It has been recently demonstrated that all-*trans* retinoic acid can induce remission in a large proportion of patients with acute promyelocytic leukemia. This is a subtype of acute myeloid leukemia mainly characterized by leucocytosis and thrombocytopenia.[121,122] At the molecular level, the existence of a t (15;17) chromosome translocation has been reported in these patients, fusing the gene PML of chromosome 15 with the gene for the RARα receptor located on chromosome 17. This generates the PML/RARα transcript in all individuals and, to a lesser extent, the RARα/PML transcript, detectable in 70% of patients. It has also been reported that the PML/RARα protein has dominant effects on RAR receptors and on the PML protein. On this basis, the RARα/PML protein blocks neutrophil differentiation and the transactivation of retinoic acid responsive genes.[123,124]

Thus, the administration of all-*trans* retinoic acid results in increased gene expression for the normal RARα receptor and in restoration of the differentiation of immature neoplastic cells into neutrophils, with consequent improvement of the coagulopathy.[125] Despite the progress in the treatment of acute promyelocyitic leukemia with the administration of all-*trans* retinoic acid, most patients acquire resistance to this retinoid. It is believed that this fact is related to the increased concentration of CRABII protein and of cytochrome P450 enzymes, resulting in greater retinoic acid catabolism and in a decrease in its plasma levels.[123]

In this respect, the use of cytochrome P450 enzyme inhibitors is being investigated, and a recent publication has reported that fluconazole administration reversed the accelerated metabolism of all-*trans* retinoic acid in patients with acute promyelocytic leukemia.[126] Another strategy proposed is the use of retinoids with low binding affinity for CRAPII protein such as 9-*cis* retinoic acid.[123]

Future directions

Natural retinoids are fundamental for the regulation of biological processes such as vision, reproduction, embryogenesis, growth, and cell differentiation. Among them, retinoic acid, formed by intracellular retinol (vitamin A) oxidation or by cleavage of β-carotene, is considered to be the major retinoid responsible for most functions of vitamin A. This is due to the ability of retinoic acid to act at the molecular level by regulating the expression of several genes. Various specific proteins and enzymes involved in retinoid and β-carotene metabolism have been identified.

However, several aspects of the metabolism of these substances still need clarification.

For example, are the mechanisms involved in the conversion of β-carotene to vitamin A at the intestinal level or other levels capable of justifying the variations reported in the literature for this conversion ratio? Are they homeostatic? Could they be coupled to β-carotene cleavage? Which of the carotenoid cleavage forms, central or eccentric, is responsible for the formation of larger amounts of retinoic acid? What mechanisms are involved in the intracellular homeostasis of all-*trans* and 9-*cis* retinoic acids? How are the retinoids with binding affinity for nuclear receptors transferred to the nucleus? What is the participation of the protein identified in rat liver in the form of a complex with β-carotene in the metabolism of this carotenoid or even of others? And finally, are there nuclear receptors for β-carotene and other carotenoids?

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