Colors with Functions: Elucidating the Biochemical and Molecular Basis of Carotenoid Metabolism

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Abstract

Carotenoids affect a rich variety of physiological functions in nature and are beneficial for human health, serving as antioxidants in lipophilic environments and blue light filters in the macula of human retina. These dietary compounds also serve as precursors of a unique set of apocarotenoid cleavage products, including retinoids. Although knowledge about retinoid biology has tremendously increased, the metabolism of retinoids' parent precursors remains poorly understood. Recently, molecular players in carotenoid metabolism have been identified and biochemically characterized. Moreover, mutations in their corresponding genes impair carotenoid metabolism and induce various pathologies in animal models. Polymorphisms in these genes alter carotenoid and retinoid homeostasis in humans as well. This review summarizes our current knowledge about the molecular/biochemical basis of carotenoid metabolism and particularly the physiological role of carotenoids in retinoid-dependent physiological processes.

Contents

INTRODUCTION

We all are familiar with carotenoids that manifest themselves as yellow to red colors of fruits, flowers, and vegetables. Carotenoids, a class of 600 related isoprenoid compounds (mostly C40) containing up to 15 conjugated double bonds, are divided into two major groups according to their chemical structures. Pure hydrocarbons such as β,β-carotene are called carotenes, whereas their oxygenated derivatives such as zeaxanthin are called xanthophylls (**Figure 1**).

Among different carotenoids, some 50 exist in the human diet, from which only about ten are present in significant amounts in human plasma. Human tissues retain considerable amounts of carotenoids. Physiological functions attributed to these compounds are associated with their capability to act as antioxidants, e.g., as free radical scavengers, in lipophilic environments such as membranes and lipoproteins (for review, see 23). Additionally, the central part of the human retina, the macula lutea, owes its yellow color to high levels of the xanthophylls, lutein and zeaxanthin (6, 113). These carotenoids are excellent absorbers of phototoxic blue light and reactive oxygen species and probably prevent light damage to the retina (for review, see 62).

Carotenoids can be transformed to apocarotenoids such as retinoids, which encompass all natural and synthetic derivatives of vitamin A (all-*trans*-retinol) (**Figure 2**). Vitamin A is the precursor for at least two essential biologically active molecules, 11-*cis*-retinal and all-*trans*-retinoic acid. 11-*cis*-Retinal binds to the protein moiety (opsin) of visual pigments that mediate phototransduction (91).

The second critical compound, all-*trans*-retinoic acid (RA), is required for a wide range of biological processes, including reproduction, embryonic development, cell differentiation, immunity, and metabolic control. This hormone-like substance is the ligand of nuclear receptors such as retinoic acid receptors (RARs) (92). RARs form heterodimeric complexes with retinoid X receptors (RXRs) that can bind 9-*cis*-retinoic acid. Besides RARs, RXRs also are obligate heterodimeric partners of other nuclear receptors, including peroxisome proliferator-activated receptors (PPARs). Several recent studies have provided evidence that retinoids can modulate the activities of these transcription factors (for review, see 144).

Vitamin A deficiency is associated with severe health problems that can cause blindness and contribute to increased mortality rates of children and pregnant women in Asia and Africa (118). However, low vitamin A intake has also been described in western societies. Fifteen percent of young individuals aged 19–24 years in the United Kingdom have total vitamin A intakes below the lower recommended nutrient intake level (24), and almost half of American

Apocarotenoids: organic isoprenoid compounds derived from oxidative cleavage of carotenoids and found in all kingdoms of nature. Examples include vitamin A and the plant hormone abscisic acid

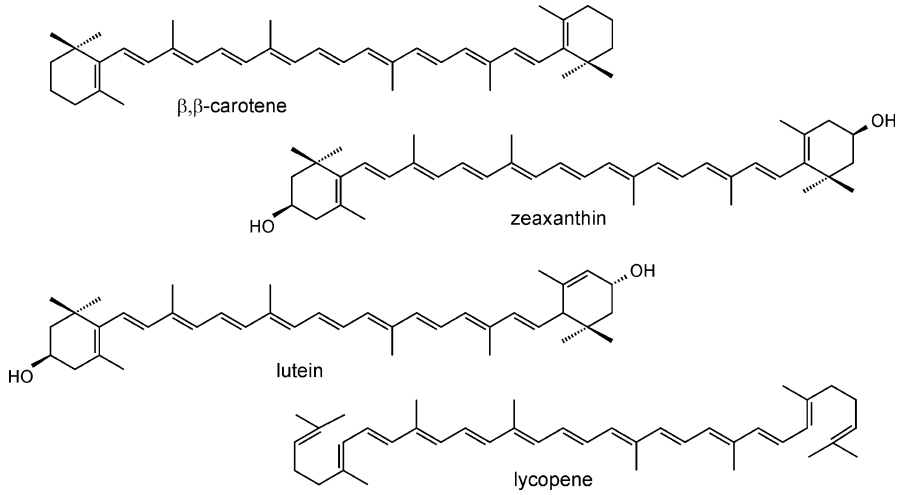

Figure 1

Chemical structure of major carotenoids in the human blood.

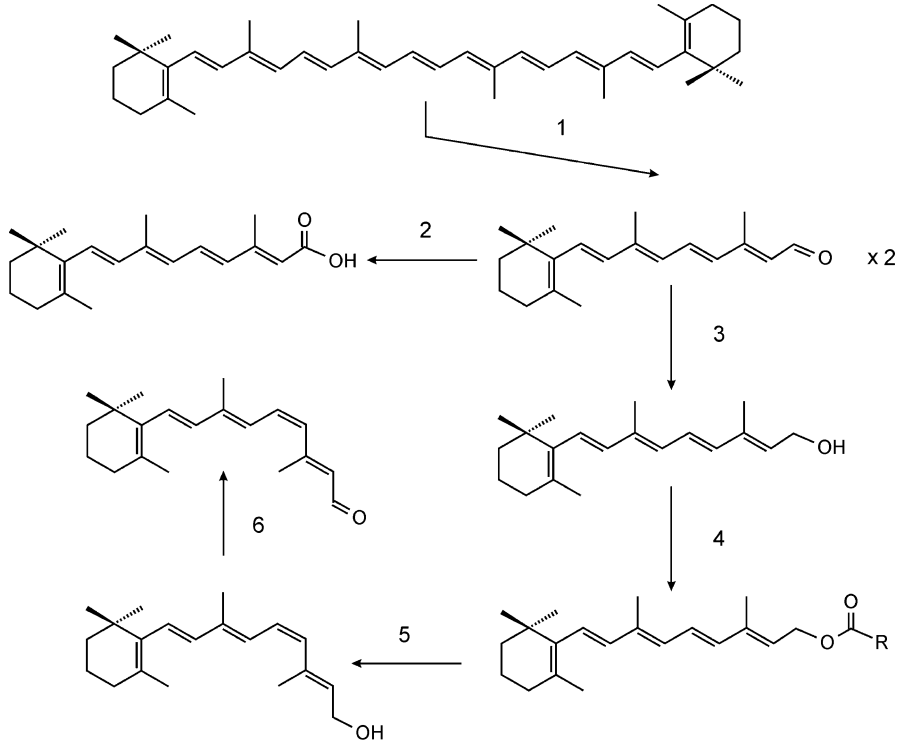

Figure 2

Enzymatic steps in β,β-carotene/retinoid metabolism in humans. (*1*) β,β-carotene is symmetrically cleaved to two molecules of all-*trans*-retinal. (*2*) all-*trans*-Retinal is oxidized to retinoic acid and/or (*3*) reduced to all-*trans*-retinol (vitamin A). (*4*) all-*trans*-Retinol is converted to retinyl esters for storage or (*5*) for the formation of 11-*cis*-retinol. (*6*) 11-*cis*-retinol is oxidized to 11-*cis*-retinal.

Retinoids: C20-apocarotenoids derived from oxidative symmetric cleavage of carotenoids (C40) that comprise all derivatives of vitamin A (all-*trans*-retinol). Their basic structure consists of a cyclic end group (β-ionone ring), a polyene side chain, and a polar end group. Alternations of end groups and modifications of the β-ionone ring create the various types of retinoids

Phototransduction: the process by which light is converted into electrical signals in photoreceptor cells of the retina. This process occurs via G-protein-coupled receptors called opsins that combine with a retinylidene chromophore (11-*cis*-retinal). When hit by a photon, 11-*cis*-retinal undergoes photoisomerization to all-*trans*-retinal, which changes the conformation of this complex that initiates a signaling cascade by activating heterotrimeric G-proteins

RA: retinoic acid

postmenopausal women may experience frank or marginal vitamin A deficiency (36). Considerable effort has been made to increase dietary carotenoid intake to combat vitamin A deficiency. Intervention studies showed that β,β-carotene can reduce night blindness and mortality rates in pregnant women in populations at risk for vitamin A deficiency (21). Other strategies involve programs for the cultivation of indigenous β,β-carotene-rich fruits and vegetables as well as genetic engineering of the carotenoid pathway in major crops such as rice, potatoes, and corn (25, 84, 137). In the future, those crops with increased β,β-carotene content may provide a sustainable source for vitamin A for the world population.

High plasma carotenoid levels have been more generally associated with a decreased risk of chronic disease, e.g., cardiovascular disease, some types of cancer, and ocular diseases, in observational studies (61). Additionally, several studies have indicated that β,β-carotene blood levels are inversely correlated with type 2 diabetes in matched controls (22, 38, 96, 138). Moreover, analysis of data from the Eye Disease Case Control (EDCC) Study Group provided evidence that individuals with high blood levels of carotenoids had a significantly lower risk of exudative age-related macular degeneration (111). This finding was confirmed by several follow up-reports, including the Age-Related Eye Disease Study (16, 108). However, intervention trials have failed to show preventive effects of carotenoids on disease incidence. Indeed, long-term supraphysiological dosing of β,β-carotene has even proven to be harmful in some cases to people at risk of disease (for recent review, see 39). These findings suggest the existence of a therapeutic window for dietary carotenoids that might be influenced by individual genetics and lifestyle.

Carotenoid metabolism can be portrayed in the following framework. These compounds must be absorbed from the intestine, transported in the circulation, and delivered to target tissues. Additionally, mechanisms must exist to eliminate carotenoids to maintain a normal physiological state. In the case of provitamin A, carotenoids must first be converted to retinoids, and these primary cleavage products must then be further metabolized for storage and/or production of biologically active retinoid derivatives. However, our understanding of the molecular and biochemical basis of carotenoid metabolism is incomplete. Without a proper molecular framework it is impossible to truly understand this process, including critical details about its regulation and its physiological impact. Lack of such knowledge has significantly hindered recommendations for carotenoid intake in health and disease.

DROSOPHILA MELANOGASTER IS AN EXCELLENT MODEL TO STUDY CAROTENOID METABOLISM

The completed human and *Drosophila* genome projects have revealed that 60% of the genes of *Drosophila* have homologs in the human genome. The fly has been successfully used as a model to study fundamental biological processes by functional genomics. Analysis of insect phototransduction cascades has significantly contributed to our understanding of the genetics of vision (45, 128). Like all animals that are endowed with the ability to detect light through visual pigments, *Drosophila* must have evolved a pathway in which dietary carotenoids are metabolically converted to chromophore. In contrast to vertebrates, carotenoid function is restricted to vision in the fly, thus making this model ideally suited for genetic dissection. In a genetic screen late in the last century, six gene loci were identified due to characteristic electroretinograms of mutants exhibiting the neither inactivation nor afterpotential mutant (*nina*) phenotype. This phenotype is caused by the complete absence of visual pigments in the compound eyes of these fly mutants (114). The molecular basis for blindness had already been elucidated for three of these *nina* mutants (*ninaA*, *ninaC*, and *ninaE*) and led to the identification of critical components involved in opsin production of fly visual pigments (for review, see 128). The *ninaB* and *ninaD*

mutants, however, feature a characteristic not found in the other *nina* mutants. Their visual performance can be rescued by feeding these flies large quantities of preformed all-*trans*-retinal (114). In the *Drosophila* mutant *ninaD*, the carotenoid content was shown to be significantly altered as compared to wild-type flies, and it was ineffective in mediating visual pigment synthesis (40). The *ninaD* gene has been identified by a candidate gene approach. In the genomic region 36E-F on chromosome 2, to which the *ninaD* mutation had been mapped (114), a nonsense mutation in a gene encoding a class B scavenger receptor was found (59). Moreover, the encoded protein shared significant sequence identity with the mammalian scavenger receptor type B, class I (SR-BI) and cluster determinant 36 (CD36), the founding member of this protein family (31). The NinaD gene encodes two splicing variant forms that differ in their C-terminal regions (59). Expression of these splicing variants in *Drosophila* Schneider cells revealed that NinaD-I was localized at the cytoplasmatic membrane, whereas the shorter splicing variant, NinaD-II, was associated with intracellular membranes (125). As expected for a critical component of carotenoid uptake, NinaD-I facilitated absorption of carotenoids into target cells, allowing the rate of zeaxanthin uptake to increase linearly in the range of 0.07−1.7 μM and plateau at higher concentrations (1.7−3.6 μM) (125).

P-element-mediated transformation of flies with a wild-type *ninaD-I* allele rescued impaired carotenoid uptake and restored visual pigment synthesis in *ninaD* mutants (59). Besides disrupted carotenoid metabolism, these *ninaD* mutants also exhibited an altered tocopherol (vitamin E) content (125). Like carotenoid uptake, tocopherol uptake was restored upon expression of the *ninaD*-I transgene in the mutant (125). Recently, another class B scavenger receptor, Santa Maria, was identified in *Drosophila*. Santa Maria shares 33% amino acid sequence identity with human SR-BI, 26% identity with mouse CD36, and 30% identity with NinaD (127). As in *ninaD* mutants, visual pigment synthesis was restored by supplementing flies with large amounts of all-*trans*-retinal. Though direct biochemical proof of Santa Maria function in cellular carotenoid uptake has yet to be demonstrated, it is likely that Santa Maria is required for chromophore production (127). Although the *ninaD* gene is mainly expressed in the gut, *santa maria* is expressed in neurons and glial cells in close vicinity to photoreceptor cells (127). Thus, Santa Maria may act downstream of NinaD in mediating carotenoid uptake into cells that produce chromophore (**Figure 3**).

The molecular player responsible for successive conversion of carotenoids into chromophore was first discovered by a cloning expression strategy that used sequence information from the plant carotenoid-cleaving enzyme VP14. Like animals, plants transform carotenoids to various apocarotenoid derivatives. The *vp14* gene encodes a protein that catalyzes the conversion of 9-*cis*-violaxanthin at the C10, C11-position to form C25 apoaldehydes and xanthoxin, a precursor of abscisic in higher plants (110). Abscisic acid is a hormone-like compound that regulates processes such as seed dormancy and responses to environmental stress. For cloning a *Drosophila* homolog, an *Escherichia coli* strain genetically engineered to produce β,β-carotene was used (123). Upon expression of a *Drosophila* carotenoid-oxygenase, this strain synthesized vitamin A at the expense of its β,β-carotene precursor, easily visible by a color shift of the bacteria from yellow to pale white. *Drosophila* cDNA encodes a protein of 620 amino acids that catalyzes cleavage of β,β-carotene at position 15,15′, resulting in formation retinaldehyde (123). Direct genetic evidence that this enzyme catalyzes the key step in retinoid production was provided by mutant analyses. The *ninaB*-mutation was cytologically mapped in the *Drosophila* genome to chromosome 3 at position 87E-F (114), coinciding with the physical location of the gene encoding the identified carotenoid cleavage. Indeed, mutations were found in two independent *ninaB* alleles that resulted in carotenoid accumulation and chromophore deficiency (121). The *ninaB* gene is expressed in glial and neuronal cells that

Nuclear receptors: a class of proteins found in the nucleus of cells. These receptors respond by working in concert with other proteins to regulate the expression of specific genes. Nuclear receptors have the ability to bind directly to DNA response elements and regulate the expression of adjacent genes; hence these receptors are classified as transcription factors. Regulation of gene expression by nuclear receptors occurs only upon binding a ligand—a molecule that affects the receptor's behavior

RARs: retinoic acid receptors

PPARs: peroxisome proliferator-activated receptors

***nina*:** neither inactivation nor afterpotential mutant

also express the Santa Maria gene, indicating that this scavenger receptor acts in conjunction with NinaB in retinoid production (127) (**Figure 3**).

Cloning and biochemical study of NinaB from the moth *Galleria mellonella* recently showed that NinaB also can act as a retinoid isomerase (86). This NinaB catalyzes an oxidative cleavage at the C15,C15′ double bond of carotenoid substrates combined with an all-*trans* to 11-*cis* isomerization of one cleavage product. Use of an asymmetric carotenoid substrate such as lutein with one β and one ε ionone ring as well as β-cryptoxanthin with one hydroxylated and one nonhydroxylated ionone ring revealed that NinaB interacts specifically with one half site of its carotenoid substrate. Thus, NinaB can convert carotenoids such as β-cryptoxathin, zeaxanthin, and lutein directly to the fly and moth chromophore, 11-*cis*-3-hydroxy-retinal. Studies showing that the 11-*cis* configuration of chromophore is a prerequisite for visual pigment synthesis (86) were supported by recent analyses indicating that NinaB is essential for visual pigment synthesis in *Drosophila* (86). Thus, genetic dissection of the pathway for chromophore production in *Drosophila* identified key players in carotenoid metabolism such as carotenoid transporters and a carotenoid-isomerooxygenase. These findings helped to identify their respective entities in vertebrates and to analyze their roles in carotenoid-dependent processes.

THREE MAMMALIAN NINAB HOMOLOGS EXERT VARIOUS FUNCTIONS IN CAROTENOID/RETINOID METABOLISM

In 1965, Goodman & Huang (42) and Olson & Hayaishi (87) characterized an enzymatic activity in cell-free homogenates from rat small intestine that converted β,β-carotene into retinaldehyde. Wyss and colleagues then cloned the first vertebrate, β,β-carotene-15,15′-monooxygenase (BCMO1), from chicken (133, 134). The vertebrate BCMO1 shares significant sequence identity with *Drosophila* NinaB and belongs to the same class of carotenoid cleavage enzymes that was first described in plants (124). Subsequently, *BCMO1* genes from mice, rats, and humans were identified, and the recombinant proteins were biochemically characterized (68, 89, 116, 100). Recombinant human BCMO1 catalyzes the cleavage of provitamin A carotenoid substrates with at least one nonsubstituted β-ionone ring, such as β,β-carotene, α-carotene, and β-cryptoxanthin, but fails to promote cleavage of nonprovitamin A carotenoids such as lycopene or zeaxanthin (68). K_m values for β,β-carotene were estimated to be in the range of 1–10 μM. BCMO1 exhibits a slightly alkaline pH-optimum, and its enzymatic activity is sensitive to chelating agents such as o-phenanthroline and α,α′-bipyridyl, indicating a dependency on ferrous iron (68, 100). The presence of ferrous iron in recombinant BCMO1 was then confirmed by inductively coupled plasma atomic emission spectrometry (94). In murine BCMO1, binding of this cofactor involves four conserved histidine residues (His172, His237, His308, and His514) and a conserved glutamine (Glu405) that are all essential for enzymatic activity (94).

Besides symmetric cleavage, an asymmetric cleavage of β,β-carotene was proposed for vitamin A production. Evidence for such an enzyme was found in cell free systems (130). A carotenoid-oxygenase, beta-carotene-9′,10′-dioxygenase (BCDO2), has been cloned from human, mouse, zebrafish, and ferrets, and recombinant ferret and mouse BCDO2 have been biochemically characterized (55, 58). Expression of BCDO2 in a β,β-carotene-accumulating *E. coli* strain revealed that it catalyzes the formation of β-10′-carotenal and β-ionone (30). Similar results were obtained with recombinant ferret BCDO2 (55). Thus, BCDO2 is a carotenoid-9′,10′-oxygenase. Besides β,β-carotene, BCDO2 also used the acyclic carotene lycopene as a substrate (55). Recombinant ferret BCDO2 cleaved 5-*cis*- and 13-*cis*-isomers of lycopene but not the all-*trans*-stereoisomer (55).

The third vertebrate family member, retinal pigment epithelium-specific protein 65-kDa (RPE65), is not a carotenoid-oxygenase but rather the long-sought retinoid isomerase in the vertebrate visual cycle. RPE65 was identified as an abundant protein in the retinal pigmented epithelium of the eyes (44). In initial studies, RPE65 was proposed to act as a retinoid binding protein (76, 135). However, three groups independently demonstrated that RPE65 is an enzyme that catalyzes the conversion of retinyl esters to 11-*cis*-retinol (50, 56, 81, 101). Like carotenoid-oxygenases, RPE65 activity depends on ferrous iron (82). In mouse models, mutations in RPE65 result in the accumulation of retinyl esters in the RPE with a resulting chromophore-deficiency (102, 107). Thus, in vertebrates, carotenoid cleavage to retinoids and the all-*trans*-to-11-*cis* isomerization of the cleavage products are catalyzed by distinct members of this protein family, whereas in insects these two reactions are catalyzed by NinaB.

Among the same species, BCMO1, BCDO2, and RPE65 share approximately 40% overall amino acid sequence identity (**Figure 4**). Recently, the crystal structure of native bovine RPE65 at 2.14 Å resolution was resolved (60). The basic RPE65 structural motif is a seven-bladed β-propeller with single strand extensions on blades VI and VII and a two-strand extension on blade III. Propeller closure occurs via 1+4 and 4+1 interactions on blades VI and VII, respectively. The top face of the β-propeller, defined by positions of the segments connecting the outer strand of one sheet with the inner strand of the next sheet, is covered by a helical cap housing the active site. The iron cofactor is located on the propeller axis near the top face and is coordinated by four conserved histidine residues and three second shell glutamate residues. Notably, each blade of the propeller contributes a single residue to the iron coordination system. The iron is accessible through a long nonpolar tunnel juxtaposing the substrate to the active center. RPE65 is stably integrated in the lipid bilayer in a monotopic fashion by virtue of several intrinsic hydrophobic residues and a palmitoylated Cys112 residue. The positions of this palmitoylation site as well as histidine and glutamate residues that coordinate the cofactor ferrous iron are well conserved among all metazoan family members. This groundbreaking structural work on bovine RPE65 will allow comparisons between different family members for identification of functional site residues that participate in the isomerization and/or oxidative cleavage reaction. Additionally, the mode of action of different family members can be further addressed. Whereas RPE65 is likely to act in an oxygen-independent manner (60), carotenoid-oxygenases such as NinaB, BCMO1, and BCDO2 depend on molecular oxygen (**Figure 4**). Experimental evidence suggests that BMO1 acts in a monooxygenase fashion (64). However, work on related plant enzymes indicates that carotenoid cleavage enzymes act as dioxygenases (109).

RPE65: retinal pigment protein of 65 kDa

REs: retinyl esters

CAROTENOID ABSORPTION IS PROTEIN MEDIATED

Two major precursors for vitamin A exist in the human diet as preformed vitamin A [primarily retinyl esters (REs)] and provitamin A carotenoids (90). REs are efficiently hydrolyzed to retinol either within the intestinal lumen (120) or at the brush border (104) (reviewed in 46). Retinol is taken up by diffusion into the enterocytes in a concentration-dependent manner (28). Within the enterocyte, absorbed retinol is esterified through the actions of the enzyme lecithin:retinol acyltransferase (LRAT), which catalyzes the transacylation of a fatty acyl group from lecithin to retinol (5, 73, 74, 105, 106). Cellular retinol-binding protein 2 (CRBP2), through binding retinol with high affinity, facilitates retinol uptake into the body and provides retinol as a substrate for LRAT (49, 88, 139). Though LRAT is the predominant enzyme for catalyzing RE formation within the intestine, studies of LRAT-deficient mice indicate that an acyl-CoA-dependent

enzyme, referred to as acyl-CoA:retinol acyltransferase (ARAT), may also participate in RE formation in intestine, mammary gland, and adipose tissues (97, 85). Ultimately, REs newly synthesized by LRAT and/or ARAT are incorporated along with other dietary lipids into nascent chylomicrons, which are then secreted from the enterocyte into the lymphatic system (11). Additionally, nonesterified retinol efflux occurs at the basolateral side of enterocytes in a process partly facilitated by the basolateral transporter, ABCA1 (28).

Absorption of carotenoids includes their release from the food matrix in the intestinal lumen, their solubilization into mixed micelles, and their uptake by intestinal mucosal cells. To study carotenoid uptake by the intestine, Earl Harrison and coworkers (29) established a highly differentiated human colonic CaCo-2 cell culture model that mimics the physiological situation. Their investigations showed that, in contrast to dietary retinoids, carotenoid absorption is saturable, indicating that this process is protein facilitated (29, 59, 119). Moreover, they also demonstrated that the all-*trans*-isomer of β,β-carotene is preferentially taken up over the *cis*-isomer (29). The receptor facilitating this absorption was identified as SR-BI, the mammalian counterpart of *Drosophila* NinaD. *SR-BI* is expressed in various tissues, including the intestine (2). This scavenger receptor was first characterized through probing cholesterol metabolism in mammals. Because SR-BI mediates the bidirectional flux of cholesterol between circulating high-density lipoproteins and target cells, cholesterol homeostasis is impaired in *SR-BI* knockout mice (1). A role for SR-BI in the absorption of dietary lipids was indicated by several studies. Blocking intestinal SR-BI function in differentiated CaCo-2 cells by antibodies, the drug ezetimide, or siRNA significantly reduced β,β-carotene uptake (26). Moreover, SR-BI has also been demonstrated to facilitate the absorption of nonprovitamin A carotenoids such as lutein and lycopene (83, 98). These findings in the CaCo-2 cell system are supported by studies in *SR-BI* knockout mice on a high-fat diet, where SR-BI was required for β,β-carotene uptake (119). Moreover, in this mouse mutant, intestinal absorption of tocopherols (vitamin E) was also decreased (99) and biliary cholesterol secretion impaired (75). In contrast, overexpression of *SR-BI* in the intestine resulted in enhanced absorption of cholesterol and fatty acids (10). Thus, SR-BI facilitates the uptake of various lipids, including carotenoids, tocopherols, and cholesterol. Because β,β-carotene uptake is not completely abolished in SR-BI deficiency, SR-BI-independent mechanisms may exist that likely involve receptors such as CD36 (119).

Furthermore, a role for SR-BI in the accumulation of carotenoids by target tissues must be considered as well. Recently, SR-BI was shown to facilitate the uptake of carotenoids in human retinal pigmented epithelial cells (27). These cells supply the retina with chromophore and carotenoids. Accumulation of carotenoids in the retina is likely supported by specific carotenoid-binding proteins. A zeaxanthin-binding protein has been molecularly and biochemically characterized that probably fulfills this function (9).

PROVITAMIN A ABSORPTION AND CONVERSION ARE UNDER NEGATIVE FEEDBACK REGULATION BY RETINOIC ACID

Upon absorption, provitamin A carotenoids are readily converted to vitamin A by the action of BCMO1 in enterocytes of the intestinal mucosa (122, 123). The primary cleavage product, retinaldehyde, is then converted to retinol and REs. These REs, along with REs produced from preformed dietary vitamin A, are packed into chylomicrons that are secreted into the blood. In humans, substantial amounts of absorbed carotenoids are not cleaved in the intestine (up to 40% of dietary intake) (18), so they, along with other lipids, become incorporated and associate with circulating lipoproteins (57). The rate of intact β,β-carotene absorption might be determined in part by the biochemical properties of the cleavage enzyme BCMO1. Addition-

ally, the protein dependency of β,β-carotene absorption suggests that regulatory mechanisms may influence this process (**Figure 5**).

Recently, the gut-specific homeodomain transcription factor ISX was identified as a repressor of intestinal *SR-BI* expression (20). SR-BI is normally found on the apical surfaces of absorptive epithelial cells, and its levels decrease from the duodenum to ileum (17, 47, 126) in contrast to the increasing duodenum-ileum gradient for ISX (20). In ISX-deficient mice, *SR-BI* expression is significantly enhanced, and its expression extends to more distal parts of the intestine (20). ISX also has been shown to repress the intestinal expression of BCMO1 (112). Animal studies already had suggested that dietary β,β-carotene and its retinoid metabolites repress intestinal *BCMO1* enzymatic activity. Because this regulation occurred at a transcriptional level and involved retinoic acid and RARs (4, 116), it was proposed that RARs and ISX independently control BCMO1 gene activity (112). However, ISX expression also is influenced by dietary retinoids, being low in vitamin A deficiency and high in vitamin A sufficiency (112). Moreover, *BCMO1* expression is not altered in ISX-deficient mice subjected to vitamin A depletion (112), suggesting instead that the effect of retinoic acid on BCMO1 expression is dependent on ISX. Recent studies in Caco-2 cells showed that *ISX* expression is induced by retinoic acid. Moreover, a retinoic acid–responsive element was identified in the *ISX* promoter to which RARs can bind (72). Furthermore, it has been shown that ISX directly suppresses *BCMO1* and *SR-BI* expression (72). These findings suggest that retinoic acid produced from dietary precursors regulates the intestinal expression of *SR-BI* and *BCMO1* by inducing ISX. Indeed, treatment of vitamin A–deficient animals with RA induced ISX expression and decreased *SR-BI* and *BCMO1* expression. Studies in BCMO1 knockout mice showed that β,β-carotene accumulation via SR-BI is repressed by retinoids (72). Thus, ISX acts as a retinoic acid-sensitive gatekeeper that controls vitamin A production. This negative feedback regulation of vitamin A production explains why β,β-carotene supplementation does not cause hypervitaminosis A (**Figure 5**). In contrast, preformed dietary retinoids can cause this problem because retinol is absorbed in a nonregulated manner. These findings also imply that there are significant differences in β,β-carotene utilization in humans with high versus low vitamin A status. In vitamin A deficiency, enhanced and distally extended expression of *SR-BI* and *BCMO1* in the small intestine may ensure that even small amounts of dietary β,β-carotene can be efficiently absorbed and used for vitamin A production. When sufficient dietary vitamin A is available, absorption of β,β-carotene is repressed at the level of scavenger receptor class B type 1 (SR-B1)-dependent absorption to avoid excess vitamin A production and toxicity.

SR-B1: scavenger receptor class B type1

ANALYSIS OF BCMO1 FUNCTION IN ANIMAL MODELS

Upon cloning BCMO1, a surprising result was that steady-state *BCMO1* mRNA levels were relatively high in peripheral nondigestive tissues. This characteristic was even reported for rodents with very low plasma carotenoid levels (58, 89, 100). In humans, analyses of *BCMO1* mRNA expression levels revealed a comparable picture (39, 136). Immunohistology showed that BCMO1 is expressed in epithelial cells in various human tissues, including the mucosal and glandular cells of stomach, small intestine, and colon; parenchymal cells in liver; cells comprising the exocrine glands in pancreas; glandular cells in prostate, endometrium, and mammary tissue; kidney tubular cells; and keratinocytes of skin squamous epithelium (69). Additionally, steroidogenic cells in testis, ovary, and the adrenal gland, as well as skeletal muscle cells, express BCMO1 (69). Moreover, BCMO1 is abundantly expressed in the retinal pigmented epithelium of the eyes (19, 136). Epithelia in general are cells that are very sensitive to vitamin A deficiency, thus indicating that BCMO1 activity may be important

for local, cell type–specific synthesis of retinoids to support vision and gene regulation.

Recently, a *BCMO1* knockout mouse model was established (50). On diets supplemented with preformed vitamin A, *BCMO1* knockout mice developed normally, and females and males were fertile, thus excluding a developmental role for BCMO1 and β,β-carotene. In contrast, these mice became vitamin A–deficient on diets providing β,β-carotene as the major source of vitamin A. Instead, they accumulated large amounts of the provitamin, not only in the blood but also in other tissues such as the liver, lung, and adipose tissues (37, 50, 71). This finding strongly supports the idea that BCMO1 is the key enzyme for vitamin A production.

Aside from grossly impaired β,β-carotene metabolism, BCMO1 knockout mice also developed pathologies not found in age-matched control animals. Female BCMO1-deficient mice had significantly lower uterus weights (71), and *BCMO1* knockout mice gained more weight in feeding studies (50, 71). *BCMO1* knockout mice also exhibited dyslipidemia and were more susceptible to diet-induced obesity (50). Moreover, total liver lipid content was elevated in BCMO1 mice, and a fatty liver phenotype was histologically evidenced by hepatocytes featuring large lipid droplets (50). Importantly, all these abnormalities were noted when mice were raised on diets supplemented with preformed retinoids.

Promoter analysis revealed that *BCMO1* is a PPAR target gene that contains a peroxisome proliferator receptor response element (PPRE) (15, 41). Site-directed mutagenesis and gel shift experiments demonstrated that this PPRE was essential for *BCMO1* promoter specificity and that PPARγ specifically binds to this element. All three PPAR isoforms—PPARα, PPARβ/δ, and PPARγ—are involved in regulating the expression of genes that regulate fatty acid metabolism (80, 43). Additionally, PPAR activity has been linked to the control of glucose metabolism and inflammation (93, 143). The finding of responsiveness of *BCMO1* to PPAR provides evidence for a possible link between the regulation of fatty acid and β,β-carotene metabolism. Interestingly, the retinal dehydrogenases, RALDH1 and RALDH2, which convert the primary β,β-carotene cleavage product retinal to RA, also have been shown to be inducible by PPARs (115). Thus, BCMO1 may influence adipocyte physiology by contributing to RA production in a tissue-specific manner. Such a cell-specific conversion of β,β-carotene to RA has been shown to be essential for embryonic development of the zebrafish (63).

In adipocytes, RA has been shown to induce lipolysis (103), mitochondrial uncoupling (3, 95), oxidative metabolism (78, 79), adipokine expression (33, 34, 54, 77), and glucose metabolism by activating both RAR and PPAR β,δ signaling (7). However, not only RA but also retinaldehyde directly derived from β,β-carotene cleavage by BCMO1 has recently been implicated in the regulation of adipocyte physiology. By binding to PPARγ and RXRs, retinal can antagonize their activities (141). Furthermore, accumulation of retinal in RALDH1-deficient mice has been shown to prevent diet-induced obesity in mice (141). Thus, activation of BCMO1 may lead to the production of retinoids that fine-tune the activities of PPARs and therefore help regulate lipid metabolism (**Figure 6**). However, a putative role of BCDO2 must be also considered in BCMO1 knockout mice. In the absence of BCMO1, increased activity of this enzyme might enhance production of other apocarotenoids that may interfere with PPAR signaling (142). Thus, further studies are required to fully understand the role of BCMO1 in mammalian biology.

ANALYSIS OF BCDO2 FUNCTION IN ANIMAL MODELS

Although a role of BCMO1 in vitamin A production has been well established, less is known about the second carotenoid-oxygenase, BCDO2. Immunocytochemistry showed that human BCDO2 is expressed in epithelial cells of

small intestine and stomach mucosa, parenchymal cells of liver, Leydig and Sertoli cells of testis, kidney tubules, adrenal gland, exocrine pancreas, and retinal pigmented epithelium and ciliary body of the eye (69). BCDO2 but not BCMO1 was detected in cardiac and skeletal muscle cells, prostate and endometrial connective tissue, and endocrine pancreas (69).

Eccentric cleavage of carotenoids has been implicated in the production of RA (129, 130). In this pathway, long-chain apocarotenoid cleavage products are truncated to RA in a stepwise process that might be mechanistically related to the β-oxidation of fatty acids (129). Additionally, recent analysis revealed that β,β-carotene-derived apocarotenoids (>C20) may exert physiological functions, e.g., β-apo-14′-carotenal was shown to inhibit PPAR signaling in both cell culture and mice (142). Notably, apocarotenoids can be formed upon supplementation of humans with β,β-carotene (53).

A critical role of BCDO2 for carotenoid metabolism is further substantiated by findings in chickens. The yellow skin color of chickens is determined by *cis*-acting and tissue-specific regulatory mutation(s) that inhibit expression of *BCDO2* in skin. In contrast to skin, *BCDO2* is normally expressed in other organs such as the liver (32). This observation in chickens indicates that BCDO2 can metabolize carotenoids in a tissue-specific manner. Moreover, the carotenoid content of cow milk was demonstrated to be significantly altered by a mutation that abolished BCDO2 function (8). In cows, a mutation in the *BCDO2* gene was discovered in exon three that resulted in a premature stop codon that abolished expression of a functional BCDO2. No adverse developmental or health effects were observed at any stage throughout the life span of BCDO2-deficient animals. *BCDO2*-deficient cows were fertile and their milk yield was normal throughout lactation. β,β-Carotene and vitamin A (all-*trans*-retinol) concentrations were also determined in serum and liver samples. Liver β,β-carotene concentrations did not differ between genotype groups, but serum β,β-carotene concentrations were higher in BCDO2-deficient cows. Interestingly, vitamin A concentrations were higher in serum, but liver vitamin A levels were lower than in wild-type cows. Whether this effect is directly due to BCDO2 action on β,β-carotene or whether altered BCMO1 activity contributes to this effect has yet to be investigated.

Aside from β,β-carotene, BCDO2 function must be also considered for the production of apocarotenoids derived from other carotenoids. Biochemical studies indicated that BCDO2 can catalyze lycopene cleavage (55, 58). In ferrets, 10′-lycopenals are produced upon lycopene supplementation (55). Moreover, studies in *BCMO1* knockout mice with elevated BCDO2 mRNA expression levels in several tissues show that lycopene tissue distribution is significantly altered compared to wild-type mice (71). Derivatives of lycopene such as apo-10′-lycopenoic acid were shown to inhibit lung tumor growth in cell culture and mouse models (66). Furthermore, lycopene supplementation inhibited hepatic carcinogenesis in rats with induced fatty livers (131). More research is needed to fully understand BCDO2 function, and it should address questions such as whether BCDO2 is mainly needed for control of carotenoid tissue levels or whether BCDO2-derived apocarotenoid cleavage products serve as precursors for signaling molecules.

GENETIC POLYMORPHISMS ALTER CAROTENOID METABOLISM IN HUMANS

Identification of molecular players in carotenoid metabolism in animal models led to the cloning and biochemical characterization of their analogous genes in humans. Like all mammalian genomes, the human genome encodes two types of carotenoid-oxygenases, BCMO1 and BCDO2, and the related retinoid isomerase, RPE65. It is well established that mutations in *RPE65* lead to a spectrum of retinal dystrophies ranging from Leber congenital amaurosis to autosomal

recessive retinitis pigmentosa (for an overview, see 117). Recently, an individual with a heterozygotic mutation in *BCMO1* was described who evidenced both elevated plasma β,β-carotene levels and low plasma retinol levels (70). At the protein level of BCMO1, this mutation resulted in replacement of a highly conserved threonine by a methionine residue (T170M). Biochemical characterization of the recombinant T170M BCMO1 protein variant showed that its activity was ≈90% lower than that of the wild-type protein. Thus, the authors concluded that haploinsufficiency of *BCMO1* causes impairment in the β,β-carotene metabolism of this patient (70).

Additionally, four different single-nucleotide polymorphisms (SNPs) in the protein-coding region of the human *BCMO1* gene were identified that result in different protein variants (**Figure 7**) (65, 70). This finding suggests variability in β,β-carotene metabolism among the human population. Indeed, intervention studies in healthy volunteers have led to the description of a low-responder phenotype and a low-converter phenotype (12). Low or poor responders are defined as individuals who show little variation in plasma β,β-carotene levels after acute or chronic supplementation. As outlined above, recent studies in human cells and mouse models indicated that β,β-carotene absorption and conversion are under negative feedback control of the β,β-carotene derivative, RA (4, 72). Hence, the low-responder phenotype may reflect a vitamin A–sufficient state in which β,β-carotene absorption is suppressed by ISX. In low converters, a low postprandial conversion efficiency occurs after β,β-carotene supplementation, as reflected by the retinyl ester/β,β-carotene ratio measured in the chylomicron fraction (12). About 45% of the western population can be classified as low converters (51, 67). This huge interindividual variability in conversion efficiency has now been reported in several studies (12, 30, 52, 67). Furthermore, pharmacokinetic studies with labeled β,β-carotene (132) indicate that low responders display a lower conversion efficiency compared to normal responders. This finding might be well explained by the above-mentioned regulation of intestinal expression of *SR-BI* (absorption) and *BCMO1* (conversion). However, two SNPs in the BCMO1 gene occur in the Caucasian population at high frequencies, with the allele frequencies for the A379V and R267S variant T allele being 24% and 42%, respectively (65). Effects of these SNPs were evaluated by testing for enzymatic activity of different recombinant BCMO1 variants. Then the results were confirmed in vivo through an intervention study with human volunteers. The latter studies indicated that women carrying either the *BCMO1* 379V or 267S+379S variants had a decreased intestinal β,β-carotene conversion efficiency (65). Given the high occurrence of these SNPs, they might account for a substantial fraction of the low-converter phenotype. Additionally, a genetic polymorphism (rs6564851) has been identified in the upstream region of the BCMO1 gene (35) (**Figure 7**). This SNP is associated with altered circulating carotenoid levels and known lipid variants associated with alpha-tocopherol levels (35). Although blood β,β-carotene and α-carotene levels were higher, lycopene, zeaxanthin, and lutein levels were significantly lower in carriers of this SNP. Even though the effect of this SNP on BCMO1 activity has yet to be clarified, the speculation that it affects *BCMO1* mRNA expression may be valid. Whether this SNP affects intestinal absorption and/or tissue-specific conversion of β,β-carotene remains to be resolved.

Additionally, SNPs in *SR-BI* and genes that encode components of lipoprotein metabolism are associated with altered plasma carotenoid levels in humans (13, 14, 48) (**Table 1**). Genetic polymorphisms in these genes may affect not only absorption rates of β,β-carotene but also the tissue distribution of β,β-carotene and other carotenoids. Notably, a polymorphism in SR-BI has already been identified as a risk factor for age-related macular degeneration (140).

Single-nucleotide polymorphisms (SNPs; pronounced *snips*): DNA sequence variations found when a single nucleotide—A, T, C, or G—in the genome differs between members of the same species (or between paired chromosomes in an individual). For example, two sequenced DNA fragments from different individuals, AAGCCTA to AAGCTTA, exhibit a difference in only a single nucleotide

Table 1 SNPs related to β,β-carotene metabolism

Gene	Location	rs-Number	Effect of the minor allele	Reference
ApoB[a]	C-516T (promoter)	rs934197	Decreased fasting β,β-carotene in men	14
BCMO1	Exon 5 (T170M)	n/a	High plasma β,β-carotene level Decreased catalytic activity in vitro	70
	Exon 8 (R267S)	rs12934922	Decrease intestinal postprandial	65
	Exon 6 + Exon 8 (R267S = A379V)	rs7501331	β,β-carotene conversion efficiency, higher fasting β,β-carotene levels in women	65
upstream of BCMO1		rs6564851	Higher plasma β,β-carotene concentration	35
LIPC[b]	C-1480T	rs1800588	Higher β,β-carotene plasma concentration in homozygous women	14
LPL[c]	Exon8 S447X	rs328	Lower fasting plasma β,β-carotene	48
SR-BI	Intron 5		Decreased fasting β,β-carotene	14

[a]apolipoprotein B.
[b]hepatic lipase.
[c]lipoprotein lipase.

CONCLUSIONS AND PERSPECTIVE

In recent years, molecular components of carotenoid metabolism have been identified and their roles in physiological processes have been studied in different animal models. Experiments in CaCo-2 cell culture systems and in mouse models demonstrate that intestinal absorption of carotenoids is protein mediated and implicate SR-BI as a key player in this process. Carotenoid cleavage oxygenases have been molecularly identified. The function of BCMO1 as the key enzyme in the production of vitamin A seems well established, as observed in *BCMO1* knockout mice and by identifying mutations and SNPs that alter β,β-carotene conversion efficiency and β,β-carotene status in humans. Moreover, a diet-responsive regulatory network that controls intestinal absorption of lipids and vitamin A production has been elucidated. In this process, intestine-specific homeodomain transcription factor ISX acts as a retinoic acid–sensitive gatekeeper that controls vitamin A production by repressing expression of *SR-BI* and *BCMO1* genes. This finding indicates that there are significant differences in β,β-carotene utilization for vitamin A production in humans with a high versus a low vitamin A status. *BCMO1* knockout mice show altered lipid metabolism and develop liver steatosis even when preformed vitamin A is made available in the diet. *BCMO1* is a PPAR target gene expressed in adipocytes and the liver, indicating a reciprocal influence between β,β-carotene metabolites and PPAR activities. These findings suggest that tissue-specific conversions of β,β-carotene to retinoids influence nuclear receptor activities that help regulate energy balance. The role of a second carotenoid-oxygenase, BCDO2, must also be considered in this process. Genetic studies in chickens and in cows showed that mutations in the *BCDO2* gene can alter carotenoid metabolism. Animal model studies also provide evidence that BCDO2 is required for the production of apocarotenoids such as lycopenals. In humans, genetic polymorphisms exist that alter carotenoid and retinoid homeostasis. In the future, this genetic variability as well as regulatory aspects must be considered when effects of carotenoids on physiological processes are evaluated. Advancing knowledge about carotenoid metabolism will contribute to the understanding of the biochemical, physiological, developmental, and medical roles of carotenoids and their numerous derivatives.

SUMMARY POINTS

1. In *Drosophila*, genetic dissection of the pathway leading from dietary carotenoids to production of visual chromophore led to identification of key components in carotenoid metabolism.
2. In mammals, including humans, homologous genes exist that encode key components of carotenoid metabolism. BCMO1 catalyzes retinoid production from provitamin A carotenoids, while a second carotenoid-oxygenase, BCDO2, catalyzes asymmetric cleavage of carotenoids to apocarotenoids. The third family member, RPE65, is not a carotenoid-oxygenase but rather a retinoid isomerase. This enzyme catalyzes the key step in the visual cycle, the conversion of retinyl esters to 11-*cis*-retinol. Recent elucidation of the bovine RPE65 crystal structure provides a molecular blueprint that will help identify critical amino acid side residues that participate in both the oxygenase and isomerase functions of these proteins.
3. Studies in highly differentiated CaCo-2 cells and mouse animal models demonstrate that intestinal carotenoid absorption is saturable and protein mediated. A critical component for this process is the scavenger receptor SR-BI, involved in the absorption of various dietary lipids including carotenoids, tocopherols, and cholesterol.
4. A diet-responsive regulatory network that controls intestinal lipid absorption and retinoid production has been elucidated. In this process, the intestine-specific homeodomain transcription factor ISX acts as a retinoic acid gatekeeper that controls vitamin A production by repressing expression of the *SR-BI* and *BCMO1* genes. These findings indicate that intestinal lipid absorption is regulated and identify vitamin A as an important dietary signal that influences this process.
5. Studies in knockout mice demonstrated that BCMO1 is the key enzyme for vitamin A production. Besides impairment in carotenoid metabolism, BCMO1-deficient mice show altered lipid metabolism. *BCMO1* is a PPAR target gene, indicating a reciprocal influence between β,β-carotene metabolites and PPAR activity in regulating fatty acid metabolism.
6. Naturally occurring mutations in the BCDO2 gene alter carotenoid and retinoid metabolism in chickens and cows. Studies in ferrets and *BCMO1* knockout mice implicate BCDO2 as well in lycopene metabolism. Animal model data show that lycopenals such as apo-10′-lycopenic acid have beneficial effects in preventing disease.
7. In humans, both mutations and SNPs in BCMO1 have been identified. Additionally, SNPs in the genes required for carotenoid transport, including *SR-BI*, alter carotenoid homeostasis. These findings provide a molecular explanation for large interindividual differences in the response to dietary carotenoids found within the human population.

FUTURE ISSUES

1. The incidence of disease related to dyslipidemia has significantly increased in the human population. Dependency of carotenoid absorption on SR-BI and the regulation of intestinal *SR-BI* expression by the vitamin A derivative RA indicate that there are substantial

differences in lipid absorption between vitamin A sufficiency and deficiency. Thus, the contribution of dietary vitamin A and β,β-carotene to the absorption of lipids including fat-soluble vitamins and cholesterol should be further addressed.

2. *BCMO1* knockout mice show several pathologies including altered lipid metabolism. This indicates that β,β-carotene is an important dietary signal that helps coordinate energy balance by influencing nuclear receptor activities. The underlying mechanism(s) remains to be elucidated.
3. The role of BCDO2 in carotenoid metabolism is still not clear. Mouse models deficient in this enzyme should be established. Research should address the question of whether BCDO2 is involved in controlling carotenoid homeostasis and/or whether BCDO2-derived apocarotenoids represent signaling molecules that impact physiological processes. Additionally, analyses of *BCMO1/BCDO2* compound knockout mice will help us understand the function of parent carotenoids in animal physiology.
4. Polymorphisms in genes responsible for carotenoid metabolism have been identified within the human population that impact carotenoid homeostasis and the conversion of carotenoids into retinoids. It is likely that genetic polymorphisms in *ISX* and *BCDO2* may also alter carotenoid homeostasis. Large genetic variability as well as variations in the regulation of carotenoid absorption/conversion must be considered when effects of carotenoids on human health are evaluated.

DISCLOSURE STATEMENT

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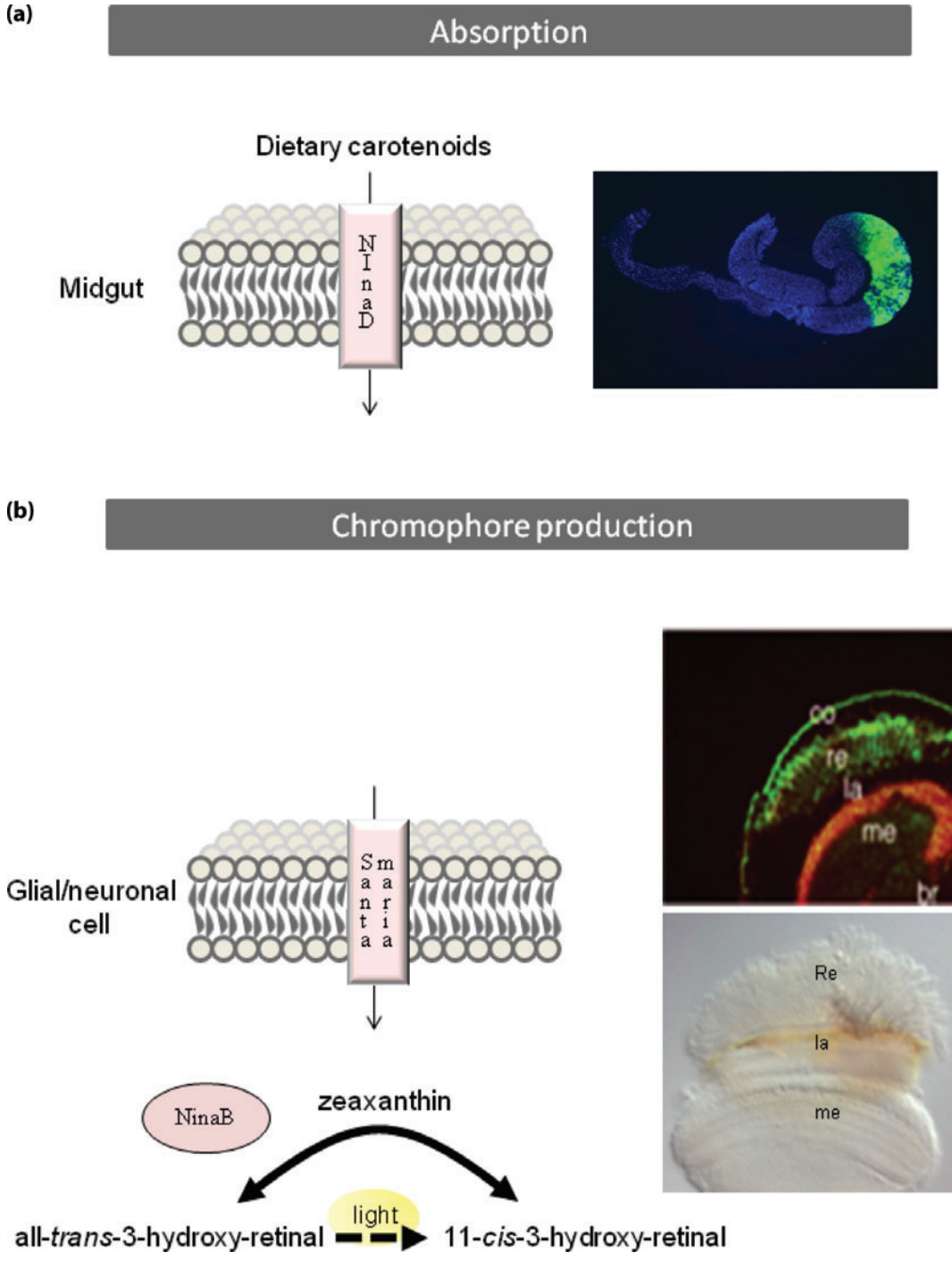

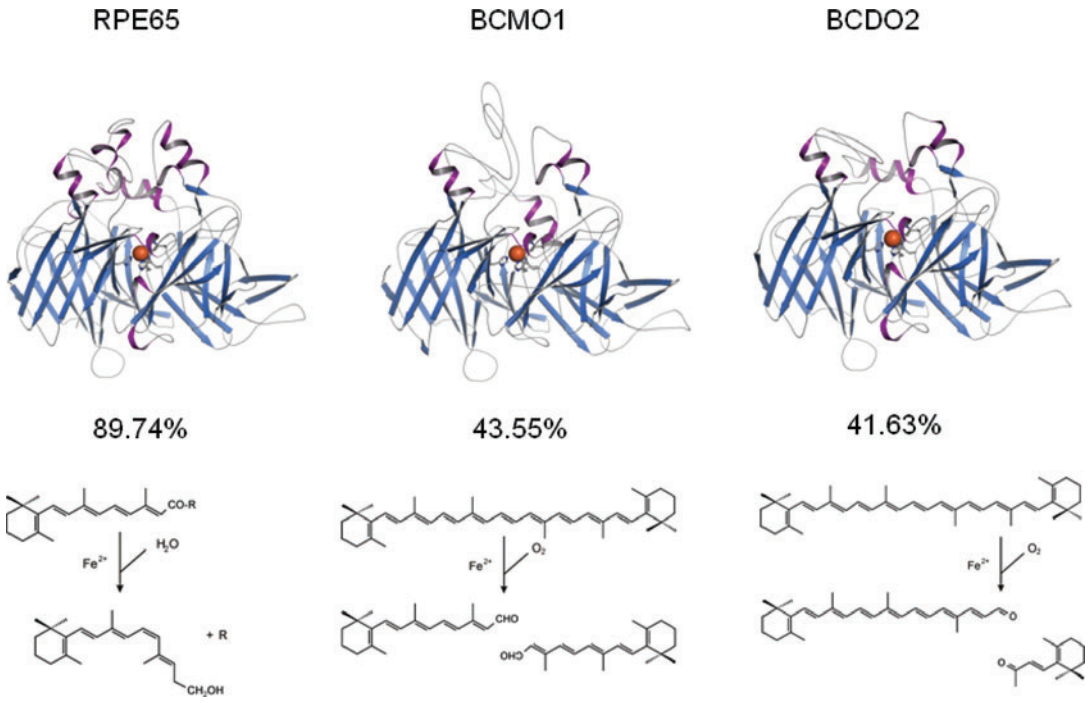

Figure 4

Three different NinaB homologs are encoded in mammalian genomes. Predictions of the molecular structures of murine RPE65, BCMO1, and BCDO2 were performed with the Swiss-Model server and the B chain from the bovine RPE65 structure. The ferrous iron is indicated in orange. Amino acid sequence homology of individual family members to bovine RPE65 is indicated. RPE65 catalyzes the conversion of retinyl palmitate to 11-*cis*-retinol and palmitate (R). The reaction involves *trans*-to-*cis* isomerization of retinol and the hydrolysis of the ester bond in a ferrous iron–dependent manner. BCMO1 catalyzes the symmetric cleavage at the C15,C15′ double bond of β,β-carotene and other provitamin A carotenoids such as β-cryptoxanthin and α-carotene to corresponding all-*trans*-retinal stereoisomers in an oxygen- and ferrous iron–dependent manner. BCDO2 catalyzes the cleavage at the C10,C9-double bond of β,β-carotene and *cis*-lycopene derivatives. In the cased of β,β-carotene, one molecule of β-10′-apo-carotenal and one molecule of b-ionone is produced. For carotenoid-oxygenases, mono- and dioxygenase reaction mechanisms have been proposed. BCD02, beta-carotene-9′,10′-dioxygenase; BCM01, β,β-carotene-15,15′-monooxygenase 1; RPE65, retinal pigment epithelium-specific protein 65-kDa.

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Figure 3

Pathway for the production of chromophore (11-*cis*-3-hydroxy-retinal) from carotenoids in *Drosophila*. (*a*) Dietary carotenoids are absorbed in a NinaD-dependent manner. The picture (*right*) shows expression of green fluorescent protein (green staining) driven by the *ninaD* promoter in the midgut of flies. Nuclei are stained with 4′,6-diamidino-2-phenylindole (DAPI) (blue staining). (*b*) For chromophore production, carotenoids are transported to glial and neuronal cells of the lamina of the optic lobes. Santa Maria facilitates the uptake of carotenoids in these cells. Absorbed carotenoids are converted to one molecule of 11-*cis*-3-hydroxy-retinal and one molecule of all-*trans*-3-hydroxy-retinal by the action of NinaB. The all-*trans*-stereoisomer is light-dependently converted to the 11-*cis*-stereoisomer. The picture (*right*) shows a schematic overview of the different layers of the optic lobes and the compound eyes of *Drosophila* (*top*). GFP expression driven by the *ninaB* promoter colocalizes with the expression of neuronal and glial markers (orange) in the lamina of the optic lobes (*bottom*, *left*). Carotenoids (yellow) accumulate in the lamina of the optic lobes in *ninaB* mutant flies that cannot convert these compounds to retinoids (*bottom*, *right*). co, cornea; re, retina; la, lamina; me, medulla.

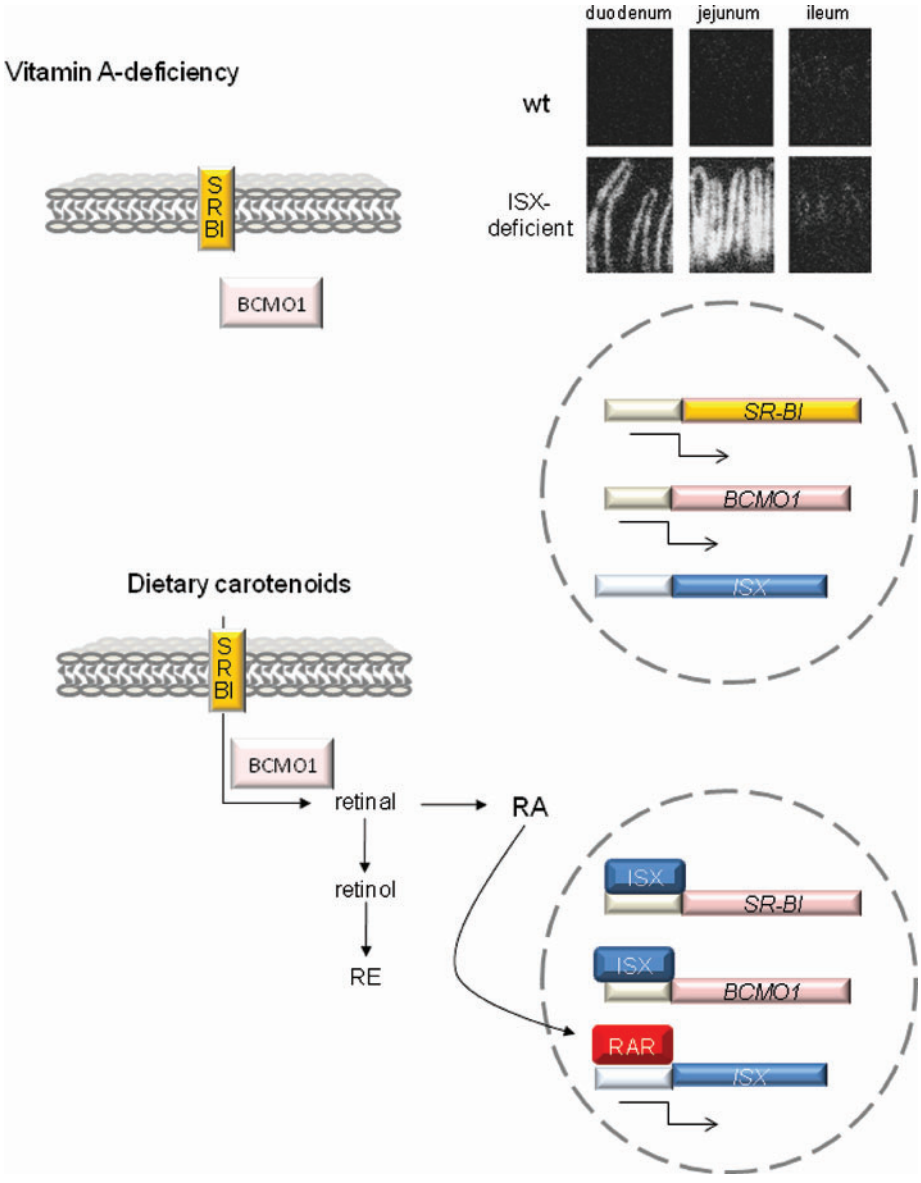

Figure 5

Negative feedback regulation of intestinal β,β-carotene absorption and conversion. (*Upper left*) In vitamin A deficiency, *SR-BI* and *BCMO1* are expressed at high levels, resulting in abundant protein levels in the duodenum and jejunum. (*Upper right*) ISX dependency of *BCMO1* expression in intestinal epithelium of the duodenum, jejunum, and ileum. In wt mice, *BCMO1* mRNA levels are low on vitamin A–sufficient diets. In ISX-deficient mice, *BCMO1* mRNA levels are highly increased. On vitamin A–sufficient diets, β,β-carotene is absorbed via SR-BI and converted to retinal via BCMO1. (*Lower left*) Retinal is reduced to retinol and esterified to RE for transport in chylomicrons. Additionally, retinal can be oxidized to RA. (*Lower right*) RA binds to RARs to induce the expression of *ISX*. In turn, ISX represses *SR-BI* and *BCMO1* mRNA expression and β,β-carotene absorption and conversion to retinal. BCM01, β,β-carotene-15,15′-monooxygenase 1; ISX, intestine-specific homeobox; RA, all-*trans*-retinoic acid; RAR, retinoic acid receptor; RE, retinyl ester; SR-BI, scavenger receptor type B, class I; wt, wild type.

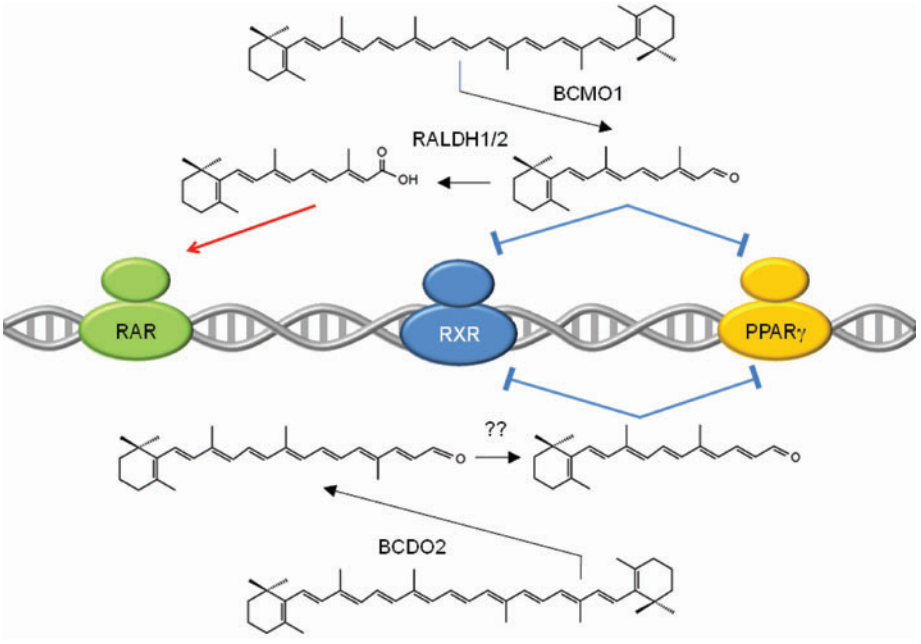

Figure 6

β,β-carotene derivatives influence nuclear receptor activities that influence energy balance. Symmetric cleavage of β,β-carotene by BCMO1 results in all-*trans*-retinal production. All-*trans*-retinal can inhibit PPARγ activity. Additionally, all-*trans*-retinal can inhibit RXR activity, the obligate heterodimeric partner of PPARs and RARs. all-*trans*-Retinal can be converted to RA by RALDH1 and RALDH2. RA via RARs control many aspects of adipocyte biology. Eccentric cleavage of β,β-carotene by BCDO2 results in the production of β-apo-10′-carotenal. Apocarotenoids such as β-apo-14′-carotenal can inhibit PPAR and RXR activities. BCD02, beta-carotene-9′,10′-dioxygenase; BCM01, β,β-carotene-15,15′-monooxygenase 1; PPAR, peroxisome proliferator-activated receptor; RA, all-*trans*-retinoic acid; RARs, retinoic acid receptors; RXR, retinoid X receptor.

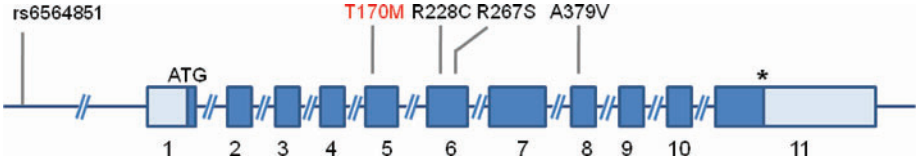

Figure 7

Schematic diagram of the physical structure of the human *BCMO1* gene. SNPs and mutations in BCMO1 gene are indicated in red. The translation start site is indicated by ATG; the stop site by an asterisk. Untranslated regions (5′ and 3′ UTR) are indicated in light blue; translated regions are in dark blue.

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Errata

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