Review
Towards a better understanding of carotenoid metabolism in animals

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Abstract
Vitamin A derivatives (retinoids) are essential components in vision; they contribute to pattern formation during development and exert multiple effects on cell differentiation with important clinical implications. All naturally occurring vitamin A derives by enzymatic oxidative cleavage from carotenoids with provitamin A activity. To become biologically active, these plant-derived compounds must first be absorbed, then delivered to the site of action in the body, and metabolically converted to the real vitamin. Recently, molecular players of this pathway were identified by the analysis of blind Drosophila mutants. Similar genome sequences were found in vertebrates. Subsequently, these homologous genes were cloned and their gene products were functionally characterized. This review will summarize the advanced state of knowledge about the vitamin A biosynthetic pathway and will discuss biochemical, physiological, developmental and medical aspects of carotenoids and their numerous derivatives.

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We are all familiar with carotenoids as the yellow to red coloring of fruits, flowers and vegetables. These colored compounds, C40 isoprenoids, are synthesized in plants, certain fungi and bacteria. Their characteristic chemical and physical properties are responsible for their light absorption as well as for the inactivation of free radicals (for a recent review, see Ref. [1]). Among the various classes of pigments found in nature, the diverse family of carotenoids is the most widespread, with important functions not only in carotenoid-producing organisms. Some animals use dietary carotenoids for coloration, well-known examples are the feathers of flamingos and the red color of salmon. However, carotenoids not only color the world around us, but are being intensively investigated currently regarding their potential to prevent chronic disease and vitamin A deficiency (VAD). Thanks to their anti-oxidative properties, beneficial effects have been reported for carotenoids in reducing the risk of coronary heart diseases, certain kinds of cancer and age-related macular degeneration (AMD) (reviewed in Ref. [2]). Most importantly, certain carotenoids are the precursors (provitamins) for the formation of vitamin A in animals.

In humans, VAD leads to night blindness in milder forms, while more severe progression results in corneal malformations, e.g., xerophthalmia. Besides visual defects, this deficiency affects the immune system, leads to infertility or causes malformations during embryogenesis. The molecular basis for these diverse effects is found in the dual role exerted by vitamin A derivatives in animal physiology.

In the entire animal kingdom, 11-cis-retinal or closely related compounds such as 11-cis-3-hydroxyretinal serve as the chromophores of the visual pigments (rhodopsin) [3,4]. Light activation of these G protein-coupled receptors is the first step in phototransduction, the process by which light is converted into a photoreceptor’s electrical response. Besides being essential for vision, in vertebrates the vitamin A derivative retinoic acid (RA) is a major signal controlling a wide range of biological processes. RA is the ligand of two...
classes of nuclear receptors, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs) [5,6] (reviewed in Refs. [7,8]). The active receptor complex is a RAR/RXR heterodimer that binds DNA regulatory sequences and regulates gene transcription in response to RA binding. RXR is not only the heterodimer partner of the RAR receptor but also an obligate partner for other nuclear receptors (for recent review, see Ref. [9]). The pleiotropic effects of vitamin A are explained by the discovery that the RA-responsive target genes are involved in a panoply of biological processes as diverse as pattern formation during embryonic development, cell differentiation and control of certain metabolic activities.

VAD is still a major problem particularly in developing countries. Vitamin A demand can be met either by preformed vitamin A or by carotenoids with provitamin A activity. Today we know that all naturally occurring vitamin A in the food chain derives from provitamin A conversion and that the world’s population mainly relies on carotenoids from staple food sources to satisfy their vitamin A need [10]. Despite the importance of provitamin A metabolism, its molecular details have remained elusive for long time. This review will focus on recent advances in this field of research. The use of genetically well-defined model organisms led to the identification of respective genes and loss-of-function analyses provided new insights into basic principles of this metabolism, e.g., of the tissue specificity of provitamin A conversion and the regulation of vitamin A homeostasis, all with substantial impact on animal physiology and human health.

1. The key step in vitamin A formation: the BCO protein and its gene

In 1930, Moore [11] provided the first evidence that a carotenoid is the precursor of vitamin A by describing β-carotene conversion in the small intestine of mammals. For this reaction, a central cleavage mechanism at the C-15'C-15' double bond for the conversion of β-carotene to vitamin A was proposed soon thereafter by Karrer et al. [12]. Then Goodman and Huang [13] and Olson and Hayashi [14] characterized the respective enzymatic activity in cell-free homogenates from rat small intestine. The β-carotene-cleavage enzyme depended on molecular oxygen and thus the enzyme was termed β,β-carotene-15,15'-dioxygenase (BCO). It was reported to be soluble, to have a slightly alkaline pH optimum and to be inhibited by ferrous iron chelators and by sulfhydryl-binding compounds, indicating that it contains a ferrous iron cofactor [15]. Subsequently, this enzyme was also characterized in different mammalian species [16] and substrate specificity was determined for different β-carotene stereoisomers [17]. Recent investigation of the mode of action of BCO provided strong evidence that oxidative cleavage at the central (15,15') double bond is catalyzed in a monooxygenase mechanism via a transient carotene epoxide [18].

In 2000, two research groups independently succeeded in cloning the key enzyme in vitamin A formation [19–21]. The approach by von Lintig and Vogt relied on sequence homology to the plant carotenoid-cleaving enzyme VP14, which catalyzes 9-cis-epoxy-carotenoid cleavage in the biosynthetic pathway of the plant growth factor abscisic acid [22]. By employing an expression cloning strategy in an Escherichia coli strain genetically engineered to produce all the enzymes needed to synthesize β-carotene de novo, they identified a β,β-carotene-15,15'-dioxygenase from the fruit fly Drosophila melanogaster. The enzymatic properties of the purified recombinant carotene oxygenase revealed that it catalyzed exclusively the centric cleavage of β-carotene (C40) to yield retinal (C20) and that it depended on ferrous iron as cofactor [19]. Direct genetic evidence that this enzyme catalyzes the key step in vitamin A formation was provided by mutant analysis. Among the various available Drosophila mutants affected in visual performance, the ninaB mutant lacks the visual chromophore, when raised on standard media with carotenoids as the sole source for vitamin A formation. The ninaB-mutation has been cytologically mapped in the Drosophila genome on chromosome 3 at position 87E–F [23], coinciding with the physical location of the Drosophila bco gene. By analyzing the molecular basis of the blindness of ninaB mutants, von Lintig et al. [24] showed that this phenotype is caused by mutations in the bco/ninaB gene, thus unequivocally demonstrating that Bco/NinaB actually catalyzes vitamin A synthesis in vivo.

Confirmation that this type of enzyme generally in metazoans catalyzes the first step in vitamin A metabolism was provided by Wyss et al. [20,25] by cloning a Bco from chicken. Their approach relied on partial protein purification and determination of peptide sequences, then using this information to synthesize corresponding degrerate oligonucleotidesprimers for PCR to generate a partial cDNA and screen a cDNA library derived from chicken small intestine. Amino acid sequence comparison between the Drosophila and chicken Bcos showed an overall similarity with several highly conserved regions and a significant similarity to some domains of the plant carotenoid oxygenase VP14 [21].

Subsequently, BCO genes from mouse and human were identified and the recombinant proteins biochemically characterized in several laboratories [26–30]. The mammalian BCO catalyzed the cleavage of carotenoid substrates with at least one unsubstituted β-ionone ring, such as β-carotene and β-cryptoxanthin, and there was no cleavage of lycopene or zeaxanthin [29]. The $K_m$ values for β-carotene were estimated to be in the range of 1–10 µM [19,26,27,29]. BCO exhibits a slightly alkaline pH optimum, and enzymatic activity is sensitive to chelating agents such as α-phenanthroline and α,α'-bipyridyl, indicating that it depends on ferrous iron [19,29]. Thus, the purified
recombinant BCOs share biochemical properties which have been already described for the native BCOs.

Purification of the recombinant BCOs as fusion proteins by affinity chromatography was achieved without the addition of detergents. This characteristic and the predicted amino acid sequences of the various BCOs indicate that we are dealing with hydrophilic, non-membrane-bound proteins. Indeed, a cytosolic localization of the native BCO was recently demonstrated for its human representative [29]. Therefore, in vitro tests for enzymatic activity must be conducted in the presence of detergents to mimic the interaction between the enzyme and its insoluble substrate. In vivo, however, the cytosolic localization of BCO may require specific binding proteins to deliver the carotenoid substrate as well as to pick up the retinoid product, since both are highly lipophilic compounds. On the product side,

Fig. 1. Proposed pathways for apo-carotenoid biosynthesis in plants and animals (for recent review, see Ref. [32] and references cited therein): (A) Abscisic acid biosynthesis. (B) Zeaxanthin oxidation in saffron styles. (C) Vitamin A biosynthesis. Abbreviations: ABA, abscisic acid; BCO, β,β-carotene-15,15'-oxygenase; VP14, 9-cis violaxanthin-oxygenase; ZCD, zeaxanthin-cleavage oxygenase.
three different types of cellular retinoid-binding proteins (CRBP I–3) have been characterized in mice (Ref. [31] and references therein). However, no direct protein–protein interaction between a recombinant murine BCO–GST fusion protein and CRBP could be detected in pull-down experiments [27]. Even though these results argue against a tight protein–protein interaction of CRBP with BCO, it seems likely that CRBPs may facilitate β-carotene cleavage by binding retinal. Hence, it remains to be elucidated whether BCO interacts tissue-specifically with a certain subset of proteins involved in retinoid metabolism. Such proteins might control the metabolic flow of the primary cleavage product retinal, either to retinol formation for vitamin A transport and storage, or in the direction of RA formation for retinoid-signaling.

To sum up, this recent research has led to the molecular identification of β,β-carotene-15,15'-oxygenases from various metazoan species. The recombinant enzymes share common biochemical properties with the native BCO from tissue homogenates. Based on its structural and biochemical properties, BCO from animals belongs to an ancient family of carotenoid modifying enzymes firstly described in plants (Fig. 1; for review, see Ref. [32]).

2. In vertebrates two additional Bco-homologous genes, Rpe65 and Bco2, exist

In the entire Drosophila genome only one bco gene is found, which is encoded by the ninaB gene [24]. In vertebrates, however, besides Bco, two additional genes, Rpe65 and Bco2, encode putative bco/ninaB homologues [19,30,33]. On the level of the deduced amino acid sequence RPE65, BCO2 and BCO share approximately 40% overall sequence identity [30]. Additionally, six histidine residues within the amino acid sequence are conserved in their positions as well as several highly conserved regions which can be considered as protein family motifs [26,30]. In humans, BCO, RPE65 and BCO2 map genomically to the chromosomal positions 16q21, 1q31 and 11q23, respectively.

RPE65 was first described as an abundant protein of the retinal pigment epithelium (RPE) with a molecular mass of 65 kDa [33], but a relationship to carotenoid/retinoid metabolism was initially not established. After the cloning and sequencing of bco/ninaB, it became evident that RPE65 belongs to the gene family of putative carotenoid-modifying enzymes. However, expression of the bovine or murine RPE65 in a β-carotene accumulating E. coli strain did not result in the formation of apocarotenals cleavage products, indicating that the RPE65 function does not include beta-carotene cleaving activity [19]. Most interestingly, mutations in RPE65 are associated with specific forms of blindness in humans, such as Leber’s congenital disease, autosomal recessive retinitis pigmentosa and rod-cone dystrophy [34,35]. A direct involvement of RPE65 in the retinoid (visual) cycle of the eyes was demonstrated by the analysis of Rpe65-deficient mice. These mice lack rhodopsin, despite the presence of the opsins apoprotein in the rod outer segments. In their eyes, 11-cis-retinal is not detectable; instead, there is an accumulation of all-trans-retinyl esters [36], intermediates of the retinoid cycle in the RPE. All-trans-retinyl esters are the substrate for the proposed isomerohydrolase which may catalyze the key step in the regeneration of the visual pigments in a combined isomerisation and ester-hydrolase reaction [37]. Even though RPE65 seems to be essential for this reaction, the recombinant RPE65 lacks isomerohydrolase activity in vitro. Recent biochemical studies suggest that RPE65 binds stereospecifically all-trans-retinyl ester and stimulates the intrinsic isomerohydrolase activity of RPE membranes [38,39]. Thus, it was proposed that RPE65 is an all-trans-retinyl ester-binding protein. Since RPE65 shares overall sequence similarities to BCO and BCO2, which both are enzymatically active proteins, it remains to be further elucidated whether this representative of the Bco-gene family is just a retinoid-binding protein or an enzymatically active component of the carotenoid/retinoid metabolism in the eyes.

The murine Bco2 gene encodes a protein of 532 amino acid residues, which shares approximately 40% overall sequence identity with both the murine BCO and RPE65. After cloning its full-length cDNA, expression of BCO2 in a β-carotene-accumulating E. coli strain revealed that it catalyzes the formation of β-10'-carotenal and β-ionone [30]. The resulting apocarotenals were identified from their chemical properties, spectral characteristics and mass spectroscopy. Thus, BCO2 is a β,β-carotene-9',10'-oxygenase specifically catalyzing the oxidative cleavage at one site of its symmetric substrate β-carotene at the C-9',C-10' double bond. The existence of this type of carotene oxygenase in other vertebrates was established by cloning this gene from human and a lower vertebrate, the zebrafish Danio rerio. The molecular identification and functional characterization of BCO2 in several vertebrate species provides strong evidence that, besides centric (C-15,C-15′), an additional eccentric (C-9',C-10') cleavage pathway for β-carotene exists in vertebrates. The existence of such a cleavage pathway for carotenoids was already proposed in several studies. Glover and Redfearn [40] provided evidence that an eccentric cleavage of β-carotene results in the formation of retinoids in mammals. In addition, the existence of such a cleavage reaction was demonstrated in cell-free homogenates [41]. Furthermore, it was shown that long-chain (β-apocarotenoids acids (~C20) as primary cleavage products from this reaction can be shortened to retinoic acid (C20). For this process a stepwise shortening reaction was proposed comparable to the β-oxidation of fatty acids [42,43]. The molecular cloning of this second, eccentrically cleaving carotene oxygenase indicates that an alternative pathway for the formation of carotenoid derivatives such as retinoids may exist in vertebrates.
To sum up, in vertebrates a small gene family of BCO-homologues exists. With the emerging number of sequences for carotenoid-cleaving enzymes from animals, but also from plants, now becoming available in the public database, this information can be used to define their catalytic domains and identify their active sites.

3. Molecular analyses of the vitamin A biosynthetic pathway

In the past few years, a large number of different components of vitamin A metabolism were molecularly identified (for review, see Ref. [44]). By reverse genetics, animal models with mutations in these genes were established. This strategy proved to be extremely powerful to learn more about individual aspects of the pleiotropic effects of this vitamin during embryonic development. Furthermore, natural mutations in the genes necessary for the metabolism of vitamin A (visual cycle) in the eyes have recently emerged as an important class of genetic defects responsible for a wide range of retinal dystrophies and dysfunctions in humans (for recent review, see Ref. [45]), e.g., as already discussed above, mutations in the BCO-homologous gene RPE65 are responsible for inherited blinding disease.

The recent cloning of BCO and BCO2 has provided the possibility to analyze their physiological functions in more detail. In the following, we will summarize recent research dealing with different aspects of provitamin A metabolism such as the impact on the formation of biologically active retinoids, provitamin A transport and body distribution as well as its tissue specificity and the regulation of this pathway.

4. Tissue-specific expression of BCO

In vertebrates, most of the vitamin A is already synthesized in epithelial cells of the intestinal mucosa by the conversion of provitamin A carotenoids and then transported to the liver for storage. Upon cloning BCO, its tissue-specific expression patterns were analyzed in several vertebrate species [25–30]. In chicken, the tissue-specific expression patterns of bco were analyzed by a combination of Northern blot and in situ hybridization experiments. Its mRNA was mainly localized in liver, in duodenal microvilli, as well as in tubular structures of the lung and the kidney [25]. In the mouse, Bco mRNA was detectable in small intestine and liver but also in kidney, testes, uterine tissues, skin, and skeletal muscle [26,27,30]. Analyses of BCO mRNA expression in humans revealed a comparable picture [29]. In addition, Yan et al. [28] reported that BCO is highly expressed in the retinal pigment epithelium of the human eyes. This RPE-specific expression of BCO in mammals was confirmed in monkeys [46].

The surprising result of all these current investigations dealing with the tissue specificity of BCO expression is that its steady-state mRNA levels are quite high in peripheral nondigestive tissues. Testes, for example, require retinoids for spermatogenesis, and vitamin A is needed for retinoid signaling in almost all tissues. Thus, BCO expression in peripheral tissues indicates that, besides an external vitamin A supply via the circulation, provitamin A may tissue-specifically impact retinoid metabolism in various cell types and tissues.

5. Provitamin A is an essential precursor for the RA signaling pathway in zebrafish embryos

It is generally assumed that retinoid-dependent physiological processes in vertebrates can be maintained by a dietary supply of preformed vitamin A. The finding that bco is expressed in a variety of different tissues raises the question whether provitamin A is an essential precursor for the formation of biologically active retinoids in vertebrate physiology. Direct evidence for such a role comes from analysis of Bco function in embryos of the zebrafish (Danio rerio) [47]. In a multitude of recent studies, this fish has proven to be a valuable model organism for the analysis of complex molecular processes in vertebrate biology. As in all vertebrate embryos, impairments in retinoid metabolism result in severe embryonic malformations due to an interference with retinoid-signaling events during development [48,49]. Lampert et al. [47] addressed the question whether Bco is needed for embryonic development in the fish. First, they demonstrated that in the zebrafish embryo bco is expressed in clearly defined spatial compartments and translated into protein. HPLC analyses for lipophilic compounds revealed the existence of both provitamin A (β-carotene) and vitamin A (all-trans-retinal) in the egg-yolk. Thus, retinoids as well as β-carotene exist as precursors for the synthesis of biologically active retinoids such as RA and 11-cis-retinal for use in zebrafish development. To test whether there is an actual requirement for the Bco-mediated β-carotene conversion to retinal, they performed loss-of-function studies. A targeted gene knock-down of Bco resulted in abnormalities of the craniofacial skeleton, hindbrain and eyes, which are all impairments well known from VAD vertebrate embryos [47]. Indeed, analyses of changes in the expression of marker genes revealed that several RA-dependent processes were severely impaired. The defects could be rescued by supplementation of RA or could also be elicited in wild-type embryos treated with Citral, an inhibitor of RA generation. These results suggest that provitamin A conversion is an essential upstream step in the pathway for RA synthesis in several specific developmental processes in the fish (Fig. 2). The use of the nontoxic provitamin for RA-signaling may provide an additional control mechanism to finely balance cellular retinoid levels.
in local tissue environments. Interestingly, embryonic expression of Bco has been also reported in mice [36]. In mammals, there is a continuous maternal supply of both vitamin A and provitamin A via the placenta. This indicates that overlapping delivery pathways exist to satisfy the embryonic demands for this vitamin. Embryonic provitamin A conversion may then assure vitamin A-dependent developmental processes under conditions when maternal vitamin A levels in the blood rapidly drop down, e.g., due to an infection disease.

Taken together, the vital role of bco in zebrafish development as well as finding Bco expression in several distinct cell types in embryonic and adult vertebrates provide strong evidence that a local, tissue-specific provitamin A conversion takes place, which influences retinoid-dependent physiological processes.

6. A putative role of class B scavenger receptors in the cellular uptake of provitamin A

The recent analyses on Bco function raise the question how provitamin A is transported within the body and delivered to different target tissues. Carotenoids are highly lipophilic molecules, suggesting that, as for other lipids, specific binding/transport proteins may exist. First insights into carotenoid transport were again provided by the analysis of a Drosophila mutant. In the blind mutant, ninaD, the carotenoid content was shown to be significantly altered as compared to wild-type flies and to be ineffective in mediating visual pigment synthesis. Molecular analyses revealed that this phenotype is caused by a defect in the uptake and body distribution of dietary carotenoids [50]. The ninaD gene encodes a cell-surface receptor with significant sequence similarity to the mammalian class B scavenger receptor, SR-BI. SR-BI plays a key role in HDL-metabolism in mammals [51] by mediating the bidirectional flux of cholesterol between lipoproteins and target cells. In ninaD flies, a nonsense mutation is found in the gene encoding this receptor, which abolished its function.

Direct functional evidence for a role of the ninaD receptor in cellular uptake of carotenoids was provided by gene rescue, using P-element mediated transformation of flies with a wild-type ninaD allele. Heat-shock induced expression of the wild-type allele in the genetic background of ninaD flies restored carotenoid uptake and visual pigment synthesis [50]. This provided genetic and functional evidence that lipoprotein-bound carotenoids are distributed to target tissues within the body by a protein-mediated transport process involving this type of scavenger receptor (Fig. 3). The existence of homologous receptors in vertebrates indicates that class B scavenger receptors may play a more general role in the cellular uptake of carotenoids from the circulating lipoprotein classes. A better under-
standing of the molecular mechanisms contributing to the body distribution of carotenoids is not only of interest with respect to retinoid metabolism, since carotenoids are important in a variety of physiological processes. For example, lutein and zeaxanthin are accumulated as macular pigments in human eyes. Beneficial effects of carotenoids—mainly due to their antioxidative properties—have been discussed in the context of several diseases. Thus, the results coming from the analyses of the ninaD mutant promise to elucidate new aspects of class B scavenger receptor functions in further research.

7. Regulation of the vitamin A biosynthetic pathway

Unlike vitamin A itself, high-dose supplementation of β-carotene in humans causes no hypervitaminosis, indicating that β-carotene cleavage to vitamin A is tightly regulated. Several investigations with animal models showed that the vitamin A status of the individual affects the enzymatic activity of BCO [52,53]. Recent analyses provided evidence that BCO regulation in the small intestine is mediated on the transcriptional level, possibly via a negative feed-back mechanism involving RA and its nuclear receptors [54]. More detailed analyses of the regulation of the murine Bco gene by Boulanger et al. [55] provided strong evidence that the murine Bco promoter contains a peroxisome proliferator response element (PPRE) as a key regulatory switch and is regulated by peroxisome proliferator-activated receptor (PPAR). PPARs constitute a sub-family of the steroid hormone receptor superfamily (for recent review, see Ref. [56]). Most of the known naturally occurring ligands of PPARs are dietary-derived fatty acids and their metabolites [57]. PPAR activates genes involved in anabolic pathways, particularly in adipose tissues, and is required for placental, cardiac and adipose tissue development [58]. RXR is the obligate heterodimeric partner of the PPAR transcription factors. The promoter analysis showed that Bco expression is positively regulated by both the PPAR/RXR heterodimer and the RXR/RXR homodimer, implying that the expression of the key enzyme for vitamin A synthesis can be up-regulated by 9-cis-RA [55]. A role of retinoid-signaling in the positive regulation of retinoid-metabolizing enzymes on the transcriptional level has also been demonstrated for the lecithin:retinol acyltransferase, LRAT [59]. Interestingly, the cellular retinol-binding protein II (Crbp2) gene is the only other gene in carotenoid and retinoid metabolism so far known to contain a PPRE. As reported for Bco, this gene is also up-regulated by ligands of PPAR but also by 9-cis-RA [60]. CRBP2 is expressed in large amounts in the small intestine of adult mammals, the major site of vitamin A synthesis. As described above, CRBP2 may act downstream of BCO in binding retinal, the primary cleavage product of provitamin A conversion. LRAT catalyzes the synthesis of retinyl esters, the storage form for vitamin A in the liver. The expression of LRAT is induced by feeding RA, but so far the details concerning its gene promoter responsive elements are missing [61].

Common mechanisms in the regulation of the genes involved in the vitamin A biosynthetic pathway may contribute to vitamin A homeostasis, and the involvement of PPARs may interlink vitamin A formation to the regulation of overall lipid metabolism. However, some recent controversial results from studies on the Bco promoter (positive regulation by 9-cis-RA) and by analyzing BCO enzymatic activity in the gut (negative regulation by RA) need further clarification. It may turn out that the latter is the result of indirect effects due to the influence of RA on additional genes involved in this process.

In sum, a better understanding of the regulation of factors dealing with the bioavailability of carotenoids, their subsequent conversion to vitamin A, and the regulatory factors interlinking this process to lipid metabolism as a whole is certainly of future importance. This interest inherently arises from the fact that carotenoids in staple foods are the major source to satisfy the world population’s vitamin A demand.

8. Centric versus eccentric cleavage: the putative role of BCO2 in retinoid metabolism

Whereas the role of BCO in the vitamin A biosynthetic pathway has been well established, the role of the second putative carotene oxygenase, BCO2, still remains somewhat elusive. There has long been a controversy over centric versus eccentric cleavage of β-carotene in the synthesis of vitamin A. Evidence that eccentric cleavage of carotenoids also occurs was provided by several investigations. Napoli and Race [62], for example, showed that, besides the formation of RA from retinal as the initial product of symmetric β-carotene cleavage, RA is directly formed from β-carotene in cell-free homogenates. As outlined above, it was shown that long-chain apocarotenoic acids (>C20) are shortened to RA in a stepwise process which is most probably mechanistically related to the β-oxidation of fatty acids [42,43]. Thus, BCO2 may catalyze the first step in an alternative pathway for RA formation. Further evidence for the existence of additional yet uncharacterized pathways for RA generation was provided by analyses of transgenic mouse embryos. By directly measuring RA generation via an RA responsive reporter transgene, it could be shown that besides the three known retinal dehydrogenases (RALDH1, -2 and -3), which catalyze the final step in RA generation, additional RA-generating systems in the heart and in the spinal cord exist [63,64].

Kiefer et al. [30] investigated the expression pattern of the eccentric carotenoid-cleaving enzyme in the mouse. Here, the Bco2 gene had an expression pattern comparable to that of Bco. The mRNA expression of both types of carotene-oxygenases in the same tissues, e.g., small intestine
and liver, confirms biochemical investigations and explains the development of this organ. Additionally, low-abundance steady-state mRNA levels of Bco2 were present in spleen, brain, lung and heart in the mouse [30]. Specific expression of BCO2 was also found in the developing heart of zebrafish embryos and in the fetal heart of humans upon analyzing a commercial multi-tissue RNA panel (A. Isken and J. von Lintig, unpublished results). These results and the existence of an alternative pathway for RA generation in the heart may indicate that BCO2 plays a particular role for the development of this organ.

Biological activities of β-apocarotenals different from retinoids have also been reported by various studies in animals (e.g., Ref. [65]). In vitro, BCO2 catalyzes, besides β-carotene cleavage, the oxidative cleavage of lycopene [30]. Favorable effects of lycopene, e.g., on certain kinds of cancers, have been repeatedly reported [66]. Thus, in addition to being a putative precursor for RA formation, in the case of β-carotene cleavage, it may be speculated that apocarotenals different from retinoids may represent biologically active substances.

To summarize, much further work needs to be done to fully understand the exact physiological functions of BCO2. These analyses must include a detailed biochemical investigation of its enzymatic properties, substrate specificity, subcellular localization and of the fate of the reaction’s primary cleavage products. Furthermore, animal models with mutations in this gene must be established to unequivocally elucidate the physiological role of this enzyme.

9. Conclusions

The molecular identification of the different metazoan carotene oxygenases has established the existence of an ancient family of non-heme iron oxygenases in animals. Via these enzymes animals have access to and are able to modulate their retinoids as needed for biological processes as diverse as vision, cell differentiation and development. With the increasing number of carotene oxygenases in the database, sequence comparison can be used to predict common structural features and to identify functional domains and active site residues. The identification of proteins involved in the transport of carotenoids in insects demonstrated that this process is protein-mediated, as already described for other lipids. The identification of these genes provides a starting point to characterize analogous genes in mammals. The advanced state of knowledge about the molecular components of the vitamin A biosynthetic pathway gained in the past few years will surely help in the worldwide public fight against VAD and will open new avenues of research, dealing with biochemical, physiological, developmental and medical aspects of carotenoids and their numerous derivatives.

References

Studies on the relative biopotencies and intestinal absorption of different apo-beta-caroteno- 


