9-20-2022

# Aster La Vista: Unraveling the Biochemical Basis of Carotenoid Homeostasis in the Human Retina

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## Recommended Citation

Bandara, S., & von Lintig, J. (2022). Aster la vista: Unraveling the biochemical basis of carotenoid homeostasis in the human retina. *BioEssays, 44,* e2200133. https://doi.org/10.1002/bies.202200133

Received: 5 July 2022 | Revised: 31 August 2022 | Accepted: 5 September 2022

DOI: 10.1002/bies.202200133

BioEssays

REVIEW ESSAY

Prospects & Overviews

# Aster la vista: Unraveling the biochemical basis of carotenoid homeostasis in the human retina

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**Funding information**
National Eye Institute, Grant/Award Numbers: EY028121, EY020551

## Abstract

Carotenoids play pivotal roles in vision as light filters and precursor of chromophore. Many vertebrates also display the colorful pigments as ornaments in bare skin parts and feathers. Proteins involved in the transport and metabolism of these lipids have been identified including class B scavenger receptors and carotenoid cleavage dioxygenases. Recent research implicates members of the Aster protein family, also known as GRAM domain-containing (GRAMD), in carotenoid metabolism. These multi-domain proteins facilitate the intracellular movement of carotenoids from their site of cellular uptake by scavenger receptors to the site of their metabolic processing by carotenoid cleavage dioxygenases. We provide a model how the coordinated interplay of these proteins and their differential expression establishes carotenoid distribution patterns and function in tissues, with particular emphasis on the human retina.

**KEYWORDS**
carotenoids, metabolism, mitochondria, transport, vision

## INTRODUCTION

Carotenoids are a common sight in nature as yellow, orange, and red colors of fruits, vegetables, and flowers. This class of pigments comprises more than 1000 related compounds and is synthesized in plants, fungi, and bacteria. A C40 backbone is the fundamental structure of these isoprenoids. The initial steps in carotenoid synthesis follow the common scheme known for isoprene synthesis in many organisms.[1] The first reaction specific to carotenoid synthesis is the condensation of two geranylgeranyl pyrophosphate (C20) molecules by phytoene synthase.[2] The double bonds of the colorless phytoene are stepwise desaturated to generate the red-colored lycopene. The terminal ends of the open-chain lycopene are converted into either $\beta$- or $\varepsilon$-ionone rings by two types of lycopene cyclases to generate $\alpha$-carotene (one $\beta$- and one $\varepsilon$-ionone ring) and $\beta$-carotene (two $\beta$-ionone rings), respectively.[3] The large diversity of carotenoids emerges from chemical modifications such as shifting of conjugated double bonds and addition of functional groups to the terminal ionone rings. Additionally, geometric isomers add variations to the chemical structures of carotenoids. Pure hydrocarbon carotenoids are called carotenes and their oxygenated derivatives are called xanthophylls.

In animals, carotenoids play important roles in vision as light filters and precursors of visual chromophore (Figure 1).[4] The xanthophylls, zeaxanthin and lutein, accumulate in high concentrations in the macula lutea of the fovea centralis of the primate eyes.[5,6] The macular pigments filter short-wavelength light, decrease chromatic aberration, and prevent light damage to the photoreceptors.[7] In bird and turtle retinas, carotenoids are concentrated in oil droplets and color tune the absorption spectra of the adjacent cone photoreceptors.[8–10]

**Abbreviations:** ACO, apo carotenoid oxygenase; BCO1, beta-carotene oxygenase 1 EC 1.13.11.63; BCO2, beta-carotene oxygenase 2 EC 1.13.11.71; CCD, carotenoid cleavage dioxygenase; GRAM, GRAM-domain containing; GSTP1, glutathione S-transferase pi isoform EC 2.5.1.18; HFL, Henle's fiber layer; LTP, lipid transfer protein; MTS, mitochondrial targeting sequence; PM, plasma membrane; SCARB1, scavenger receptor class B member 1; StARD3, StAR related lipid transfer domain containing 3.

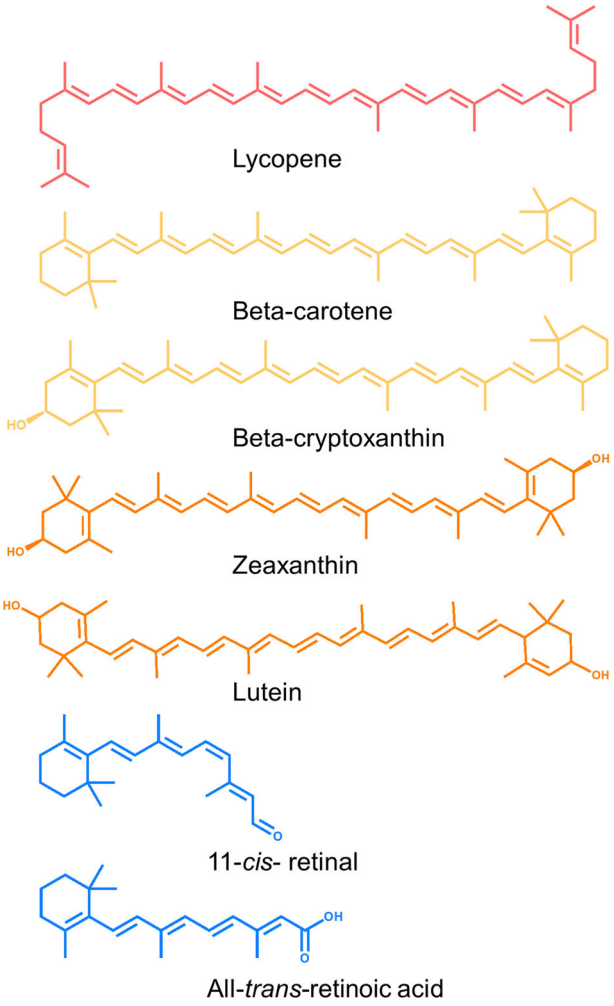


**FIGURE 1** Chemical structures of major carotenoids and retinoids of humans

The bright colors of carotenoids are important signals and expedite communication even over evolutionary borders. They attract pollinators such as birds and insects and advertise ripe fruits in exchange for seed dispersal. Carotenoids serve as ornaments in feathers and bare parts of the skin of amphibians, reptiles, and birds.[11,12] They are an important color trait that maintains ranks in schools of fish and influences the mating choice of birds.[13,14] Even some tropical bats display yellow carotenoids in bare skin parts, indicating that the pigments' role in visual communication is conserved in mammalian species.[15]

Though vertebrates acquire carotenoids exclusively from the diet, they metabolically convert their chemical structures and generate a set of unique metabolites.[16] For instance, birds and fish convert yellow carotenoids to red keto-carotenoids such as echinenone and astaxanthin[17–19] and some birds can even synthesize crimson and burgundy colored carotenoids.[20]

Another critical metabolite of carotenoids are apocarotenoids.[21] They derive from oxidative cleavage of double bonds in the carbon backbone of the carotenoid molecules. Apocarotenoids serve as signaling molecules and chromophores in all kingdoms of living nature.[22] In vertebrates, carotenoids such as $\beta$-carotene are precursors for vitamin A (all-*trans*-retinol). Vitamin A is converted to at least two biologically active molecules, 11-*cis*-retinal and all-*trans*-retinoic acid[16] 11-*cis*-Retinal is the chromophore of G protein coupled receptors that mediate phototransduction in the retina.[23] All-*trans*-retinoic acid binds to ligand-activated transcription factors of the nuclear hormone receptor class and regulates gene expression.[24,25] This function establishes carotenoids as hormone precursors in processes as diverse as cell differentiation, embryonic development, immunity, and metabolism.[26–28]

## CAROTENOID TRANSPORTERS AND METABOLIZING ENZYMES

The hydrophobic nature of carotenoids provides a barrier for diffusion in the aqueous environment of the body. This property requires a mechanism for the transportation of the pigments in the extra- and intracellular space of the body. A nonsense mutation in the coding region of a blind Drosophila mutant led to the discovery of a gene that encodes a membrane protein that facilitates the cellular uptake of carotenoids.[29,30] Later, orthologous genes were identified in vertebrates such as the scavenger receptor class B member 1(*SCARB1*) and cluster determinant 36(CD36).[31] Initially described as components in respectively, cholesterol and fatty acid metabolism, class B scavenger receptors were shown to mediate membrane transport of carotenoids and other fat soluble vitamins.[32]

The scavenger receptors' role in carotenoid metabolism was characterized in cell lines of the intestine.[33–35] In the intestine, dietary carotenoids and fat-soluble vitamins are extracted from the food matrix and incorporated into mixed micelles with bile salts, cholesterol, fatty acids, and phospholipids. Experiments in *Scarb1*$^{-/-}$ mice established that the receptor is critical for uptake of carotenoids from the diet.[36] The need for a protein-mediated uptake mechanism for carotenoids was corroborated by genetic analyses in salmon[37] and canary birds.[38] The white recessive breed of the common canary (*Serinus canaria*) displays a splice donor site mutation in the gene encoding the SCARB1 receptor and lacks the yellow carotenoid pigmentation.[38] In humans, genetic polymorphisms in the *SCARB1* gene are associated with lower carotenoid concentration in the serum and ocular tissues.[32] Consistently, it was shown that class B scavenger receptors also are involved in the cellular uptake of the pigments from circulating lipoproteins into peripheral tissues, including the eyes.[31,39,40]

The molecular details of carotenoid uptake into cells are still incomplete. The crystal structure of the SCARB1 protein is not available, therefore we modelled the structure based on the sequence using SWISS-MODEL tool and CD36(PDB ID – 5LGD) as the template.[41] According to the model (Figure 2A), the SCARB1 structure consists of a large extracellular domain, which mainly follows an antiparallel $\beta$-barrel scaffold. Three short alpha helices cover the top of the $\beta$-barrel core and two other short helices connect the

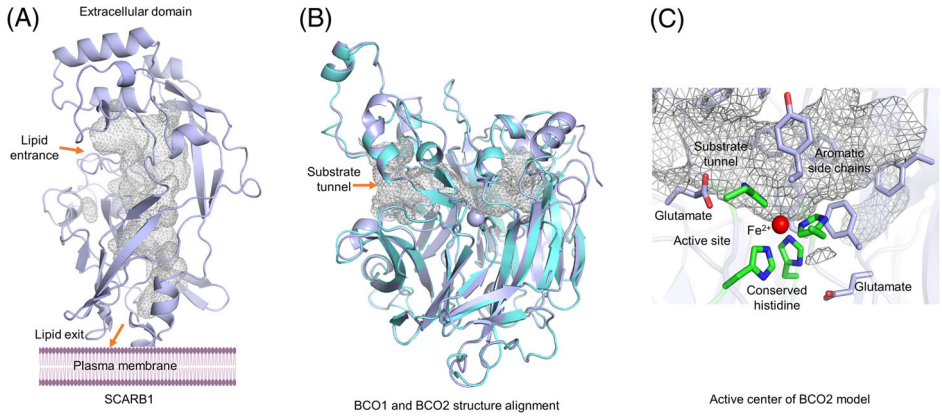


**FIGURE 2** Homology models of class B scavenger receptor and carotenoid cleavage dioxygenases. (A) Extracellular domain model of human SCARB1 receptor with proposed lipid entering and exiting tunnel highlighted with a grey color mesh (B) Structure alignment of BCO1 and BCO2 homology model (RMSD ~ 0.1 Å), BCO1 is shown with cyan and BCO2 is shown with purple color. The enzymes' substrate tunnel is shown as grey color mesh. (C) Active site of BCO2 with parts of the substrate-binding cavity is displayed as grey color mesh. The active center contains a ferrous iron (highlighted in red color) that is coordinated by four conserved histidine residues (green) and glutamate residues (sky blue). Additionally, aromatic amino side chains are highlighted that interact with the carotenoid substrates

extracellular domain with the N-terminal and C-terminal alpha helices that are embedded in the membrane. Variations in the length of the $\beta$-sheets create an uneven $\beta$-barrel core with one extended side compared to the other side. This irregularity forms the entrance of a wide hydrophobic cavity for lipid transport. The elongated cavity of the receptor can accommodate long chain carotenoids and other lipids before loading them into the membrane bilayer. The model of SCARB1 implicates that additional proteins must exist that deliver carotenoids from the plasma membrane (PM) to other cellular compartments as well as to metabolizing enzymes for further processing of the pigments to biologically active metabolites, including vitamin A.

The next critical players of carotenoid metabolism are carotenoid cleavage dioxygenases (CCDs).[42,43] These non-heme iron oxygenases convert carotenoids to apocarotenoids and control carotenoid homeostasis in tissues.[44–46] Mammalian genomes encode three members of this protein family.[47] Among them are two canonical CCDs: $\beta$-carotene oxygenase 1 – EC1.13.11.6(BCO1) and $\beta$-carotene oxygenase 2 – EC 1.13.11.71(BCO2). The third family member is the retinoid isomerase retinal pigment epithelium specific 65 kDa protein (RPE65).[48] RPE65 is vital for chromophore metabolism in the eyes and we refer to a recent review that describes RPE65's chemistry and biology.[49] The cytosolic enzyme BCO1 cleaves carotenoids and apocarotenoids (> C20) with at least one unsubstituted $\beta$-ionone ring at C15-C15' position.[44,50,51] The cleavage results in the formation of all-*trans*-retinal and a second short chain apocarotenoid (< C20) in case of apocarotenoid cleavage.[44] A tight regulation of BCO1 activity in the intestine prevents hypervitaminosis A when $\beta$-carotene is abundant in the diet.[16,52] This regulation was examined in mice and involves the intestine specific homeodomain transcription factor ISX.[53] ISX suppresses the mRNA expression of *Bco1* and *Scarb1* gene in response to retinoic acid signaling.[54] As a result, carotenoid absorption and vitamin A production in the intestine is controlled by a negative feedback loop.[36]

In contrast to BCO1, the mitochondrial enzyme BCO2 displays broad substrate specificity for various carotenoids and apocarotenoids and cleaves across the C9'-C10' position.[46,55,56] Three dimensional crystal structures of BCO1 and BCO2 are not available at this time. Therefore, we used homology modelling for BCO1 and BCO2 using 3D structures of apocarotenoid oxygenases (ACOs)[57] and RPE65.[58] Additionally, a recently solved structure of a bacterial ACO with bound apocarotenoid substrate provided atomic insights about the arrangement between substrate and enzyme.[59] Generally, the CCD scaffold is a seven-bladed $\beta$-propeller structure connected with alpha helices and loops on the top (Figure 2B). CCDs require ferrous iron ($Fe^{2+}$) as cofactors. The metal is coordinated by four conserved histidine residues and three glutamate residues. The substrate-binding cavity of CCDs mainly consists of hydrophobic amino acids that position the scissile bond of carotenoids to the ferrous iron in the active center (Figure 2C). Albeit the overall fold is conserved between BCO1 and BCO2, their substrate specificities are distinct. Our recent work showed that BCO2 has a bipartite substrate-binding cavity that is wide enough to accommodate a large panoply of carotenoid and apocarotenoid substrates. The cleavage of a carotenoid by BCO2 occurs in a stepwise process via a long-chain apocarotenoid intermediate.[60] The narrow entrance of the substrate tunnel of BCO1 selects unsubstituted $\beta$-ionone rings and contributes to the selectivity of vitamin A production.[61]

The significance of CCDs for carotenoid and vitamin A homeostasis has been demonstrated by several groups. A knockout mouse model for BCO1 displays hypercarotenemia and vitamin A deficiency when raised on $\beta$-carotene containing diet.[45] Mutations that affect BCO1 gene regulation can lead to hypervitaminosis A in mice.[52,62] In humans, non-synonymous single nucleotide polymorphisms (A379V and R267S) in the *BCO1* gene diminish the activity of the enzyme.[63] Another study showed that common genetic variations in the *BCO1* gene are associated with blood carotenoid levels and macular pigment density in the human population,[64,65] likely because of the

BCO1-dependent negative feedback regulation of vitamin A production and carotenoid absorption in the intestine.[36] Genetic variants and polymorphism in BCO2 have been reported primarily in non-primates such as rabbits,[66] sheep,[67] and bovine.[68] A partially recessive W80X mutation in the bovine *BCO2* gene affects fat and milk color. Similarly, a nonsense mutation in the sheep *BCO2* gene results in a yellow fat phenotype. Furthermore, in domestic chicken, the yellow skin phenotype is caused by genetic polymorphism in *BCO2*.[69] Additionally, color polymorphisms in lizards are associated with the *BCO2* gene.[70]

In humans, the contribution of BCO2 to carotenoid metabolism has been the subject of a controversy.[71] However, mounting evidence indicates that BCO2 is an active enzyme and plays a critical role in carotenoid homeostasis.[71] A recent study focused on the three main splice variants of human BCO2 encoding 579(BCO2a), 545(BCO2b), and 539(BCO2c) amino acid variants. The variants differ with regard to their N-terminal portion of the proteins. These encode mitochondrial target sequences, which are removed during mitochondrial import.[71,72] Importantly, recombinant human BCO2 without mitochondrial target sequence was soluble and enzymatically active.[72] The same study showed that a primate specific four amino acid insertion in BCO2 does not abolish enzymatic activity of this CCD. Considering all the above findings, BCO2 function is critical for carotenoid homeostasis of cells and is well conserved among vertebrates.

## DIFFERENTIAL EXPRESSION OF BCO2 AND COLORATION

Carotenoids are highly concentrated in some cell types and tissues of vertebrates. The yellow skin color in chicken and reptiles as well as the sexual dichromatism in birds rely on various degrees of carotenoid accumulation.[69,70,73] However, not only the total amount of carotenoids differ between tissues but also their patterns of distribution within tissues. For instance, the distribution of carotenoids in the human retina in central versus peripheral regions are opposed.[74] Recent genetic studies suggest that BCO2 is a major contributor in the tissue distribution of carotenoids.[71,72] *BCO2* gene is associated with sexual dichromatism in birds, including the European serin and canary birds.[73] A recent study showed that genetic variations in BCO2 determines keto-carotenoid accumulation in *Ranitomeya sirensis*. The frog exists as red or yellow colored variants. Red frogs either display a low expression of *BCO2* or express a splice variant with no catalytic activity when compared to yellow frogs.[75] Similarly, *BCO2* expression contributes to skin color difference in European lizards[70] and in chicken.[69] Eventually, single cell transcriptomics revealed that *BCO2* is one of the most regulated genes in cone photoreceptors of the human retina with high expression in the periphery and low expression in the central retina.[76] Thus, differential expression of *BCO2* is seemingly a common mechanism that contributes to the cellular distribution patterns of carotenoids in tissues of vertebrate species.

## CAROTENOID BINDING PROTEINS

Cells acquire carotenoids by class B scavenger receptors that deposit the pigments into the PM. Carotenoid processing by BCO2 occurs in mitochondria.[46,77] Additionally, other carotenoid metabolizing enzymes exist in the organelles, such as carotenoid hydroxylases.[17,18] Therefore, mechanisms must exist that transport carotenoids from the site of entry to the organelles for enzymatic processing.[78] In principle, the transportation of carotenoids can be attained by two mechanisms, known as vesicular transport and non-vesicular transport via binding proteins. Vesicular transport of carotenoids involves the packaging of the lipids into various lipoprotein classes such as chylomicrons, very low density lipoproteins, low density lipoproteins, and high density lipoproteins as well as the receptor mediated uptake of the lipoproteins' cargo into cells of different tissues as recently reviewed elsewhere.[16,79] Representative, the structure of Apo-lipoprotein A1, a major component of high denisty lipoproteins, is displayed in Figure 3. The non-vesicular transport of carotenoids involves specific binding proteins that transfer the pigments from one to another membrane within cells. Previously, carotenoid binding proteins have been identified in lobster and silkworm.[80–82] In the human retina, glutathione S-transferase pi isoform (GSTP1) was recognized as zeaxanthin binding protein[83] and StAR related lipid transfer domain containing 3 (StARD3) as lutein binding protein[84] (Figure 3). StARD3 is a homolog of the carotenoid binding protein of the silkworm.[82] GSTP1 and StARD3 bind respectively zeaxanthin and lutein with dissociation constants in the low micromolar range.[83,84] However, their mode of carotenoid binding has not been fully established at the atomic level. GSTP1 is a globular enzyme that does not display a specific lipid-binding cavity (Figure 3). StARD3 transports cholesterol from endosomes to mitochondria and other membranes.[85] Its lipid-binding cavity is too short to accommodate a long and rigid carotenoid molecule (Figure 3), indicating that a larger portion of the carotenoid exists outside of the binding cavity.[86]

Many lipid transfer proteins (LTP) adopt the characteristic StAR-related lipid-transfer (StART) domain fold. Our recent study showed that a novel class of LTP, the Aster proteins, can bind and transport carotenoids.[87] The crystal structure of murine Aster A with bound cholesterol was solved and provided critical insights into the proteins' lipid binding properties.[88] Their lipid-binding cavity is large enough to house an elongated rigid carotenoid molecule (Figure 3). Aster proteins belong to the stARkin protein superfamily, and it was recently shown that the LTP Anchored at a Membrane contact site/Lipid transfer at contact site are a family of sterol transfer proteins in yeast.[89,90] The mammalian orthologous of these sterol transfer proteins were identified as GRAM-domain-containing (GRAMD1/2/3) proteins and some of these proteins locate at membrane contact sites between the endoplasmic reticulum (ER) and mitochondria as well as contact sites between ER and PM.[91] The GRAMD1/2/3 proteins display an N terminal GRAM domain, which directly interacts with phospholipids in the PM. A C-terminal transmembrane helix is anchored in the ER. The middle StART-like lipid transfer domain is absent in the GRAMD2 and

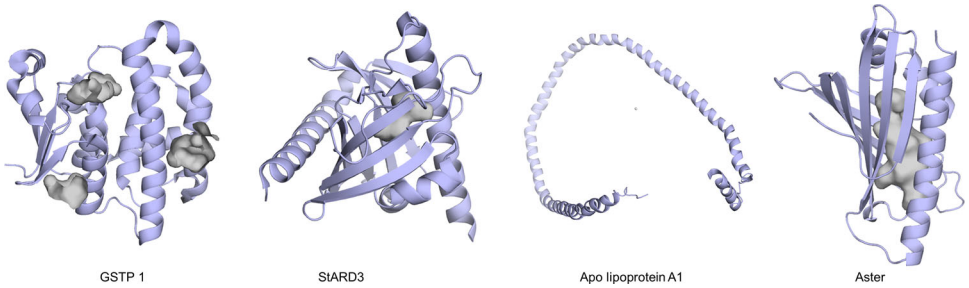


**FIGURE 3** Carotenoid binding proteins of mammals. The structures are shown as monomers and the putative lipid cavities inside the protein are displayed in grey color. Left to right – GSTP1, zeaxanthin binding protein of the primate retina (PDB ID - 5X79), StARD3, lutein binding protein of the primate retina (PDB ID - 5I9J). Apo-lipoprotein A, extra cellular carotenoid binding protein (PDB ID -1AV1). StARD lipid binding domain of Aster (GRAM1D) proteins (PDB ID -6GQF). The lipid binding cavities of the proteins are shown with 7Å cavity detection radius and visualized using Pymol software

GRAMD3 family members, suggesting that they lack lipid transferring capability. In contrast, GRAMD1 gene products, Aster A, Aster B, and Aster C, contain a StART-like domain. A recent study showed that Aster proteins are crucial for non-vesicular cholesterol transport from PM in to the ER.[88] The researchers also showed that Aster B is required for sterol homeostasis in the adrenal gland. Consequently, Aster B knockout mice have impaired steroidogenesis.[88] A follow up study with Aster proteins discovered that inhibition of Aster proteins by small molecules hindered the transport of lipoprotein-derived cholesterol from PM to the ER.[92]

Since Aster proteins work downstream of SCARB1[88] that facilitates sterol and carotenoid uptake, we hypothesized that they also transfer carotenoids from PM to membranes of other cellular compartments. Interestingly, GRAMD1B possesses a mitochondrial target sequence,[93] which may assist carotenoid transport to mitochondria where the BCO2 enzyme resides. To test our hypothesis, we established a novel expression system for the StART-like domain of Aster proteins in a zeaxanthin synthesizing *E. coli* strain.[94,95] Our analysis revealed that the recombinant StART-like domain of murine Aster proteins A and B extracted carotenoids from the bacterial membranes. The carotenoprotein complexes displayed a yellow color as well as characteristic spectral properties indicative for carotenoid binding. Furthermore, we analyzed whether the apo-Aster proteins can bind carotenoids in in vitro assays. We were able to achieve binding curves with a model carotenoid solubilized in Triton X-100 detergent. Two tryptophan residues that reside in close proximity to the binding cavity gave us a possibility to perform tryptophan fluorescence quenching assays to monitor the binding. The assays revealed hyperbolic binding curves with a dissociation constant in the micromolar range. We also showed that carotenoids compete with cholesterol for the same binding site in Aster proteins using a fluorescent cholesterol analog named 22-NBD cholesterol.[87]

To gain insight into the in vivo role of GRAMD1 in carotenoid transport, we correlated *Gramd1* gene expression and carotenoid distribution in mouse tissues. Our quantitative reverse transcription-polymerase chain reaction (qRT-PCR) data of mouse tissues showed that *Gramd1b* has high tissue specific expression in the adrenal gland and testis. *Gramd1a* did not show such tissue specific expression patterns and was expressed in various tissues at comparable level. Next, we determined the carotenoid distribution in tissues of the *Bco2* gene knockout (Bco2$^{-/-}$) mice.[46] These mice accumulate zeaxanthin in tissues when subjected to feeding with this carotenoid.[77,78] As expected high levels of carotenoids accumulated in the adrenal glands with high levels of GRAMD1b expression. Similarly, heart and testis with high *Gramd1b* mRNA expression showed carotenoid concentration over serum levels. These experiments indicated that carotenoids can compete with cholesterol for their transport proteins, SCARB1 and GRAMD1B, and are enriched in similar tissues.

## GRAMD1B AND OCULAR CAROTENOID TRANSPORT

Interestingly, *Gramd1b* expression and carotenoids accumulation was low in the murine retina that unlike to primates do not accumulate carotenoids.[96] Even the BCO2-deficient mouse retina displays carotenoid concentrations that are below serum concentrations,[36,71] indicating that rodents lack a system to concentrate carotenoids in the eyes. By contrast, human retina with macula pigment concentration express high levels of GRAMD1b as revealed by our analysis of RNA and protein samples from donor eyes.[87] Immunohistochemistry confirmed that GRAMD1B protein is expressed in central and peripheral parts of the human eyes. GRAMD1B was detected in both rod and cone photoreceptors but also in other cell types of the retina.[87]

The pattern of GRAMD1B immunostaining in the retina and mechanism of action is consistent with a role as carotenoid transport protein. Previous studies reported that carotenoids are delivered by SCARB1 to the retinal pigment epithelium.[31] From these cells of the outer retinal blood barrier, carotenoids are transported to the retina by the interphotoreceptor retinoid-binding protein.[97] Our finding of GRAMD1B staining in the photoreceptor outer segments suggests that it acts downstream of interphotoreceptor retinoid-binding protein in this transport process and helps photoreceptors to acquire carotenoids. Additionally, the expression of GRAMD1B in ganglion cells of the retina implies that GRAMD1B also contributes to carotenoid transport at vessels of the inner blood-retinal barrier. In fact, it has been previously

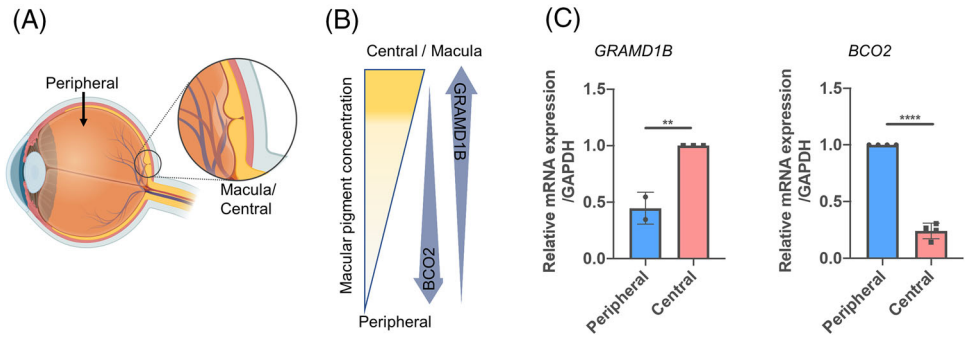


**FIGURE 4** Macula pigment distribution in the human eye. (A) Scheme of the human eye. (B) Scheme correlating macular pigment distribution with *BCO2* and *GRAMD1B* gene expression in peripheral and central parts of the retina. (C) *GRAMD1B* and *BCO2* mRNA expression in central and peripheral regions of the retina of human donor eyes (for details see Ref. [87])

shown that carotenoids are present in various layers of the human retina.[98]

## DIFFERENTIAL EXPRESSION OF BCO2 AND GRAMD1B IN THE HUMAN RETINA

The macular pigment concentration is about 100 fold higher in the fovea region than in the peripheral retina.[99] The distribution of zeaxanthin and lutein was recently established by an elegant confocal Raman spectroscopy approach.[98] Zeaxanthin is amassed in the fovea centralis of the human eyes[5] (Figure 4A) and enriched in the Henle's fiber layer (HFL) of the fovea.[5] The HFL contains bundles of unmyelinated cone photoreceptor axons that connect to ganglion cells in the outer plexiform layer of the retina. In contrast, lutein distributes more diffuse in the central retina and at lower concentration. A recent study suggests that inactivity of BCO2 contributes to this pattern of distribution of carotenoids in the primate retina.[100] However, such inactivity would lead to an accumulation of the pigments throughout the retina. More recently, differential BCO2 expression was implicated in this process, being high in peripheral and low in central cone photoreceptors.[76] A regulated expression of an enzymatically active BCO2 in the retina provides the advantage that chemically modified carotenoids can be removed from cells to prevent oxidative stress and harm to cellular structures.[101,102] In fact, we previously showed that *BCO2* mRNA expression is induced by oxidative stress and that the enzyme converts carotenoid oxidation products.[72,103] Therefore, we followed on the latter observation of a differential expression of BCO2 in the retina[76] and added GRAMD1B as an additional player to the scenario. We hypothesized that GRAMD1B transport supplements macular pigments to cells and increases their concentration in central regions. In peripheral regions, however, mitochondrial BCO2 cleaves the macular pigments to apo-carotenoids and discard them. In fact, GRAMD1B expression level in the human retina correlated with this model [87] being higher in central than in peripheral parts of the retina.[87] Moreover, our study showed that *BCO2* expression was opposed to *GRAMD1B* expression, being significantly higher in peripheral retina when compared to the central retina (Figure 4B,C). Therefore, we conclude that differential expression of *BCO2* and *GRAMD1B* provide a mechanism that establishes the distribution of carotenoids in the human retina. Furthermore, we propose that induction of *BCO2* expression by oxidative stress in diseased retinas contribute to macular pigment turnover. Interestingly, data from the Carotenoids in Age-Related Eye Disease Study supports our proposal. Genetic polymorphism in *BCO2* were unrelated to macular pigment density (BCO2 expression is very low in central parts of a healthy retina)[65] but related to higher odds of age related macular degeneration that is known to be promoted by oxidative stress.[104,105]

The zeaxanthin and lutein binding proteins GSTP1 and StARD3, respectively, likely act downstream of GRAMD1B and retain the macula pigments in the respective cell types of the human retina.[83,84] Accordingly, a recent study revealed that overexpression of human GSTP1 increased the zeaxanthin concentration by 40% in the retina of *Bco2*$^{-/-}$ mice.[106] However, enormous quantities of these proteins would be required if macular pigments exclusively exist bound to GSTP1 and StARD3 in the central retina. Therefore, additional retention mechanisms, that is, membranous structures, may concentrate the pigments in the macular region of the fovea.

## FUTURE DIRECTIONS AND UNANSWERED QUESTIONS

The recent advances of our understanding of carotenoid uptake, transport, and metabolism provide a molecular framework for carotenoid distribution and accumulation in tissues. However, many questions remain open and need to be clarified. What are the dynamics between the different involved protein components? What regulatory mechanism control carotenoid metabolism and integrate it into the entire lipid homeostasis. Particularly, the intracellular transport of carotenoids by Aster proteins from PM to mitochondria remains to be established in biochemical detail. Furthermore, the mechanisms for carotenoid concentration in different tissues and cell types remain to be defined. Are StARD3 and GSTP1 the only players in this process or does the HFL contains specific membranous structures that retain carotenoids in large quantities? Finally, rare inherited

mutations exist in *GRAMD1B*, including Arg189Trp and Arg198X that are linked with the intellectual disability.[107,108] However, it has not been established whether the same mutations affect macular pigment density and distribution in the eyes. It will be fascinating to further study the interaction between Aster proteins and the established protein components of carotenoid metabolism in health and disease.

## ACKNOWLEDGMENTS

The authors wish to thank the members of the von Lintig laboratory for insightful comments and discussions. This research was supported by grants from the National Eye Institute (EY028121 and EY020551).

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## DATA AVAILABILITY STATEMENT

Data supporting this review paper are available in the Proceedings of the National Academy of Sciences https://doi.org/10.1073/pnas.2200068119 and the other cited articles.

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**How to cite this article:** Bandara, S., & von Lintig, J. (2022). Aster la vista: Unraveling the biochemical basis of carotenoid homeostasis in the human retina. *BioEssays*, *44*, e2200133. https://doi.org/10.1002/bies.202200133