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Mechanisms of vitamin A metabolism and deficiency in the mammalian and fly visual system

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Abstract

Vitamin A deficiency can cause human pathologies that range from blindness to embryonic malformations. This diversity is due to the lack of two major vitamin A metabolites with very different functions: the chromophore 11-*cis*-retinal (vitamin A aldehyde) is a critical component of the visual pigment that mediates phototransduction, while the signaling molecule all-*trans*-retinoic acid regulates the development of various tissues and is required for the function of the immune system.

Since animals cannot synthesize vitamin A *de novo*, they must obtain it either as preformed vitamin A from animal products or as carotenoid precursors from plant sources. Due to its essential role in the visual system, acute vitamin A deprivation impairs photoreceptor function and causes night blindness (poor vision under dim light conditions), while chronic deprivation results in retinal dystrophies and photoreceptor cell death. Chronic vitamin A deficiency is the leading cause of preventable childhood blindness according to the World Health Organization. Due to the requirement of vitamin A for retinoic acid signaling in development and in the immune system, vitamin A deficiency also causes increased mortality in children and pregnant women in developing countries.

Drosophila melanogaster is an excellent model to study the effects of vitamin A deprivation on the eye because vitamin A is not essential for *Drosophila* development and chronic deficiency does not cause lethality. Moreover, genetic screens in *Drosophila* have identified evolutionarily conserved factors that mediate the production of vitamin A and its cellular uptake. Here, we review our current knowledge about the role of vitamin A in the visual system of mammals and *Drosophila melanogaster*. We compare the molecular mechanisms that mediate the uptake of dietary vitamin A precursors and the metabolism of vitamin A, as well as the consequences of vitamin A deficiency for the structure and function of the eye.

Graphical Abstract

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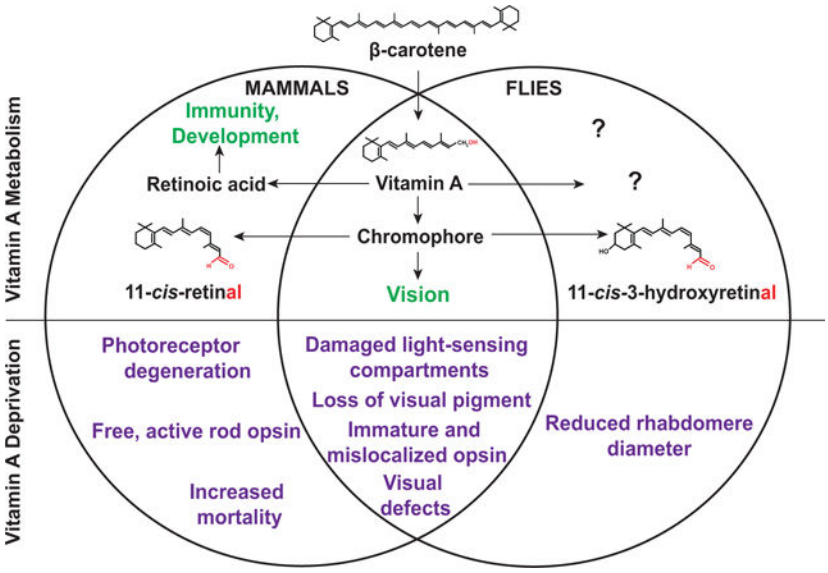

Keywords

vision; photoreceptor; vitamin A; rhodopsin; chromophore; carotene; retinoic acid; visual pigment; rhabdomere; outer segment; rod; cone; visual cycle

1. Carotenoid-based chromophores, visual pigments, and other eye pigments

Carotenoids are organic pigments that are produced by plants, algae, and photosynthetic bacteria (von Lintig, 2010). They produce the colors of various fruits and vegetables that range from bright yellow to dark red. Carotenoids are a diverse group of lipophilic isoprenoids that can be divided into two main classes: the carotenes, which lack oxygen atoms, and the xanthophylls, which contain oxygen atoms. Some carotenoids have provitamin A activity, which means that they can be converted to vitamin A in our body. Since animals are unable to synthesize carotenoids, they need to obtain vitamin A either as preformed vitamin A from animal products or synthesize it from plant-derived precursors such as β-carotene (von Lintig, 2012). In mammals, β-carotene is converted in the gut to retinal (vitamin A aldehyde), which can be converted to retinol (vitamin A) or retinyl ester (Fig. 1).

The 11-*cis*-retinal chromophore (Fig. 1), or a closely related variant (e.g. 3,4-didehydroretinal in fish and amphibians or 11-*cis*-3-hydroxyretinal in some insects), is the basis of vision in the animal kingdom (Tsin and Santos, 1985; Vogt, 1984; Wald, 1968; Wald and Brown, 1956; Yau and Hardie, 2009). It is covalently attached via a Schiff base linkage to a specific opsin protein and thereby forms the visual pigment (Brown and Wald, 1964; Wald, 1968) (Fig. 2). Visual pigments are G protein-coupled seven transmembrane receptors that are embedded in the membranes of specialized light-sensing photoreceptor compartments, which are called outer segments in humans (Fig. 2) and rhabdomeres in flies (Kumar and Ready, 1995; Nickell et al., 2007). The wavelength sensitivity of a visual

pigment is determined by interactions between the retinal chromophore and specific amino acids of its opsin partner (Kefalov et al., 2010; Salcedo et al., 2009; Zheng et al., 2015). Animals therefore commonly express different opsins that form different visual pigments in specific photoreceptor subtypes (Rister and Desplan, 2011). For instance, the human eye expresses a rod opsin (Rhodopsin) for vision at dim light conditions and three cone opsins that are sensitive to different wavelengths of light, while the *Drosophila* eye expresses six Rhodopsins with different wavelength sensitivities (Rister et al., 2013). The expression of visual pigments with different wavelength sensitivities in different photoreceptor subtypes is a prerequisite for color vision (Briscoe and Chittka, 2001).

While the visual pigment largely determines a photoreceptor's wavelength sensitivity, the sensitivity can be shaped by the addition of carotenoid-based sensitizing or filtering pigments (Kirschfeld, 1979; Kirschfeld et al., 1977; Sharkey et al., 2020). In contrast to the visual pigment, these additional pigments are photostable, i.e. they are not chemically modified upon the absorption of light. For instance, birds use carotenoid-based filter pigments, which are stored in oil droplets in front of the visual pigment-containing outer segments, to fine-tune the spectral sensitivity of their short wavelength-sensitive cones and to enhance color vision (Toomey et al., 2016). In flies, the UV-sensitizing pigment 3-hydroxyretinol has been proposed to be attached via hydrogen bonds to the visual pigment Rhodopsin 1 (Rh1) (Kirschfeld and Vogt, 1986) to add UV sensitivity to Rh1's maximal sensitivity in the blue-green range of the spectrum (Kirschfeld et al., 1977; Vogt and Kirschfeld, 1984). Another example for photostable carotenoid pigments in flies are the UV-sensitizing pigment and the blue-absorbing pigment that shape the sensitivity of the visual pigment Rh4 in 'yellow R7' photoreceptors (Hardie, 1986) to a maximal response in the UV range (Kirschfeld, 1979; Sharkey et al., 2020). The blue-absorbing pigment is a mixture of the xanthophylls lutein and zeaxanthin (Hardie, 1986), which function as a yellow filter (Kirschfeld, 1979). Lutein and zeaxanthin also produce the color of the 'yellow' spot (macula lutea) in the central human retina, which contains the fovea that mediates high resolution color vision. In the macula, the xanthophyll pigments serve as filters that absorb phototoxic UV as well as blue light and thereby improve visual performance (Renzi and Hammond, 2010; Stringham and Hammond, 2007). In addition, these pigments have been proposed to protect the macula due to their antioxidant properties (Ahmed et al., 2005).

2. Carotenoid uptake and vitamin A metabolism in mammals and flies

In this section, we review and compare the molecular mechanisms that mediate carotenoid uptake, vitamin A metabolism, chromophore formation, and visual pigment formation in mammals and in flies.

2.1. Carotenoid uptake and vitamin A metabolism in mammals

The generation of the visual chromophore and the visual pigment requires mechanisms for carotenoid uptake, transport, metabolism, delivery to target cells, and storage (Fig. 2). Vitamin A (retinol) and its derivatives (retinal and retinoic acid, Fig. 1) can be generated from plant-based precursors such as the provitamin A carotenoid β-carotene (von Lintig, 2010), which is highly lipophilic and therefore requires special mechanisms for uptake and

transport. β-carotene is absorbed in the small intestine by the Scavenger Receptor class B type I (SR-BI) (van Bennekum et al., 2005) (Fig. 2). The first essential metabolic step is the breakdown of β-carotene by the β-carotene-15,15'-dioxygenase BCO1 to two molecules of all-*trans*-retinal (Hessel et al., 2007; Lindqvist and Andersson, 2002; von Lintig and Vogt, 2000; von Lintig and Wyss, 2001; Wyss et al., 2000). All-*trans*-retinal is then converted to all-*trans*-retinol and ultimately to all-*trans*-retinyl ester by the Lecithin Retinol Acyl-Transferase LRAT for transport (Batten et al., 2004; Wongsiriroj et al., 2008). The all-*trans*-retinyl esters are packed into chylomicrons for secretion into the lymph; once they reach the bloodstream, most of the all-*trans*-retinyl esters are taken up by the liver and stored there (Paik et al., 2004) (Fig. 2). In the liver, all-*trans*-retinyl ester is hydrolyzed back to all-*trans*-retinol (Goodman et al., 1965). All-*trans*-Retinol can then again be esterified to all-*trans*-retinyl ester by LRAT for storage (Batten et al., 2004) or bind to the Retinol Binding Protein RBP4 to be transported via the circulatory system to retinoid-metabolizing tissues such as the eye (Goodman, 1980).

Chromophore synthesis and response to light in mammals—In the eye (Fig. 2), RBP4-bound all-*trans*-retinol is taken up by the retinal pigment epithelium through the cell surface receptor and essential vitamin A transporter Stimulated by Retinoic Acid 6 (STRA6) (Kawaguchi et al., 2007). LRAT converts the received all-*trans*-retinol to all-*trans*-retinyl ester, which is used to produce the 11-*cis*-retinal chromophore of the visual pigment in a multistep process in the retinal pigment epithelium (von Lintig, 2012) (Fig. 2): All-*trans*-retinyl ester is converted and re-isomerized to 11-*cis*-retinol by the enzyme Retinal Pigment Epithelium 65 (RPE65) (Redmond et al., 1998). 11-*cis*-Retinol is subsequently oxidized to 11-*cis*-retinal by the Retinol Dehydrogenases RDH5 and RDH11 (Haeseleer et al., 2002). Since 11-*cis*-retinal is highly lipophilic, it requires the Cellular Retinaldehyde-binding Protein (CRAL-BP) that facilitates the transport to the outer segments of the photoreceptors (Saari et al., 2001) (Fig. 2). There, the 11-*cis*-retinal chromophore covalently binds to the opsin protein via a Schiff base linkage and thereby forms the visual pigment (Rhodopsin in rod photoreceptors) (Wald, 1968).

The visual pigment is a light-sensitive G protein-coupled receptor (Palczewski et al., 2000) that initiates phototransduction (Yau and Hardie, 2009), the amplification and conversion of a single photon response into an electrical signal that can be interpreted by the brain. Upon absorption of a photon, the 11-*cis*-retinal chromophore is isomerized from *cis* to *trans*, which causes a conformational change of the opsin to its photoactivated state, Metarhodopsin (Yau and Hardie, 2009). This activates the G protein and initiates the phototransduction cascade (Arshavsky et al., 2002), which ultimately results in a change in the membrane potential of the photoreceptor.

To prevent saturation of the photoreceptor and to maintain a high temporal resolution, Metarhodopsin is rapidly inactivated through GRK1-mediated phosphorylation and Arrestin binding (Yau and Hardie, 2009). As the mammalian visual pigment is photoisomerized ('bleached') (Wald and Brown, 1956) and Metarhodopsin is enzymatically hydrolyzed, the opsin dissociates from the all-*trans*-retinal (Fig. 2). The free opsin stays in a low-level activity state (Fain et al., 2001) and continuously triggers the phototransduction cascade in the absence of the chromophore (Fain, 2006). Especially after exposure to a bright light

source, this ‘bleaching adaptation’ can significantly reduce the sensitivity of the rod photoreceptors to subsequent light stimulation (Fain et al., 2001; Lamb and Pugh, 2004; Pepperberg, 2003). The binding of the chromophore to the opsin therefore suppresses the prolonged, constitutive activity of the opsin and promotes the recovery of light sensitivity.

Chromophore regeneration in the visual cycle in mammals—To sustain vision after the dissociation of all-*trans*-retinal from the visual pigment, the chromophore needs to be regenerated (‘recycled’) in the mammalian eye in an energy-consuming process. Moreover, since the aldehyde group of the dissociated all-*trans*-retinal is highly reactive, the free chromophore needs to be rapidly cleared to avoid cellular damage (Maeda et al., 2008; Saari, 2016). Chromophore regeneration is achieved through a series of light-independent enzymatic steps in the so-called visual cycle (Kiser et al., 2014; Wald, 1935).

In the mammalian rod visual cycle (Fig. 3), the first step is the reduction of all-*trans*-retinal in the outer segments to all-*trans*-retinol by the Retinol Dehydrogenases RDH8 and RDH12 (Parker and Crouch, 2010). All-*trans*-retinol is then transported to the retinal pigment epithelium, where it gets esterified by LRAT (Batten et al., 2004). The retinyl esters can either be used to store vitamin A in lipid droplets called retinosomes (Imanishi et al., 2004) or be hydrolyzed and re-isomerized to 11-*cis*-retinol by RPE65. 11-*cis*-Retinol is subsequently oxidized in a final enzymatic step to 11-*cis*-retinal and re-transported to the outer segments, where it is again used for visual pigment synthesis. Taken together, the mammalian visual cycle ensures a sufficient supply of the chromophore to maintain vision and photoreceptor health; it also removes toxic products such as all-*trans*-retinal.

Another pathway for chromophore regeneration potentially involves a more recently identified RPE-retinal G protein-coupled Receptor (RGR)-dependent process (Chen et al., 2001; Morshedian et al., 2019; Zhang et al., 2019). RGR is a nonvisual opsin that has been suggested to act as a photoisomerase in a photic visual cycle that converts all-*trans*-retinal to 11-*cis*-retinal (Chen et al., 2001). However, the proposed role of RGR in a separate photic visual cycle is controversial because RGR has been shown to promote, in a light-independent manner, the conversion of retinyl esters to 11-*cis*-retinal by enhancing isomerohydrolase activity in the classical visual cycle (Wenzel et al., 2005).

As the chromophore regeneration by the classical visual cycle is insufficient to maintain light sensitivity of mammalian photoreceptors under bright daylight conditions, where cones are more important than rods (the latter are specialized in dim light vision), an attractive model is that RGR plays a role in an alternative cone visual cycle (Mata et al., 2002; Morshedian et al., 2019). This additional visual cycle has been proposed to involve Müller cells that take up all-*trans*-retinol that is released by rods and cones and subsequently oxidized to all-*trans*-retinal by RDH10. Upon absorption of a photon, all-*trans*-retinal is isomerized by RGR to 11-*cis*-retinal. RDH10 reduces 11-*cis*-retinal to 11-*cis*-retinol, which is then absorbed by the cones and oxidized to 11-*cis*-retinal by an unknown retinal dehydrogenase. 11-*cis*-Retinal binds to cone opsins via Schiff linkage to form the visual pigment. The absorption of a photon converts 11-*cis*-retinal to all-*trans*-retinal, which is released from the bleached cone opsin. RDH8 reduces all-*trans*-retinal to all-*trans*-retinol,

which is then taken up by Müller cells; this completes this second visual cycle (Morshedian et al., 2019).

Mutations in key components of the visual cycle and vitamin A metabolism cause blinding eye diseases, either due to impaired chromophore synthesis or the accumulation of toxic products (Travis et al., 2007). For instance, mutations in *RPE65* (Marlhens et al., 1997), *LRAT* (Thompson et al., 2001), or *RDH12* (Perrault et al., 2004) cause Leber Congenital Amaurosis, an inherited eye disease that is characterized by early-onset retinal degeneration as well as severe visual impairment or blindness from infancy. Insights into the underlying mechanisms have been gained from knockout mouse models: For instance, *Rpe65*−/− mutant mice accumulate all-*trans*-retinyl esters (Fig. 3), lack 11-*cis*-retinoids, and display age-dependent photoreceptor degeneration (Redmond et al., 1998; Znoiko et al., 2005).

Retinoic acid signaling controls vitamin A production in mammals through negative feedback—Mammals use vitamin A not only for the generation of the chromophore, but also for the synthesis of the important signaling molecule retinoic acid that binds to nuclear receptors, which are ligand-regulated transcription factors that directly control gene expression upon activation (Cunningham and Duester, 2015). In the eye, retinoic acid is generated by the oxidation of retinal by Retinaldehyde Dehydrogenase (RALDH). Retinoic acid binds to the transporter Cellular Retinoic Acid Binding Protein (CRABP) that facilitates its transport into the nucleus (Kam et al., 2012). In the nucleus, retinoic acid binds to a heterodimer of the nuclear receptors Retinoic Acid Receptor (RAR) and Retinoic Acid X Receptor (RXR) (Huang et al., 2014; Zhang et al., 2015). The heterodimer binds to retinoic acid response elements in the promoters of target genes; the binding of retinoic acid to the RAR-RXR heterodimer causes corepressor release, coactivator recruitment, and target gene activation (Wei, 2003).

With respect to vitamin A metabolism, retinoic acid signaling plays a key role in controlling both the intestinal absorption of β-carotene as well as the subsequent conversion to vitamin A through negative feedback (von Lintig, 2012) (Fig. 4A). The uptake of dietary β-carotene leads to the synthesis of retinoic acid, which binds the RAR-RXR heterodimer and induces transcription of *ISX* (Lobo et al., 2010). *ISX* encodes the transcription factor Intestine Specific Homeobox (ISX) (Seino et al., 2008) that acts as a ‘gatekeeper’ (Lobo et al., 2010) of carotenoid metabolism in the small intestine by transcriptionally repressing *SR-BI* and *BCO1*. The consequence is the inhibition of β-carotene absorption and cleavage, respectively (Fig. 4A). The strength of ISX-mediated repression depends on the amount of vitamin A precursors in the diet: an abundance of β-carotene leads to an increase in retinoic acid that induces the repressor ISX and thus repression of vitamin A metabolism by negative feedback (Lobo et al., 2013).

Conversely, under conditions of vitamin A deprivation (Fig. 4B), the lack of β-carotene and retinoic acid production causes a lack of expression of the repressor ISX and consequently an increased expression of SR-BI and BCO1, which both promote vitamin A production (Lobo et al., 2010). This negative feedback regulation by retinoic acid ensures that the absorption of β-carotene and the production of vitamin A match the requirements of the body (Lobo et al., 2013).

2.2. Carotenoid uptake and vitamin A metabolism in *Drosophila melanogaster*

Like mammals, *Drosophila melanogaster* cannot synthesize vitamin A *de novo* and employs evolutionarily conserved key factors for carotenoid uptake and vitamin A metabolism (Fig. 5). Dietary carotenoids are taken up in the *Drosophila* midgut by the homolog of SR-BI, Neither inactivation nor afterpotential D (NinaD) (Kiefer et al., 2002; Voolstra et al., 2006). Unlike mammals, however, flies do not immediately metabolize the carotenoids in the gut; instead, they reach the head via the hemolymph. In the head, the cellular uptake is mediated by another scavenger receptor, Santa-Maria (Scavenger receptor acting in neural tissue and majority of Rhodopsin is absent) (Wang et al., 2007) that is co-expressed in both neuronal and glial cells with NinaB (von Lintig and Vogt, 2000). NinaB is a β-carotene 15,15'-dioxygenase that combines the oxygenase and isomerase functions of its mammalian homologs BCO1 and RPE65, respectively. Hydroxylation yields the xanthophyll zeaxanthin, which is oxidatively cleaved by NinaB into one molecule of 11-*cis*-3-hydroxyretinal and one molecule of all-*trans*-3-hydroxyretinal (Oberhauser et al., 2008). It is unclear in which specific cell type(s) of the *Drosophila* head this step takes place, but it has been suggested that it involves extraretinal neurons of the central nervous system (Gu et al., 2004; Yang and O'Tousa, 2007). The enrichment of *ninaB* expression in the head and its lack of expression in the body (Yang and O'Tousa, 2007) contrasts the broad expression of its mammalian homologs in various tissues such as kidney, liver, testes, and muscle (Lindqvist and Andersson, 2002; Redmond et al., 2001). This difference in spatial expression is most likely due to the additional involvement of the mammalian homologs in retinoic acid signaling that regulates the development of various organs, which is not the case in flies (see below).

Chromophore synthesis and response to light in *Drosophila*—11-*cis*-Retinal, which results from the NinaB-mediated cleavage of carotenoids such as zeaxanthin (Oberhauser et al., 2008), can be directly used to generate the chromophore 11-*cis*-3-hydroxyretinal. The chromophore then covalently binds to the opsin protein and forms the visual pigment called Rhodopsin (e.g. Rhodopsin 1, Rh1) (Fig. 5). Like in mammalian photoreceptors, the absorption of light causes the photoisomerization of the chromophore from the 11-*cis* to the all-*trans* configuration and converts Rhodopsin to Metarhodopsin (Hardie and Juusola, 2015). In contrast to mammalian Metarhodopsin, *Drosophila* Metarhodopsin does not bleach and all-*trans*-retinal remains bound to the opsin (Stavenga et al., 2017). Metarhodopsin can be rapidly re-isomerized to Rhodopsin by absorbing a photon of a specific wavelength (Hamdorf and Rosner, 1973; Ostroy et al., 1974; Stavenga et al., 2017). For instance, blue light converts Rh1 to Metarhodopsin and red light converts it back to Rh1 (Fig. 5).

Chromophore regeneration in *Drosophila melanogaster*—The *Drosophila* visual pigment does not bleach, but some Metarhodopsin is slowly degraded and releases all-*trans*-3-hydroxyretinal (Stavenga and Hardie, 2011) (Figs. 5 and 6). Another source of free all-*trans*-3-hydroxyretinal is the NinaB-mediated cleavage of zeaxanthin (Fig. 5 and see above). All-*trans*-3-hydroxyretinal from both sources can be converted to 11-*cis*-3-hydroxyretinal in a blue light-, Pdh-, and Rdhb-dependent manner (Schwemer, 1984; Wang et al., 2010; Wang et al., 2012) (Fig. 6). Since the fly visual pigment does not bleach, the fly visual cycle serves to regenerate the chromophore for maintaining Rhodopsin expression in

adult flies. Consistent with this rationale, vitamin A-deprived flies that are supplemented with all-*trans*-retinal generate the chromophore and visual pigment when exposed to blue light; however, they fail to do so when kept in the dark (Satoh et al., 2005). The underlying mechanism remains to be elucidated.

The *Drosophila* pigment cells of the retina play a role in chromophore synthesis and regeneration that parallels the function of the retinal pigment epithelium in mammals (Wang et al., 2010) (Fig. 5). The pigment cells express the retinoid-binding protein PDA is not apparent (Pinta) that functions similarly to CRAL-BP in mammals (Saari et al., 2001) (Wang and Montell, 2005). Since Pinta preferentially binds all-*trans*-retinol, it has been proposed to either sequester all-*trans*-retinol to enrich vitamin A in pigment cells and/or to facilitate its oxidation to all-*trans*-retinal (Wang and Montell, 2005). The Photoreceptor dehydrogenase Pdh (Wang et al., 2010), which is functionally equivalent to mammalian RDH (Parker and Crouch, 2010), reduces all-*trans*-3-hydroxyretinal to all-*trans*-3-hydroxyretinol (Fig. 6). All-*trans*-3-hydroxyretinol is isomerized and oxidized by Rdhb to 11-*cis*-3-hydroxyretinal, which is transported to the photoreceptors and binds to the opsin to form the visual pigment (Wang et al., 2010).

The *Drosophila* visual cycle differs from the mammalian visual cycle in that retinal is converted to retinol in the pigment cells that are equivalent to the mammalian retinal pigment epithelium, rather than in the rhabdomeres that are equivalent to the outer segments. Moreover, retinyl esters (O'Byrne and Blaner, 2013) appear neither to be used for storage nor for transport of vitamin A in flies (Wang et al., 2010). The fly visual cycle also seems to have a rather low efficiency (Stavenga et al., 2017) and serves the *de novo* formation of chromophore when the adult eye is carotenoid deprived, and not as an indispensable chromophore recycling system like in mammals (Wang et al., 2010).

Retinoic acid signaling in *Drosophila melanogaster*—Mammals use vitamin A to synthesize retinoic acid, an important signaling molecule in various tissues (Kam et al., 2012). However, retinoic acid does not play a comparable role in *Drosophila*. While there is a *Drosophila* homolog of mammalian RXR called Ultraspiracle (Usp) (King-Jones and Thummel, 2005), Usp lacks the residues in the ligand binding domain that confer affinity for retinoic acid (Oro et al., 1990) and does not bind the RXR ligand 9-*cis*-retinoic acid (Bonneton et al., 2003). Usp's natural ligand has not been unambiguously identified (Beck et al., 2009; Bonneton et al., 2003; Clayton et al., 2001), but it is well established that Usp has nuclear hormone receptor activity and forms a heterodimer with the Ecdysone Receptor (EcR) (King-Jones and Thummel, 2005). The hormone 20-hydroxyecdysone binds to the EcR-Usp heterodimer and controls major developmental transitions through ecdysone receptor response elements in target genes. While there is no known homolog of RAR in *Drosophila*, Usp can also heterodimerize with Dhr3, the *Drosophila* homolog of the RAR-related Orphan Receptor (ROR) (King-Jones and Thummel, 2005). While RORα binds to cholesterol derivatives (Bitsch et al., 2003; Kallen et al., 2002), RORβ binds to all-*trans*-retinoic acid (Stehlin-Gaon et al., 2003); however, Dhr3 has only a 35% similarity to human RORβ's ligand binding domain (King-Jones and Thummel, 2005). In summary, these data suggest that flies lack canonical retinoic acid signaling.

Two studies suggested that vitamin A metabolites are required for *apolipophorin* (also known as *retinoid and fatty acid binding glycoprotein*) (Shim et al., 1997) and *Rh1* expression (Picking et al., 1996). The latter is controversial because reduced *Rh1* mRNA levels were only observed on Northern blots when Sang's medium, but not other media that lack sources of vitamin A and cause dramatically reduced Rh1 protein levels, was used for vitamin A deprivation (Picking et al., 1996). It is also unclear whether the proposed effects are direct or indirect. Retinoic acid might serve other functions: For instance, one study showed the requirement for retinoid biosynthesis for *Drosophila* tissue regeneration (Halme et al., 2010) and another identified a retinoid-dependent carboxypeptidase that mediates the degradation of misfolded Rhodopsin (Huang et al., 2018).

3. Consequences of vitamin A deprivation for vision in mammals and *Drosophila*

In this section, we review and compare the structural and functional consequences of dietary or genetic vitamin A deprivation in mammals and flies.

Effects of vitamin A deprivation on mammalian photoreceptor morphology and function

In humans, vitamin A deficiency can be caused by suboptimal diets, eating disorders, or chronic impairment of tissues that mediate vitamin A absorption. Vitamin A deprivation causes multiple ocular and extraocular diseases such as xerophthalmia, Bitot's spot, keratitis, and keratomalacia (Sommer, 2008). Chronic vitamin A deprivation is the leading cause of preventable childhood blindness according to the World Health Organization (https://www.who.int/data/nutrition/nlis/info/vitamin-a-deficiency).

Since vitamin A is critical for human vision as a precursor of the visual chromophore (Saari, 2016), insufficient vitamin A intake causes a lack of visual pigment. Consequently, a common initial symptom of acute vitamin A deficiency is impaired rod function and night blindness, i.e. compromised vision in dimly lit environments (Dowling and Wald, 1958, 1960). In contrast to the fly eye (see below), chromophore deficiency does not necessarily cause a concomitant loss of the rod opsin in the mammalian eye: while *Rpe65* mutant mice are unable to generate 11-*cis*-retinal (Fig. 3) and their rods have disorganized outer segments that lack Rhodopsin, the opsin protein is still present and correctly localized in the severely functionally impaired rods (Redmond et al., 1998). *Rpe65* mutant rods slowly degenerate, potentially due to the large amounts of free opsin that constitutively triggers the phototransduction cascade and impairs cellular calcium homeostasis (Cornwall and Fain, 1994; Fain, 2006; Melia et al., 1997). Injection of 11-*cis*-retinal in the *Rpe65* mutant mice causes Rhodopsin regeneration and improves rod function (Ablonczy et al., 2002).

In contrast to the presence of free rod opsin, cone opsin synthesis and trafficking are defective in *Rpe65* mutants (Rohrer et al., 2005). Moreover, cone degeneration begins much earlier and progresses faster than rod degeneration (Rohrer et al., 2005; Znoiko et al., 2005). Cone opsin localization, cone function, and cone survival are improved by early administration of 11-*cis*-retinal (Rohrer et al., 2005; Znoiko et al., 2005), suggesting that the

lack of the chromophore causes the underlying defects. However, it is not known whether dietary vitamin A deficiency leads to comparable cone defects in the mammalian retina.

Since vitamin A is also the precursor of the major signaling molecule retinoic acid, which is indispensable for mammalian development and the functioning of the immune system (Sommer, 2008), chronic deprivation is difficult to study in mammalian model systems. To circumvent lethality, retinoic acid can be added to a vitamin A-depleted diet to rescue the developmental function and to limit vitamin A deprivation to the eye (Katz et al., 1993). This approach is possible because retinoic acid can neither be reduced to retinal nor to retinol in mammals (Dowling and Wald, 1960; Katz et al., 1993). Rats whose eyes are vitamin A deprived in this manner have dramatically reduced Rhodopsin levels (Dowling and Wald, 1960; Katz et al., 1993) and the size of the rod outer segments is reduced by 50% (Katz et al., 1993). These early defects, which eventually progress to photoreceptor degeneration, can be rescued by the injection of all-*trans*-retinol (Katz et al., 1993). Consistent with the deficiency in retinal and Rhodopsin, deprived rats require higher light intensities to reach electroretinogram (ERG) responses that are comparable to the non-deprived control rats (Katz et al., 1993).

In summary, the study of vitamin A deprivation in nocturnal mammals (mice and rats) yielded major insights into the consequences for rod structure and function. Because it is technically challenging to make mammalian eyes vitamin A deficient and, to our knowledge, there is a lack of an established model to study vitamin A deficiency in a diurnal mammal, the molecular consequences (particularly in cones) are still understudied.

Effects of vitamin A deprivation on *Drosophila* photoreceptor morphology and function

Since flies do not require vitamin A for their development or immune responses, chronic deprivation studies are conveniently feasible either through dietary deprivation (Figs. 7A–B) or genetic deprivation (Figs. 7D–F). The latter can be achieved by the mutation of genes that are essential for vitamin A metabolism but whose loss also does not cause lethality. When flies are chronically vitamin A deprived throughout their development, their light-sensing compartments, the rhabdomeres, are abnormally shaped and often improperly spaced (Fig. 7). Moreover, the surface area of the rhabdomeres is dramatically reduced (Lee et al., 1996) (Fig. 7). These defects resemble the structural damage in vitamin A-deprived mammalian outer segments. The size defect can be rescued by feeding the flies with carrot juice (Sapp et al., 1991). Moreover, like deprived rat photoreceptors, deprived *Drosophila* photoreceptors also show a dramatic decrease in Rhodopsin levels (Harris et al., 1977; Nichols and Pak, 1985). Without the chromophore, the opsin cannot complete the secretory pathway and instead remains in an immature, glycosylated state in the endoplasmic reticulum (Huber et al., 1994; Ozaki et al., 1993). This immature opsin is degraded faster in vitamin A deficient photoreceptors than in vitamin A replete photoreceptors (Huber et al., 1994). Taken together, chromophore deficiency in flies causes defective opsin maturation and trafficking, which resembles chromophore deficient mammalian cone opsins but contrasts the expression and proper localization of free mammalian rod opsin.

Like mammalian vitamin A deficient eyes, deprived *Drosophila* eyes show abnormal ERG responses due to their lack of visual pigment. For instance, the ERGs of both *ninaD* and

ninaB mutants lack the prolonged depolarizing afterpotential (PDA), which is reflected in their names (*nina* stands for '*neither inactivation nor afterpotential*') (Pak et al., 2012). The PDA is generated by a strongly depolarizing blue-light stimulus that converts a substantial amount (>20%) (Pak et al., 2012) of Rhodopsin to Metarhodopsin, saturates the response of the photoreceptors, and renders them unresponsive to another stimulus. In non-deprived flies, the PDA persists in the dark for several minutes after the stimulus is switched off, because the Metarhodopsin molecules outnumber the Arrestin molecules that are required for the termination of the response (Dolph et al., 1993). Due to the bistable nature of the fly visual pigment (see above), the PDA can be terminated by reconverting Metarhodopsin to Rhodopsin with an orange or red stimulus (Hamdorf and Rosner, 1973; Ostroy et al., 1974; Stavenga et al., 2017). The absence of the PDA in *ninaD* mutants is consistent with their lack of Rhodopsin - that would be required to overcome Arrestin levels - due to the lack of the chromophore.

In summary, *Drosophila melanogaster* is a powerful model to study the effects of vitamin A deficiency on the eye because vitamin A is not required for fly development or survival. However, little is known about the molecular consequences of vitamin A deprivation beyond opsin maturation. Moreover, like in mammalian models, most fly studies were focused on the rod-equivalent photoreceptor subtype (outer photoreceptors) and we know very little about how the cone-equivalent photoreceptor subtypes are affected.

Conclusions

The comparison of mammalian and fly models of vitamin A metabolism and deficiency reveals that similar mechanisms underlie the formation of the chromophore and the visual pigment, which includes evolutionarily conserved genes for absorption and processing of dietary β-carotene. Moreover, vitamin A deprivation causes a dramatic loss of the chromophore and the visual pigment, as well as similar structural and functional photoreceptor defects in rod or rod-equivalent photoreceptors in both model systems. The dependency of cone opsin maturation and fly Rhodopsin maturation on the retinal chromophore is also similar.

However, the mechanisms of vitamin A distribution and storage, the light response of the visual pigment (bleaching and chromophore release in mammals), and the role of the visual cycle are very different. Moreover, mammals show a much broader range of functions of vitamin A due to its role in retinoic acid-mediated gene regulation, while the role of the related nuclear receptors in flies is substantially divergent. It will be interesting to see whether more specialized roles of retinoic signaling in flies will be found in future studies. Moreover, it needs to be addressed in more detail in both model systems how different photoreceptor subtypes are affected by vitamin A deficiency.

In summary, mammalian and fly models have revealed deep insights into the molecular mechanisms of vitamin A metabolism and how the mutation of key players causes a variety of human pathologies. Given the remarkable recent progress, this field will continue to provide critical insights into the molecular basis of vision and inspire the development of therapies to prevent and treat degenerative eye diseases.

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Highlights

- Comparison of mammalian and fly models to study the role of vitamin A in vision.
- Vitamin A deprivation causes similar molecular and functional defects in the eye.
- Shared mechanisms for chromophore and visual pigment formation.
- Mechanisms for vitamin A distribution and storage are different.
- Light response of the visual pigment and role of the visual cycle also differ.

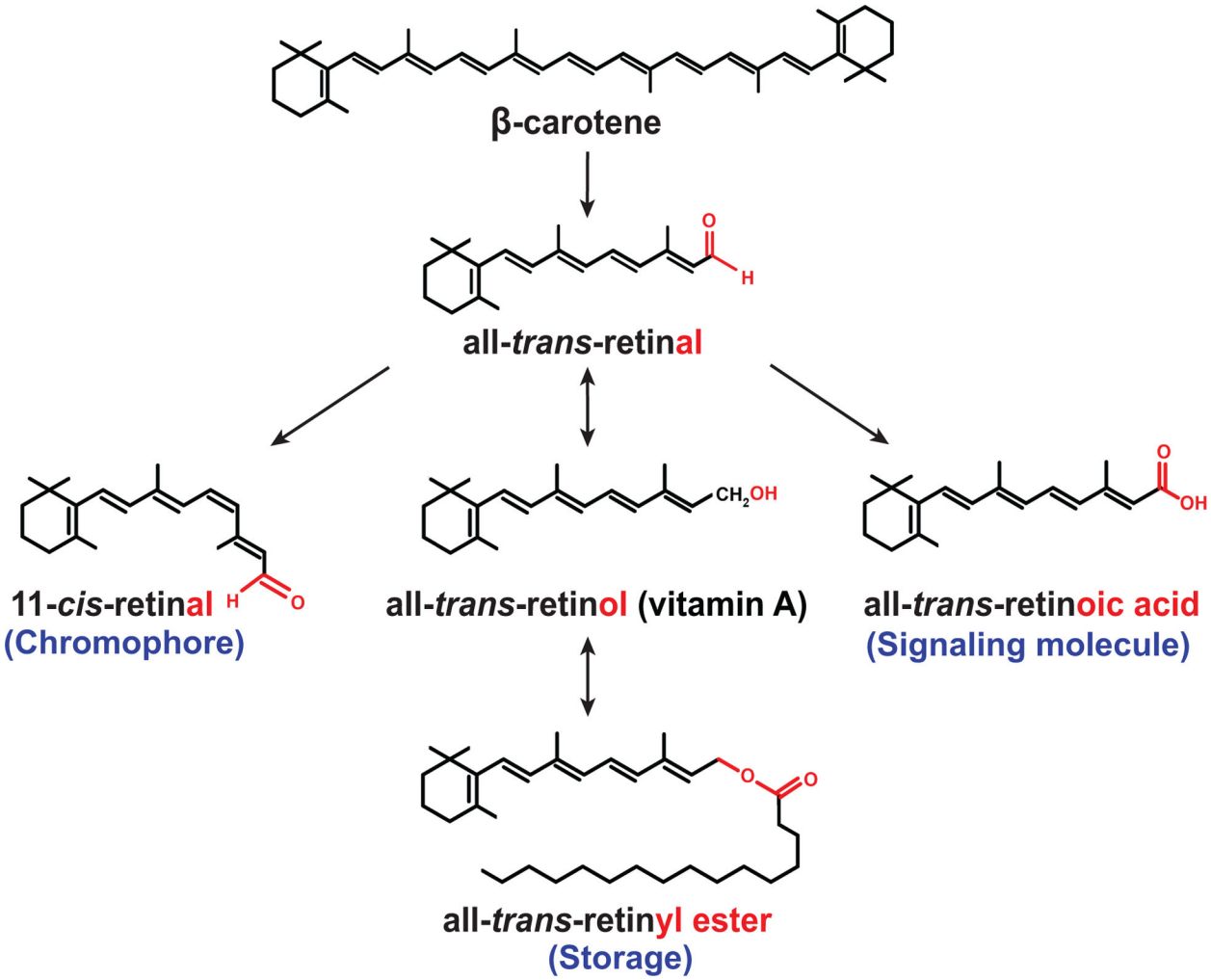

Figure 1. Major carotenoid and retinoid molecules in mammalian vitamin A metabolism.

The oxidative cleavage of the dietary precursor β-carotene generates all-*trans*-retinal (vitamin A aldehyde), which can be isomerized to the visual chromophore 11-*cis*-retinal. Oxidation of all-*trans*-retinal results in the signaling molecule all-*trans*-retinoic acid, while reduction of all-*trans*-retinal generates all-*trans*-retinol (vitamin A) that can be esterified to all-*trans*-retinyl ester, an important transport and storage form of vitamin A in mammals.

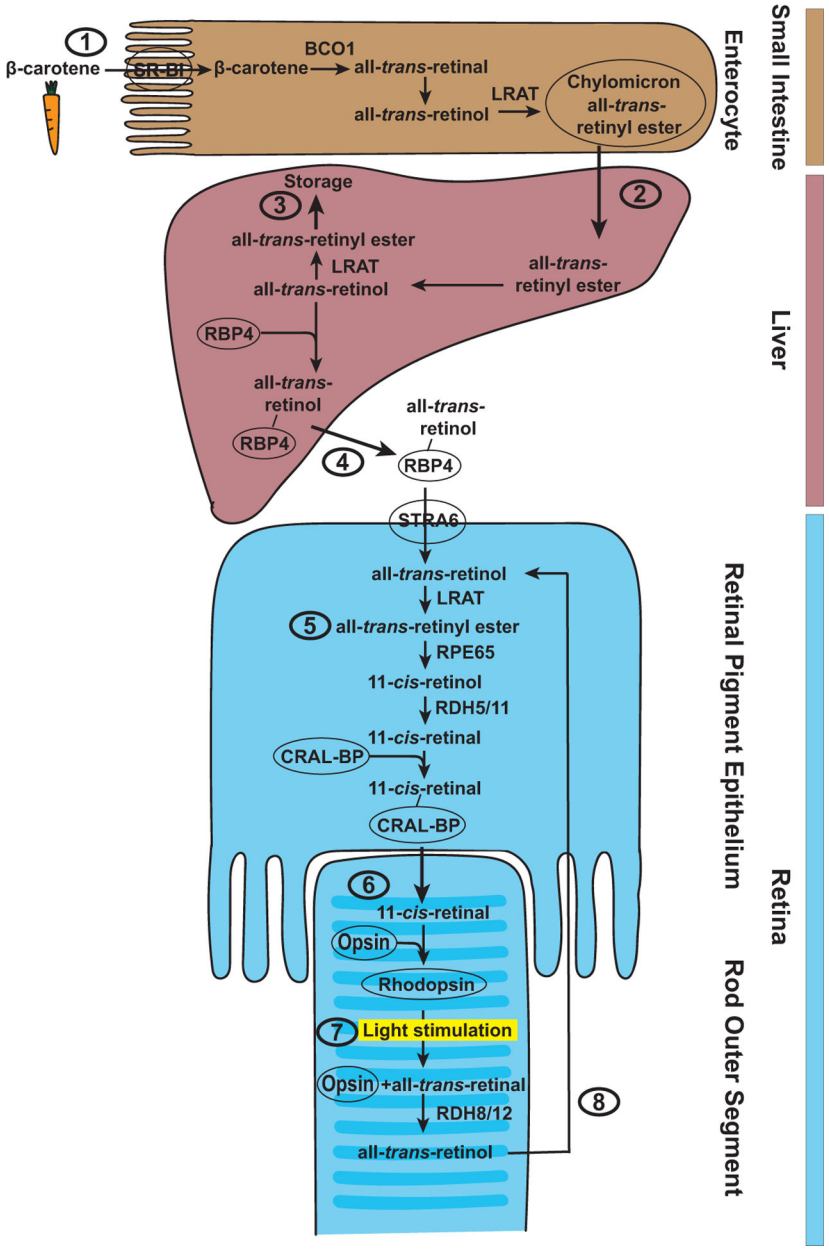

Figure 2. Carotenoid uptake and vitamin A metabolism in mammals.

(1) β-carotene is absorbed in the enterocytes of the small intestine by SR-BI and broken down to two all-*trans*-retinal molecules by BCO1. All-*trans*-retinal is converted to all-*trans*-retinol and esterified by LRAT to all-*trans*-retinyl esters for transport in chylomicrons. (2) In the liver, all-*trans*-retinyl ester is hydrolyzed to all-*trans*-retinol, which can again be esterified into all-*trans*-retinyl ester by LRAT for storage (3) or bind to RBP4 for transport to the eye (4), where it is taken up in the retinal pigment epithelium by STRA6. (5) There, LRAT converts all-*trans*-retinol to all-*trans*-retinyl ester, which is converted in a series of enzymatic steps involving RPE65, RDH5, and RDH11 to the 11-*cis*-retinal chromophore. (6) CRAL-BP transports the chromophore to the outer segments of the photoreceptors, where it covalently binds to the opsin protein and forms the visual pigment Rhodopsin. (7)

Light stimulation causes isomerization of the chromophore from *cis* to *trans*, a conformational change of the opsin, and dissociation of all-*trans*-retinal from the opsin. (8) Free all-*trans*-retinal is reduced to all-*trans*-retinol by RDH8 and RDH12 and transported to the retinal pigment epithelium.

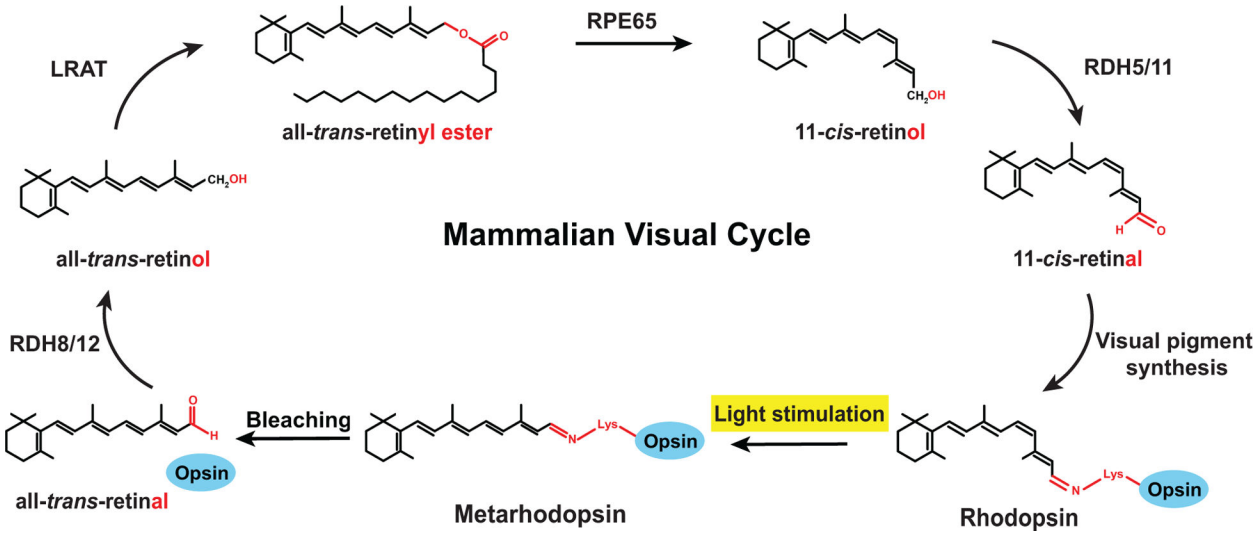

Figure 3. Mammalian visual cycle.

In the mammalian visual pigment, the 11-*cis*-retinal chromophore is covalently bound to the opsin (blue). Light stimulation causes a *cis* to *trans* isomerization (bleaching), hydrolysis of the Schiff base linkage, and release of all-*trans*-retinal. Chromophore regeneration is initiated by reduction of all-*trans*-retinal by RDH8/12 to all-*trans*-retinol and its transport to the retinal pigment epithelium. All-*trans*-retinol is esterified by LRAT to all-*trans*-retinyl ester, which is isomerized by RPE65 to 11-*cis*-retinol; the latter is oxidized by RDH5/11 to yield the 11-*cis*-retinal chromophore.

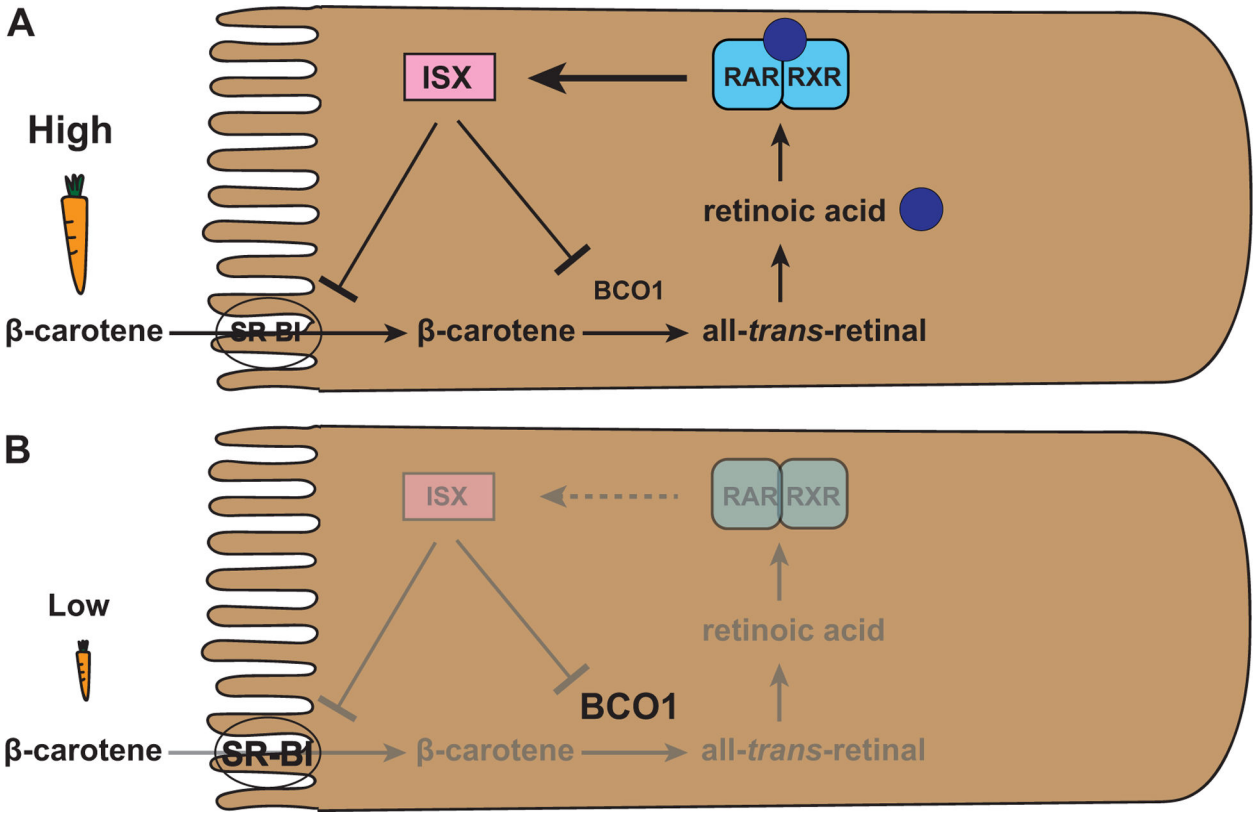

Figure 4. Retinoic acid signaling controls vitamin A production in mammals.

(A) Abundance of dietary β-carotene leads to the synthesis of retinoic acid that binds the RAR-RXR heterodimer and induces the expression of ISX. ISX is a repressor of SR-BI and BCO1 that are required for β-carotene uptake and processing, respectively. This retinoic acid based negative feedback mechanism limits vitamin A production.

(B) Vitamin A deprivation results in a lack of retinoic acid and thus a loss of activation of the repressor ISX. The lack of ISX leads to an increased expression of SR-BI and BCO1 and thus increased vitamin A production through increased β-carotene uptake and processing, respectively.

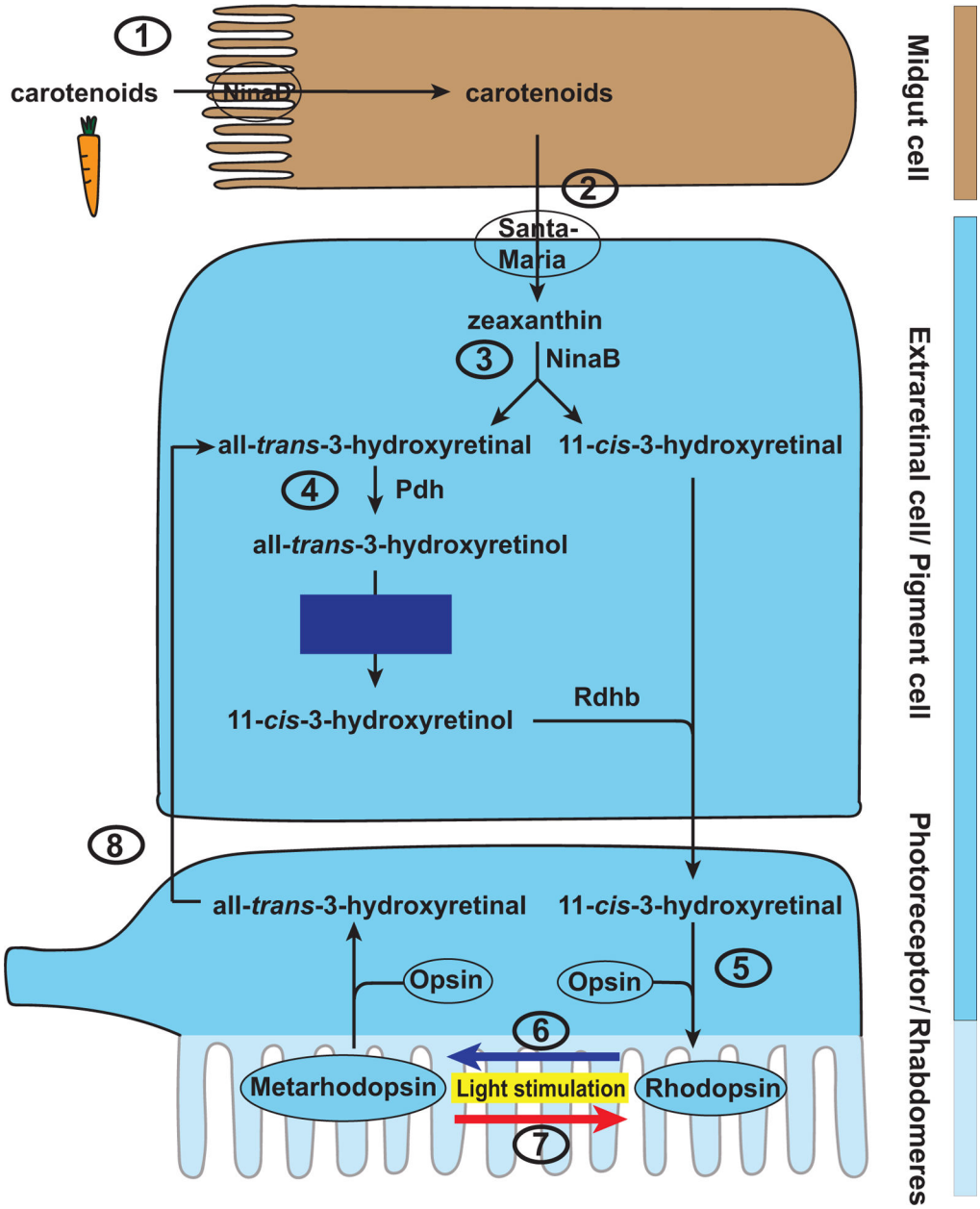

Figure 5. Carotenoid uptake and vitamin A metabolism in *Drosophila*.

(1) Dietary carotenoids are taken up in the *Drosophila* midgut by NinaD, the homolog of SR-BI. (2) Carotenoids reach the head via the hemolymph and are taken up in unknown extraretinal cells by another scavenger receptor, Santa-Maria. (3) Carotenoids are converted to zeaxanthin that is cleaved by NinaB, a homolog of BCO1 and RPE65, to 11-*cis*-3-hydroxyretinal and all-*trans*-3-hydroxyretinal. (4) The latter is reduced and isomerized to 11-*cis*-3-hydroxyretinol in a Pdh- and blue light-dependent manner. Oxidation by Rdhb generates the 11-*cis*-3-hydroxyretinal chromophore. (5) The chromophore covalently binds to the opsin protein and thereby forms the visual pigment Rhodopsin. (6) The absorption of light causes a photoisomerization from *cis* to *trans* and converts Rhodopsin to its activated form, Metarhodopsin. Note that all-*trans*-3-hydroxyretinal remains bound to the opsin.

Metarhodopsin can be reconverted to Rhodopsin by absorption of a photon of a specific wavelength (7). Some Metarhodopsin is degraded and the released all-*trans*-3-hydroxyretinal (8) can be recycled to 11-*cis*-3-hydroxyretinal in pigment cells.

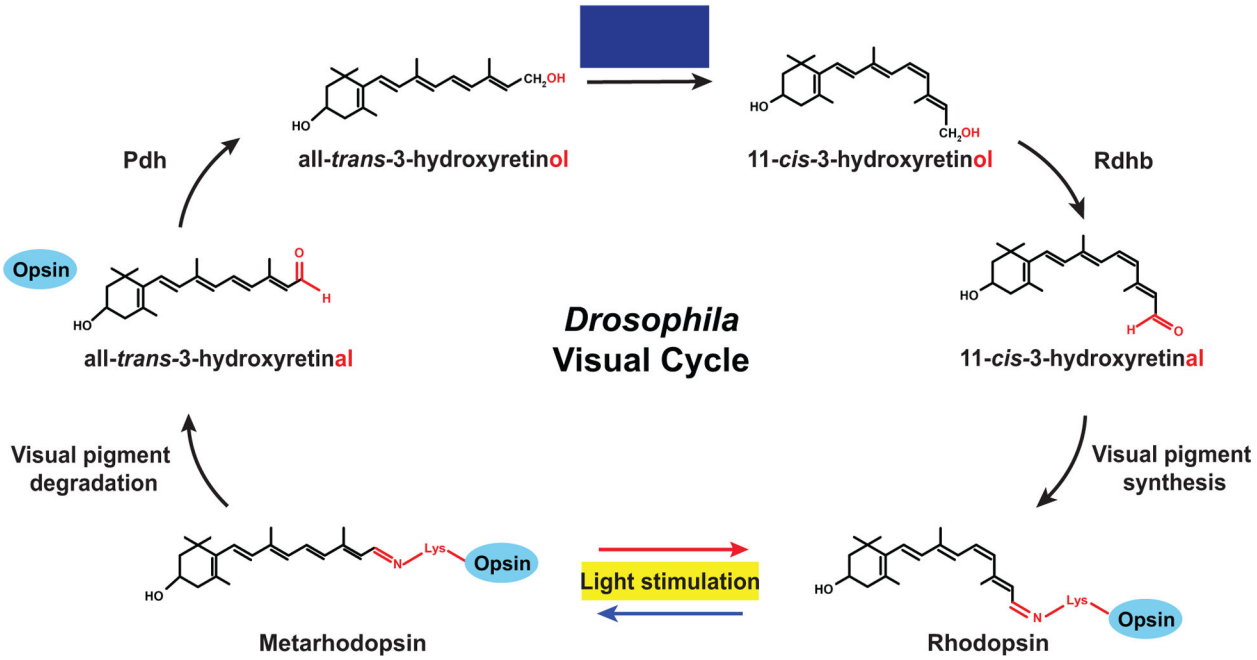

Figure 6. *Drosophila* visual cycle.

In the *Drosophila* visual pigment, the 11-*cis*-3-hydroxyretinal chromophore is covalently bound to the opsin (blue). After absorption of light and the resulting *cis* to *trans* isomerization, the chromophore remains bound to the opsin. Some of the activated visual pigment (Metarhodopsin) is slowly degraded and releases all-*trans*-3-hydroxyretinal, which is reduced to all-*trans*-3-hydroxyretinol by Pdh. All-*trans*-3-hydroxyretinol is isomerized in a blue light-dependent manner to 11-*cis*-3-hydroxyretinol, which is oxidized by Rdhb to the 11-*cis*-3-hydroxyretinal chromophore that is used for visual pigment synthesis.

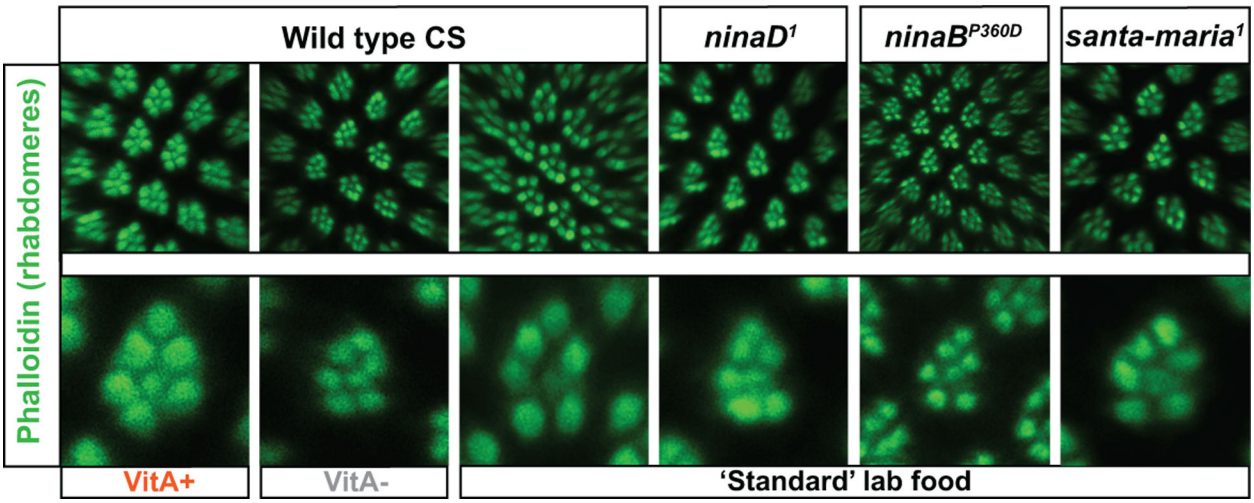

Figure 7. Dietary and genetic vitamin A deprivation in *Drosophila*.

The light-sensing compartments (rhabdomeres) of adult *Drosophila* photoreceptors are labeled with Phalloidin (green). Scale bars, 10 μm.

(A-C) Dietary vitamin A deprivation causes structural rhabdomere damage.

(A) and (C) Wild type Canton S flies that were raised on minimal medium with β-carotene supplementation (VitA+) or 'standard' lab food have normally sized and shaped rhabdomeres (green).

(B) Wild type CS flies that were vitamin A deprived on minimal medium without β-carotene supplementation (VitA-) have dramatically reduced, abnormally shaped, and improperly spaced rhabdomeres.

(A'-C') High magnification view of single unit eyes. Seven rhabdomeres are visible; note the reduction of the cross-sectional diameter, improper spacing, and abnormal shape caused by dietary vitamin A deprivation (B').

(D-F) Genetic vitamin A deprivation through mutation of genes that are critical for vitamin A metabolism also causes rhabdomere damage (compare to wild type in C). All mutants were raised on 'standard' lab food.

(D'-F') High magnification view of single mutant unit eyes. Seven rhabdomeres are visible; note the reduction of the cross-sectional diameter, improper spacing, and abnormal shape in all mutants (compare to wild type in C'). See text for details.