E-Article

The Vitamin A–Redox Hypothesis: A Biochemical Basis for Honest Signaling via Carotenoid Pigmentation

Geoffrey E. Hill* and James D. Johnson

Department of Biological Sciences, Auburn University, Auburn, Alabama 36849

Submitted October 26, 2011; Accepted July 15, 2012; Electronically published October 5, 2012

abstract: Trade-offs in resource allocation have been widely stated as the means by which the honesty of ornamental traits is maintained, but an alternative to this resource trade-off hypothesis is that production of ornamentation is linked to the biochemical efficiency of vital cellular processes. Carotenoids are antioxidants, potentially tying carotenoid-based coloration to the oxidative state of an organism, and some carotenoids are also precursors for vitamin A, which regulates numerous cellular processes. We present a biochemical model for regulation of ornamental coloration based on interdependencies of carotenoid and retinoid biochemistry. We propose that vitamin A regulatory mechanisms, redox systems, and carotenoid pigmentation pathways link carotenoid coloration to oxidative state and to a host of important aspects of performance, such as immune function. The activity of β-carotene ketolase, which catalyzes the oxidation of yellow carotenoids into red carotenoids, is responsive to the states of vitamin A pools and redox systems such that coloration is a direct reflection of the physiological state of an animal. According to the vitamin A–redox hypothesis, feather coloration is associated with a range of performance measures because performance emerges from functionality of the same basic cellular processes that regulate pigmentation. We present the vitamin A–redox hypothesis as a testable alternative hypothesis to the resource trade-off hypothesis for the maintenance of honesty of carotenoid pigmentation.

Keywords: sexual selection, condition-dependent trait, oxidative state, plumage coloration, β-carotene ketolase, 4-keto-carotenoid, cardueline finch.

Introduction

The indicator model of sexual selection proposes that individuals in better condition have greater expression of showy traits such as bold colors, elaborate songs, or long tails than individuals in lower condition (Zahavi 1977; Hamilton and Zuk 1982; Kodric-Brown and Brown 1984). According to this theory, ornamental traits are used in choosing mates and assessing rivals because the higher condition that is associated with bigger, more elaborate, and more brilliant ornaments is indicative of better performance (Hamilton and Zuk 1982; Andersson 1986). These concepts of condition and honest signaling lie at the heart of sexual selection theory, but for most traits in most animals there is no unambiguous and comprehensive definition of condition and no clear mechanisms to link ornament expression to performance (Hill 2011).

Until recently, the most widely cited definition of condition in the evolutionary and physiological ecology literatures was "the pool from which resources are allocated" (Rowe and Houle 1996). This definition of condition is the foundation of the resource trade-off hypothesis, which proposes that individuals trade off allocation of energy or other limiting resources such as pigment molecules between body maintenance and ornament production such that only males with large resource reserves can both maintain body function and produce elaborate ornamentation. More recently, condition was defined as the capacity to maintain vital cellular processes such that ornamentation links to performance via shared pathways regulated by condition (shared pathway hypothesis; Hill 2011). Because most aspects of performance that are associated with ornament expression—immunocompetence, oxidative state, energy balance, muscular or neural performance, and so on—are the result of processes that occur at the cellular level, ornamental traits as signals of cellular processes is an intuitively appealing idea. For most ornaments, however, too little is known about the biochemical processes that affect production for the shared pathway hypothesis to be evaluated (Metcalfe and Alonso-Alvarez 2010).

Here we provide a hypothesis for the cellular pathways that link carotenoid coloration to individual condition and performance. Carotenoid coloration is one of the best-studied ornamental traits, and it is widely proposed to be condition dependent in a range of vertebrate taxa (Hill 2006; Lopez et al. 2009). Carotenoid pigments produce brilliant yellow, orange, and red coloration in the skin, bills, eyes, and feathers of animals (Fox 1976), which are among the most striking visual displays in nature. Unlike other pigments used as animal colorants, carotenoids can-

* Corresponding author; e-mail: ghill@auburn.edu.

Am. Nat. 2012. Vol. 180, pp. E127–E150. 0003-0147/2012/18005-53420$15.00.
DOI: 10.1086/667861

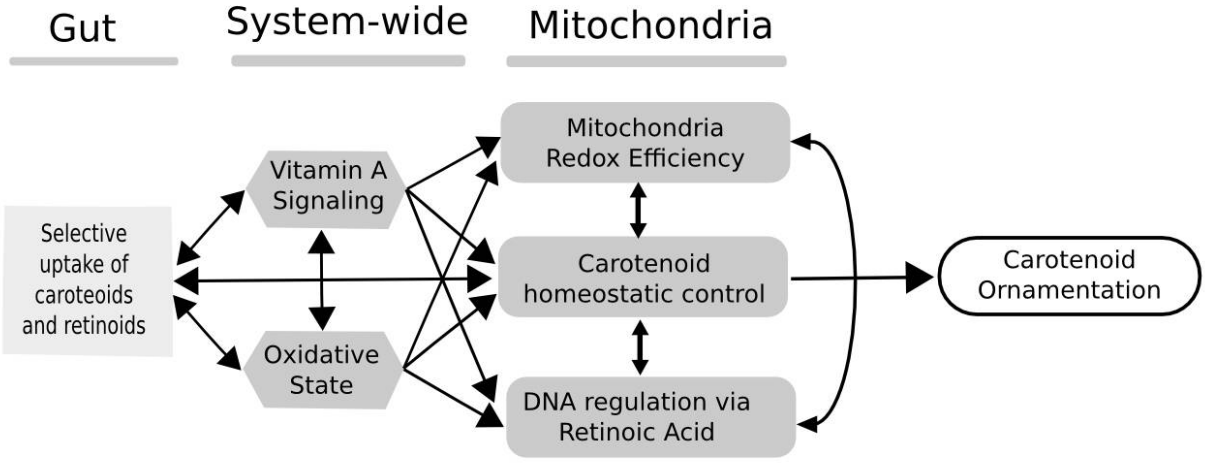


Figure 1: A generalized schematic representing the functional links between carotenoid ornamentation and the basic cellular processes that underlie the vitamin A–redox hypothesis. Uptake of carotenoids and retinoids is closely regulated to maintain a homeostatic vitamin A and redox state within the organism. Redox and vitamin A systems, which can be affected by a wide range of environmental perturbations from disease to toxins, in turn dictate the efficiency of the critical biochemical processes in the mitochondria, the regulation of a myriad of genetically controlled processes through the signaling molecular all-trans retinoic acid, and the pathways needed to produce ornamental carotenoid pigmentation. It is the interdependencies of vitamin A and carotenoid pathways that link carotenoid coloration to cellular redox homeostasis and functionality of physiological systems. There is potential for resource limitation and allocation trade-offs involving carotenoids and vitamin A to play a role in determining signal honesty, but the intimate links between coloration and vital cellular processes mean that carotenoids can honestly signal individual condition without the need for resource trade-offs.

not be synthesized de novo by animals and so must be acquired through the diet (Goodwin 1984). Although no vertebrate can synthesize carotenoid pigments, some vertebrates endogenously convert yellow dietary carotenoids into red keto-carotenoids. For most species of vertebrates, including all songbirds, the primary carotenoid pigments available in the diet are yellow; red carotenoid pigments are produced through the oxidation of yellow pigments (McGraw 2006). Some dietary carotenoids, most importantly β-carotene and β-cryptoxanthin, also serve as precursors for vitamin A (retinol), and carotenoid cleavage is an important source of vitamin A for vertebrates (von Lintig 2010).

Birds are among the most colorful class of vertebrates, and a large literature has developed demonstrating that environmental circumstances during feather growth including access to carotenoid pigments (Hill 1992; McGraw et al. 2006), nutrition (Hill 2000; Hill et al. 2009) and exposure to parasites (Thompson et al. 1997; Hill et al. 2004) can alter feather pigmentation. Moreover, experimental studies have found complex interactions between carotenoid supplementation, pathogen exposure, immuno-stimulation, hormone levels, oxidative state, and skin or feather coloration (Peters 2007; Alonso-Alvarez et al. 2008; Peters et al. 2011). The overall pattern that emerges from these studies is that carotenoid pigmentation is somehow associated with fundamental redox pathways and the immune system, but the mechanisms for such links remain poorly understood (McGraw et al. 2010; Svensson and Wong 2011).

The Vitamin A–Redox Hypothesis

As a means to explore the mechanisms for honest signaling via carotenoid coloration, we present a hypothesis of the cellular pathways for carotenoid pigments in vertebrates as they fulfill roles as colorants, antioxidants, and vitamin A precursors (fig. 1). We propose that it is the interdependencies of these basic cellular processes—and particularly the heretofore unappreciated importance of β-carotene and β-cryptoxanthin as vitamin A precursors and the role of vitamin A in modulating cellular processes—that link carotenoid coloration to oxidative state and to a host of important aspects of individual performance such as immune function, vision, growth, lipogenesis, glycolysis, and energy homeostasis. By this model, the information content of carotenoid coloration derives primarily from these shared pathways. We present the vitamin A–redox hypothesis (fig. 1) as a testable alternative to the resource

trade-off hypothesis as an explanation for the maintenance of honest signaling via carotenoid coloration, although the vitamin A–redox hypothesis and the resource trade-off hypothesis are not necessarily mutually exclusive. Each might correctly describe the same ornament system in different contexts. Our goal is to stimulate new thinking regarding the links between carotenoid pigmentation and condition and to advocate the inclusion of more detailed biochemical pathways in hypotheses for the mechanisms of honest signaling. In this way, we present our model as a starting point for future tests of the signal content of carotenoid-based coloration in animals and how and why such color signals evolved and are maintained.

Vitamin A and Carotenoids

Because the biochemistry of vitamin A, carotenoids, and related molecules is likely unfamiliar to many biologists interested in animal ornamentation, we begin with a basic overview of the chemistry these classes of molecules (fig. 2).

Basic Biochemistry of Carotenoids

Carotenoids play important biochemical roles in all animals and must be acquired in the diet because they cannot be synthesized de novo (Goodwin 1984). Carotenoids in the tissues of animals are characterized by alternating double bonds that extend across a carbon backbone consisting of eight individual isoprene units. The majority of dietary carotenoids have six-carbon end-rings (known as ionone rings) with one double bond per ring. If the double bond is in the C5-C6 position, the end ring is considered a β-ionone ring; if the double bond is in the C4-C5 position, the end ring is considered an ε-ionone ring. Chemically, these end rings behave differently and are generally, but not always, acted upon by specialized β- and ε-ionone-specific enzymes (fig. 2).

The alternating, conjugated double bonds that span carotenoids from one end ring to the other lower the overall energy of electrons, giving carotenoids their chemical and physical properties. Low-energy electrons make carotenoids sensitive to visible light, hence their prolific occurrence in and around the photosystems of plants, algae, and bacteria. Carotenoids actively interact with radicals in the membranes in which they reside undergoing both electron abstraction and electron transfer reactions (Edge et al. 1997; Truscott 2001; Liu et al. 2008; Böhm et al. 2011). The most fundamental radical reaction carried out by carotenoids involves the one-electron reduction of a radical thereby quenching the latter and creating a positively charged carotenoid radical (CAR•+). Whether these reactions are antioxidant or pro-oxidant depends on the redox potential of the partner with which the carotenoid radical interacts (Böhm et al. 2011). Vitamin A, with only one-half the conjugated electrons of carotenoids, is also capable of quenching radicals and thereby may also serve as an antiradical partner in membranes (Livrea and Tesoriere 1998; Palace 1999).

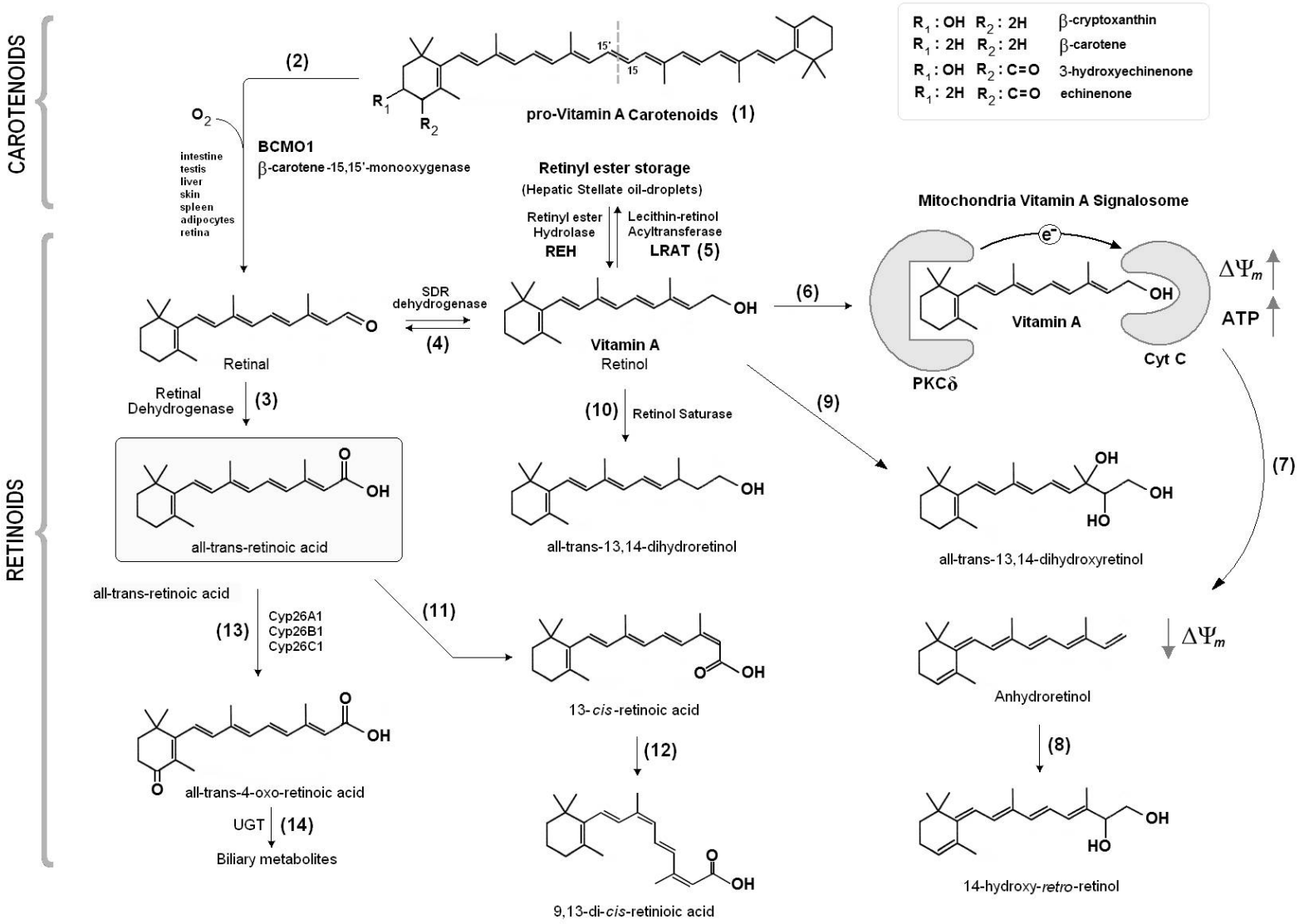
The molecular structure and charge of carotenoids determines how they interact with reactive oxygen species (ROS). Carotenes such as β-carotene, which have no functional groups, are highly reduced compared to carotenoids with ketone and hydroxyl functional groups such as astaxanthin. Carotenes serve as free radical scavengers within the lipid bilayer and typically function as antioxidants, but they may turn pro-oxidant with increasing oxygen levels or changes in local redox conditions (Edge et al. 1997; Truscott 2001). Astaxanthin, in contrast, sits nearly perpendicular within the bilayer, permitting it to interact with the cytosol on both sides of the membrane. This orientation makes oxidized carotenoids such as astaxanthin more readily accessible to cytosoltic antioxidants such as ascorbate and glutathione (Truscott 2001). Within the bilipid membrane, astaxanthin may also interact with α-tocopherol and ubiquinone (Edge et al. 1988; Böhm et al. 1997). Astaxanthin is also capable of directly oxidizing superoxide, a property shared only with ubiquinone (Martínez et al. 2008; Galano et al. 2009). In general, the superoxide-quenching capability of carotenoids correlates with the redness (Martínez et al. 2008; Galano et al. 2009).

Basic Biochemistry of Retinoids

Retinoids are a class of apocarotenoids that include retinol, otherwise known as "vitamin A," and other similar molecules that do not have vitamin A activity, such as retinal, retinoic acid, and retinyl esters (fig. 2). Retinoids also includes a host of synthetic molecules that show bioactive retinoid behavior in vivo. Hereafter we will use "retinoid" to refer to natural apocarotenoids.

Vitamin A, like carotenoids, is lipophilic (hydrophobic) and has antioxidant properties with physiological consequences (Monaghan and Schmitt 1932; Ciaccio et al. 1993; Tesoriere et al. 1993; Palace et al. 1999). Vitamin A is also susceptible to auto-oxidation in the presence of oxygen, leading to products that can be especially detrimental to the cell and most importantly to mitochondria (Olson 1990; Murata and Kawanishi 2000; Krinsky and Johnson 2005; Gelain and Moreira 2008; Pasquali et al. 2008, 2009*a*, 2009*b*, 2010). If excessive vitamin A is consumed in the diet, which is rare in wild animals, hypervitaminosis A toxicity may develop (Klamt et al. 2003*a*, 2003*b*; Senoo et al. 2010). The key point is that too much vitamin A can be as damaging to cellular processes as too little.

Because vitamin A and related retinoids are lipophilic


CAROTENOIDS
RETINOIDS
(2)
O2
BCMO1
β-carotene-15,15'-monooxygenase
intestine
testis
liver
skin
spleen
adipocytes
retina
pro-Vitamin A Carotenoids (1)
R1 : OH R2 : 2H β-cryptoxanthin
R1 : 2H R2 : 2H β-carotene
R1 : OH R2 : C=O 3-hydroxyechinenone
R1 : 2H R2 : C=O echinenone
Retinyl ester storage
(Hepatic Stellate oil-droplets)
Retinyl ester Hydrolase REH
Lecithin-retinol Acyltransferase LRAT (5)
Mitochondria Vitamin A Signalosome
e-
ΔΨm
ATP
Vitamin A
Cyt C
PKCδ
SDR dehydrogenase
(4)
Retinal
Vitamin A
Retinol
(6)
Retinal Dehydrogenase
(3)
(10)
Retinol Saturase
(9)
(7)
all-trans-retinoic acid
all-trans-13,14-dihydroretinol
all-trans-13,14-dihydroxyretinol
all-trans-retinoic acid
(13)
Cyp26A1
Cyp26B1
Cyp26C1
(11)
13-cis-retinoic acid
ΔΨm
Anhydroretinol
(12)
(8)
all-trans-4-oxo-retinoic acid
UGT
(14)
Biliary metabolites
9,13-di-cis-retinioic acid
14-hydroxy-retro-retinol

as well as susceptible to oxidation, they are transported by small carrier proteins within cells (Sun 2012). These small carrier proteins include cellular retinol binding proteins (CRBPI, CRBPII, and CRPBIII) and cellular retinoic acid binding proteins (CRABPI, CRABPII, and CRABPIII), all members of the fatty acid binding protein superfamily of lipophilic carriers (Selvaraj et al. 2008). These proteins facilitate intracellular trafficking of retenoids, and they protect bound retinoids during storage and transport by providing a protective protein shell in which they reside. Retinol transport by the various isoforms of CRBP is a highly regulated process and is critical to vitamin A homeostasis (Senoo et al. 2010). Generally, retinoid carrier proteins exceed in concentration the level of unesterified retinoids within the cell in order to protect both the cell and the retinoid from spurious reactions but also to increase the controlled flux of these active signaling molecules between sites of action. Vitamin A is stored in hepatic stellate or extrahepatic oil droplets after being esterified by lecithin:acyl retinol transferase to fatty acids such as palmitate, stearate, or linoleic (fig. 2; Moise et al. 2007; Senoo 2007; Senoo et al. 2010).

Vitamin A is the principal parent compound of bioactive retinoids such as all-trans-retinoic acid (fig. 2), which serve primarily as transcriptional ligands controlling and modulating gene expression (D'Ambrosio et al. 2011). All-trans-retinoic acid is largely responsible for the regulation of its own sequestration, production, and destruction, indicating the strict control of this bioactive signaling molecule (Napoli 2012). In addition to vitamin A's primary role as a DNA transcriptional regulator, a growing body of evidence now supports nongenomic roles for vitamin A (Gelain et al. 2006; Acin-Pérez et al. 2010*a*, 2010*b*; Berry et al. 2011). The majority of these nongenomic processes remain to be proven physiologically, although growing evidence indicates that vitamin A may play significant roles as antioxidants and modulators of electron transport in mitochondria; for instance, cytochrome c and vitamin A may form a functional complex known as a signalosome (Acin-Pérez et al. 2010*a*; Hoyos et al. 2012; fig. 2). Additional nongenomic roles have been characterized as physiologically important, for example in post-translational modification of proteins (retinylation of proteins having significant effects on protein-lipid interactions; Takahashi and Breitman 1994; Tucci et al. 2008; Senatore et al. 2010), mitochondrial energy and redox homeostasis (Chiu et al. 2008), and as a plasma-circulating signaling complex bound to retinol binding protein 4 regulating energy homeostasis and insulin levels in target tissues (Berry et al. 2011; Berry and Noy 2012).

Sources of Vitamin A

Vitamin A is an essential micronutrient. It can be sequestered directly from the diet, and dietary retinol is encountered primarily in the form of retinyl esters that are hydrolyzed by intestinal brush-border esterases. Retinol is then transported into the enterocyte where it is re-esterified with palmitic or stearic acid for transport to developing chylomicrons or stored locally within retinyl esters oil droplets (Sun 1997; Napoli 2012).

Alternatively, vitamin A can be synthesized de novo from the symmetrical cleavage (cleaved at the 15,15′ position) of β-carotene or other pro-vitamin A carotenoids by β,β-carotene-15,15′-carotenoid oxygenase (BCMO1), an enzyme found predominately active within hepatic stellate cells (Amengual et al. 2011*a*; fig. 2). An alternative pathway for vitamin A synthesis, currently considered a minor pathway, is the asymmetric cleavage of β-carotene by β, β-carotene-9′,10′-oxygenase (BCO2). In the BCO2 pathway, β-carotene (or other pro–vitamin A carotenoid) is cleaved asymmetrically into β-ionone (a C12 fragment) and β-10′-apocarotenal (C28). β-10′-apocartonal can be oxidized and trimmed in the β-oxidation cycle of mito-

Figure 2: The principal pathways linking retinoids and pro–vitamin A carotenoids. If one or both of the β-ionone rings of the carotenoid (*1*) is unmodified, it can serve as a precursor for the production of retinal, which can be directly converted to retinol and stored as retinyl esters. Retinol can serve in a host of physiologically supportive roles and is the precursor of all-trans-retinoic acid, a prolific and critical modulating ligand of gene transcription. The production of retinal following symmetric pro–vitamin A carotenoid cleavage (*2*) by BCMO1 may be directly converted to all-trans-retinoic acid (*3*) or to vitamin A (retinol) (*4*). The latter can then be esterified for storage (*5*) by lecithin:retinol acyltransferase (LRAT) and released from storage by retinol ester hydrolase (REH). The mitochondrial signalosome (*6*) provides redox support for maintaining mitochondrial membrane potential and stimulation of the Krebs cycle using vitamin A as cofactor (Hoyos et al. 2012). Vitamin A can also be converted to all-trans-13,14-dihydroxyretinol (*9*), anhydroretinol (*7*), and 14-hydroxy-retroretinol (*8*), which control cell proliferation (Moise et al. 2007). Anhydroretinol has been implicated as an antagonist to retinol in the control of mitochondria redox potential. In addition, vitamin A can be saturated by retinol saturase (*10*) producing all-trans-13,14-dihydroxyretinol, which may serve as precursor to other retinoids or as a sink for retinoids (Moise et al. 2004). Retinal is converted in an irreversible step to all-trans-retinoic acid (*3*), the principle regulating ligand of retinoic acid receptor and retinoic X receptor nuclear receptors. Another retinol, 9-cis-retinol (not shown), is limited to expression in the β cells of the pancreas and may originate biosynthetically from the absorption of 9-cis-β-carotene, while 9,13-di-cis-retinoic acid (*12*) remains uncharacterized. Not shown is 11-cis-retinal, which serves as a photoreceptor chromophore in the vision cycle. Finally all-trans retinoic acid is biologically deactivated by p450 CYP26 (*13*) and excreted following glucuronidation by UDP-glucuronosyltransferase (UGT) after which the metabolites pass through the bile for excretion.

chondria to yield retinal and subsequently vitamin A (fig. 2). Currently there is little to support the idea that this latter pathway is biologically significant for production of vitamin A (Amengual et al. 2011*a*).

Dietary carotenoids that are assimilated for use as animal colorants or precursors to animal colorants fall into two classes. The first class contains pro-vitamin A carotenoids, which include the dietary carotenoids β-carotene, β-cryptoxanthin, and α-carotene (fig. 2). All pro-vitamin A carotenoids have at least one unmodified β-ionone end-ring, a substrate recognition feature for cleavage by BCMO1. β-cryptoxanthin, for example, has only one hydroxylated β-ionone end-ring, so it can be cleaved to produce one molecule of vitamin A. The second class of carotenoids, called xanthophylls, have both end-rings oxygenated and cannot serve as vitamin A precursors (fig. 2). Lutein and zeaxanthin are the most common xanthophylls with no pro-vitamin A activity in the diets of most vertebrates. Pro–vitamin A carotenoids are a major source of vitamin A in many vertebrates (Packer et al. 2005), although the relative importance of dietary carotenoids versus dietary retinoids as sources of vitamin A is unknown for any wild animal.

Physiology of Vitamin A

Vitamin A plays a key role in a dazzling array of basic life processes. Vitamin A is best known for its role in the visual cycle (Wald 1968; Rando 1990; Peterson et al. 2005) and as a transcriptional activator binding to retinoic acid response element in the promoter regions of genes. Vitamin A has been found to participate in embryonic development (Johnson and Scadding 1991; Cupp et al. 1999), postnatal growth (Antipatis et al. 2000; Sun et al. 2006), spermatogenesis (Hogarth and Griswold 2010), testosterone synthesis and release (Chaudhary et al. 1989; Tucci et al. 2008; Munetsuna et al. 2009), mitochondrial thermogenic and redox uncoupling and mitochondrial complex I and II redox homeostasis (Alvarez et al. 1995; Barber et al. 2000; Estornell et al. 2000; Korichneva et al. 2003; Ballinger 2005), energy homeostasis (Chiu et al. 2008; Acin-Pérez et al. 2010*b*), immunity (Mora et al. 2008; Mullin 2011), glycolysis (Hoyos et al. 2000; Yang et al. 2005; Acin-Pérez et al. 2010*a*), intracellular signaling (Buck et al. 1991), adipogenesis and lipogenesis (Amengual et al. 2011*a*; Reichert et al. 2011; Yasmeen et al. 2012), insulin regulation (Driscoll et al. 1997; Berry et al. 2011), modulation of the receptor for advanced glycation end products (Gelain et al 2011), apoptosis (Klamt et al. 2008; Livingston and Klasing 2008), cell differentiation (Wu and Zhao 1993; Park et al. 1997), cell proliferation (Naves et al. 2001), regulation of ATP-binding cassette transporters (ABCA1; Costet et al. 2003), B lymphocyte activation (Ertesvag et al. 2007; Maruya et al. 2011), T-cell activation (Iwata et al. 2003; Li and Li 2007), triglyceride and cholesterol metabolism (Standeven et al. 1996; Wuttge et al. 2001), and stimulation of the microbial commensal immunity (Hall 2010). All of these processes are integrally associated with the control of core homeostatic redox processes, many of which have been implicated in redox-based diseases.

Vitamin A and Redox Regulation

Vitamin A has recently been shown to play a significant physiological role in redox homeostasis. Vitamin A deficiency in laboratory rats led to increases in oxidative damage to mitochondria, although the underlying cause could not be assigned to transcriptional activity or known antioxidant properties of vitamin A (Barber et al. 2000; Estornell et al. 2000). It was recently discovered that vitamin A plasma concentrations in vivo (1–2 μM) are necessary for the coactivation of protein kinase C-δ (PKCδ) serving as a signal transducer upstream of the pyruvate dehydrogenase complex within the mitochondria matrix (Acin-Perez et al. 2010*a*; Hoyos et al. 2012). It was proposed that retinol directly participates in electron transfer between cytochrome c and PKCδ, thereby implicating vitamin A as a cofactor in the homeostatic control of the redox state of mitochondria, modulating the flux of acetyl CoA into the Krebs cycle. Conflicting results have been presented by other researchers (see Hoyos et al. 2012) regarding the proposed pathway of the PKCδ cascade (it would require, e.g., a yet-undiscovered phosphatase), but the stimulatory effects of vitamin A as a cofactor in the redox activation of PKC-γ resulting in increased acetyl-CoA flux into the Krebs cycle appear well founded (Acin-Perez et al. 2010*b*; Hoyos 2000, 2012). Recently Berry and Noy (2012) uncovered a signaling role for vitamin A, whereby it triggers hormone-like responses on the surface of vascular endothelia cells. These and other recent findings have shed new light on potential core regulatory roles of vitamin A in energy homeostasis (Chen et al. 1999; Korichneva et al. 2003; Chiu et al. 2008; Hoyos et al. 2012).

Retinol is the precursor for all-trans-retinoic acid that regulates DNA transcriptional activity of more then 500 genes, many of which are integral to core energetic pathways that maintain cellular redox equilibrium. All-trans-retinoic acid serves as a DNA transcriptional activator/repressor in numerous fundamental processes (such as lipogenesis and adipogenesis) under strict redox control, either at the protein or DNA ligand receptor level (see Zolfaghari and Ross 2003; Schonfeld and Wojtczak 2008; Park et al. 2009). Retinoic acid signaling appears early in chordate evolution, at the vertebrate-invertebrate boundary, having a proposed link with hox gene expression (Marlétaz et al. 2006; Albalat 2009; fig. 2). Other retinoids,

such as 13,14-didehydroretinol, 14-hydroxy-retro-retinol, and anhydroretinol have bioactive roles independent of all-trans-retinoic acid (Moise et al. 2007; Chiu et al. 2008; fig. 2).

In considering how carotenoids function in the bodies of vertebrates beyond their role as colorants, evolutionary and physiological ecologists have focused on the immunostimulatory and antioxidant properties of carotenoids (Cohen and McGraw 2009; Svensson and Wong 2011). The role of provitamin A carotenoids as a primary dietary source for vitamin A has been almost completely overlooked by biologists studying ornamental coloration, but carotenoids, retinoids, and ROS are fundamentally interdependent within the bodies of vertebrates. Here we present a biochemical model in which we hypothesize that it is the connections and interdependencies between vitamin A systems, redox processes, and production of ornamental pigments that primarily link carotenoid coloration to performance. In our opinion, the signaling properties of carotenoids simply cannot be understood without due consideration of the physiological requirements for the acquisition, storage, and mobilization of vitamin A and its bioactive metabolites in controlling vital homeostatic redox states within the organism and in regulating carotenoid bioavailability and the transport of carotenoids to peripheral tissues. Our model focuses most specifically on animal species that display red ketolated carotenoids because in these animals the connections between carotenoid and retinoid systems are most pervasive. We do not, however, limit the hypothesis to red taxa. Species that use exclusively the non-pro-vitamin A carotenoids lutein and zeaxanthin and their metabolic derivatives in ornamentation are still subject to vitamin A regulation of carotenoid uptake, transport, and metabolism, as described below. The vitamin A–redox hypothesis potentially applies to all animals that have carotenoid-based color displays.

Our hypothesis is drawn primarily from studies on retinoid, carotenoid, and ROS pathways as they relate to vitamin A homeostasis and redox balance conducted on model organisms such as *E. coli*, *Drosophila*, *Mus*, and humans. This research was published primarily in the biochemical and biomedical literatures. A few studies came from the poultry literature. Except for the carotenoid content of food, plasma, and the integument (reviewed in McGraw 2006), virtually no studies of the mechanisms for pigmentation in songbirds or any vertebrate with ornamental carotenoid coloration have been undertaken. Many of the mechanisms by which carotenoids are taken up, transported, and deposited and many of the pathways that connect carotenoid biochemistry to redox pathways and vitamin A homeostasis are conserved across eumetazoans and certainly across vertebrates (Packer et al. 2005; Moise et al. 2007; von Lintig 2010). Thus, the hypothesized pathways we present are in all cases physiologically and phylogenetically reasonable. Our vitamin A–redox hypothesis is meant to be a starting point for studies of vitamin A and carotenoid processes in birds and other ornamented vertebrates.

The Biochemical Basis for the Vitamin A–Redox Hypothesis

The heart of the vitamin A–redox hypothesis is interactions and interdependencies between the physiological processes in the uptake, transport, mobilization, and metabolism of retinoids and carotenoids, including both pro–vitamin A carotenoid and non-pro–vitamin A carotenoids. Vitamin A reserves meet a constant and vital requirement within an organism for the regulation of genomic transcription through the action of specific retinoids that are products of vitamin A, most notably all-trans-retinoic acid (fig. 2). Nongenomic roles of vitamin A also appear to be critical for cellular homeostasis, but this area of vitamin A biochemistry is in its infancy. Current work on the control of oxidative phosphorylation by vitamin A (Chiu et al. 2008; Acin-Perez et al. 2010; Hoyos et al. 2012) provides compelling evidence for nonclassical transcriptional regulation by vitamin A in cellular homeostasis.

The mobilization of vitamin A stores for the biosynthesis of all-trans-retinoic acid involves two dehydrogenation steps, both under the genetic control of all-trans-retinoic acid (fig. 2; Napoli 2012). The first step is a reversible dehydrogenation converting retinol to retinal. The second step is a committed and irreversible oxidation to retinoic acid (fig. 2). CRBP-I, the vitamin A intracellular carrier protein, shuttles vitamin A to and from these early conversion reactions. For this reason CRBP-I becomes a critical player in accounting for the movement of and intracellular storage (outside of oil droplets) of vitamin A and its metabolites. Normally, all-trans-retinoic acid is maintained at nanomolar levels because of its potentially deleterious teratogenic influence on gene promoters (Mark et al. 2006; Pan and Baker 2007). On a timescale of hours, organisms consume vitamin A to control gene activity and to carry on other nongenomic activities. Vitamin A circulation and recirculation rates are high in plasma, but if circulating vitamin A levels drop, as might result from an immune challenge, then rapid mobilization of vitamin A from hepatic stellate or other extrahepatic stores is required to maintain strict concentrations levels in plasma (1–2 μM; Ross and Zolfaghari 2004). Recently it has been demonstrated that vitamin A storage levels in hepatic stellate cells was the principal determinant in vitamin A disposal rates (Cifelli et al. 2008).

The role of vitamin A, most notably its role as precursor to all-trans-retinoic acid, requires the cooperation of nu-

merous cofactors and supporting systems, many of which are predicted to be redox sensitive; hence, the biochemical efficiency of acquiring, sequestering, storing, and transporting vitamin A should be indicative of the overall condition of the organism. Those organisms in good condition (sensu Hill 2011), which operate in a favorable environment where vitamin A stores are not limiting, should perform well. When faced with challenges such as disease or oxidative stress, these healthy organisms will be better equipped to mobilize vitamin A from its retinyl ester stores to maintain the efficient and timely processing required to neutralize the challenge. Those organisms which are otherwise less well equipped, perhaps with lower storage reserves of vitamin A, will experience lower levels of biochemical efficiency and hence a lower capacity to quickly mobilize and transport vitamin A from its stores in hepatic stellate cells to extrahepatic tissue where it is needed. We advance the hypothesis that those organisms that are most capable of sequestering, storing, and mobilizing vitamin A are also those best able to signal this condition through oxidative reactions that convert carotenoids into ornamental signals. We further hypothesize that the successful oxidation of ornamental pigments reflects the overall biochemical efficiency of the underlying vital pathways and that the oxidation is not necessarily controlled by the availability of carotenoids or vitamin A in the environment (Hill 2011).

It has been long established that xanthophylls play a modulatory role in immunology, likely as antioxidants, but the mechanisms for such modulation remain uncertain. Furthermore, xanthophylls, at least in cells that express the asymmetric mitochondrial cleavage carotenoid oxygenase BCO2, are known to accumulate within the membranes of mitochondria. Amenugal et al. (2011*b*) report that carotenoids initiate pro-oxidant effects at the level of mitochondria and are deleterious under the conditions reported in BCO1 knockout and wild-type mice. Nevertheless, we propose that birds, which accumulate carotenoids in hepatic mitochondria (Mayne and Parker 1986), may be better able to utilize carotenoids as antioxidants and potentially modulate electron transport within mitochondria. So long as carotenoids are maintained within a favorable concentration window within mitochondria—with the aid of BCO2, which appears to remove oxidized or otherwise irreversibly destroyed carotenoids—carotenoids may provide a redox benefit, especially in cooperation with active ER mitochondria-associated membranes (MAM). This is an area of carotenoid physiology that has been neglected but holds much promise for illuminating the roles of carotenoids in mediating oxidative stress.

The Vitamin A–Redox Hypothesis Applied to Cardueline Finch Feather Coloration

The above overview of the interactions between retinol, mitochondrial redox state, tissue homeostasis, and carotenoids hints at how carotenoid pigmentation in vertebrates might signal fundamental aspects of cellular functionality. To make such a hypothesis applicable to the study of ornamental traits, a more specific hypothesis for ornamentation, vitamin A balance, and oxidative state is needed. Here we present a hypothesis for carotenoid coloration of feathers in one family of songbirds: family Fringillidae, the cardueline finches. An exclusive focus on plumage coloration in cardueline finches allows us to avoid having to consider the many individual cases of carotenoid use across vertebrates, which cannot be accommodated in a single manuscript. The same or similar carotenoid pathways, however, will also be found not only in other families of songbirds and across class Aves, but throughout subphylum vertebrata in species with carotenoid-based coloration. Most importantly, the same basic connections between redox and vitamin A pathways that make carotenoid coloration in cardueline finches an honest signal of individual condition will occur in every animal with carotenoid-based coloration. Thus our specific model for carotenoid pigmentation in cardueline finches can serve as a general model for control of carotenoid pigmentation in animals.

The Biochemical Basis for Red and Yellow Coloration

Among cardueline finches, there are three basic mechanisms for production of carotenoid pigmentation (McGraw 2006), which can be thought of as three tiers of ornamentation. The first tier, which involves simplest mechanism, is the deposition of the yellow dietary carotenoids lutein and zeaxanthin, unmodified, into feathers, bills, and skin. This mechanism accounts for yellow feather coloration in chaffinches and some grosbeaks (genera *Mycerobas*, *Fringilla*, *Coccothraustes*, *Hesperiphona*, and *Pyrrhula*; Stradi 1998; McGraw 2006). The second tier of ornamentation involves the oxidation of dietary lutein and zeaxanthin into 3-dehydrolutein and canary xanthophyll A or B. This yellow-to-yellow carotenoid pathway produces the yellow feather coloration found in many canaries, siskins, and goldfinches as well in as some bullfinches and grosbeaks (genera *Serinus*, *Carduelis*, *Pyrrhoplectes*, *Rhynchostruthus*, and *Pyrrhula*; Stradi et al. 1995). The third tier of ornamentation, involving the most complex mechanisms of production, is the oxidation of yellow dietary carotenoids into red keto-carotenoid pigments. This mechanism accounts for the red plumage of rosefinches, bullfinches, redpolls, crossbills, and some

grosbeaks and siskins (genera *Carpodacus*, *Uragus*, *Pyrrhula*, *Rhodopechys*, *Loxia*, *Carduelis*, *Haematospiza*, and *Pinocola*; Stradi et al. 1996, 1997; Inouye et al. 2001).

In every cardueline finch with red feather coloration for which the pigment composition of feathers has been quantified, the predominant red keto-carotenoid is 3-hydroxy-echinenone (Stradi et al. 1996, 1997; Stradi 1998; Inouye et al. 2001; see fig. 2 for the structure of 3-hydroxy-echinenone and other carotenoids). The 3-hydroxy-echinenone is formed from dietary β-cryptoxanthin through the ketolation of one of the two β-ionone rings. If both ionone rings are ketolated the red pigment adonirubin is formed (Stradi et al. 1996; McGraw 2006). Because it is a more highly oxidized and conjugated carotenoid, adonirubin creates a more intense red hue than 3-hydroxy-echinenone. Adonirubin occurs in much reduced concentrations compared to 3-hydroxy-echinenone in the feathers of cardueline finches. Secondary sources of red coloration in cardueline finches are the red pigments echinenone and canthaxanthin, formed from the ketolation of one or both rings, respectively, of dietary β-carotene. Astaxanthin is formed from zeaxanthin when both end rings are ketolated (McGraw 2006). Other songbirds use astaxanthin or canthaxanthin instead of 3-hydroxy-echinenone as their primary red feather pigment (McGraw 2006). Our focus on cardueline finches dictates a focus on the ketolation of β-cryptoxanthin to 3-hydroxy-echinenone. We will begin by presenting a cellular biochemical model for the production of red keto-carotenoids from yellow dietary pigments (fig. 3), and we will then consider mechanisms for less complex pathways for yellow feather pigmentation in cardueline finches.

The Vitamin A–Redox Hypothesis Applied to Red Finches

We propose that the associations between red bill, skin, and feather coloration and performance measures such as immunocompetence arise through the interdependencies of vitamin A systems, oxidative pathways, and the metabolism of dietary pro–vitamin A carotenoids by a yet-to-be-described β-carotene ketolase enzyme (fig. 3). The β-carotene ketolase is the enzyme that catalyzes the C4 ketolation of β-cryptoxanthin into 3-hydroxy-echinenone, a red keto-carotenoid. The key features of the mechanism that we propose is that production of red pigments for feather, bill, and skin coloration is directly linked to vitamin A homeostatic processes because the dietary pigments needed for production of red pigments for ornamentation are also vitamin A precursors. The production of red pigments will likely be sensitive to any environmental challenge that depletes vitamin A pools or that alters delicate redox balances that control the efficient operation of vital energy and redox centers. To achieve optimal performance at a cellular level, which is observable at the organismal level as characteristics such as immunocompetence, stamina, and cognition, finches must effectively regulate the uptake, storage, distribution, and use of carotenoids and vitamin A. In presenting this model, we will consider the route that carotenoid pigments take in moving from the lumen to feathers, the biochemical transformations that occur along this route, and the key mechanisms that control the pathways involved (fig. 3).

Absorption and Transport of Carotenoids and Retinoids

The process leading to ornamental coloration begins with the uptake of carotenoids from ingested food in the form of micelles in the intestinal lumen into enterocytes (cells at the interface of the gut and lumen; Kotake-Nara and Nagao 2011; fig. 3). Efficiency of the uptake of ingested carotenoids is unmeasured in cardueline finches, but in humans it is relatively low and is variable for different carotenoids in different dietary sources (Chitchumroonchokchai et al. 2004). Before intestinal absorption at the lumen, esterified xanthophylls are hydrolyzed to free carotenoids, most likely by the action of the pancreatic cholesterol esterase (EC 3.1.1.13; Granado-Lorencio et al. 2006).

Uptake of carotenoid molecules from the lumen occurs primarily by facilitated transport with limited passive diffusion (During et al. 2005; Yonekura and Nagao 2007; Kotake-Nara and Nagao 2011; Harrison 2012). Facilitated transport of carotenoids, as well as cholesterol and other lipids, occurs at the interface of the lumen and enterocytes and is under the control of several transmembrane transport proteins. Transporters that are known to bind carotenoids in vertebrate species include protein scavenger receptor class B member 1 (SR-B1), cluster determinant 36 (CD36), and members of the ABC transporter family including ABCG5/G8 complex, which has been implicated in transporting lutein in the chicken (Kreiger 1999; Reboul et al. 2005; Herron et al. 2006; During et al. 2008).

The important role of SR-B1 in regulating carotenoid uptake has been demonstrated in both the human retina (During and Harrison 2007) and in the guts of *Drosophila* (Kiefer et al. 2002), domestic mice (van Bennekum et al. 2005; Lobo et al. 2010), and humans (Borel et al. 2011). Animals do not at all times maximize uptake of carotenoids, as has sometimes been assumed in the ecology literature (Hill 1992); rather, protein-facilitated transport appears to be both selective and highly regulated (Reboul et al. 2005; Nagao 2011). Facilitated diffusion of dietary carotenoids into enterocytes is complex and the details are only beginning to emerge (Nagao 2011). SR-B1 appears to be primarily responsible for the uptake of carotenes, although lutein absorption by SR-B1 in humans has been

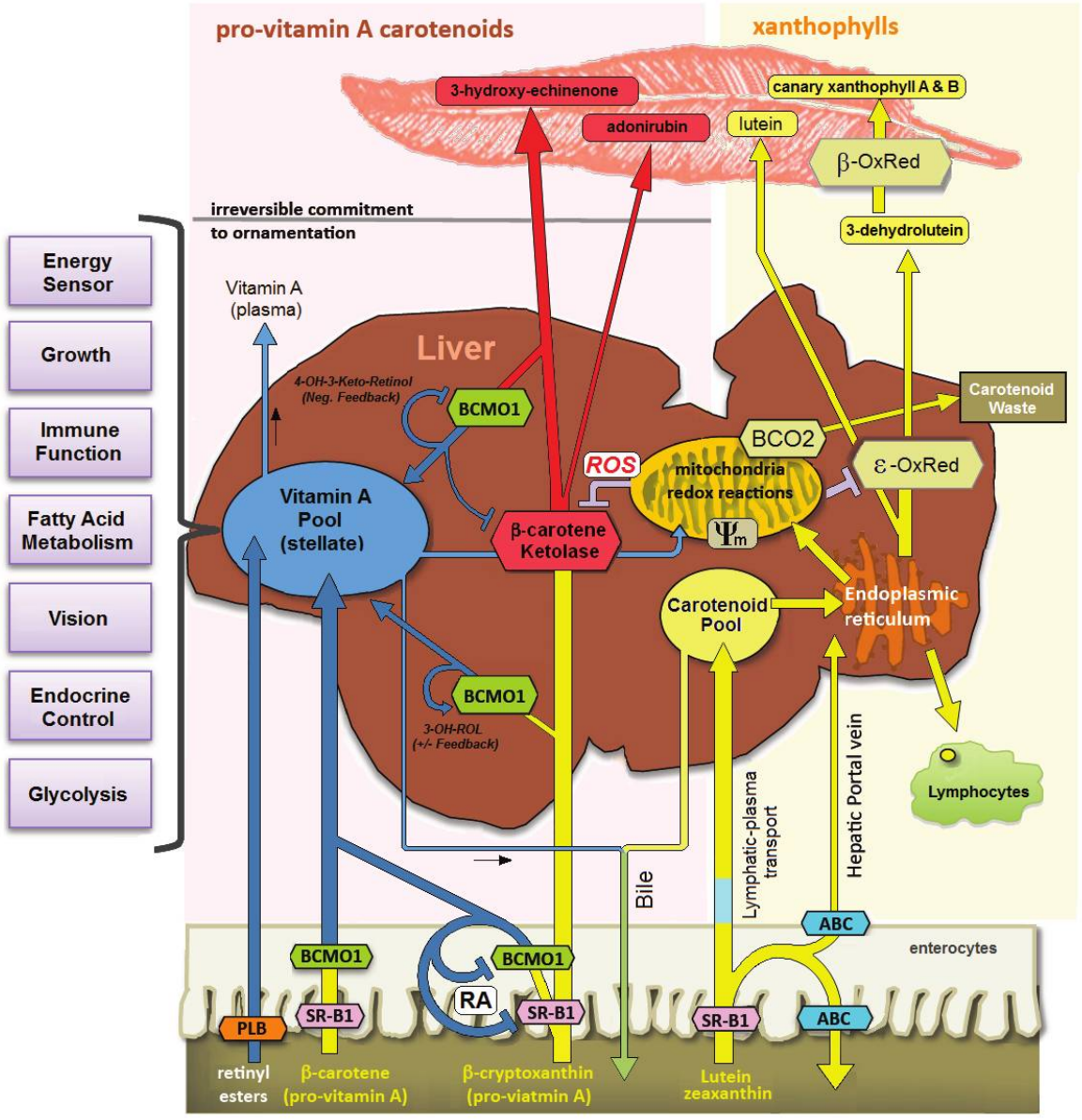


Figure 3: The vitamin A–redox hypothesis applied to carotenoid and retinoid pathways in a cardueline finch (family Fringillidae) with red feather coloration. Carotenoid uptake from the lumen into enterocytes is controlled by the scavenger receptor B1 (SR-B1), which is regulated by local pools of vitamin A via retinoic acid (RA). Carotenoids may also enter by diffusion or be facilitated by transporters such as cluster determinant 36 (CD36), Niemann-Pick C1-like protein, as well as other yet unidentified protein transporters (Reboul and Borel 2011; Sakudoh et al. 2010). β-carotene is cleaved within enterocytes of the gut to retinol and either stored locally as retinyl esters or packaged along with other lipids into chylomicrons for transport to the liver via the lymphatics (thoracic duct) for further processing and packaging. β-cryptoxanthin is the primary precursor for red feather pigmentation while simultaneously serving as a source for vitamin A. Dietary vitamin A is also taken up in the form of retinyl esters facilitated by phospholipase B (PLB). Vitamin A is transported to the plasma from the vitamin A pool to maintain strict plasma vitamin A levels. Depending on the state of vitamin A pools and local redox requirements, as determined by levels of reactive oxygen species (ROS), β-cryptoxanthin is either converted to the red ketocarotenoid 3-hydroxy-echinenone or cleaved by β,β-carotene-15,15′-carotenoid oxygenase (BCMO1) to retinol and 3-hydroxy-retinol, the latter likely serving as a feedback transcriptional regulator of BCMO1 and possibly the β-carotene ketolase as well. Under stressful conditions, the vitamin A–redox hypothesis predicts that 3-hydroxy-echinenone is repartitioned back into the vitamin A pool by BCMO1. When β-cryptoxanthin is converted to

reported (Reboul et al. 2005; Harrison 2012). In addition, SR-B1 has been demonstrated to be a bidirectional transporter moving lipids into enterocytes and reverse transporting them back into the lumen (Reboul and Borel 2011). Other ABC transporters, such as cluster determinant 36, may play a significant role in total carotenoid absorption and reverse transport, but the overall contributions of these proteins to total carotenoid absorption remain unknown (Kotake-Nara and Nagao 2011).

Carotenoids have also been found in biliary fluids in humans (but not in rats or ferrets), so the biliary tree may constitute a recycling loop for carotenoids and retinoids between the liver and the lumen of the intestine (Leo et al. 1995; fig. 3). Carotenoid absorption via deoxygenated hepatic portal venous circulation may materially affect the overall mass balance and success of absorptive processes at the level of the intestinal lumen, as was demonstrated in the extrahepatic circulation of cholesterol (Yu et al. 2002). The importance of this route of carotenoid circulation is supported by the fact that up to 30% of circulating high-density lipoprotein (HDL) is formed via this route. In chickens, the hepatic portal vein is a significant pathway for the absorption of fatty acids from the intestines (Noyan et al. 1964). Earlier, Bloom et al. (1951) found that short-chain fatty acids (<C12) were preferentially carried to the liver by the portal hepatic circulation suggesting that unesterified carotenoids may also follow this route. These results support the proposition that a fraction of carotenoids may be absorbed into the hepatic circulation, but to date, carotenoid transport has not been characterized in either the biliary fluids or hepatic portal circulation of birds.

Within the enterocyte, β-carotene and β-cryptoxanthin, the major pro-vitamin A carotenoids in the diets of songbirds, are either packaged into chylomicrons (where they are assembled into lipoproteins) for transport to the liver via the lymphatic and circulatory systems or they are cleaved locally by the enzyme BCMO1 into retinal as the first step toward retinol production (von Lintig and Vogt 2000; Wyss 2004; Moise et al. 2005). At the level of the intestine, SR-B1 and BCMO1 are subject to negative feedback by all-trans retinoic acid (Boulanger et al. 2003; Lobo et al. 2010; fig. 3). By storing vitamin A esters locally, enterocytes can supply the necessary levels of vitamin A required to maintain adequate real-time retinoic acid signaling and control of membrane transporters and other systems, including localized immune responses.

The storage and bioactivation of retinoids provide communication links between the vitamin A system of the body and carotenoid uptake into enterocytes. Through such feedback control of SR-B1 and BCMO1, retinol regulates the absorption and use of carotenoids (fig. 3). Feedback control by retinol can originate locally in the enterocytes themselves or it can originate in the liver (Lobo et al. 2010). Importantly for understanding the production of ornamental coloration, carotenoid absorption and cleavage is a regulated process geared toward the maintenance of both vitamin A homeostasis and beneficial levels of circulating carotenoids, not necessarily toward maximizing quantities of total carotenoids.

SR-B1 facilitates not only absorption of β-carotene and β-cryptoxanthin but also the absorption of lutein and, potentially, zeaxanthin, two of the most abundant dietary carotenoids that are not vitamin A precursors (Reboul et al. 2005). Because lutein and zeaxanthin are not pro–vitamin A, they follow a pathway that overlaps with, but is largely independent of, the pro–vitamin A dietary carotenoids (fig. 3). Some carotenoids, and particularly lutein and zeaxanthin, are reverse transported back into the lumen from enterocytes via ABCA1 (Herron et al. 2006). The active transport of carotenoids out of enterocytes and into the gut by ABC-like transporters requires ATP, so not only are there circumstances in which organisms absorb less than maximal carotenoids, there are conditions under which individuals expend energy to move absorbed carotenoids out of the body. This observation of actively exporting carotenoids back into the intestinal lumen is consistent with some recent observations of the cost of excess carotenoids (Huggins et al. 2010; Amengual et al. 2011*b*), but it stands in contrast to ideas that animals always maximize carotenoid absorption as a means to maximize carotenoid pools for ornamental coloration (Hill 2002). The activity of SR-B1, BCMO1, and ABC trans-

adonirubin or deposited within keratinizing feathers, it is irreversibly lost to the vitamin A pool. Uptake of the dietary xanthophylls lutein and zeaxanthin is also regulated by SR-B1 and potentially other uncharacterized transport proteins and may be further controlled through reverse transport into the intestinal lumen via ATP-binding cassette transporter (ABC) or SR-B1 (Reboul and Borel 2011). Dietary xanthophylls that remain in the body are moved to carotenoid pools, primarily in the liver, from where they can be used locally as antioxidants; moved into pathways to become feather pigments; stored in adipose tissue or in small quantities in lymphocytes; diverted to biliary circulation for excretion; or cleaved by β, β-carotene-9′,10′-oxygenase (BCO2) to be removed as waste. Through oxidation reactions catalyzed by one or more, likely two, unknown oxidoreductases (β-OxRed and ε-OxRed), lutein can be converted to 3-dehydrolutein or canary xanthophyll A or B. Carotenoids enter the endoplasmic reticulum from internal endomembrane systems processing incoming chylomicrons or via internal oil droplets and are processed out through the secretory pathway or to the mitochondria. The pathways taken by both red and yellow feather pigments are linked to both vitamin A homeostasis and redox systems such that feather coloration is a direct indicator of how well these systems are functioning.

porters are likely regulated by feedback mechanisms that are responsive to both local and systemwide states of vitamin A pools and redox systems. By our model, environmental conditions that induce stress, such as pathogen invasion, poor nutrition, extreme temperature, or exposure to toxins, will disrupt system homeostasis and affect the expression of these carotenoid-transporting proteins in enterocytes.

Genetic variation also plays a role in the uptake, cleavage, and reverse-transport of carotenoids. In studies of humans, allelic variation in SR-B1 resulted in different quantities of both pro–vitamin A and non-pro–vitamin A carotenoids in circulation (Borel et al. 2005, 2011), and allelic variations in BCMO1 have been correlated with decreased intestinal β-carotene conversion (Leung et al. 2009) as well as varying levels of circulating β-carotene, zeaxanthin, and lutein (Ferrucci et al. 2009; Lietz et al. 2012). Moreover, allelic variation in the ATP-binding cassette ABCG5 affected levels of both lutein and cholesterol in circulation (Herron et al. 2006). Other allelic variations in lipid transport proteins such as those that occur in ABCA1 can have associated impacts on cotransporters such as lipoproteins that carry carotenoids (Attie 2007). The implications of these human studies are that genetic variation can directly affect animal coloration through actions on carotenoid uptake, storage, transport, and cleavage mechanisms.

Pro–vitamin A carotenoids that are not cleaved are packaged into chylomicrons along with other nonpolar molecules for further processing in the liver (Borel et al. 2005). Alternatively, they may be reverse transported back into the lumen by ABC transporters, but no studies to date have assessed the reverse processing of pro–vitamin A carotenoids, which may be a significant route only under rare conditions of hypervitaminosis A. We do not include reverse transport of pro–vitamin A carotenoids in figure 3 or figure 4. In the liver, carotenoids accumulate in oil droplets in hepatocytes (Blomhoff 1994; Cooper 1997), and pro-vitamin A carotenoids are transported to hepatic stellate cells, where further retinol production and storage occurs (Shmarakov et al. 2010; von Lintig 2010). Hepatocytes are the primary site of accessible carotenoid storage in vertebrates including songbirds, but pigment profiles of carotenoid oil droplets in either hepatocytes or stellate cells are lacking. Storage of xanthophylls versus pro–vitamin A across liver cell types remains to be characterized. Because BCMO1 has been found to be more active in hepatic stellate cells, it is presumed that pro–vitamin A carotenoids would be preferentially stored in stellate oil droplets.

Carotenoid Metabolism

In red cardueline finches, most β-carotene is presumably cleaved in enterocytes into retinol, leaving primarily β-cryptoxanthin, lutein, and zeaxanthin as intact carotenoids. These pigments follow one of two pathways (fig. 3). β-cryptoxanthin is oxidized into the red pigment 3-hydroxy-echinenone (approximately 85%) or adonirubin (3-hydroxy-β,β-carotene-4,4′-dione; approximately 15%; Inouye et al. 2001) by a β-carotene ketolase as follows:

$$\text{ß-cryptoxanthin} \xrightarrow[\text{ketolase}]{\text{ß-carotene}} \text{3-hydroxy-echinenone} \xrightarrow[\text{ketolase}]{\text{ß-carotene}} \text{adonirubin}$$

Oxidation to 3-hydroxy-echinenone does not irreversibly remove these carotenoids from the vitamin A pool because 3-hydroxy-echinenone remains a pro–vitamin A carotenoid. In contrast, further oxidation of 3-hydroxy-echinenone to adonirubin irreversibly removes it from the vitamin A pool (fig. 3).

In vertebrates, the β-carotene ketolase remains uncharacterized (Pointer et al. 2012), so the site of keto-carotenoid formation is unknown. Evidence suggests that the primary site of ketolation in songbirds is the liver (del Val et al. 2009*a*, 2009*b*). The vitamin A–redox hypothesis predicts that avian ketolase should operate within the cell in a highly regulated biochemical environment under strict redox control. The location of the ketolase should coincide with a preexisting pathway through the cell that includes the transport of other similar lipids such as cholesterol and fats.

The vitamin A–redox hypothesis predicts that under low-stress and high-energy conditions the ketolase will be active. Under such conditions, vitamin A processes should be operating in an efficient manner providing the necessary control over multitissue high-energy genomic and redox systems. In such favorable conditions, β-cryptoxanthin is oxidized to 3-hydroxy-echinenone for feather pigmentation and β-carotene and some β-cryptoxanthin molecules are mobilized into vitamin A stores. Under conditions of stress, vitamin A stores (retinyl esters within stellate cells) are expected to be mobilized, and the β-carotene ketolase is predicted to be downregulated. Under such circumstances, the body turns to depleting retinol pools and sequestering incoming dietary retinol and pro–vitamin A carotenoids to maintain cellular function. If retinol stores are depleted by chronic stressful conditions, the survival of the organism becomes threatened. This hypothesized mechanism links the redness of feather coloration directly to vitamin A regulation and ROS levels and makes feather redness a reflection of how well vital cellular processes are proceeding.

It is interesting to speculate that both 3-hydroxy-retinol and 4-keto-3-hydroxy-retinol may serve as feedback regulators on the activity of the β-carotene ketolase and BCMO1 (fig. 3). By ketolating the oxygenated ring of β-cryptoxanthin and using 3-hydroxy-echinenone to color their feathers, male cardueline finches retain flexibility in vitamin A production that is lost by birds that use other

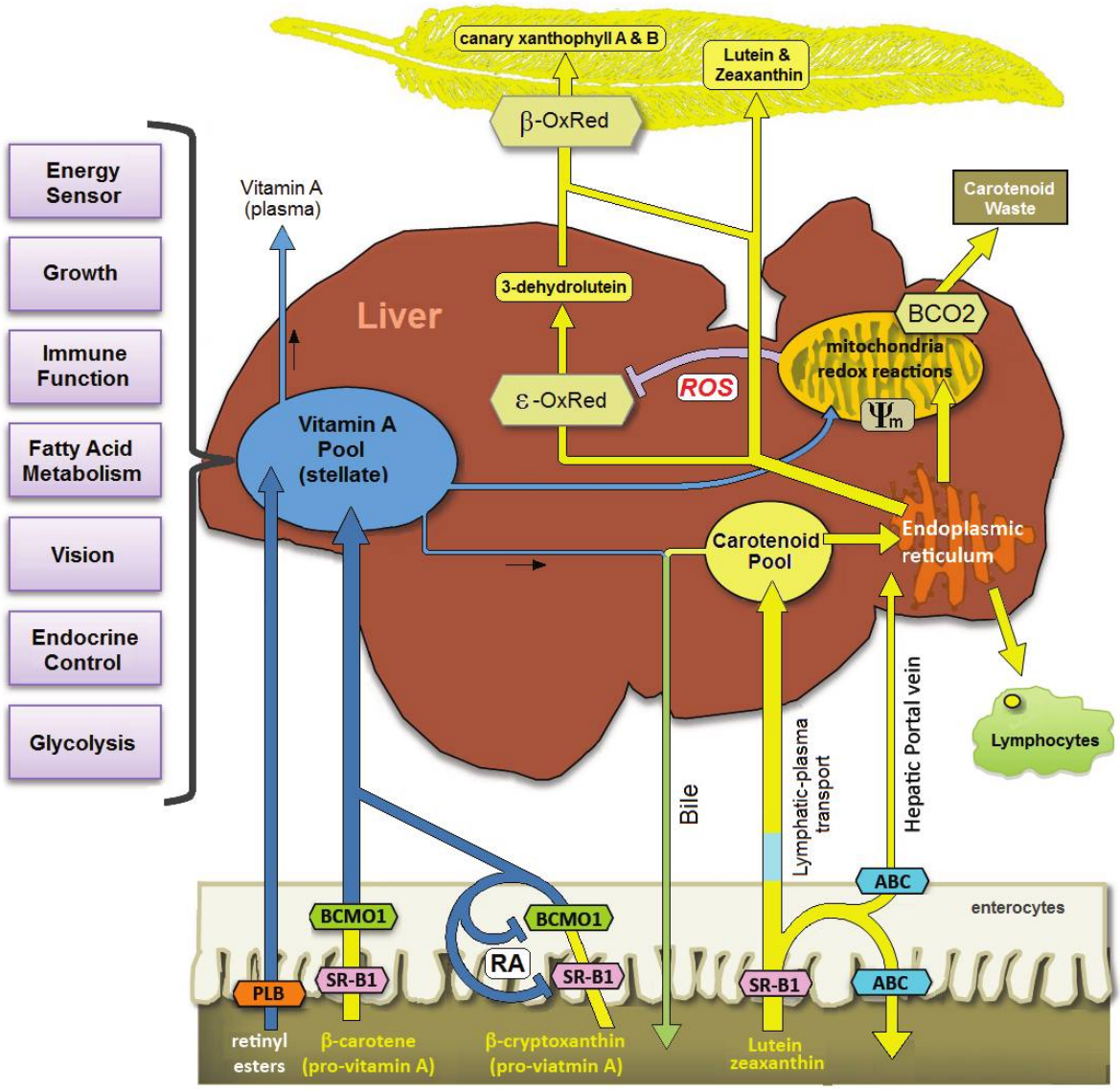
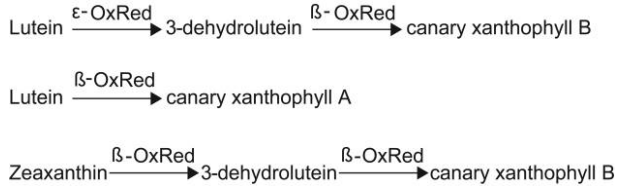


Figure 4: The vitamin A–redox hypothesis applied to carotenoid and retinoid pathways in a cardueline finch (family Fringillidae) with yellow feather coloration. The proposed mechanisms within these pathways are similar to those shown in figure 3, except all pro-vitamin A carotenoids are available for conversion to vitamin A by β,β-carotene-15,15′-carotenoid oxygenase (BCMO1). The unknown oxidoreductases (β-OxRed and ε-OxRed) that convert lutein and zeaxanthin to 3-dehydrolutein, canary xanthophyll A, and canary xanthophyll B are proposed to be under redox control and are expected to be sensitive to local production of reactive oxygen species (ROS). Whether there is direct competition for carotenoid pigments for use as antioxidants, in lymphocytes, or for some other supportive functions versus use of pigments for ornamentation remains to be demonstrated.

keto-carotenoids such as astaxanthin or canthaxanthin, which cannot be reconverted into vitamin A. The cleaving of 3-hydroxy-echinenone to produce vitamin A should occur only with a concomitant need for offsetting potential decreases in vitamin A storage and for the maintenance of plasma vitamin A. To date, the status of vitamin A stores within stellate cells has not been investigated in stress-challenged birds. Our hypothesis predicts that these stores will be depleted rather quickly (over days).

In red finches, lutein and zeaxanthin, which are the common dietary carotenoids that are not provitamin A, follow a second set of metabolic pathways (fig. 3). They are either moved into carotenoid storage pools in hepatocytes in the liver, mobilized to mitochondria where they

can become oxidized and destroyed, or they are dehydrogenated into 3-dehydrolutein, canary xanthophyll B, and canary xanthophyll A as follows:



The enzyme responsible for the oxidation of these xanthophylls remains unidentified; hence, they are labeled ε- or β-OxRed indicating unknown oxidoreductases specific to the epsilon or beta ring oxidations. The location of the ε-OxRed in the liver would explain why 3-dehydrolutein is observed in plasma circulation in birds (Khachik et al. 2002; McGraw and Schuetz 2004).

The β-OxRed, in contrast, is proposed to oxidize β-ionone rings to form 3-dehydrolutein and canary xanthophyll B from zeaxanthin and canary xanthophyll A and B from lutein and 3-dehydrolutein. The β-OxRed may reside in the feather follicles (McGraw 2004) to which 3-dehydrolutein as well as lutein and zeaxanthin is transported through circulation (figs. 3, 4). If β-OxRed occurs at the site of pigment deposition in the feather follicles, it would explain why canary xanthophylls are rarely detected in circulation (McGraw et al. 2005). Beyond precursors and products, all of these pathways are speculative. The identities of the oxidoreductase(s) and their location(s) remain to be definitely demonstrated in birds.

Uses of Xanthophylls

The yellow pigments in the bodies of finches—zeaxanthin, lutein, 3-dehydrolutein, and canary xanthophylls A and B—appear to be used as (1) feather pigments, (2) antioxidants in various tissues such as retina, or (3) as antioxidants in lymphocytes. Based on studies of retinal tissue (Chucair et al. 2007) and the preferential expression of BCO2 within mitochondria (Lobo et al. 2012), the primary redox support of xanthophylls appears to be the quenching of intracellular ROS, particularly in the environment of the endoplasmic reticulum associated with mitochondria (MAM) and, as proposed earlier, serving as antioxidants within the mitochondria. Although Amengual et al. (2011*b*) have demonstrated that xanthophylls can be deleterious to mitochondria in laboratory mice, we believe that these results are a consequence of high concentrations of carotenoids involved in this study, which may interfere with mitochondria redox reactions. In addition, canary xanthophylls are excellent Michael addition acceptors, which are known to deplete glutathione stores in mitochondria (Rao et al. 1988; Schlosser et al. 1990; Nagi and Almakki 2009). We propose that at lower concentrations, xanthophylls may provide a yet uncharacterized benefit in the mitochondria of vertebrates such as reptiles and birds.

Smaller quantities of lutein and zeaxanthin are deposited in oil droplets within lymphocytes (Puppels et al. 1993; Ramanauskaite et al. 1997; Schut et al. 1997), where they presumably act as antioxidants during immune activities that generate ROS. The quantities of carotenoids that are deposited in lymphocytes and whether such deposition involves primarily lutein and zeaxanthin or also pro–vitamin A carotenoids remains to be determined. β-carotene has, however, been shown to be taken up by lymphocytes in vitro, which may indicate a pro–vitamin A role of this carotenoid within immune cells (Schut et al. 1997). Moreover, ROS produced in the mitochondria as well as retinoids can also affect the proliferation and activity of lymphocytes (Iwata et al. 2003; Ertesvag et al. 2007; Li and Li 2007; Chiu et al. 2008; Maruya et al. 2011), so the effects of carotenoids on lymphocytes can potentially originate either directly from activity of internally deposited carotenoids or indirectly from the effects of carotenoids on vitamin A pools and redox systems (Ruhl 2007; figs. 3, 4).

Three Tiers of Ornament Elaboration

Considering all of these uses for and interactions among pro–vitamin A carotenoids and carotenoids that are not pro–vitamin A (xanthophylls) and bearing in mind the distinction between pathways that produce red pigments versus pathways that produce yellow pigments, we can speculate about the significance of a three-tier ornamentation scheme in red cardueline finches. This three-tier scheme is primarily an empirical observation, but we can interpret this hierarchy of ornamentation from the perspective of the vitamin A–redox hypothesis.

The highest tier of ornamentation involves the pathways by which the ketolase converts β-cryptoxanthin to red keto-carotenoids. These are the pathways that are essential for male finches to produce red feather pigments and maximum ornamentation, but they are the pathways that are most sensitive to redox perturbations that disrupt retinol storage and transport or intracellular redox balance, either locally or throughout the body. The oxidation of carotenoids to C4 ketolated products may take one of two routes. The first route is hydroxylation of canary xanthophylls that have undergone keto-enol isomerization, followed by C4 hydroxylation and the production of C4 ketolated products (e.g., astaxanthin from canary xanthophyll B). However, the absence of canary xanthophylls in plasma circulation is an argument against this biosynthesis route. The second route would proceed by double hydroxylation of the β-cryptoxanthin directly at the C4

position, which would then spontaneously dehydrate to the ketolated product.

The second tier—oxidation of zeaxanthin and lutein—becomes apparent when the ketolase is downregulated either directly or indirectly by redox or vitamin A imbalance. With downregulation of the ketolase, the oxidation systems responsible for the production of canary xanthophylls become apparent. Whether the systems for producing canary xanthophylls are actually activated in concert with downregulation of the ketolase or are simply revealed with loss of ketolated carotenoids remains to be determined. The redox changes that cause the loss of ketolase activity may create conditions for the production of canary xanthophylls. For instance, there may be upregulation of a specific SR-B1 scavenger protein on the cell surface of feather germs where canary xanthophylls are predicted to be synthesized. The lowest tier of ornamentation is the deposition of dietary lutein or zeaxanthin unmodified into feathers, which results in drab yellow plumage. This lowest tier should occur if the bird is subject to chronic or severe stress that disrupts oxidative balance and disrupts the production of canary xanthophylls (ε- and β-OxRed).

If we are correct in our interpretation, this tiered response reflects the interdependencies of vitamin A homeostasis, oxidative state, and ketolase activity. In this way, feather coloration of red cardueline finches, and in particular the hue of the red feathers, is a precise reflection of the redox and vitamin A state of an individual bird during molt. In this way, coloration is an honest signal of the functionality of biochemical systems that are essential to body function. For animals, there may be no better signal of individual condition in the true sense of functionality of vital cellular processes than keto-carotenoids deposited in the integument.

The Vitamin A–Redox Hypothesis Applied to Yellow Finches

The majority of cardueline finches are yellow, never depositing keto-carotenoids in their feathers, and the mechanisms by which most cardueline finches produce yellow feather coloration (fig. 4) are essentially the same as the second- and third-tier mechanisms described above for red species. In yellow cardueline finches, β-cryptoxanthin and β-carotene are not used as feather pigments or pigment precursors; they are cleaved into vitamin A, rejected at the level of the enterocyte via ABC transporters, or excreted from hepatic stores via BCO2 cleavage or other phase I and II transport systems (Mein et al. 2011). For species of cardueline finches that use canary xanthophylls as their primary feather pigments, the non-pro–vitamin A carotenoids—lutein and zeaxanthin—are oxidized into 3-dehydrolutein and canary xanthophyll A and B (McGraw et al. 2001), as described above. In species that use unmodified dietary lutein and zeaxanthin, these dietary pigments are simply moved to feather follicles.

Some of the key interactions between pigmentation, vitamin A homeostasis, and oxidative state are lacking in the color-producing mechanisms of yellow finches compared to red finches (fig. 4). Nevertheless, vitamin A still regulates many core pathways, including control over carotenoid uptake and transport. Vitamin A also plays a role as an antioxidant and in mitochondrial maintenance (Chiu et al. 2008), serving to preserve mitochondrial membrane redox potential, thereby assisting in redox maintenance. All pro–vitamin A carotenoids, however, are available for vitamin A pools. Environmental perturbations can impact the production of yellow coloration at a number of levels, including effects on carotenoid uptake via SR-B1 or reverse transport via ABC transporters in the gut as well as downregulation of the unknown OxRed enzymes for those species that use canary xanthophylls A and B. Understanding how the differences in connectivity between vitamin A processes, oxidative state, and carotenoid pathways between red and yellow bird species translate into differences in the signal content of their color displays will require more detailed understanding of the biochemical processes involved in each pigment strategy as well as experimental studies that directly test for the costs and trade-offs involved in the different pigment systems.

Discussion

Based on an expansive biochemical and biomedical literature, we propose that mechanisms of carotenoid pigmentation are linked to vitamin A homeostasis and oxidative state through the pathways by which pro–vitamin A carotenoids are converted to vitamin A, through the regulation of carotenoid uptake and reverse transport at the gut and through the mediation of redox balance via vitamin A and carotenoids homeostasis. The vitamin A connections that we highlight in this article are essential missing elements to all current models of the signal function of carotenoid coloration (reviewed in Hill and McGraw 2006). The only previous mention of vitamin A within the context of carotenoid coloration of vertebrates was a study in which circulating vitamin A was positively correlated with carotenoid-based bill coloration of breeding spotless starlings (*Sturnus unicolor*; Navarro et al. 2010). In this article on starlings, the mechanisms by which vitamin A might relate to bill coloration were not explained.

In the literature focused on how carotenoid-based coloration functions as a signal, there is a nearly universal assumption that signal honesty is maintained by resource limitation and allocation trade-offs (Andersson 1994; Loz-

ano 1994; Hill 2006; reviewed in Svensson and Wong 2011). With regard to carotenoid coloration, the limiting resource is typically proposed to be carotenoid pigments (Hill 2006). In this literature, the challenge of acquiring full ornamental pigmentation was first attributed to an overall scarcity of carotenoids in the diets of ornamented animals and the difficulty of accruing sufficient carotenoids to fully pigment integumentary structures (Endler 1983; Hill 1992). In more recent literature, the key limitation of color production is more often proposed to result from trade-offs between allocation of carotenoids for deposition as colorants versus use of carotenoids as free radical scavengers in the control of oxidative stress (Lozano 1994; Peters et al. 2011). A few researchers proposed that carotenoids are not typically limiting in the diets of vertebrates and that physiological constraints keep some birds from fully expressing coloration (Hudon 1994; Bortolotti et al. 1996; Thompson et al. 1997). Without specific mechanisms for how physiological processes limit coloration (but see von Schantz et al. 1999), the focus has remained on resource limitation and resource trade-offs (Rowe and Houle 1996).

Here, we provide a detailed mechanism for control of ornamental carotenoid coloration by which coloration directly reflects the functionality of vital cellular processes. In this model, the honesty of the signal arises from the inexorable connection between the pathways that produce red carotenoid pigments and the pathways that maintain vitamin A and cellular redox homeostasis; in other words, biochemistry of carotenoid pigmentation supports the shared pathway hypothesis (Hill 2011). Inadequate intake of dietary pigments and trade-offs in the allocation of pigments for maintenance versus ornamentation may, in some cases, play important roles in limiting ornament production, for instance, for young and inexperienced birds who forage poorly (McGraw et al. 2006), but according to our model we would not expect resource limitation to be the primary mechanism maintaining signal honesty. In species with red coloration produced by ketocarotenoids derived from yellow dietary pigments, production of ornamental carotenoid coloration can occur only when vitamin A and redox pathways are in balance. In our view, ornamental coloration does not materially compete with cellular processes for resources—it directly reflects how well such cellular processes are functioning.

Our vitamin A–redox hypothesis accommodates the observations that magnitude of infection by parasites (Brawner et al. 2000; McGraw and Hill 2000; Hill et al. 2004) and quality of nutrition (Hill and Montgomerie 1994; Hill 2000; McGraw et al. 2005) at the time of molt affect the carotenoid coloration produced by male finches. Perturbations such as poor nutrition, parasites, immunostimulation, temperature stress, captivity, low social status, and other components of somatic state are expected to reduce the condition of an individual (Hill 2011), thereby upsetting the processes that maintain vitamin A homeostasis and redox state. The genotype and epigenetic state of an organism can likewise affect condition and hence the processes of feather pigmentation (Hill 2011). Any deviation from normal redox levels or any depletion of vitamin A pools should lead to a reduction in feather pigmentation. Based on our model, we propose that physiological state is ultimately what is being signaled through pigmentation.

There has been special interest among ecologists regarding possible connections between carotenoid access and immunocompetence. In particular, it has been proposed that there is a direct trade-off between use of carotenoids to enhance immune defense and allocation of carotenoids to integumentary structures for ornamentation (the pigment allocation hypothesis; Lozano 1994). Studies of several bird species have demonstrated associations between either carotenoid supplementation and immunocompetence or stimulation of the immune system and carotenoid ornamentation (reviewed in Horak et al. 2007; McGraw et al. 2011; Sepp et al. 2011). Given that lymphocytes carry carotenoid stores (Puppels et al. 1993; Ramanauskaite et al. 1997) presumably to aid in immune defense, there is the potential for a direct trade-off between allocation of carotenoids to lymphocytes versus ornamentation. However, the amount of carotenoids allocated to lymphocytes is likely to be very small compared to the quantities allocated to ornaments and other vital cellular processes. We propose, therefore, that the observed effects of carotenoids on the immune system as well as the effects of immuno-stimulation on carotenoid ornamentation are consequences of the interdependencies of carotenoid pathways, vitamin A homeostasis, and the redox state of the organism rather than a consequence of allocation trade-offs of carotenoid resources. According to our vitamin A–redox hypothesis, production of maximal ornamentation as well as maintenance of an effective immune system both depend on highly functional redox and vitamin A systems.

A fundamental problem with current empirical support for the resource trade-off hypothesis with respect to carotenoid pigmentation is that experiments purporting to demonstrate trade-offs in allocation of carotenoid resources (reviewed in McGraw et al. 2010), though on the surface compelling, do not show that a true trade-off is involved. As outlined in this article, there exist numerous potential physiological complexities in the interactions of the immune system, redox state, vitamin A systems, and carotenoid pigment pathways that have not been considered in tests of carotenoid resource allocation. For example, according to the resource trade-off hypothesis, the decrease in ornament production brought about by an

immunological challenge is the direct result of proportioning carotenoid resources toward immunological and antioxidant functions and away from ornamentation. Experiments of this type typically demonstrate the loss of color in response to an immune challenge, and authors attribute the loss in coloration to reallocation of carotenoids. However, an alternative interpretation for loss of color following an immune challenge is that the treatment affected vitamin A homeostasis or components of the mechanisms by which carotenoids are utilized including uptake, transport, metabolism, or deposition (as illustrated in figs. 3, 4). Allocation trade-offs may occur at local cellular and subcellular levels, but this type of local resource partitioning is not what is tested in experiments or hypothesized in the current literature on carotenoid coloration. Ultimately, demonstration of resource trade-off will require the coupling of observed loss of coloration with material loss of pigment for ornamentation equal to a gain in immunological cells such as lymphocytes or macrophages.

The vitamin A–redox hypothesis and the resource allocation hypothesis are not necessarily mutually exclusive; both could be correct explanations for the link between carotenoid ornamentation and individual condition for the same ornamental traits in the same species of birds but in different contexts. For instance, in environments in which pro– and/or nonvitamin A carotenoids are particularly scarce or for individual birds that forage poorly and consume few carotenoids, resource trade-offs might play a role in ornament production. In environments in which adequate carotenoids are available, resource trade-offs may play no role in ornamentation. It may prove, however, that carotenoid pigments are rarely limiting in the natural diets of vertebrates (Hudon 1994) and hence that resource trade-offs are rarely a factor in carotenoid signaling. Carefully designed experiments will be required to distinguish between the resource allocation hypothesis and the vitamin A–redox hypothesis. The most fundamental test to distinguish these hypotheses is to provide abundant precursor carotenoids (e.g., β-cryptoxanthin) to males of a red cardueline finch species during molt and then to subject the birds to stress such as captivity. The resource allocation hypothesis predicts full pigmentation if resources remain abundant. The vitamin A–redox hypothesis predicts reduced coloration regardless of pigment resources because of disruptions to the vitamin A and redox balance as a result of stress. In addition, the vitamin A–redox hypothesis predicts that any stress challenge that leads to a loss of stored vitamin A reserves should have a negative impact on ornamentation.

Our model, for the first time, presents an explanation for why different bird species use different red keto-carotenoids to pigment their feathers. The primary pigment strategy of cardueline finches and some other red songbirds is to produce 3-hydroxy-echinenone from β-cryptoxanthin. Other bird species produce their primary red pigments by oxidizing lutein or zeaxanthin to α-doradexanthin and astaxanthin, respectively, or β-carotene to canthaxanthin or β-cryptoxanthin to adonirubin (McGraw 2006). The oxidation of β-cryptoxanthin to 3-hydroxy-echinenone involves the ketolation of only one of two β-ionone rings and produces the only red keto-carotenoid found in birds that retains pro–vitamin A activity. All other keto-carotenoids that are used as feather colorants produce a more saturated red pigment than 3-hydroxy-echinenone because they are more highly oxidized carotenoid molecules and hence are irrevocably lost from the vitamin A pool once they are produced. Species that use 3-hydroxy-echinenone as their primary red feather pigments retain flexibility in augmentation of their vitamin A pool from red pigments. Given such flexibility, species that use 3-hydroxy-echinenone should be more adaptable and better able to deal with environmental perturbations than species that irreversibly create pigments with no vitamin A activity. Such species should also have more variable expression of plumage coloration than species that use keto-carotenoids that are not vitamin A precursors.

Our biochemical model leaves the basis for honest signaling in species with yellow pigmentation of integumentary structures somewhat unresolved. The pathways involved with uptake, transport, and deposition of both dietary carotenoids and canary xanthophylls A and B are certainly sensitive to oxidative state. Moreover, vitamin A mechanisms regulate the uptake, reverse transport, and circulating levels of carotenoids. Production of canary xanthophyll A and B via oxidation of dietary pigments is also likely to be subject to catalytic control and/or negative feedback from ROS. Thus, there is the potential for yellow feather coloration to primarily signal redox state and vitamin A equilibrium but with different interdependencies than for ketolase-derived coloration.

The coloration of species that use unmodified dietary pigments as plumage colorants has the most direct link to dietary absorption and distribution of carotenoid pigments. Studies on great tits (*Parus major*) have shown convincingly that changes in dietary lutein across environments leads to population-wide changes in feather coloration (Slagsvold and Lifjeld 1985; Partali et al. 1987; Isaksson 2009). These observations suggest that access to carotenoids is a key component to color production in some species that use unmodified pigments. Even in species using unmodified dietary pigments, however, pigmentation is linked to key cellular processes via control, distribution, and elimination of carotenoids by vitamin A, the homeostasis of which is reflected in the overall energy and redox state of the organism.

We present our models for regulation of carotenoid pigmentation as a starting point for a more complete understanding of the evolution and function of carotenoid coloration in vertebrates. Virtually all of the specific pathways and mechanisms that we propose remain to be demonstrated in vertebrates with ornamental coloration, but animals with ornamental coloration must uptake, transport, metabolize, eliminate, and deposit carotenoids. Several studies in recent years have shown connections between coloration of vertebrates, carotenoid access, and oxidative state, but this literature has presented perplexing patterns that sometimes appear contradictory (Cohen and McGraw 2009; Costantini et al. 2010; Svensson and Wong 2011). We anticipate that some of these previously inexplicable patterns will begin to make sense as biologists include vitamin A pathways in their studies, more carefully consider the fundamental differences between carotenoids that are vitamin A precursors and those that are not, and appreciate the different interdependencies of retinoids, carotenoids, and oxidative state that are involved with different forms of ornamental coloration. Ultimately, a better understanding of the biochemical and cellular mechanisms involved with pigment utilization will be needed before the basis for honest signaling via carotenoid coloration can be fully comprehended.

Acknowledgments

This work was supported by National Science Foundation grant IOS-0923600 to G.E.H. W. Hood provided valuable input on the article and helped create figures 3 and 4. The Hill and Hood lab groups helped organize the ideas presented. We would like to thank P. H. Homann, A. R. Moise, and T. Slagsvold for helpful input or discussions during the preparation of this manuscript. Special thanks to C. Alonso-Alvarez who, through three review cycles, read and carefully considered every idea presented in this long and complex article and made dozens of insightful suggestions for improving the manuscript. It was a Herculean effort that substantially improved our article.

Literature Cited

Acin-Perez, R., B. Hoyos, J. L. Gong, V. Vinogradov, D. A. Fischman, M. Leitges, B. Borhan, A. Starkov, G. Manfredi, and U. Hammerling. 2010*a*. Regulation of intermediary metabolism by the PKCδ signalosome in mitochondria. FASEB Journal 24:5033–5042.

Acin-Perez, R., B. Hoyos, F. Zhao, V. Vinogradov, D. A. Fischman, R. A. Harris, M. Leitges, et al. 2010*b*. Control of oxidative phosphorylation by vitamin A illuminates a fundamental role in mitochondrial energy homoeostasis. FASEB Journal 24:627–636.

Albalat, R. 2009. The retinoic acid machinery in invertebrates: ancestral elements and vertebrate innovations. Molecular and Cellular Endocrinology 313:23–35.

Alonso-Alvarez, C., L. Perez-Rodriguez, R. Mateo, O. Chastel, and J. Vinuela. 2008. The oxidation handicap hypothesis and the carotenoid allocation trade-off. Journal of Evolutionary Biology 21: 1789–1797.

Alvarez, R., J. de Andrés, P. Yubero, Viñas, O., T. Mampel, R. Iglesias, M. Giralt, and F. Villarroya. 1995. A novel regulatory pathway of brown fat thermogenesis. Journal of Biological Chemistry 270: 5666–5673.

Amengual, J., E. Gouranton, Y. G. J. van Helden, S. Hessel, J. Ribot, E. Kramer, B. Kiec-Wilk, et al. 2011*a*. Beta-carotene reduces body adiposity of mice via BCMO1. PLoS ONE 6:e20644, doi:10.1371/journal.pone.0020644.

Amengual, J., G. P. Lobo, M. Golczak, H. N. M. Li, T. Klimova, C. L. Hoppel, A. Wyss, K. Palczewski, and J. von Lintig. 2011*b*. A mitochondrial enzyme degrades carotenoids and protects against oxidative stress. FASEB Journal 25:948–959.

Andersson, M. 1986. Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. Evolution 40:804–816.

———. 1994. Sexual selection. Princeton University Press, Princeton, NJ.

Antipatis, C., G. Grant, and C. J. Ashworth. 2000. Moderate maternal vitamin A deficiency affects perinatal organ growth and development in rats. British Journal of Nutrition 84:125–132.

Attie, A. D. 2007. ABCA1: at the nexus of cholesterol, HDL and atherosclerosis. Trends in Biochemical Science 32:172–179.

Ballinger S. W. 2005. Mitochondrial dysfunction in cardiovascular disease. Free Radical Biology and Medicine 38:1278–1295.

Barber, T., E. Borras, L. Torres, C. Garcia, F. Cabezuelo, A. Lloret, F. V. Pallardo, and J. R. Viña. 2000. Vitamin A deficiency causes oxidative damage to liver mitochondria in rats. Free Radical Biology and Medicine 29:1–7.

Berry, D. C., H. Jin, A. Majumdar, and N. Noy. 2011. Signaling by vitamin A and retinol-binding protein regulates gene expression to inhibit insulin responses. Proceedings of the National Academy of Sciences of the USA 108:4340–4345.

Berry, D. C., and N. Noy 2012. Signaling by vitamin A and retinol-binding protein in regulation of insulin responses and lipid homeostasis. Biochimica et Biophysica Acta 1821:168–176.

Blomhoff, R. 1994. Transport and metabolism of vitamin A. Nutrition Reviews 52:13–23.

Bloom, B. I., L. L. Chaikoff, and W. O. Reinhardt. 1951. Intestinal lymph as pathway for transport of absorbed fatty acids of different chain lengths. American Journal of Physiology 166:451–455.

Böhm, F., E. J. Edge, D. J. Land and T. G. Truscott. 1997. Carotenoids enhance vitamin E antioxidant efficiency. Journal of the American Chemical Society 119:621–622.

Böhm, F., E. J. Edge, and G. Truscott. 2011. Interactions of dietary carotenoids with activated (singlet) oxygen and free radicals: potential effects for human health. Molecular Nutrition & Food Research 55:1–12.

Borel, P., F. S. de Edelenyi, S. Vincent-Baudry, C. Malezet-Desmoulin, A. Margotat, B. Lyan, J. M. Gorrand, N. Meunier, S. Drouault-Holowacz, and S. Bieuvelet. 2011. Genetic variants in BCMO1 and CD36 are associated with plasma lutein concentrations and macular pigment optical density in humans. Annals of Medicine 43: 47–59.

Borel, P., J. Drai, H. Faure, V. Fayol, C. Galabert, M. Laromiguiere,

and G. Le Moel. 2005. Recent knowledge about intestinal absorption and cleavage of carotenoids. Annales De Biologie Clinique 63:165–177.

Bortolotti, G., J. J. Negro, J. L. Tella, T. A. Marchant, and D. M. Bird. 1996. Sexual dichromatism in birds independent of diet, parasites and androgens. Proceedings of the Royal Society B: Biological Sciences 263:1171–1176.

Boulanger, A., P. McLemore, N. G. Copeland, D. J. Gilbert, N. A. Jenkins, S. S. Yu, S. Gentleman, and T. M. Redmond. 2003. Identification of beta-carotene 15,15′-monooxygenase as a peroxisome proliferator-activated receptor target gene. FASEB Journal 17: 1304–1306.

Brawner, W. R., III, G. E. Hill, and C. A. Sundermann. 2000. Effects of coccidial and mycoplasmal infections on carotenoid-based plumage pigmentation in male house finches. Auk 117:952–963.

Buck, J., F. Derguini, E. Levi, K. Nakanishi, and U. Hammerling. 1991. Intracellular signaling by 14-hydroxy-4,14-retro-retinol. Science 254:1654–1656.

Chaudhary, L. R., J. C. Hutson, and D. M. Stocco. 1989. Effect of retinol and retinoic acid on testosterone production by rat leydig-cells in primary culture. Biochemical and Biophysical Research Communications 158:400–406.

Chen, Y. Q., J. Buck, and F. Derguini. 1999. Anhydroretinol induces oxidative stress and cell death. Cancer Research 59:3985–3990.

Chitchumroonchokchai, C., S. J. Schwartz, and M. L. Failla. 2004. Assessment of lutein bioavailability from meals and a supplement using simulated digestion and Caco-2 human intestinal cells. Journal of Nutrition 134:2280–2286.

Chiu, H. J., D. A. Fischman, and U. Hammerling. 2008. Vitamin A depletion causes oxidative stress, mitochondrial dysfunction, and PARP-1-dependent energy deprivation. FASEB Journal 22:3878–3887.

Chucair, A. J., N. P. Rotstein, J. P. SanGiovanni, A. During, E. Y. Chew, and L. E. Politi. 2007. Lutein and zeaxanthin protect photoreceptors from apoptosis induced by oxidative stress: relation with docosahexaenoic acid. Investigative Ophthalmology & Visual Science 48:5168–5177.

Ciaccio, M., M. Valenza, L. Tesoriere, A. Bongiorno, R. Albiero, and M. A. Livrea. 1993. Vitamin A inhibits doxorubicin-induced membrane lipid peroxidation in rat tissues in vivo. Archives of Biochemistry and Biophysics 302:103–108.

Cifelli, C. J., J. B. Green, Z. Want, S. Yin, R. M. Russell, G. Tang, and M. H. Green. 2008. Kinetic analysis shows that vitamin A disposal rate in humans is positively correlated with vitamin A stores. Journal of Nutrition 138:971–977.

Cohen, A. A., and K. J. McGraw. 2009. No simple measures for antioxidant status in birds: complexity in inter- and intraspecific correlations among circulating antioxidant types. Functional Ecology 23:310–320.

Cooper, A. D. 1997. Hepatic uptake of chylomicron remnants. Journal of Lipid Research 38:2173–2192.

Costantini, D., M. Rowe, M. W. Butler, and K. J. McGraw. 2010. From molecules to living systems: historical and contemporary issues in oxidative stress and antioxidant ecology. Functional Ecology 24:950–959.

Costet, P., F. Lalanne, M. C. Gerbod-Giannone, J. R. Molina, X. Fu, E. G. Lund, L. J. Gudas and A. R. Tall. 2003. Retinoic acid receptor-mediated induction of ABCA1 in macrophages. Molecular Cellular Biology 23:7756–7766.

Cupp, A. S., J. M. Dufour, G. Kim, M. K. Skinner, and K. H. Kim. 1999. Action of retinoids on embryonic and early postnatal testis development. Endocrinology 140:2343–2352.

del Val, E., J. C. Senar, J. Garrido-Fernandez, M. Jaren, A. Borras, J. Cabrera, and J. J. Negro. 2009*a*. Hepatic conversion of red carotenoids in passerine birds. Naturwissenschaften 96:989–991.

———. 2009*b*. The liver but not the skin is the site for conversion of a red carotenoid in a passerine bird. Naturwissenschaften 96: 797–801.

D'Ambrosio, D. N., R. D. Clugston, and W. S. Blaner 2011. Vitamin A metabolism: an update. Nutrients 3:63–103.

Driscoll, H. K., C. D. Adkins, T. E. Chertow, M. B. Cordle, K. A. Matthews, and B. S. Chertow. 1997. Vitamin A stimulation of insulin secretion: effects on transglutaminase mRNA and activity using rat islets and insulin-secreting cells. Pancreas 15:69–77.

During, A., H. D. Dawson, and E. H. Harriosn. 2005. Carotenoid transport is decreased and expression of the lipid transporters SR-BI, NPC1L1, and ABCA1 is downregulated in caco-2 cells treated with ezetimibe. Journal of Nutrition 135:2305–2312.

During, A., S. Doraiswamy, and E. H. Harrison. 2008. Xanthophylls are preferentially taken up compared with β-carotene by retinal cells via a SRBI-dependent mechanism. Journal of Lipid. Research 49:1715–1724.

During, A., and E. H. Harrison. 2007. Mechanisms of provitamin A (carotenoid) and vitamin A (retinol) transport into and out of intestinal Caco-2 cells. Journal of Lipid Research 48:2283–2294.

Edge, E. J., D. Land, L. McGarvey, L. Mulroy and T. G. Truscott. 1988. Relative one-electron eduction potentials of carotenoid radical cations and the interactions of carotenoids with the vitamin E radical cation. Journal of the American Chemical Society 120: 4087–4090.

Edge, E. J., D. J. McGarvey, and T. G. Truscott. 1997. The carotenoids as anti-oxidants: a review. Journal of Photochemistry and Photobiology B 41:189–200.

Endler, J. A. 1983. Natural and sexual selection on color patterns in poeciliid fishes. Environmental Biology of Fishes 9:173–190.

Ertesvag, A., H. C. Aasheim, S. Naderi, and H. K. Blomhoff. 2007. Vitamin A potentiates CpG-mediated memory B-cell proliferation and differentiation: involvement of early activation of p38MAPK. Blood 109:3865–3872.

Estornell, E., J. R. Tormo, P. Mañin, J. Renau-Piqueras, J. Timoneda, and T. Barber. 2000. Effects of vitamin A deficiency on mitochondria function in rat liver and heart. British Journal of Nutrition 84:927–934.

Ferrucci, L., J. R. B. Perry, A. Matteini, M. Perola, T. Tanaka, K. Silander, N. Rice, et al. 2009. Common variation in the beta-carotene 15,15′-Monooxygenase 1 gene affects circulating levels of carotenoids: a genome-wide association study. American Journal of Human Genetics 84:123–133.

Fox, D. L. 1976. Animal biochromes and structural colours. University of California Press, Berkeley, CA.

Galano, A., R. Vargas, and A. Martínez. 2009. Carotenoids can act as antioxidants by oxidizing the superoxide radical anion. Physical Chemistry Chemical Physics 12:193–200.

Gelain, D. P., M. Cammarota, A. Zanotto-Filho, R. B. Oliveira, F. Dal-Pizzol, I. Izquierdo, L. R. Bevilaqua, and J. C. Moreira. 2006. Retinol induces the ERK1/2-dependent phosphorylation of CREB through a pathway involving the generation of reactive oxygen species in cultured Sertoli cells. Cell Signalling 18:1685–1694.

Gelain, D. P., M. A. de Bittencourt Pasquali, F. F. Caregnato, and J. C. Fonseca Moreira. 2011. Vitamin A (retinol) up-regulates the

receptor for advanced glycation endproducts (RAGE) through p38 and Akt oxidant-dependent activation. Toxicology 289:38–44.

Gelain, D. P., and J. C. Moreira. 2008. Evidence of increased reactive species formation by retinol, but not retinoic acid, in PC12 cells. Toxicology in Vitro 22:553–558.

Goodwin, T. W. 1984. The biochemistry of carotenoids. Vol. 2. Animals. Chapman & Hall, New York.

Granado-Lorencio, F., B. Olmedilla-Alonso, C. Herrero-Barbudo, I. Blanco-Navarro, B. Perez-Sacristan, and S. Blazquez-Garcia. 2006. In vitro bioaccessibility of carotenoids and tocopherols from fruits and vegetables. Food Chemistry 102:641–648.

Hall, J. A. 2010. Vitamin A metabolism and commensal stimulation in the promotion of mucosal immunity. PhD diss. University of Pennsylvania, Philadelphia, PA.

Hamilton, W. D., and M. Zuk. 1982. Heritable true fitness and bright birds: a role for parasites? Science 218:384–386.

Harrison, E. H. 2012. Mechanisms involved in the intestinal absorption of dietary vitamin A and provitamin A carotenoids. Biochimica et Biophysica Acta 1821:70–77.

Herron, K. L., M. M. McGrane, D. Waters, I. E. Lofgren, R. M. Clark, J. M. Ordovas, and M. L. Fernandez. 2006. The ABCG5 polymorphism contributes to individual responses to dietary cholesterol and carotenoids in eggs. Journal of Nutrition 136:1161–1165.

Hill, G. E. 1992. Proximate basis of variation in carotenoid pigmentation in male house finches. Auk 109:1–12.

———. 2000. Energetic constraints on expression of carotenoid-based plumage coloration. Journal of Avian Biology 31:559–566.

———. 2002. A red bird in a brown bag: the function and evolution of ornamental plumage coloration in the house finch. Oxford University Press, New York.

———. 2006. Environmental regulation of ornamental coloration. *In* G. E. Hill and K. J. McGraw, eds. Bird coloration. Vol. 1. Mechanisms and measurements. Harvard University Press, Cambridge, MA.

———. 2011. Condition-dependent traits as signals of the functionality of vital cellular processes. Ecology Letters 14:625–634.

Hill, G. E., K. L. Farmer, and M. L. Beck. 2004. The effect of micoplasmosis on carotenoid plumage coloration in male house finches. Journal of Experimental Biology 207:2095–2099.

Hill, G. E., W. R. Hood, and K. A. Huggins. 2009. A multifactorial test of the effects of carotenoid access, food intake and parasite load on the production of ornamental feathers and bill coloration in American goldfinches. Journal of Experimental Biology 212: 1225–1233.

Hill, G. E., and K. J. McGraw, eds. 2006. Bird coloration. Vol. 1. Mechanisms and measurements. Harvard University Press, Cambridge, MA.

Hill, G. E., and R. Montgomerie. 1994. Plumage colour signals nutritional condition in the house finch. Proceedings of the Royal Society B: Biological Sciences 258:47–52.

Hogarth, C. A., and M. D. Griswold. 2010. The key role of vitamin A in spermatogenesis. Journal of Clinical Investigation 120:956–962.

Horak, P., L. Saks, M. Zilmer, U. Karu, and K. Zilmer. 2007. Do dietary antioxidants alleviate the cost of immune activation? an experiment with greenfinches. American Naturalist 170:625–635.

Hoyos, B., R. Acin-Perez, D. A. Fischman, G. Manfredi, and U. Hammerling. 2012. Hiding in plain sight: uncovering a new function of vitamin A in redox signaling. Biochimica et Biophysica Acta 1821:241–247.

Hoyos, B., A. Imam, R. Chua, C. Swenson, G. X. Tong, E. Levi, N. Noy, and U. Hammerling. 2000. The cysteine-rich regions of the regulatory domains of Raf and protein kinase C as retinoid receptors. Journal of Experimental Medicine 192:835–845.

Hudon, J. 1994. Showiness, carotenoids, and captivity: a comment on Hill (1992). Auk 111:218–221.

Huggins, K. A., K. J. Navara, M. T. Mendonca, and G. E. Hill. 2010. Detrimental effects of carotenoid pigments: the dark side of bright coloration. Naturwissenschaften 97:637–644.

Inouye, C. Y., G. E. Hill, R. Montgomerie, and R. D. Stradi. 2001. Carotenoid pigments in male house finch plumage in relation to age, subspecies, and ornamental coloration. Auk 118:900–915.

Isaksson, C. 2009. The chemical pathway of carotenoids: from plants to birds. Ardea 97:125–128.

Iwata, M., Y. Eshima, and H. Kagechika. 2003. Retinoic acids exert direct effects on T cells to suppress T(h)1 development and enhance T(h)2 development via retinoic acid receptors. International Immunology 15:1017–1025.

Johnson, K. J., and S. R. Scadding. 1991. Effects of vitamin A and other retinoids on the differentiation and morphogenesis of the integument and limbs of vertebrates. Canadian Journal of Zoology 69:263–273.

Khachik, F., F. F. de Moura, D. Y. Zhao, C. P. Aebischer, and P. S. Bernstein. 2002. Transformations of selected carotenoids in plasma, liver, and ocular tissues of humans and in nonprimate animal models. Investigative Ophthalmology & Visual Science 43: 3383–3392.

Kiefer, C., E. Sumser, M. F. Wernet, and J. von Lintig. 2002. A class B scavenger receptor mediates the cellular uptake of carotenoids in *Drosophila*. Proceedings of the National Academy of Sciences of the USA 99:10581–10586.

Klamt, F. F. Dal-Pizzol, E. A. Bernard, and J. C. Morerira. 2003*a*. Enhanced UV-mediated free radical generation; DNA and mitochondria damage caused by retinol supplementation. Photochemical Photobiology 2:856–860.

Klamt, F., F. Dal-Pizzol, D. P. Gelain, R. S. Dalmolin, R. B. de Oliveira, M. Bastiani, F. Horn, et al. 2008. Vitamin A treatment induces apoptosis through an oxidant-dependent activation of the mitochondrial pathway. Cell Biology International 32:100–106.

Klamt, F., F. Dal-Pizzol, R. Roehrs, R. B. de Oliveira, R. Dalmolin, J. A. Henriques, H. H. de Andrades, A. L. de Paula Ramos, J. Saffi, and J. C. Moreira. 2003*b*. Genotoxicity, recombinogenicity and cellular preneoplastic transformation induced by vitamin A supplementation. Mutation Research. 539:117–125.

Kodric-Brown, A., and J. H. Brown. 1984. Truth in advertising: the kinds of traits favored by sexual selection. American Naturalist 124:309–323.

Korichneva, I., J. Waka, and U. Hammerling. 2003. Regulation of the cardiac mitochondrial membrane potential by Retinoids. Journal of Pharmacology and Experimental Therapeutics 305:426–433.

Kotake-Nara, E., and A. Nagao. 2011. Absorption and metabolism of xanthophylls. Marine Drugs 9:1024–1037.

Krieger, M. 1999. Charting the fate of the"good cholesterol": identification and characterization of the high-density lipoprotein receptor SR-BI. Annual Review of Biochemistry 68:523–558.

Krinsky, N. J., and E. J. Johnson. 2005. Carotenoid actions and their relation to health and disease. Molecular Aspects of Medicine 26: 459–516.

Leo, M.A., S. Ahmed, S. I. Aleynik, J. H. Siegel, F. Kasmin, and C.

S. Lieber. 1995. Carotenoids and tocopherols in various hepatobiliary conditions. Journal of Hepatology 23:550–556.

Leung, W. C., S. Hessel, C. Meplan, J. Flint, F. Oberhauser, F. Tournniaire, J. E. Hesketh, J. von Lintig, and G. Lietz. 2009. Two common single nucleotide polymorphisms in the gene encoding (beta)-carotne 15,15′-monoxygenase alter (beta)-carotene metabolism in female volunteers. FASEB Journal 23:1041–1053.

Li, T. X., and Y. Li. 2007. Synergistic effect of zinc and vitamin A on T cell functions. Biomedical and Environmental Sciences 20: 131–134.

Lietz, G., A. Oxley, W. Leung, and J. Hesketh. 2012. Single nucleotide polymorphisms upstream from the b-carotene 15,15′-monoxygenase gene influence provitamin A conversion efficiency in female volunteers. Journal of Nutrition 142:161S-165S.

Liu, D., Shi, J., A. C. Ibarra, Y. Kakuda, and S. J. Xue. 2008. The scavenging capacity and synergistic effects of lycopene, vitamin E, vitamin C, and β-carotene mixtures on the DPPH free radical. Food Science Technology 41:1344–1349.

Livingston, K. A., and K. C. Klasing. 2008. Inadequate vitamin A during development increases bursacyte apoptosis in chicks. Poultry Science 87:50–51.

Livrea, M. A., and L. Tesoriere. 1998. Antioxidant activity of vitamin A within lipid environments. *In* P. J. Quinn and V. E. Kagan, eds. Subcellular biochemistry. Plenum, New York.

Lobo, G. P., J. Amengual, G. Palczewski, D. Babino, and J. von Lintig. 2012. Mammalian carotenoid-oxygenases: key players for carotenoid function and homeostasis. Biochimica et Biophysica Acta 1821:78–87.

Lobo, G. P., S. Hessel, A. Eichinger, N. Noy, A. R. Moise, A. Wyss, K. Palczewski, and J. von Lintig. 2010. ISX is a retinoic acid-sensitive gatekeeper that controls intestinal beta, beta-carotene absorption and vitamin A production. FASEB Journal 24:1656–1666.

Lopez, P., M. Gabirot, and J. Martin. 2009. Immune challenge affects sexual coloration of male Iberian wall lizards. Journal of Experimental Zoology A 311A:96–104.

Lozano, G. A. 1994. Carotenoids, parasites, and sexual selection. Oikos 70:309–311.

Mark, M., N. B. Ghyselinch, and P. Chambon. 2006. Function of retinoid receptors: lessons from genetic and pharmacological dissections of the retinoic acid signaling pathway during mouse embryogenesis. Annual Review of Pharmacology and Toxicology 46: 451–480.

Marlétaz F., L. Z. Holland, V. Laudet, and M. Schubert. 2006. Retinoic acid signaling and the evolution of chordates. International Journal of Biological Sciences 2:38–47.

Martínez, A., M. A. Rodríguez-Gironés, A. Barbosa, and M. Costas. 2008. Donator acceptor map for carotenoids, melatonin and vitamins. Journal of Physical Chemistry A 112:9037–9042.

Maruya, M., K. Suzuki, H. Fujimoto, M. Miyajima, O. Kanagawa, T. Wakayama, and S. Fagarasan. 2011. Vitamin A-dependent transcriptional activation of the nuclear factor of activated T cells c1 (NFATc1) is critical for the development and survival of B1 cells. Proceedings of the National Academy of Sciences of the USA 108: 722–727.

Mayne, S. T., and R. S. Parker. 1986. Subcellular distribution of dietary β-carotene in chick liver. Lipids 21:164–169.

McGraw, K. J. 2004. Colorful songbirds metabolize carotenoids at the integument. Journal of Avian Biology 35:471–476.

———. 2006. Mechanics of carotenoid coloration. *In* G. E. Hill and K. J. McGraw, eds. Bird coloration. Vol. 1. Measurements and mechanisms. Harvard University Press, Cambridge, MA.

McGraw, K. J., A. A. Cohen, D. Costantini, and P. Horak. 2010. The ecological significance of antioxidants and oxidative stress: a marriage between mechanistic and functional perspectives. Functional Ecology 24:947–949.

McGraw, K. J., and G. E. Hill. 2000. Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. Proceedings of the Royal Society B: Biological Sciences 267:1525–1531.

McGraw, K. J., G. E. Hill, and R. S. Parker. 2005. The physiological costs of being colourful: nutritional control of carotenoid utilization in the American goldfinch (*Carduelis tristis*). Animal Behaviour 69:653–660.

McGraw, K. J., G. E. Hill, R. Stradi, and R. S. Parker. 2001. The influence of carotenoid acquisition and utilization on the maintenance of species-typical plumage pigmentation in male American goldfinches (*Carduelis tristis*) and northern cardinals (*Cardinalis cardinalis*). Physiological and Biochemical Zoology 74:843–852.

McGraw, K. J., P. M. Nolan, and O. L. Crino. 2006. Carotenoid accumulation strategies for becoming a colourful house finch: analyses of plasma and liver pigments in wild moulting birds. Functional Ecology 20:678–688.

———. 2011. Carotenoids bolster immunity during moult in a wild songbird with sexually selected plumage coloration. Biological Journal of the Linnean Society 102:560–572.

McGraw, K. J., and J. G. Schuetz. 2004. The evolution of carotenoid coloration in estrildid finches: a biochemical analysis. Comparative Biochemistry and Physiology B 139:45–51.

Mein, J. R., G. G. Dolnikowski, H. Ernst, R. M. Russell, and X. D. Wang. 2011. Enzymatic formation of apo-carotenoids from the xanthophyll carotenoids lutein, zeaxanthin and beta-cryptoxanthin by ferret carotene-9′,10′-monooxygenase. Archives of Biochemistry and Biophysics 506:109–121.

Metcalfe, N. B., and C. Alonso-Alvarez. 2010. Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. Functional Ecology 24: 984–996.

Moise, A. R., V. Kiksa, Y. Imanishi, and K. Palczewski 2004. Identification of all-*trans*-retinol:all-*trans*-13,14-dihydroretinol saturase. Journal of Biological Chemistry 279:50230–50242.

Moise, A. R., V. Kuksa, W. S. Blaner, W. Baehr, and K. Palczewski. 2005. Metabolism and transactivation activity of 13,14-dihydroretinoic acid. Journal of Biological Chemistry 280:27815–27825.

Moise, A. R., N. Noy, K. Palczewski, and W. S. Blaner. 2007. Delivery of retinoid-based therapies to target tissues. Biochemistry 46:4449–4458.

Monaghan, B. R., and F. O. Schmitt. 1932. The effects of carotene and of vitamin A on the oxidation of linoleic acid. Journal of Biological Chemistry 96:387–395.

Mora, J. R., M. Iwata, and U. H. von Andrian. 2008. Vitamin effects on the immune system: vitamins A and D take centre stage. Nature Reviews Immunology 8:685–698.

Mullin, G. E. 2011. Vitamin A and immunity. Nutrition in Clinical Practice 26:495–496.

Munetsuna, E., Y. Hojo, M. Hattori, H. Ishii, S. Kawato, A. Ishida, S. A. J. Kominami, et al. 2009. Retinoic acid stimulates 17 beta-estradiol and testosterone synthesis in rat hippocampal slice cultures. Endocrinology 150:4260–4269.

Murata, M., and S. Kawanishi. 2000. Oxidative DNA damage by

vitamin A and its derivative via superoxide generation. Journal of Biological Chemistry 275:2003–2008.

Nagao, A. 2011. Absorption and metabolism of dietary carotenoids. Biofactors 37:83–87.

Nagi, M. N., and H. A. Almakki. 2009. Thymoquinone supplementation induces quinone reductase and glutathione transferase in mice liver: possible role in protection against chemical carcinogenesis and toxicity. Phytotherapy Research 23:1295–1298.

Napoli, J. L. 2012. Physiolgoical insights into all-trans-retinoic acid biosynthesis. Biochimica et Biophysica Acta 1821:152–167.

Navarro, C., T. Perez-Contreras, J. M. Aviles, K. J. McGraw, and J. J. Soler. 2010. Beak colour reflects circulating carotenoid and vitamin A levels in spotless starlings (*Sturnus unicolor*). Behavioral Ecology and Sociobiology 64:1057–1067.

Naves, M. M. V., E. R. Silveira, M. L. Z. Dagli, and F. S. Moreno. 2001. Effects of beta-carotene and vitamin A on oval cell proliferation and connexin 43 expression during hepatic differentiation in the rat. Journal of Nutritional Biochemistry 12:685–692.

Noyan, A., W. J. Lossow, B. Nathan, and I. L. Chaikoff. 1964. Pathway and form of absorption of palmitic acid in the chicken. Journal of Lipid Research 5:538–541.

Olson, J. A. 1990. Biochemistry of vitamin A and carotenoids. *In* A. Sommer and K. P. West, eds. Vitamin A deficiency: health, survival, and vision. Oxford University Press, New York.

Packer, L., K. Kraimer, U. Obermuller-Jevic, and H. Sies. 2005. Carotenoids and retinoids: molecular aspects and health issues. American Oil Chemists' Society, Champaign, IL.

Palace V. P., N. Khaper, Q. Qin, and P. K. Singal. 1999. Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease. Free Radical Biology and Medicine 26:746–761.

Pan, J., and K. M. Baker. 2007. Retinoic acid and the heart. Vitamins and hormones 75:257–283.

Park, C. K., Y. Ishimi, M. Ohmura, M. Yamaguchi, and S. Ikegami. 1997. Vitamin A and carotenoids stimulate differentiation of mouse osteoblastic cells. Journal of Nutritional Science and Vitaminology 43:281–296.

Park, U. H., H. S. Han, E. Um, X. H. An, E. J. Kim, and S. J. Um. 2009. Redox regulation of transcriptional activity of retinoic acid receptor by thioredoxin glutathione reductase (TGR). Biochemical and Biophysical Research Communications 390:241–246.

Partali, V., S. Liaaenjensen, T. Slagsvold, and J. T. Lifjeld. 1987. Carotenoids in food-chain studies. 2. The food-chain of *Parus* spp monitored by carotenoid analysis. Comparative Biochemistry and Physiology B 87:885–888.

Pasquali, M. A., D. P. Gelain, M. R. de Oliveira, G. A. Behr, L. L. da Motta, R. F. da Rocha, F. Klamt, and J. C. Moreira. 2009*a*. Vitamin A supplementation for different periods alters oxidative parameters in lungs of rats. Journal of Medicinal Food 12:1375–1380.

———. 2009*b*. Vitamin A supplementation induces oxidative stress and decreases the immunocontent of catalase and superoxide dismutase in rat lungs. Experimental Lung Research 35:427–438.

Pasquali, M. A., D. P. Gelain, A. Zanotto-Filho, L. F. de Souza, R. B. Oliveira, F. Klamt, and J. C. Moreira. 2008. Retinol and retinoic acid modulate catalase activity in Sertoli cells by distinct and gene expression-independent mechanisms. Toxicology in Vitro 22: 1177–1183.

Pasquali, M. A., C. E. Schnorr, L. B. Feistauer, D. P. Gelain, and J. C. Moreira 2010. Vitamin A supplementation to pregnant and breastfeeding female rats induces oxidative stress in the neonatal lung. Reproductive Toxicology 30:452–456.

Peters, A. 2007. Testosterone and carotenoids: an integrated view of trade-offs between immunity and sexual signalling. Bioessays 29: 427–430.

Peters, A., S. Magdeburg, and K. Delhey. 2011. The carotenoid conundrum: improved nutrition boosts plasma carotenoid levels but not immune benefits of carotenoid supplementation. Oecologia (Berlin) 166:35–43.

Peterson, R., L. Wynkoop, and P. Di Raddo. 2005. On the importance of vitamins in maintaining good health: vitamin A and vision. Abstracts of Papers of the American Chemical Society 229:U549.

Pointer, M. A., M. Prager, S. Andersson, and N. I. Mundy. 2012. A novel method for screening a vertebrate transcriptome for genes involved in carotenoid binding and metabolism. Molecular Ecology Resources 12:149–159.

Puppels, G. J., H. S. P. Garritsen, J. A. Kummer, and J. Greve. 1993. Carotenoids located in human lymphocyte subpopulations and natural-killer-cells by Raman microspectroscopy. Cytometry 14: 251–256.

Ramanauskaite, R. B., I. Segers Nolten, K. J. deGrauw, N. M. Sijtsema, L. van der Maas, J. Greve, C. Otto, and C. G. Figdor. 1997. Carotenoid levels in human lymphocytes, measured by Raman microspectroscopy. Pure and Applied Chemistry 69:2131–2134.

Rando, R. R. 1990. The chemistry of vitamin-A and vision. Angewandte Chemie 29:461–480.

Rao, R., D. N., N. Takahashi, and R. P. Mason. 1988. Characterization of a glutathione conjugate of the 1,4-benzosemiquinone-free radical formed in rat hepatocytes. Journal of Biological Chemistry 263:17981–17986.

Reboul, E., L. Abou, C. Mikail, O. Ghiringhelli, M. Andre, H. Portugal, D. Jourdheuil-Rahmani, M. J. Amiot, D. Lairon, and P. Borel. 2005. Lutein transport by Caco-2 TC-7 cells occurs partly by a facilitated process involving the scavenger receptor class B type I (SR-BI). Biochemical Journal 387:455–461.

Reboul, E. L., and P. Borel 2011. Proteins involved in uptake, intracellular transport and basolateral secretion of fat-soluble vitamins and carotenoids by mammalian enterocytes. Progress in Lipid Research 50:388–402.

Reichert, B., R. Yasmeen, S. Jeyakumar, F. Yang, T. Thomou, H. Alder, D. Duester, et al. 2011. Concerted action of aldehyde dehydrogenases influences depot-specific fat formation. Molecular Endocrinology 25:799–809.

Ross, A. C., and R. Zolfaghari. 2004. Regulation of hepatic retinol metabolism: perspectives from studies on vitamin A status. Journal of Nutrition 134:269S–275S

Rowe, L., and D. Houle. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. Proceedings of the Royal Society B: Biological Sciences 263:1415–1421.

Ruhl, R. 2007. Effects of dietary retinoids and carotenoids on immune development. Proceedings of the Nutrition Society 66:458–469.

Sakudoh, T., T. Iizuka, J. Narukawa, H. Sezutsu, I. Kobayashi, S. Kuwazaki, Y. Banno, A. Kitamura, H. Sugiyama, and N. Takada. 2010. A CD36-related transmembrane protein is coordinated with an intracellular lipid-binding protein in selective carotenoid transport for cocoon coloration. Journal of Biological Chemistry 285: 7739–7751.

Schonfeld, P., and L. Wojtczak. 2008. Fatty acids as modulators of the cellular production of reactive oxygen species. Free Radical Biology and Medicine 45:231–241.

Schlosser, M. M., R. D. Shurina, and G. F. Kalf. 1990. Prostaglandin H synthase catalyzed oxidation of hydroquinone to a sulfhydryl-

binding and DNA-damaging metabolite. Chemical Research in Toxicology 3:333–339.

Schut, T. C. B., G. J. Puppels, Y. M. Kraan, J. Greve, L. L. J. VanderMaas, and C. G. Figdor. 1997. Intracellular carotenoid levels measured by Raman microspectroscopy: comparison of lymphocytes from lung cancer patients and healthy individuals. International Journal of Cancer 74:20–25.

Selvaraj, S. R., V. Bhatia, and U. Tatu. 2008. Oxidative folding and assembly with transthyretin are sequential events in the biogenesis of retinol binding protein in the endoplasmic reticulum. Molecular Biology of the Cell 19:5579–5592.

Senatore, V., E. Cione, A. Gnoni, and G. Genchi. 2010. Retinoylation reactions are inversely related to the cardiolipin level in testes mitochondria from hypothyroid rats. Journal of Bioenergetics and Biomembranes 42:321–328.

Senoo, H. 2007. Vitamin A-storing cell (stellate cell) system. Pages 59–70 *in* I. T. Loessing, ed. Vitamin A: new research. Nova Science, New York.

Senoo, H., K. Yoshikawa, M. Morii, M. Miura, K. Imai, and Y. Mezaki. 2010. Hepatic stellate cell (vitamin A-storing cell) and its relative: past, present and future. Cell Biology International 34:1247–1272.

Sepp, T., U. Karu, E. Sild, M. Manniste, and P. Horak. 2011. Effects of carotenoids, immune activation and immune suppression on the intensity of chronic coccidiosis in greenfinches. Experimental Parasitology 127:651–657.

Shmarakov, I., M. K. Fleshman, D. N. D'Ambrosio, R. Piantedosi, K. M. Riedl, S. J. Schwartz, R. W. Curley, et al. 2010. Hepatic stellate cells are an important cellular site for beta-carotene conversion to retinoid. Archives of Biochemistry and Biophysics 504: 3–10.

Slagsvold, T., and J. T. Lifjeld. 1985. Variation in plumage color of the great tit *Parus major* in relation to habitat, season and food. Journal of Zoology 206:321–328.

Standeven, A. M., R. L. Beard, A. T. Johnson, M. F. Boehm, M. Escobar, R. A. Heyman, and R. A. Chandraratna. 1996. Retinoid-induced hypertriglyceridemia in rats is mediated by retinoic acid receptors. Fundamental Applied Toxicology 33:264–271.

Stradi, R. 1998. The colour of flight: carotenoids in bird plumage. University of Milan Press, Milan.

Stradi, R., G. Celentano, M. Boles, and F. Mercato. 1997. Carotenoids in bird plumage: the pattern in a series of red-pigmented Carduelinae. Comparative Biochemistry and Physiology B 117:85–91.

Stradi, R., G. Celentano, E. Rossi, G. Rovati, and M. Pastore. 1995. Carotenoids in bird plumage. I. The carotenoid pattern in a series of Palearctic Carduelinae. Comparative Biochemisty and Physiology B 110:131–143.

Stradi, R., E. Rossi, G. Celentano, and B. Bellardi. 1996. Carotenoids in bird plumage: the pattern in three *Loxia* species and in *Pinicola enucleator*. Comparative Biochemistry and Physiology B 113:427–432.

Sun, G., S. E. H. Alexson, and E. H. Harrison. 1997. Purification and characterization of a neutral, bile salt-independent retinyl ester hydrolase from rat liver microsomes. Journal of Biological Chemistry 272:24488–24493.

Sun, H. 2012. Membrane receptors and transporters involved in the function and transport of vitamin A and its derivates. Biochimica et Biophysica Acta 1821:99–112.

Sun, S., P. Yao, F. Song, and L. Liu. 2006. Effects of vitamin A on development and growth of mice embryos: involvement of HOXC-4. Drug Metabolism Reviews 38:51–52.

Svensson, P. A., and B. B. M. Wong. 2011. Carotenoid-based signals in behavioural ecology: a review. Behaviour 148:131–189.

Takahashi, N., and T. R. Breitman. 1994. Retinoylation of proteins in mammalian cells. Pages 257–273 *in* R. Blomhoff, ed. Vitamin A in health and disease. Dekker, New York.

Tesoriere L., M. Ciaccio, A. Bongiorno, A. Riccio, A. M. Pintaudi, and M. A. Livrea. 1993. Antioxidant activity of all-trans-retinol in homogeneous solution and in phosphatidylcholine liposomes. Archives of Biochemistry and Biophysics 307:217–223.

Truscott, T. G. 2001. Synergistic effects of antioxidants in vitamins. Pages 68–79 *in* P. Walter and D. H. Hornig, eds. Function of vitamins beyond recommended dietary allowances. Darger, Basel.

Thompson, C. W., N. Hillgarth, M. Leu, and H. E. McClure. 1997. High parasite load in house finches (*Carpodacus mexicanus*) is correlated with reduced expression of a sexually selected trait. American Naturalist 149:270–294.

Tucci, P., E. Cione, and G. Genchi. 2008. Retinoic acid-induced testosterone production and retinoylation reaction are concomitant and exhibit a positive correlation in Leydig (TM-3) cells. Journal of Bioenergetics and Biomembranes 40:111–115.

van Bennekum, A., M. Werder, S. T. Thuahnai, C. H. Han, P. Duong, D. L. Williams, P. Wettstein, G. Schulthess, M. C. Phillips, and H. Hauser. 2005. Class B scavenger receptor-mediated intestinal absorption of dietary ss-carotene and cholesterol. Biochemistry 44: 4517–4525.

von Lintig, J. 2010. Colors with functions: elucidating the biochemical and molecular basis of carotenoid metabolism. Annual Review of Nutrition 30:35–56.

von Lintig, J., and K. Vogt. 2000. Filling the gap in vitamin A research: molecular identification of an enzyme cleaving beta-carotene to retinal. Journal of Biological Chemistry 275:11915–11920.

von Schantz, T., S. Bensch, M. Grahn, D. Hasselquist, and H. Wittzell. 1999. Good genes, oxidative stress and condition-dependent sexual signals. Proceedings of the Royal Society B: Biological Sciences 266:1–12.

Wald, George. 1968. Molecular basis of visual excitation. Science 162: 230–239.

Wu, R., and Y. H. Zhao. 1993. Regulation of mucous cell-differentiation by vitamin-A in human primary airway epithelial culture. American Review of Respiratory Disease 147:A465-A465.

Wuttge, D. M., A. Romert, U. Eriksson, H. Torma, G. K. Hansson, and A. Sirsjo. 2001. Induction of CD36 by all-trans retinoic acid: retinoic acid receptor signaling in the pathogenesis of atherosclerosis. FASEB Journal 15:1221–1223.

Wyss, A. 2004. Carotene oxygenases: a new family of double bond cleavage enzymes. Journal of Nutrition 134:246S–250S.

Yang, Q., T. E. Graham, N. Mody, F. Preitner, O. D. Peroni, J. M. Zabolotny, K. Kotani, L. Quadro, and B. B. Kahn. 2005. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 436:356–362.

Yasmeen, R., S. M. Jeyakumar, B. Reichert, F. Yang, and O. Ziouzenkova. 2012. The contribution of vitamin A to autocrine regulation of fat depots. Biochimica et Biophysica Acta 1821:190–197.

Yonekura, L., and A. Nagao. 2007. Intestinal absorption of dietary carotenoids. Molecular Nutrition & Food Research 51:107–115.

Yu J. Y., S. L. DeRuiter, and D. L. Turner. 2002. RNA interference by expression of short-interfering RNAs and hairpin RNAs in mammalian cells. Proceedings of the National Academy of Sciences of the USA 99:6047–6052.

Zahavi, A. 1977. The cost of honesty (further remarks on the handicap principle). Journal of Theoretical Biology 67:603–605.
Zolfaghari, R., and A. C. Ross. 2003. Recent advances in molecular cloning of fatty acid desaturase genes and the regulation of their expression by dietary vitamin A and retinoic acid. Prostaglandins Leukotrienes and Essential Fatty Acids 68:171–179.

Associate Editor: Elizabeth Adkins-Regan
Editor: Mark A. McPeek

Male house finch (*Carpodacus mexicanus*). Photograph by Geoffrey E. Hill.