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Vitamin A (Retinoid) Metabolism and Actions: What We Know and What We Need to Know About Amphibians

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Abstract

Vitamin A status is an important consideration in the health of both wild and captive amphibians. Data concerning whole body vitamin A homeostasis in amphibians are scarce, although these animals have been used as experimental models to study the actions of vitamin A in vision, limb regeneration and embryogenesis. The available data suggest that many aspects of vitamin A biology in amphibians are similar to the canonical characteristics of vitamin A metabolism and actions established in mammals. This is consistent with the evolutionary conservation of these important biological processes. Amphibians must obtain vitamin A in their diet, with captive animals being prone to vitamin A deficiency. There is still much to be learned about vitamin A biology in amphibians that can only be achieved through rigorous scientific research. Improved understanding of amphibian vitamin A biology will aid the conservation of endangered amphibians in the wild, as well as the successful maintenance of ex situ populations.

Keywords

retinol; retinoic acid; vitamin A deficiency; hypervitaminosis A; carotenoid

INTRODUCTION

An improved understanding of vitamin A biology in amphibians is important when considering both captive and wild populations. In captivity, amphibians are dependent on feeder species to obtain their required dietary vitamin A. Inadequate dietary vitamin A intake and resultant vitamin A deficiency are a threat to the health of captive populations. With regard to amphibians in the wild, it is well recognized that many amphibian populations are in decline [Stuart et al., 2004], and it has been suggested that altered vitamin A homeostasis may be a contributing factor [Cohen, 2001].

Vitamin A is an essential micronutrient in higher animals, meaning it must be obtained in the diet to maintain health and prevent disease development. Amphibians are not different in this regard, and cannot synthesize provitamin A carotenoids or vitamin A [Densmore and Green, 2007]. By definition, the term vitamin A refers to all-*trans*-retinol; however the term vitamin A is often used colloquially to broadly refer to all retinol metabolites, including

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retinyl esters and retinoic acid. The term retinoid, which is often used synonymously with vitamin A, refers to all natural and synthetic chemicals that bear a structural resemblance to vitamin A [Sporn et al., 1976].

Vitamin A biology has been well studied in mammals, particularly in humans and in experimental models such as rats and mice; however, there are also certain research areas where the importance of vitamin A signaling has been studied in great depth using amphibian models. This work primarily includes studies into the molecular basis of vision, limb regeneration, and embryogenesis. With regard to vision, the Nobel laureate George Wald frequently used eyes from amphibians in his pioneering work elucidating the molecular basis of visual excitation, a process whose central requirement is the vitamin A metabolite, 11-*cis*-retinal [Wald, 1935, 1955; Palczewski, 2012]. To date, amphibians are still used as important models of vitamin A physiology in the visual cycle [Gonzalez-Fernandez, 2002; Solessio et al., 2009]. In addition to studying vision in amphibians, there is an extensive literature that stretches back more than 100 years describing vitamin A actions in the regeneration of appendages in amphibians [Stocum and Cameron, 2011]. While this work primarily focuses on limb regeneration, it also includes other appendages such as individual digits, fins and tails [Monaghan and Maden, 2013]. The most frequently studied experimental models in this field are the urodele amphibians, particularly the axolotl [Nye et al., 2003; Frobisch and Schubin, 2011], although other amphibians such as the African clawed frog (*Xenopus laevis*) have also been investigated [Suzuki et al., 2006]. One of the most important themes to emerge from this research is the central role of retinoic acid in the regeneration process [Maden and Hind, 2003; Blum and Begemann, 2013], reflecting its crucial role in embryonic limb development [Lewandoski and Mackem, 2009]. In addition to limb development, vitamin A signaling has been shown to be important for several other aspects of embryogenesis, with amphibian species serving as important models for much of this work, particularly *X. laevis* [Kraft et al., 1995; Maden, 2008; Harland and Grainger, 2011].

The work cited above gives only a brief introduction to the many publications concerning vitamin A activity using amphibians as experimental models. From this survey of the literature, it may seem that vitamin A metabolism is relatively well understood in amphibians, but this is not the case. Indeed, in the contexts described above, amphibians have been used as model systems: a convenient means to answer specific, basic scientific questions. Thus, there is a great depth of knowledge in specific areas of vitamin A actions in amphibians; however, few studies have focused on vitamin A homeostasis in amphibians as their primary goal. The purpose of this review is to summarize the available information concerning whole body vitamin A homeostasis in amphibians and to highlight areas for future research.

General Overview of Vitamin A Metabolism

First, we will provide a brief and general overview of vitamin A biology and metabolism (Fig. 1). It should be noted that this information has been gained primarily from studies of humans and rodents. Also note that this biology has been demonstrated to hold for most mammals, especially those of domesticated species, which are the best studied of the other

mammalian species. More limited information obtained from the study of birds, reptiles, amphibians, and fish suggests that the central dogma regarding vitamin A biology and homeostasis in mammals is conserved across these different vertebrate phyla. We direct the reader to the recent reviews by D'Ambrosio et al. [2011] and Shirakami et al. [2012] as sources for obtaining a more extensive and detailed understanding of basic vitamin A biology.

Within the body, the most abundant forms of vitamin A are all-*trans*-retinyl esters, -retinol, -retinal and -retinoic acid, although numerous other low abundance vitamin A species have been reported in the literature [D'Ambrosio et al., 2011; Shirakami et al., 2012]. The chemical structures of these vitamin A forms and that of the major dietary provitamin A carotenoid, β-carotene, are provided in Figure 2. Aside from vision, where 11-*cis*-retinal is the chromophore of the visual pigment rhodopsin [Wald, 1935, 1955; Palczewski, 2012], the all-*trans*-vitamin A isomers are the most physiologically important forms of vitamin A and account for 99% of all vitamin A present in the body. Throughout this review, when we refer to a vitamin A metabolite without specifically stating an isomeric configuration, this should be taken to indicate the all-*trans*-isomer.

Retinoic acid accounts for most of the actions of vitamin A within the body. It is a potent transcriptional regulator that acts through its cognate nuclear hormone receptors, the retinoic acid receptors (RARs; Fig. 1) [Mangelsdorf et al., 1994; Al Tanoury et al., 2013]. There are three distinct RARs (α, β, and γ), encoded by three distinct genes, which in the presence of retinoic acid interact with the transcriptional machinery to modulate rates of gene transcription for retinoic acid-responsive genes [Mangelsdorf et al., 1994; Al Tanoury et al., 2013]. The RARs are members of the steroid/thyroid/retinoid superfamily of nuclear hormone receptors. Thus, the molecular basis for vitamin A's actions in the body is mechanistically similar to those of steroids like estrogen, testosterone, thyroid hormone, and vitamin D. Transcription of over 500 distinct genes is proposed to be retinoic acid-responsive [Balmer and Blomhoff, 2002]. For more information regarding the molecular actions of the RARs or other steroid/thyroid/retinoid superfamily members, the reader is referred to two recent reviews by Al Tanoury et al. [2013] and Pawlak [2012].

Retinol is a precursor for retinal and retinoic acid synthesis. Specific enzymes (retinol dehydrogenases) are able to catalyze the oxidation of retinol to retinal, which can then be oxidized by other enzymes (retinal dehydrogenases) to retinoic acid (Fig. 1). Retinol is also a transport form of vitamin A that is secreted from the liver bound to serum retinol-binding protein (RBP). In the fasting circulation, retinol bound to RBP is the predominant vitamin A species [D'Ambrosio et al., 2011; Shirakami et al., 2012]. The existence of RBP allows for the mobilization of stored vitamin A from the liver [Quadro et al., 1999]. When dietary vitamin A and provitamin A carotenoid intake is insufficient, hepatic stores of retinol are secreted bound to RBP in order to maintain tissue vitamin A levels. When circulating levels of retinol (vitamin A) are reported, these primarily reflect retinol-RBP. Only when hepatic vitamin A stores become exhausted do circulating retinol levels rapidly become undetectable.

Retinyl esters are primarily a storage form for vitamin A. These are formed through esterification of retinol with a long chain acyl-group, primarily those of palmitic, oleic, linoleic, and stearic acids (Fig. 1) [Blaner et al., 2009; D'Ambrosio et al., 2011; Shirakami et al., 2012]. Within the healthy well-nourished liver, the major site for vitamin A storage in the body, retinyl esters are the predominant vitamin A species. Retinyl esters are also present, often in relatively high concentrations, in the postprandial circulation [D'Ambrosio et al., 2011; Shirakami et al., 2012]. Dietary vitamin A is packaged as retinyl ester in nascent chylomicrons by the intestinal epithelium along with other dietary lipids for uptake into the body [D'Ambrosio et al., 2011; Abumrad and Davidson, 2012; Shirakami et al., 2012]. Thus, following a vitamin A-rich meal, one would expect to observe relatively high levels of retinyl esters in the circulation, along with retinol-RBP.

Since animals cannot synthesize de novo vitamin A, ultimately all vitamin A in the body is derived from the cleavage of provitamin A carotenoids that are synthesized in plants, fungi and bacteria [Olson and Krinsky, 1995; von Lintig, 2010]. Only some carotenoids have provitamin A activity; the prime example of which is β-carotene. Within higher animals, β-carotene can be cleaved into two molecules of retinal through the actions of the enzyme carotene-15,15′-monooxygenase [von Lintig, 2010]. Thus, most species that have been studied can acquire vitamin A from the diet as either provitamin A carotenoids or preformed vitamin A, which was formed from a provitamin A carotenoid by an animal lower on the food chain.

Vitamin A Metabolism in Amphibians: What We Know

One of the difficulties in undertaking a review of the literature of vitamin A biology in amphibians arises from the diverse nature of the available literature (e.g., peer-reviewed publications, conference proceedings, theses and book chapters). A substantial portion of this literature is old and many of these early studies employed methodologies that are now recognized as being prone to introducing artifacts into experimental measures, possibly resulting in erroneous data. Some of the more recent studies have been published as conference proceedings or in other formats without peer review. A small portion of the available literature also fails to provide sufficient information regarding how or what vitamin A species were measured, and what information the authors are actually reporting, that is, total vitamin A (retinol+retinyl ester), or simply vitamin A (retinol). We have attempted to summarize the available literature where we are satisfied that sufficient information has been provided to allow for assessment of the qualitative and quantitative validity of the data. Another important caveat when considering vitamin A biology in amphibians is that the majority of research has been performed in the order anura (frogs), with little focus on the orders caudata (salamanders, newts) and gymnophina (caecilians). Thus, these latter two orders represent a vastly understudied area of vitamin A biology in amphibians.

It is clear from the literature that most of the genes encoding proteins important for vitamin A metabolism and mediating vitamin A actions have been evolutionarily conserved. This is undoubtedly a reflection of the importance of vitamin A in maintaining a healthy organism across different classes of vertebrates, as well as in invertebrates such as *Drosophila*

melanogaster and *Caenorhabditis elegans* [Beckett and Petkovich, 1999]. Consistent with this notion, many of the key proteins involved in vitamin A metabolism and signaling have been identified and cloned in model amphibian species, particularly *X. laevis* and the Western clawed frog (*X. tropicalis*). We note that these conserved proteins include all the components necessary for vitamin A metabolism and signaling, including the three RARs needed for mediating vitamin A-transcriptional activity, enzymes needed for retinol esterification to retinyl ester, retinol oxidation to retinal and subsequently to retinoic acid, enzymes needed for the catabolism of retinoic acid, and vitamin A binding proteins needed for vitamin A transport within the circulation and within the cell. Since this information was obtained through direct sequencing of the respective species DNA, these identifications are unequivocal. These conserved sequences provide much confidence that the general mechanisms discussed above for vitamin A metabolism and actions that were identified in mammals also hold for amphibians.

When we consider the existing literature describing tissue vitamin A levels in amphibians, data reported before the routine application of high performance liquid chromatography (HPLC) to measure vitamin A (prior to the early 1980s) should be interpreted with caution, as many older techniques (i.e., fluorometric analysis and the Carr-Price reaction) were prone to experimental error. Care should also be taken when interpreting more recent reports of tissue vitamin A levels, with the reader critically assessing the experimental design and analytical methods. A case in point concerns a trio of reports from the TOXEN research center in Quebec, Canada [Berube et al., 2005; Boily et al., 2005; Boily et al., 2009]. In this series of reports, modern HPLC protocols were used to measure vitamin A levels in wild bullfrogs (*Rana catesbeiana*) to assess responses to agricultural contamination. A critical review of these data reveal that the reported tissue vitamin A levels differed by more than an order of magnitude in samples from the same species, collected at the same locations, 1 year apart. The reason for this discrepancy is unclear, but should give the critical reader pause for thought. While it is possible that these differences reflect biological variability in the wild, they may also reflect unidentified experimental errors. This example serves to highlight the difficulty in conducting rigorous scientific research in wild amphibians, as well as the need for reader caution when interpreting the existing literature.

Given this caveat, the question remains: what do we know about vitamin A homeostasis in amphibians? It is clear that most of the genes known to be important in vitamin A metabolism and signaling are evolutionarily conserved in amphibians. As described below, the literature suggests that many of the key characteristics of vitamin A homeostasis identified in mammals hold true for amphibians. For example, in a survey of tissues collected from the Common frog (*R. temporaria*), the liver was found to contain the highest levels of vitamin A, with relatively lower levels found in the kidney and eyes, and no detectable levels of vitamin A measured in the fat bodies, pancreas, testes, tongue, stomach, skin, and skeletal muscle [Morton and Rosen, 1949]. Thus, similar to many mammals, the liver of amphibians seems to be the primary site of vitamin A storage in the body. A closer examination of vitamin A in the liver of the Leopard frog (*Rana spp*) revealed that the major fraction of vitamin A stored in the liver is in the form of retinyl ester [Futterman and Andrew, 1964]. Detailed analysis of the fatty acyl composition of retinyl ester in the same

study revealed that the two most abundant retinyl ester isoforms in the frog liver were retinyl palmitate and retinyl oleate; thus, the abundance of retinyl ester in the liver and its fatty acyl composition is comparable to mammals and fish [Futterman and Andrew, 1964]. This observation has been confirmed in wild and captive bullfrogs (*R. catesbeina*), for which retinyl palmitate has also been shown to be the most abundant retinyl ester isoform found in the liver [Tsin et al., 1984; Boily et al., 2009].

An additional central feature of vitamin A homeostasis is the distribution of vitamin A in the circulation bound to RBP. The available data on this subject obtained from *R. catesbeina* indicates that, similar to mammals, vitamin A is transported bound to RBP [Shidoji and Muto, 1977]. Consistent with this notion are the high levels of both nucleotide (~60%) and amino acid (~63%) sequence identity between RBP in humans and frogs (*X. laevis*) [McKearin et al., 1987]. While the literature suggests that amphibians use RBP to distribute vitamin A in the circulation, reliable data describing actual circulating retinol levels have not been widely reported, and the normal range of circulating retinol in vitamin A-sufficient amphibians has not been defined. Circulating levels of retinol in frogs (*X. laevis*) are approximately equal in males and females, and reported to be around 0.1 nmol/ml in captive animals [Azuma et al., 1993]. Although, in another example of the high variability in reported data, the circulating retinol levels in captive Peurto rican crested toads (*Peltophrne lemur*) ranged between 0.91 and 0.98 nmol/ml [McComb, 2010].

The reader should be reminded that circulating retinol levels are tightly regulated and kept relatively constant except in times of vitamin A deficiency. Thus, relatively low circulating levels of retinol would be indicative of severe vitamin A deficiency; however, marginal vitamin A deficiency may be overlooked if this is the only metric used. In this regard, hepatic vitamin A levels would be more indicative of vitamin A deficiency prior to a drop in circulating vitamin A levels, although there is a paucity of reference data to make a judgment on what constitutes normal hepatic vitamin A content. Hepatic retinol levels for wild amphibians have been reported in several species [Pessier, 2013], although corresponding values for captive animals and those displaying signs of vitamin A deficiency are generally lacking.

Dietary Vitamin A Intake in Amphibians

An important issue to consider regarding vitamin A homeostasis in amphibians is how this micronutrient is obtained from their diet. An important distinction needs to be made here between nutrient sources for wild amphibians and for those kept in captivity. Although hard data is lacking, reports suggest that wild amphibians are capable of eating at least hundreds of invertebrates per day, and that they consume a diverse array of prey species [Jones et al., 2006; DuRant and Hopkins, 2008]. Providing adequate nutrition to captive amphibians has its challenges, particularly since it is often necessary to provide these animals with live feeder invertebrates, as they prefer to consume moving prey. It is clear from nutrient analyses of mass-produced feeder species that many are lacking in certain essential micronutrients, particularly vitamin A [Pennino et al., 1991; Barker et al., 1998; Finke, 2002]. In order to overcome these deficiencies, it is common practice to supplement feeder species via dusting with a nutrient-rich powder, or by gut-loading. As with any nutrient, an

important consideration when feeding captive amphibians is how much vitamin A should be provided in the diet, both to prevent vitamin A deficiency as well as to avoid the effects of hypervitaminosis A through the consumption of excess vitamin A. As yet, there are no established nutritional guidelines concerning the recommended daily amount of vitamin A that should be consumed by amphibians in order to maintain adequate health—a priority for future research.

Another aspect of dietary vitamin A intake in amphibians is the form that it is provided in the diet. There is some evidence to suggest that amphibians cannot cleave dietary β-carotene to yield physiologically available vitamin A. An important distinction must be made between β-carotene's properties as a provitamin A and its other roles, such as pigmentation. It is clear that dietary carotenoids contribute to the distinct pigmentation of amphibians, with deficient animals in captivity displaying faded coloration [Ogilvy et al., 2012]. Several different carotenoids, including β-carotene, have been detected in the tissues of various amphibian species, including the Northern leopard frog (*R. pipiens*) and the Common frog (*R. temporaria*) [Morton and Rosen, 1949; Khachik et al., 2002], suggesting that it can be absorbed from the diet. Despite the detection of β-carotene in amphibian tissues, there is no current evidence to suggest that they can convert it to vitamin A. For example, liver and small intestine tissue homogenates obtained from Cane toads (*Bufo Marinus*) and Cuban tree frogs (*Osteopilus septentrionalis*) are reported to be unable to cleave β-carotene [McComb, 2010]. Similarly, Tiger salamanders (*Amblystoma tigrinum*) fed a diet rich in β-carotene did not accumulate hepatic vitamin A stores and were presumed to be unable to cleave dietary β-carotene [Collins et al., 1952]. This apparent inability to cleave β-carotene is significant when maintaining amphibians in captivity, as Chiricahua Leopard Frogs (*R. chiricahuensis*) fed a diet containing only β-carotene developed squamous metaplasia of the conjunctiva, which is indicative of vitamin A deficiency [Wright, 2006]. On the other hand, the reproductive success of captive Strawberry Poison Frogs (*Oophaga Pumilio*) was improved when feeder flies were supplemented with mixed carotenoids, including β-carotene [Dugas et al., 2013]. These examples serve to highlight the fact that carotenoids are an important component of the amphibian diet, but should not necessarily be relied upon to maintain adequate vitamin A intake and status. In this regard, dietary supplementation with preformed vitamin A is likely required, as discussed below. While the extant data suggests that amphibians do not cleave dietary β-carotene, it is interesting to note that amphibian species harbor copies of the carotenoid cleavage enzymes in their genome. This scenario is similar to observations in carnivores; for example, the domestic cat (*Felis catus*) genome contains a predicted copy of beta-carotene 15,15′-monooxygenase 1, but these animals have been reported to absorb β-carotene without cleaving it [Schweigert et al., 2002].

Vitamin A Deficiency in Amphibians

Vitamin A deficiency is seemingly a frequent occurrence in captive amphibians and appears to be related to the low vitamin A content of feeder species as described above. There are several clinical manifestations of amphibian vitamin A deficiency; however, the most commonly occurring problem is squamous metaplasia of the mucus-secreting epithelia. Similarly, squamous metaplasia has long been associated with vitamin A deficiency in mammals [Wolbach and Howe, 1925; De Luca et al., 1985]. This is particularly common in

the amphibian mouth, leading to a condition called lingual squamous metaplasia, colloquially referred to as short tongue syndrome, but it can also affect other tissues such as the conjunctiva and reproductive organs [Wright, 2006; Pessier, 2013].

Short tongue syndrome is characterized by difficulty in amphibians capturing their prey and is associated with lethargy, weight loss and death if untreated. This syndrome has been reported in numerous amphibian species including captive pig frogs (*R. gryillo*) [Elkan, 1968], Kihansi spray toads (*Nectophrynoides asperginis*) [Lee et al., 2006], Panamanian golden frogs (*Atelopus zeteki*) [Poole, 2006], Wyoming toads (*B. baxteri*), Rocky mountain boreal toads (*B. boreas boreas*), Woodhouse's toads (*B. woodhousii*), and African foam-nesting frogs (*Chiromantis xeramplina*) [Pessier, 2013; Sim et al., 2010]. Importantly, the occurrence of short tongue syndrome has been linked to low tissues levels of vitamin A in Wyoming toads (*B. baxteri*), and an enriched diet containing high levels of vitamin A has been shown to resolve the feeding problems associated with short tongue syndrome in deficient frogs [Li et al., 2009; Pessier, 2013). Similarly, treatment with vitamin A was able to resolve swellings on the lower eyelids of vitamin A deficient frogs [Wright, 2006; Pessier, 2013]. Care must be taken when providing dietary supplementation to vitamin A deficient amphibians to ensure adequate nutrient availability; for example, rapid loss of powdered supplements from crickets has been shown to interfere with the efficacy of vitamin A supplementation [Li et al., 2009]. Specifically, the whole-body vitamin A level of deficient African foam-nesting frogs (*C. xeramplina*) was not significantly increased by providing these animals with crickets powdered with a nutritional supplement understood to contain high amounts of vitamin A [Sim et al., 2010]. On the other hand, topical administration of a water-soluble emulsion of retinyl palmitate increased whole-body vitamin A levels by approximately four-fold [Sim et al., 2010].

Hypervitaminosis A in Amphibians

While vitamin A deficiency is relatively well recognized in amphibians, hypervitaminosis A is also described in the literature. Although many studies describing hypervitaminosis A are in amphibians deliberately given excess vitamin A for experimental purposes, this condition can arise in captive amphibians supplemented with too much vitamin A. For example, hypervitaminosis seems to frequently occur in *X. laevis* that are fed mammalian liver tissue or whole rodent pups, both of which have relatively high vitamin A levels compared to typical invertebrate feeder species [Densmore and Green, 2007]. The effects of excess dietary vitamin A may affect the skin (causing ulceration or excess shedding) and liver (causing fibrosis or hepatocellular degeneration), as well as weight loss [Pessier, 2013].

Many studies into the effects of hypervitaminosis A have come from experiments where excess vitamin A has been provided to amphibians at different stages of their life cycle, with the majority of these studies conducted in the larval stage of amphibian development. For example, *X. laevis* tadpoles exposed to excess amounts of vitamin A in their feeding mixture (2.0 mg, twice weekly) suffered from loss of their rostral tentacles, tail resorption, and developmental delay [Weissmann, 1961]. There was also a striking effect on the intestine, such that the tadpoles experienced chronic diarrhea. Follow-up studies from the same group using retinoic acid instead of vitamin A gave the same result, with the added observation

that the skin became hyperpigmented and hemorrhagic [Weissmann et al., 1963], although interestingly these tadpoles appeared to have accelerated rather than delayed development. Thus, vitamin A and retinoic acid excess have similar effects during tadpole morphogenesis, an observation which is consistent with the conclusion that the transcriptionally active retinoic acid metabolite of vitamin A, when in excess, induces abnormal development. The importance of vitamin A signaling in vision and limb development is underscored by the observation that exposure of *X. laevis* tadpoles to excess dietary retinoic acid gives rise to developmental abnormalities in the limb and eye, which are dependent on the timing and dose of retinoic acid exposure [Alsop et al., 2004]. It should also be highlighted that there are likely species-specific differences in the effects of excess vitamin A exposure; for example, *B. melanostictus* tadpoles exposed to retinyl palmitate (15, 20, 30 IU/ml) in their rearing solution displayed abnormal development of the keratinized epidermis of the jaws, whereas tadpoles of *R. cyanphlyctis* were unaffected [Jangir et al., 1994]. This is not unlike different mouse strains that show marked differences in their responses to excessive retinoic acid exposure.

What We Need to Know: Areas of Future Study

It is obvious that much work needs to be done before there will be a solid foundation for understanding vitamin A biology in amphibian species. There is a clear need for rigorous and systematic study of vitamin A biology in amphibians; however, because resources for these studies are limited, decisions must first be made as to which amphibian species are best for study. There are thousands of amphibians species recognized to science, thus a consensus must be reached regarding which are the most relevant, most representative, and/or the most important species to be investigated. Currently, most research has been conducted on anurans; however, the other two orders of amphibians (caudata and gymnophina) should also be considered.

In our opinion, systematic studies into basic amphibian retinoid biology should be performed in frogs of the genus *Xenopus*, specifically *X. leavis* and *X. tropicalis*. Studies into these species would be advantageous for numerous reasons. For example, the genome of *X. tropicalis* has recently been sequenced [Hellsten et al., 2010], making it more amenable to genetic studies. Similarly, many of the genes in the retinoid metabolism pathway have also been characterized in *X. laevis.* The broad application of *Xenopus* in previous studies concerning retinoid biology in amphibians (see examples above) is also advantageous, providing a starting point for future work. Furthermore, *Xenopus* are already a model amphibian species for many fields in the life sciences, such as embryology, immunobiology, toxicology, and endocrinology [Brown, 2004; Rollins-Smith et al., 2004; Huang et al., 2005; Takase et al., 2007; Robert and Cohen, 2011; Berg, 2012]. Thus, there is an existing expertise regarding their maintenance and experimental manipulation in captivity, as well as an established supply chain to support research efforts. To compliment this work in *Xenopus*, we would envisage that confirmatory studies would also be performed in exemplar endangered species, so species specific nuances regarding retinoid biology could be identified as required.

With regard to specific research priorities, several themes emerge. With respect to the maintenance of amphibians in captivity, it would be helpful to determine the dietary vitamin A requirement of model amphibians that supports successful reproduction and the maintenance of good health. These requirements could then be used to establish guidelines regarding the recommend dietary intake of vitamin A for amphibians in captivity. As part of this process, it would also be useful to determine the normal tissue concentrations of circulating and hepatic retinol, retinyl ester and retinoic acid levels in healthy, well-nourished amphibians. These levels could then be used as benchmarks to unequivocally assess the vitamin A status of both captive and wild amphibian populations. Note, we strongly believe that the methodologies and experimental approaches employed in the study of vitamin A levels in amphibians need to be standardized and the nomenclature utilized in these studies made uniform. It is our view that modern HPLC or liquid chromatography tandem mass spectrometry instrumentation must be employed for this purpose. With further regard to dietary vitamin A intake, it should be definitively established which, if any, amphibians are able to cleave dietary β-carotene into vitamin A (see above).

The concept of normal tissue concentrations of retinoid raises a philosophical question regarding the definition of normal; specifically, what vitamin A concentrations in amphibians should be considered normal? Is normal reflected by blood and tissue vitamin A levels measured for captive amphibians routinely fed known amounts of vitamin A in their diets? Or is normal reflected by blood and tissue vitamin A levels measured in wild amphibians? Furthermore, which of these levels are optimal for maintaining amphibians in captivity? It is clear to us based on our studies in humans and rodents that vitamin A biology in these species evolved in a manner so as to allow for the most efficient utilization and retention of vitamin A. Mice living in vivariums, fed a vitamin A-sufficient chow diet throughout their lives have much greater blood retinol and hepatic retinyl ester levels than free-living mice in the wild [William S. Blaner, unpublished data]. Similarly, humans living in modern industrial societies possess much higher levels of blood retinol and hepatic retinyl esters than humans living in less developed and more primal settings [Ribaya-Mercado et al., 2004; Valentine et al., 2013]. As more systematic data become available, it will become necessary for informed decisions to be made regarding what should be considered “normal” for amphibians.

A final important point concerns the dissemination of scientific research into amphibians. Investigators studying vitamin A biology in amphibians should submit their findings to peer-reviewed scientific journals for critical evaluation. Proper publication of experimental data gives the reader confidence that the reported data has withstood the rigors of peer review and can be trusted. Furthermore, manuscripts published in established journals will benefit from a wider circulation and ease of access for interested readers.

SUMMARY

Vitamin A metabolism and signaling is an evolutionarily conserved system that is essential to maintain adequate health in many species including amphibians. Certain aspects of vitamin A biology have been well-studied in a small number of amphibian species, specifically vision, limb regeneration and embryonic development. On the other hand, there

is a relative paucity of data concerning basic, whole-body vitamin A homeostasis in amphibians, hampered by limited resources and a high degree of species diversity. An improved knowledge of vitamin A homeostasis in amphibians will aid the conservation of endangered species in the wild, as well as the successful maintenance of species in ex situ populations.

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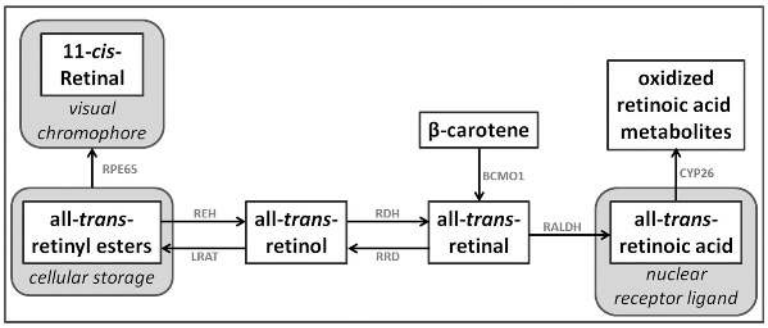

Fig. 1.

Generalized scheme for the metabolism of vitamin A. This figure shows a simplified pathway representing the metabolism of vitamin A. Note, retinyl esters, retinol, and β-carotene can all be taken into the body from the diet. Vitamin A (all-*trans*-retinol) can be stored in the body in the form of retinyl esters, which are synthesized by lecithin retinol acyltransferase (LRAT). In times of dietary vitamin A-insufficiency, retinyl ester stores may be hydrolyzed by retinyl ester hydrolases (REH) to yield physiologically available all-*trans*-retinol. The metabolic intermediate all-*trans*-retinal can be generated via the cleavage of β-carotene by carotene-15,15′-oxygenase (BCMO1), or through the oxidation of all-*trans*-retinol, mediated by retinol dehydrogenases (RDH). Note, the reduction of all-*trans*-retinal to all-*trans*-retinol is also possible, and is mediated by retinol reductases (RRD). Within the retinal pigment epithelium cells of the eye, all-*trans*-retinyl ester is hydrolyzed and isomerized to form 11-*cis*-retinal by RPE65, which is essential for phototransduction. The major active metabolite of dietary vitamin A is all-*trans*-retinoic acid, which is made from all-*trans*-retinal by retinal dehydrogenases (RALDH). Within the nucleus, all-*trans*-retinoic acid binds to nuclear receptors to control the expression level of vitamin A-responsive genes. When all-*trans*-retinoic acid is no longer needed, it is catabolized by specific cytochrome enzymes (CYP26) and then eliminated from the body.

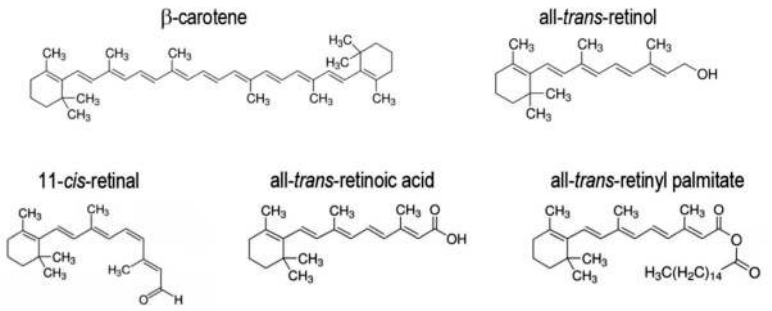

Fig. 2.

Chemical structures of different retinoids. The chemical structure of the major provitamin A carotenoid, β-carotene, is shown alongside all-*trans*-retinol (vitamin A), 11-*cis*-retinal, all-*trans* retinoic acid, and all-*trans* retinyl palmitate.