Subcellular Biochemistry 81

Mary Ann Asson-Batres
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The Biochemistry of Retinoid Signaling II

The Physiology of Vitamin A - Uptake, Transport, Metabolism and Signaling

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Preface

Vitamin A and its active derivative, retinoic acid (RA), have been known for a century to be essential for all steps of life, from embryo to adult. Over this time span, the biochemical properties of vitamin A and vitamin A derivatives and their mechanisms of action at the subcellular, cellular, and systems level have been deciphered by countless groups of researchers working in laboratories around the world. The field continues to produce novel insights into the actions of vitamin A, which have turned out to be surprisingly diverse and elegantly controlled by an amazing complexity of biosynthetic and regulatory processes that are beautifully orchestrated to execute its distribution and conversion to physiologically active derivatives. To update and synthesize this spectacular array of disparate findings, the editorial staff of Springer invited us to produce a multivolume book series to recapitulate all the historical and recent discoveries that define vitamin A biology. This volume represents the second in that series.

The first volume was published in 2014. It covered topics related to the structure and biochemistry of nuclear retinoic acid receptors, their interactions with retinoic acid, and their role as transcription factors as well as signaling moieties in the cytoplasm, regulating the expression of target genes and intermediates involved in cell growth, differentiation, development, and organogenesis.

In this second volume, topics cover vitamin A uptake, transport, and storage; the enzymatic conversion of vitamin A into retinoic acid; the formation and regeneration of the retinaldehyde chromophore that facilitates vision; and, along a very different vein, processes where vitamin A is active as a molecule in its own right.

Volume II is divided into nine chapters, each contributed by an author, whose area of expertise has had an impact on his/her respective field. All chapters follow a similar format which begins with a general introduction to the area, followed by descriptions of (a) the earliest findings and history of the area, (b) key findings that contributed to the development of the field, and (c) research that defines our current understanding of the area. Each chapter concludes with the author's perspective on the relevance and future directions for the field.

Chapter 1 covers the nomenclature and chemistry of provitamin A carotenoids and vitamin A derivatives, named retinoids. Chapters 2, 3, and 4 focus on vitamin A

uptake, transport, storage, and mobilization. Chapters 5, 6, and 7 address the mechanism of synthesis of RA from vitamin A, its shuttling into the nucleus, and its catabolism. Chapter 8 presents and discusses recent findings highlighting a novel mechanism of vitamin A action, distinct from that mediated by retinoic acid, wherein vitamin A acts directly to activate kinase signalosomes that are involved in lipid metabolism and energy homeostasis. Chapter 9 provides an up-to-date summation of foundational and new data describing the well-known and essential role of vitamin A in vision.

We thank all of the authors for their efforts and enthusiasm in preparing this volume. They comprehensively reviewed the literature and provided stimulating ideas for the future. We also thank and acknowledge Meran Owen for his invitation to put together this Retinoic Acid Signaling book series; his assistant, Tanja van Gaans for her help and assistance in bringing the volume to completion; and Springer Publishing for its support of this project.

It is our hope that this second volume, along with the first, will serve as a solid introduction for all interested readers and as a strong reference for all current and future scientists working in the field of vitamin A biology.

Nashville, TN, USA — Mary Ann Asson-Batres
Illkirch, France — Cecile Rochette-Egly
November 24, 2016

In Memoriam

Proteins that bind vitamin A and its derivatives facilitate the many actions of retinoids. The initial recognition of this concept grew out of research carried out by DeWitt Goodman and colleagues at Columbia University, who identified retinol-binding protein (RBP) and who proposed that it was the major transporter of vitamin A in the bloodstream [2], and Frank Chytil and colleagues at Vanderbilt University, who identified a "*macromolecular fraction… capable of binding [3H] retinol in vitro and which differs from the serum component*" [1]. This early body of work has driven much of the research that is highlighted in the chapters that comprise this volume.

David Ong

David Ong joined Frank Chytil's laboratory in late 1973 and proceeded to purify the "macromolecular component" that Frank and his teammates had identified. This molecule turned out to be the first cellular retinol-binding protein (CRBP) [3]. The discovery of CRBP stimulated a flurry of research by Chytil, Ong, and others that subsequently led to the identification and characterization of all of the retinoid-binding proteins that we now know are the transporters and mediators of vitamin A's storage, metabolism, and downstream actions. Fittingly, Dave and Frank shared the Osborne and Mendel Award from the American Institute of Nutrition (now the ASN) in 1983 for their revolutionary work identifying and characterizing the cellular retinoid-binding proteins.

Frank Chytil passed away on July 7, 2014, and Dave Ong passed away just one short year later, on April 25, 2015. We invited Dave to contribute a chapter describing the history and biology of the cellular retinol-binding proteins and his and Frank's contributions to this field, but he declined in favor of enjoying his newly entered state of retirement, offering instead to serve as a consultant to Joe Napoli, the author of Chap. 2. Despite his many insightful comments and generosity in sharing firsthand information, Dave felt he hadn't done enough to warrant coauthorship on Joe's chapter and asked simply to be acknowledged.

Noa Noy

Just before publication, we were very sorry to learn that Noa Noy became gravely ill and, quite unexpectedly, passed away on October 18th, 2016. Noa was a truly innovative researcher in the field of retinoids and **her work** spanned two important areas of inquiry as detailed here in Chaps. 3 and 7. She was a very good friend and colleague, and she will be missed by all the 'retinoids' community.

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Contents

Chapter 1
Carotenoids and Retinoids: Nomenclature, Chemistry, and Analysis

Earl H. Harrison and Robert W. Curley, Jr.

Abstract Carotenoids are polyenes synthesized in plants and certain microorganisms and are pigments used by plants and animals in various physiological processes. Some of the over 600 known carotenoids are capable of metabolic conversion to the essential nutrient vitamin A (retinol) in higher animals. Vitamin A also gives rise to a number of other metabolites which, along with their analogs, are known as retinoids. To facilitate discussion about these important molecules, a nomenclature is required to identify specific substances. The generally accepted rules for naming these important molecules have been agreed to by various Commissions of the International Union of Pure and Applied Chemistry and International Union of Biochemistry. These naming conventions are explained along with comparisons to more systematic naming rules that apply for these organic chemicals. Identification of the carotenoids and retinoids has been advanced by their chemical syntheses, and here, both classical and modern methods for synthesis of these molecules, as well as their analogs, are described. Because of their importance in biological systems, sensitive methods for the detection and quantification of these compounds from various sources have been essential. Early analyses that relied on liquid adsorption and partition chromatography have given way to high-performance liquid chromatography (HPLC) coupled with various detection methods. The development of HPLC coupled to mass spectrometry, particularly LC/MS-MS with Multiple Reaction Monitoring, has resulted in the greatest sensitivity and specificity in these analyses.

Keywords Carotenoid • Retinoid • Nomenclature • Vitamin A chemistry • Retinoid structure

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Abbreviations

CTCL	cutaneous T-cell lymphoma
HPLC	high performance liquid chromatography
HWE	Horner-Wadsworth-Emmons modification
IUB	International Union of Biochemistry
IUPAC	International Union of Pure and Applied Chemistry
LC/MS	liquid chromatography/mass spectrometry
LC/MS-MS	liquid chromatography/mass spectrometry-mass spectrometry
MRM	multiple reaction monitoring
RAL	retinal
RA	retinoic acid
RAR	retinoic acid receptor
RXR	retinoid X receptor
ROL	retinol
TTNPB	tetrahydro-tetramethyl-napthalenyl-propenyl-benzoic acid
UV	ultraviolet

Introduction

Carotenoids are synthesized in plants and in certain microorganisms such as some bacteria, algae, and fungi. They are a group of pigments that are widespread in nature and responsible for the yellow/orange/red/purple colors of many fruits, flowers, birds, insects, and marine animals. Over 600 carotenoids have been isolated from natural sources and new ones continue to be discovered or synthesized. All carotenoids are derived from the basic linear structure of lycopene that contains 40 carbon atoms and an extended system of 13 conjugated double bonds. Carotenoids derive from this parent structure by cyclization at one or two ends of the chain and by dehydrogenation and/or oxidation.

The carotenoids and retinoids are all biosynthesized beginning with activated forms (pyrophosphates) of the five carbon molecule "isoprene" (see Fig. 1.1). The product of "head-to-tail" condensing of two of these units produces a "monoterpene". When three of these isoprene units are combined, the important sesquiterpene relay compound farnesyl pyrophosphate is produced. Using different enzymes and pathways, this sesquiterpene is converted into, for example, the sterols, the dolichols and other triterpenes. Four of these units combine in a similar manner to produce the diterpene geranylgeranyl pyrophosphate which is the precursor of quinones such as ubiquinone, the carotenoids, and other diterpenes [24].

Carotenoids are fundamentally important in the evolution and ecology of many taxa. Because they absorb light in some part of the visible spectrum, carotenoids are colored and carotenoid-based coloration is used by both plants and animals as

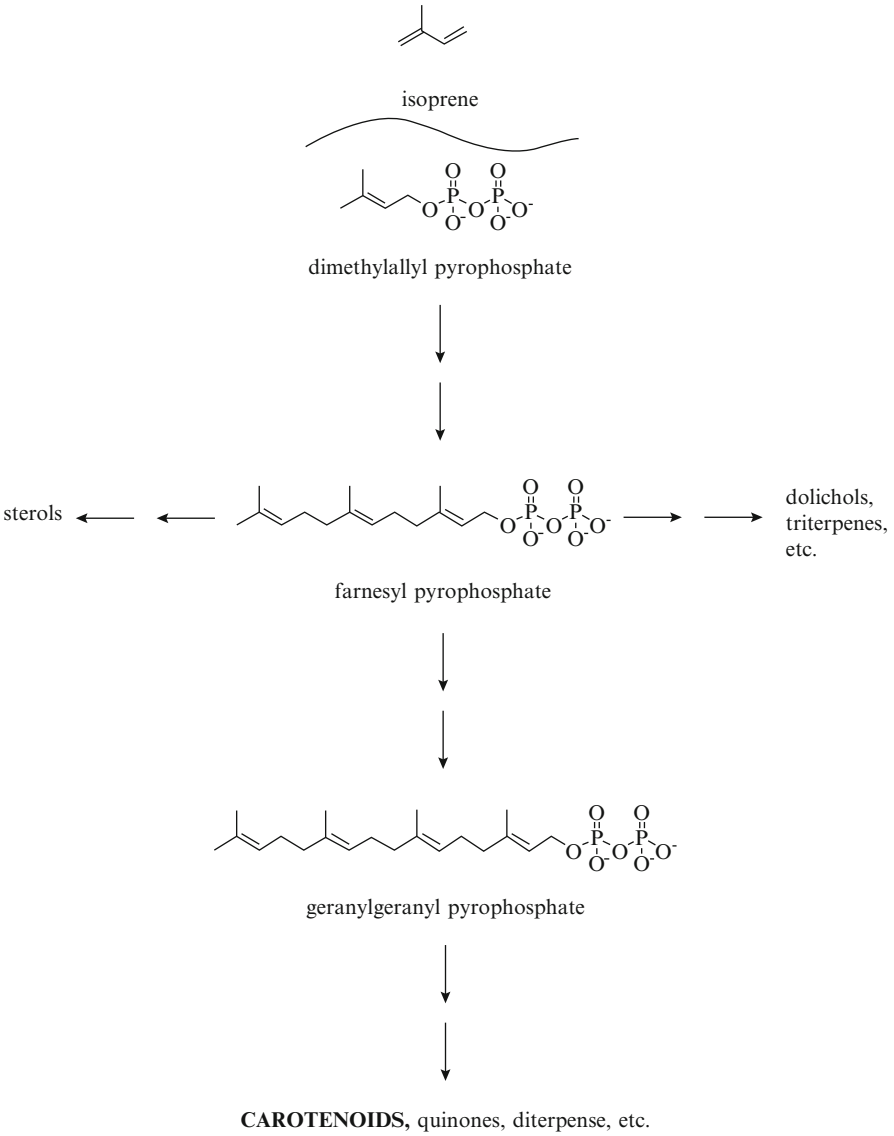

Fig. 1.1 Biosynthesis of carotenoids and other terpenoids

attractants (e.g. of pollinators for plants and of mates for animals) and to communicate fitness [2] (Fig. 1.2).

One of the principal functions of the carotenoids in the plant and animal kingdoms is as antioxidants. Singlet oxygen is a high energy reactive form of molecular oxygen which can be produced from ground state triplet oxygen by light-induced photosensitization as well as other chemical reactions. Other damaging reactive

Fig. 1.2 Carotenoids and mate choice. Male American goldfinch, *Carduelis tristis*, an avian species in which females choose mates based on variation in the extent of carotenoid-based plumage coloration

oxygen species include peroxyl and hydroxyl radicals. The carotenoids can protect cellular components from the damaging reactions of photo-oxidation and reactive oxygen species by multiple mechanisms including: 1) their very large molar absorption coefficient for light that allows them to protect directly against photo-oxidation; 2) their ability to quench directly highly reactive singlet oxygen; and 3) their loss of protons in response to interactions with reactive species that produces a much less reactive radical center in the carotenoid molecule which is stabilized by the polyene network [26]. Indeed, all three of these mechanisms are involved in the role that carotenoids play in the fundamental process of photosynthesis (see [3]) and in their putative roles as antioxidants in human health (see [40]).

Some of the carotenoids are metabolically converted to the essential nutrient vitamin A or retinol [18]. Vitamin A is an essential vitamin for higher animals, including humans. The vitamin is needed for normal embryogenesis and development and for vision, immunity, reproduction, and the maintenance of differentiated epithelial tissues. Vitamin A is the generic term used for all naturally occurring compounds containing a β-ionone ring, other than the carotenoids, that exhibit qualitatively the biological activity of retinol. In [34] Sporn et al. coined the term "retinoid" to refer to the natural and synthetic chemical derivatives of retinol and retinoic acid, regardless of whether they have vitamin A activity.

Retinol is a C20 isoprenoid (or a diterpenoid). It can be derived from metabolic conversion of some dietary carotenoids, which are C40 isoprenoids (or a tetraterpenoid). Such carotenoids are termed provitamin A carotenoids. In order to exhibit a provitamin A activity, the carotenoid molecule must have at least one unsubstituted β-ionone ring and the correct number and position of methyl groups in the polyene chain.

In this chapter we discuss the nomenclature and classification of retinoids and carotenoids as well as the chemical synthesis and quantitative analysis of these two classes of compounds.

Retinoid/Carotenoid Nomenclature and Classification

Retinoids

Karrer [23] established the structure of the dietary component in fat ("fat soluble A", now "vitamin A"), that McCollum and Davis had first discovered was essential for growth in mammals [31]. Soon after, Wald isolated a substance from frog and mammal eyes that he called "retinene" [38], and Morton suggested that the substance that Wald had isolated from eyes was the aldehyde of vitamin A which he called "retinaldehyde" [32]. In 1960, the International Union of Pure and Applied Chemistry (IUPAC) published recommendations on the nomenclature of the vitamins and proposed that the parent retinoids should be known as retinol (ROL; **1**; Fig. 1.3), retinal (RAL; **2**; Fig. 1.3) and retinoic acid (RA; **3**; Fig. 1.3) [7]. These names recapitulated the importance of these substances for vision in the retina and also made use of the suffixes normally used in organic chemistry to indicate one is dealing with an alcohol ("-ol"), aldehyde ("-al"), or carboxylic acid ("-oic acid") oxidation state at the polar terminus of the molecule. The accepted numbering scheme for the positions in these molecules is shown in Fig. 1.3. It should be noted that when there might be confusion between the molecule retinal and the adjective retinal (pertaining to the retina), that the use of retinaldehyde is still recommended.

The names for various vitamin A metabolites, and the accepted numbering convention for the positions within the molecules differ substantially from the systematic names and numbering system that would be used if one were to apply the accepted rules of nomenclature for organic chemicals. For example, using the latter nomenclature, RA with its carboxylic acid carbon as position 1 would be: (all-*E*)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid.

In the early 1980s the Joint Commission on Biochemical Nomenclature of the IUPAC-International Union of Biochemistry (IUB) issued the recommendations on the nomenclature of retinoids. At that time [6], retinoids were defined as compounds composed of 4 isoprene units joined head-to-tail such that the products were monocyclic compounds with 5 conjugated double bonds and a functional group at the terminus of the acyclic portion of the molecules.

Following the Commision's recommendations, if the functional group at the 15-position is changed, the remainder of the hydrocarbon is referred to as the "retinyl" radical and the new functional group identified, for example there are esters of retinol known as retinyl acetate (**4** Fig. 1.3) and retinyl palmitate (**5**; Fig. 1.3). Changes to the state of hydrogenation of the parent structure are denoted by indicating the position(s) involved and "hydro" for addition of hydrogen or "dehydro" for removal of hydrogen. For example, the ROL derivative originally termed "vitamin A_2" would be 3,4-didehydroretinol (**6**; Fig. 1.3).

In the parent retinoids, all of the double bonds are in a *trans*, or *E*, configuration. Changes from this starting stereochemistry are described by using the lowest numbered carbon in the double bond effected and identifying it as now being *cis*, or