VITAMIN RECEPTORS

Vitamins as Ligands in Cell Communication

EDITED BY

Krishnamurti Dakshinamurti

Vitamin receptors: *Vitamins as ligands in cell communication*

EDITED BY

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Vitamins are essential micronutrients available to animal organisms through the diet. This book takes a fresh approach to vitamin-binding proteins, with emphasis on the nature of the binding of the vitamin ligand to a protein and its sequela. The role of vitamin-binding proteins as initiators of the metabolic response is evaluated. Experts in the field from around the world present a state-of-the-art account of their work on the interaction of vitamins with specific intracellular systems through the appropriate binding proteins and how this interaction results in the biological action of vitamins. This is the first comprehensive book dealing with the subject. The book will be of interest to research workers and postgraduate students in the fields of biochemistry and nutrition.

Intercellular and intracellular communication

General editor: Professor B. Cinader

Vitamin receptors
Vitamins as ligands in cell communication

Intercellular and intracellular communication

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PREFACE

Contemporary research has allowed us to discern a linguistic structure in biological interactions in which molecules in solution and in or on membranes react with one another, sequentially, and thus form sentences of a language. Interlocking ligand–receptor systems constitute an intracellular and intercellular communication system in which molecules, such as cytokines, hormones, metabolic products, vitamins and foreign macromolecules can serve as words and convey signals – messages – through combination with membrane structures, i.e. receptors. These signals can initiate synthesis of other factors which can combine with receptors and form the sentences of the molecular language. Thus, molecular language consists of sequential interactions between receptors and ligands, which are either cell-bound or secreted by one cell and taken up by membrane structures of another cell. A succession of these interactions at the membranes of cells and organelles coordinate cell metabolism within the same and between different cells and organs.

The analogy between language and molecular communication can also be detected in the evolutionary diversification of words, i.e. in the existence of superfamilies of molecules, having a common two-chain structure and involved in recognition of different ligands. Members of these families show homologies in a variety of cell types and among cells of different animals, from invertebrates to vertebrates.

Within cells, some types of organelles acquire macromolecules from the cytoplasm. The site-specificity of these acquisitions depends on receptors, which recognize appropriate proteins by signals that may consist of amino acid sequences or of post-translational modifications.

In communication, occurring between different types of cells, receptors can be activated through soluble factors, and hence at a distance. Receptor–ligand interaction can also occur between membranes of

different cell types, i.e. via adhesion molecules that play a role in structural development of organs, exemplified by neural cell adhesion and embryological development under the influence of 'master' cells.

Once a ligand has combined with the membrane receptor of a cell, a change in membrane configuration can ensue and activate a series of metabolic events. Alternatively, ligands and receptors can enter the cell through pathways which start in 'coated pits', and can then be delivered to lysosomes for degradation or be recycled to the surface. This process of endocytosis mediates and regulates the entrance and half-life of various types of molecules, such as growth factors, viruses, toxins and nutrients.

Cell communication is regulated by activation events and by processes which limit the period during which a given stimulus can affect biochemical reactions that are initiated via a particular receptor. This limitation is achieved by processes such as endocytosis, recycling, and affinity changes in receptors, and through disassociation of macromolecular complexes with which the ligand binding site is associated.

Function of each cell type involves internal exchange between cytoplasm and various membrane-bound compartments, i.e. organelles. The function of each organ involves communication between different cell types; that of the organism as a whole involves communication between different organs.

The vast majority of molecules involved in biological sentences, whether receptors or soluble ligands, are autologous. However, the impact of external waves of energy can trigger the sentences of the neuronal system and external molecules can trigger sentences in the immune and nutritional systems.

Past volumes of this series have dealt with various aspects of molecular language, reviewing hormone receptors, cellular interactions in plants, tumor biology, neurology and psychiatry. The fifth volume of our series examined the steps by which molecules can enter the cell interior and be transported with and without receptor to the locations within the cell in which they interact or in which they are being disposed of. It thus dealt with traffic within the cell and with the interrelation between pathways of endocytosis and exocytosis. The present (sixth) volume of this series considers vitamins in terms of the processes by which they enter the system and the receptors by which they affect and activate the system. It deals with nutrients, i.e. with molecules, synthesized in the external world, and hence with an instance of communication initiated by encounters with externally generated molecules. This particular feature is common to the nutritional and the immune systems.

Vitamin receptors deals with chemically diverse molecules, water- and

lipid-soluble vitamins, taken up in the gastrointestinal tract through specific saturable membrane components. It surveys binding mechanisms, intracellular transport and, in some instances, transport to the nucleus and the interaction with sections of the genome, resulting in transcription of specific proteins. So far, there is little indication of a common ancestral receptor molecule from which vitamin receptors have descended; they may be derived from different lines of descent. There are indications that some vitamin receptors may have common molecular antecedents with receptors of the endocrine system. The identification of receptor structures remains an important challenge for the future development of analysis of the language of communication in which vitamins are involved.

Bernhard Cinader

INTRODUCTION

This volume attempts to assemble the current information on the ligand (vitamin) – receptor (binding protein) interaction as it applies to each of the vitamins, from the site of enteric absorption to the site of vitamin function. For the absorption and transport of most vitamins, specific proteins, which have a recognition factor incorporated into them, are involved. At the plasma membrane of the cell, a second recognition factor has to function before internalization of the vitamin. Intracellular vitamin-binding proteins and their interaction with cellular systems resulting in the biological action of the vitamins provides the last step in the successive stages of communication. Thus, we have the concept of a ligand, an extraorganismic molecule, reacting with a specific protein entity, the receptor, facilitating absorption and transport of the ligand to its site of action with the resultant sequence of physiological events to which we refer as 'vitamin function'. The term 'receptor', in the context of vitamins, is used to refer to proteins with a recognition function and not to receptors in the traditional pharmacological sense. It includes proteins which bind to the vitamin ligand and function as extracellular or intracellular transporters as well as the smaller number of vitamin-binding nuclear proteins. In the latter case, vitamins might act as small molecular modulators of protein–DNA interaction, a situation analogous to that of some hormones. The designation 'receptors' for these categories of vitamin-binding proteins appears to be appropriate as it connotes a function, more than ligand binding.

Vitamins are required by all protozoans and metazoans that have been studied. They do not conform to any single chemical classification. Some vitamins, such as retinol (vitamin A), cholecalciferol (vitamin D), α-tocopherol (vitamin E) and phylloquinone (vitamin K), are lipid-soluble; others, such as thiamine (vitamin B_1), riboflavin (vitamin B_2), niacin,

pyridoxine (vitamin B_6), panthothenic acid, lipoic acid, biotin, folic acid, cyanocobalamin (vitamin B_{12}) and ascorbic acid (vitamin C), are water-soluble. While the unlimited availability of vitamins may have permitted the evolutionary loss of the ability of animals to synthesize vitamins, the ability to absorb vitamins from the environment or from digested food is essential. The modes of uptake, intra- and intercellular transport of these two broad categories of vitamins are different, based on their solubility characteristics. Lipoprotein binders are implicated in the transport of the lipid-soluble vitamins. Among the water soluble-vitamins the bindings of cyanocobalamin and folate, respectively, to specific proteins were recognized early. The intestinal absorption of other water-soluble vitamins was assumed to be through simple diffusion. Increasing evidence has accumulated to indicate that the absorption of many water-soluble vitamins in the gastrointestinal tract is through saturable mechanisms. In addition, the concentrations of many water-soluble vitamins in the brain are much higher than in plasma. This is true of placental transport as well, implicating specific transporters for individual vitamins across the blood–brain and placental barriers, respectively.

Absorption of vitamins by most cells occurs through membrane-associated transport systems which are present in low concentrations. These receptor-like proteins may consist of an extracellular vitamin-binding domain linked to a transmembrane anchor. The fertilized egg and embryo obtain vitamins from the external environment using cell-surface vitamin receptor/transporter proteins. The vitamins are used directly without storage. In contrast, the eggs of birds and reptiles contain both the vitamins and their respective vitamin-binding proteins deposited in the yolk for transport to the oocyte.

The physiological effects of vitamins follow their entry into the cell, metabolic transformation and association with specific apoenzymes, resulting in accentuation of a metabolic pathway. In this respect vitamins are metabolic initiators. Where the vitamin function is other than as a coenzyme or prosthetic group of an enzyme its effect is through regulation of cellular protein synthesis. The mechanisms of action of lipid-soluble vitamins are similar to those of the steroid hormones. Water-soluble vitamins such as biotin and folic acid also seem to have a similar function.

The transcriptional patterns of selected genes are often altered by external signals that the cell receives. Thus changes in nutritional supplies, interaction with hormones, viral infection or exposure to environmental factors such as metals, light, high temperature or radiation can either induce or repress the transcription of specific genes or gene sets. In

many cases the regulation of transcription (up or down) is mediated by alterations in the availability of essential transcription factors or by change in the factors' ability to bind to specific regulatory sequence motifs.

The nuclear vitamin D receptor has been characterized as belonging to the steroid family of receptors. Retinol and retinoic acid and their respective binding proteins may serve unique vital functions within different tissues and organs at different times during development. The large number of retinoic acid receptor isoforms expressed in a subtype-specific fashion during development and in differentiated tissues is consistent with the diversity of retinoid action. Folate binding proteins might be involved in transmission of messages for cell division as the accumulation of folate is essential for purine and thymidine synthesis. Biotin has been shown to mimic the actions of insulin in inducing glucokinase mRNA and repressing phosphoenolpyruvate carboxykinase mRNA. The effect of biotin is seen in the starved as well as the diabetic animal in a way that does not involve the synthesis or secretion of insulin. The presence of nuclear binding proteins for biotin and pyridoxal phosphate is indicated, although their functions are not yet known.

We are still at a preliminary stage in our exploration of the groups of vitamin binding proteins. This volume aims to focus attention on the protein-bound vitamin function. The role of some vitamins as the prosthetic group of an enzyme or as a coenzyme participating in enzymatic catalysis is well recognized. This traditional aspect of vitamin function is not considered here. The emphasis is rather on the nature of the binding of the vitamin ligand to a protein, and its sequela.

Krishnamurti Dakshinamurti

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1

Serum and Cellular Retinoid-Binding Proteins

Dianne Robert Soprano

Introduction

Retinoids include both the natural forms of vitamin A and a large number of synthetic analogues which may or may not display the biological activity of vitamin A. The major natural forms of vitamin A include retinol, retinal and retinoic acid (see Figure 1). All of these forms of vitamin A have a conjugated double bond system which renders them extremely hydrophobic and thus quite water insoluble.

Vitamin A is an essential nutrient in the diet for normal growth (Wolback & Howe, 1925, 1933), differentiation (Wolback & Howe, 1925, 1933), reproduction (Thompson *et al.*, 1964) and vision (Wald, 1968). Vitamin A from the diet is absorbed and transported to the liver. Once in the liver, vitamin A can either be stored or transported to individual cells of target tissues where vitamin A elicits its biological functions. The target tissues include not just the eye but virtually all tissues of the body. In addition, once within the target cell, vitamin A must be transported to its appropriate site(s) of action. Finally, vitamin A may be transported from the target tissue back to the liver or to another target tissue. In each case, an extremely hydrophobic molecule must be transported through an aqueous environment in order to perform its physiological roles.

Since the isolation of the first retinoid binding protein, retinol-binding protein (RBP) in 1968 (Kanai *et al.*, 1968), much information has been obtained related to the identification, characterization and most recently the molecular biology of a number of proteins which bind retinoids. These binding proteins include serum RBP (Goodman, 1984; Soprano & Blaner, 1993); two cellular retinol-binding proteins, CRBP-I (Chytil & Ong, 1984) and CRBP-II (Ong, 1984); two cellular retinoic acid-binding

proteins, CRABP-I (Chytil & Ong, 1984) and CRABP-II (Bailey & Siu, 1988; Giguère *et al.*, 1990*a*); four nuclear retinoic acid receptors, α-RAR (Giguère *et al.*, 1987; Petkovich *et al.*, 1987; Zelent *et al.*, 1989), β-RAR (Zelent *et al.*, 1989; Benbrook *et al.*, 1988; Brand *et al.*, 1988), γ-RAR (Zelent *et al.*, 1989; Krust *et al.*, 1989; Giguère *et al.*, 1990*b*) and RXR (Mangelsdorf *et al.*, 1990); and two unique retinoid binding proteins, cellular retinal-binding protein, CRALBP (Futterman *et al.*, 1977), and interphotoreceptor retinol-binding protein, IRBP (Bridges, 1984), found only in visual tissue. It is becoming more apparent that except for retinyl esters the important biologically active forms of vitamin A may always be bound both intracellularly and extracellularly to an appropriate binding protein in order for vitamin A to be transported through aqueous environments.

Figure 1.1. Chemical formulae for major naturally occurring retinoids. (*a*) All-*trans* retinol; (*b*) all-*trans* retinal; (*c*) all-*trans* retinoic acid.

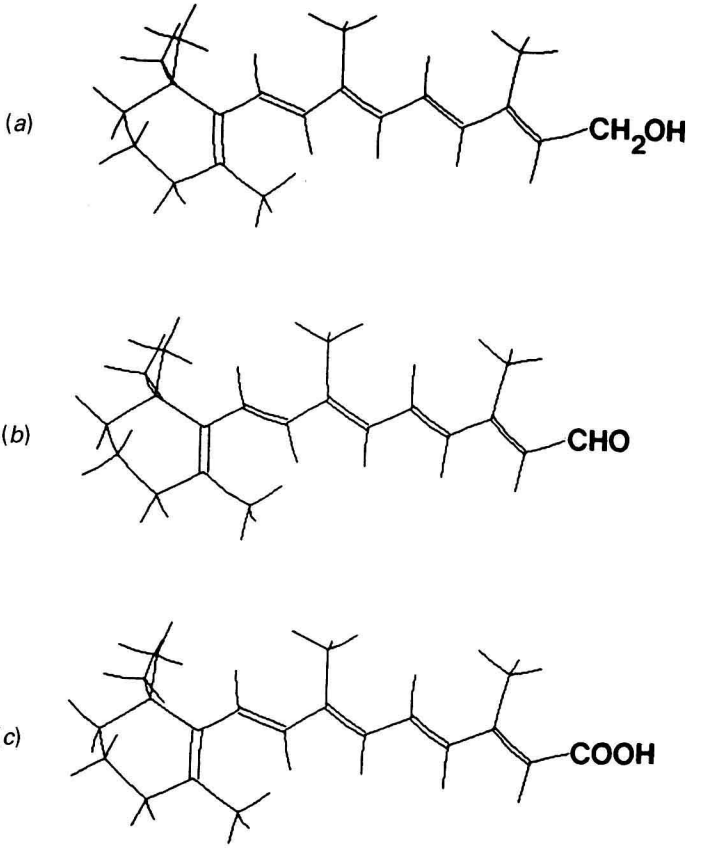

Table 1.1. *Retinoid-binding proteins*

Name	M_r	Endogenous ligand	Site of action	Function
Retinol-binding protein, RBP	21 000	all-*trans* retinol	blood	intercellular transport of retinol
Cellular retinol-binding protein, type I, CRBP-I	15 700	all-*trans* retinol	within cells of vitamin A responsive tissues	intracellular transport of retinol
Cellular retinol-binding protein, type II, CRBP-II	15 600	all-*trans* retinol, all-*trans* retinal	intestinal mucosa cells	absorption and transport of dietary vitamin A within enterocytes
Cellular retinoic acid-binding protein, type I, CRABP-I	15 500	all-*trans* retinoic acid	within cells of vitamin A responsive tissues	intracellular transport of retinoic acid
Cellular retinoic acid-binding protein, type II, CRABP-II	15 500	all-*trans* retinoic acid	developing embryo and adult skin cells	intracellular transport of retinoic acid

The focus of this chapter will be on RBP, CRBP-I, CRBP-II, CRABP-I and CRABP-II and their role in the intercellular and intracellular transport of vitamin A. Table 1 contains a summary of the general properties of each of these retinoid-binding proteins. I will discuss each of these retinoid-binding proteins in the context of their role in the normal metabolism of vitamin A within experimental animals and humans. (The RARs are believed to be involved in the transactivation of retinoic-acid-responsive genes similar to steroid or thyroid hormone receptors and are the subject of Chapter 2 in this volume.) Finally, the discussion of the specialized retinoid-binding proteins found in visual tissue is beyond the scope of this chapter; however, the topic has been reviewed by Chader (1989) and Bridges (1984).

Role of retinoid-binding proteins in the absorption of vitamin A

The natural sources of vitamin A in the diet include retinyl esters, derived from animal sources, and certain plant carotenoid pigments, such as β-carotene. Within the intestinal mucosal cells β-carotene