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Signaling by retinol and its serum binding protein

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ABSTRACT

Vitamin A, retinol, circulates in blood bound to retinol-binding protein (RBP) which, in turn, associates with transthyretin (TTR) to form a retinol–RBP–TTR ternary complex. At some tissues, retinol-bound (holo-) RBP is recognized by a membrane protein termed STRA6, which transports retinol from extracellular RBP into cells and, concomitantly, activates a JAK2/STAT3/5 signaling cascade that culminates in induction of STAT target genes. STRA6-mediated retinol transport and cell signaling are critically inter-dependent, and they both require the presence of cellular retinol-binding protein 1 (CRBP1), an intracellular retinol acceptor, as well as a retinol-metabolizing enzyme such as lecithin: retinol acyltransferase (LRAT). STRA6 thus functions as a "cytokine signaling transporter" which couples vitamin A homeostasis and metabolism to cell signaling, thereby regulating gene transcription. Recent studies provided molecular level insights into the mode of action of this unique protein.

1. Introduction

Vitamin A was recognized as an essential factor in foods about a century ago [1,2] and a substantial body of knowledge on its mechanisms of action and biological functions has since accumulated [3]. It is usually believed that the parental vitamin A, retinol, is biologically inert and that it exerts its biological activities only by giving rise to active metabolites including 11-*cis*-retinal, critical for vision, and all-*trans*-retinoic acid (RA) (Fig. 1), which regulates gene transcription by activating several members of the nuclear receptor family of ligand-controlled transcription factors [4–7]. Vitamin A is stored in the body in the form of retinylesters (Fig. 1) and its major storage site is in stellate cells in the liver [8]. The vitamin is secreted from the hepatic pool into the circulation bound to retinol-binding protein (RBP), a member of the lipocalin family of proteins [9,10]. Lipocalins share a low sequence identity but a highly conserved overall fold. They are comprised of an eight-stranded antiparallel β-sheet that is folded over itself to form a β-barrel which constitutes the ligand binding pocket. The amino termini of lipocalins wrap around the back of the barrel, 'capping' that side of the pocket. In contrast, the front of the β-barrel is open, providing an entryway for the ligand which is flanked by a single loop. Retinol binds to RBP with the β-ionone ring innermost and the hydroxyl head-group reaching to the protein surface where it is coordinated to a water molecule at the pocket entrance ([11,12], Fig. 2).

In blood, holo-RBP is associated with another protein termed transthyretin (transporter of thyroxin and retinol, TTR), a 56 KDa homotetramer which, in addition to binding RBP, also serves as a thyroid hormone carrier. The major sites of synthesis of TTR are the choroid plexus in the brain and the liver, and the protein is found in plasma and in cerebrospinal fluid [13]. Under normal physiological conditions, vitamin A circulates in plasma within a retinol:RBP:TTR ternary complex which forms at 1:1:1 molar ratio. It is believed that the association with TTR serves to prevent loss of the low molecular weight (21 KDa) RBP by glomerular filtration in the kidneys. Notably, association of RBP with TTR requires the presence of retinol, and the complex dissociates following loss of the ligand [14]. The reported 3-dimensional crystal structure of the complex of holo RBP with TTR [15] reveals that association with TTR blocks the entrance to the ligand-binding pocket of RBP (Fig. 3). Notably, although RBP can bind retinal and retinoic acid with an affinity similar to that displayed by retinol, it does not bind to TTR in the presence of these retinoids [16]. It seems that the larger head groups of retinal and retinoic acid interfere with binding of RBP to its serum partner protein.

Binding to RBP allows the poorly-soluble retinol to circulate in plasma, but the vitamin dissociates from the protein prior to uptake into cells. Due to its lipophilic nature, free retinol can readily enter cells by diffusion through the plasma membrane [17]. In addition, in some tissues, the vitamin can also be internalized by an integral plasma membrane protein termed stimulated by retinoid acid gene 6 (STRA6), a largely hydrophobic protein which is predicted by computer modeling to contain 11 trans-membrane helices, a number of loops, and a large cytosolic C-terminal tail (see Fig. 5). STRA6 binds extracellular RBP and facilitates transport of retinol from its plasma

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carrier into cells [18]. In the adult, STRA6 is expressed in blood–organ barriers, retinal pigment epithelial of the eye, brain, adipose tissue, spleen, kidney, testis, and female genital tract [19,20].

2. STRA6 is both a retinol transporter and a cytokine signaling receptor

While STRA6 functions as a retinol transporter, studies of mice lacking the receptor established that it is not important for maintaining retinol availability in most tissues either during embryonal development or in the adult. The exception is the eye, where the expression level of the receptor is exceptionally high [21]. Hence, it was reported that the retinoid content of many tissues is indistinguishable in *Stra6*-null and WT mice fed regular chow diet [22]. Moreover, while RBP is critical for development in mice fed vitamin A-deficient diet (VAD) [23], *Stra6*-null fetuses born from dams fed a VAD do not exhibit any hallmarks of fetal vitamin A deficiency syndrome [22]. It was further shown that the rate of uptake of retinol from circulating holo-RBP, and the retinoid content of STRA6-expressing tissues in adult mice fed a vitamin A deficient diet, when holo-RBP is the sole source of retinol, were only modestly reduced in *Stra6*-null mice. These observations strongly support the conclusion that the major fraction of cellular uptake of retinol from circulating holo-RBP occurs in a transporter-independent fashion, likely by diffusion across the plasma membrane, at rates that are sufficient for physiological needs. The exception is the eye where, due to a very high expression level of the receptor in retinal pigment epithelium, ablation of STRA6 leads to severe depletion of retinoid stores. Notably however, even in this organ, morphological changes and reduction in visual function in *Stra6*-null mice are mild [21]. These observations suggest that STRA6 may have physiological function(s) other than serving as a retinol transporter.

Indeed, our recent studies revealed that, in addition to mediating retinol transport, STRA6 functions as signaling receptor that, upon binding holo-RBP, activates a JAK/STAT cascade [24]. Extracellular polypeptides such as cytokines, hormones, and growth factors often function by activating cognate cell surface receptors that transduce a signaling cascade mediated by tyrosine kinases called Janus kinases (JAK) and by their associated transcription factors Signal Transducers and Activators of Transcription (STAT). In turn, activated STATs reprogram gene expression and thus regulate multiple aspects of cellular behavior [25–27]. Binding of such extracellular ligands to their cognate cytokine receptors results in phosphorylation and activation of receptor-associated JAK which, in turn, phosphorylates a tyrosine residue in the cytosolic domain of the receptor. As STATs contain an SH2 domain that recognizes the resulting phosphotyrosine, these transcription factors are recruited to activated receptors where they are phosphorylated by JAK. Subsequently, STATs form dimers that translocate to the nucleus where they function as transcription factors. STATs thus regulate gene expression in response to a myriad of cytokines, hormones, and growth factors.

JAK/STAT signaling is "switched off" by several types of regulators. The signal can be dampened by dephosphorylation of activated receptors, JAKs, and STATs [26], and by inhibition of the transcriptional activity of STATs [28,29]. Additional important negative regulators of these pathways are encoded by the direct STAT target genes called Suppressors of Cytokine Signaling (SOCS). Following their upregulation by STAT, SOCS function as components of negative feedback loops that inhibit JAK/STAT signaling by competing with STATs for binding to phosphotyrosines in activated receptors, by blocking the catalytic activity of JAK, and by recruiting

Fig. 2. The three dimensional crystal structure of holo-RBP. The human holo-RBP structure (PDB ID 1BRP) was rendered using Pymol (http://www.pymol.org/). The structure shows the eight stranded antiparallel β-sheet folded over itself to form a β-barrel. Retinol (white) is encapsulated by the barrel with the β-ionone ring buried in the binding pocket and the alcohol group is at the protein surface.

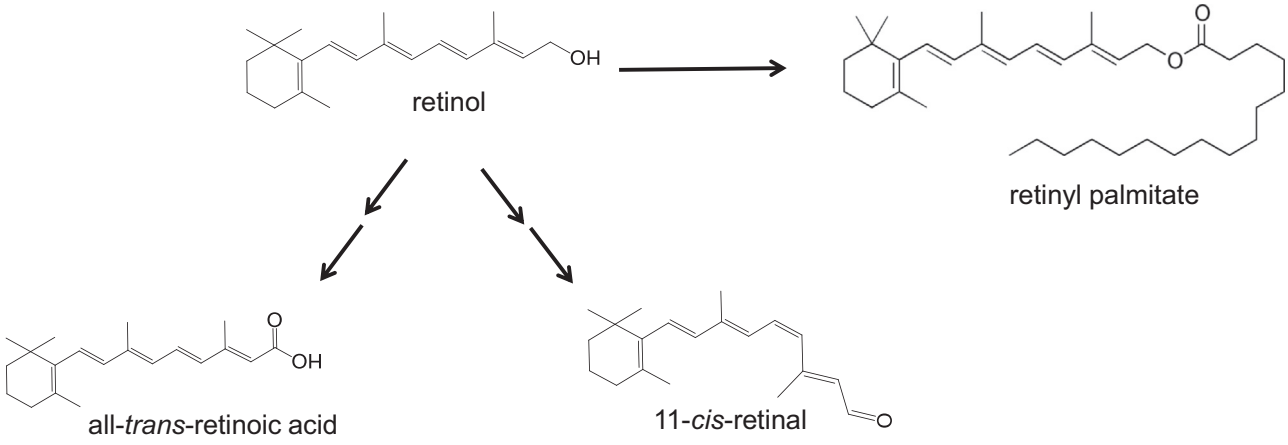

Fig. 1. Chemical structures of vitamin A, retinol, and some of its metabolites . In cells, retinol can be metabolically converted to its storage species retinyesters. Retinol can also be metabolically transformed to active metabolites including all-*trans*-retinoic acid, which regulates gene transcription, and 11-*cis*-retinal, which serves as a cofactor for the visual chromophore rhodopsin and is critical for vision.