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Vitamin A in regulation of insulin responsiveness: mini review

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Vitamin A, retinol, circulates in blood bound to retinol-binding protein (RBP4) which, in turn, associates with another serum protein, transthyretin (TTR), to form a ternary retinol-RBP4-TTR complex. At some tissues, retinol-bound (holo-) RBP4 is recognised by a receptor termed stimulated by retinoic acid 6 (STRA6) which transports retinol into cells. This mini-review summarises evidence demonstrating that, in addition to functioning as a retinol transporter, STRA6 is also a signalling receptor which is activated by holo-RBP4. The data show that STRA6-mediated retinol transport induces receptor phosphorylation, in turn activating a Janus kinases2/signal transducers and activators of transcription (STAT)3/5 cascade that culminates in induction of STAT target genes. STRA6-mediated retinol transport and cell signalling are inter-dependent, and both functions critically rely on intracellular retinol trafficking and metabolism. Hence, STRA6 couples 'sensing' of vitamin A homeostasis and metabolism to cell signalling, allowing it to control important biological functions. For example, by inducing the expression of the STAT target gene suppressor of cytokine signalling 3, STRA6 potently suppresses insulin responses. These observations provide a rationale for understanding the reports that elevation in serum levels of RBP4, often observed in obese mice and human subjects, causes insulin resistance. The observations indicate that the holo-RBP4 /STRA6 signalling cascade may comprise an important link through which obesity leads to insulin resistance and suggest that the pathway may be a novel target for treatment of metabolic diseases.

Vitamin A: Cytokine signalling: Retinol binding protein: Janus kinases/signal transducers and activators of transcription: Insulin response

Vitamin A was recognised as an essential dietary factor necessary for growth about a century ago[1,2]. The vitamin is critical for embryonic development, and in the adult, it is necessary for vision and immunity and plays key roles in regulation of metabolism and cell growth, differentiation and survival. Upon its absorption by intestinal enterocytes, retinol is esterified to retinyl esters which are incorporated into chylomicrons and secreted to the circulation to be taken up by the liver[3]. The liver stores the vitamin and provides it to the body in times of insufficient dietary vitamin A intake. Retinol is secreted from the liver into blood bound to retinol-binding protein (RBP; encoded for by the *RBP4* gene) which delivers it to extrahepatic tissues[4]. Indeed, in RBP-null mice, vitamin A is sequestered in the liver and animals rapidly become deficient if it is not provided in the food[5].

While the liver comprises the main site of RBP4 synthesis, some extra-hepatic tissues, including lung, adipose tissue, kidney, testis, brain and retinal pigment epithelium in the eye also express and secrete RBP. In blood, retinol-bound RBP4 (holo-RBP) is associated with

Abbreviations: CRBP, cellular retinol-binding protein; JAK, Janus kinases; RBP, retinol-binding protein; STAT, signal transducers and activators of transcription; STRA6, stimulated by retinoic acid 6; TTR, transthyretin.

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another protein, transthyretin (TTR), to form a ternary TTR-RBP4-retinol complex.

Retinol can spontaneously dissociate from RBP4 and, due to its hydrophobic nature, readily moves into cells by diffusion through the plasma membranes[6–9]. In addition, at some tissues, holo-RBP4 is recognised by a plasma membrane receptor termed stimulated by retinoic acid 6 (STRA6), that transports retinol from the binding protein into cells[10]. In the adult, STRA6 is expressed in blood–organ barriers, retinal pigment epithelium cells of the eye, brain, spleen, kidney, testis, female genital tract and adipose tissue but not in the liver or in the colon[11,12]. Surprisingly, characterization of STRA6-null mice showed that the receptor is not necessary for maintaining proper retinoid content of tissues other than the eye, and that its ablation does not disrupt physiological functions that critically depend on vitamin A either during embryonic development or in the adult[13–15]. Furthermore, ablation of STRA6 does not impair embryonic development even when dams were fed a vitamin A deficient diet throughout pregnancy[13]. Hence, the contribution of STRA6 to retinol uptake by most cells is modest, and the receptor is not essential for vitamin A homeostasis in tissues other than the eye. These observations suggest that STRA6 has important biological functions other than to transport retinol into cells.

STRA6, a cytokine signalling receptor activated by holo-retinol-binding protein

We recently discovered that STRA6 functions as a surface signalling receptor[13,16–19]. Such receptors are activated by extracellular cytokines, hormones and growth factors and they transduce signalling cascades by activating protein kinases termed Janus kinases (JAK) and their associated transcription factors called signal transducers and activators of transcription (STAT). Upon their activation, STAT move to the nucleus where they regulate the transcription of specific target genes which contain STAT response elements in the regulatory regions[20–22].

We found that treatment of STRA6-expressing cells with holo-RBP4 leads to phosphorylation of STRA6, in turn triggering recruitment and activation of JAK2 and, in a cell-specific manner, STAT3 or STAT5[16]. STRA6 recruits STAT through an amino acid sequence in the receptor's intracellular domain that contains a consensus phosphotyrosine motif[12]. Holo-RBP4 thus functions as a classical cytokine to activate a STRA6/JAK2/STAT3/5 pathway. Remarkably, unlike other cytokine receptors, STRA6 is activated not simply by binding its ligand but by the act of transporting retinol. Consequently, STRA6 signalling critically depends on STRA6-mediated retinol transport. Our data further showed that, in turn, retinol transport cannot proceed if STRA6 phosphorylation is impaired[13,18]. The two functions of STRA6 are thus critically inter-dependent. Moreover, we found that STRA6 not only binds holo-RBP4 but also directly associates with the intracellular RBP termed CRBP1. Hence, the receptor does not

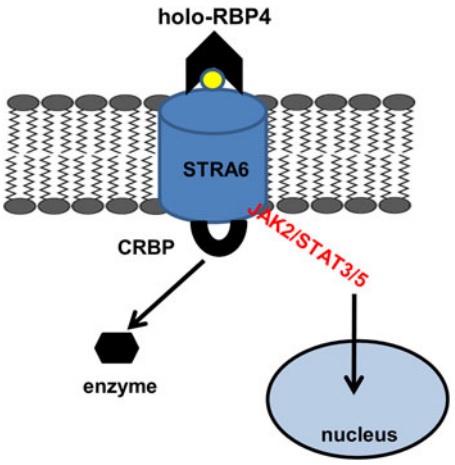

Fig. 1. (Colour online) Model for the retinol-binding protein (RBP4)/STRA6 pathway. STRA6 binds extracellular holo-RBP4 and transports retinol to receptor-associated cellular retinol-binding protein (CRBP). Retinol transfer activates STRA6, thereby triggering a signalling cascade mediated by the kinase Janus kinases 2 (JAK2) and its associated transcription factors signal transducers and activators of transcription 3 (STAT3) or STAT5. Activated STAT translocates to the nucleus where it induces target gene expression. Upon binding retinol, CRBP dissociates from STRA6 and delivers retinol enzymes that can metabolise it.

transport vitamin A into the cytoplasm but, instead, directly transfers it from extracellular RBP4 to intracellular CRBP1, bypassing the need for the lipophilic vitamin to dissociate into the aqueous milieu of the cytosol[18]. Upon binding retinol, CRBP1 dissociates from STRA6 and delivers the vitamin to a retinol-metabolizing enzyme. One such enzyme is Lecithin:retinol acyltransferase, which catalyses the conversion of retinol to its storage species retinylesters. Lecithin:retinol acyltransferase receives its substrate directly from CRBP1[23], unloads retinol from CRBP1 and, by metabolizing it, maintains an inward-directed retinol concentration gradient allowing continuing retinol uptake[19,24]. As retinol transport is required for activation of STRA6 signalling, both the cellular RBP and the retinol metabolizing enzyme are necessary for enabling receptor phosphorylation[18,19]. A model for the mechanism of action of STRA6 is shown in Fig. 1.

As mentioned earlier, holo-RBP4 circulates in blood bound to TTR. We found that although TTR does not interfere with the movement of retinol into cells by free diffusion, it prevents holo-RBP4 from binding to STRA6. TTR thus inhibits both STRA6-mediated retinol transport and STRA6-initiated signalling[17]. As the binding affinity of holo-RBP4 to TTR and STRA6 are similar[10,25], STRA6 can function only when its expression level in particular cells is very high, allowing it to locally compete with TTR for holo-RBP, or when serum level of holo-RBP4 exceeds that of TTR. The high expression level of STRA6 in the retinal pigment epithelium thus allows the receptor to significantly contribute to vitamin A uptake by the eye[15]. Interestingly, it was reported that serum level of RBP4 is elevated in obese mice and human subjects[17,26,27] but the level of

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TTR is not[17]. Under these circumstances, the high RBP : TTR ratio in blood may lead to hyperactivation of STRA6. It was also reported that increasing the blood level of RBP4 either by feeding mice a high fat-containing diet or by direct injection of the protein causes insulin resistance[17,26–28]. These observations raise the intriguing possibilities that RBP-induced activation of STRA6 may be involved in regulation of insulin responses, and that such an activity may underlie the well-established but incompletely understood link between obesity and insulin resistance.

Involvement of retinol-binding protein/STRA6 signalling in regulation of insulin responses

The discovery of the holo-RBP/STRA6 signalling cascade and the observations that this pathway activates STAT suggest a possible mechanism through which RBP4 may control insulin responses. A prominent STAT target in the insulin-responsive tissues adipose tissue and muscle is the gene that encodes suppressor of cytokine signalling 3[29], a potent negative regulator of the insulin receptor. Indeed, we found that holo-RBP4 suppresses insulin receptor in cultured cells and that the effect required activation of STRA6, JAK2 and STAT5 as well as up-regulation of suppressor of cytokine signalling 3[16]. In accordance, administration of RBP4 to mice activated STRA6, JAK2 and STAT5, up-regulated suppressor of cytokine signalling 3 and decreased the phosphorylation status of insulin receptor in adipose tissue and muscle, but not in liver, a tissue that does not express STRA6[16]. Additional experiments showed that STRA6-null mice are completely protected from insulin resistance caused by administration of RBP4 and partially protected from obesity-induced glucose intolerance. It was recently reported in regard to this that even partial reduction on STRA6 only in adipose tissues improves insulin responsiveness in obese mice[30]. These observations conclusively demonstrate that STRA6 mediates RBP4-induced suppression of insulin signalling and that elevated RBP4 levels in blood of mice contribute to the well-documented but incompletely understood link through which obesity leads to insulin resistance.

Concluding remarks and future directions

The discovery that retinol in conjunction with its blood carrier RBP4 can control cellular functions by activating STRA6-mediated cell signalling reveals a new mechanism by which vitamin A exerts its biological functions. The observations show that holo-RBP4/STRA6 signalling regulates insulin responses, but the complete spectrum of the biological activities of the path remain to be clarified. Interestingly, it has been reported that STRA6 is up-regulated in several human cancers[11]. STRA6 and its associated components may thus be involved in oncogenic activities. While available information indicates that STRA6 can trigger a JAK2/STAT3/5 cascade, it is possible that, like other cytokine receptors, STRA6 can also activate other signalling pathways. The complete spectrum of the signalling networks that may be activated by this receptor and cross-talk between this pathway and others remain to be explored. The observations open a new window on vitamin A biology, and they suggest that STRA6 may be a druggable target for novel approaches for therapy of metabolic disease and perhaps other pathologies.

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Conflict of Interest

None.

Authorship

The author was solely responsible for all aspects of preparation of this paper.

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