Biochimica et Biophysica Acta 1821 (2012) 206–212

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

Review

Chromatin remodeling and epigenetic regulation of the *CrabpI* gene in adipocyte differentiation ☆

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ARTICLE INFO

Article history:
Received 27 January 2011
Received in revised form 7 March 2011
Accepted 14 March 2011
Available online 22 March 2011

Keywords:
CRABPI
Retinoic acid
Thyroid hormone
RIP140. TRAP220
Chromatin remodeling

ABSTRACT

Retinoic acid (RA) acts by binding to nuclear RA receptors (RARs) to regulate a broad spectrum of downstream target genes in most cell types examined. In cytoplasm, RA binds specifically to cellular retinoic acid binding proteins I (CRABPI), and II. Although the function of CRABPI in animals remains the subject of debate, it is believed that CRABPI binding facilitates RA metabolism, thereby modulating the concentration of RA and the type of RA metabolites in cells. The basal promoter of the *CrabpI* gene is a housekeeping promoter that can be regulated by thyroid hormones (T3), DNA methylation, sphinganine, and ethanol acting on its upstream regulatory region. T3 regulation of *CrabpI* is mediated by the binding of thyroid hormone receptor (TR) to a TR response element (TRE) approximately 1 kb upstream of the basal promoter. Specifically, in the adipocyte differentiation process, T3 regulation is bimodal and closely associated with the cellular differentiation status: T3 activates *CrabpI* in predifferentiated cells (e.g., mesenchymal precursors or fibroblasts), but suppresses this gene once cells are committed to adipocyte differentiation. These disparate effects are functions of T3-triggered differential recruitment of coregulatory complexes in conjunction with chromatin looping/folding that alters the configuration of this genomic locus along adipocyte differentiation. Subsequent sliding, disassembly and reassembly of nucleosomes occur, resulting in specific changes in the conformation of the basal promoter chromatin at different stages of differentiation. This chapter summarizes studies illustrating the epigenetic regulation of *CrabpI* expression during adipocyte differentiation. Understanding the pathways regulating *CrabpI* in this specific context might help to illuminate the physiological role of CRABPI *in vivo*. This article is part of a special issue entitled: Retinoid and Lipid Metabolism.

1. Introduction

All-*trans* retinoic acid (RA) is the major retinoid regulating various biological processes, and it exerts pleiotropic effects in animals. Because its derivatives are potential therapeutics for managing diseases including cancers [1–5], RA is also important clinically. The biological activities of RA are mediated primarily by binding to nuclear RA receptors (RARs) that regulate a range of downstream target genes in most cell types examined [6,7]. Before entering the nucleus, RA can bind specifically to two cytoplasmic RA binding proteins that belong to a family of intracellular lipid-binding proteins: cellular retinoic acid binding proteins I (CRABPI) and II (CRABPII) [reviewed in [8,9]. Molecular and biochemical studies showed that CRABPI binding facilitates RA metabolism in the cytoplasm, thereby modulating the concentration of RA, as well as the type of its metabolites within cells [8–12]. Very differently, a more recent study showed that CRABPII binding to RA delivers the hormone to nuclear RARs for gene regulation [13]. Both proteins, especially CRABPI, appear to be highly conserved across species: Human CRABPI has only one amino acid substitution relative to the bovine, rat and mouse proteins, whereas CRABPII tolerates a greater range (~10%) of sequence variation among the four species. Their tight binding to RA (reported Kd is in the low to sub nM range) [14,15] and sequence conservation during evolution suggests that these proteins might have critical functions. However, despite their presumed physiological importance, *CrabpI*-, *CrabpII*-, and even *CrabpI*/*CrabpII*-double knockout mice appeared grossly normal [16,17]. But it remains to be seen whether these mutant animals' tolerance to an abnormal vitamin A status, especially hypovitaminosis A, has changed. It is possible that metabolism of endogenous retinoids and/or their extensive crosstalk with other closely related nutrients/hormones (e.g., lipids) could complicate the interpretation and extrapolation of data when comparing studies performed in animals and in experimental systems reconstituted *in vitro*. Some of these difficulties stem from the fact that the specific

Abbreviations: 3C, Chromosome conformation capture; ChIP, chromatin immunoprecipitation; CRABP, cellular retinoic acid binding protein; EC, embryonal carcinoma; LM-PCR, ligation mediated polymerase chain reaction; MNase, micrococcal nuclease; RA, retinoic acid; RAR, retinoic acid receptor; RIP140, receptor interacting protein 140; TIS, transcription initiation site; TR, thyroid hormone receptor; TRAP220, thyroid hormone receptor activating protein 220; TRE, thyroid hormone response element

☆ This article is part of a special issue entitled: Retinoid and Lipid Metabolism.

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doi:10.1016/j.bbalip.2011.03.003

physiological roles of, and the biological context relevant to, the two CRABPs have yet to be established (see Section 2.1). Interestingly, recent reports have shown polymorphism in CRABPII in lipid profile, such as low-density lipoprotein cholesterol levels [18].

With regard to gene regulation, *CrabpI* responds directly to thyroid hormones (T3/T4) but also uses complex regulatory mechanisms, as shown in studies *in vivo* and *in vitro* [19,20; also see below]. In contrast, *CrabpII* appears to respond primarily to RA activation [21,22]. This chapter summarizes studies on the regulatory mechanisms underlying *CrabpI* gene expression in the specific biological system of fibroblast–adipocyte differentiation, because in this cell differentiation model, T3 is a common agent used in differentiation cocktails [23]. To address the issue of physiological relevance, studies of *CrabpI* in this system were conducted in an entirely endogenous context. Understanding the pathways regulating *CrabpI* in this specific context should help illuminate the physiological role of CRABPI *in vivo*.

2. Regulation of the *CrabpI* gene

2.1. Dispute regarding CRABPI functions

After the first CRABPI cDNA was cloned [24,25], biochemical studies using systems reconstituted *in vitro* established CRABPI as a specific, high-affinity, cytoplasmic RA binding protein [reviewed in [26]. It was hypothesized that CRABPI binding reduces the concentration of biologically active RA available to the nucleus for gene regulation and simultaneously inactivates RA, reducing its toxicity. Early studies in which CRABPI was manipulated/mutated in RA-responding cells supported the general notion that high levels of CRABPI desensitize the responsiveness of cells to RA, i.e., such cells are more resistant to RA induction of gene expression [10–12]. Consistent with the notion that binding to CRABPI reduces the cellular availability of RA for general growth, studies in transgenic mice showed that globally over-expressing CRABPI produced specific pathologies in growth and reproduction, as well as abnormalities of the liver and spleen [27,28]. In contrast, mutating CRABPI (specifically, with regard to its RA-binding affinity) eliminated its ability to desensitize the response of cells to RA [12].

Another proposal suggested that CRABPI binding helps to convert RA to derivatives required for specific biological processes. RA can be metabolized to more polar products (e.g., 4-OH-RA, 4-oxo RA, 5,6-epoxy RA, 20-OH-RA, etc.) [29,30] primarily by an RA-metabolizing cytochrome p472 belonging to the CYP26 class [31]. Some of these metabolites can regulate gene expression by binding to RARs, suggesting possible physiological roles [32,33]. Whether metabolic conversion of RA by CYP26 absolutely requires CRABPI is unclear, but it has been shown that RA catabolism in CYP26-positive cells can be enhanced by elevating cellular CRABPI level [34]. Thus, most data support the notion that CRABPI expression could modulate the types of biologically active retinoids in cells.

RA is an essential nutrient and a potent morphogen. Some of its metabolites can also be morphogens [35–37]. Based on this, as well as on the functions described above, it was predicted that CRABPI is so crucial to normal development and/or survival that animals lacking this protein would be unable to survive or, if so, would show severe developmental defects. Surprisingly, *CrabpI* knockout mice exhibited either no, or very minor, alterations in phenotype in laboratory environments [16,17]. However, mounting evidence suggests that genetically altered mutant animals usually adopt compensatory mechanisms that might prevent a protein's physiological role from being fully revealed in knockout animals. Given the complicated metabolism of RA and its intricate relationship with many other hormones, nutrients and signaling pathways, it is likely that such alternative pathways/mechanisms might be used to compensate for the complete loss of CRABPI. Therefore, the physiological role for CRABPI in the context of whole animals remains to be determined.

Studies of *CrabpI* gene regulation have nevertheless yielded some interesting clues to the protein's physiological role. During the fibroblast/adipocyte differentiation process, *CrabpI* regulation is subject to epigenetic factors such as nutritional and/or hormonal status, as well as to changes in cell-autonomous factors [38,39]. These factors drastically alter the chromatin conformation of *CrabpI* and enhance its plasticity, expanding the range of contexts in which it can be regulated. Accordingly, CRABPI might play a role in certain biological contexts where crosstalk of nutrients and hormones is important. Understanding how epigenetic factors affect the regulation of *CrabpI* might help to define the context in which CRABPI is physiologically important and relevant.

2.2. Chromatin elements and factors/signals regulating CrabpI

Initial *in vitro* studies supported a role for CRABPI in titrating intracellular RA concentrations for gene regulation [8,9]. This suggests that cells sensitive to RA exert specific controls over the *CrabpI* gene to regulate CRABPI protein levels as needed. This was confirmed by studies of *CrabpI* in cell models where RA is known to be important for differentiation (e.g., embryonal carcinoma [EC] cells, embryonic fibroblast/adipocyte differentiation) [10–12,19,38].

The EC model is a classical cell differentiation model known for its sensitivity to RA [19]. Depending on the concentration of RA provided, EC cultures immediately take on various differentiation pathways. These stem cells typically thus produce abundant CRABPI to prevent accidental surges in RA availability to RARs, which might be detrimental to normal propagation. However, because the culture can simultaneously adopt multiple pathways, interpreting the data from studies using ECs to address the role of CRABPI in modulating RA availability can be complicated and challenging.

In contrast, the fibroblast/adipocyte differentiation model [40] provides a well-defined differentiation system where information about the physiological requirements for hormones/nutrients and changes in cell-autonomous factors (including CRABPI) can all be integrated. Another advantage of the adipocyte differentiation model is its requirement for, and sensitivity to, hormonal, dietary, and genetic factors. Specifically, fibroblast/adipocyte differentiation is highly sensitive to RA and T3: RA exerts a suppressive effect in early differentiation, whereas T3 is stimulatory [40,41].

Studies of *CrabpI* regulation using cell cultures (EC and 3T3 fibroblast) and transgenic mice have identified a specific basal promoter region located within a 172-base-pair region upstream of the transcription initiation site (TIS) [42,43] (Fig. 1). This promoter is a TATA-less and GC-rich classical housekeeping gene promoter. The activity is driven by transcription factor Sp1 binding to its cognate site (GC box) buried within multiple GC islands. As such, it is subject to epigenetic regulation by DNA methylation [42,44]. In addition to DNA methylation, factors such as RA, T3/T4, sphinganine, and ethanol [19,20,45,46] all can regulate *CrabpI* by acting on its upstream regulatory region (Fig. 1), although the mechanisms underlying the actions of sphinganine and alcohol are not understood. T3 and T4 (with or without RA) regulate *CrabpI* by binding to thyroid hormone receptor (TR)/retinoid X receptor heterodimers that act on a DR4-type thyroid hormone response element (TRE) located approximately 1 kb upstream of the Sp1-binding GC box [38,39]. Importantly, this particular TRE was the first to be identified as a "negative TRE" because, under certain culture conditions, it mediates gene suppression by T3/T4 directly. Further studies established the bifunctionality of this TRE by demonstrating that T3 can activate or repress *CrabpI* in the same culture under various conditions or in different states of differentiation. Specifically, T3 activates *CrabpI* in precursor cells but suppresses this gene once cells are committed to adipocyte differentiation; whereas in terminally differentiated adipocytes this gene is entirely silenced [47]. The bi-functionality of this TRE and the bimodal response of *CrabpI* gene to T3 form the basis, at least partially, for the