# Chapter 6
# Cellular Retinoic Acid Binding Proteins: Genomic and Non-genomic Functions and their Regulation

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**Abstract** Cellular retinoic acid binding proteins (CRABPs) are high-affinity retinoic acid (RA) binding proteins that mainly reside in the cytoplasm. In mammals, this family has two members, CRABPI and II, both highly conserved during evolution. The two proteins share a very similar structure that is characteristic of a "β-clam" motif built up from10-strands. The proteins are encoded by two different genes that share a very similar genomic structure. CRABPI is widely distributed and CRABPII has restricted expression in only certain tissues. The *CrabpI* gene is driven by a housekeeping promoter, but can be regulated by numerous factors, including thyroid hormones and RA, which engage a specific chromatin-remodeling complex containing either TRAP220 or RIP140 as coactivator and corepressor, respectively. The chromatin-remodeling complex binds the DR4 element in the *CrabpI* gene promoter to activate or repress this gene in different cellular backgrounds. The *CrabpII* gene promoter contains a TATA-box and is rapidly activated by RA through an RA response element. Biochemical and cell culture studies carried out in vitro show the two proteins have distinct biological functions. CRABPII mainly functions to deliver RA to the nuclear RA receptors for gene regulation, although recent studies suggest that CRABPII may also be involved in other cellular events, such as RNA stability. In contrast, biochemical and cell culture studies suggest that CRABPI functions mainly in the cytoplasm to modulate intracellular RA availability/concentration and to engage other signaling components such as ERK activity. However, these functional studies remain inconclusive because knocking out one or both genes in mice does not produce definitive phenotypes. Further studies are needed to unambiguously decipher the exact physiological activities of these two proteins.

**Keywords** CRABPI • CRABPII • RARs • Retinoic acid • Differentiation

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## Abbreviations

| | |
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| AP2 | Activating Protein 2 |
| COUP | Chicken Ovalbumin Upstream Promoter |
| CRABP | Cellular Retinoic Acid Binding Protein |
| DR | Direct Repeat |
| ERK | Extracellular Signal-Regulated MAP kinase |
| ESC | Embryonic Stem Cell |
| GCNF | Germ Cell Nuclear Factor |
| LRH-1 | Liver Receptor Homologue-1 |
| PPAR | Peroxisome Proliferator Activated Receptor |
| RAR | Retinoic Acid Receptor |
| RXR | Retinoid X Receptor |
| RA | Retinoic Acid |
| SF-1 | Steroidogenic Factor 1 |
| SP1 | Specific Protein 1 |
| TR2 | Testis Receptor 2 |
| TRAP220 | Thyroid hormone Receptor-Associated Protein 220 |
| RIP140 | Receptor Interacting Protein 140 |

## Introduction

For more than a century, vitamin A and retinoids have been known to participate in crucial processes in different organisms, including humans. Among different retinoids, retinoic acid (RA) has attracted considerably more attention because it very potently and directly affects almost all types of animal tissues/cells. Early interest in the field focused on identification of RA biological targets in cells. The first such targets that were described were the cytosolic binding proteins, known as cellular RA binding protein I and II (CRABPI and CRABPII) [36]. Because gene knockout of either CRABPI or CRABPII did not produce obvious developmental or physiological defects in laboratory mice, interest in CRABPs gradually diminished after initial identification and characterization studies in the early 90s. However, the notion that CRABPs are not important because laboratory animals can survive without them is difficult to reconcile with the fact that the proteins are highly conserved throughout evolution. More recently, studies using newer techniques have begun to provide compelling evidence that CRABPs are performing important. These results have reignited enthusiasm in the field about CRABPs. Nonetheless, the decades-long query regarding the exact physiological function of CRABPs remains unsettled. Thus, the jury is still out and there remains a continuing need to study CRABPs in a physiological context.

## History

### *Discovery of CRABPI and CRABPII*

Observation that RA had a distinct binding profile in animal tissue fractions as compared with retinol-binding fractions [6] suggested there were binding proteins with specificity for RA. This was confirmed by subsequent biochemical studies in which radioisotope-labeled RA was reacted with tissue extracts. Ong and Chytil were the first to utilize classical sedimentation methods to partially purify a rat testis fraction that retained $^{3}$H-RA binding activity, and determined its molecular weight to be 14,500 [35, 46]. These careful binding studies confirmed the specificity of this fraction toward RA, showing that the ligand could not be competed out even with a 200-fold molar excess of cold retinal, retinol, or oleic acid. They found the partially purified protein bound to RA has a fluorescence excitation spectrum with lambda max at 350 nm, which is different from that of cellular retinol-binding protein (CRBP) bound to retinol, which has a lambda max of 334 nm [6]. In these initial studies, RA-binding proteins were not only found in the testis, but also in several other tissues such as brain, eye, ovary, and uterus. These initial biochemical characterization studies were important in leading the way for further protein purification and, subsequently, molecular cloning of the cDNAs for CRABPs. The cloning experiments conclusively demonstrated the existence of CRABPI [50, 51, 59] and CRABPII [3, 5, 22] in rodents and humans.

CRABPs were identified long before the discovery of nuclear RA receptors (RARs). The studies performed at that time during the 1980's revealed that CRABPs are present essentially in the cytosol and would serve as shuttles transferring the ligand into the nucleus.

### *Genetic Analysis*

Gene and amino acid sequence comparisons place CRABPs in the same family under the super family of intracellular lipid binding proteins [18, 27]. Amino acid sequence analyses have shown that the two CRABPs have a sequence conservation of approximately 72 % for the human proteins [3]. Further studies have revealed that both *crabpI* and *crabpII* genes are conserved across different vertebrate animal species, including mouse, human, rat, frog, bird and fish [40], which indicates these proteins are likely to have conserved functions.

### *Protein Structure*

The crystal structure of bovine and mouse CRABPI was first determined for the protein complexed with its natural ligand, RA (holo-CRABP) [25]. A year later, the crystal structure of apo-CRABPI (protein without ligand) was resolved and

compared with the holo-CRABPI structure [52]. The human CRABPII crystal structure was determined for the protein complexed with a synthetic retinoid [25]. The structure of CRABPII is very similar to that of CRABPI.

The CRABP structures are very similar to other intracellular lipid binding proteins in that they share a very similar *β-clam* motif consisting of two β-sheets built up from10-strands. The ligand, RA, is sandwiched inside the clam with the acidic group situated innermost [52]. Both proteins prefer binding all-*trans* RA (atRA) compared to 9-*cis*-RA, but they bind RA very differently [33]. CRABPI specifically and tightly binds atRA with a Kd in the low- to sub-nM range [20, 32, 47]. The lowest reported value is less than 0.4 nM [32]. In contrast, the Kd of CRABPII towards atRA is within the nM range and can be as high as 65 nM [5, 18, 27, 32].

## Development of the Field

Although they share structural similarities, classical biochemical studies and gene expression patterns suggest that CRABPI and II behave differently and thus, likely have distinct physiological functions with regard to RA transport, metabolism, and signaling.

## CRABPI

### *Localization of CRABPI*

CRABPI is primarily localized in the cytoplasm and is found in most adult tissues [10, 18]. However, in certain cell types, CRABPI has been detected only at embryonic stages [18], and was reported to be associated with mitochondria [45]. Of note, some groups detected CRABPI in the nucleus [21], raising the hypothesis that this protein might also interfere with RAR's functions.

### *Biological Activities and Mechanism of Action of CRABPI*

Classical biochemical studies have suggested that CRABPI mediates RA catabolism by cytochrome p450 enzymes [31]. It has been proposed that RA bound to CRABPI is a better substrate for the RA-metabolizing cytochrome p450s than free RA and that this more efficient interaction could increase the levels of polar metabolites such as 4-OH and 4-Oxo RA. However, the mechanism for the latter process is not clear, and the physiological impact remains uncertain.

In fact, mostly homogeneous distribution of CRABPI in the cytoplasm suggested the protein primarily functions in various cytosolic processes, such as cell signaling.

Therefore our group recently embarked on systematic studies aimed at carefully dissecting the potential downstream physiological targets of apo- and holo-CRABPI. Embryonic stem cells (ESCs) were used because they represent a standard model for addressing RA's pleiotropic effects. Indeed, RA is known for profoundly triggering differentiation and/or apoptosis in these cells. Moreover, CRABPI is very abundant in ESCs, while CRABPII is almost non-detectable. Our early work determined that, in ESCs, RA rapidly stimulates the post-translational phosphorylation and sumoylation of transcription factors that are important for ESC proliferation, and that this activity does not involve RARs, thus highlighting non-canonical, non-genomic effects of RA in the cytoplasm [14, 24, 38]. More recently, by employing CRABPI knock down, we identified a specific and rapid, RA-responsive process that was dependent on CRABPI, and not on RAR. This CRABPI-dependent effect involved the activation of the cytoplasmic kinase pathway ERK1/2, which is crucial for ESCs cell cycle regulation. Indeed, the RA-activated, (ERK1/2) was found to stabilize the levels of the cyclin-dependent kinase, p27, leading to a delay in cell cycle progression, while at the same time, facilitating cell differentiation.

Based on these reports, we can propose the model shown in Fig. 6.1 which posits that an ESC, when exposed to RA, exploits both immediate cytosolic (via CRABPI) and delayed nuclear (via RARs) targets to prepare its genome for proper differentiation programming [42]. The cell cycle decision is critical for stem cells because it

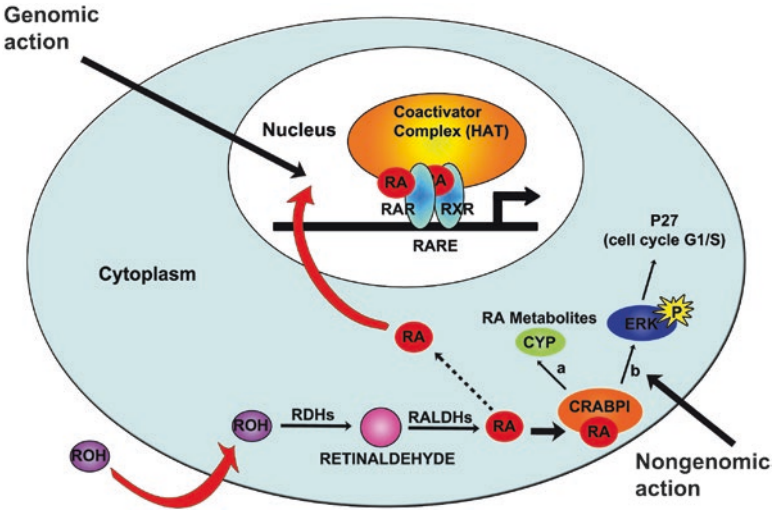


**Fig. 6.1** A proposed model of CRABPI function. In cells sensitive to the genomic activity of RA such as stem cells, CRABPI functions to a) direct accidentally surged RA to its metabolic enzymes such as the cytochrome p450 system (CYP) to reduce RA toxicity to stem cells, or b) to activate cytoplasmic signaling such as ERK that in turns controls cell cycle progression regulator p27 to ensure effective cell differentiation program. In these cells, a limited amount of RA is delivered to the nucleus for its genomic function (depicted by a *broken arrow*)

indicates the difference between continuing to proliferate versus committing to a differentiation program. In the nucleus, RA regulates RA-responding genes, mediated by RARs/RXRs, to direct ESCs toward specific differentiation programs. In the cytoplasm, RA elicits rapid effects, mediated by CRABPI, to place a hold on cell cycle progression. This short window of cell cycle delay is important for a stem cell's "commitment" to proceed to proper differentiation because only when the cytosolic signaling pathway is readily activated and RA has entered the nucleus to activate RARs/RXRs, can the cell move on and commit to a specific differentiation program. How CRABPI-RA can perform such an intriguing function in the cytoplasm remains to be elucidated. Additionally, as proposed by others, CRABPI may also direct excess RA to the cytochrome p450 system for catabolism to guard against potential RA toxicity.

Alternative proposals for the actions of CRABPI have been also reported. For example, the detection of CRABPI in the nucleus [21] prompted the hypothesis that CRABPI may interact with RARs to influence their function in the nucleus (see review in [34]). Accordingly, other *in vitro* studies suggested that CRABPI establishes an RA gradient in the nucleus that passively delivers RA to RARs. This proposed mechanism of RA transfer from CRABPI to RAR is very different than the more direct mechanism of RA transfer proposed for CRABP II (see below). Intriguingly, overexpressing CRABPI failed to affect the transcriptional activity of RARs in Cos-1 cell [16] but reduced RA responses in embryonic cells [11, 67]. Thus the significance of CRABPI in the nucleus remains unclear. However, it is important to recognize that all these experiments were conducted in different cellular backgrounds with different experimental models and detection methods.

Nevertheless, a consensus seems to emerge that CRABPI expression levels matter to cells that are responsive to RA signals (such as cells of embryonic origin) in terms of gene regulation and cellular behavior. This is most strongly supported by several animal studies conducted by several groups including ours [41, 65, 66]. While *CrabpI* gene knockout mice appeared grossly normal [15, 23], ectopic overexpressing of CRABPI in two transgenic mouse models induced abnormalities in adult lung and liver [61, 65, 66], and caused defective liver lens fiber differentiation and pancreatic tumorigenesis [41]. These transgenic animal data strongly suggest that CRABPI should not be abnormally expressed in animals. Our conclusion based on these results is that the level of CRABPI expression is important for maintaining a normal cellular response to RA. The unsolved question is, why does CRABPI matter if animals deficient in CRABPI survive?

## *The CRABPI Gene and Its Regulation*

The *crabpI* gene (Fig. 6.2) promoter does not contain the TATA box that is typically seen in housekeeping genes, but does include binding sites for the major transcription factor Specific Protein 1 (SP-1), a pair of overlapping direct repeat (DR) 4/DR5 elements (now defined as Thyroid hormone response element, or TRE) that are

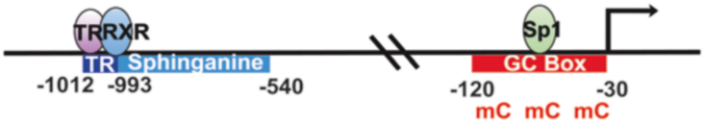


**Fig. 6.2** The mouse *crabpI* gene promoter. The gene is driven by a TATA-less promoter, which contains mainly multiple GC boxes for Sp1 binding to activate basal promoter. This basal promoter is subjected to regulation by extensive DNA methylation, which accounts for basal gene repression in non-expressing cells. Approximately 1 kb upstream of this promoter lies a DR4/DR5-containing thyroid hormone response element (TRE) for regulation by hormones like T3/T4 and RA [13]. This regulatory region is also subjected to modulation by lipid signals such as sphinganine. Hormonal regulation of this gene depends upon hormone-induced chromatin juxtaposition of this TRE to GC box region (see Fig. 6.4)

responsible for its regulation by RA [60] and thyroid hormones [65, 66], a region responsive to lipid signals such as sphinganine [64] and ethanol [10], and a region enriched in DNA methylation that is known to be important in heterochromatin formation for gene silencing [58].

We have employed the mouse embryonal carcinoma cell (similar to ESC) and fibroblast-adipocyte differentiation (where RA is one important factor for commitment) models to study the physiological regulation of the *crabpI* gene. In both experimental models, the *crabpI* gene is regulated by thyroid hormones and RA through the thyroid hormone response element (TRE) [57]. These studies have exploited extensive biochemical methodology to determine whether regulation occurs at the chromatin level. We have examined the endogenous chromatin conformation of the *crabpI* gene locus, particularly its promoter and a contiguous upstream regulatory region because this segment of the mouse *crabpI* gene has been validated as physiologically functional in a transgenic reporter mouse model [62, 63]. Based on these experiments, we concluded that the *crabpI* gene is rapidly activated by thyroid hormones in undifferentiated preadipocytes, and that this activity is mediated by the Thyroid hormone Receptor-Associated Protein 220 (TRAP220)-containing activating mediator complex [37]. In this hormone-activating phase (Fig. 6.3), the enhancer region of the *crabpI* gene is looped to juxtapose its basal promoter region and, through nucleosome sliding, the transcription initiation site-spanning nucleosome falls off the chromatin, thereby opening its transcription initiation site for active gene transcription. Then, in cells stimulated to differentiate into adipocytes, the *crabpI* gene negatively responds to the same hormonal input (Fig. 6.3). In this hormone-repressive phase, chromatin remodeling on the *crabpI* gene requires the repressive remodeling machinery that contains the corepressor named Receptor Interacting Protein 140 (RIP140) whose expression level is highly elevated in differentiating adipocytes [39]. During the repressive chromatin remodeling phase, DNA methylation and heterochromatin begins to form on the c*rabpI* gene promoter, rendering this gene gradually silenced in differentiated adipocytes [56]. Thus in this model, regulation of the *crabpI* gene is bimodal, depending on the differentiation status of the cells. Such a tight regulation of the *crabpI* gene is in line with the identified function of CRABPI in the stem cell model. In pre-committed

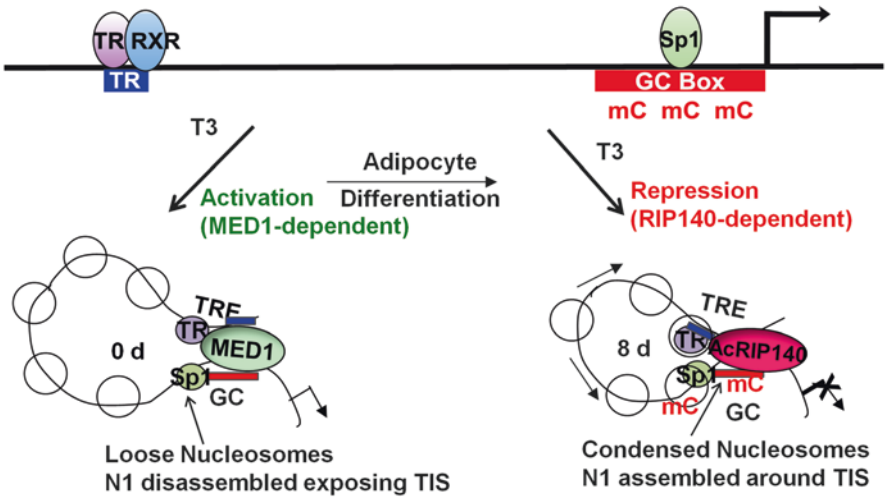


**Fig. 6.3** Chromatin remodeling of *crabpI* gene in adipocyte differentiation. In adipocyte differentiation before cells are committed to adipocyte lineage, the *crabpI* gene is initially activated by thyroid hormones and RA (*left*) which induce chromatin juxtaposition, mediated by the Mediator (MED1) complex, of TRE region with the GC box region. This shortens the distance between the upstream regulatory region and the basal promoter, and simultaneously recruits chromatin remodeling machinery to cause nucleosome sliding and disassembly of N1 nucleosome from the transcription initiation site, resulting in rapid gene activation. As cells are committed to the adipocyte lineage and in the presence of T3/T4 or RA, corepressor RIP140 expression is elevated to replace MED1, which maintains the chromatin fold but recruits chromatin repressive enzymes such as histone deacetylases, heterochromatin protein 1 and DNA methyl transferases to condense this chromatin. At this stage, the CRABPI gene is completely silenced and can no longer be activated

stem cells CRABPI is needed (to reduce free RA), whereas in post-committed cells CRABPI is no longer needed and therefore, the gene is repressed.

## CRABPII

### *Localization*

During embryogenesis, CRABP II is widely expressed [44], whereas in the adult, available data suggest the protein is expressed in a more restricted pattern in tissues that are sensitive to RA, such as skin, ovary, uterus, choroid plexus, and the olfactory epithelium [2, 18, 27, 28].

CRABPII has been detected both in the cytosol and in the nucleus [21] and has been observed to traffic from the cytoplasm to the nucleus [16, 17]. Noy and colleagues reported that RA binding induces the formation of a nuclear translocation signal in the CRABPII molecule which can function as a coactivator [49, 68]. Majumbar's laboratory determined that the sumoylation state of CRABPII is also important for its nuclear localization [29].

## *Biological Activities and Mechanism of Action*

Unfortunately, mutant mice lacking expression of CRABPII or both CRABPII and CRABPI genes display no obvious developmental or adult abnormalities [26]. However, one cannot exclude that this could be due to compensatory mechanisms in a fully protected, optimal laboratory environment. Thus, other approaches have been used to shed light on the potential functions of CRABPII, at least *in vitro*.

*In vitro* studies have shown that CRABPII channels RA to RAR in a "direct collisional process" that facilitates RAR transcriptional activity [12, 49]. RA-reporter assays have indicated that CRABPII (but not CRABPI), enhances the induction of RA target genes. Co-immunoprecipitation assays and gel-shift assays have further shown that CRABPII interacts directly with RAR/RXR complexes in solution [16, 17]. It was concluded from these studies that CRABPII serves as a coactivator for RARs (Fig. 6.4).

It must be stressed that most of these studies were conducted using RA-reporter assays and engineered cell lines, leaving open questions concerning the physiological relevance of the reported interpretations. One most intriguing point is that, many RA responsive cells, including ESCs, do not express CRABPII. Therefore, it would seem that a potential co-activator function of CRABPII is only needed in specific cell types.

Very recently, an interesting finding was reported that CRABPII also displays non-transcriptional activities in the absence of RA [55]. Surprisingly these studies revealed that CRABPII directly interacts with HuR, an ubiquitously expressed protein which binds mRNAs and protects them against degradation. This interaction markedly increases the affinity of HuR for some target transcripts exemplified by the apoptotic peptidase activating factor 1 (Apaf-1), which is involved in apopttic responses. Consequently, the stability and the expression levels of the transcripts are increased. Then upon RA binding, CRABPII dissociates from HuR and translocates to the nucleus, where it delivers RA to RAR. Although the spectrum of genes whose expression is regulated by HuR in cooperation with CRABPII remains to be identified, the data establish that the tumor suppressive activity of CRABPII is exerted both by its ability to deliver RA to RAR, resulting in induction of RAR-targeted growth inhibitory genes, and by its involvement in HuR-mediated stabilization of proapoptotic transcripts. In conclusion, CRABPII seems to regulate gene expression not only via regulating transcription, but also via posttranscriptional processes.

## *The Gene and Its Regulation*

The *crabpI* and *crabpII* genes share approximately 75 % sequence homology and have a very similar gene structure in that both contain 4 exons led by a short 5'-untranscribed region. The conservation in the gene structures and the similarity

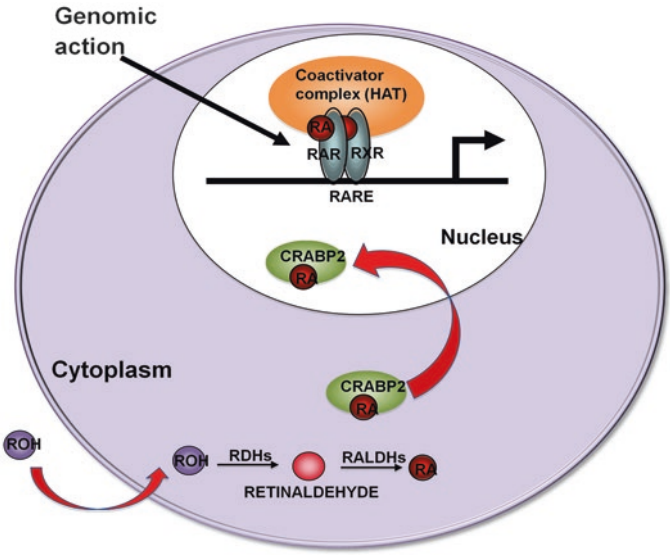


**Fig. 6.4** A proposed model of CRABPII function. In cells regulated RA, CRABPII functions to capture RA from the cytoplasm, deliver RA to the nuclear RAR/RXR to activate RA target genes

of the 5' region would suggest similar regulation. Surprisingly, these two genes are regulated very differently and exhibit distinct expression profiles [36, 44].

The *crabp*II gene (Fig. 6.5) contains a typical TATA-containing promoter and is regulated primarily by RA-responding direct repeat (DR) elements [4]. In RA-induced cell differentiation models, the human *crabp*II gene is regulated by a functional DR5 located approximately 5.6 kb upstream whereas the mouse *crabp*II gene is regulated by a DR1 and a DR2 located approximately 1.1 kb upstream [19]. Studies of the *CrabpII* gene regulation utilized various cellular models including embryonal carcinoma cells [19], neuroblastoma cells [43], skin [53], uterus [28], cancer cells [30, 54] and adipocytes [8, 9]. Interestingly, these studies reported that the *crabp*II gene can be up- or down-regulated in response to various signals or hormones upon binding of transcription factors to other specific response elements located in the promoter. In this context, adipocytes differentiation is an interesting model to study the regulation of CRABPII. Indeed CRABPII is highly expressed in preadipocytes and it has been shown that it sensitizes preadipocytes to RA-induced inhibition of differentiation. Interestingly, RA inhibited differentiation if administered within a short time frame following its induction but failed to do so when administered later in the program. This failure has been attributed to the down regulation of CRABPII upon binding of the glucocorticoid receptor and of the CCAAT/enhancer-binding protein αlpha (C/EBPα) to specific response elements, during the process of adipocyte differentiation and in mature adipocytes, respectively (Fig. 6.5) [8]. Thus, down regulation of CRABPII appears critical to allow adipogenesis to proceed. In contrast, during myogenic transformation the *crabp*II gene is activated by MyoD and Sp1 binding to its basal promoter [69].

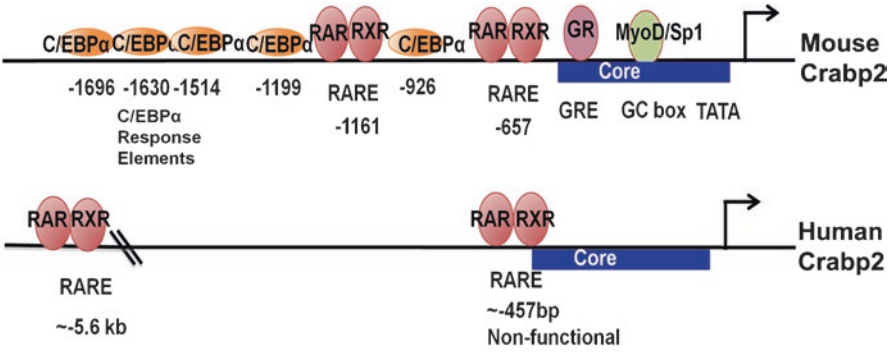


**Fig. 6.5** The *crabpII* gene promoter. The basal Promoter is from −459 through −4 and contains TATA sequence and GC box. In adipocyte differentiation, this gene is repressed by glucocorticoid (through GRE) and CCAAT/enhancer-binding protein. In myogenic transformation, this gene is activated by MyoD and Sp1 binding to its basal promoter. On the human gene (*lower*), approximately 5.6 kb upstream region lies a RA-response element (RARE) that mediates major RA induction of this gene

In conclusion, a general theme emerges that the *crabpII* gene, like the *crabpI* gene, is subject to extensive positive and negative hormonal regulation. However, it has not been reported yet whether regulation of CRABPII expression involves promoter recruitment of the same positive and negative coregulators (TRAP220 and RIP240) as described for CRABPI.

## Current State of the Field

As it currently stands, reports of CRABPI and CRABPII functions are primarily based upon *in vitro* experiments. Biochemical characterization of these proteins suggests their roles must be for very different aspects of RA signaling. CRABPII is thought to play an active role in gene expression via facilitating RA delivery to RARs into the nucleus and via stabilizing transcripts. In contrast, CRABPI would rather direct RA to the nucleus passively and would participate in cytosolic signaling via ERK1/2 activation.

Most importantly, at the gene level, both CRABPI and CRABPII can be regulated by several transcription factors in response to a variety of signals, indicating a need for both proteins to maintain proper expression levels in certain physiological contexts. The physiological regulation of the *crabpI* gene is better understood because specific alterations in the chromatin have been reported and considered in animal models. On the contrary, regulation of the *crabpII* gene requires further validation studies in vivo.

However, questions remain concerning the physiological relevance of these cytosolic proteins since whole body gene knockout approaches have revealed little specific information about the function of these two proteins in animals. Only CRABPI function has been examined in animals using over-expressing transgenic mouse models, and the results suggest that the level of CRABPI is important for normal animal physiology in adults.

## Relevance and Future Directions

The CRABPI and II proteins were identified more than four decades ago, a long time before RARs. As both proteins are highly conserved during evolution and across species throughout the animal kingdoms, it was conceivable that they should be constrained for specific physiological needs. However, disappointingly, due to the lack of obvious phenotypes of mice deficient in either one or both genes, the functional roles of these two proteins is still debated. This has been quite discouraging and disappointing for the field.

In fact one cannot exclude that in a fully protected, optimal laboratory environment, the lack of phenotype could be due to compensatory mechanisms that spare mutant mice from lethality or deleterious effects. Thus, other approaches should be used to shed light on the physiological relevance of CRABPs.

Recent studies have begun to elucidate the functional roles of CRABPs. With regard to the function of CRABPI, it becomes increasingly evident that it mediates non-canonical effects of RA, i.e. the activation of kinase pathways. Now the question is how CRABPI performs such a function and whether this function occurs in other cell types than stem cells.

Concerning CRABPII, engineered experimental systems highlighted the role of this protein as a nuclear cofactor for RARs. The intriguing point is that, in contrast to RARs, CRABPII is not expressed in all RA-responsive cells. Such an observation raised several questions: why do only certain RA-responding cells employ CRABPII as a cofactor and is CRABPII required for only certain RA-regulated genes? In this context it is worth noting that in cells that do not express CRABPII, RA has been found to bind another type of fatty acid binding protein exemplified by FABP5 [48]. The striking observation is that FABP5 does not channel RA to RARs, rather it channels to another nuclear receptor, PPARβ/δ [1]. Consequently other genes involved in cell growth, survival and lipid metabolism are regulated.

Such a diversion from RAR to PPARβ/δ has been observed in mammary carcinoma cells with an aberrantly high FABP5/CRABPII ratio and has been correlated to RA resistance [48]. Most interestingly, *in vivo*, in a model of obese mice, RA has been shown to suppress obesity and insulin resistance via both PPARβ/δ and RARs [7]. All these data indicate that depending on the cell type, RA can bind other proteins than CRABPs and thus can regulate a wider subset of genes. All these recent data increase the complexity of the mechanism of action of RA and open new avenues in the field of the cellular retinoic acid binding proteins.

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