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Chromatin remodeling and epigenetic regulation of the *Crabp1* gene in adipocyte differentiation

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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 *Crabp1* 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 *Crabp1* 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 *Crabp1* 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 *Crabp1* expression during adipocyte differentiation. Understanding the pathways regulating *Crabp1* in this specific context might help to illuminate the physiological role of CRABPI *in vivo*.

Keywords

CRABPI; retinoic acid; thyroid hormone; RIP140; TRAP220; chromatin remodeling

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].

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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 K_d 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 hypo-vitaminosis 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 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, *Crabp1* 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 *Crabp1* gene regulation have nevertheless yielded some interesting clues to the protein's physiological role. During the fibroblast/adipocyte differentiation process, *Crabp1* 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 *Crabp1* 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 *Crabp1* might help to define the context in which CRABPI is physiologically important and relevant.

2.2 Chromatin elements and factors/signals regulating *Crabp1*

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 *Crabp1* gene to regulate CRABPI protein levels as needed. This was confirmed by studies of *Crabp1* 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 *Crabp1* 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 *Crabp1* 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 *Crabp1* 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 *Crabp1* in the same culture under various conditions or in different states of differentiation. Specifically, T3 activates *Crabp1* 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 *Crabp1* gene to T3 form the basis, at least partially, for the epigenetic control of *Crabp1* promoter (see Section 3). Regulation of the biological activity of both the basal promoter and the T3/T4-regulated region via this TRE has been confirmed in transgenic mouse models using a *lacZ* reporter that recapitulated the endogenous *Crabp1* gene activity and its T3 response, at least in mouse embryos [43, 48].

3. Epigenetic regulation of the *Crabp1* gene

3.1 Indicators of epigenetic regulation of *Crabp1*

Earlier studies to determine the regulatory sequences and *trans*-acting factors affecting *Crabp1* gene expression showed that specific DNA methylation was important [44], particularly methylation of specific GC sequences within the region 1 kb upstream of the TIS. Subsequent studies of transgenic mouse embryos carrying the *Crabp1* reporter confirmed that the gene promoter could be altered in the embryos by changes in nutritional and hormonal factors (*e.g.*, vitamin A, T3) in the mothers. Intriguingly, the responses also seemed to be related to the stage of embryonic development [43, 48].

Sensitivity to DNA methylation [44] implicates epigenetic mechanisms in the control of this gene, and the bimodal response of *Crabp1* in cells at different developmental stages provides a cellular context to examine such regulation. In predifferentiated cells (fibroblasts), *Crabp1* is constitutively active (albeit at basal levels) and produces a constant amount of CRABPI [38, 39, 49], presumably to titrate RA in precursor cells that are more sensitive to fluctuations in RA concentration. Activation of *Crabp1* in these cells by T3 presumably enhances this protective mechanism by increasing the cells' capacity to titrate RA under certain conditions (*e.g.*, a surge in T3 and RA). In contrast, in committed cultures, T3 treatment represses *Crabp1*, ultimately silencing it completely in fully differentiated adipocytes [50]. Since the exact functional role for CRABPI in adipocytes is still unclear, there are several scenarios where this type of biphasic regulation may become physiologically relevant during adipogenesis [51]. For instance, in precursor cells, CRABPI-free RA can be inhibiting to cell proliferation or CRABPI-bound RA can provide certain unknown functions; hence the *Crabp1* gene is induced in these cells. Once cells are

committed to adipocyte differentiation, CRABPI-free RA can be a facilitating factor for the differentiation process; reducing CRABPI level in these committed, differentiating adipocytes would enhance cellular sensitivity to RA. Alternately, the committed differentiating adipocytes may not need a strong effect from certain unknown CRABPI-RA functions, therefore the *Crabpl* gene is gradually suppressed in the committed cells. In terminally differentiated adipocyte, the unknown CRABPI-RA mediated effects may not be needed, or the cell can now tolerate a bigger fluctuation in CRABPI-free RA concentration, therefore CRABPI is no longer needed and *Crabpl* becomes entirely silenced (Fig. 2). In this model, epigenetic regulation of *Crabpl* is tied intricately to the status of the cells in terms of their sensitivity to, and potential need for, RA.

3.2 Potential mechanisms of *Crabpl* epigenetic regulation

Chromatin remodeling in which a gene's responses to regulatory factors are altered without affecting its DNA sequence represents one type of epigenetic regulation. Nucleosomes are the smallest structural units of chromatin, and nucleosome arrays are generally formed on silenced genes. They can also be assembled on actively transcribed genes, but in such cases the gene's TIS is usually not fully embedded in the nucleosomes. Upregulation of transcriptionally active genes would likely involve altering the positions of the nucleosomes or disassembling the array entirely to allow full activation of transcription. Conversely, in gene silencing, the actively transcribed chromatin segment is likely to be condensed, *e.g.*, by compacting into heterochromatin, which usually indicates permanent gene silencing.

3.2.1 Tools for studying epigenetic regulation—Several methods can be used to study the process of chromatin remodeling on a specific genomic segment [49]:

- Chromatin conformation is usually first assessed biochemically by micrococcal nuclease (MNase) digestion mapping, which determines whether a specific chromatin segment assembles arrays of nucleosomes or if it exists in an “open” configuration without forming nucleosomes.
- Restriction accessibility assesses if a specific chromatin region is accessible to restriction enzymes, indicative of an open chromatin conformation. Changes in the pattern of restriction accessibility suggest possible remodeling of the chromatin segment.
- Nucleosome scanning can roughly map the nucleosomal positions on organized chromatin segments.
- Ligation-mediated polymerase chain reaction (LM-PCR) is used to determine the precise positions of nucleosomes on an organized chromatin segment, providing a high-resolution map of the array. Changes in nucleosome positioning on chromatin as assessed by this technique provide the most convincing evidence for chromatin remodeling.
- Chromatin immunoprecipitation (ChIP) and repeated ChIP can detect specific activating or repressing chromatin marks, as well as the recruitment of specific chromatin remodeling machineries (usually multiple subunits). These methods are commonly adopted to determine the status of a specific chromatin segment and to detect the factors involved in chromatin remodeling.
- Cross-ChIP is a newly developed technique used to detect high-order chromatin conformations such as loops and folds formed by protein factors that interact with otherwise separated chromatin segments.
- Chromosome conformation capture (3C) detects physical associations between chromatin segments that might otherwise be physically separated or even on

different chromosomes [52]. Such associations usually are formed by the actions of proteins that recruit different chromatin segments to a specialized chromatin segment or hub.

These procedures can be performed in combination, as needed. They are used to examine the process of remodeling in chromatin collected from cells in different states of differentiation or from different tissues. They are also used to identify the machineries responsible for remodeling, and to monitor and validate the altered activating/repressing chromatin marks resulting from such remodeling processes. Importantly, these allow the examination of specific chromatin remodeling events at a defined genomic locus in an entirely endogenous and physiologically relevant context.

3.2.2 *Crabp1* chromatin remodeling and its epigenetic regulation during adipocyte differentiation—Initial studies examined the conformation of chromatin in the endogenous *Crabp1* gene promoter/regulatory region in precursor cells (*i.e.*, before differentiation). In these cells, *Crabp1* gene is constitutively active and forms a linear nucleosome array that spans its entire 5' upstream regulatory region, including the TRE site [49] (Fig. 3). The absence of nucleosomes on the TIS of *Crabp1* in these precursor cells is consistent with the constitutive basal transcription of the *Crabp1* gene in these cells [Fig. 2]. This provides the baseline for subsequent studies of chromatin remodeling of *Crabp1* throughout adipocyte differentiation.

In the model proposed here, T3 regulation of *Crabp1* gene is bimodal, depending upon the differentiation status of the cultures (see Section 3.1; Figs. 2 and 3). Remodeling of this chromatin segment in the adipocyte differentiating cultures was examined using a series of molecular and biochemical assays. MNase assays first detected a nucleosome array on the *Crabp1* chromatin locus not only in the precursor cells, but also throughout differentiation, *i.e.* from committed cells to terminally differentiated adipocytes [51]. However, restriction accessibility studies detected different restriction patterns on the chromatin in cells at different differentiation states, suggesting that chromatin remodeling had occurred during differentiation.

Nucleosome scanning and LM-PCR examined nucleosome positioning on the *Crabp1* locus in proliferating precursor cells (*i.e.*, with constitutive basal *Crabp1* activity), in T3-treated precursor cells (where *Crabp1* activity is further activated), and in T3-treated differentiated cells (where *Crabp1* activity becomes silenced) [49, 50]. In the proliferating precursor cells, the original five-nucleosome array (N5 to N1, from 5' to 3') covers the TRE to the 5' border of the TIS, leaving the TIS accessible to RNA polII for transcription initiation (Fig. 3). However, upon T3 treatment, the nucleosomes of this array slide from the center (*i.e.*, the N3 nucleosome) toward both ends of the array. Also, N1 disassembles such that TIS is surrounded by an open chromatin, thus enhancing *Crabp1* gene transcription in these precursor cells. In T3-treated committed cells, the nucleosome array slides even further and N1 reassembles; intriguingly, the nucleosomes are positioned even further apart from each other in the new array. Moreover, this region is decorated with more compacted, repressed chromatin marks, such as H3K9-me3 and H3K29-me3 modifications [50]. Thus, T3 stimulation of *Crabp1* in precursor cells results in nucleosome sliding and disassembly of the nucleosome nearest the TIS, whereas T3-triggered *Crabp1* suppression in committed and differentiated adipocytes involves reassembly of the N1 nucleosome and further sliding of these nucleosomes. The lengthening of the internucleosomal distance on the array suggests that the assembly's higher-order structure has been altered such that the nucleosomes are no longer evenly spaced (see below).

ChIP studies confirmed the presence of activating chromatin marks in precursor cells consistent with active *Crabp1* transcription in T3-treated proliferating precursor cultures. ChIP data also verified that repressive chromatin marks consistent with gene silencing are present in terminally differentiated cells. Interestingly, cross-ChIP studies demonstrated an unusual high-order chromatin configuration on the *Crabp1* locus only in T3-treated cells (Fig. 4A). Without T3 treatment, the *Crabp1* promoter/regulatory region assembles a regular linear nucleosome array, whereas in T3-treated precursor cells, before the differentiation cocktail is added, the segment from the TRE to the GC box adopts a folded structure by forming a chromatin-TRAP220 Mediator complex that also associates with T3-bound TR and Sp1. In this complex, T3-bound TR and Sp1 are the anchor proteins that recruit the two chromatin segments by binding to TRE and the GC box, respectively. T3 induces the association between TR and TRAP220 (a subunit of the Mediator complex that associates constitutively with Sp1), thus triggering formation of the chromatin fold/loop by connecting the otherwise distant TR-TRE to the Sp1-GC box. It is tempting to speculate that by forming this fold, regulatory signals such as T3 can be transmitted more efficiently and rapidly to the TIS near the GC box (Fig. 1) because they can be propagated without transmitting through the five nucleosomes. Presumably the sliding of nucleosomes toward the ends of the loop relieves the tension generated by forming this structure. Consequently, the sliding nucleosome array pushes away the downstream terminal nucleosome, facilitating its rapid disassembly and opening up the TIS for further activation of transcription. This looping, coupled with the nucleosome sliding/disassembly process, appears to form the basis for chromatin remodeling in the *Crabp1* regulatory segment, and accounts for *Crabp1* gene induction by T3 in precursor cells (Fig. 4A).

As described above, the *Crabp1* gene undergoes two modes (phases) of regulation by T3 in the fibroblast/adipocyte differentiation process: initial activation by T3 in precursor cells followed by suppression by T3 in cells committed to differentiation (Fig. 2). It is intriguing that the same genomic segment undergoes different chromatin remodeling processes to provide opposing outcomes for the same gene in response to the same hormone. ChIP and cross-ChIP assays confirmed that the chromatin fold of the *Crabp1* promoter/regulatory region detected in T3-treated precursor cells is retained in the T3-treated differentiating cultures, all the way from committed cells to terminally differentiated adipocytes. However, on this chromatin fold, TRAP220 is gradually replaced by corepressor RIP140, which can also interact directly with TR in a T3-dependent manner [47, 53]. LM-PCR and nucleosome scanning showed that the spacing of the five nucleosomes increases as cell differentiation proceeds, although the central N3 nucleosome retains its original position. In terminally differentiated adipocytes, N1 entirely covers the TIS, and this region is decorated strongly with repressive chromatin marks, including those for heterochromatin (*e.g.*, G9a, HP1a, HP1c and H1). These results indicate that when T3 acts as a repressive signal in the cell differentiation phase, chromatin remodeling not only reassembles the nucleosome to cover the TIS but also involves compaction of the chromatin fold. To relieve the tension generated by the compaction, the nucleosomes must move further apart. Presumably, this assists formation of heterochromatin on the promoter in the terminally differentiated adipocytes where CRABPI may no longer be needed, concluding the second phase of chromatin remodeling on the *Crabp1* promoter during adipocyte differentiation.

It is important to note that endogenous TRAP220 expression levels remain constant in cultures at different stages of differentiation, whereas endogenous RIP140 levels increase gradually as cells undergo terminal differentiation [49]. This underlines the importance of physiological context (*e.g.*, different developmental states and/or tissues) with respect to the cellular machinery critical for forming the chromatin folds on *Crabp1*. The difference between TRAP220 and RIP140 resides in their abilities to recruit different histone-modifying enzymes: TRAP220 recruits activating enzymes like histone acetyl transferases,

whereas RIP140 recruits repressive enzymes such as histone deacetylases and methyl transferases. The bimodal response of *Crabp1* to T3 thus appears to result from dynamic changes in cell-autonomous factors that trigger recruitment of different coregulatory components to the same genomic segment to generate distinct chromatin marks in cells at different stages of development, differentiation or maturation. Environmental factors — in this case, T3 — provide the external cues to activate the different epigenetic programs that regulate *Crabp1* in cells with very different nuclear contexts.

Regarding genome plasticity, several potentially relevant phenomena are noticed, such as the increasingly appreciated diverse effects of T3 [54] and the lipogenic versus thermogenic switch during adipogenesis [51, 55]. T3 not only governs energy expenditure by activating thermogenic pathways as numerous classical studies have demonstrated [review in Ref. 54], but also can enhance lipogenesis as more recently discovered [55]. On the other hand, at least in the *in vitro* fibroblast-adipocyte differentiation model, while white adipocytes, which are lipogenic, dominate the terminally differentiated culture, a phenotypic switch to brown adipocytes, which are mostly thermogenic, could happen following certain gene manipulation [56]. Could these also involve some forms of epigenetic regulation of critical genes? Again, answers to this question would most likely have to take into account the entire biological context of the experimental systems, such as the developmental/differentiating states when specific cell autonomous factors may fluctuate, and the availability of specific external cues that may change.

4. Future perspectives

Studies of *Crabp1* gene regulation have provided important clues regarding the specific contexts in which this gene's activity is altered. In precursor cells that are sensitive to differentiation triggers, *Crabp1* uses activating chromatin remodeling in a specific nuclear context where appropriate factors such as TRAP220 are in place. In committed or terminally differentiated adipocytes, *Crabp1* adopts repressive chromatin remodeling because changes in nuclear factors (specifically, elevated RIP140 levels) favor formation of heterochromatin on this locus. Although environmental factors such as hormones can provide cues that stimulate remodeling of *Crabp1* chromatin in precursor cells, the ultimate control resides in the dynamics of crucial cell-autonomous factors. Thus, cells at different developmental stages can regulate their *Crabp1* gene expression to optimize the benefits of the protein to the cell's specific physiological context. Precursor cells that require more CRABPI to reduce CRABPI-free RA would activate the *Crabp1* gene. Committed cells benefit from the availability of CRABPI-free RA, and terminally differentiated adipocytes may be resistant to CRABPI-free RA. Therefore, these cells repress or silence *Crabp1* by engaging repressive chromatin remodeling, resulting in a heterochromatin conformation at this locus. The challenge in future studies will be to identify other factors that might also be involved in regulating *Crabp1* in different physiological contexts.

Classical studies of gene regulation address DNA sequence, *trans*-acting factors, and regulatory signals from hormones and signal transduction pathways. These are typically examined in reconstituted cell culture models, and therefore provide only the basic elements of a puzzle that should be addressed in a specific physiological context. Using adipocyte differentiation as a model has revealed the epigenetic basis for bimodal regulation of the *Crabp1* gene. Given that *Crabp1* is regulated not only by DNA methylation and hormones but also by factors modulating membrane signals and signal transduction, it is tempting to speculate that such factors might contribute to its epigenetic regulation in other cellular contexts. It is possible that other epigenetic factors trigger forms of chromatin remodeling on the *Crabp1* gene locus entirely different from chromatin looping elicited by T3.

Initial speculations regarding epigenetic regulation of *Crabp1* were made almost two decades ago, but confirmation of this phenomenon and dissection of its machinery were not possible until technical breakthrough enabling the study of chromatin in an endogenous context. The concept of context-dependent regulation arising from these studies suggests that there is a close intertwining and cross-regulation of *Crabp1* by retinoids and other hormones and nutrients. Nevertheless, numerous questions remain. For example, it would be interesting to understand how retinoid metabolism affects committed cells' survival and clonal expansion, as well as their ability to differentiate, *i.e.*, does the capacity to metabolize RA provide an indication of a cell's potential for differentiation? Also, because *Crabp1* expression is rapidly elevated in the presence of T3 in precursor cells, cellular retinoid metabolism presumably becomes more active when such cells are exposed to T3. It is unknown, however, if other retinoid metabolites are required for precursor cell proliferation, differentiation potential, or function. Finally, the precise relationship between T3, RA and adipocyte differentiation in animals remains to be established.

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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

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Figure 1.

The regulatory and promoter region of mouse *Crabp1* gene. The TRE (TR/RXR binding site) is located approximately 1 kb upstream of the housekeeping promoter that contains five GC boxes for Sp1 binding. The basal promoter can become heavily methylated at its GC sequences. Sphinganine can also regulate this promoter through a small region down stream of the TRE region. T3 signal can be transmitted through TRE which relays the information to GC box as indicated by a curved arrow.

Figure 2.

Bimodal regulation of *Crabpl* gene by T3 in adipocyte differentiation. T3 can activate or repress this gene. In precursor cells that may be inhibited by CRABPI-free RA (unbound to CRABPI), this gene is activated by T3, which requires TRAP220 (phase I). Elevated CRABPI level can be protective for these precursor cells. In committed cells (phase II), this gene is repressed by T3, which requires gradually elevated RIP140. CRABPI level is gradually lowered in these cells which are no longer sensitive to CRABPI-free RA, or they may benefit from certain facilitating effects of CRABPI-free RA in the culture.

Figure 3.

T3-induced chromatin remodeling of the mouse *Crabp1* gene regulatory region. Middle panel: In untreated precursor cells, the regulatory region is assembled into a linear evenly spaced nucleosome array. The TRE and GC boxes span N1 through N5. Upper panel: T3 treatment before differentiation (in precursor cells) triggers nucleosomes sliding from the center where N3 is assembled toward both up- and down-stream regions due to formation of a chromatin fold between the TRE (N5) and Sp1 (N1) sites (see Fig. 4). This also disassembles N1, leaving TIS in an open conformation to activate this gene (heavy arrow). Lower panel: T3 treatment post-differentiation (in committed and terminally differentiated adipocytes) triggers further sliding of nucleosomes as a result of compacting of the chromatin fold (see Fig. 4). N1 reassembles to cover TIS, suppressing this gene expression (indicated by a cross) in terminally differentiated adipocytes.

Figure 4.

The model of bimodal chromatin remodeling on the *Crabp1* regulatory region. Upper panel depicts linear arrangement of this chromatin segment flanked by TRE and GC box. Upon T3 treatment, this segment forms a fold anchored by TRAP220-TR-Sp1 complex that binds to both TRE and GC box. Lower panel depicts the models of T3-induced chromatin fold in pre-differentiation phase (left, activation) and post-differentiation phase (right, repression). The formation of this chromatin fold requires either TRAP220 (during pre-differentiation) or RIP140 (during post-differentiation). The fold in the repression phase (post-differentiation) is more compact than that in the activation phase (pre-differentiation), and may become heterochromatinized.