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Induction of histone acetylation on the CRBPII gene in perinatal rat small intestine

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Abstract

The expression of genes associated with lipid and vitamin A metabolism is elevated when the small intestinal mucosa is maturing rapidly during the perinatal period. We have previously reported that cellular retinol-binding protein type II (CRBPII) mRNA levels rise abruptly in the rat small intestine during this period. In this study, we examined whether the acetylation of histones H3 and H4 is involved in the intestinal expression of CRBPII during the perinatal stage. The expression of cyclin D1 and cyclin B1 genes, which are markers of cell proliferation, decreased markedly during the perinatal period, whereas expression of CRBPII as well as villin, a marker of intestinal maturation, increased rapidly. Using a ChIP assay, we showed rapid induction of acetylation of the histones H3 and H4 which interacted with the promoter/enhancer region of the CRBPII gene at this time. The binding of CBP and p300, which have histone acetyltransferase activity, as well as binding of retinoid X receptor α (RXRα) increased on the CRBPII promoter/enhancer region during the perinatal period. These results suggest that CRBPII gene expression during the perinatal period is associated with abrupt acetylation of histones H3 and H4 followed by the binding of CBP/p300 and RXRα.

Keywords: Histone acetylation; CRBPII; CBP/p300; Intestine; Development

1. Introduction

During the postnatal period when rat pups are nourished by their mother's milk, the small intestine undergoes rapid differentiation and morphological change [1]. Expression of several genes, including those associated with fatty acid metabolism, is known to be elevated in the small intestine during this perinatal period [2,3]. The expression of several structural proteins including villin, a marker of intestinal maturation and differentiation, also increases during this period [4]. These results indicate that the small intestine matures and adapts to nutrients after birth. In our previous study, we demonstrated that expression of CRBPII, which is a retinol and retinal binding protein which may play an important role in intestinal vitamin A transport and metabolism [5–7], is elevated during the perinatal period [8].

Recent studies have suggested that an abrupt change in gene expression, which accompanies maturation and differentiation of myeloid cells, is regulated by histone modifications such as acetylation, methylation and phosphorylation [9]. In particular, the acetylation of histones H3 and H4 is thought to play a central role in the regulation of transcription, because hyperacetylation of histones H3 and H4 is associated with the euchromatin region of the genome [10]. Recent studies have demonstrated that the acetylation of histones H3 and H4 on the promoter/enhancer region of genes induces the binding of transcriptional machinery including coactivators [11], the SWI/SNF complex [12], nuclear receptors [13], and RNA polymerase II, on their target genes, and that the acetylation of histones H3 and H4 is accompanied by changes in gene expression and tissue differentiation during the developmental stage [14].

Previous studies have suggested that the expression of the CRBPII gene is regulated by the RXR dimer with PPARα, a subtype of a nuclear receptor for fatty acids and prostanoids, through fatty acids [15,16], and with the nuclear receptors for

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retinoic acid [17] i.e., RAR and RXR. Our own studies have previously shown that p300, a coactivator known to act as histone acetyltransferase, is expressed in the small intestine, and that p300 strongly activates PPAR signaling [18]. Because RXRα, RARα and RARβ are detectable during the perinatal period [8], it is likely that the signaling of retinoic acid and nuclear receptors is involved in the expression of the CRBPII gene during the perinatal period through recruitment of RXR and CBP/p300 triggered by acetylation of histones H3 and H4. However, it is unclear whether the acetylation of histones H3 and H4 on the CRBPII gene, as well as recruitment of RXR and CBP/p300 is modulated during the perinatal period.

In this study, we focused on the acetylation status of histones H3 and H4 as well as the binding of CBP, p300, RXR and RNA polymerase on the promoter/enhancer region of the CRBPII gene during the perinatal period. The results of this study provide the first evidence that an abrupt change in gene expression in the small intestine during the perinatal period is accompanied not only by recruitment of RXR, CBP/p300 and RNA polymerase, but also by acetylation of histones H3 and H4.

2. Materials and methods

2.1. Animals

Pregnant Sprague–Dawley rats were obtained from Japan SLC (Hamamatsu, Japan). Rats were obtained at different stages of pregnancy for collection of samples from embryos and pups at different developmental stages; rats carrying 11-day embryos (E11) were obtained for collection of samples at E16. Rats carrying 15-day embryos were obtained for collection of samples at E19. Rats carrying 17-day embryos were obtained for collection of samples from 0-day pups (defined as the day when pups were born), 1-day pups (defined as the day after pups were born) and 3-day pups. The pregnant rats were kept in our own labs between purchase and killing. After pups were born, they were kept with their dams and their dams were given free access to water and a standard laboratory chow diet (MF, Oriental Yeast, Tokyo, Japan) throughout the experimental period. All rats were housed under a light/dark cycle with 12-h darkness from 19:00 to 07:00. Twelve rat embryos at the age of E16 and a further 12 at E19 were harvested from their dams between 10:00 and 12:00. Six rat pups at each of ages 0, 1 and 3 days after birth were killed by decapitation between 10:00 and 12:00. The experimental procedures used in the present study met the guidelines of the animal usage committee of the University of Shizuoka.

2.2. Quantitative RT-PCR analysis

The entire small intestine was flushed with ice-cold 0.9% NaCl solution. A portion (100 mg) of the small intestine was used for total RNA extraction. Total RNA was isolated using TRIzol reagent (Invitrogen, Tokyo, Japan) according to the manufacturer's protocol. Total RNA (1 μg) was subjected to reverse transcription using Superscript II reverse transcriptase (Invitrogen). CRBPII, β-actin, histone H3, histone H4, cyclin D1, cyclin B1, villin, CBP, p300 and 18S rRNA cDNAs were amplified by real time RT-PCR in a Light Cycler system (Roche Diagnostic Co., Tokyo, Japan) using the SYBR Green Kit (Takara, Tokyo, Japan). The sequences of the primers are shown in Table 1. Amplification conditions were as follows: pre-activation at 95 °C for 15 min, denaturation at 94 °C for 15 s, annealing at 62 °C for 25 s, and extension at 72 °C for 10 s. The products of each gene amplified by real-time RT-PCR were confirmed to be single band and specific using a melting curve, which indicates the temperature at which PCR products re-anneal after double strand DNA products release single strands at high temperature (95 °C). We also confirmed that the products amplified from all primer sets were not amplified without cDNA from total RNA of Caco-2 cells. The CT-value of each gene calculated by real-time RT-PCR was converted to a signal intensity by the delta-run method, which assumes that the difference of 1 cycle between samples produces a 2-fold difference. The signals of each gene were then normalized to 18S rRNA signals and were converted to relative ratios against the signal of each gene at E16. The CT-values at 3 days after birth were 18.6 for CRBPII, 29.9 for villin, 21.0 for histone H3, 22.4 for histone H3, 24.5 for cyclin D1, 27.2 for cyclin B1, 16.7 for β-actin, 23.9 for CBP and 24.4 for p300.

2.3. The chromatin immunoprecipitation (ChIP) assays

The mucosa removed from the entire small intestine for ChIP assays was pooled for the twelve rat embryos at each of the gestational sampling times (E16, E19). Similarly, mucosa from the six rat pups at 0, 1 and 3 days after birth was pooled for each day. The mucosa samples were incubated with a fixation solution (1% formaldehyde, 4.5 mM HEPES pH 8.0, 9 mM NaCl, 0.09 mM EDTA,

Table 1
Primers used for real time RT-PCR and amplification of DNAs after chromatin immunoprecipitation

Gene	Forward (5′–3′)	Reverse (5′–3′)	Accession no.
rCyclinB1	atcagttaaccgaaacaaaagctc	ccaattctcatgcagtatgctatc	NM_171991
rCyclinD1	aggggatgtgagagaagaagtatg	atgaaagatacaaagcaacgtgaa	NM_171992
rp300	caacagaatagccctggattaagt	atcatacctgctgtggactgagta	AB066220
rCBP	catgaatgctaacttcaaccagac	ttgagcctgattcattaagctatg	AB066219
rβ-actin	gtaaagacctctatgccaacacagt	atgatcttgatcttcatggtgcta	NM_031144
rCRBPII	gttccgcaactatgacctagattt	tcacttctttttgaacacttgttcg	NP_036772
r18s	gaacgcgtgcatttatcaga	gatcggcccgaggttatcta	X01117
rVillin	caacttctatgagggagactgctac	tagtcatccagctgtgtggtataga	NM_024401
rHistone H3	gtaaagcacccaggaaacaact	gtttcttcacccctccagtaga	BC086580
rHistone H4	atgtctggccgaggcaaa	gttgtcgcgcaggacttt	AY936209
rSI −100/+100	gccttacttacaaaaacatctccac	tgctgttactatgatgaagaggaca	
rSI −300/−100	acatttaaattccccttcacttttc	taaagacaaggaagtcaaggatttg	
rSI −700/−500	ttttgttgaaaaatacagggatactc	tttgttctgctagcaacaattaaata	
rSI −900/−700	tacttttttctaatggcagaggaaca	agttagagcaagctagaacaatatgc	
rCRBPII −100/+100	gcagtaacgtttaacacagtctcc	ccttcatgtagccttcaaagttct	
rCRBPII −300/−100	gagtccagtcagcctaaaatgag	aagttcaacaggtctgtaggcagt	
rCRBPII −700/−500	ctgtcttttacaagtcaccattgc	taacagactcccctacacacacac	
rCRBPII −1100/−900	acaattaaccgaccttcttgtctc	cttgcatgcatgaatataaggaaa	