Induction of the BCMO1 Gene during the Suckling–Weaning Transition in Rats Is Associated with Histone H3 K4 Methylation and Subsequent Coactivator Binding and Histone H3 Acetylation to the Gene

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Summary The cells involved in nutrient absorption in the small intestine of rats undergo rapid maturation during the suckling–weaning transition period, i.e., 2–4 wk after birth. During this period, the serum thyroid hormone level is increased. However, the molecular mechanisms involved in the regulation of β-carotene 15,15′-monooxygenase 1 (BCMO1) gene expression in the small intestine remain unknown. In this study, we found that jejunal β-carotene 15,15′ dioxygenase activity and the gene expression of BCMO1 were significantly increased during this transition period between days 13 and 27 after birth. A chromatin immunoprecipitation assay revealed that di- and tri-methylation of histone H3 at lysine 4 (K4) and the binding of thyroid hormone receptor (TR) α-1 binding on the promoter/enhancer and/or transcribed regions of the BCMO1 gene were enhanced from the earlier stage of weaning (i.e., 20 d after birth), prior to an enhancement of the acetylation of histone H3 and the binding of coactivator (SRC-1 and CBP) to the promoter/enhancer and/or transcribed regions of the BCMO1 gene, which was apparent at 27 d after birth. These results suggest that histone H3 K4 methylation and TRα-1 binding on the BCMO1 gene during the suckling–weaning transient period in rats predisposes to subsequent coactivator recruitment and histone H3 acetylation on the gene.

Key Words histone modification, TRα-1, BCMO1, postnatal development, jejunum

β-Carotene is a precursor of vitamin A that is cleaved into two retinal molecules by β-carotene 15,15′ dioxygenase (EC 1.13.11.21), which was cloned as β-carotene 15,15′-monooxygenase 1 (BCMO1) (1–3). β-Carotene is absorbed via the small intestine and is broken down into retinal by BCMO1 in rats. In mammals, several studies have reported the presence of the activity of β-carotene 15,15′ dioxygenase and the mRNA expression of the BCMO1 gene in the small intestine as well as in other tissues such as the liver, lung, kidney, brain and testis (4–9). It has been reported that BCMO1 gene expressions in the small intestine, lung and testis are down-regulated by retinoic acid (RA) (1–3). However, the molecular mechanisms involved in the induction of the BCMO1 gene in the small intestine during developmental periods remain unknown.

A recent study has demonstrated that BCMO1 mRNA is upregulated by thyroid hormone in human intestinal Caco-2 BB1 cells (10), but it is unclear whether this is also true in vivo. It is known that the serum concentrations of thyroid hormones such as l-triiodothyronine (T3) and l-tetraiodothyronine (thyroxine, T4) increase during the suckling–weaning transition period from 13 to 27 d after birth (11). Thyroid hormones transmit their signals via the nuclear receptor thyroid hormone receptor (TR), and recent studies have shown that the TRα subtype, but not the TRβ subtype, is important for intestinal maturation (12–15). TRα has since been shown to have two alternative splicing variants, TRα-1 and TRα-2. TRα-1 has high affinity for T3, and its target genes are upregulated by binding of TRα-1 with T3 to the promoter region of the target genes (16). We have recently demonstrated that the expression of other thyroid hormone-responsive genes, including hexose transporters (GLUT5, SGLT1 and GLUT2), is increased during this period, and that administration of T3 between days 12 and 17 and between days 16 and 21, but not between days 22 and 27, upregulated these genes. We also found that TRα-1, but not TRα-2, showed a transient increase in expression during this period (17). Furthermore, we demonstrated that T3 increased GLUT5 gene expression, enhanced the binding of TRα-1 to the cis-element thyroid hormone responsive element located on the promoter/enhancer region of the GLUT5, and increased its transactivity in intestinal Caco-2 cells (18). These
results suggest that the transient induction of TRα-1 along with an elevated serum T3 level in the small intestine during the suckling–weaning transition period is involved in the regulation of thyroid hormone-responsive genes during this period. Considering that β-carotene is provided from the diet after weaning, the activity and mRNA levels of BCMO1 would increase during this transition period, and it is likely that TRα-1 is involved in the induction.

In terms of the signals mediated by nuclear receptors including TRα-1, recent studies have demonstrated that nuclear receptors enhance the expression of their target genes by binding to histone acetyltransferase (HAT), which catalyzes histone acetylation. Many HATs have been identified, including general control of amino acid synthesis (GCN5), p300/CBP-associated factor (PCAF) and CREB binding protein (CBP). Among these HATs, CBP is responsible for ligand-dependent transcriptional control by nuclear receptors (19). In addition, steroid receptor coactivator (SRC)-1, which is known to bind nuclear receptors and CBP (20), is important to transmit the signals of thyroid hormones, since SRC-1 binds TRα-1 in the presence of the T3, and the expression is induced by T3 signals (21). The results indicate that CBP and SRC-1, which are frequently termed as the coactivators, may be used as molecules for intermediating signals of TRα-1 for the intestinal genes, but it is still to be elucidated. The complexes formed by CBP and SRC-1, along with nuclear hormone receptors including TRα-1, induce the acetylation of histones H3 and H4 on the promoter/enhancer region of various genes. The acetylation of histones induces the binding of transcriptional machinery, consisting of SWI/SNF complex, transcriptional factors, and RNA polymerase II on their target genes. The acetylation of histone H3 and H4 is accompanied by changes in gene expression during development (22, 23). Moreover, recent studies have demonstrated that mono-, di- or tri-methylation of histone H3 at lysine residue 4 (K4) is important for the initial activation of transcription, because di-/tri-methylation of histone H3 K4 is induced prior to the acetylation of histones and transactivation of genes; methylation of histone H3 K4 induces acetylation of histones (24, 25). Accordingly, methylation of histone H3 K4 facilitates subsequent histone acetylation by the CBP (24, 26). In addition, several studies have indicated that tri-methylation of histone H3 K4 induces the recruitment of mRNA transcription and elongation complexes onto target genes. This occurs through the binding of proteins containing plant homeodomains (PHD), chromodomains, Tudor domains or WD40 repeat domains to methylated histones (27, 28). However, it is still unknown whether a thyroid hormone-responsible gene in the small intestine is regulated by histone H3 K4 methylation, histone H3 acetylation or coactivator binding followed by the TR binding.

Based on these earlier findings, we hypothesized that the activity and mRNA expression of BCMO1 are increased during the suckling–weaning transition period, and that these events involve TRα-1, SRC-1 and CBP bindings and histone H3 acetylation and histone H3 K4 methylation on the promoter/enhancer and transcribed regions.

MATERIALS AND METHODS

Animals. Sprague-Dawley pregnant rats carrying 10-d embryos were obtained from Japan SLC, Inc. (Hamamatsu, Japan). After the pups were born, they were kept with their mothers, and both mothers and pups were given free access to a standard laboratory chow diet (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) throughout the experimental period. To examine jejunal enzyme activity during the developmental period, we collected samples as follows. Fetuses were collected on embryonic day 17 (4 d before birth) and day 19 (2 d before birth). Rat pups of both sexes, were killed at the ages of 0, 3, 7, 14, 17, 21 and 28 d between 13:00 and 14:00. In another experiment, pups were killed at the age of 5 (suckling period), 13, 20 and 27 d after birth in order to examine jejunal gene expression. The experimental procedures used in the present study met the guidelines of the Animal Usage Committee of the University of Shizuoka.

Preparation of intestinal samples. The entire small intestine was removed and the jejunoileum was divided into two segments of equal length. The proximal half of the jejunoileum (regarded as the jejunum in the present study) was flushed with ice-cold 0.9% NaCl solution. Then, 300–500 mg of tissue was homogenized in two volumes (vol/wt) of ice-cold 50 mM N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (Hepes)-KOH buffer solution (pH 7.4) containing 1.15% KCl, 1 mM ethylenediaminetetraacetic acid (EDTA) and 0.1 mM dithiothreitol (DTT) using a Teflon-glass homogenizer. After centrifugation of the homogenates at 10,000 g for 30 min at 4°C, the resulting supernatant was centrifuged at 105,000 g for 1 h at 4°C. The cytosolic supernatant was then applied to a Sephadex G-25 column (PD-10, GE Healthcare, Tokyo) equilibrated with 10 mM Hepes-KOH buffer, pH 7.4, containing 100 mM EDTA, 50 mM KCl and 0.1 mM DTT. The protein fraction eluted in the void volume was divided into 0.5-mL aliquots to determine enzyme activity. The protein concentration was measured using Lowry’s method (29).

In another experiment, a 1-cm segment (100 mg) was excised from the middle region of the jejunal loop and immediately used for RNA extraction. The remaining part of the jejunal loop was used for chromatin immunoprecipitation (ChIP) assays.

β-Carotene 15,15′ dioxygenase activity assay. β-Carotene 15,15′ dioxygenase activity was determined using the method described by Tajima et al. (30), with some modifications. Cytosolic protein (0.5 mg) was first pre-incubated at 37°C for 10 min in the presence of 0.1 mM N-Tris-(hydroxymethyl) methylglycine (Tricine)-KOH buffer, pH 8.0, containing 6 mM cholic acid and 0.5 mM DTT. The enzyme reaction was started by the addition of 80 μL of substrate solution containing 75 μM all-trans β-carotene, 0.1 mM α-tocopherol and 0.45% Tween-40 in a total volume of 0.4 mL. The reac-
The addition of 100 mL of butylated hydroxytoluene (BHT). The resulting mixture was centrifuged at 7000 rpm for 10 min to obtain a clear hexane phase. The total hexane phase was then combined and evaporated to dryness under nitrogen. The residue was dissolved in 50 mL of methanol for high-performance liquid chromatography (HPLC). The HPLC analysis was performed according to Tajima et al. (33).

The reaction mixture was incubated under dim red light for 1 h at 37°C. The reaction was stopped by adding 0.4 mL of methanol. The o-ethyloxide derivative was prepared essentially according to van Kuijk et al. (31). Briefly, 100 μL of 2.5 mM o-ethylhydroxylamine hydrochloride in 0.5 M potassium phosphate buffer, pH 6.5, followed by the addition of 100 μL of methanol containing 125 μg/mL cholesterol. After incubation for 10 min at 25°C, a known amount of purified retinol acetate was added as an internal standard, and the products including retinal (o-ethyl) oxime were extracted twice from the reaction mixture into n-hexane containing 100 μg/mL butylated hydroxytoluene (BHT). The resulting mixture was centrifuged at 700 × g for 10 min to obtain a clear hexane phase. The total hexane phase was then combined and evaporated to dryness under nitrogen. The residue was dissolved in 50 μL of methanol for high-performance liquid chromatography (HPLC). The HPLC analysis was performed according to Tajima et al. (30). The retinal (o-ethyl) oxime represented approximately 100% of the total o-ethyl-oximes produced by the β-carotene 15,15′-dioxygenase.

Quantitative RT-PCR analysis. Total RNA was extracted by the acidified guanidine thiocyanate method, as described by Chomczynski and Sacchi (32). The RNA samples (1.5 μg) were converted into cDNA using SuperScript™ II reverse transcriptase (Invitrogen, Tokyo, Japan) according to the manufacturer’s instructions. To quantify the mRNA levels of each gene, real-time PCR amplification was performed with a Light-Cycler system (Roche Molecular Biochemicals, Tokyo, Japan). Amplifications were carried out in a total volume of 10 μL containing 10 μM each of the gene-specific primers, cDNA and SYBR Premix Ex Taq (Takara, Shiga, Japan) with the following conditions: pre-activation at 95°C for 5 min, denaturation at 95°C for 10 s, annealing and extension at 65°C for 10 s, and extension at 72°C for 10 s. The cycle threshold (CT) values of each gene and 18S rRNA detected by real-time RT-PCR were converted to signal intensities by the delta-delta method. This method calculates the difference of 1 CT value as a 2-fold difference between the signal for each gene and the signal for a gene for normalization (18S rRNA) using the formula [2′CT for each gene − CT for 18S rRNA]. The primer sequences are listed in Table 1.

Chromatin immunoprecipitation (ChIP) assays. The mucosa was removed from the jejunum for the ChIP assays were fixed with 1% formaldehyde and sonicated as previously described (33). ChIP assays were performed, as previously described (33), using 1 μg of anti-acetyl-histone H3 (Millipore, Tokyo, Japan), anti-acetyl-histone H4 (Millipore), anti-trimethyl-histone H3 and H4 (Abcam), anti-dimethyl-histone H3 K4 (Abcam), anti-trimethyl-histone H3 K4 (Millipore), anti-TRα1 (Santa Cruz Biotechnology, CA, USA), anti-SRC-1 (Millipore) and anti-CBP (Santa Cruz Biotechnology) antibodies or normal rabbit IgG. The sequences of the primer sets are listed in Table 1. The size of the PCR products amplified by each primer set in PCR and ChIP assays ranged from 50 to 150 bp.

Table 1. The sequences of oligonucleotide primers used in this study.

| Region on the BCMO1 gene | Sequence                                                                                     | Accession No. |
|--------------------------|---------------------------------------------------------------------------------------------|---------------|
| BCMO1 − 9,000            | 5′-GGCTGCTCCTGGAACCTTGTTG-3′                                                               | NM_053648.2   |
| BCMO1 − 3,000            | 5′-CTTGTTAGTCGCCGTTGCC-3′                                                                 | X01117        |
| BCMO1 − 1,000            | 5′-ACTCTTGATTGCTGCTGTTG-3′                                                                 |               |
| BCMO1 − 500              | 5′-CATATTACAGGCGTCTCCT-3′                                                                   |               |
| BCMO1 + 1                | 5′-ACAGATGGAGGAGATCAAGGA-3′                                                                 |               |
| BCMO1 + 500              | 5′-TGGATGTGTTCTTTCCCACC-3′                                                                  |               |
| BCMO1 + 2,000            | 5′-TCAGTTTTGTGATGGATCTGG-3′                                                                 |               |
| BCMO1 + 5,000            | 5′-TACTTGGCTGCTCCACCTGGTGT-3′                                                              |               |
| BCMO1 over 4,000         | 5′-GGGAAGCTGGAGAAGAGT-3′                                                                    |               |
| BCMO1 over 6,000         | 5′-TTTGGATGGACATGGAGTAAG-3′                                                                 |               |
| BCMO1 mRNA               | 5′-CCGCAAGACCCATTGATACT-3′                                                                 |               |
| 18S rRNA                 | 5′-CTTGTTAGTCGCCGTTGCC-3′                                                                   |               |
RESULTS

Changes in \( \beta \)-carotene 15,15’ dioxygenase activity and BCMO1 gene expression in the rat jejunum during the prenatal and postnatal periods

We examined the changes in \( \beta \)-carotene 15,15’ dioxygenase activity in developing rats. Jejunal \( \beta \)-carotene 15,15’ dioxygenase activity was low between the perinatal and the suckling period, but started to increase from 17 d after birth. By 21 and 28 d after birth, its activity was 6.4-fold \((p<0.01)\) and 27-fold \((p<0.01)\), respectively, greater than the baseline level observed between days −4 and 14 (Fig. 1A). To investigate whether BCMO1 gene expression is associated with the induced
activity of β-carotene 15,15′ dioxygenase during the suckling–weaning transition, we performed real-time RT-PCR to determine the mRNA level of BCMO1. Jejunal BCMO1 mRNA expression was low during the suckling period between postnatal days 5 and 13. However, its expression gradually increased during the weaning period, and was 4.7-fold (\(p<0.05\)) and 8.8-fold (\(p<0.05\)) greater on days 20 and 27, respectively, than that on day 5 (Fig. 1B).

Changes in the mono-, di- and tri-methylation of histone H3 K4 on the promoter/enhancer and transcribed regions of the jejunal BCMO1 gene in rats during the suckling–weaning transition

To investigate whether the mono-, di- and tri-methylation of histone H3 K4 on the rat BCMO1 gene change during the suckling–weaning transition, we performed ChIP assays using their antibodies in the jejunum of developing rats (5–27 d after birth). We used primer sets targeting specific regions of the BCMO1 gene to detect the ChIP signals (Fig. 2A). The ChIP signals detected for control IgG were less than 0.009% per input at all regions of the BCMO1 gene (data not shown). The ChIP signals of dimethylated histone H3 K4 on the promoter/enhancer were elevated by 3.3-fold (\(p<0.05\)) and 2.4-fold (\(p<0.05\)) at 27 d after birth (Fig. 2B). The ChIP signals for trimethylated histone H3 K4 were significantly greater on the transcription region (+500) of the BCMO1 gene both at 20 d (4.3-fold, \(p<0.05\)) and at 27 d (6.7-fold, \(p<0.05\)) than that at 5 d after birth (Fig. 2D).

Changes in the acetylation of histones H3 and H4 on the promoter/enhancer and transcribed regions of the jejunal BCMO1 gene in rats during the suckling–weaning transition

The acetylation levels of histone H3 on the transcription regions (+500 and +2,000 bp) of BCMO1 gene were elevated by 3.3-fold (\(p<0.05\)) and 2.4-fold (\(p<0.05\)), respectively, at 27 d, compared with those at 5 d after birth (Fig. 3A). The acetylation of histone H4 on the transcription regions tended to increase over time, but remained low between 13 and 27 d after birth (Fig. 3B).

Changes in the binding of TRα-1, SRC-1 and CBP to the BCMO1 gene in rat jejunum during the suckling–weaning transition

To investigate whether TRα-1 is recruited to the rat BCMO1 promoter/enhancer region during the suckling–weaning transition, we performed ChIP assays using an antibody for TRα-1. The ChIP signals for TRα-1 on the rat BCMO1 promoter/enhancer region (−1,000 and −500 bp upstream of the transcription start site) were increased between 13 and 20 d of age, and the signals at −1,000 bp and −500 bp at 20 d of age were 3.7-fold (\(p<0.05\)) and 4.0-fold (\(p<0.05\)), respectively, greater than that observed at 5 d after birth (Fig. 4A). We also examined whether the binding of SRC-1 and CBP to the BCMO1 promoter/enhancer regions are altered during the suckling–weaning transition period. The ChIP sig-
nal for SRC-1 binding was significantly greater on the enhancer region (~500 bp) of the BCMO1 gene at 27 d (4.0-fold, p<0.05) than at 5 d after birth (Fig. 4B). Similarly, the binding of CBP to the upstream region of the BCMO1 gene tended to increase postnatally, and the signal for CBP binding at ~500 bp was significantly greater (3.0-fold, p<0.05) at 27 d than at 5 d after birth (Fig. 4C).

**DISCUSSION**

In this study, we first demonstrated that the activity and mRNA expression of BCMO1 increased during the suckling–weaning transition period between days 13 and 27 after birth, which is the period that serum thyroid hormones are elevated (11). Interestingly, we found that TRα-1 binding to the promoter/enhancer region of BCMO1 gene was increased between days 13 and 27, and these signals on day 20 were significantly greater than the other days. We previously reported that the expression of TRα-1 increased during the suckling–weaning transition, particularly between postnatal days 13 and 20, a time when the serum T3 concentration is greatly increased, and that the expression of TRα-1 declined subsequently by the end of weaning (i.e., day 27) (17). This study is the first that demonstrates that an ontogenetic induction of intestinal genes during the suckling–weaning transient period is directly regulated by TRα-1 binding to the promoter/enhancer region. However, typical sequences of the thyroid hormone responsive elements were not found around the promoter/enhancer region, i.e., between −1,000 bp and −500 bp, of the rat BCMO1 gene. It is known that thyroid hormone responsive elements are variable. Thus, it should be examined in further experiments employing DNase I foot printing and reporter assays whether thyroid hormone responsive elements are located on the promoter/enhancer region of the rat BCMO1 gene.

In addition, it is possible that other transcriptional factors are concerned with jejunal induction of the BCMO1 gene during the suckling–weaning transient period. It has been reported that BCMO1 gene expression in the small intestine, lung and testis was down-regulated by retinoic acid (RA) (1–3). In addition, a previous study has demonstrated that the peroxisome proliferator-activated receptor (PPAR) response element is located upstream of the mouse BCMO1 gene, and that PPARγ, one of the PPAR subtypes, isolated from the nuclear extract of the colon cell line TC7 cells, is bound to the PPRE (34). Previous studies have also demonstrated that jejunal expression of genes related to nutrient absorption such as cellular retinol-binding protein, type II (CRBPII) and liver-type fatty-acid binding protein (L-FABP) are regulated by another subtype of PPAR, i.e., PPARα (35). During the postnatal period, the PPARα gene is highly expressed during the suckling–weaning transient period from 13 to 20 d after birth (36). Thus, PPARα may be also concerned with the induction of the jejunal BCMO1 gene confined to the period between days 13 and 20 after birth. Further studies should examine whether PPARs as well as the nuclear receptors for retinoids such as RAR and RXR are involved in the induction of the BCMO1 gene during the suckling–weaning transient period.

In this study, we found that di- and tri-methylation of histone H3 K4 on the promoter/enhancer and transcribed regions of the BCMO1 gene increased remarkably from day 13 to 27. It should be noted that a significant increase in di- and tri-methylation of histone
H3 K4 was observed as early as on day 20, prior to enhancements of the acetylation of histone H3 on the transcribed regions and the binding of coactivators (SRC-1 and CBP) on the promoter/enhancer regions of the BCMO1 gene. Recent studies have demonstrated that methylation of histone H3 K4 facilitates subsequent histone acetylation, which is directed by CBP (24, 26). Thus, it is likely that the induction of coactivator bindings and histone H3 acetylation on the BCMO1 gene is caused by increased histone H3 K4 methylations. Several nuclear proteins containing PHD, chromodomains, Tudor domains or WD40 repeat domains have been shown to bind to methylated histones and recruit histone acetyl-transferase including CBP (27, 28). In addition, histone methyl-transferases such as Set proteins and MLL proteins are capable of methylating histone H3 K4 (24, 28). Further studies should identify the histone H3 methyltransferase responsible for the BCMO1 gene induction and the binding proteins for methylated histone H3 K4 on the BCMO1 gene responsible for transmitting signal of the histone H3 K4 methylation to that of histone H3 acetylation.

It should be noted that the enhanced binding of TRα-1 to the BCMO1 gene was diminished by day 27, although the mRNA levels of the BCMO1 gene as well as histone H3 acetylation and histone H3 K4 methylations were continuously elevated to a maximal level attained although the mRNA levels of the BCMO1 gene as well as and the histone H3 K4 methylation on the BCMO1 gene were continuously elevated to a maximal level attained although the mRNA levels of the BCMO1 gene as well as histone H3 K4 methylations. Several nuclear proteins containing PHD, chromodomains, Tudor domains or WD40 repeat domains have been shown to bind to methylated histones and recruit histone acetyl-transferase including CBP (27, 28). In addition, histone methyl-transferases such as SET proteins and MLL proteins are capable of methylating histone H3 K4 (24, 28). Further studies should identify the histone H3 methyltransferase responsible for the BCMO1 gene induction and the binding proteins for methylated histone H3 K4 on the BCMO1 gene responsible for transmitting signal of the histone H3 K4 methylation to that of histone H3 acetylation.

It should be noted that the enhanced binding of TRα-1 to the BCMO1 gene was diminished by day 27, although the mRNA levels of the BCMO1 gene as well as histone H3 acetylation and histone H3 K4 methylations were continuously elevated to a maximal level attained on day 27. This result suggests that TRα-1 binding to the BCMO1 gene is prerequisite to the induction of the BCMO1 gene during the suckling–weaning transition period (i.e., between days 13 and 20), but is not required for sustaining a maximal level of gene expression. We found in this study that not only histone H3 acetylation, but also histone H3 K4 di- and tri-methylations are enhanced in the transcribed region (+1, +500, and +2,000) during the suckling–weaning transition period. Previous studies have demonstrated that methylation of H3 K4 is important for the initial activation of transcription because di-/tri-methylation of histone H3 K4 is induced prior to the acetylation of histones and transactivation of genes. Indeed, methylation of histone H3 K4 induces acetylation of histones (24, 25). Acetylation of histones leads to the recruitment of transcription complexes onto the promoter/enhancer region and mRNA elongation complexes to the transcribed region close to the transcription initiation site through binding of bromodomain-containing proteins to acetylated histones (22, 23, 37). Thus, histone H3 K4 methylation is likely to induce histone acetylation and subsequent binding of transcriptional complexes and RNA polymerase II onto the BCMO1 gene. It should be examined in further studies whether the bindings of transcriptional complexes and RNA polymerase II onto the jejunal BCMO1 gene are altered during the suckling–weaning period.

In conclusion, we have demonstrated for the first time in this study that TRα-1 is directly bound to the BCMO1 gene and the in vivo binding is associated with the induction of the BCMO1 gene during the suckling–weaning transition period, and that the TRα-1 binding and the histone H3 K4 methylation on the BCMO1 gene are followed by enhanced coactivator binding and histone H3 acetylation on the promoter/enhancer and/or transcribed regions of the gene.

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