Transcriptional Regulation of Tal2 Gene by All-trans Retinoic Acid (atRA) in P19 Cells

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TAL2 is a transcription factor required in the normal development of mouse brain. In a previous study, we demonstrated that the expression of Tal2 gene is induced by the complex of all-trans retinoic acid (atRA) and retinoic acid receptor α (RARα) in mouse embryonal carcinoma P19 cells. atRA is also known to be important in inducing P19 cells to differentiate into the neural lineage. Therefore, we believe that the function of TAL2 in neural differentiation may be clarified by utilizing P19 cells. As the atRA-RARα complex induced the expression of Tal2, we focused on the regulatory region that is involved in its transcription. The atRA-RARα complex occupies a characteristic retinoic acid response element (RARE) located in the promoter of target genes. Therefore, we searched for RARE on the mouse Tal2 and found that a RARE-like element was located in the intron. We also found that a TATA-box-like element was located in the 5′-region of Tal2. Involvement between transcriptional activity and the TATA-box-like element was confirmed in the luciferase assay, and TATA-box binding protein was bound to this element upstream of Tal2 in P19 cells. atRA signaling activated the transcription through the RARE-like element, and RARs was bound to this element on Tal2 in P19 cells. In addition, the interaction between these elements on Tal2 was shown in the chromatin immunoprecipitation assay. These results suggest that the transcription of Tal2 is coordinately mediated by two distal regulatory elements.

Key words Tal2 gene; retinoic acid receptor α (RARα); TATA-box binding protein (TBP); retinoic acid response element (RARE); TATA-box

TAL2 is a transcription factor required for the normal development of mouse brain. The expression of Tal2 gene is observed in the diencephalon, mesencephalon, and metencephalon of the developing mouse brain. Tal2-null mutant mice are viable at birth and initially appear normal. However, they develop signs of running and die between 13 and 32 d after birth. Therefore, Tal2 is thought to play a pivotal role in development of the brain.

We found that Tal2 expression is altered in P19 cells after addition of all-trans retinoic acid (atRA) and suspension culture for cell aggregation. Moreover, we showed that its expression is induced by atRA. P19 cells are a line of pluripotent embryonal carcinoma and appear to differentiate into derivatives of three germ layers—endoderm, mesoderm, or ectoderm—depending on the inducers and culture conditions, using the same mechanisms as normal embryonic cells. atRA treatment and cell aggregation have an important role in the induction of neural differentiation in P19 cells.6–8 Because these cells have been used as a model in studies of neural differentiation, we believe that the function of Tal2 in development may be clarified by utilizing P19 cells.

atRA, which is a metabolite product of vitamin A, is one of the most important morphogens, and is a signal molecule involved in neural differentiation.9–11 atRA is also known to be capable of inducing embryonic stem cells and embryonal carcinoma cells to differentiate into neural lineages.12,13 atRA functions as the activating ligand for retinoic acid receptors (RARs), and atRA-RAR complexes regulate the expression of over 500 target genes as a transcription factor.14–16 RARs are a member of the nuclear receptor superfamily, and consist of three isotypes: α, β, and γ. In response to atRA signaling, RARs occupy characteristic retinoic acid response elements (RAREs) located in the promoter regions of target genes. RAREs are classically described as direct repeats of the hexameric motif (A/G)G(G/T)TCA separated by 1, 2, or 5 nucleotides (referred to as DR1, DR2, or DR5, respectively).19–21 Recently, the recurrent motif (A/G)G(G/T)G(C/A), which differs from the classical consensus motif (A/G)G(G/T)TCA at position 5, with a G instead of a C, was reported from the alignment of several RAREs.20

In a previous study, we also showed that RARα was involved in the induction of Tal2 in P19 cells after the addition of atRA and suspension culture for cell aggregation. As the atRA–RARα complex induced the expression of Tal2, we focused on the regulatory region involved in its transcription. Therefore, we searched for a RARE motif on Tal2 and found a RARE-like element in the intron of Tal2. Moreover, a TATA-box-like element, which might participate in transcription as core promoter recognized by basal transcriptional factors, was mapped in the 5′-region of Tal2. Subsequently, we investigated the relationship between the transcriptional activities and these elements, and the association between these elements and bound proteins. In addition, we examined the interaction between these elements on Tal2 in P19 cells.

MATERIALS AND METHODS

Cell Culture and Neural Differentiation P19C6, a sub-
clone of the P19 mouse embryonic carcinoma cell line, was used in this study. P19C6 was provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. P19 cells were cultured in α-MEM (Sigma-Aldrich, St. Louis, MO, U.S.A.) supplemented with 10% fetal bovine serum (FBS) (Life Technologies, Carlsbad, CA, U.S.A.) and 2 mM L-glutamine (Kanto Chemical, Tokyo, Japan). To induce neural differentiation, cells were aggregated in a suspension culture dish (SUMILON, Tokyo, Japan) at a seeding density of 2×10^5 cells/mL in the presence of 1 μM atRA (Sigma-Aldrich) dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich). DMSO concentration in culture condition was 0.01%.

5'-Rapid Amplification of cDNA Ends (RACE) of Tal2

RNAs were isolated from P19 cells according to the manufacturer’s instructions. To identify the 5’-ends of Tal2, the SMARTer RACE cDNA Amplification Kit (TaKaRa Bio, Shiga, Japan) was used according to the manufacturer’s instructions. The 5’-end of Tal2 was amplified by Ex taq (TaKaRa) and a mouse Tal2 gene-specific primer, 5'-AGAGGCTCACAATGACTGAATCTCTGGAAGGCTGGAACCAAGCTTT-3'. Subsequently, polymerase chain reaction (PCR) products were cloned into the pGEM-T Easy vector (Promega, Madison, WI, U.S.A.) for sequencing.

Western Blotting

P19 cells were treated with atRA for 0, 3, 6, 12 and 24 h in suspension culture. Treated cells were washed with phosphate buffered saline (PBS) and then harvested by centrifugation. Samples were resuspended in lysis buffer (62.5 mM Tris–HCl [pH 6.8], 10% glycerol, 2% sodium dodecyl sulfate (SDS), 0.05 mg/mL bromophenol blue) and boiled for 5 min. They were then separated by SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to an Immobilon-P transfer membrane (Merck Millipore, Darmstadt, Germany) in transfer buffer (25 mM Tris, 192 mM glycine, 10% methanol) using the Trans-Blot Turbo Transfer System (Bio-Rad, Hercules, CA, U.S.A.). After blocking with 5% skim milk in 0.1% TBS-T (10 mM Tris–HCl [pH 7.4], 150 mM NaCl, 0.1% Tween 20) for 1 h at room temperature, membranes were incubated with anti-RARα antibody, anti-TFIID (TBP) antibody (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.), and anti-β-Actin antibody (MEDICAL & BIOLOGICAL LABORATORIES, Nagoya, Japan). Subsequently, membranes were incubated with secondary antibody conjugated to horse-radish peroxidase (Cell Signaling Technology, Danvers, MA, U.S.A.), and were detected with the Immobilon Western chemiluminescent horse-radish peroxidase (HRP) substrate.

Luciferase Reporter Assay

The 5’-region of Tal2 was amplified from mouse genomic DNA, and was cloned into the firefly luciferase reporter plasmid, pGL4.10[luc2P] vector (Promega). P19 cells (8×10^5 cells) were seeded onto 12-well plates and transfected with vectors using Lipofectamine 2000 (Life Technologies) according to the manufacturer’s instructions. Medium was replaced at 5 h after transfection with atRA or DMSO in suspension culture. After a further 20 h, luciferase activity was measured using the Dual-Glo Luciferase Assay System (Promega) according to the manufacturer’s instructions. Subsequently, the reporter vector containing the Tal2 gene region from −240 to +73 was used to analyze the TATA-box-like element. For this purpose, a reporter vector lacking this element (−240ΔTATA) was also constructed. Luciferase activity was performed in the same manner as for the 5’-region of Tal2.

For analysis of the TATA-box-like element upstream of Tal2, we utilized the CheckMate/Flexi Vector Mammalian Two-Hybrid System (Promega). The firefly luciferase reporter plasmid pGL4.3l[luc2P/PL4UAS/Hygro] vector was digested with Nhel and HindIII to remove the adenovirus major late promoter. The following oligonucleotides were inserted into pGL4.3l digested with Nhel and HindIII: TATA(Tal2), 5’-GGT ACC TGC GGT GTCT ATCTAA A GCT GTG TGC GAC GAG AC CCG TCCTGATGCAA GGCCAGG GCCGCAA AAGCTTT-3’, and TATA(Tal2)Mut, 5’-GCT AGCGCCG CTTG GCG GCCGCAA G GCT GTG TGC GAC GAG ACC CTG GTG CTC ACAA GGC GAG G CGCA ACAA AGCTTT-3’. Mutated bases are shown in boldface. In cotransfection experiments with MyoD fused to VP16 expression vector, Id fused to GAL4 expression vector and reporter vector, P19 cells (4×10^5 cells) were seeded onto 24-well plates and were transfected with these vectors using Lipofectamine 2000 (Life Technologies) according to the manufacturer’s instructions. Luciferase activity was assayed by Dual-Glo Luciferase Assay System at 24 h after cotransfection.

For analysis of the RARE-like element in the intron of Tal2, RARA fused to VP16 (VP16-RARA) was cloned into the pcAGGS vector. Moreover, to remove GAL4-binding sites, the pGL4.3l vector was digested with KpnI and Nhel, and the following oligonucleotides were inserted into pGL4.3l digested with KpnI and Nhel: DR5(Tal2)×3, 5’-GTTACCTGA ACTTGG ACTCAC CTGTGA ACTTTG ACTCAC CTGTGA ACTTTG ACTCAC CTGTGA GC-3’; and DR5(Tal2)Mut×3, 5’-GTTACC AAA ACTTGG ACTCAC CTGTGA AAAAAA ACTTTG ACTCAC CTGTGA AAA ACTTGG ACTCAC CTGTGA GCT AGC-3’. The vector contained three tandem repeats of the RARE-like element (underlined). Mutated bases in DR5(Tal2)Mut are shown in boldface. The luciferase activities in P19 cells transfected with both the VP16-RARA expression vector and the reporter vector were measured in the same manner as cotransfection experiments with the TATA-box-like element. Luciferase activities from the RARE-like element with atRA signaling were measured in P19 cells.

All experiments were carried out in triplicate, and firefly luciferase activity was normalized using Renilla luciferase activity. Statistical significance was determined by t test (*p<0.05).

Preparation of Nuclear Proteins and Electrophoretic Mobility Shift Assay (EMSA)

Nuclear extracts were prepared as described previously. Briefly, P19 cells (1×10^7 cells), which were treated with 1 μM atRA for 0 and 3 h in suspension culture, were harvested by centrifugation. The cell pellet was resuspended in buffer A (10 mM N-(2-hydroxyethyl)-piperazine-N’-2-ethanesulfonic acid (HEPES)–KOH [pH 7.9], 10 mM KCl, 1.5 mM MgCl_2, 0.5 mM dithiothreitol (DTT), Complete Miniti ethylenediaminetetraacetic acid (EDTA)-free protease inhibitors [Roche Diagnostics, Basel, Switzerland]) and incubated on ice for 10 min. Nuclei were collected from the lysates by centrifugation at 10000×g for 5 min. Pellets were then resuspended in buffer C (20 mM HEPES [pH 7.9], 420 mM NaCl, 1.5 mM MgCl_2, 0.2 mM EDTA, 25% glycerol, 0.5 mM DTT, Complete Mini EDTA-free protease inhibitors [Roche]) and incubated on ice for 30 min. Supernatants were collected from centrifugation at 20000×g for 2 min. The protein concentration of nuclear extracts was measured using the Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad).

EMSA was also performed as described previously.
RESULTS AND DISCUSSION

Regulatory Region of Tal2 Gene  

ataRA signaling regulates the transcription of target genes through the binding of RARs to RARE, and is involved in the induction of Tal2 in P19 cells. Thus, we aimed to predict the regulatory region of Tal2, and searched for the RARE motif located within ±10 kb from both the transcription start site (TSS) and the end of Tal2, with reference to previous studies. Mouse Tal2 consists of two exons and an intron. Although RARE has not been mapped in the 5'- or 3'-flanking regions of Tal2, we found a RARE-like motif, which has been reported, in the intron of Tal2. This motif was designated “DR5(Tal2)” in this paper. The direct repeat of the hexameric motif at this site is separated by 5 nucleotides (Fig. 1A). 

Chromatin Immunoprecipitation (ChIP) Assay  

ChIP assay was performed by SimpleChIP Plus Enzymatic Chromatin IP Kit (Magnetic Beads) (Cell Signaling Technology) according to the manufacturer’s instructions. This assay was used for P19 cells (4×10⁶ cells for the RARE-like element or 2×10⁵ cells for the TATA-box-like element) treated with atRA for 3 h in suspension culture. The isolated chromatin DNA was subjected to PCR analysis using AmpliTaq Gold 360 Master Mix (Life Technologies). The following gene specific primers were used to amplify: TATA(Tal2), 5'-TTCTTCTCCTCTCAGGCTCCTTTG-3' (sense) and 5'-TCTCCTAAGCAGCAGAGACGTG-3' (antisense); and TATA(Tal2) Mut, 5'GGCCTTTGATAGGCTCCTTCTAAGCAGAGACGTG-3'. Mutated bases are shown in boldface. Nuclear extracts (10 µg) were incubated for 30 min at room temperature. Samples were separated by electrophoresis on 5% native PAGE. Gels were analyzed using the Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, U.S.A.). For supershift analysis, anti-RARα antibody (ActiveMotif, Carlsbad, CA, U.S.A.) or anti-TFID (TBP) antibody (Santa Cruz Biotechnology) was added to nuclear extract before addition of the probe, and then incubated at room temperature for 1 h.

Briefly, Alexa 680-labeled probes containing following sequences were used for EMSA: TATA(Tal2), 5'-Alexa680-GCGGTGTCTCTATTAAAGCTGTTGGGCAAGCA-3'; TATA(Tal2)Mut, 5'-Alexa680-GCCTGCTTCCGGTAAAGGCTGTTGGGCAAGCA-3'; DR5(Tal2), 5'-Alexa680-CTAGGGGATAGGCTCCTTCTAAGCAGAGACGTG-3'; and DR5(Tal2)Mut, 5'-Alexa680-CTAGGGGATAGGCTCCTTCTAAGCAGAGACGTG-3'. Mutated bases are shown in boldface. Nuclear extracts (10 µg) were incubated for 10 min on ice in reaction buffer (10 mM Tris–HCl [pH 7.5], 50 mM NaCl, 0.5 mM EDTA, 0.5 mM DTT, 1 mM MgCl₂, and 2 mM poly(dI–dC)·poly(dI–dC)). Alexa 680-labeled probe was then added to nuclear extracts. These mixtures were incubated for 30 min at room temperature. Samples were separated by electrophoresis on 5% native PAGE. Gels were analyzed using the Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, U.S.A.). For supershift analysis, anti-RARα antibody (ActiveMotif, Carlsbad, CA, U.S.A.) or anti-TFID (TBP) antibody (Santa Cruz Biotechnology) was added to nuclear extract before addition of the probe, and then incubated at room temperature for 1 h.

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upstream T nucleotide is most commonly at −31 or −30 relative to TSS.26–28)

We assumed that TATA(Tal2) in 5′-region and DR5(Tal2) in the intron were involved in the transcription of Tal2. It is known that TATA-box binding protein (TBP) binds to the TATA-box, and that RARα, which is involved in the induction of Tal2, binds to RARE. Thus, we examined the expression of these proteins in P19 cells with atRA treatment in suspension culture (Fig. 1C). As a result, it was observed that these proteins remained unchanged for 24 h after atRA treatment.

**Relationship between TATA(Tal2) and Transcription of Tal2**
We constructed reporter vectors containing the 5′-region of Tal2 and examined the transcriptional activity of these regions in P19 cells (Fig. 2A). Luciferase activity increased in P19 cells transfected with these constructs, as compared with empty vector. However, their activity with atRA treatment was almost the same with DMSO treatment. Under adhesion conditions, luciferase activity was observed in P19 cells transfected with these reporter vectors, similar to suspension culture (data not shown). TATA(Tal2) was contained in these vectors and might be involved in the transcription. The TATA-box is the predominant DNA element of core promoters that directs transcriptional initiation, and is recognized and bound by TBP, which is a subunit of the TFIIID complex in eukaryotes, with RNA polymerases and associated factors.26,29)

Indeed, the deletion of TATA(Tal2) from the 5′-region was decreased the luciferase activity (Fig. 2B).

Therefore, to examine the transcriptional activity of TATA(Tal2), we utilized the two-hybrid system adapted for use in mammalian cells.30,31) In this system, the association between one protein fused to the yeast GAL4 DNA-binding domain and the other protein fused to the herpes simplex virus VP16 activation domain promote the assembly of RNA polymerase II complexes at the TATA-box and increase the transcription of the firefly luciferase reporter gene. Two positive controls that encode Id fused to GAL4 (GAL4-Id) and MyoD fused to VP16 (VP16-MyoD) were contained in this system, and we utilized the vectors expressing these proteins to verify the transcriptional activity of TATA(Tal2). Moreover, we constructed the reporter vectors containing TATA(Tal2) instead of a minimal adenoviral promoter downstream of five GAL4 binding sites. These constructs were cotransfected into P19 cells, and the luciferase activity was measured. As a result, the luciferase activity from the reporter vector containing TATA(Tal2) was significantly increased in P19 cells that expressed both VP16-MyoD and GAL4-Id as compared with the reporter vector containing TATA(Tal2)Mut, including mutations in TATA(Tal2) (Fig. 2C). These results indicate...
that TATA(Tal2) functions as a core promoter involved in transcription.

Next, to examine the binding of TBP to TATA(Tal2), we prepared the nuclear extracts from P19 cells treated by atRA in suspension culture and performed electrophoretic mobility shift assay (EMSA) using Alexa 680-labeled oligonucleotide probes containing TATA(Tal2) or TATA(Tal2)Mut. Although the pattern was different between these probes, several shifted complexes, which represented these probes bound to nuclear extracts, were detected. These complexes at 0 h were same as those at 3 h (data not shown). Therefore, binding of TBP to the Alexa 680-TATA(Tal2) probe was verified by supershift analysis of EMSA with the nuclear extract at 3 h (Fig. 3A).

As a result, the disappearance of a shifted complex was confirmed by supershift analysis with anti-TBP antibody (black arrowhead in Fig. 3A), and it was confirmed that TBP binds to TATA(Tal2). Furthermore, we examined whether TBP binds to TATA(Tal2) in P19 cells by chromatin immunoprecipitation (ChIP) analysis (Fig. 3B). Chromatin from P19 cells 3 h after atRA treatment in suspension culture, which was immunoprecipitated with anti-TBP antibody or normal rabbit IgG, was subjected to PCR.

Fig. 3. Binding of TBP to TATA(Tal2)

(A) P19 cells were treated by atRA in suspension culture for 3 h and nuclear proteins were extracted from these cells. To verify the binding of TBP to TATA(Tal2), supershift analysis using anti-TBP antibody was performed. The disappearance of signals that indicated complexing with TBP and TATA(Tal2) was confirmed by analysis using Alexa 680-labeled TATA(Tal2) probes (black arrowhead). (B) The binding of TBP to the TATA-box-like element in P19 cells was verified by ChIP assay. Purified chromatin from P19 cells at 3 h after atRA treatment in suspension culture, which was immunoprecipitated with anti-TBP antibody or normal rabbit IgG, was subjected to PCR.
Fig. 4. Transcriptional Activity of DR5(Tal2) in P19 Cells

(A) The scheme of the constructed reporter vector. (B) P19 cells were cotransfected with the reporter vector containing DR5(Tal2)×3, DR5(Tal2)Mut×3 or GAL4-binding domain as a control, and the vector expressed constitutive active RARα (VP16-RARA). Luciferase activity was detected in P19 cells expressing VP16-RARA. Data represent means±S.E. of three independent experiments. *p<0.05. (C) Reporter vectors containing DR5(Tal2)×3 or DR5(Tal2)Mut×3 were transfected into P19 cells, followed by treatment with atRA in suspension culture. Luciferase activity was detected in these cells treated by atRA in suspension culture. As a control, reporter vector containing GAL4-binding domain was used. Data represent means±S.E. of three independent experiments. *p<0.05.

Fig. 5. Binding of RARα to DR5(Tal2)

(A) P19 cells were treated with atRA in suspension culture at 0 h and 3 h. After extraction of nuclear protein from these cells, the binding of nuclear extracts to DR5(Tal2) was examined by EMSA using Alexa 680-labeled probes. A signal that indicated complexing with RARα and DR5(Tal2) was detected (black arrowhead) in nuclear extracts at 3 h. White arrowhead indicates the non-specific interaction. (B) To confirm the binding of RARα to DR5(Tal2), supershift analysis using anti-RARα antibody was performed. A shifted signal (gray arrowhead) that indicated complexing with RARα and DR5(Tal2) was detected by the analysis using Alexa 680-labeled DR5(Tal2) probes. White arrowhead indicates the non-specific interaction. (C) Binding of RARα to RARE-like element in P19 cells was verified by ChIP assay. Purified chromatin from P19 cells at 3 h after atRA treatment in suspension culture, which was immunoprecipitated with anti-RARα antibody or normal rabbit IgG, was subjected to PCR.
characteristic shifted complex with Alexa 680-DR5(Tal2) was
detected in nuclear extracts at 3 h (black arrowhead in Fig.
5A) compared with 0 h. This complex was not observed in the
nuclear extracts with Alexa 680-DR5(Tal2) Mut. As RARα is
continuously expressed in P19 cells (Fig. 1C), this result may
be correlated with the activation of Tal2 by atRA signaling.
Moreover, to confirm the binding of RARα to Alexa 680-DR5(Tal2), supershift analysis with anti-RARα antibody
was performed. Nuclear extracts at 3 h were used in this anal-
ysis. A specific shifted complex was detected in the nuclear
extracts with Alexa 680-DR5(Tal2) and anti-RARα antibody
(gray arrowhead in Fig. 5B). The addition of anti-RARα anti-
body did not change the mobility of any complexes including
the complex (white arrowhead in Fig. 5) in the nuclear ex-
tracts with Alexa 680-DR(Tal2) Mut. Therefore, these results
indicate that RARα binds to DR5(Tal2). Subsequently, we
examined by ChIP assay whether RARα bound to DR5(Tal2)
in P19 cells (Fig. 5C). Similarly to anti-TBP antibody, chro-
matin from P19 cells at 3 h after atRA treatment in suspension
culture was immunoprecipitated with anti-RARα antibody,
and purified binding DNA was analyzed by PCR. PCR ex-
periments using specific primers showed that anti-RARα anti-
body, but not IgG, immunoprecipitated the region containing
DR5(Tal2) in P19 cells, whereas negative controls were not
immunoprecipitated with anti-RARα antibody. Therefore,
these results indicate that RARα binds to DR5(Tal2) located
in the intron of Tal2 in P19 cells.

Taken together, the RARE-like element “DR5(Tal2)” in the
intron of Tal2, which consists of a direct repeat of the hexa-
meric motif separated by 5 bp, responded to constitutively ac-
tive RARα and atRA signaling, and bound with RARα, which
is a receptor for atRA, in P19 cells. These results suggest
that DR5(Tal2) is involved in the transcription of Tal2 by atRA
signaling in P19 cells.

Interaction between TATA(Tal2) and DR5(Tal2)  In this
paper, we showed that TATA(Tal2) in the 5′-region of Tal2
functioned as a core promoter and DR5(Tal2) in the intron of
Tal2 responded to the atRA-RARα signaling. It is known that
RARs heterodimerize with retinoid X receptors (RXRs) and
bind to RARE. In the absence of atRA, the heterodimer of
RAR and RXR (RAR/RXR) is thought to constitutively bind
to RARE and repress transcription through associations with
the corepressors, nuclear receptor corepressor (NCoR) and
silencing mediator for retinoid and thyroid hormone receptors
(SMRT). Moreover, these corepressors interact with HDAC3.
HDACs are able to deacetylate lysine residues of the N-termi-
nal tails of histone and prevent transcription. Upon binding of
atRA, RAR/RXR undergoes a conformational change result-
ing in the release of corepressor complexes, and associates
with coactivators such as SRCs and CBP/p300. The coactiva-
tors induce chromatin remodeling and facilitate the assembly
of a transcription preinitition complex containing TBP.11,34,35)
Therefore, it is anticipated that TATA(Tal2) and DR5(Tal2)
interact through RARα, coactivators and a transcription pre-
initition complex containing TBP, and participate in the tran-
scription of Tal2 in P19 cells (Fig. 6A).

We utilized ChIP assay to examine the interaction be-
tween TATA(Tal2) and DR5(Tal2) in P19 cells (Fig. 6B). In
this assay, chromatin immunoprecipitated with anti-RARα
antibody was analyzed by PCR using specific primers for

Fig. 6. Interaction between TATA(Tal2) and DR5(Tal2)

(A) The scheme of the transcriptional regulation of Tal2 that we anticipated. (B) The interaction between TATA(Tal2) and DR5(Tal2) was examined by ChIP assay. P19
cells were treated with atRA for 0 h or 3 h in suspension culture. Purified chromatin immunoprecipitated with anti-RARα antibody was subjected to PCR using specific
primers that were targeted to TATA(Tal2), and purified chromatin immunoprecipitated with anti-TBP antibody was subjected to PCR using specific primers that were
targeted to DR5(Tal2).
TATA(Tal2). Also, chromatin immunoprecipitated with anti-TBP antibody was analyzed by PCR using specific primers for DR5(Tal2). Consequently, it was confirmed that RARα was associated with TATA(Tal2), and that TBP was associated with DR5(Tal2). Therefore, our data suggested that DR5(Tal2) and TATA(Tal2) interacted through RARα and TBP in P19 cells, and support the possibility that TATA(Tal2) and DR5(Tal2) are regulatory elements which coordinate to promote the transcription of Tal2 in P19 cells.

CONCLUSION

Because atRA signaling is important for neural differentiation, we believe that Tal2 induced by atRA commits target cells to the neural lineage. In this study, we focused on the regulatory region involved in the transcription of Tal2. Our data suggest that transcription of Tal2 is mediated by two distal regulatory elements: the TATA-box-like element “TATA(Tal2)” and the RARE-like element “DR5(Tal2).” There is a distance of approximately 5kb between these elements. It is thought that TATA(Tal2) upstream of exon 1 functions as a core promoter, and that DR5(Tal2) located in the intron of Tal2 is bound with RARα and responds to atRA signaling. To clarify the interaction between TATA(Tal2) and DR5(Tal2) in the transcription of Tal2, it is important to identify the cofactors, which are involved in the transcription of Tal2. Further work is underway to clarify the transcriptional regulation of Tal2 by atRA signaling in P19 cells.

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Conflict of Interest The authors declare no conflict of interest.

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