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Induced Expression, Localization, and Chromosome Mapping of a Gene for the TBP-Interacting Protein 120A

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TBP-interacting protein 120A (TIP120A) is a novel eukaryotic transcriptional regulator and has been suggested to be involved in the general regulation of transcription because of its ability to potentiate transcription of all classes of genes and to interact with common transcriptional machineries. In the present study, we investigated the expression of the *tip120a* gene. TIP120A transcripts were expressed abundantly in the heart and liver, moderately in the brain and skeletal muscle, and only slightly in the spleen and lung. This ubiquitous expression pattern was similar to that of TBP. Gene expression of TIP120A in the rat liver was not stimulated by hepatocarcinogenesis or liver regeneration. TIP120A was thus suggested not to be a growth-related protein. On the other hand, in P19 mouse embryonal carcinoma cells, TIP120A expression was elevated upon retinoic acid treatment, which induces differentiation. Notably, the foci-like nuclear localization pattern of TIP120A was transformed into a speckle-like pattern. The level of TIP120A was also elevated in such stem-like cells as F9 and HL60 after each differentiation procedure, retinoic acid and DMSO, respectively. In HEp-2 cells, TIP120A was observed as a limited number of nuclear foci, and the localization coincided with that of the PML oncogenic domain. FISH detection revealed that the human *tip120a* gene was located at 12q14, the position to which a myopathic type scapuloperoneal syndrome locus also mapped. Our study suggests that, contrary to an early assumption, TIP120A is involved in tissue-specific and/or differentiation-related gene expression.

Key Words: **TIP120A; P19; F9; HL60; POD; differentiation; transcription factor.**

Abbreviations used: TIP120; TBP-interacting protein 120; POD, PML oncogenic domain; PML, promyelocytic leukemia; HCC, hepatocellular carcinomas; SP, scapuloperoneal syndrome; SPMD, scapuloperoneal muscular distrophy.

[1] The accession number of the rat TIP120A (previously submitted as TIP120) in the EMBL/DDBJ/GenBank is D87671.

TBP-interacting protein 120A, TIP120A (previously referred to as TIP120), was isolated as a TBP-interacting protein by *in vitro* procedures (1). We also identified another TIP120 family protein, TIP120B, that is specifically expressed in muscle tissues (2). Although TIP120A does not contain any of the typical motifs found in transcription regulatory factors and general transcription factors, it potentiates all classes of eukaryotic gene expression at the preinitiation step of transcription (3, 4). TIP120A is associated with TBP and some transcription-relating proteins (Makino *et al.*, manuscript in preparation), both of which are included in all classes of eukaryotic transcription apparatus (3–6). Actually, TIP120A facilitates the activities of RNA polymerase-I, -II, and -III (4). Notably, one of the common subunits of RNA polymerases, RPB5, bound to TIP120A (4). However, it remains to be clarified how TIP120A gene expression alters during cellular activity. On the basis of its mechanistic properties and of the genes it affects, TIP120A has been assumed to be involved in coordinated gene expression for multiple genes when cell growth and/or differentiation are induced by stimuli. The behavior of TIP120A in such cell dynamism has not yet been investigated. In this study, we investigated TIP120A gene expression during cell growth, carcinogenesis and differentiation.

MATERIALS AND METHODS

Northern blotting. A membrane on which poly(A)$^+$ RNAs (2 μg) from various rat tissues had been blotted was purchased from Clontech. This membrane had been confirmed by β-actin probe to be blotted with the same amounts of each RNA. The probe DNA included the whole *tip120a* cDNA sequence (1). Northern blotting was performed by using QuikHyb hybridization solution (Stratagene) for 1 h. RNA extraction and preparation of poly(rA) RNAs were previously described (7). Two μg of poly(A)$^+$ RNA or 10 μg of total RNA was blotted in each lane.

Hepatocarcinogenesis and liver regeneration of rats. The Solt-Farber protocol was applied to obtain rat hepatocellular carcinomas (HCCs) (8, 9). Male Wistar rats were i.p.-injected with 200 mg

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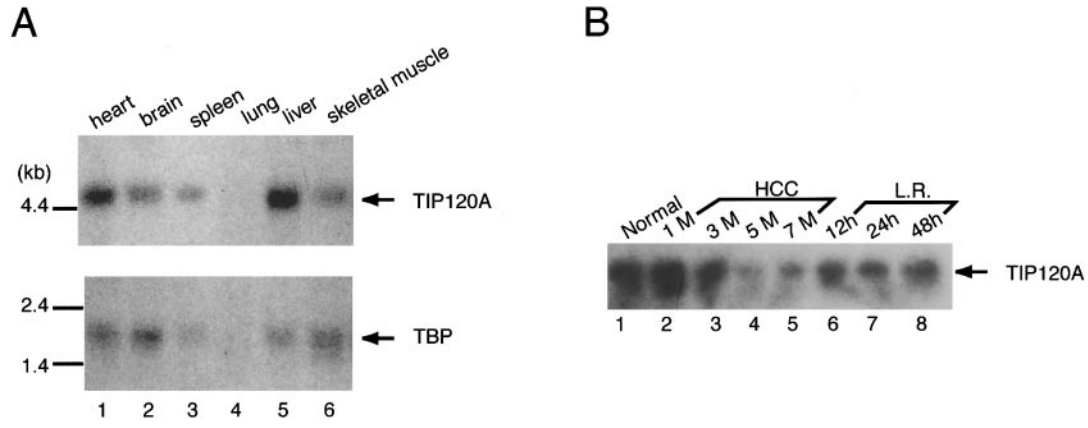

FIG. 1. Expression of TIP120A in rat tissues. (A) Multiple tissue Northern (MTN) blot membrane (Clontech) was hybridized with *tip120a* (top) and *tbp* probes (bottom). Positions of the specific signals are indicated by arrows. Lanes contain RNAs from rat heart (lane 1), brain (lane 2), spleen (lane 3), lung (lane 5), liver (lane 4), or skeletal muscle (lane 6). (B) Expression of TIP120A transcripts during hepatocellular carcinogenesis (HCC) and liver regeneration (L.R.). Lane 1; normal rat liver. Lanes 2–5, liver RNAs at 1 to 7 months (M) after initiation of the Solt-Farbar protocol. Lanes 6–8, RNAs from regenerating liver at various period after partial hepatectomy.

diethylnitrosoamine/kg. After the rats had been fed a basal diet for 2 weeks, oral administration of 0.02% 2-acethylaminofluorene was initiated. One week later, 70% partial hepatectomy was performed. Hyperplastic nodules were found in the rat livers after a few months and the rats died of HCC in 6–12 months as reported previously (10).

Cell culture and differentiation. P19 mouse embryonal carcinoma cells were cultured in α-MEM (Gibco) supplemented with 10% fetal bovine serum. For retinoic acid (RA) treatment that conducts differentiation, all-trans-retinoic acid (Sigma), was added into the medium at a final concentration 0.5 μM (11). Another mouse embryonal carcinoma cell F9 was grown in DMEM. The human promyelocytic leukemia cells HL60 were cultured in RPMI1640 (Gibco). F9 cells were differentiated by RA (0.2 μM), dibutyl cyclic AMP (0.1 mM), and isobutyl methylxanthine (0.1 mM) (12). In the case of HL60 cell differentiation, we used 1.3% dimethyl sulfoxide as previously described (13). Human HEp-2 cells (14) were grown in DMEM (Gibco).

Western blotting. Proteins in cells were extracted by the method of Schreiber *et al.* (15). Proteins (50 μg) were analyzed by 7.5% SDS-PAGE and specific proteins were detected alkaline phosphatase method as described previously (3, 4).

Transfection and transient luciferase assay. For the transient luciferase assay, P19 cells were transfected with the luciferase reporter plasmid followed by the adenovirus VA1 promoter (150 ng). After transfection, the cells were transferred to new dishes and further cultured for 48 h. The luciferase activity in 200 μl of reaction cocktail was measured with a Luciferase Assay System (Promega) and TD20/20 luminometer (Terner Designs). Obtained values were normalized by protein concentration.

Antibodies and indirect immunofluorescence staining. Anti-TIP120A antibody (1) and anti-PML antibody (14) have been described previously. Indirect immunofluorescence staining of TIP120A in P19 cells was performed as described (14, 16) using affinity-purified anti-TIP120A antibody. Cells were fixed with cold acetone/methanol. The air-dried cells were hydrated twice in cold PBS, blocked with 2% normal goat serum, then incubated with anti-TIP120A antibody for 60 min. FITC-conjugated donkey anti-rabbit IgG (H + L) (Jackson) was used as the second antibody. The cells were then stained for DNA with 1 μg/ml of DAPI (4′, 6-diamidino-2-phenylindole) (Wako). For double immunofluorescence staining of HEp-2 cells, permeabilized cells on coverslips were incubated with anti-TIP120A antibody and biotinylated anti-PML antibody under the same conditions as described above. The cells were incubated with Texas-Red-conjugated secondary monoclonal antibody (Jackson Lab.) and streptavidin-FITC (Amersham) for 1 h.

FISH mapping. Human lymphocytes cultured in α-MEM with 10% fetal calf serum were treated with phytohemagglutinin and synchronized by BrdU treatment (0.18 mg/ml). The block was released in the normal medium with thymidine (2.5 μg/ml). Cells were harvested and slides were made by using a standard procedure (17). The isolated rat TIP120A cDNA was biotinylated with dATP using BioNick Labeling Kit (BRL). The procedure for FISH detection was performed as previously (17). The chromosomes in the slide were stained with DAPI. FISH signals and DAPI banding patterns were recorded separately on photographs, and the assignment of the FISH-mapping was achieved by superimposing the FISH signals with the DAPI-banded chromosomes.

RESULTS

Gene Expression of TIP120A in Various Cell States

We investigated the gene expression of TIP120A in various rat tissues by Northern blotting. TIP120A was found to be expressed abundantly in the heart and liver, moderately in the brain and skeletal muscle, and only slightly in the spleen and lung (Fig. 1A). Obviously, no correlation was observed between cell proliferation and TIP120A expression because TIP120A transcripts were low in the spleen and lung, whereas other tissues have poor proliferating indexes. We also investigated the gene expression of TBP, and found that expression patterns of both genes were roughly similar. These results imply the cooperative action of TIP120A and TBP and are consistent with the fact of physical interaction between TIP120A and TBP (1).

Hepatocytes do not proliferate normally. However, rat liver is a good material with which to study carcinogenesis and cell proliferation in natural tissue, because chemical hepatocarcinogenesis and liver regeneration can be easily performed. We generated HCCs in rats by the Solt-Farber protocol. During this proce-

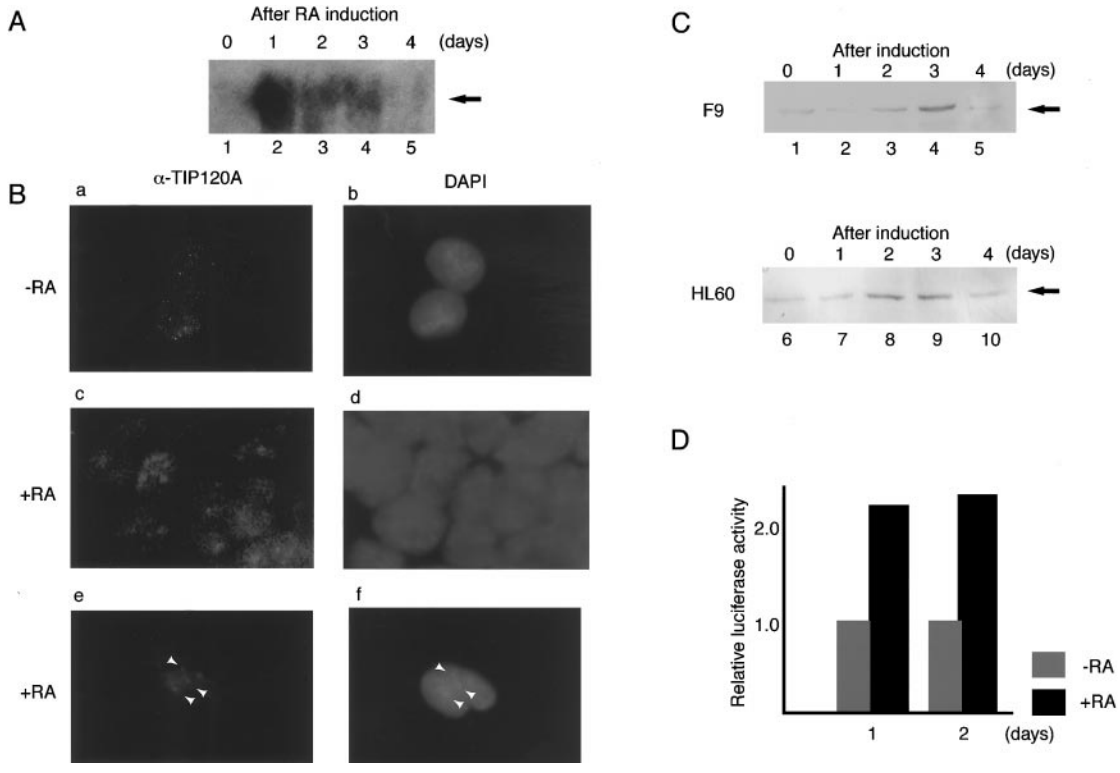

FIG. 2. Expression and localization of TIP120A in mouse P19 cells and the effect of retinoic acid (RA). (A) Induction of TIP120A mRNA expression in RA-treated P19 cells. RNAs were analyzed by Northern blotting. RNAs from untreated cells (lane 1) and 1 day (lane 2), 2 days (lane 3), 3 days (lane 4), and 4 days (lane 5) after treatment with RA. Cells were exposed to RA for only the initial 48 h. (B) Alteration of TIP120A localization in P19 cells after RA treatment. (a, c, and e) TIP120A-staining with anti(α)-TIP120A antibody; (b, d, and f) DAPI-staining; (a and b) before RA treatment; (c–f) after RA treatment (at 48 h). (e and f) Higher magnification and longer exposure in photography of RA-treated cells. Arrowheads indicate collocations of TIP120A speckles and DNA-concentrated regions. (C) TIP120A is overexpressed in other types of cells upon differentiation. F9 cells (top) and HL60 cells (bottom) were differentiated by each procedure and TIP120A proteins were determined by Western blotting by the use of TIP120A antibody. Lanes 1 and 6, native cells. Lanes 2–5 and 7–10, cells treated for 1–4 days, respectively. Arrows; position of TIP120A. (D) Promoter activity of the luciferase-expression vector carrying the adenovirus VA1 promoter in the native (gray columns) and RA-treated (solid columns) in P19 cells. Cell extracts were prepared 1 or 2 days after transfection. Activities are present as ratios to that of the native cells at each day.

dure, the animals developed hepatomas within 6 months and died within 12 months. Gene expression of TIP120A decreased significantly at 5 months after the induction of hepatocarcinogenesis (Fig. 1B). At this stage, more than 50% of the rat livers were occupied by hepatomas (data not shown). This result suggests that gene expression of TIP120A is repressed in HCCs. We further prepared regenerating livers and determined the level of TIP120A mRNAs therein. No apparent change was seen in the TIP120A mRNAs in the regenerating rat livers (Fig. 1B). Thus, it was found that TIP120A gene expression did not become elevated during liver regeneration.

Expression and Localization Pattern of TIP120A during Cell Differentiation

RA treatment is known to arrest the proliferation of P19 embryonic carcinoma cells and induce them to undergo differentiation. In RA-treated P19 cells, a number of genes are known to be stimulated although another group of gene such as UTF-1 and Oct-3 are repressed (12, 18, 19). After prolonged culture, RA-treated P19 cells differentiate into neural cells or muscle cells, depending on the culture conditions. We determined the level of TIP120A gene expression in the early stage (within the first few days) of the cultivation of RA-treated P19 cells. During this period, cells did not express neural marker proteins yet. The gene expression of TIP120A, however, was stimulated (around 3 to 4-fold) in the RA-treated cells at the first few days after RA treatment (Fig. 2A, lanes 2–4). Notably, its elevated expression declined to the basal level on day 4 upon RA treatment (Fig. 2A, lane 5). Then, the intracellular localization of TIP120A protein was investigated by means of indirect immunostaining (Fig. 2B). Before RA treatment, TIP120A proteins were observed in the nuclei as sharp dots (= foci) (Fig. 2B, panel a). However, RA-treatment altered the staining profile dramatically, *i.e.* foci transformed into enlarged speckles (Fig. 2B, panel c). Taken together with results of

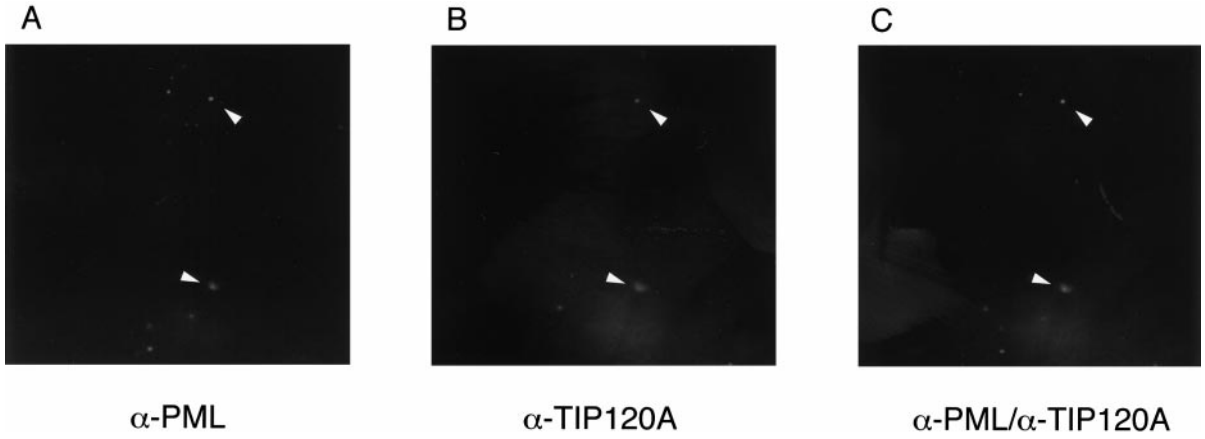

FIG. 3. Localization of PML and TIP120A proteins in HEp-2 cells. HEp-2 cells were subjected to (A) double immunofluorescence labeling (biotinylated anti(α)-PML antibody and streptavidin-FITC) to detect endogenous PML proteins and to (B) indirect immunostaining using anti (α)-TIP120A antibody and Texas red-conjugated mouse monoclonal antibody. The above two images were superimposed (C). Arrows indicate the co-stained positions in C.

Fig. 2A, this suggests that the expression of TIP120A protein as well as its transcripts was increased in P19 cells by treatment with RA. Careful observation revealed that many TIP120A speckles were colocalized with the concentrated DAPI-stained regions of the treated cells (arrowheads, Fig. 2B, panels e and f). These observations imply that, in RA-treated P19 cells, TIP120A is concentrated near chromatin which might lead elevated gene transcription.

Above observations provided a possibility that *tip120a* is a differentiation-responsive gene. We examined these phenomenon in other types of cells such as F9 and HL60 by Western blotting, which are known to differentiate to parietal endoderm (20) and macrophage or neutrophil (21), respectively. F9 cells contain small amount of TIP120A protein (Fig. 2C, lane 1). When the cells were treated with RA, increased TIP120A protein was observed on day 3 (Fig. 2C, lane 3). However, the amounts of TIP120A dropped on day 4 (Fig. 2C, lane 5). The TBP, as a control protein, did not change during differentiation protocol (data not shown). Moreover, HL60 cells also resulted in a weak but significant increase of TIP120A in day 2 and 3 upon differentiation (Fig. 2C, lanes 8 and 9), and also declined on day 4 (lane 10). TIP120A is thus thought to be a differentiation-related protein. We investigated alteration of the transcriptional capability of the treated P19 cells by using an adenovirus VA1 promoter-driven luciferase reporter plasmid, because the VA1 promoter was demonstrated to be stimulated by TIP120A (4). We found that the VA1 gene expression was activated by RA treatment (Fig. 2D), suggesting that accumulation of TIP120A is involved in transcriptional stimulation in the RA-treated cells.

Human HEp-2 cells were examined for the localization of TIP120A foci. Figure 3B demonstrates that these cells contained limited numbers (2–3 foci) of the stained foci. This result suggests that TIP120A plays a specific role in these cells. HEp-2 cells have been known to contain POD (PML oncogenic domain) (14). We were able to reproduce this observation because individual HEp-2 cells had a few clear POD foci (Fig. 3A). Interestingly, some, but not all, PODs were colocalized with TIP120A (Fig. 3C). These results suggest that some TIP120A proteins in the POD play a specific role in HEp-2 cells.

Chromosome Mapping of Human TIP120A

Normal human lymphocytes were analyzed for chromosomal position of *tip120a* with a *tip120a* cDNA probe. Under the hybridization condition, *tip120a* did not cross-react with *tip120b* (data not shown). The FISH revealed a pair of positive signals on one of the chromosomes (Fig. 4A). From the assay of 10 independent photographs, the location of the probe sequence was assigned at the long arm of the q14 region of chromosome 12 (Fig. 4). We thus assigned the *tip120a* gene in this region. It is notified that a human disease such as SPMD (scapuloperoneal muscular dystrophy) has been mapped at 12q13.3–q15 (22).

DISCUSSION

TIP120A Is a Differentiation-Related Protein

TIP120A is able to activate all classes of promoter both *in vivo* and *in vitro* (4). On the basis of mechanistic analyses, TIP120A was suggested to stimulate preinitiation complex formation via interaction with TBP and RNA polymerases (4). Hence, TIP120A has been assumed to influence the expression of a large number of genes. This mechanism may explain rather ubiquitous expression of this gene and coordinated gene expression, which is believed to occur when cells

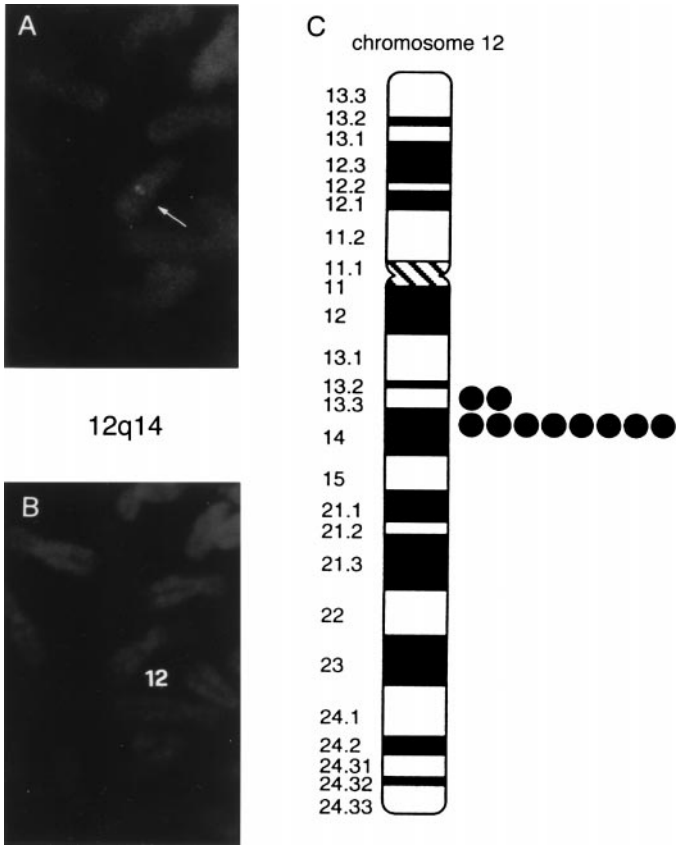

FIG. 4. Position of the TIP120A gene in human chromosomes. Human chromosomes were analyzed by FISH detection using rat *tip120a* sequence probe. (A) FISH observation for the *tip120a* probe. An arrow indicates the position of the specific signal. (B) DAPI-staining for identical chromosomes in A and identification of chromosome 12. (C) Schematic representation of human chromosome 12 and summary of FISH detection of 10 independent samples. Positions of the signals are indicated with dots.

are exposed to growth stimuli, differentiation inducers, and morphogens. We investigated how TIP120A is expressed in rat tissues, and found that TIP120A expression was not necessarily correlated with proliferating tissues since non-proliferating tissues (e.g., brain, liver, and muscle) contained considerable amounts of TIP120A transcripts (Fig. 1A). Moreover, neither carcinogenic transformation nor regeneration of rat liver increased TIP120A transcripts (Fig. 1B). Liver regeneration is known to be a model system of normal liver growth. Therefore, we conclude that TIP120A is not a growth-related factor.

On the other hand, the gene expression of TIP120A was markedly induced in P19 cells upon RA treatment (Fig. 2A). Immunostaining revealed that TIP120A localization pattern in the nucleus was altered by RA treatment from sharp foci to large speckles (Fig. 2B). We also noted that TIP120A protein was increased in RA-treated P19 cells. This induction is further confirmed by the results using other stem-like cells such F9 and HL60. Like P19, these cells also expressed increased amounts of TIP120A proteins by the induction of by RA in a few days after treatment, and returned to a basal level thereafter (Fig. 2C). Thus, it appears that TIP120A is temporally required for differentiation at least in these cells.

We found that TIP120A overexpression resulted in the growth arrest of P19 cells (S. Yogosawa, manuscript in preparation). Therefore, we propose that TIP120A may have a potential to arrest cell growth positively and promote cell differentiation. It is not exactly known whether the TIP120A increased in RA-treated P19 cells is responsible for transcriptional activation. However, the adenovirus VA1 promoter was stimulated in RA-treated P19 cells. We have found that ectopic TIP120A expression resulted in the elevation of VA1 promoter-driven luciferase activity (4). Therefore, we think that elevated level of TIP120A can participate in the induction of a set of genes in treated P19 cells.

Although the function of the POD in gene expression has not been well elucidated, its localization pattern in HL60 cells was reported to be altered from sharp foci to lager speckles by RA treatment (23, 24). Moreover, the POD is also known to be a region in which viruses replicate (16). HIV-1 growth was also reported to be related to the POD (25). Perhaps, TIP120A might be also efficiently used in viral replication.

Does TIP120A Participate in a Specific Cellular Function?

At present, we do not know which genes were specifically regulated by TIP120A or in what kind of gene induction does TIP120A participate. P19 is a bipotential embryonic carcinoma cell that can be differentiated into neural- or muscle-type cells by RA. TIP120A was also stimulated by RA in F9 cells which differentiate into parietal endoderm. Therefore, TIP120A might be involved in muscle-specific gene expression or the differentiation itself. The tissue distribution pattern of TIP120A in the rat was consistent with the above assumption since the transcripts were enriched in those tissues (Fig. 1A). More interestingly, chromosome mapping alludes a possible correlation between TIP120A and a human disease such as scapuloperoneal muscular distrophy (SPMD) (Fig. 4). SPMD is a muscular type scapuloperoneal syndrome (SP) (22). The SPs exhibit heterogeneous neuromuscular diseases which are characterized by weakness in the distribution of shoulder-girdle and peroneal muscle. Observations that TIP120A overexpression in cultured cells resulted in cell-growth arrest or cell death and that TIP120A was included in a complex containing cell-cycle regulatory factors (S. Yogosawa, manuscript in preparation) also tend to support the above notion. It seems strange that TIP120B, another TIP120 family protein, has been demonstrated to be a muscle-specific

protein (2). Perhaps, TIP120A and TIP120B might work cooperatively or complementarily in muscle development.

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