Journal of Molecular Neuroscience

ISSN0895-8696/99/12:131–145/$13.75

Identification of 3′UTR Region Implicated in Tau mRNA Stabilization in Neuronal Cells

Stella Aronov, Ruth Marx, and Irith Ginzburg*

Department of Neurobiology, Weizmann Institute of Science, Rehovot 76100, Israel

Received February 2, 1999; Revised March 1, 1999; Accepted March 2, 1999

Abstract

Tau, a neuronal microtuble-associated protein (MAP) plays an important role in the formation and maintenance of neuronal polarity. Tau mRNA is a stable message and exhibits a relatively long half-life in neuronal cells. The regulation of mRNA stability is a crucial determinant in controlling mRNA steady-state levels in neuronal cells and thereby influences gene expression. The half-lives of specific mRNAs may be dependent on specific sequences located at their 3′untranslated region (UTR), which in turn, may be recognized by tissue-specific proteins.

To identify the sequence elements involved in tau mRNA stabilization, selected regions of the 3′UTR were subcloned downstream to c-*fos* reporter mRNA or to the coding region of the tau mRNA. Using stably transfected neuronal cells, we have demonstrated that a fragment of 240 bp (H fragment) located in the 3′UTR can stabilize c-*fos* and tau mRNAs. Analysis of stably transfected cells indicated that the transfected tau mRNAs are associated with the microtubules of neuronal cells, suggesting that this association may play a role in tau mRNA stabilization. This step may be a prerequisite in the multistep process leading to the subcellular localization of tau mRNA in neuronal cells.

Index Entries: Tau mRNA; 3′untranslated region; message stabilization; microtubules; PC12 cells; LAN-1 cells.

Introduction

The posttranscriptional regulation of mRNA stability plays an important role in eukaryotic gene expression by modulating the cytoplasmic abundance of individual mRNAs. Although the importance of mRNA stability has been recognized, the structures and mechanisms involved are just beginning to emerge. It has been established that mRNA decay is a controlled process dependent on specific *cis*-acting sequences and *trans*-acting factors acting in a tissue-specific manner (Amara et al.,

*Author to whom all correspondence and reprint requests should be addressed.

1994). The half-lives of different mRNAs in a given cell may vary considerably, and may cover a wide spectrum. mRNAs exhibiting short half-lives of <1 h, e.g. of c-*myc* (Wisdom and Lee, 1991), c-*fos* (Shyu et al., 1991), and MATa1 (Parker and Jacobson, 1990), encoding for protooncogenes and cycle control factors, have been documented. On the other hand, mRNAs that accumulate in terminally differentiated cells with half-lives of >50 h, e.g., of crystalline (Li and Beebe, 1990) collagen (Hamalainen et al., 1985; Maatta et al., 1995) and a-globin (Aviv et al., 1976; Lodish and Small, 1976; Weiss and Liebhaber, 1994), have been reported.

The sequences that have been found to be involved in mRNA stabilization include the Poly(A) tail (Jacobson and Peltz, 1996) and specific sequences located within the 3'-UTR and/or 5'-UTR (Leibold and Munro, 1988; Casey et al., 1989). Stabilization signals located within the 3'-UTR of mRNAs have been reported for specific messages, including amyloid precursor protein mRNA (D'arcangelo and Halegoua, 1993; Rajagopalan et al., 1998), ribonucleotide reductase R2 mRNA (Amara et al., 1994), transferrin receptor mRNA (Leibold and Munro, 1988), a-globin (Weiss and Liebhaber, 1994; Wang et al., 1996; Wang and Leibhaber, 1996), and GAP-43 (Kohn et al., 1996; Tsai et al., 1997). It is clear that although common sequences may be present in different mRNAs transcripts, they still may be differentially regulated. These differences may reflect, in part, the presence of additional sequences affecting the secondary structure of the regions and binding of protein factors that are cell-type- or stage-specific (Brewer, 1991; Crosby et al., 1991).

Tau microtubule-associated proteins (MAPs) constitute a heterogeneous family (55–62 kDa) of neural specific proteins, found primarily in the axon, that are developmentally regulated. The function of tau in the stabilization of microtubules (MTs), which are important for both establishing and maintaining neuronal morphology, has been demonstrated (Kosik, 1997). Tau mRNA is a stable message, exhibiting a relatively long half-life (Sadot et al., 1995). It has been documented that the accumulation of tau mRNA in brain precedes the accumulation of tau protein by 2 and 10 d in the cerebrum and cerebellum, respectively (Mangin et al., 1989). In PC12 cells the half-life of tau mRNA increases by threefold following neuronal growth factor (NGF)-induced differentiation (Sadot et al., 1995).

Our previous studies using the *in situ* hybridization approach demonstrated that unlike tubulin mRNA, which is confined to cell bodies, or MAP2 mRNA, which extends into dendrites, tau mRNA enters the proximal portion of the axon (Litman et al., 1993). This localization process involves *cis*-acting sequences located in the 3'UTR region of tau mRNA, binding of specific *trans*-acting proteins, and is associated with the neuronal MTs (Litman et al., 1994; Behar et al., 1995). Thus, it has been suggested that the cytoskeleton is involved in the multistep localization process, which includes translocation, anchoring, and stabilization of mRNA (Bogucka-Glotzer and Ephrussi, 1996; Bassell and Singer, 1997).

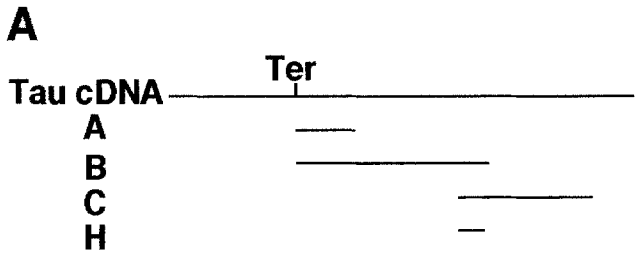
In the present work, we have identified the 3'UTR region, which confers tau mRNA stability. This region increases the stability of c-*fos* reporter mRNA following tansfection into PC12 cells. In addition, we have identified a region of 240-bp sequence (fragment H), which affects tau mRNA stabilization both in PC12 and LAN-1 neuronal cell lines. Finally, we demonstrated the association of this region with MTs, suggesting that the MTs are instrumental in the stabilization process that may be a prerequisite for the localization of tau mRNA in neuronal cells.

Materials and Methods

Cell Cultures

Rat adrenal pheochromocytoma (PC12) cells (Greene and Tischler, 1976) were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 8% horse serum and 8% fetal calf serum (FCS) in 8% CO_2 incubator at 37°C. For NGF treatment, $1.2–1.5 \times 10^6$ cells were plated on collagen-coated 90-mm plates and grown in DMEM supplemented with 1% horse serum, 2 m*M* glutamine, and 50 µg/mL gentamycine. For induction of the cells 50 ng/mL of 7S NGF (Alomone Labs, Jerusalem, Israel), was added every 2 d.

Human neuroblastoma (LAN-1) cells (Lopresti et al., 1992) were grown in RPMI-1640 medium, supplemented with 10% FCS, 2 m*M* glutamine, 50 U/mL penicillin, and 50 µg/mL streptomycin in a 5% CO_2 incubator at 37°C. For Bt_2AMP treatment, cells were plated at a density of $0.6–1 \times 10^6$ cells on 90-mm plates and 1 m*M* of Bt_2AMP, which was added every 2 d.



B

Fragment H

GGCGCCATCGTGGATGGAGTCCGTGTGTCCTGGAGATTACCCTGGACACCTCTG**CTT**
TTTTTTTTTTACTTTAGCGGTTGCCTCCTAGGCCTGACTCCTTCCCATGTTGAACTGG
AGGCAGCCAAGTTAGGTGTCAATGTCCTGGCATCAGTATGAACAGTCAGTAGTCCCA
GGGCAGGGCCACACTTCTCCCATCTTCTGCTTCCACCCCAGCTTGTGATTGCTAGCC
TCCCAGAGCT

Fig. 1. **(A)**. Schematic representation of tau mRNA subregions employed for the stability experiments. Fragment A corresponds to the first 282 nucleotides of the 3' UTR (Kosik et al., 1989). Fragments B, C, and H correspond to nucleotides 1124–2800 (1676 bp), 2519–3869 (1350 bp), and 2519–2760 (240 bp), respectively, on the whole tau cDNA clone (Sadot et al., 1994). **(B)** The 241-bp sequence of fragment H. The bold characters represent 21 bp of the AU-rich region.

Plasmid Constructs

All constructs included either the coding region of c-*fos*, used as a reporter gene (Greenberg and Ziff, 1984), or tau coding region (Sadot et al., 1994), ligated to fragments from tau 3'UTR in PC1Neo vector (Promega).

Fos constructs included tau 3' UTRs (Fig. 1; A corresponds to the first 282 bp of tau 3'UTR following the termination codon, and corresponds to nucleotides 1124–1406 (Kosik et al., 1989). Fragments B–1395 bp and C–1351 bp correspond to nucleotides 1124–2799 and 2519–3869, respectively (Sadot et al., 1994).

Tau constructs contained hemagglutinine (HA) flag (30 bp) fused in frame to the N-terminus of tau coding region. HA-tau coding construct included the tau coding region only, HA-tau coding-B included the tau-coding region linked to fragment B, and HA-tau coding-H included tau-coding region linked to 241 bp of the 3'UTR sequences (2519–2760), (Sadot et al., 1994) (Fig. 1).

Stable Transfections

PC12 cells (2×10^6) were transfected using the lipofectin reagent (BRL). After 48 h, the medium was replaced by fresh medium containing 400 μg/mL neomycin (G418). The medium was changed every 4 d, and pools of resistant transformed cells were obtained at the end of 2 wk, which were further processed to obtain single transfected cell clones.

Transfections into LAN-1 cells were performed by the $CaPO_4$ procedure using BES buffer (Sambrook et al., 1989), and the selection was performed as described above.

RNA Isolation and RNase Protection Assay

Total RNA was extracted from PC12 and LAN-1 cells utilizing RNAzol B according to the manufacturer's instructions (Cinna Biotecx Laboratories Inc.). For the analysis of RNA isolated from cells tansfected with c-*fos* constructs, the probe included the junction fragment of the human c-*fos* and tau 3'-noncoding region. For RNA isolated from PC12 cells transfected with tau constructs, the probe included the fragment containing HA flag linked to the first 250 bp of the tau coding region, thus allowing one to distinguish between the endogenous and transfected tau mRNAs. RNA probes were transcribed by T3 RNA polymerase from the blue-

script plasmids containing either tau, c-*fos*, or glyceraldehyde-phosphate dehydrogenase (GAPDH) inserts in the presence of [^{32}P] UTP 800 Ci/mmol (Amersham). Total RNA (40 μg) was annealed to 250,000 cpm of [^{32}P]-labeled antisense RNA probe for 5 min at 85°C in 80% formamide, 40 m*M* piperazine-*N*,*N*-bis-ethane-sulfonic acid (PIPES) buffer (pH = 6.4), 400 m*M* NaCl, and 1 m*M* ethylene diamine tetra-acetic acid (EDTA). Hybridization was allowed to continue for at least 16 h at 45°C. Single-stranded RNA was digested for 30 min at 4°C with 100 μg/mL of RNase A (Sigma) and 800 U/mL RNase T_1 (BRL). The RNases were inactivated by incubation with 250 μg/mL proteinase K and 0.3% sodium dodecyl sulfate (SDS) for 20 min at 37°C. The protected RNA hybrids were extracted with a phenol–chloroform–isoamyl alcohol mixture. Following ethanol precipitation, the RNA was dissolved in 80% formamide, incubated for 5 min at 85°C, and electrophoresed on a 6% acrylamide urea gel. The gel was dried, and the autoradiogram film was exposed for 1 d at –70°C using an intensifying screen. The intensity of the radioactive protected bands was analyzed by the aid of a computerized video imaging system (Biological Detection Systems, Pittsburgh, PA).

mRNA Stability Analysis

The half-lives of *fos* and tau mRNAs were measured after treatment with actinomycin D (Sigma), 5–10 μg/mL, an inhibitor of transcription. Typically, total RNA was isolated from control cells, or cells treated with actinomycine D for 5, 17, 20, or 25 h. Twenty to 40 μg of RNA were analyzed by RNase protection assay, using specific probes as described above.

Cell Fractionation and RNA Preparation

Total RNA was extracted from PC12 cells and LAN-1 cells under conditions that preserve preexisting MTs and allow the quantitative separation of in vivo assembled MTs from unassembled tubulin (Black and Baas, 1989; Litman et al., 1993). Generally, cultures were rinsed twice with Ca^{2+} and Mg^{2+} free PBS, scraped, and homogenized in buffer A: 100 m*M* PIPES buffer (pH 6.9), 0.5% Triton X-100, 1 m*M* $MgSO_4$, 25 m*M* KCl, a mixture of protease inhibitors (1 m*M* phenylmethyl sulfonyl fluoride [PMSF], 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 10 μg/mL pepstatin [Sigma]), in the presence of MT-stabilizing reagents (4 *M* glycerol, 5% v/v dimethylsulfoxide [DMSO], 10 n*M* Taxol, 1 m*M* EGTA), 20 m*M* vanadyl sulfate ribonucleoside complex (BRL), and 25 μg/mL yeast tRNA (Sigma). Following centrifugation at 12,000*g* in Eppendorf centrifuge, the supernatant was removed and centrifuged at 32,000*g* in a Beckman airfuge for 30 min at room temperature to yield assembled MTs and unassembled tubulin fractions. RNA was isolated and analyzed as described above.

Colchicine Treatment

In order to study the involvement of MTs in tau mRNA stabilization, transfected induced and noninduced PC12 and LAN-1 cells were treated with 50 μ*M* colchicine (Sigma) for the indicated time periods. At the end of incubation time, total RNA was isolated and analyzed by RNase protection assay, as described above.

Statistical Analysis

The intensity of protected fragments, as visualized with the aid of a computerized video imaging system (Biological Detection Systems), was measured and expressed as percent of control. The data are presented as the integrated intensity value multiplied by the area occupied by the signal and standarized in comparison to GAPDH signals (a protected fragment of 100 bp). For each experiment, a single exponential decay curve was used to obtain the $t_{1/2}$ value, using the mathematical function $f(x) = ae - bx$. The b value was resolved by the Sigma Plot 4.11 computer program (Jandel Scientific) and $t_{1/2}$ was calculated as the ratio of $0.69/b$. The figures and interactions of the function were obtained using the Sigma Plot 4.11 computer program. Data were analyzed by ANOVA, followed by Bartlett's test for homogeneity of variance. Bonferroni's test of multiple comparisons was applied as the post hoc test.

Results

Identification of Tau 3′UTR Region that Stabilize c-fos Reporter mRNA

Previous studies have indicated that the stability of tau mRNA is increased following NGF treatment of PC12 cells (Sadot et al., 1995). In order to

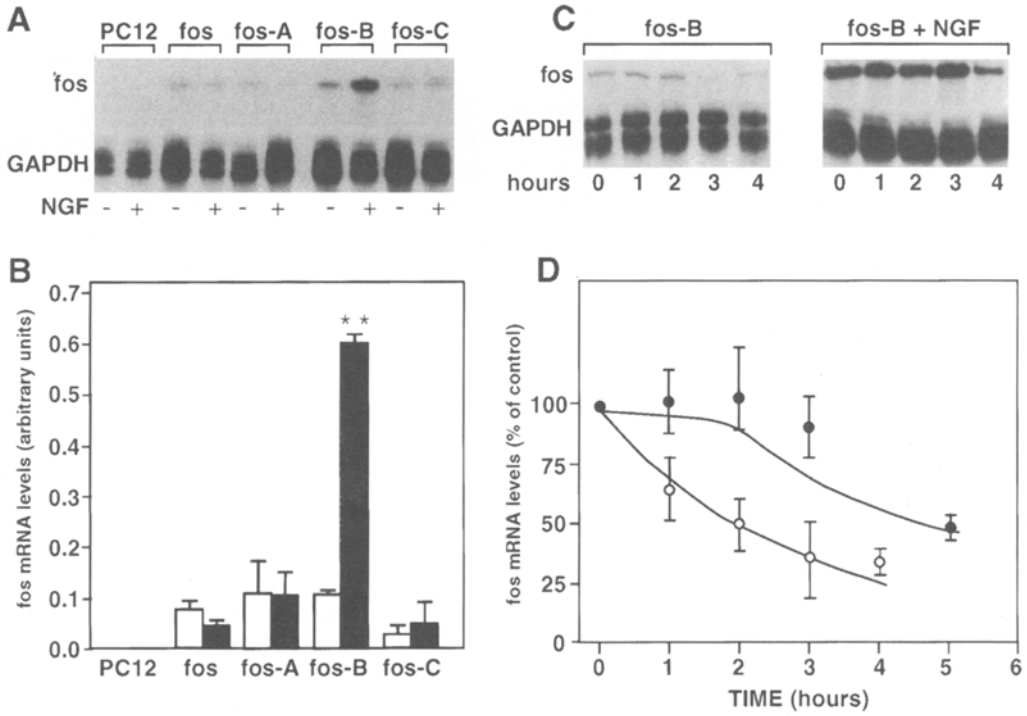


Fig. 2. The steady-state levels **(A, B)** and half-lives **(C, D)** of transfected c-*fos* mRNA constructs in PC12 cells. (A) RNase protection assay using c-*fos* as a probe. RNA extracted from control PC12 cells and from stable transfected PC12 lines with c-*fos* coding region and constructs *fos*-A, *fos*-B, and *fos*-C, untreated and treated for 6 d with NGF. The GAPDH probe (100 bp) was used as a standard for equal loading control (a representative experiment). (B) Quantitative analysis of four experiments expressed in arbitrary units. ❑ shows c-*fos* mRNA levels in noninduced cells, ■ shows the levels of c-*fos* mRNA in NGF-induced transfected cells. Data are mean ± SEM of four experiments. Asterisks mark a significant difference in tau mRNA levels ($^{**}p < 0.01$). (C) The half-life of *fos*-B mRNA in transfected PC12 lines. RNase protection assay using c-*fos* as a probe. The GAPDH probe (100 bp) was used as a standard for equal loading control (a representative experiment). (D) Quantitative analysis of four experiments expressed as percent of total RNA level present before and after the addition of the actinomycin D. The half-life of *fos*-B mRNA in induced PC12 cells was 5.8 h (●) compared to 2 h in noninduced cells (○).

identify sequence elements involved in tau mRNA stabilization, selected regions of the 3'UTR (Fig. 1) were subcloned downstream of the coding region of a human c-*fos* cDNA. C-*fos* was previously shown to have a short half-life, with $t_{1/2}$ of about 30 min both in 3T3 and PC12 cells (Curran and Morgan, 1985; Bartel et al., 1989; Haby et al., 1994), and thus, it was assumed that any stabilization effects could be detected.

Four stable lines of PC12 cells tranfected with constructs containing the c-*fos* coding region or c-*fos* linked to tau 3'UTR subregions-A (*fos*-A), B (*fos*-B), and C (*fos*-C) were isolated. The transfected PC12 cell lines expressing c-*fos* constructs did not show any morphological differences following NGF induction, as compared to control nontransfected differentiated PC12 cells (data not shown).

RNase protection assays using human c-*fos* as a probe were performed to determine the steady-state levels of c-*fos* mRNA linked to tau 3'UTRs in the stable transfected PC12 lines, untreated or treated with NGF for 6 d (Fig. 2A,B). PC12 cell lines transfected with *fos*-B, showed a significant increase of sixfold in the steady-state levels of c-*fos* mRNA following treatment with NGF. Other transfected PC12 lines with c-*fos* coding region alone, *fos*-A, or *fos*-C did not show any significant differences in the levels of c-*fos* mRNA following NGF treatment (Fig. 2B). Nontransfected PC12 cells did not show any background signal with human c-*fos* as a probe.

Fragments B and C overlap in a region H (Fig. 1), which in the following experiment was found to be sufficient for tau stabilization. However, fragment C does not show any effect on c-*fos* mRNA, which may suggest that the additional region in fragment C or the mRNA structure transcribed in these cells may interfere with or not facilitate *fos*-mRNA stabilization. These results suggested that sequences present in fragment B may affect the stability of c-*fos* mRNA in transfected PC12 cells in response to NGF induction.

The Half-Life of c-fos Linked to Tau 3′UTR mRNA in PC12 Cells

The effect of fragment B on the half-life of c-*fos* mRNA in transfected PC12 cells was tested following actinomycin D treatment of cells grown in the presence or absence of NGF. For these experiments, we chose *fos*-B-transfected PC12 cell line, since this transfected line showed maximal levels of *fos* mRNA following NGF treatment. Actinomycin D was added to NGF-treated or untreated *fos*-B transfected cells, and RNA was extracted at the indicated time-points (Fig. 2C,D). RNase protection assays were performed, and the $t_{1/2}$ of *fos*-B mRNAs were calculated from the decay curves, after normalization using GAPDH mRNA signal (which yields a protected fragment of 100 bp). The results demonstrated that in NGF-treated PC12 cells, the half-life of *fos*-B mRNA increased by more than twofold, as compared to noninduced cells, transfected with *fos*-B ($t_{1/2}$ = 5.8 h or 2 h for *fos*-B + NGF and *fos*-B respectively) (Fig. 2D). These results indicate that the sequences present in fragment B affect the half-life of c-*fos* (of $t_{1/2}$ = 30 min) (Greenberg and Ziff, 1984). Moreover, the stabilization effect is enhanced by NGF treatment. The specificity of the effect to neuronal cells was tested when nonneuronal 3T3 cells were transfected with constructs that contain c-*fos* alone and *fos*-B sequences. The half-lives of *fos*-B and *fos* mRNAs in transfected 3T3 cells treated with actinomycin D were not significantly different from endogenous *fos* mRNA ($t_{1/2}$ = 30 min) when tested by RNase protection assay (data not shown).

Identification of Sequences of 3′UTR Involved in Stabilization of Tau mRNA

To test the stabilization effect of 3′UTR sequences on tau mRNA, three constructs were prepared. All the constructs contained the HA tag sequence (30 bp) linked to the N-terminus of the tau coding region (cod), the tau coding region linked to fragment B (cod-B), which showed the maximal effect on c-*fos*, and fragment H (cod-H), subregion of fragment B (Fig. 1). Fragment H contains an AU-rich region, which was previously demonstrated to bind neuronal proteins that were enriched in the microtubule fraction (Behar et al., 1995). Recently, these proteins were identified as belonging to the Elav-like proteins involved in mRNA stabilization (Chung et al., 1997; Aranda et al., 1999).

The steady-state levels of cod, cod-B, and cod-H mRNAs in transfected PC12 cells were measured using RNase protection assays. The probe used in these experiments included the sequence of the HA tag and allowed the concomitant identification of endogenous and transfected tau mRNA of 250 and 280 bp of protected fragment, respectively (Fig. 3 A,B). The results show an increase in the steady-state levels of cod-B and cod-H mRNAs by 3.2- and 4-fold, respectively, as compared to cod signal, following NGF treatment. This increase is similar to the known induction of tau mRNA in NGF-treated PC12 cells. These results suggest that sequences present in fragment B and in H, which is part of fragment B, are involved in the stabilization of tau mRNA and are more pronounced in response to NGF induction.

The half-lives of cod, cod-B, and cod-H mRNAs in transfected PC12 cells were tested following actinomycin D treatment (Fig. 3 C,D). Actinomycin D was added to NGF-treated or untreated cells, and RNA was extracted at the indicated time-points and analyzed by RNase protection assays. The $t_{1/2}$ of tau mRNAs was calculated from the decay curves, after normalization with GAPDH mRNA signal. The results demonstrated that in noninduced PC12 cells, the half-lives of cod-B mRNA and cod-H mRNA were increased by 2- and 4.5-fold as compared to cells transfected by cod alone ($t_{1/2}$ = 3.5, 7, or 16 h for cod, cod-B, and cod-H, respectively) (Fig. 3C). After 6 d of NGF treatment, the half-life of cod-B mRNA increased to 20 h and of cod-H mRNA increased to 35 h in comparison with cod, which increased to 11 h (Fig. 3D). These results indicate that the sequences present in fragment B and in subfragment H affect the half-life of tau mRNA, which is prolonged by NGF treatment. The increase in the half-lives of the transfected

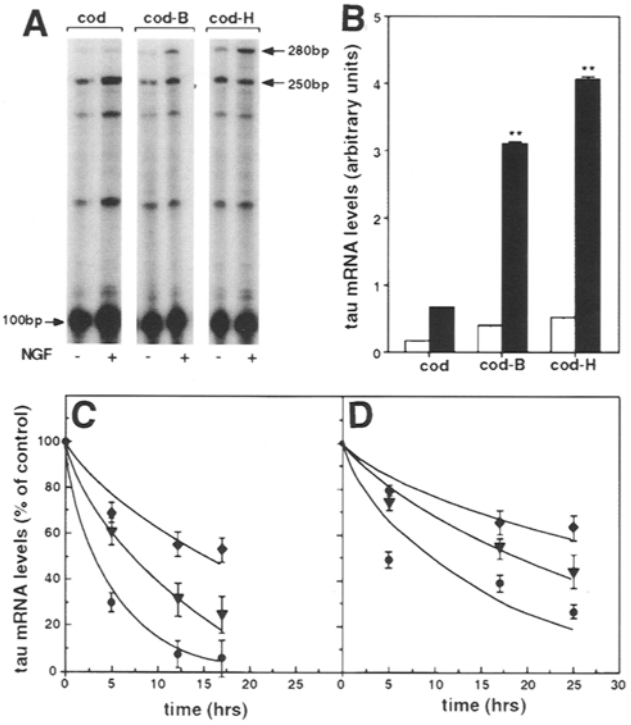


Fig. 3. The steady-state levels **(A, B)** and half-lives **(C, D)** of transfected tau mRNA constructs in PC12 cells. (A) RNase protection assay using HA-tau coding fragment as a probe. RNA extracted from stable transfected PC12 lines with cod, cod-B, and cod-H tau mRNAs constructs untreated and treated for 6 d with NGF. The GAPDH probe (100 bp) was used as a standard for equal loading control (a representative experiment). (B) Quantitative analysis of four experiments expressed in arbitrary units. ❑ Shows the transfected tau mRNAs levels innon induced cells, whereas ■ shows the levels of tau mRNAs in NGF-induced transfected cells. Data are mean ± SEM of four experiments. Asterisks mark a significant difference in tau mRNA levels. (**$p < 0.01$). (C, D) The half-life of cod (●), cod-B (▼), and cod-H (◆) tau mRNAs in transfected PC12 lines. The RNA was extracted and analyzed by RNase protection assay using HA-tau probe. Quantitative analysis of four experiments expressed as percent of total RNA level present before and after the addition of the actinomycin D. (C) The half-lives of cod, cod-B, and cod-H were 3.5, 7, and 16 h, respectively, for tau mRNAs in noninduced transfected PC12 lines, and (D) the half-lives of cod, cod-B, and cod-H were 11, 20, and 35 h, respectively, for tau mRNAs in induced transfected PC12 lines.

mRNAs may therefore contribute to the increase in their steady-state levels observed in Fig. 3A,B.

To verify that the stabilization effect is not limited to PC12 cells, the effect of the 3'UTR fragments on tau mRNA levels and half-lives was tested in LAN-1 human neuroblatoma cells, which differentiate and extend neurites following Bt_2AMP induction (Lopresti et al., 1992). LAN-1 cells were transfected with the same constructs (cod, cod-B, and cod-H), and stable transfected lines were isolated. The steady-state levels of tau mRNA in the three lines, untreated and treated for 4 d with Bt_2AMP, were measured using RNAase protection assays (Fig. 4A). The steady state levels of the three expressed mRNAs were increased following Bt_2AMP treatment. Cod-B and cod-H mRNA levels showed a significant increase of 2.5- and 3.5-fold as compared with cod alone (Fig. 4B).

The half-lives of cod, cod-B, and cod-H mRNAs were analyzed following actinomycin D treatment of transfected LAN-1 cells, untreated or treated with Bt_2AMP. The results demonstrated that in LAN-1 cells the half-lives of cod-B mRNA and cod-H mRNA increased by three- and fourfold, respectively, as compared to cells transfected with cod alone. The $t_{1/2}$ of cod, cod-B, and cod-H were 4.6, 14, and 19 h, respectively (Fig. 4C). Following induction with Bt_2AMP, the half-lives of the cod-B and cod-H mRNAs increased to 24 and 30 h in comparison with cod, which increased to 10.8 h (Fig. 4D). The similarity of the results obtained in both PC12 and LAN-1 neuronal cells demonstrates the involvement of 3'UTR in tau mRNA stabilization, regardless of the cell systems employed. Moreover, in both cell lines, tau mRNA half-life is increased following neuronal differentiation, which further confirms the involvement of tau mRNA in neurite outgrowth.

Tau mRNA Association with the MTs

Previous work using primary neuronal cell cultures demonstrated that tau mRNA is associated with the MTs (Litman et al., 1994). The following experiments were performed in order to examine whether the association with MTs, through the 3'-UTR region, in addition to the Poly(A) region, may contribute to the mechanism affecting tau mRNA stability (Figs. 5 and 6).

Cellular fractionation of noninduced and NGF-induced transfected PC12 cells into the MT (pellet)

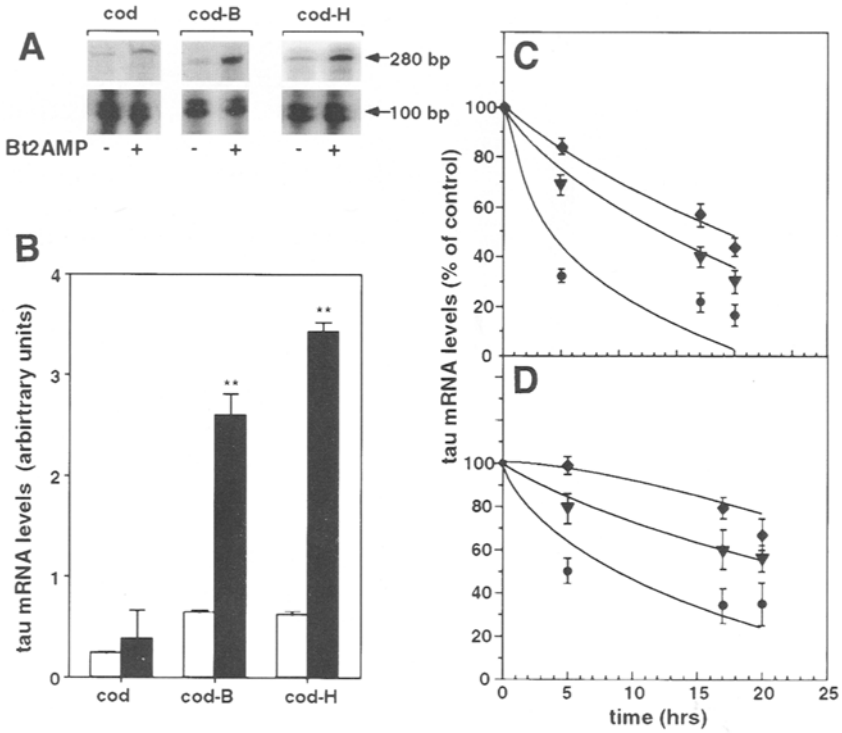


Fig. 4. The steady-state levels **(A, B)** and half-lives **(C, D)** of transfected tau mRNA constructs in LAN-1 cells. (A) RNase protection assay was performed using HA-tau probe. RNA extracted from stable transfected LAN-1 lines containing cod, cod-B, and cod-H tau mRNAs constructs untreated and treated for 4 d with Bt_2AMP. The GAPDH probe (100 bp) was used for equal loading control (a representative experiment). (B) Quantitative analysis of four experiments expressed in arbitrary units. ❑ shows transfected tau mRNAs levels in noninduced cells, whereas ■ shows the levels of tau mRNAs in Bt_2AMP-induced cells. Data are mean ± SEM of four experiments. Asterisks mark a significant difference in tau mRNA levels. (**$p < 0.01$). (C, D) The half-life of cod (●), cod-B (▼), and cod-H (◆) tau mRNAs in transfected LAN-1 lines. RNase protection assay was performed using HA-tau probe. Quantitative analysis of four experiments expressed as percent of total RNA level present before and after the addition of the actinomycin D. (C) The half-lives of cod, cod-B, and cod-H were 4.6, 14, and 19 h, respectively, for tau mRNAs in noninduced transfected LAN-1 lines, and (D) the half-lives of cod, cod-B, and cod-H were 10.8, 24, and 30 h, respectively, for tau mRNAs in induced transfected LAN-1 lines.

and cytoplasmic (supernatant) fractions was performed under the fractionation condition, which preserves the cellular assembled MT fraction. RNA was extracted from the corresponding MTs and cytoplasmic fractions and analyzed by RNase protection assay using tau-specific probes. Tau mRNA was found primarily in the MT fraction in all the cell lines tested, regardless of their state of differentiation as compared to GAPDH (Fig. 5A), which is found mostly in the soluble supernatant fraction (Burgin et al., 1990; Litman et al., 1994). These results are in accord with our previous experiments, which demonstrated the association of tau mRNA with microtubules of neuronal cells (Litman et al., 1994).

A quantitative analysis of the proportions of tau mRNA transcribed from constructs containing fragments B or H, isolated from the supernatant-soluble and assembled MTs fractions, is shown in Fig. 5B. In all the experiments performed, between 70 and 95% of tau mRNA was found in the pellet fraction. These results indicated that in all transfected PC12 cell lines, tau mRNA is associated with the MT system, and the level of tau mRNA associated with the pellet fraction is higher in cells transfected with constructs containing fragment B or H as compared to the cells transfected with a construct containing the coding region only. Moreover, the fraction associated with MTs is increased fol-

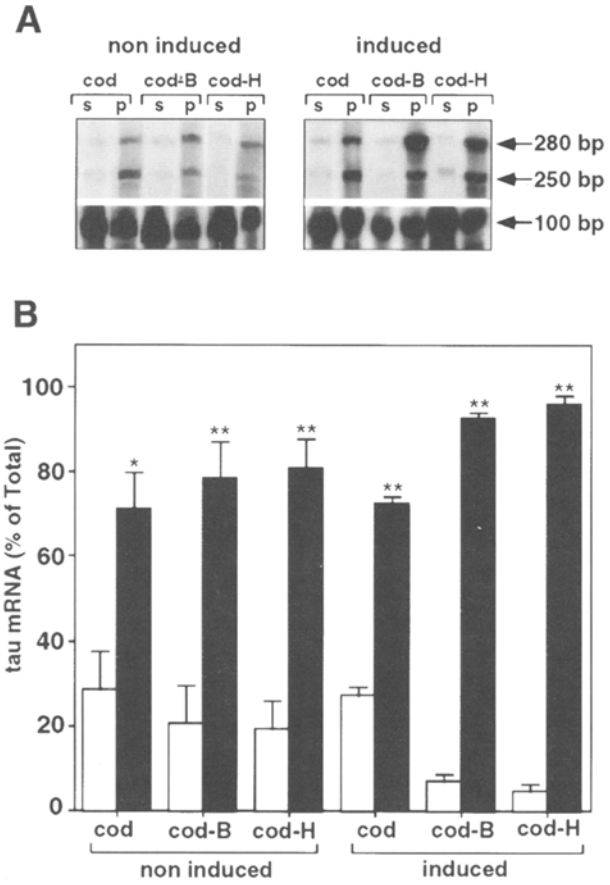


Fig. 5. Association of transfected cod, cod-B, and cod-H tau mRNAs with the MT fraction prepared from PC12 cells. **(A)** RNase protection assay was performed on RNA isolated from soluble supernatant (S) and assembled MTs pellet (P) fractions isolated from PC12 cells transfected with cod, cod-B, and cod-H constructs untreated or treated for 6 d with NGF. The GAPDH probe (100 bp) was used for equal loading control (representative experiment). **(B)** Quantitative analysis of four experiments expressed as percent of total RNA level. ❑ Shows the level of transfected tau RNAs isolated from soluble supernatant, whereas ■ shows the levels of tau mRNA isolated from assembled MTs pellet fraction. Data are mean ± SEM of four experiments. Asterisks mark a significant difference of the tau mRNA found in the pellet as compared to the supernatant fraction ($*p < 0.05$; $**p < 0.01$).

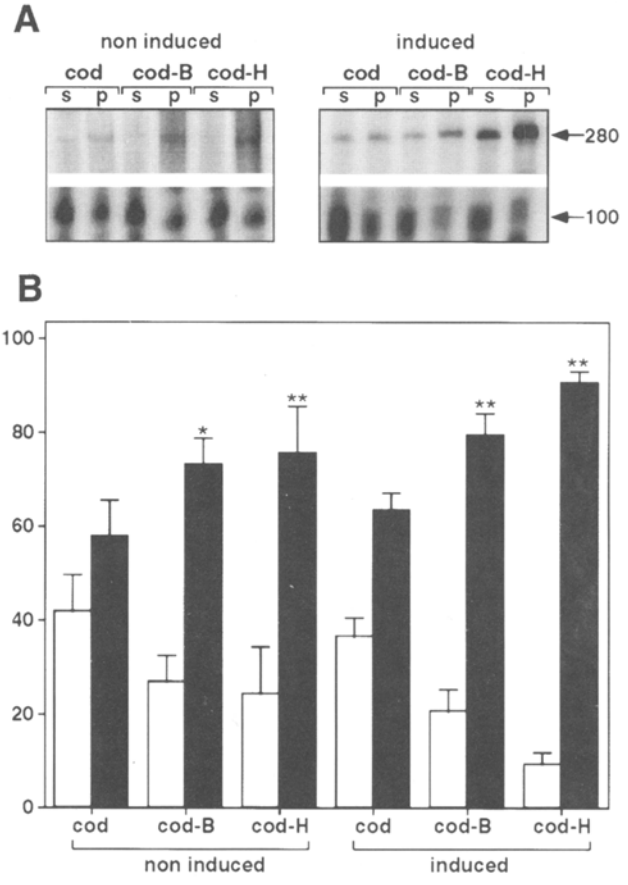


Fig. 6. Association of transfected cod, cod-B, and cod-H tau mRNAs with the MT fraction prepared from LAN-1. **(A)** RNase protection assay was performed on RNA isolated from soluble supernatant (S) and assembled MTs pellet (P) fractions isolated from LAN-1 cells transfected with cod, cod-B, and cod-H constructs untreated or treated for 4 d with Bt_2AMP. The GAPDH probe (100 bp) was used for equal loading control (represantative experiment). **(B)** Quantitative analysis of four experiments and expressed as percent of total RNA level. ❑ Shows the level of transfected tau RNAs isolated from soluble supernatant, whereas ■ shows the levels of tau mRNA isolated from assembled MTs pellet fraction. Data are mean ± SEM of four experiments. Asterisks mark a significant difference of the tau mRNA found in the pellet as compared to the supernatant fraction ($*p < 0.05$; $**p < 0.01$).

lowing NGF treatment, as demonstrated in a representative experiment shown in Fig. 5A. These results are in agreement with the results shown in Fig. 3, indicating increased steady-state levels and half-lives of cod-B and cod-H tau mRNAs in transfected PC12 cell lines following NGF-induced differentiation.

Similar results were observed in transfected LAN-1 cell lines (Fig. 6A, B). The results indicate

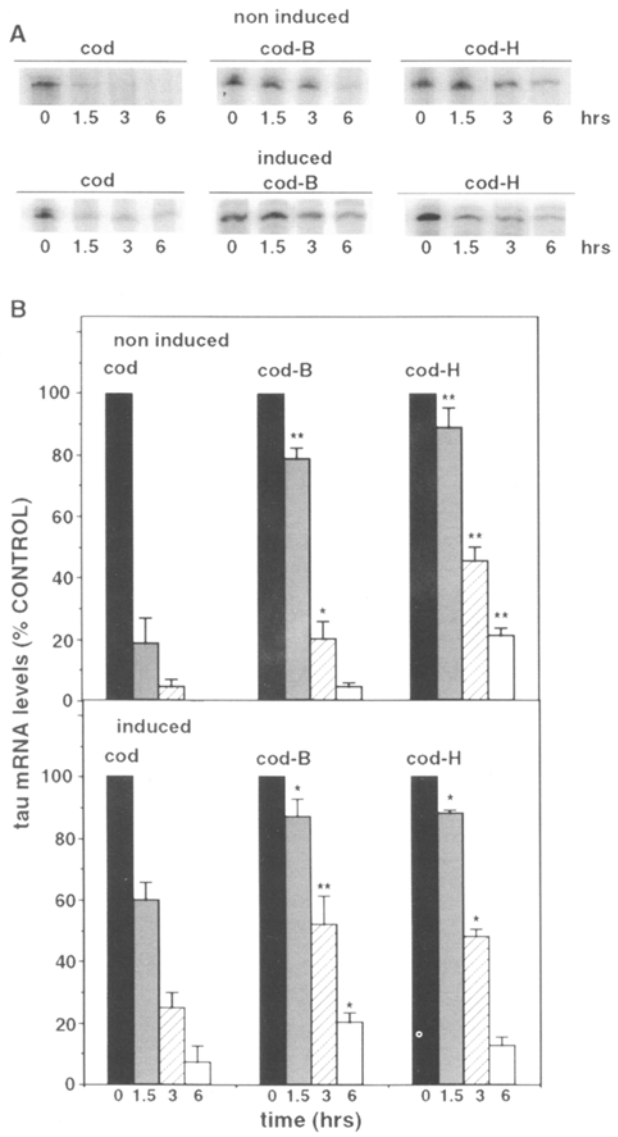


Fig. 7. Effect of colchicine treatment on tau mRNA levels in transfected LAN-1 cells. Induced and noninduced transfected LAN-1 cells with tau constructs containing the cod, cod-B, and cod-H were treated with colchicine for 1.5, 3, and 6 h. **(A)** RNase protection assay was performed using HA-tau probe. **(B)** Quantitative analysis of four experiments expressed as percent of total RNA level present before and after the addition of the colchicine. Data are mean ± SEM of four experiments showing a significant difference in tau mRNA levels following treatment with colchicine for the specified time (hours). Asterisks mark a significant difference (*$p < 0.05$; **$p < 0.01$).

that the total level of tau mRNA is increased following induction (Fig. 6A), and a higher proportion is associated with the MT fraction (Fig. 6B). These experiments are in agreement with the results shown in Fig. 4. which showed increased steady-state levels and half-lives for the constructs, including fragment B or H. In addition, the proportion of tau mRNA associated with MT fraction is higher in both PC12 and LAN-1 cells transfected with cod-H construct, which may suggest a higher affinity between the mRNA species and MTs, beyond the association via the Poly(A) tail, similar to our previous findings for endogenous tau mRNA in primary neuronal cell cultures (Litman et al., 1994).

To analyze further the protective effect of the association on tau mRNA with MTs, transfected LAN-1 cells were treated with colchicine, which depolymerizes the MTs. Cells were treated for the indicated time periods with colchicine, and the levels of tau mRNAs were tested by RNase protection assays and normalized to GAPDH mRNA signals (Fig. 7).

The results demonstrate that treatment with colchicine reduces the levels of tau mRNAs in all the three LAN-1-transfected cell lines tested. In noninduced LAN-1 cells transfected with coding region only, colchicine treatment caused rapid decrease of the tau mRNA levels (after 1.5 and 3 h), whereas in cells transfected with cod-B or cod-H, the messages levels were more stable by four to five times. After 6 h of colchicine treatment, the signal of tau coding mRNA disappeared completely (Fig. 7A), but in cells transfected with cod-B or cod-H, a residual level was observed after 3 or 6 h of treatment. In induced LAN-1 cells following 1.5, 3, and 6 h of colchicine treatment, the levels cod-B and cod-H were 2–2.5 times higher as compared to the level observed in cells transfected with cod alone (Fig. 7 B). These results demonstrate that treatment with colchicine reduces the levels of tau mRNAs in all cell lines tested. However, the 3′-UTR sequences of fragment B and H of 3′UTR render the messages more stable, even under the depolymerization conditions of MT, and thus suggest that these regions play a role in the association with MT.

Discussion

Tau, a neuronal MAP, plays an important role in the formation and maintenance of neuronal polar-

ity. The expression of tau is regulated transcriptionally (Sadot et al., 1996) and posttranscriptionally by stabilization and subcellular localization of tau mRNA. We have previously demonstrated that the tau gene promoter is responsive to NGF stimulation in PC12 cells (Sadot et al., 1996). However, the increased levels of tau mRNA and proteins observed during neurite outgrowth (Drubin et al., 1988; Caceres and Kosik, 1990) are also contributed by tau mRNA stabilization (Sadot et al., 1995). In the present study, we have identified the region in the tau 3'UTR that affects tau mRNA stability. We have shown that fragment B derived from tau 3'UTR confers stability to heterologous c-*fos* when used as a reporter mRNA, in transfected PC12 cells (Fig. 2). Similar stabilization effects was observed when this region, which contains 1400 bp and is about one-third of the total 3'UTR, was linked to the tau coding region and transfected into PC12 cells. Further experiments demonstrated that the shorter region of 240 nucleotides fragment-H is sufficient for tau mRNA stabilization (Figs. 3 and 4). This effect is not limited to NGF-induced transfected PC12 cells, since similar tau mRNA stabilization was observed in Bt_2AMP-induced transfected LAN-1 cells (Figs. 5 and 6). Our results also demonstrate that tau mRNA is associated with the MTs and that this association is more pronounced in cells transfected with constructs that contain fragment B or H (Figs. 5–7). These results are in line with our previous data, which demonstrated that tau mRNA is associated with neuronal MTs (Litman et al., 1993). The association of tau mRNA with neuronal MTs was of a tighter nature than that observed for tubulin, neurofilament, and GAPDH mRNAs, which may be bound through their Poly(A) tails to the cytoskeleton (Litman et al., 1993, 1994).

Fragment H is part of the tau 3'UTR associated with tau mRNA axonal localization, and was previously shown to be involved in binding of trans-acting neuronal protein factors (Behar et al., 1995). Recently, we have identified the 43-dDa RNA binding protein as a member of the Elav-like protein family, which binds to the AU-rich domain located in this fragment (Aranda and Ginzburg, 1999) and is implicated in mRNA stabilization in vivo (Fan and Steitz, 1998). The levels of these proteins are increased in PC12 on NGF-induced differentiation, which correlates well with the increased stabilization of tau mRNA observed on neuronal differentiation (Dobashi et al., 1998). The present data define the *cis*-acting sequences located in the tau 3'UTR, which are involved in stabilization of mRNA, and this region contains the AU-rich region. Indeed, for tau mRNA, which is targeted into the proximal segment of the axon (Litmann et al., 1993), the process of RNA stabilization is an important control step, as a prerequisite for its translocation.

Recent studies have demonstrated a wide spectrum of functions, including subcellular localization and stabilization, mediated by the 3'UTR of mRNAs (Jackson, 1993; St. Johnston, 1995; Jacobson and Peltz, 1996; Gao, 1998). Several different mechanisms have been proposed for eukaryotic mRNA degradation (Beelman and Parker, 1995), which are initiated by deadenylation followed by binding of *trans*-acting proteins to AU-rich elements (Chen and Shyu, 1995; Alonso et al., 1997) that may designate the RNA for rapid degradation. However, our experiments suggest that the region that contains an AU-rich domain is involved in tau mRNA stabilization. Thus, the binding of the proteins to the AU-rich domain may restrict access of the degradation machinery to the mRNA in a regulated manner. Recent in vivo studies relate the U-rich regulatory signals to stabilization of target mRNAs (Hamalainen et al., 1985; Jain et al., 1997; Fan and Steitz, 1998; Peng et al., 1998).

In addition to the AU-rich domains, pyrimidine-rich domains, which were suggested to act as stability determinants, are found in tau fragment H (Holcik and Liebhaber, 1997). These pyrimidine-rich clusters conform to the concensus sequence $(C/U)CCAN_xCCC(U/A)Py_xUC(C/U)CC$, which was observed in the highly stable mRNAs encoding for α-globin, lipoxygenase, α-collagen, and tyrosine hydroxylase. However, the nature of the binding proteins to this region in tau is not yet clear.

Computer simulation of RNA folding of the 240 bp of tau mRNA has revealed a stable secondary structure around the AU-rich motif (data not shown). Similar secondary structures correlating with mRNA stability have been demonstrated for ribonucleotide reductase R2 mRNA (Amara et al., 1994), α-globin mRNA (Wisdom and Lee, 1991), and α_2-adrenergic receptor mRNA (Yang et al., 1997). Several studies have shown that AU-rich elements, containing distinct sequence motifs, regulate mRNA stability (Chen and Shyu, 1995; Chung

et al., 1997). The heterogeneity in both AU-rich and pyrimidine-rich motifs reinforces the idea that the secondary structure of the 3'UTR is important, and probably the combination of the motifs that bind *trans*-acting proteins in a tissue-specific manner is the functional entity.

The involvement of the cytoskeleton in mRNA localization and its function in protection against degradation has been demonstrated (Bogucka-Glotzer and Ephrussi, 1996; Bassell and Singer, 1997). Electron microscopy studies, as well as the application of specific drugs that disrupt the cellular cytoskeleton, have identified that in neurons the majority of Poly(A) mRNAs colocalize with MTs. This is in contrast to fibroblast cells where the large proportion of Poly(A) mRNA associates with actin filaments (Bogucka-Glotzer and Ephrussi, 1996; Bassell and Singer, 1997). We have previously demonstrated the association of tau mRNA with the MTs, and in this study, we have demonstrated the contribution of tau 3'UTR to this association (Figs. 5–7). The binding of *cis*-sequences located in 3'UTR region may play an additional role beyond binding of the Poly(A) region, which is important for the stabilization, translocation, and/or anchoring of specific mRNAs. The binding of the complex may involve additional proteins to form the RNA-protein particles, which were recently described in the RNA localization pathway (Bogucka-Glotzer and Ephrussi, 1996). These particles are localized in close proximity to MTs and may vary in their protein composition between subcellular domains, i.e., axons and dendrites of neuronal cells (Antic and Keen, 1998).

In addition to tau mRNA, the posttranscriptional regulation by mRNA stabilization has been demonstrated for several neuronal genes. These include growth-associated protein (GAP43) (Kohn et al., 1996; Tsai et al., 1997; Chung et al., 1997), neurofilament (Ikenaka et al., 1990), tyrosine hydroxylase (Grima et al., 1985; Czyzyk-Krzeska and Beresh, 1996), and amyloid precursor protein (Zaidi and Maher, 1994). A common sequence motif residing in the 3'UTR of these mRNA includes a uridine-rich regulatory element, which may bind tissue-specific RNA binding proteins that were recently observed both in the nucleus and in the cytoplasm. These proteins may be involved in RNA splicing in the nucleus, whereas their cytoplasmic location may suggest additional roles, such as control of mRNA stability and/or translation (Good, 1997). Since this effect is frequently induced by various growth factors, we hypothesize that subtle changes in protein levels may be controlled posttranscriptionally by mRNA stabilization and thus may play a role in neuronal differentiation and plasticity.

The finding that tau MAP is the major structural components of neurofibrillary tangles, a hallmark lesion in Alzheimer's brain, has stimulated molecular studies to decipher the regulation of tau expression and segregation in neuronal cells (Geodert et al., 1991; Kosik, 1994). Tau MAP is a neuronal specific protein located primarily in the axon. It has been documented that tau protein levels are higher in Alzheimer's disease brains than in age-matched control brains (Khatoon et al., 1992). This increase, which may result from changes in the transcription of the gene and/or the stability of the message, cause tau protein to lose its axonal localization, i.e., tau protein can also be detected in the somatodendritic compartment (Bancher et al., 1989; Goedert et al., 1991). Thus, a misregulation of the factors that control tau expression may cause the MT breakdown and cell death observed in Alzheimer brain.

Acknowledgments

This work was supported by a grant from the Ministry of Science Israel and the French Ministry of Research and Technology. We acknowledge the advice and help of L. Behar throughout this project.

References

Alonso A. D. C., Grundke-Iqbal I., Barra H. S., and Iqbal K. (1997) Abnormal phosphorylation of tau and mechanism of Alzheimer neurofibrillary degeneration: Sequestering of MAP1 and 2 and the dissasembly of microtubules by the abnormal tau. *Proc. Natl. Acad. Sci. USA* **94,** 298–303.

Amara F. M., Chen F. Y., and Wright J. A. (1994) Phorobol ester modulation of a novel cytoplasmic protein binding activity at the 3'-untranslated region of mammalian ribonucleotie reductase R2 mRNA and role in message stability. *J. Biol. Chem.* **269,** 6709–6715.

Antic D. and Keen J. D. (1998) Messenger ribonucleoprotein complexes containing human ELAV pro-

teins: interactions with cytoskeleton and translational apparatus. *J. Cell Sci.* **111,** 183–197.

Aranda G. E., Behar L., Chung S., Furneaux H., and Ginzburg I. (1999) Embryonic lethal abnormal vision-like RNA-binding proteins regulate neurite outgrowth and tau expression in PC12 cells. *J. Neurosci.* **19,** in press.

Aviv H., Voloch Z., Bastos R., and Levy S. (1976) Biosynthesis and stability of globine mRNA in cultured erthroleukemic friend cells. *Cell* **8,** 495–503.

Bancher C., Brunner C., Lassmann H., Budka H., Jellinger K., Wiche G., et al. (1989) Accumulation of abnormally phosphorylated tau precedes the formation of neurofibrillary tangles in Alzheimer's disease. *Brain Res.* **447,** 90–99.

Bartel D. P., Sheng M., Lau L. F., and Greenberg M. E. (1989) Growth factors and membrane depolarization activate distinct programs of early response gene expression: dissociation of fos and jun induction. *Genes Dev.* **3,** 304–313.

Bassell G. and Singer R. H. (1997) mRNA and cytoskeletal filaments. *Curr. Opinion Cell Biol.* **9,** 109–115.

Beelman C. A. and Parker R. (1995) Degradation of mRNA in eukaryotes. *Cell* **81,** 179–183.

Behar L., Marx R., Sadot E., Barg J., and Ginzburg I. (1995) Cis-acting signals and trans-acting proteins are involved in tau mRNA targeting into neurites of differentiating neuronal cells. *Int. J. Dev. Neurosci.* **13,** 113–127.

Black M. M. and Baas P. W. (1989) The basis of polarity in neurons. *Trends Neurosci.* **12,** 211–214.

Bogucka-Glotzer J. and Ephrussi A. (1996) mRA localization and the cyoskeleton. *Cell Dev. Biol.* **7,** 357–365.

Brewer G. (1991) An A+U-rich element RNA-binding factor regulates c-myc mRNA stability in vitro. *Mol. Cell. Biol.* **11,** 2460–2466.

Burgin K. E., Waxham M. N., Rickling S., Westgate S. A., Mobley W. C., and Kelly P. T. (1990) In situ hybridization histochemistry of Ca2+/calmodulin-dependent protein kinase in developing rat brain. *J. Neurosci.* **10,** 1788–1798.

Caceres A. and Kosik S. (1990) Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons. *Nature* **343,** 461–463.

Casey J. L., Koeller D. M., Ramin V. C., Klausner R. D., and Harford J. B. (1989) Iron regulation of transferrin receptor mRNA levels requires iron-responsive elements and a rapid turnover determinant in the 3' untranslated region of the mRNA. *EMBO J.* **8,** 3693–3699.

Chen C.-Y. A. and Shyu A.-B. (1995) AU-rich elements: characterization and importance in mRNA degradation. *TIBS* **20,** 465–470.

Chung S., Perrone-Bizzozero N., Kohn D. T., and Furneaux H. (1997) The Elav-like proteins bind to a conserved regulatory element in the 3'UTR of GAP-43 mRNA. *J. Biol. Chem.* **272,** 6593–6598.

Crosby S. D., Puetz J. J., Simburger K. S., Fahrner T. J., and Milbrandr J. (1991) The early response gene NGFI-C encodes a zinc finger transcriptional activator and is a member of the GCGGGGGCG (GSG) element-binding protein family. *Mol. Cell. Biol.* **11,** 3835–3841.

Curran T. and Morgan J. I. (1985) Superinduction of c-fos by nerve growth factor in the presence of peripherally active benzodiazepines. *Science* **229,** 1265–1268.

Czyzyk-Krzeska M. F. and Beresh J. E. (1996) Characterization of the hypoxia-inducible protein binding site within the pyrimidine-rich tract in the 3'-untranslated region of the tyrosine hydroxylase mRNA. *J. Biol. Chem.* **271,** 3293–3299.

D'arcangelo G. and Halegoua S. (1993) A branched sighaling pathway for nerve growth factor is revealed by src-, ras-, and raf-mediated gene inductions. *Mol. Cell Biol.* **13,** 3146–3155.

Dobashi Y., Mitsuhiko S., Wakata Y., and Kameya T. (1998) Expression of HuD protein is essential for initial ohase of neuronal differentiation in PC12 cells. *Biochem. Biophys. Res. Commun.* **244,** 226–229.

Drubin D. G., Kobayashi S., Kellog D., and Kirschner M. W. (1988) Regulation of microtubule protein levels during cellular morphogenesis in nerve growth-factor treated PC12 cells. *J. Cell Biol.* **106,** 1583–1591.

Fan X. C. and Steitz J. A. (1998) Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases the in vivo stability of ARE-containing mRNAs. *EMBO J.* **17,** 3448–3460.

Gao F.-B. (1998) Messenger RNAs in dendrites: localization, stability, and implications for neuronal function. *BioEssays* **20,** 70–78.

Goedert M., Sisodia S. S., and Price D. L. (1991) Neurofibrillary tangles and b-amyloid deposits in Alzheimer's Disease. *Curr. Opinion Neurobiol.* **1,** 441–447.

Good P. (1997) The role of elav-like genes, a conserved family encoding RNA-binding proteins, in growth and development. *Semin. Cell Dev. Biol.* **8,** 557–584.

Greenberg M. E. and Ziff E. B. (1984) Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene. *Nature* **311,** 433–438.

Greene L. A. and Tischler A. (1976) Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *Proc. Natl. Acad. Sci. USA* **73,** 2424–2428.

Grima B., Lamouroux A., Blanot F., Biguet N. F., and Mallet J. (1985) Complete coding sequence of rat tyrosine hydroxylase mRNA. *Proc. Natl. Acad. Sci. USA* **82,** 617–621.

Haby C., Lisovoski F., Aunis D., and Zwiller J. (1994) Stimulation of the cyclic GMP pathway bo NO induces expression of the immediate early genes c-fos and junB in PC12 cells. *J. Neurochem.* **62,** 496–501.

Hamalainen L. O., Karinen J., and Kivirikko K. (1985) Synthesis and degradation of type 1 procollagen mRNA in cultured human skin fibroblasts and the effect of cortisol. *J. Biol. Chem.* **260,** 720–726.

Holcik M. and Liebhaber S. A. (1997) Four highly stable eukaryotic mRNAs assemble 3′ untranslated region RNA-protein complexes sharing cis and trans components. *Dev. Biol.* **94,** 2410–2414.

Ikenaka K., Nakahira K., Takayama C., Wada K., Hatanaka H., and Mikosiba K. (1990) Nerve growth factor rapidly induces expression of the 68-kDa neurofilament gene by posttranscriptional modification in PC12h-R cells. *J. Biol. Chem.* **265,** 19,782–19,785.

Jackson R. J. (1993) Cytoplasmic regulation of mRNA function: the importance of the 3′ untranslated region. *Cell* **74,** 9–14.

Jacobson A. and Peltz S. W. (1996) Interrelationships of the pathway of mRNA decay and translation in eukariotic cells. *Ann. Rev. Biochem.* **65,** 693–739.

Jain R. G., Andrews L. G., McGowan K. M., Pekala P. H., and Keene J. D. (1997) Ectopic expression of Hel-N1, an RNA binding protein, increases glucose transporter (GLUT1) expression in 3T3-L1 adipocytes. *Mol. Cell. Biol.* **17,** 954–962.

Khatoon S., Grundek-Iqbal I., and Iqbal K. (1992) Brain levels of mircotubule-associated protein tau are elevated in Alzheimer's disease: a radioimmuno-slot-blot assay for nanograms of the protein. *J. Neurochem.* **59,** 750–753.

Kohn D. T., Tsai K.-C., Cansino V. V., Neve R. L., and Perrone-Bizzozero N. I. (1996) Role of highly conserved pyrimidine-rich sequences in the 3′ untranslated region of the GAP-43 mRNA in mRNA stability and RNA-protein interactions. *Mol. Brain Res.* **36,** 240–250.

Kosik K. S., Orecchio L. D., Bakalis S., and Neve R. L. (1989) Developmentally regulated expression of specific tau sequences. *Neuron* **2,** 1389–1397.

Kosik K. S. (1994) The Alzheimer's disease Sphinx: A riddle with plaques and tangles. *J. Cell Biol.* **127,** 1501–1504.

Kosik K. S. (1997) *Brain Microtubule Associated Protein* (Avila J., Brandt R., and Kosik K. S., eds.). Academic, Harwood, pp. 43–52.

Leibold E. A. and Munro H. N. (1988) Cytoplasmic protein binds in vitro to a highly conserved sequence in the 5′ untranslated region of ferritin heavy- and light-subunit mRNAs. *PNAS* **85,** 2171–2175.

Li X. A. and Beebe P. C. (1990) Messenger RNA stabilization in chicken lens development: a re-examination. *Dev. Biol.* **146,** 239–241.

Litman P., Barg J., Rindzoonski L., and Ginzburg I. (1993) Subcellular localization of tau mRNA in differentiating neuronal cell culture: implications for neuronal polarity. *Neuron* **10,** 627–638.

Litman P., Barg J., and Ginzburg I. (1994) Microtubules are involved in the localization of tau mRNA in primary neuronal cell culture. *Neuron* **13,** 1463–1474.

Lodish H. F. and Small B. (1976) Different life times of reticulocyte messenger RNA. *Cell* **7,** 59–69.

Lopresti P., Poluha W., Poluha D. K., Drinkwater E., and Ross A. H. (1992) Neuronal differentiation triggered by blocking cell proliferation. *Cell Growth Differ.* **3,** 627–635.

Maatta a., Ekholm E., and Penttinen R. P. (1995) Effect of the 3′ untranslated region on the expression level and mRNA stability of a1 (1) collagen gene. *Biochim. Biophys. Acta* **1260,** 294–300.

Mangin G., Couchie D., Charriere-Bertrand C., and Nunez J. (1989) Timing of expression of t and its encoding mRNAs in the developing cerebral neocortex and cerebellum of the mouse. *J. Neurochem.* **53,** 45–50.

Parker R. and Jacobson A. (1990) Translation of a 42-nucleotide segment within the coding region of the mRNA encoded by the MATa1 gene are involved in promoting rapid mRNA decay in yeast. *Proc. Natl. Acad. Sci. USA* **87,** 2780–2784.

Peng S. S., Chen C. Y., Xu N., and Shyu A. B. (1998) RNA stabilization by the AU-rich element binding protein, HuR, an ELAV protein. *EMBO J.* **17,** 3461–3470.

Rajagopalan L. E., Westmark C. J., Jarzembowski J. A., and Malter J. S. (1998) hnRNP C increases amyloid precursor protein (APP) production by stabilizing APP mRNA. *Nucleic Acids Res.* **26,** 3418–3423.

Sadot E., Marx R., Barg J., Behar L., and Ginzburg I. (1994) Complete sequence of 3′-untranslated region

of tau from rat central nervous system. *J. Mol. Biol.* **241,** 325–331.

Sadot E., Barg J., Rasouly D., Lazarovici P., and Ginzburg I. (1995) Short- and long-term mechanisms of tau regulation in PC12 cells. *J. Cell Sci.* **108,** 2857–2864.

Sadot E., Heicklen-Klein A., Barg J., Lazarovici P., and Ginzburg I. (1996) Identification of a tau promoter region mediating tissue-specific-regulated expression in PC12 cells. *J. Mol. Biol.* **256,** 805–812.

Sambrook J., Fritsch E. F., and Maniatis T. (1989) *Molecular Cloning. A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Shyu A.-B., Belasco J. G., and Greenberg M. E. (1991) Two distinct destbilizing elements in the c-fos message trigger deadenylation as a first step in rapid mRNA decay. *Gene Dev.* **5,** 221–231.

St. Johnston D. (1995) The intracellular localization of messenger RNAs. *Cell* **81,** 161–170.

Tsai K.-C., Cansino V. V., Kohn D. T., Neve R. L., and Perrone-Bizzozero N. I. (1997) Post-transcriptional regualtion of the GAP-43 gene by specific sequences in the 3' UTR of the mRNA. *J. Neurosci.* **17,** 1950–1958.

Wang S., Browning K. S., and Miller W. A. (1996) A viral sequence in the 3'-untranslated region mimics a 5' cap in facilitating translation of uncapped mRNA. *EMBO J.* **16,** 4107–4116.

Wang X. and Leibhaber S. A. (1996) Complementary change in cis determinants and trans factors in the evolution of an mRNA stability complex. *EMBO J.* **15,** 5040–5051.

Weiss I. M. and Liebhaber S. A. (1994) Erythroid cell-specific determinants of a-globin mRNA stability. *Mol. Cell. Biol.* **14,** 8123–8132.

Wisdom R. and Lee W. (1991) The protein-coding region of c-myc mRNA contains a sequence that specifies rapid mRNA turnover and induction by protein synthesis inhibitors. *Genes Dev.* **5,** 232–243.

Yang Q., McDermott P. J., Duzic E., Pleij W. A., Sherlock J. D., and Lanier S. M. (1997) The 3'-untranslated region of the a_{2c}-adrenergic receptor mRNA impedes translation of the receptor of the receptor message. *J. Biol. Chem.* **272,** 15,466–15,473.

Zaidi S. H. E. and Malter J. S. (1994) Amyloid precursor protein mRNA stability is controlled by a-29 base element in the 3'-untranslated region. *J. Biol. Chem.* **269,** 24,007–24,013.