Binding of a protein to an AU-rich domain of tumour necrosis factor α mRNA as a 35 kDa complex and its regulation in primary rat astrocytes

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Newcastle disease virus (NDV) induces tumour necrosis factor α (TNF α) gene transcription and increases the mRNA stability. NDV stabilizes TNF α mRNA by preventing poly(A) shortening in a protein kinase C-dependent manner. TNF α 3'-untranslated region (UTR) contains an AU-rich domain (ARD) with seven AUUUA pentamers, a motif implicated in poly(A) removal and mRNA degradation. In this report, protein binding to TNF α ARD and the effects of NDV and kinases on ARD-binding activity were investigated in primary rat astrocytes. Both nuclear and cytoplasmic extracts contained proteins binding to centrally located 27 nt AUUUAUUUUUUUUUUUUUUUUUUUAUUA, within TNF α ARD. Portions of ARD with a single AUUUA did not show ARD-binding activity. The ARD-protein complexes migrated as two bands on electrophoretic mobility-shift assay. The slower moving complexes appeared either as a broader

band or doublets. The UV cross-linked ARD-protein complexes, however, migrated as a single 35 kDa band on SDS/PAGE. In cytoplasmic extracts treated with alkaline phosphatase there was a decrease in the faster moving complex and an increase in the slower moving complex, whereas NDV infection produced the reverse effect. In addition, the faster moving complex was decreased when cytoplasmic extracts from NDV-infected cells were treated with protein phosphatase 1 or 2A. Neither NDV infection nor phosphatase treatment affected the mobility pattern of nuclear extracts. The data indicate that a protein of molecular mass less than 35 kDa binds to a segment of TNF α ARD containing primarily UUAUUUAUU motifs, and the ARD-binding activity in cytoplasmic compartment is post-transcriptionally modified.

INTRODUCTION

The steady-state level of mRNA accumulation is determined from the rate of its biosynthesis and degradation. Stability of mRNA can be regulated as a physiological response within the cell [1–3] and also by external stimuli, such as Ca²⁺, hormone, cytokine, virus and inflammatory mediators [4–11]. Although mechanisms involved in post-transcriptional mRNA regulation are complex, the mRNA itself contains sequence-specific information which determines its stability [reviews, 12–14]. A group of unstable mRNAs with a half-life ($t_{1/2}$) of approx. 15–60 min, which include mRNAs of oncogenes and cytokines, carries AU-rich domains (ARDs) in their 3'-untranslated region (UTR) [12–14]. Single or multiple copies of ARD function as the mRNA-stabilizing element, as shown by *in vivo* and *in vitro* experiments using deletion and mutation analysis of the RNA, as well as by utilizing chimaeric gene constructs [15–18].

We have previously shown that infection of primary astrocytes by Newcastle disease virus (NDV) induces tumour necrosis factor α (TNF α) gene transcription and stabilization of TNF α mRNA with prolonged $t_{1/2}$ [8,19]. Whereas protein kinase C (PKC) inhibitors partially inhibit TNF α gene transcription, the mRNA stabilization mediated by NDV is effectively abolished by various PKC inhibitors in astrocytes [8,19–21]. Kinase-dependent stability of TNF α mRNA was also shown in human monocytes where treatment with okadaic acid to inhibit protein phosphatases led to TNF α mRNA stabilization [22]. We have also demonstrated that the regulated step in PKC-dependent

TNF α mRNA stabilization involves deadenylation of the poly(A) tail [21]. The poly(A) tail is protected from deadenylation by poly(A)-binding protein, and poly(A) shortening is believed to be required for endo- or exo-nucleases to attack the mRNA for degradation [23–26]. Poly(A) deadenylation and mRNA degradation can be regulated by sequence-specific elements within ARD [27,28], and the nonamer UUAUUUAUU appears to be the key AU-rich sequence motif that mediates mRNA degradation [29].

ARD-binding proteins of molecular mass between 16 and 65 kDa have been identified that can bind to a variety of AU-rich elements present in unstable mRNAs, such as those encoding interferon β , TNF α , interleukin (IL)-2, c-Fos, c-Jun, c-Myc and granulocyte-macrophage colony-stimulating factor [9,30–36]. In addition to the ARD in regions of untranslated mRNA, ARD located within the coding sequences also serves as a binding site for the ARD-binding proteins and functions as an instability element [9]. At present, it is unknown whether kinase-dependent stabilization of unstable mRNAs, such as that described for TNF α , occurs through a modification of proteins that bind to the ARD.

In the present study, we analysed protein binding to TNF α 3'-UTR in primary rat astrocytes. We identified a protein of approx. 35 kDa that binds to a 27 nt segment within 3'-UTR ARD. On electrophoretic mobility shift assay (EMSA), the ARD-protein complexes migrated as two bands, in which the slower moving complexes were noted as a broader band or a doublet. This mobility pattern was altered when cells were

Abbreviations used: ARD, AU-rich domain; DTT, dithiothreitol; EMSA, electrophoretic mobility-shift assay; FCS, fetal calf serum; IL, interleukin; NDV, Newcastle disease virus; PKC, protein kinase C; PP, protein phosphatase; PTP, protein tyrosine phosphatase; TNF α , tumour necrosis factor α ; UTR, untranslated region; DMEM, Dulbecco's modified Eagle's medium; moi, multiplicity of infection.

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infected with NDV and when cytoplasmic extracts were treated with phosphatases.

MATERIALS AND METHODS

Primary cultures of rat astrocytes and treatment with NDV

Astrocytes were purified from stratified cultures of mixed glial cells from neonatal Sprague–Dawley rat brains using the method of McCarthy and DeVellis with modification as described [37,38]. More than 95% of cells at the final step of isolation expressed glial fibrillary acidic protein, an astrocyte marker, and less than 2% were positive for CD11b, the iC3b receptor expressed on microglia but not on astrocytes [21,39]. Astrocyte monolayers were grown in 75 cm² flasks (Corning, Corning, NY, U.S.A.) in Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 containing 10% fetal calf serum (DMEM/FCS). The New Jersey La Sota strain of NDV (ATCC, Rockville, MD, U.S.A.) was grown in fertilized chicken eggs and the viral titres were determined as described [38]. The stock suspension was diluted with DMEM before each experiment. Astrocytes preincubated in serum-free DMEM for 3 h were infected with NDV at a multiplicity of infection (moi) of 30, also in serum-free DMEM [38].

Chemicals and reagents

A protein kinase inhibitor H-7 [40] was purchased from Seikagaku America CO. (Rockville, MD, U.S.A.). Recombinant protein phosphatases PP-1, PP-2A and PP-2B and protein tyrosine phosphatase 1B (PTP-1B) were obtained from Upstate Biotechnology (Lake Placid, NY, U.S.A.).

Northern-blot analysis of TNF α mRNA

The effect of kinase inhibitor, H-7, on NDV-induced TNF α mRNA stability was determined as previously reported [8]. In brief, cells kept in serum-free medium for 30 min were infected with 30 moi of NDV for 2 h in DMEM, and then an equal volume of DMEM/FCS was added for an additional 4 h of incubation. An RNA polymerase II inhibitor α -amanitin (5 μ g/ml) was then added to the culture with or without 60 μ M H-7 and cells were further incubated. At different time points after α -amanitin addition, total RNA was extracted and the relative levels of TNF α mRNA were determined by Northern-blot analysis [8]. A 1.1 kb *EcoRI*–*PstI* fragment of murine TNF α cDNA (kindly provided by Dr. B. Beutler, University of Texas, Dallas, TX, U.S.A.) and 1.4 kb *HindIII* fragment of β -actin cDNA were used to probe the mRNA.

Preparation of nuclear and cytosolic extracts

Astrocytes were collected by scraping and centrifuged at 600 *g* (2000 rev./min) for 10 min at 4 °C. Cytoplasmic and nuclear extracts were prepared as described previously [41]. In brief, cell pellets (1×10^7) were suspended in 0.2 ml of lysis buffer [10 mM Hepes, pH 7.6, 3 mM MgCl₂, 40 mM KCl, 2 mM dithiothreitol (DTT), 5% glycerol, 0.5% Nonidet P40, 8 μ g/ml leupeptin and 0.5 mM PMSF] and the suspension was kept on ice for 15 min. Nuclei were pelleted at 3000 *g* (12000 rev./min) for 10 min at 4 °C in a Beckman Microfuge and the supernatants were used as cytoplasmic extracts. To obtain nuclear extracts, the pelleted nuclei were washed in wash buffer (10 mM Hepes, pH 7.6, 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM PMSF and 1 mM DTT) and then suspended in extraction buffer (20 mM Hepes, pH 7.6, 0.42 M NaCl, 25% glycerol, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM PMSF and 1 mM DTT). The mixtures were stirred for

30 min at 4 °C, then centrifuged at 3000 *g* for 10 min at 4 °C. The supernatants were dialysed against extraction buffer containing 0.1 M KCl instead of 0.42 M NaCl. Protein concentration was determined by BCA protein assay (Pierce Chemical Co., Rockford, IL, U.S.A.). Samples were stored at –70 °C and used within 10 days.

Construction of plasmids and preparation of RNA probes

Plasmids containing the ARD of TNF α 3'-UTR were prepared by inserting double-stranded oligonucleotides, 5'-GAGTG TCGAC ATTTA TATTT GCACT TATTA TTTAT TATTT ATTTA TTATT TATTT ATTTG CTTAT GAATG TATTT ATCTA GAGAG T-3', into the *XbaI*–*SalI* site of the polylinker region of pGEM4 plasmid (Promega, Madison, WI, U.S.A.). Oligonucleotides were prepared with the *SalI*–*XbaI*- and *EcoRI*–*SalI*-recognition sequences at the 5' and 3' end respectively. *Bgl*/II and *Ssp*I segment (296 bp) of mouse TNF α cDNA 3'-UTR containing ARD was also inserted into the polylinker region of pGEM4. Plasmids containing an IL-6 ARD were prepared by inserting a sequence, 5'-ATTTA TTGAT AATTT AAATA AGTAA ACTAT AAGTT AATTT ATGAT TGATA TTTA-3', into pGEM3. Plasmids linearized with *XbaI* or *SalI* were used as templates for RNA synthesis using the Trans Probe Kits from Pharmacia (Piscataway, NJ, U.S.A.) as described by the manufacturer. RNA was purified by a series of phenol, chloroform and ethanol precipitations or by low-melting-point agarose-gel electrophoresis, followed by phenol, chloroform and ethanol precipitation. RNA transcripts were labelled with [α -³²P]UTP (800 Ci/mmol) to yield a specific radioactivity of approx. 3×10^8 c.p.m./ μ g of RNA.

Analysis of RNA–protein complex by EMSA and SDS/PAGE

Cytoplasmic or nuclear extracts (10 μ g of protein) were incubated at room temperature for 20 min with labelled RNA probes (4 ng) in a binding buffer (10 mM Hepes, pH 7.6, 40 mM KCl, 3 mM MgCl₂, 2 mM DTT, 5% glycerol and 0.5% Nonidet P40). The mixtures were further incubated for 10 min with heparin (30 μ g/ml) and yeast RNA (50 μ g/ml), in a final volume of 15 μ l. RNA not associated with proteins was digested with RNase T1 (30 units) (Boehringer-Mannheim, Indianapolis, IN, U.S.A.) for 20 min at room temperature. The RNase-resistant complexes were resolved by non-denaturing PAGE (6% gel) in 0.4 $\times$ Tris/borate/EDTA buffer at a constant voltage of 250 V. To cross-link proteins to RNA, RNA/protein mixtures were transferred to a 96-well plate and the samples were irradiated at 254 nm UV (250 mJ) in a Stratalinker UV apparatus. The samples were digested with RNase T1, then with 1 mg/ml RNase A for 15 min at 37 °C, and were analysed by SDS/PAGE (10% gel) under reducing conditions. In some experiments RNA probes and proteins were allowed to bind in cytoplasmic or nuclear extracts, and digested with RNases as described. The RNase-resistant complexes were resolved in low-melting-point agarose gel, UV-cross-linked *in situ*. The protein–RNA bands identified by autoradiography were excised, resuspended in Laemmli buffer and analysed by SDS/PAGE (10% gel) under reducing conditions. The binding specificity was determined by incubating the extracts with unlabelled competitor RNA for 10 min at room temperature in binding buffer before adding the labelled probe. Competitor RNA included poly(A), poly(U), poly(G), poly(C) ribopolymers (Sigma), a 66 nt ARD segment homologous to the probe, and three smaller segments derived from the 66 nt ARD probe.

Effects of phosphatases and NDV

In order to assess directly the effect of phosphorylation on ARD–protein complex-formation, cytoplasmic and nuclear extracts were incubated with acid phosphatase (10 mg/ml), alkaline phosphatase [agarose-bound enzyme was used according to the manufacturer's (Sigma) instructions] for 30 min in binding buffer before the addition of the labelled probe and analysed by EMSA. In addition, to determine whether the ARD-binding properties induced by NDV are affected post-transcriptionally, cytoplasmic and nuclear fractions of astrocytes infected with NDV in the presence or absence of 60 μ M H-7 were examined by EMSA. The NDV-stimulated nuclear and cytoplasmic extracts were also treated with PP-1 (0.2 unit), PP-2A (0.2 unit), PP-2B (250 ng) or PTP-1B (10 μ l of PTP-1B–agarose) for 30 min before the addition of the labelled probe.

RESULTS

TNF α mRNA stability analysed by Northern blotting

Astrocytes were stimulated with NDV to induce expression of the TNF α gene. Transcription was blocked by α -amanitin, and cells were incubated in the presence or absence of H-7 (60 μ M), then the levels of TNF α mRNA were determined by Northern blot. In the presence of H-7, TNF α was rapidly degraded to undetectable level by 3 h, whereas the mRNA decay was much slower in the absence of H-7 (Figure 1).

Identification of specific RNA-binding protein to TNF α mRNA

The 3'-UTR of TNF α mRNA spans nts +1203 to +1626 and the ARD spans nts +1292 to +1357, as shown in Figure 2. These sequences were used as probes and inhibitors in binding assays for EMSA. The ARD probe consists of 65 nts containing seven AUUUA motifs and *Sal*I and *Xba*I sites at the 5' and 3' border respectively. This ARD region was further divided into three segments and these smaller segments were used as probes or inhibitors for the RNA–protein complex-formation (Figure 2). Both nuclear and cytoplasmic fractions formed RNase T1-resistant complexes with the 3'-UTR probe (Figure 3, left). When ARD was used as a probe, two complexes were resolved in unstimulated cells, a faster complex as a narrow band and a slower and broader complex (Figure 3, centre). Both methods used for preparing the RNA probe produced a similar RNA–protein-binding pattern. The 74 nt ARD in IL-6 3'-UTR used for comparison showed that this IL-6 probe also formed RNA–

Figure 2 Probes used in the binding experiments

Probes used in the binding experiments are shown. A probe within 3'-UTR of TNF α is composed of 425 nts spanning +1203 to +1626. ARD is composed of nts +1292 to +1357. Three smaller probes derived from the ARD are shown. Probes 1 and 3 are underlined and probe 2 is double underlined.

Figure 3 RNA EMSA using astrocyte extracts and TNF α 3'-UTR, TNF α ARD and IL-6 ARD

Radiolabelled *in vitro*-synthesized RNA representing TNF α 3'-UTR, TNF α ARD (TNF AU-rich) or IL-6 ARD (IL6 AU-rich) was incubated with nuclear (N) or cytosolic (C) extracts (10 μ g of protein), as described in the Materials and methods section. Reaction mixtures were digested with RNase T1, and electrophoresed on 6% polyacrylamide gel under non-denaturing conditions. Binding to 32 P-labelled TNF α ARD (+1292/+1357) generated two complexes. Binding to TNF α 3'-UTR (+1203/+1626), IL-6 ARD (+841/+896) and probe alone (P) is also shown.

Figure 1 Effects of kinase inhibitor H-7 on NDV-induced TNF α mRNA decay

Primary rat astrocytes were infected with 30 moi of NDV for 6 h. Cells were further incubated with 5 μ g/ml α -amanitin and with (+) or without (–) 60 μ M H-7 for the indicated time periods. Relative TNF α mRNA expression was determined by Northern-blot analysis using 10 μ g of total RNA per lane. Unstimulated and stimulated astrocytes with NDV for 6 h are also shown. β -Actin mRNA, used as a control, was not affected by virus or kinase inhibitors in this experiment.

binding complexes with astrocyte nuclear and cytoplasmic extracts; however, the mobilities of these protein complexes significantly differed from those of the TNF α ARD–protein complexes (Figure 3, right).

The binding specificity for TNF α ARD probe was studied by inhibition of complex-formation with unlabelled ribopolymers. Preincubation of the extracts with 250 ng (about 60-fold excess) of poly(A) and poly(U) showed that the binding was inhibited

Figure 4 Inhibition of ARD binding in nuclear and cytosolic extracts by poly(U)

Nuclear (N) and cytoplasmic (C) extracts were pretreated with 250 ng of unlabelled ribohomopolymers before incubation with 4 ng of labelled TNF α ARD probe. Extracts not treated with inhibitors (none) and probe alone (P) are also included.

Figure 5 Identification of the complex-forming sites in TNF α ARD

RNA-binding assays and EMSA were carried out with cytosolic extracts (10 μ g) and 4 ng of 32 P-labelled ARD (T) or probes 1–3 (see Figure 2). In a competition assay, ARD (T) was used as a probe and unlabelled ARD fragments 1, 2 and 3 as competitors. Experiments with nuclear extracts gave similar results (not shown).

only with poly(U) (Figure 4). Even at 1 μ g, poly(A), poly(C) and poly(G) failed to inhibit ARD–protein complex-formation (results not shown). The binding specificity was shown by almost complete inhibition of the binding with 20 molar excess of

Figure 6 Detection of ARD-binding proteins by UV cross-linking and SDS/PAGE

The ARD–protein complexes were cross-linked by UV irradiation (+), then digested with RNase T1 or RNase T1 followed by RNase A. Samples, including probe alone (P) and extracts incubated with probes, but not UV-cross-linked (–), were analysed by SDS/PAGE (10% gel). RNase T1 treatment generated a 45 kDa RNA–protein complex. Additional treatment using RNase A reduced the complex to a 35 kDa band.

unlabelled homologous ARD (Figure 5). The specific binding site within ARD was then examined with three smaller oligonucleotides derived from ARD (see Figure 2) used as probes (probe 1, 2 and 3) or competitors at 20 molar excess (Figure 5). Whereas probe 1 and probe 3 each containing a single AUUUA motif failed to produce complex-formation with cytoplasmic extracts, probe 2 generated two well-defined bands. Probe 2 which contained five overlapping AUUUA motifs, three of them as a UUAUUUAUU nonamer, was able to form RNase-resistant complexes. Identical experiments with nuclear extracts produced similar results (not shown).

Identification of ARD-binding proteins by UV cross-linking

When nuclear or cytoplasmic extracts were treated with proteinase K, complex-formation with ARD was completely abolished (results not shown). To characterize the ARD-binding proteins, the RNA–protein complexes were UV-cross-linked and the ARD–protein adducts were analysed by SDS/PAGE (10% gel). UV-cross-linked RNase-T1-resistant ARD–protein complexes present in nuclear and cytoplasmic extracts migrated as a single band with a molecular mass of 45 kDa (Figure 6). Further digestion with RNase A reduced the mass of the complex to 35 kDa. Similar results were obtained by cross-linking the complexes in an agarose gel (not shown). Extracts from NDV-stimulated astrocytes produced an identical size complex (results not shown).

Effects of phosphatase treatment of cytosolic extracts on formation of ARD–protein complexes

To determine whether ARD–protein complex-formation is affected by the state of phosphorylation of the ARD-binding protein, cytoplasmic and nuclear extracts were treated with phosphatases, then the mobility pattern of ARD–protein complexes was examined. Alkaline phosphatase completely abolished the formation of the faster moving complex with cytoplasmic extracts, whereas the effect of acid phosphatase was minimal

Figure 7 Effect of acid phosphatase and alkaline phosphatase on ARD–protein complex mobility

Cytosolic (C) or nuclear (N) extracts were treated with acid phosphatase or alkaline phosphatase before the addition of the ARD probe and EMSA.

Figure 8 Effects of phosphatases on NDV-induced ARD–protein complexes

Top, Astrocytes infected with NDV for 0.5, 1, 2 and 3 h in the absence or presence of H-7. Cytoplasmic extracts were then examined for the mobility pattern of the ARD–protein complexes. Bottom, Nuclear and cytoplasmic extracts from astrocytes infected with NDV for 1 and 3 h were treated with PP-1, PP-2A or PP-2B for 30 min before ARD binding and EMSA.

(Figure 7). Complex-formation with nuclear extracts was minimally affected by alkaline phosphatase. Cytoplasmic extracts from NDV-infected astrocytes produced an increase in the faster moving complex at 1 h after infection and this effect was significantly reduced when cells were infected with NDV in the presence of H-7 (Figure 8, top). Treatment of NDV-stimulated cytoplasmic but not nuclear extracts with PP-1 or PP-2A also reduced faster migrating complex (Figure 8, bottom). Treatment

with PP-2B and PTP-1B (not shown) was not effective. PP-1 and PP-2A are highly specific phosphatases for serine and threonine residues, whereas PP-2B [42] and PTP-1B [43] dephosphorylate tyrosine residues.

DISCUSSION

This work is the first to explore systematically protein binding to the 3'-UTR region of TNF α . We have shown that the nuclear and cytoplasmic extracts from serum or NDV-stimulated astrocytes contain proteins that bind to an ARD of TNF α 3'-UTR and that these ARD–protein complexes migrate as two bands in EMSA. By UV cross-linking and SDS/PAGE, we have identified a 35 kDa ARD–protein complex after extensive RNase treatment. Thus the molecular mass of the TNF α ARD-binding protein would be less than 35 kDa, because some RNA is still covalently linked. The competition assays have shown that the binding to ARD was uridine-specific and was restricted to a centrally located segment of the ARD, which contains five overlapping AUUUA motifs, primarily as a UUAUUUAUU nonamer motif. A single AUUUA motif within ARD did not show ARD-binding activity, when examined directly by using these sequences as targets or indirectly as inhibitors. The results are consistent with a recent report that demonstrates UUAUUUAUU as the key AU-rich sequence motif that mediates poly(A) deadenylation in mRNA decay [29].

Previously, a number of ARD-binding proteins have been identified [9,30–36,44]. The molecular mass of these ranged between 30 and 45 kDa, except the 59 and 65 kDa [9,45] proteins that bind the c-Myc and interferon β ARDs respectively. Brewer's group demonstrated that a 37 and 40 kDa protein binding to c-Myc and granulocyte–macrophage colony stimulating factor 3'-UTR are both phosphoproteins and immunologically cross-reactive [36,45]. The deduced sequence of the cloned 37 kDa protein showed two RNA-recognition motifs and possible domains of protein–protein interaction [36]. Whether TNF α ARD-binding protein is related to the other ARD-binding protein is unknown at present. It is also interesting to note that a 35 kDa ARD-binding protein is induced on stimulation by β -adrenergic receptor agonist, and induction of this complex destabilizes β -adrenergic receptor transcript, an otherwise stable mRNA [46,47].

The effects of NDV and phosphatases on the mobility of ARD–protein complexes were examined because TNF α mRNA induced by NDV is stabilized through a PKC-dependent pathway [8,21]. Formation of faster moving complex was increased in astrocytes by NDV, but this complex-formation was significantly reduced by H-7, a kinase inhibitor, and also when NDV-stimulated cytoplasmic extracts were treated with serine and threonine phosphatases. These data indicate that the ARD-binding protein can be post-transcriptionally modified in a kinase-dependent manner only in the cytoplasmic compartment. Although the effect of protein phosphorylation on mRNA stability is not yet known, it is possible that phosphorylation of ARD-binding protein may affect the affinity of ARD binding and/or interaction of ARD-binding protein with other proteins such as poly(A)-binding protein and endo- and exo-nucleases. This speculation is consistent with our finding that PKC activity is required to prevent poly(A) tail degradation to achieve TNF α mRNA stability [21].

Stabilization of TNF α mRNA in astrocytes may be biologically significant, since TNF α , which is increased in the spinal fluid of multiple sclerosis patients, is predominantly localized in astrocytes within actively demyelinating lesions [48–50]. In addition, the requirement of TNF α in immune-mediated demyelination

has been shown in animal models [51,52]. Thus sustained production of TNF α by glial cells after virus infection through the mechanism of mRNA stabilization can be a significant factor during the course of inflammatory diseases affecting the central nervous system.

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REFERENCES

- Gay, D. A., Sisodia, S. S. and Cleveland, D. W. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5763–5767
- Boado, R. J. and Pardridge, W. M. (1993) *J. Neurochem.* **60**, 2290–2296
- Klausner, R. D., Rouault, T. A. and Harford, J. B. (1993) *Cell* **72**, 19–29
- Wodnar-Filipowicz, A. and Moroni, C. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 777–781
- Petersen, D. D., Kock, S. R. and Granner, D. K. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7800–7804
- Peppel, K., Vinci, J. M. and Baglioni, C. (1991) *J. Exp. Med.* **173**, 349–355
- Stephens, J. M., Carter, B. Z., Pekala, P. H. and Malter, J. S. (1992) *J. Biol. Chem.* **267**, 8336–8341
- Lieberman, A. P., Pitha, P. M. and Shin, M. L. (1990) *J. Exp. Med.* **172**, 989–992
- Raj, N. B. K. and Pitha, P. M. (1993) *FASEB J.* **7**, 702–710
- Shirazi, Y., Rus, H. G., Macklin, W. B. and Shin, M. L. (1993) *J. Immunol.* **150**, 4581–4590
- Lindsten, T., June, C. H., Ledbetter, J. A., Stella, G. and Thompson, C. B. (1989) *Science* **244**, 339–343
- Brawerman, G. (1987) *Cell* **48**, 5–6
- Ross, J. (1988) *Mol. Biol. Med.* **5**, 1–14.
- Sachs, A. B. (1993) *Cell* **74**, 413–421
- Shaw, G. and Kamen, R. (1986) *Cell* **46**, 659–667
- Shyu, A. B., Belasco, J. G. and Greenberg, M. E. (1991) *Genes Dev.* **5**, 221–231
- Whittemore, L. A. and Maniatis, T. (1990) *Mol. Cell. Biol.* **10**, 1329–1337
- Binder, R., Hwang, S. P. L., Ratnasabapathy, R. and Williams, D. L. (1989) *J. Biol. Chem.* **264**, 16910–16918
- Fisher, S. N., Kim, Y.-U. and Shin, M. L. (1994) *J. Immunol.* **153**, 3210–3217
- Chung, I. Y., Kwon, J. and Benveniste, E. N. (1992) *J. Immunol.* **149**, 3894–3902
- Lieberman, A. P., Pitha, P. M. and Shin, M. L. (1992) *J. Biol. Chem.* **267**, 2123–2126
- Sung, S., Walters, J. A. and Fu, S. M. (1992) *J. Exp. Med.* **176**, 897–901
- Brewer, G. and Ross, J. (1988) *Mol. Cell. Biol.* **8**, 1697–1708
- Bernstein, P. and Ross, J. (1989) *Trends Biochem. Sci.* **14**, 373–377
- Peppel, K. and Baglioni, C. (1991) *J. Biol. Chem.* **266**, 6663–6666
- Decker, C. J. and Parker, P. (1993) *Genes Dev.* **7**, 1632–1643
- Wilson, T. and Treisman, T. (1988) *Nature (London)* **336**, 396–399
- Lowell, J. E., Rudner, D. Z. and Sachs, A. B. (1992) *Genes Dev.* **6**, 2088–2099
- Zubiaga, A. M., Belasco, J. G. and Greenberg, M. E. (1995) *Mol. Cell. Biol.* **15**, 2219–2230
- Bohjanen, P. R., Petryniak, B., June, C. H., Thompson, C. B. and Lindsten, C. B. (1991) *Mol. Cell. Biol.* **11**, 3288–3295
- Gillis, P. and Malter, J. S. (1991) *J. Biol. Chem.* **266**, 3172–3177
- You, Y., Chen, C. Y. A. and Shyu, A. B. (1992) *Mol. Cell. Biol.* **12**, 2931–2940
- Vakalopoulou, E., Schaack, J. and Schenk, T. (1991) *Mol. Cell. Biol.* **11**, 3355–3364
- Malter, J. S. (1989) *Science* **246**, 664–666
- Schuler, G. D. and Cole, M. D. (1988) *Cell* **55**, 1115–1122
- Zhang, W., Wagner, B. J., Ehrenman, K., Schaefer, A. W., DeMaria, C. T., Crater, D., DeHaven, K., Long, L. and Brewer, G. (1993) *Mol. Cell. Biol.* **13**, 7652–7665
- McCarthy, K. D. and DeVellis, J. (1980) *J. Cell. Biol.* **85**, 890–902
- Shin, M. L., Lieberman, A. P. and Fisher, S. N. (1993) *Methods Neurosci* **17**, 18–34
- Lieberman, A. P., Pitha, P. M., Shin, H. S. and Shin, M. L. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6348–6352
- Hidaka, H. and Tanaka, T. (1987) *Methods Enzymol.* **139**, 570–582
- Lee, K. A. W., Bindereif, A. and Grenn, M. R. (1988) *Gene Anal. Tech.* **5**, 22–31
- Pallen, C. J. and Wang, J. H. (1984) *J. Biol. Chem.* **259**, 6134–6141
- Frangioni, J. V., Beahm, P. H., Shifrin, V., Jost, C. A. and Neel, B. G. (1992) *Cell* **68**, 545–560
- Alberta, J. A., Rundell, K. and Stiles, C. D. (1994) *J. Biol. Chem.* **269**, 4532–4538
- Brewer, G. (1991) *Mol. Cell. Biol.* **11**, 2460–2466
- Lee, N. H., Earle-Hughes, J. and Fraser, C. M. (1994) *J. Biol. Chem.* **269**, 4291–4298
- Huang, L., Tholanikunnel, B. G., Vakalopoulou, E. and Malbon, C. C. (1993) *J. Biol. Chem.* **268**, 25769–25775
- Sharief, M. K. and Hentges, R. (1991) *N. Engl. J. Med.* **325**, 467–472
- Hofman, F. M., Hinton, D. R., Johnson, K. and Merrill, J. E. (1989) *J. Exp. Med.* **170**, 607–612
- Selmaj, K., Raine, C. S., Cannella, B. and Brosnan, C. F. (1991) *J. Clin. Invest.* **87**, 949–954
- Ruddle, N. H., Bergman, C. M., McGrath, K. M., Lingenheld, E. G., Grunnet, M. L., Padula, S. J. and Clark, R. B. (1990) *J. Exp. Med.* **172**, 1193–1200
- Selmaj, K., Raine, C. S. and Cross, A. H. (1991) *Ann. Neurol.* **30**, 694–700