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FAST TRACK

Single-Stranded Nucleic Acid-Binding Protein, Purα, Interacts With RNA Homologous to 18S Ribosomal RNA and Inhibits Translation In Vitro

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Abstract Purα is a highly conserved, eukaryotic sequence-specific DNA- and RNA-binding protein involved in diverse cellular and viral functions including transcription, replication, and cell growth. Purα exerts its activity in part by interacting with other viral and cellular proteins. One such protein is the human immunodeficiency virus (HIV) type I regulatory protein Tat. Earlier studies have demonstrated that this interaction is mediated by Purα-associated RNA (PARNA) and that RNA immunopurified from mammalian expressed Purα was capable of reconstituting the interaction between these two proteins. In the current study, we characterize four RNA species which were immunopurified with Purα. Northern blot analysis with one of the PARNAs revealed a highly abundant signal of ~2.0 kilobases (kb) present in all cell lines tested. Sequence analysis of each of the four PARNA clones revealed a high homology to different regions of the human 18S ribosomal RNA sequence. Based on this homology, we investigated the influence of Purα on translation. Luciferase assays were performed after coupled in vitro transcription/translation reactions with a vector containing a luciferase reporter construct and increasing concentrations of BSA, GST, and GST-Purα. Inclusion of GST-Purα in these reactions resulted in a dose-dependent inhibition of luciferase activity. Similar inhibition was observed with in vitro translation reactions performed with in vitro transcribed luciferase RNA and increasing concentrations of GST-Purα. In control experiments, inclusion of increasing concentrations of GST-Purα with luciferase protein resulted in no effect on luciferase activity. Taken together, these data demonstrate that Purα inhibits translation reactions in vitro. Moreover, this Purα-mediated inhibition of translation can be abrogated by HIV-1 Tat protein. J. Cell. Biochem. 83: 355–363, 2001.

Key words: Purα; translation; PARNA; RNA; human immunodeficiency virus; Tat

Human Purα (GenBank accession no M96684) is a sequence-specific DNA- and RNA-binding protein initially cloned through its affinity for a sequence element present at many eukaryotic origins of DNA replication [Bergmann and Johnson, 1992; Bergmann et al., 1992]. This 322 amino acid protein is composed of a modular structure with a central repeat region, a glycine-rich amino terminus, and a glutamine-glutamate-rich carboxy terminus. Purα is the founding member of the pur family of proteins which currently consists of Purα, Purβ, and Purγ. Since its initial description, Purα has been demonstrated to be involved in diverse cellular and viral functions including gene transcription, replication, and cell growth [reviewed in Gallia et al., 2000].

Purα acts as both a transcriptional activator and repressor of cellular genes. Purα has been shown to activate many cellular genes including myelin basic protein (MBP) [Haas et al., 1993, 1995; Tretiakova et al., 1998], the β-subunit of the neuronal nicotinic acetylcholine receptor [Du et al., 1997], the neuron-specific FE65 gene [Zambrano et al., 1997], and the transforming growth factor β 1 [Thatikunta et al., 1997]. In avian fibroblasts infected with the rous sarcoma virus, a Pur element functions as an enhancer

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for the clusterin gene [Herault et al., 1993]. Other studies have suggested that Purα may function as a transcriptional repressor. More specifically, Purα has been shown to be a transcriptional repressor of the human *fas* promoter [Lasham et al., 2000] and has been implicated in repression of the murine vascular smooth muscle (VSM) α-actin gene [Kelm et al., 1997].

In addition to its activity in modulating diverse cellular genes, Purα is also involved in regulating several human viruses including the JC virus (JCV) and the human immunodeficiency virus (HIV), type 1. Purα regulates both transcription and replication of JCV, an opportunistic polyomavirus and etiological agent of the demyelinating disease progressive multifocal leukoencephalopathy (PML) [Major et al., 1992]. Purα has been shown to stimulate JCV early gene transcription and decrease the ability of the viral regulatory protein, large T-antigen, to increase JCV late gene transcription [Chen and Khalili, 1995]. This latter effect is mediated by the physical interaction between Purα and T-antigen [Gallia et al., 1998]. Purα has also been shown to modulate JCV early and late gene transcription through its association with the Y-box binding protein YB-1 [Safak et al., 1999]. In addition to its effects on JCV transcription, Purα has been shown to suppress JCV DNA replication in glial cells [Chang et al., 1996].

PML emerges most frequently in patients with the acquired immunodeficiency syndrome in comparison to other immunocompromised conditions. This suggests that the presence of HIV-1 in the central nervous system can contribute to the pathogenesis of PML. Studies have indicated a direct intercommunication between HIV-1 and JCV mediated through the HIV-1 regulatory protein Tat [Chowdhury et al., 1993]. Although Tat does not interact directly with JCV DNA sequences, the Tat-responsive region of JCV contains a Purα binding site and Purα and Tat synergistically activate the JCV late gene promoter [Krachmarov et al., 1996]. Moreover, Purα has been shown to interact with the HIV-1 Tat protein [Krachmarov et al., 1996, Gallia et al., 1999b; Wortman et al., 2000]. Interestingly, the interaction between Purα and the HIV-1 Tat protein is mediated by RNA associated with Purα [Gallia et al., 1999b]. In that study we immunopurified Purα from a transiently transfected human malignant glioblastoma/astrocytoma cell line and using reverse transcription-polymerase chain reaction (RT-PCR) techniques, isolated several Purα-associated RNA (PARNA) species. One of these clones was able to reconstitute the interaction between Purα and Tat. In the current study, we characterize four PARNA species and report their sequences as homologous to distinct regions of human 18S ribosomal RNA (rRNA). Based on the association of Purα with RNA which is involved in cellular translation, we investigated the role, if any, of Purα on translation. Here, we demonstrate that Purα is capable of inhibiting translation in vitro. Moreover, we show that the HIV-1 Tat protein can abrogate the Purα-mediated inhibition of translation.

METHODS

Plasmids and Cell Cultures

PARNAs were isolated using reverse-transcription followed by polymerase chain reaction assays as previously described [Gallia et al., 1999b]. Clones were screened for the presence of inserts by restriction enzyme digestion. Clones 10, 11, 14, and 47 contained inserts, were designated PARNA clones 10, 11, 14, and 47, respectively, and further analyzed. PARNA clone 14 was subcloned into *Eco*RI-digested pCNDA3 (Invitrogen, Carlsbad, CA) generating pCDNA3-PARNA clone 14. The plasmid pGL3 T7-luciferase was constructed by digesting pCDNA3-TAR, a vector which contains the HIV-1 TAR sequence cloned into the *Hind*III/*Bam*HI sites of pCDNA3, with *Sma*I and *Bam*HI. This *Sma*I/*Bam*HI fragment, which contains the T7 promoter and the HIV-1 TAR sequence, was then subcloned into *Sma*I/*Bgl*II digested pGL3-basic vector (Promega, Madison, WI), generating pGL3-T7-TAR. This construct was subsequently digested with *Hind*III, releasing the TAR fragment, and the vector containing the T7 promoter and the luciferase gene was religated generating pGL3-T7-luciferase. The sequence of all plasmids and PARNA clones was verified by DNA sequencing using an ABI automatic sequencer.

Rat glioma (C6), African green monkey kidney (CV-1), human cervical adenocarcinoma (HeLa), and human glioblastoma (U-87 MG and T98-G) cells were obtained from the American Type Culture Collection (Manassas, VA) and maintained at 37°C in Dulbecco's modified Eagle medium (GIBCO, Grand Island, NY)

supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified incubator containing 5% CO_2.

RNA Isolation/Northern Blotting

Total RNA was isolated from C6, CV-1, HeLa, T98-G, and U-87 MG cells using an acid phenol extraction as previously described [Queen and Baltimore, 1983]. Briefly, cells were washed twice with 1× PBS and lysed with a solution containing 50 mM sodium acetate (pH 5.1) and 1% SDS. Cell lysates were scraped into tubes containing an equal volume of prewarmed acid phenol equilibrated with 50 mM sodium acetate (pH 5.1) and were incubated at 60°C for 15 min. The aqueous phase was extracted with neutral phenol/chloroform [equilibrated with 50 mM Tris (pH 7.4)], followed by chloroform extraction and ethanol precipitation. After *DNase*I digestion to remove endogenous DNA from the samples, 20 μg of total RNA were analyzed on 1.2% agarose 0.4% formaldehyde, 1 × morpholinepropanesulfonic acid gels and hybridized to probe generated from the 338 PARNA clone 14 cDNA after transfer to nylon membrane as previously described [Ausubel et al., 1989].

Proteins

GST fusion proteins were expressed and purified as described previously [Gallia et al., 1999b]. Briefly, bacteria containing GST fusion proteins were grown overnight at 37°C in Luria Bertani medium containing 100 mg/L ampicillin. The following morning, the cells were diluted 1:10 in fresh medium, grown to an absorbance at 595 nm of 0.6–0.7 and induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside for 3 h at 37°C. Cells were then pelleted at 6,500*g* at 4°C and subsequently resuspended in NETN buffer containing 20 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA, and 0.5% Nonidet P-40. After sonication on ice, the bacterial lysate was centrifuged at 40,000*g* at 4°C to remove insoluble material. Glutathione-Sepharose beads (Amersham Pharmacia Biotech, Piscataway, NJ) were added to the supernatant and after a 3 h incubation at 4°C, the beads were pelleted and washed three times with 50 volumes of NETN buffer each time. Proteins were eluted from the glutathione resin at room temperature in elution buffer containing 15 mM reduced glutathione and 5 mM dithiothretiol in 50 mM Tris-Cl, pH 8.0. Eluted proteins were dialyzed against 20 mM Tris-Cl, pH 7.5, 50 mM NaCl, 0.5 mM DTT, 0.2 mM phenylmethylsulfonyl fluoride, 0.2 mM EDTA, and 10% glycerol at 4°C. The integrity and purity of the GST fusion proteins were analyzed by SDS–PAGE followed by Coomassie Blue staining. BSA was obtained commercially (New England BioLabs, Beverly, MA).

Luciferase Reactions

Coupled in vitro transcription–translation reactions were performed with *Xba*I linearized pGL3-T7-luciferase and various amounts of exogenously added BSA, GST, and GST-Purα using the TNT-coupled transcription–translation wheat germ extract following the manufacturers instructions (Promega). An equal volume aliquot of each reaction was assayed for luciferase activity according to the manufacturer's recommendations (Promega). Alternatively, in vitro transcribed luciferase RNA was incubated with various amount of BSA, GST, or GST-Purα in coupled transcription–translation extract and subsequently an equal volume of the reaction was assayed for luciferase activity. Experiments were performed at least in triplicate with qualitatively similar results. Relative light units were converted into fold activity for graphical representation.

In Vitro Transcription Reactions

In vitro transcribed RNA, used in the luciferase assays shown in Figure 4A, was generated from *Bam*HI linearized pGL3-T7-luciferase according to the recommendations of the manufacturer (Promega). pGL3-T7 DNA was removed by digestion with *DNase*I following the transcription reactions according to the recommendations of the manufacturer (Promega).

RESULTS

Purα Associates with RNAs Homologous to 18S RNA

We have previously isolated human PARNA sequences [Gallia et al., 1999b]. These sequences were isolated by immunopurifying transiently transfected Purα from the human glioblastoma cell line U-87 MG and subsequently isolating RNA from the Purα immunocomplexes and using that RNA as templates for RT-PCR assays. Four clones were obtained and designated as PARNA clones 10, 11, 14, and 47. Northern blot analysis was performed using

PARNA clone 14 and a variety of tissue culture cell lines including human glioblastoma (U-87 MG and T98-G), rat glioma (C6), human cervical adenocarcinoma (HeLa), and African green monkey kidney (CV-1) cells. As shown in Figure 1, PARNA clone 14 detected a highly abundant RNA species in an area corresponding to 18S rRNA in all cell lines analyzed. This observation also demonstrated that the RNA species isolated was indeed expressed in eukaryotic cells. Sequence analysis of these four clones and comparison with known sequences using advanced BLASTN searches revealed that each clone had a high similarity or identity to various regions of the human 18S rRNA sequence (GenBank accession no X03205). Figure 2A shows the graphical alignment of the four PARNA cDNA clones in relationship to the entire 18S rRNA sequence. Clones 10 and 14 represent overlapping clones; clones 11 and 47 are distinct from the other RNA clones isolated, but are homologous to 18S rRNA. PARNA clone 10 (GenBank Accession number AF397157) consists of 551 base pairs (bp) with 99% identity to the 3′ end of the human 18S rRNA sequence (Fig. 2B). PARNA clone 11 (GenBank Accession number AF397158) is 585 bases in length and overall has a 97% identity with the 5′ end of the human 18S rRNA sequence. Interestingly, this clone possess a 18 base sequence not found in the 18S rRNA sequence. Excluding this 18 base sequence, there is only one nucleotide difference between this clone and the human rRNA sequence (Fig. 2C). PARNA clone 14 (GenBank Accession number AF397159) consists of 338 bp, the first 257 of which are 100% identical to the human 18S rRNA sequence. Interestingly, the last 89 bases located at the 3′ end of this clone are 100% identical to the opposite strand of the human 18S rRNA sequence (Fig. 2A). PARNA clone 47 (GenBank Accession number AF397160) consists of 179 bases with 100% identity to the human 18S rRNA sequence.

Purα Inhibits Translation In Vitro

Based on the observation that all of the RNA species immunopurified with Purα were homologous to 18S rRNA, we next investigated the influence of Purα on translation. In the first series of experiments, we performed coupled in vitro transcription–translation reactions using pGL3-T7-luciferase, a vector containing

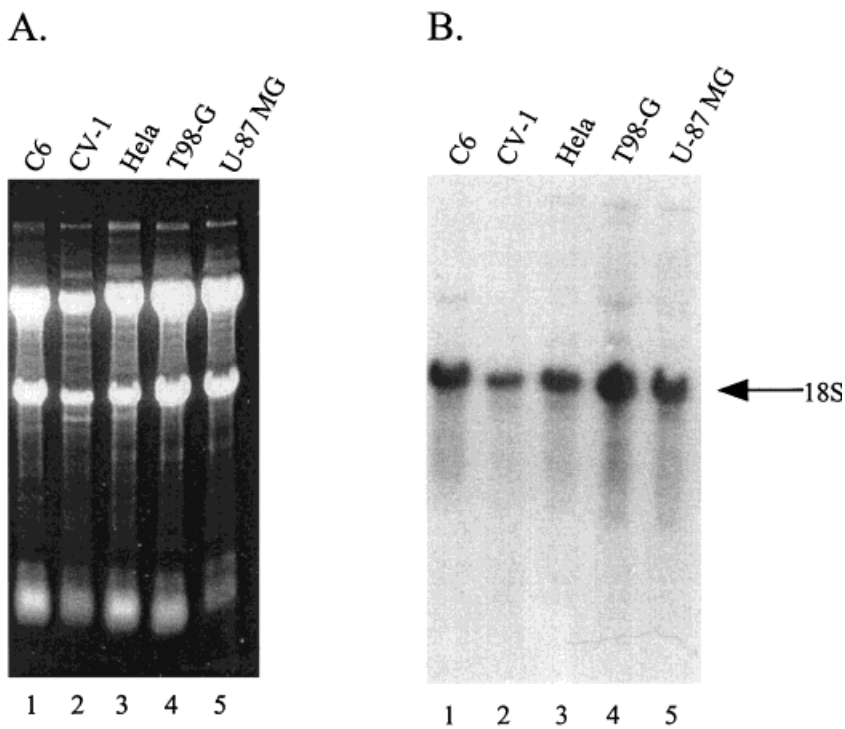
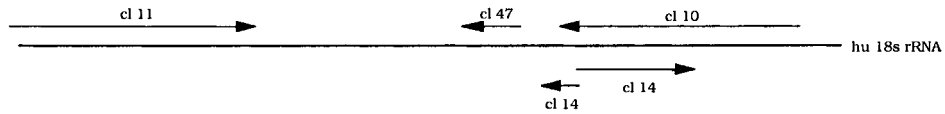


Fig. 1. Northern blot analysis using PARNA clone 14. Total RNA was extracted from C6 (rat glioma), CV-1 (African green monkey kidney), HeLa (human cervical adenocarcinoma), and T98-G and U-87 MG (human glioblastoma) cells, separated on a denaturing formaldehyde-agarose gel (**A**) and hybridized to labeled PARNA clone 14 DNA (**B**). The position of 18S rRNA is shown by the arrow.

A.



B.

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PARNA c10:   1 CCCCGCCAGGGCCGTGGGCCGACCCCGGCGGGGCCGATCCGAGGGCCTCACTAAACCATCCAATCGGTAGTAGCGACGGGCGGTGTGTAC 90
               | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
18S rRNA: 1782 CTCCGCCAGGGCCGTGGGCCGACCCCGGCGGGGCCGATCCGAGGGCCTCACTAAACCATCCAATCGGTAGTAGCGACGGGCGGTGTGTAC 1693

PARNA c10: 61  AAAGGGCAGGGACTTAATCAACGCAAGCTTATGACCCGCACTTACTGGGAATTCCTCGTTCATGGGGAATAATTGCAATCCCCGATCCCC 180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
18S rRNA: 1692 AAAGGGCAGGGACTTAATCAACGCAAGCTTATGACCCGCACTTACTGGGAATTCCTCGTTCATGGGGAATAATTGCAATCCCCGATCCCC 1603

PARNA c10: 181 AGC-C-TATGGGGTTCAACGGGTTACCCGCGCCTGCCGGCGTAGGGTAGGCACACGCTGAGCCAGTCAGTGTAGCGCGCGTGCAGCCCCG 268
               | | |  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
18S rRNA: 1602 ATCACGAATGGGGTTCAACGGGTTACCCGCGCCTGCCGGCGTAGGGTAGGCACACGCTGAGCCAGTCAGTGTAGCGCGCGTGCAGCCCCG 1513

PARNA c10: 269 GACATCTAAGGGCATCACAGACCTGTTATTGCTCAATCTCGGGTGGCTGAACGCCACTTGTCCCTCTAAGAAGTTGGGGGACGCCGACCG 358
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
18S rRNA: 1512 GACATCTAAGGGCATCACAGACCTGTTATTGCTCAATCTCGGGTGGCTGAACGCCACTTGTCCCTCTAAGAAGTTGGGGGACGCCGACCG 1423

PARNA c10: 359 CTCGGGGGTCGCGTAACTAGTTAGCATGCCAGAGTCTCGTTCGTTATCGGAATTAACCAGACAAATCGCTCCACCAACTAAGAACGGCCA 448
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
18S rRNA: 1422 CTCGGGGGTCGCGTAACTAGTTAGCATGCCAGAGTCTCGTTCGTTATCGGAATTAACCAGACAAATCGCTCCACCAACTAAGAACGGCCA 1333

PARNA c10: 449 TGCACCACCACC-ACGGAATCGAGAAAGAGCTATCAATCTGTCAATCCTGTCCGTGTCCGGGCCGGGTGAGGTTTCCCGTGTTGAGTCAA 537
               |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
18S rRNA: 1332 TGCACCACCACCCACGGAATCGAGAAAGAGCTATCAATCTGTCAATCCTGTCCGTGTCCGGGCCGGGTGAGGTTTCCCGTGTTGAGTCAA 1243

PARNA c10: 538 ATTAAGCAGGCCCT 551
               |||||||| ||
18S rRNA: 1242 ATTAAGCACTCCTG 1229
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C.

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PARNA c11: 1   AACGAACGGGACTCTGGCTACCTGGTTGATCCTGCCAGTAGCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCTAAGTACGCACGGC 90
                                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
18S rRNA:  1                     TACCTGGTTGATCCTGCCAGTAGCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCTAAGTACGCACGGC 72

PARNA c11: 91  CGGTACAGTGAAACTGCGAATGGCTCATTAAATCAGTTATGGTTCCTTTGGTCGCTCGCTCCTCTCCTACTTGGATAACTGTGGTAATTC 180
               |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
18S rRNA:  61  CGGTACAGTGAAACTGCGAATGGCTCATTAAATCAGTTATGGTTCCTTTGGTCGCTCGCTCCTCTCCCACTTGGATAACTGTGGTAATTC 162

PARNA c11: 181 TAGAGCTAATACATGCCGACGGGCGCTGACCCCCTTCGCGGGGGGGGATGCGTGCATTTATCAGATCAAAACCAACCCGGTCAGCCCCTCT 270
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
18S rRNA:  121 TAGAGCTAATACATGCCGACGGGCGCTGACCCCCTTCGCGGGGGGGATGCGTGCATTTATCAGATCAAAACCAACCCGGTCAGCCCCTCT 252

PARNA c11: 271 CCGGCCCCGGCCGGGGGGCGGGCGCCGGCGGCTTTGGTGACTCTAGATAACCTCGGGCCGATCGCACGCCCCCCGTGGCGGCGACGACCC 360
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
18S rRNA:  241 CCGGCCCCGGCCGGGGGGCGGGCGCCGGCGGCTTTGGTGACTCTAGATAACCTCGGGCCGATCGCACGCCCCCCGTGGCGGCGACGACCC 342

PARNA c11: 361 ATTCGAACGTCTGCCCTATCAACTTTCGATGGTAGTCGCCGTGCCTACCATGGTGACCACGGGTGACGGGGAATCAGGGTTCGATTCCGG 450
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
18S rRNA:  301 ATTCGAACGTCTGCCCTATCAACTTTCGATGGTAGTCGCCGTGCCTACCATGGTGACCACGGGTGACGGGGAATCAGGGTTCGATTCCGG 432

PARNA c11: 451 AGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCGACCCGGGGAGGTAGTGACGAAAA 540
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
18S rRNA:  421 AGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCGACCCGGGGAGGTAGTGACGAAAA 522

PARNA c11: 541 ATAACAATACAGGACTCTTTCGAGGCCCTGTAATTGGAATGAGTC 585
               |||||||||||||||||||||||||||||||||||||||||||
18S rRNA:  481 ATAACAATACAGGACTCTTTCGAGGCCCTGTAATTGGAATGAGTC 567
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Fig. 2. Purα associates with human RNA sequences homologous to 18S rRNA. **A**: Graphical alignment of the four PARNA cDNA clones with the human 18S rRNA sequence. Clones 10 and 14 are partially overlapping; clones 11 and 47 are distinct from the other clones. The arrows depict the direction of the clone sequenced. (**B** and **C**) Sequence alignment of the PARNA cDNA clones 10 (B) and 11 (C) with the 18S rRNA sequence. The 18S rRNA sequence is shown at the bottom of each alignment.

the T7 promoter upstream of the luciferase reporter gene, and 0.1, 0.5, 2.5, and 7.5 μM concentrations of purified BSA, GST, and GST-Purα. As shown in Figure 3, inclusion of BSA at any concentration (lanes 2–5) did not affect the amount of luciferase activity observed. Similarly, inclusion of increasing amounts of GST (Fig. 3, lanes 6–9) had no effect on the amount of luciferase activity observed. Inclusion of Purα, however, resulted in a dose-dependent inhibition of luciferase activity (Fig. 3, lanes 10–13). This observation is consistent with an inhibitory effect of Purα on translation. Several other possibilities, however, could also explain this observation. Subsequent experiments were performed to decipher the exact step affected by Purα in this reaction.

As Purα has been shown to both activate and suppress transcription, we next evaluated which part of the coupled transcription–translation reaction was affected by Purα. To this end, we prepared luciferase mRNA in vitro and after *DNase*I digestion, used this mRNA in translation reactions containing increasing amounts of BSA, GST, and GST-Purα. As shown in Figure 4A, neither BSA (compare lane 1 with 2–5) nor GST (compare lane 6 with 7–10) had any affect on luciferase activity. Conversely, inclusion of GST-Purα in the translation reaction resulted in a dose-dependent decrease in luciferase activity (Fig. 4A, compare lane 11 with 12–15). To evaluate any direct influence of

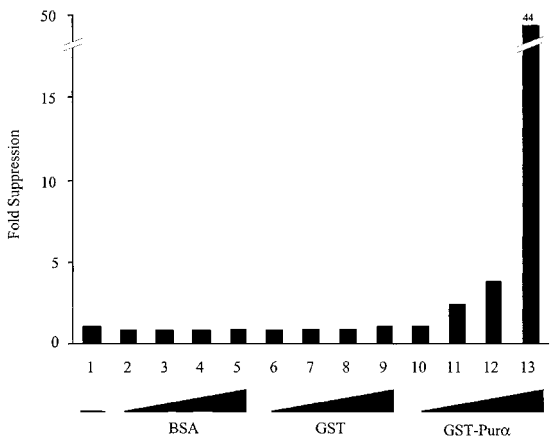

Fig. 3. Purα inhibits coupled in vitro transcription–translation reactions. Luciferase assays were performed after coupled in vitro transcription/translation reactions containing pGL3-T7-luciferase and 0.1, 0.5, 2.5, and 7.5 μM BSA (**lanes 2–5**), GST (**lanes 6–9**), and GST-Purα (**lanes 10–13**) purified proteins. **Lane 1** contains no exogenously added protein. Fold suppression is depicted.

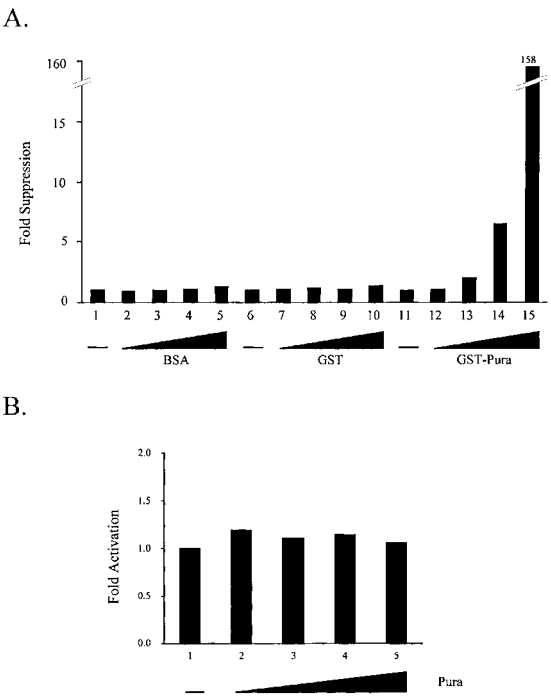

Fig. 4. Purα inhibits in vitro translation. **A**: In vitro translation reactions were performed with pGL3-T7-generated luciferase RNA in the absence (**lanes 1**, **6**, and **11**) or presence of 0.1, 0.5, 2.5, and 5.0 μM of BSA (**lanes 2–5**), GST (**lanes 7–10**), and GST-Purα (**lanes 12–15**) purified proteins. Fold suppression is depicted. **B**: Purα has no direct effect on luciferase activity. Luciferase assays were performed with a equal amount of in vitro translated luciferase protein in the absence (lane 1) or presence of 0.1, 0.5, 2.5, and 7.5 μM GST-Purα protein (lanes 2–5). Fold activation is depicted.

Purα on luciferase activity itself, in vitro translated luciferase protein was incubated with increasing amounts of purified GST-Purα protein. As shown in Figure 4B, GST-Purα had no direct effect on luciferase protein activity (compare lanes 1 with 2–6). Thus, the effect of Purα observed in Figures 3 and 4A is not due to any direct influence on luciferase activity per se. Taken together, these data demonstrate that Purα suppresses translation in vitro.

HIV-1 Tat Protein Abrogates Purα-Mediated Suppression of Translation

We have previously demonstrated that the RNA derived from clone 14 was able to reconstitute the interaction between Purα and the HIV-1 Tat protein [Gallia et al., 1999b]. Moreover, this RNA was able to enhance the transcriptional activation of the HIV-1 promoter by Purα and HIV-1 Tat in glial cells [Gallia et al., 1999b]. Based on these previous observations, we next examined the influence of the

HIV-1 Tat protein on the Purα-mediated translational inhibition. As shown in Figure 5, 5.0 μM BSA and GST had no effect on luciferase activity (lanes 2 and 3). Inclusion of 5.0 μM GST-Purα resulted in over a 25-fold inhibition of luciferase activity. Full length GST-Tat protein demonstrated no effect on luciferase activity (lanes 5–7). Interestingly, increasing amounts of GST-Tat protein in reaction mixtures containing a constant amount of GST-Purα resulted in a dose-dependent decrease of the Purα-mediated inhibitory effect on luciferase activity (lanes 8–10). This demonstrates that the HIV-1 Tat protein abrogates the suppression of translation mediated by Purα.

DISCUSSION

Numerous studies have demonstrated that Purα interacts with RNA molecules and that RNA modulates both structural and functional activities of Purα. Tretiakova et al. [1998] demonstrated that Purα purified from mouse brain extract was associated with a cellular RNA, called PU-RNA, with significant homology to 7SL RNA. This PU-RNA was further shown to inhibit the interaction between Purα and its target PUR element-containing regulatory motif of the myelin basic protein gene promoter. Purα has also been shown to interact with neural BC1 RNA [Kobayashi et al., 2000].

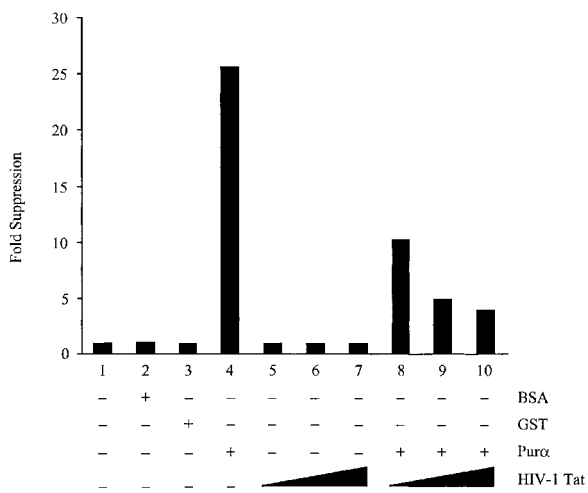

Fig. 5. The HIV-1 Tat protein abrogates Purα-mediated inhibition of translation. Luciferase assays were performed after coupled transcription/translation reactions containing pGL3-T7-luciferase plasmid and 5.0 μM BSA (**lane 2**), 5.0 μM GST (**lane 3**), 5.0 μM GST-Purα (**lane 4**, **8–10**), and 10 nM (**lanes 5** and 8), 50 nM (**lanes 6** and 9) or 100 nM (**lanes 7** and 10) HIV-1 Tat protein. **Lane 1** contains no exogenously added protein. Fold suppression is depicted.

Additionally, Purα (and Purβ) has been shown to neural link BC1 RNA to microtubules through dendrite-targeting RNA motifs located in the 5′ region of the BC1 RNA leading the authors to suggest a role for these proteins in dendritic translocation of BC1 RNA [Ohashi et al., 2000]. In addition to these mammalian RNA molecules, Purα interacts with the HIV-1 regulatory RNA element TAR [Chepenik et al., 1998]. Moreover, this study demonstrated that overexpression of Purα in human glial cells increased transcription from the HIV-1 long terminal repeat via a TAR-dependent mechanism.

In addition to nucleic acid interactions, Purα binds to a variety of regulatory proteins. Many of these interactions are RNA-dependent, including the self-association of Purα [Gallia et al., 1999a] as well as the association between Purα and the HIV-1 regulatory Tat protein [Gallia et al., 1999b; Wortman et al., 2000]. We previously demonstrated that mammalian RNA was able to reconstitute the interaction between Purα and Tat [Gallia et al., 1999b]. In this report, we characterize RNA species immunopurified from Purα after transient transfection into a human glioblastoma cell line. Interestingly, three of the RNA clones were homologous to, and one clone identical to, various regions of human 18S rRNA. Interestingly, PARNA clone 14 possesses sequences identical to both strands of the 18S RNA sequence. Based on the fact that Purα interacted with RNA species homologous to 18S rRNA, we evaluated what, if any, role Purα may play in translation. Our results demonstrate that Purα inhibits translation reactions in vitro. Although this is the first evidence that Purα has a direct role in the translation process, several recent studies are noteworthy in light of our findings [Kelm et al., 1999b; Li et al., 2001].

Kelm et al. [1999b] demonstrated that Purα, Purβ, and the mouse Y-box binding protein, MSY1, interacted with a mRNA sequence derived from a region of the mouse VSM α-actin exon 3. This sequence was identified by its structural similarity to a region of the VSM gene 5′ promoter which contains a sequence whose opposite strands interact with pur or MSY1 proteins [Kelm et al., 1999a]. When this exon 3-derived sequence was placed in the 5′ untranslated region of a reporter mRNA, translation was suppressed. Interestingly, translational efficiency was restored by mutations in the exon

3-derived sequence which impaired mRNA binding of Purα, Purβ, and MSY1 [Kelm et al., 1999b]. Other mRNA-binding proteins, however, with physiologic roles unrelated to translational regulation have been demonstrated to function as translational repressor proteins when their RNA binding sites were placed in the 5′ UTR of reporter mRNA [Stripecke et al., 1994]. Nonetheless, the study by Kelm et al. [1999b] is interesting in light of our current studies demonstrating a Purα-mediated translational inhibition in vitro.

Although Purα was originally isolated from nuclei of mammalian cells, several studies have demonstrated that at least a fraction of the Purα cellular content is contained within the cytoplasm (Li et al., 2001) [Osugi et al., 1996]. Interestingly, a recent study by Li et al. [2001] examining the subcellular localization of Purα within the brain reported that Purα co-fractionated with free and membrane-bound polysomes. This study, although providing no experimental data addressing the functional role in translation, suggests Purα may play a role in translation.

Purα is involved in diverse cellular and viral functions including transcription, replication, and cell growth. The experiments presented herein demonstrate that Purα is also involved in inhibiting cellular translation, at least in vitro. Future experiments will be necessary to fully elucidate the mechanism and significance of Purα, and potentially other pur protein family members, in mammalian translation as well as other cell functions.

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REFERENCES

Ausubel F, Brent R, Kingston R, Moore D, Seidman JG, Smith JA, Struhl K. 1989. Current protocols in molecular biology. New York: John Wiley and Sons Inc.

Bergmann AD, Johnson EM. 1992. The HeLa Pur factor binds single-stranded DNA at a specific element conserved in gene flanking regions and origins of DNA replication. Mol Cell Biol 12:1257–1265.

Bergmann AD, Ma ZW, Johnson EM. 1992. Sequence of cDNA comprising the human pur gene and sequence-specific single-stranded-DNA-binding properties of the encoded protein. Mol Cell Biol 12:5673–5682.

Chang CF, Gallia GL, Muralidharan V, Chen NN, Zoltick P, Johnson EM, Khalili K. 1996. Evidence that replication of human neurotropic JC virus DNA in glial cells is regulated by the sequence-specific single-stranded DNA-binding protein Purα. J Virol 7:4150–4156.

Chen NN, Khalili K. 1995. Transcriptional regulation of human JC polyomavirus promoters by cellular proteins YB-1 and Purα in glial cells. J Virol 69:5843–5848.

Chepenik LG, Tretiakova AP, Krachmarov CP, Johnson EM, Khalili K. 1998. The single-stranded DNA binding protein, Pur-α, binds HIV-1 TAR RNA and activates HIV-1 transcription. Gene 210:37–44.

Chowdhury M, Kundu M, Khalili K. 1993. GA/GC-rich sequence confers Tat responsiveness to human neurotropic virus promoter, JCVL, in cells derived from central nervous system. Oncogene 8:887–892.

Du Q, Tomkinson AE, Gardner PD. 1997. Transcriptional regulation of neuronal nicotinic acetylcholine receptor genes: a possible role for the DNA-binding protein Purα. J Biol Chem 272:14990–14995.

Gallia GL, Safak M, Khalili K. 1998. Interaction of the single-stranded DNA-binding protein Purα with the human polyomavirus JC virus early protein T-antigen. J Biol Chem 273:32662–32669.

Gallia GL, Darbinian N, Johnson EM, Khalili K. 1999a. Self-association of Purα is mediated by RNA. J Cell Biochem 75:1–15.

Gallia GL, Darbinian N, Tretiakova A, Ansari SA, Rappaport J, Brady J, Wortman MJ, Johnson EM, Khalili K. 1999b. Association of HIV-1 Tat with the cellular protein, Purα, is mediated by RNA. Proc Natl Acad Sci USA 96:11572–11577.

Gallia GL, Johnson EM, Khalili K. 2000. Purα: a multifunctional single-stranded DNA-and RNA-binding protein. Nucleic Acids Res 28:3197–3205.

Haas S, Gordon J, Khalili K. 1993. A developmentally regulated DNA-binding protein from mouse brain stimulates myelin basic protein gene expression. Mol Cell Biol 13:3103–3112.

Haas S, Thatikunta P, Steplewski A, Johnson EM, Khalili K, Amini S. 1995. A 39-kD DNA-binding protein from mouse brain stimulates transcription of myelin basic protein gene in oligodendrocytic cells. J Cell Biol 130: 1171–1179.

Herault Y, Chatelain G, Brun G, Michel D. 1993. The PUR element stimulates transcription and is a target for single strand-specific binding factors conserved among vertebrate classes. Cell Mol Biol Res 39:717–725.

Kelm RJ Jr., Elder PK, Strauch AR, Getz MJ. 1997. Sequence of cDNAs encoding components of vascular actin single-stranded DNA-binding factor 2 establish identity to Purα and Purβ. J Biol Chem 272:26727–26733.

Kelm RJ Jr., Cogan JG, Elder PK, Strauch AR, Getz MJ. 1999a. Molecular interactions between single-stranded DNA-binding proteins associated with an essential MCAT element in the mouse smooth muscle α-actin promoter. J Biol Chem 274:14238–14245.

Kelm RJ Jr., Elder PK, Getz MJ. 1999b. The single-stranded DNA-binding proteins, Purα, Purβ, and MSY1 specifically interact with an exon 3-derived mouse vascular smooth muscle α-actin messenger RNA sequence. J Biol Chem 274:38268–38275.

Kobayashi S, Agui K, Kamo S, Li Y, Anzai K. 2000. Neural BC1 RNA associates with Purα, a single-stranded DNA

and RNA binding protein, which is involved in the transcription of the BC1 RNA gene. Biochem Biophys Res Commun 277:341–347.

Krachmarov CP, Chepenik LG, Barr-Vagell S, Khalili K, Johnson EM. 1996. Activation of the JC virus Tat-responsive transcriptional control element by association of the Tat protein of human immunodeficiency virus 1 with cellular protein Purα. Proc Natl Acad Sci 93:14122–14177.

Lasham A, Lindridge E, Rudert F, Onrust R, Watson J. 2000. Regulation of the human *fas* promoter by YB-1, Purα and AP-1 transcription factors. Gene 252:1–13.

Li Y, Koike K, Ohashi S, Funakoshi T, Tadano M, Kobayashi S, Anzai K, Shibata N, Kobayashi M. 2001. Purα protein implicated in dendritic RNA transport interacts with ribosomes in neuronal cytoplasm. Biol Pharm Bull 24:231–235.

Major EO, Amemiya K, Tornatore CS, Houff SA, Berger JR. 1992. Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clin Microbiol Rev 5:49–73.

Ohashi S, Kobayashi S, Omori A, Ohara S, Omae A, Muramatsu T, Li Y, Anzai K. 2000. The single-stranded DNA- and RNA-binding proteins Pur α and Pur β link BC1 RNA to microtubules through binding to the dendrite-targeting RNA motifs. J Neurochem 75:1781–1790.

Osugi T, Ding Y, Tanaka H, Kuo CH, Do E, Irie Y, Miki N. 1996. Involvement of a single-stranded DNA binding protein, ssCRE-BP/Purα, in morphine dependence. FEBS Lett 391:11–16.

Queen C, Baltimore D. 1983. Immunoglobulin gene transcription is activated by downstream sequence elements. Cell 33:741–748.

Safak M, Gallia GL, Khalili K. 1999. Reciprocal interaction between two cellular proteins, Purα and YB-1, modulates transcriptional activity of JCV CY in glial cells. Mol Cell Biol 19:2712–2723.

Stripecke R, Oliveira CC, McCarthy JEG, Hentze MW. 1994. Proteins binding to 5′ untranslated region sites: a general mechanism for translational regulation of mRNAs in human and yeast cells. Mol Cell Biol 14: 5898–5909.

Thatikunta P, Sawaya BE, Denisova L, Cole C, Yusibova G, Johnson EM, Khalili K, Amini S. 1997. Identification of a cellular protein that binds to Tat-responsive element of TGFβ-1 promoter in glial cells. J Cell Biochem 67:466–477.

Tretiakova A, Gallia GL, Shcherbik N, Jameson B, Johnson EM, Amini S, Khalili K. 1998. Association of Purα with RNAs homologous to 7 SL determines its binding ability to the myelin basic protein promoter DNA sequence. J Biol Chem 273:22241–22247.

Wortman MJ, Krachmarov CP, Kim JH, Gordon RG, Chepenik LG, Brady JN, Gallia GL, Khalili K, Johnson EM. 2000. Interaction of HIV-1 Tat with Purα in nuclei of human glial cells: characterization of RNA-mediated protein–protein binding. J Cell Biochem 77:65–74.

Zambrano N, DeRenzis S, Minopoli G, Faraonio R, Donini V, Scaloni A, Cimino F, Russo T. 1997. DNA-binding protein Purα and transcription factor YY1 function as transcription activators of the neuron-specific FE65 gene promoter. Biochem J 328:293–300.