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Self-Association of Purα Is Mediated by RNA

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Abstract The 322 amino acid cellular protein, Purα, is a sequence-specific single-stranded DNA-binding protein implicated in control of transcription and replication. Previous studies have demonstrated that the interaction between Purα and its target DNA sequence results in the formation of multimeric complexes. In this study, we demonstrate that Purα can self-associate in the absence of DNA. This self-association, while independent of DNA, is mediated by RNA. Through in vitro studies with bacterially expressed glutathione S-transferase fusion proteins, and the synthetic peptides corresponding to various central regions of Purα, the domain which is important for the self-association of Purα is localized to acidic leucine-rich repeats. Interestingly, these repeats have previously been shown to interact with the human immunodeficiency virus 1 (HIV-1) Tat protein and in this study we demonstrate that Tat is able to disrupt the self-association of Purα. We have recently cloned a Purα associated-RNA, PU-RNA, and here we show that PU-RNA can specifically reconstitute the self-association of Purα. RNA not only mediates the self-association of Purα, but also modulates the ability of Purα to interact with its target DNA sequence. Electrophoretic mobility shift assays performed with and without RNase treatment demonstrate that RNA inhibits the interaction between Purα and its target DNA sequence. Moreover, we demonstrate that the self-association of Purα can be reconstituted by a specific oligonucleotide encompassing the Purα binding site. The implications of these findings with respect to Purα's role in transcription and replication are discussed. J. Cell. Biochem. 74:334–348, 1999. © 1999 Wiley-Liss, Inc.

Key words: Purα; HIV-1 tat protein; transcription; replication; self-association; RNA

Purα, a 322 amino acid cellular protein, is a single-stranded DNA-binding protein with specific affinity for a purine-rich element comprised of repeats of (GGN)n [Bergemann and Johnson, 1992; Bergemann et al., 1992]. These so-called PUR elements are present in several gene flanking regions as well as initiation zones of eukaryotic DNA replication [Bergemann and Johnson, 1992]. As such, Purα has been implicated in control of both gene transcription and DNA replication. Indeed, since the initial characterization and cloning of Purα, several laboratories have cloned genes encoding proteins which have revealed identity to Purα [Du et al., 1997; Haas et al., 1995; Jurk et al., 1996; Kelm et al., 1997; Osugi et al., 1996; Tada and Khalili, 1992; Zambrano et al., 1997].

Several studies have demonstrated that Purα is a transcriptional activator of both cellular as well as viral genes. Purα has been shown to activate several promoters including the myelin basic protein promoter [Haas et al., 1993, 1995], the JC virus (JCV) early gene promoter [Chen and Khalili, 1995], the human immunodeficiency virus I (HIV-1) long terminal repeat (LTR) [Chepenik et al., 1998], and the neuron-specific FE65 gene promoter [Zambrano et al., 1997]. In addition, Purα has been demonstrated to interact with other gene regulatory elements and has thus been implicated in the expression of the neuronal nicotinic acetylcholine receptor gene promoter [Du et al., 1997], the single-stranded cAMP response element [Osugi et al., 1996], and the vascular smooth muscle α-actin promoter [Kelm et al., 1997].

In addition to its role in gene transcription, Purα has also been implicated in control of DNA replication. There are several lines of evidence supporting a role for this protein in DNA replication. PUR elements are repeated within zones of initiation of DNA replication [Bergemann and Johnson, 1992]. Moreover, Purα has been demonstrated to interact with a PUR ele-

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ment upstream of the c-myc gene, near the center of a region implicated as an initiation zone for chromosomal DNA replication [Bergemann et al., 1992]. Purα has also been shown to interact with viral origins of DNA replication as well, including the JCV and the Bovine Papillomavirus (BPV) origins of replication [Chang et al., 1996; Jurk et al., 1996]. Although the biological activity of Purα on eukaryotic and BPV replication is yet undescribed, perhaps the most direct evidence supporting a role for Purα in DNA replication emerges from the effect of Purα on JCV DNA replication. Chang et al. [1996] demonstrated that overexpression of Purα suppresses replication of JCV DNA in glial cells.

In addition to its role in transcription and replication, Purα may also play a role in the control of cell growth. Johnson et al. [1995] have demonstrated that Purα interacts with the hypophosphorylated form of the retinoblastoma tumor suppressor gene product, pRb. pRb is an integral protein involved in progression of the cell cycle [Weinberg, 1995]. The hypophosphorylated form of pRb complexes with a variety of proteins in the G0 and G1 phases of the cell cycle including transcription factors such as E2F. Hyperphosphorylation of pRb in late G1 results in the release of these transcription factors from pRb. E2F, free from pRb, is subsequently able to activate genes necessary for progression through G1 and entry into the S phase of the cell cycle. Interestingly, Purα interacts with the same region of pRb as does E2F and several other proteins [Johnson et al., 1995]. Although the functional consequence of Purα's interaction with pRb is yet undescribed, the selective association of Purα with the hypophosphorylated form of a protein so intimately involved in cell cycle progression suggests a potentially crucial role. Interestingly, patients with acute myeloblastic leukemia and myelodysplastic syndrome display deletions in the gene encoding Purα [Lezon-Geyda et al., 1997], located at human chromosome band 5q31 [Ma et al., 1995].

With respect to the association between Purα and its target DNA sequence, previous studies have demonstrated that the interaction between Purα and its target sequence results in the formation of multimeric complexes [Johnson et al., 1995; Krachmarov et al., 1995; Muralidharan et al., 1997]. In this study, we examine the ability of Purα to interact in solution in the absence of DNA. Our results demonstrate that while Purα can associate in the absence of DNA, it's self-association is mediated by RNA. Moreover, a recently isolated Purα-associated RNA [Tretiakova et al., 1998], PU-RNA, is capable of reconstituting the self-association of Purα. We further demonstrate that RNA not only mediates the self-association of Purα, but also influences the ability of Purα to interact with its target DNA sequence.

MATERIALS AND METHODS

Electrophoretic Mobility Shift Assay (Band Shift Assay)

Oligonucleotide probes were end-labeled with [γ-^{32}P] using T4 polynucleotide kinase and then gel purified. Protein samples were incubated with 50,000 cpm of labeled probe in a 20 µl reaction containing 12 mM HEPES (pH 7.9), 4 mM Tris (pH 7.5), 60 mM KCl, 5 mM $MgCl_2$, 0.8 mM dithiothreitol (DTT), and 1 µg poly[dI-dC] as nonspecific competitor. Reactions performed in the presence of RNase contained 10µg/ml of DNase-free RNase (Boehringer-Mannheim Biochemicals, Indianapolis, IN). The reaction mixtures were incubated at 4°C for 30 min and complexes were resolved by electrophoresis on 0.5X Tris-borate-EDTA (TBE) 6% native polyacrylamide gels. Electrophoresis was carried out at 180 V for 3–4 h at 4°C. For experiments performed with synthetic peptides, reactions were carried out in the presence of RNase as described above, and 100 or 500 ng of the synthetic peptides derived from various regions of Purα.

Plasmid Constructs, Oligonucleotides, and Synthetic Peptides

The expression vector pEBVHis B-Purα (pEBV-Purα) contains the coding region of the Purα gene fused 3' to a histidine epitope tag under control of the Rous Sarcoma Virus promoter. pEBV-Purα was constructed by first subcloning the EcoRI fragment containing the coding region of Purα from pGEX-1λT-Purα [Johsnon et al., 1995] into EcoRI digested pCDNA3 (Invitrogen, La Jolla, CA) generating pCDNA3-Purα. pCDNA3-Purα was subsequently cut with BamHI and XhoI and the BamHI/XhoI fragment containing the Purα coding sequence was ligated into BamHI/XhoI cut pEBVHis B (Invitrogen). GST-Purα and GST-Purα deletion mutants containing amino acids 85–322, 167–322, 216–322, 274–322, and 1–215

have previously been described [Johnson et al., 1995]. Other GST-Purα C-terminal deletion mutants containing amino acids 1–174, 1–154, 1–123, and 1–71 were generated by digesting pGEX-1λT Purα with BamHI/HincII, BamHI/ScaI, BamHI/PvuII, and BamHI/Eco47III, respectively and subsequently ligating the BamHI/blunt end restriction enzyme fragments into BamHI/SmaI digested pGEX-2T (Pharmacia, Gaithersburg, MD). The plasmid, pMAL-Purα, encoding a maltose-binding protein (MalBP)-Purα fusion protein, has been previously described [Chen et al., 1995]. pCDNA3-PU-RNA used to generate in vitro PU-RNA transcripts has been described previously [Tretiakova et al., 1998]. All plasmids were verified by DNA sequencing by using Sequenase (United States Biochemical Corporation, Cleveland, OH).

Oligonucleotides were prepared commercially by Oligos Etc. (Guilford, CT). The following oligonucleotides derived from the JC viral origin of DNA replication were used:

VG-1: 5′-AGCTTGGAGGCGGAGGCGGCCTC-GGCG -3′

DD-1: 5′-CGCCGAGGCCGCCTCCGCCTCC-AAGCT -3′.

Synthetic peptides were prepared commercially by Research Genetics. The following peptides derived from various regions of Purα were used:

Peptide A: H-LTLSMSVAVERFDYLGDFIE-HY-OH

Peptide B: H-QNKRFYLDVKQN-OH

Peptide C: H-IALPAQGLIEFRDALAKLID-DY-OH

GST Affinity Chromatography Assay

For in vitro binding assays, either 200μg of cellular extract containing a histidine epitope tagged Purα or 5μl (except where noted) of [^{35}S]-labeled in vitro transcribed-translated Purα was incubated with 5μg of *E. coli* purified GST, GST-Purα, or GST-Purα deletion mutants immobilized on glutathione sepharose beads in 300μl of lysis buffer 150 buffer (LB 150 containing 50 mM Tris-HCl, pH 7.4; 150 mM NaCl; 5 mM EDTA; 0.1% NP-40) plus 1 μg/μl BSA for 1 h at 4°C with continuous rocking. After incubation, the beads were pelleted and washed five times with LB 150 buffer. Bound proteins were eluted with Laemmli sample buffer, heated to 95°C, and fractionated by SDS-PAGE. Purα was detected by either autoradiography or immunoblot analysis with anti-T7 monoclonal antibody recognizing the histidine epitope tag (Novagen, Madison, WI). For experiments with cell extracts, 25μg of whole cell extract was loaded as controls. One-tenth of the amount of [^{35}S]-labeled Purα was likewise loaded as control. Where indicated, Purα was pretreated with 10μg/ml of soluble DNase-free RNase (Boehringer-Mannheim) or 50 units of RNase-free DNase I (Boehringer-Mannheim) for 30 min at room temperature. In the experiment shown in Figure 5, 6μl of [^{35}S]-labeled Purα was incubated with 5μg of GST or GST-Purα in the absence or presence of 2, 4, 8, or 16μl of [^{35}S]-labeled HIV-1 Tat protein. Experiments in which it was necessary to remove RNase from the binding reaction, [^{35}S]-labeled Purα was treated with 10 units of insoluble RNase (RNase conjugated to agarose beads) (Sigma, St. Louis, MO) per μl of protein for 2 h at room temperature. After treatment with insoluble RNase, the RNA free [^{35}S]-Purα-containing extract was separated from the RNase agarose bead conjugate by first microcentrifugation followed by filtration through a 0.2μm cellulose acetate filter Spin-X column (Costar, Cambridge, MA). In experiments that utilized [^{35}S]-labeled Purα pretreated with insoluble RNase, all reactions were supplemented with 40 units of RNase-inhibitor (Boehringer-Mannheim) and 5 mM DTT. In experiments performed with the addition of synthetic peptides, reactions were carried out in the absence of RNase and in the presence of either 0.1 or 1.0μg of each synthetic peptide as indicated. For reconstitution experiments, 2.5μg of either PU-RNA, tRNA, VG-1 DNA oligonucleotide, or DD-1 DNA oligonucleotide were added to the binding reactions as indicated in the text.

Extract Preparation and Western Blot (Immunoblot) Analysis

The human astrocytic cell line, U-87 MG (ATCC, Manassas, VA) was transfected with 30μg of pEBV-B or pEBV-Purα. Forty-eight h after transfection, cells were washed three times with 1X PBS and lysed in lysis 150 buffer containing 50 mM sodium fluoride, 1 mM phenylmethylsulfonyl fluoride, 1μg/ml leupeptin, and 1μg/ml aprotinin for 20 min on ice. Lysates were scraped and collected into microfuge tubes, vortexed briefly, and microcentrifuged for 20 min at 4°C. Proteins were transferred to supported nitrocellulose membranes (Schleicher &

Schnell, Keene, NH) in Western transfer buffer (192 mM glycine, 25 mM Tris base, and 20% methanol). For immunoblot analysis, the membranes were first blocked for 30 min in 10% nonfat dry milk in PBS-T (1X PBS and 0.1% Tween-20) and then incubated for 1 h with a 1:3,000 dilution of an antibody which recognizes the histidine epitope tag fused to Purα (anti-T7). After washing, the membranes were incubated for 1 h with a horseradish peroxidase-linked goat anti-mouse antibody at room temperature. Antibody detection was achieved by enhanced chemiluminescence (ECL) according to the manufacturer's recommendations (Amersham, Arlington Heights, IL).

In Vitro Transcription and Translation

[^{35}S]-labeled Purα was synthesized in vitro from XbaI linearized pCDNA3-Purα using the TNT coupled transcription-translation wheat germ extract (Promega, Madison, WI) according to the manufacturer's instructions.

In Vitro Transcription

PU-RNA was transcribed in vitro using T7 RNA polymerase. Transcription reactions (20μl) contained 0.5 μg of linearized DNA templates, 40 mM Tris (pH 7.5), 6 mM $MgCl_2$, 10 mM DTT, 4 mM spermidine, 20 units of RNase inhibitor, 0.5 mM of ATP, GTP, CTP, and UTP, and 20 units of T7 RNA polymerase (Boehringer-Mannheim). Reactions were incubated for 1 h at 37°C followed by the addition of 20 units of RNase-free DNase I and subsequently incubated for 20 min at 37°C.

Protein Purification

GST fusion proteins were expressed and purified as previously described [Smith and Johnson, 1988]. Briefly, bacteria were grown overnight at 37°C in Luria Bertani (LB) medium supplemented with 100 mg/l ampicillin. The following morning, the cells were diluted 1:10 in fresh LB medium containing 100 mg/l ampicillin, grown to an optical density of 0.6–0.7 at 595 nm, and induced for 2.5 h at 37°C with 0.6 mM isopropyl-β-D-thiogalactopyranoside (IPTG). Bacteria were pelleted at 7,000*g* at 4°C, resuspended in NETN buffer (20 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA, and 0.5% NP-40) containing 1μg/ml aprotinin, 1μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride (PMSF). After sonication on ice, the bacterial lysate was centrifuged at 40,000*g* at 4°C to remove insoluble material. Glutathione-sepharose beads (Pharmacia) were incubated with the supernatant for 3 h at 4°C. Beads were pelleted and washed with 20 volumes of NETN buffer three times. The integrity and purity of the fusion proteins were analyzed by SDS-PAGE followed by Coomassie blue staining. Maltose binding protein (MalBP) and MalBP-Purα were expressed and purified on amylose affinity columns. Briefly, 10 ml overnight cultures were grown at 37°C in LB medium supplemented with 100 mg/l ampicillin. The following morning, the overnight cultures were inoculated into 1 L of LB containing 100 mg/l ampicillin and 2 gm of glucose. The cells were grown to an optical density of 0.4–0.6 and induced for 2 h at 37°C with 0.3 mM IPTG. Bacteria were pelleted at 7,000*g* at 4°C, resuspended in column buffer (20 mM Tris, pH 7.4, 200 mM NaCl, 1 mM EDTA, and 1 mM DTT) containing 1 mg/ml lysozyme, 1μg/ml aprotinin, 1μg/ml leupeptin, and 1 mM PMSF. After sonication on ice, insoluble material was removed by centrifugation at 40,000*g* at 4°C. MalBP and MalBP-Purα were purified on amylose affinity columns according to the manufacturer's instructions (New England Biolabs, Beverly, MA).

RESULTS

Purα Interacts Specifically With Its Target Sequence and Forms Multimeric Complexes

The single-stranded DNA target of Purα is comprised of GGN sequence repeats, called PUR elements. Previous studies have demonstrated that the interaction between Purα and these purine-rich PUR sequences results in the formation of multimeric complexes [Johnson et al., 1995; Krachmarov et al., 1995; Muralidharan et al., 1997]. In the first series of experiments, electrophoretic mobility shift assays were performed with the purine-rich strand of the JCV origin of DNA replication, VG-1, and a maltose-binding protein-Purα fusion protein (MalBP-Purα). As shown in Figure 1A, incubation of MalBP-Purα with the labeled VG-1 oligonucleotide resulted in several complexes with distinct electrophoretic mobilities, labeled complex a, b, and c (lanes 2–4). No DNA protein complex was detected upon incubation of the control MalBP with the VG-1 probe (Fig. 1A, lane 5), suggesting that the MalBP is not responsible for the association of MalBP-Purα to the VG-1 sequence. The formation of three MalBP-Purα/

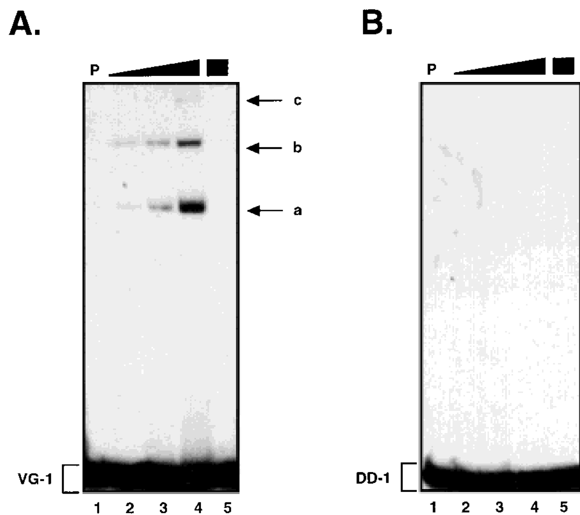

Fig. 1. Purα interacts specifically with a purine-rich sequence and forms multimeric complexes. **A**: VG-1, a purine-rich single-stranded oligonucleotide, was incubated with 10, 50, and 250 ng of MalBP-Purα (**lanes 2–4**, respectively) or 250 ng of MalBP (**lane 5**). The three resulting MalBP-Purα/VG-1 nucleoprotein complexes, designated complex a, b, and c, are indicated by arrows. **B**: The pyrimidine-rich single strand complement of VG-1, termed DD-1, was incubated with 10, 50, and 250 ng of MalBP-Purα (**lanes 2–4**, respectively) or 250 ng of MalBP (**lane 5**). **Lane 1** contains probe alone (P). Excess free probe is indicated by brackets at the bottom of each panel.

VG-1 nucleoprotein complexes with distinct electrophoretic mobilities suggests that Purα binds to the VG-1 sequence in multimeric forms. This is consistent with previous studies demonstrating that Purα interacts with the single-stranded sequence element derived from the myelin basic protein promoter, MB1, in both monomeric and dimeric forms [Muralidharan et al., 1997]. Incubation of MalBP-Purα with the pyrimidine-rich complement of the VG-1 sequence, called DD-1, did not result in any complex formation (Fig. 1B, lanes 2–4), demonstrating the specificity of the interaction between Purα and the VG-1 sequence. Taken together, these results demonstrate that Purα interacts specifically with the purine-rich strand of the JCV origin of DNA replication and that this interaction results in the formation of multimeric complexes.

Self Association of Purα Is Mediated by RNA

To determine whether Purα is capable of self-association in the absence of DNA, GST affinity chromatography assays were performed. In the first series of experiments, the human astrocytic U-87 MG cell line was transiently transfected with a control plasmid (pEBV-B) or a plasmid encoding Purα fused to an amino terminal histidine epitope tag (pEBV-Purα). Extracts derived from these transiently transfected cells were incubated with GST or GST-Purα immobilized on glutathione-sepharose beads. After washing, proteins retained on the beads were analyzed by immunoblot analysis with an antibody which recognizes the histidine epitote tag. As shown in Figure 2A, the histidine-tagged Purα was specifically retained on the sepharose column containing GST-Purα (lane 6), but not on the column containing GST alone (lane 5). No interaction was observed with the control transfected cell extract (lanes 3 and 4). To provide additional evidence for the self-association of Purα, in vitro transcribed-translated [^{35}S]-labeled Purα was incubated with GST or GST-Purα immobilized on glutathione-sepharose beads. After washing, binding was assessed by SDS-PAGE followed by autoradiography. As shown in Figure 2B, [^{35}S]-labeled Purα was specifically retained on the sepharose column containing GST-Purα (lane 3), but not on the column containing GST alone (lane 2). To exclude any interference by or dependence on DNA in this protein-protein interaction, ethidium bromide (100 μg/ml) was included in the reaction [Lai amd Herr, 1992]. As shown in Figure 2B, [^{35}S]-labeled Purα was able to interact with GST-Purα in the presence of ethidium bromide (lane 4) demonstrating the independence of this interaction on DNA. Taken together, these results indicate that Purα self-associates, and that this self-association is independent of the presence of DNA.

Since Purα also interacts with RNA [Chepenik et al., 1998; Herault et al., 1995; Tretiakova et al., 1998], the dependence of RNA in the self-association of Purα was examined. To address this question, U-87 MG cells were transiently transfected with pEBV-Purα. Extract derived from these transiently transfected cells was either treated with soluble DNase or RNase and then incubated with GST or GST-Purα immobilized on glutathione-sepharose beads. After washing, proteins retained on the beads were analyzed by immunoblot analysis with an antibody which recognizes the histidine epitope tag. As shown in Figure 2C and consistent with our previous results, treatment of the cellular extract with DNase had no effect on the self-association of Purα (compare lanes 5 and 6). Treatment of cellular extract with RNase, however, abrogated the self-association (Fig. 2C,

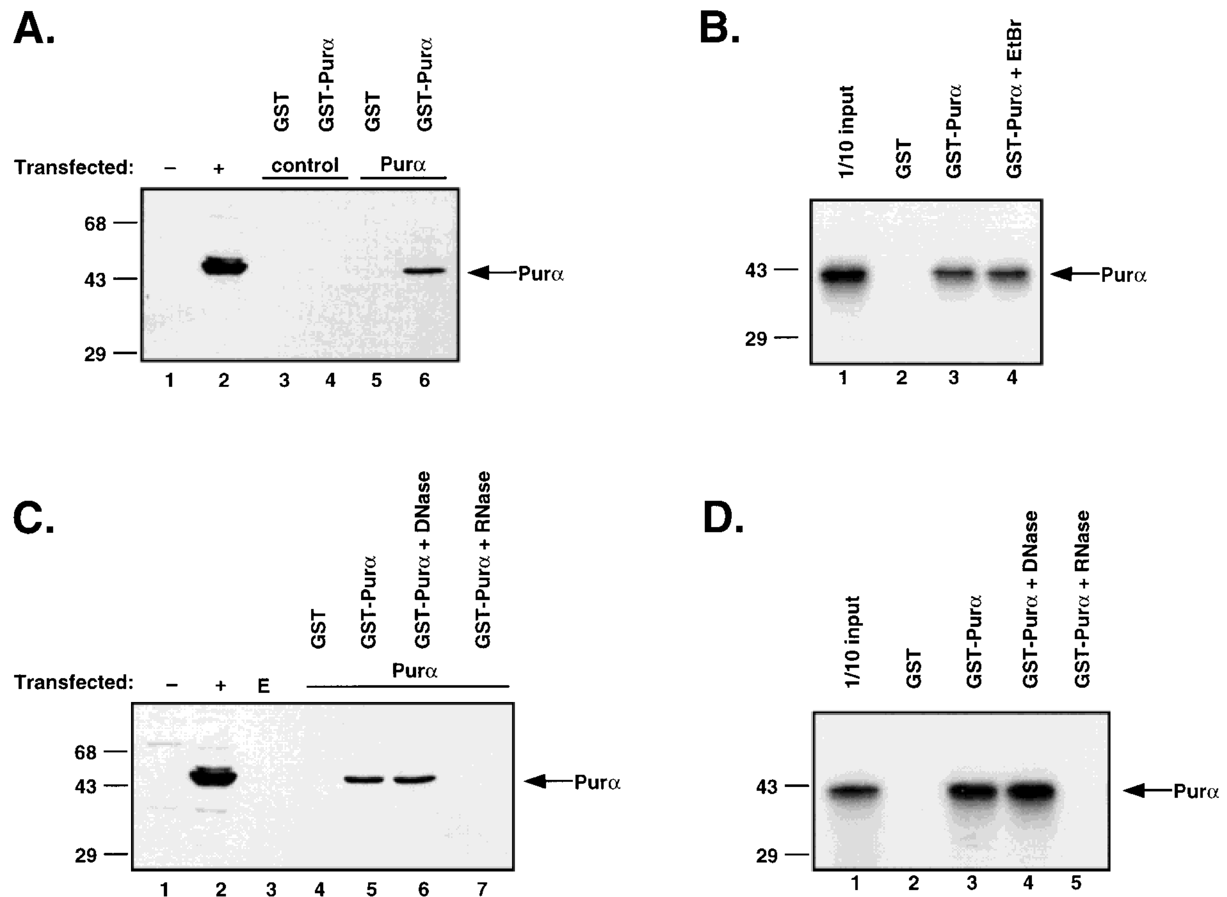

Fig. 2. Self-association of Purα is independent on DNA but is mediated by RNA. **A**: The human astrocytic cell line, U-87 MG, was transfected with a control plasmid (pEBV-B) or a plasmid encoding Purα fused to an epitope tag (pEBV-Purα). Cellular extract from control and Purα transfected cells was incubated with GST (**lanes 3 and 5**) or GST-Purα (**lanes 4 and 6**) immobilized on glutathione-sepharose beads. Bound proteins were fractionated by SDS-PAGE and detected by immunoblot analysis with an antibody recognizing the epitope tag (anti-T7). Lanes 1 and 2 represent direct immunoblot analysis of cells mock transfected with pEBV-B (**lane 1**) or transfected with pEBV-Purα (**lane 2**). **B**: [^{35}S]-labeled Purα produced by in vitro transcription-translation was incubated with pEBV-B GST (**lane 2**) or GST-Purα (**lanes 3 and 4**) either in the absence (lane 3) or presence (lane 4) of ethidium bromide. **Lane 1** contains one-tenth the amount of [^{35}S]-labeled Purα used in the binding reaction. Bound proteins were fractionated by SDS-PAGE and visualized by autoradiography. **C**: Cellular extract from U-87 MG cells transfected with Purα (pEBV-Purα) was incubated with GST (**lane 4**), or GST-Purα (**lanes 5–7**), either in the absence (lane 5) or presence of DNase (lane 6) or RNase (lane 7). Bound proteins were fractionated by SDS-PAGE and detected by immunoblot analysis with anti-T7 antibody which recognizes the epitope tag fused to Purα. **Lane 3** is an empty lane (E). As in A, **lanes 1 and 2** represent direct immunoblot analysis of cells mock transfected with pEBV-B (lane 1) or transfected with pEBV-Purα (lane 2). **D**: [^{35}S]-labeled Purα was incubated with GST (**lane 2**) or GST-Purα (**lanes 3–5**) either in the absence (lane 3) or presence or DNase (lane 4) or RNase (lane 5). **Lane 1** contain one-tenth the amount of [^{35}S]-labeled Purα used in the binding reactions. Bound proteins were fractionated by SDS-PAGE and visualized by autoradiography. In all panels, arrows indicate Purα and the positions of molecular mass markers (in kilodaltons) are shown on the left.

compare lanes 5 and 7) indicating that the self-association of Purα is mediated by RNA. Additionally, in vitro transcribed-translated [^{35}S]-labeled Purα was either treated with soluble DNase or RNase and then incubated with GST or GST-Purα immobilized on glutathione-sepharose beads. After washing, binding was assessed by SDS-PAGE followed by autoradiography. As shown in Figure 2D, whereas treatment with DNase had no effect on the self-association of Purα (compare lanes 3 and 4), treatment with RNase abrogated the self-association of Purα (compare lanes 3 and 5). To exclude the possibility that RNase selectively degrades the [^{35}S]-labeled Purα, controls reactions were run after treating in vitro translated Purα and cellular extract containing epitope tagged Purα with DNase and RNase. Neither DNase nor RNase affected the stability of Purα (data not shown) suggesting that the abroga-

tion of the self-association of Purα upon RNase treatment is not due to the degradation of Purα. Taken together, these results demonstrate that the self-association of Purα is mediated by RNA.

Localization of the Purα Self-Association Region

Structurally, Purα is composed of several modular domains (Fig. 3C) [Bergemann et al., 1992]. The central region contains three aromatic and basic repeats (class I) interspersed with two acidic leucine-rich repeats (class II). In addition to this central repeat region, Purα possess an amino terminal glycine-rich region, an amphipathic α-helix, and a glutamate-glutamine-rich region near the carboxy terminus. To identify the region(s) responsible for Purα self-association, a series of N- and C-terminal deletion mutants fused to GST were utilized. In vitro transcribed-translated [^{35}S]-labeled Purα was incubated with GST, GST-Purα, and the various GST-Purα deletion mutants immobilized on glutathione-sepharose beads. After washing, binding was assessed by autoradiography. As shown in Figure 3A, removal of the amino terminal 84 amino acids encompassing the glycine-rich region of Purα and the first class I repeat, GST-Purα (85–322), retained the ability to bind [^{35}S]-labeled Purα (lane 4). Similarly, removal of the amino terminal 166 amino acids, GST-Purα (167–322), retained the ability to bind [^{35}S]-labeled Purα (Fig. 3A, lane 5). Further amino terminal deletion mutants, GST-

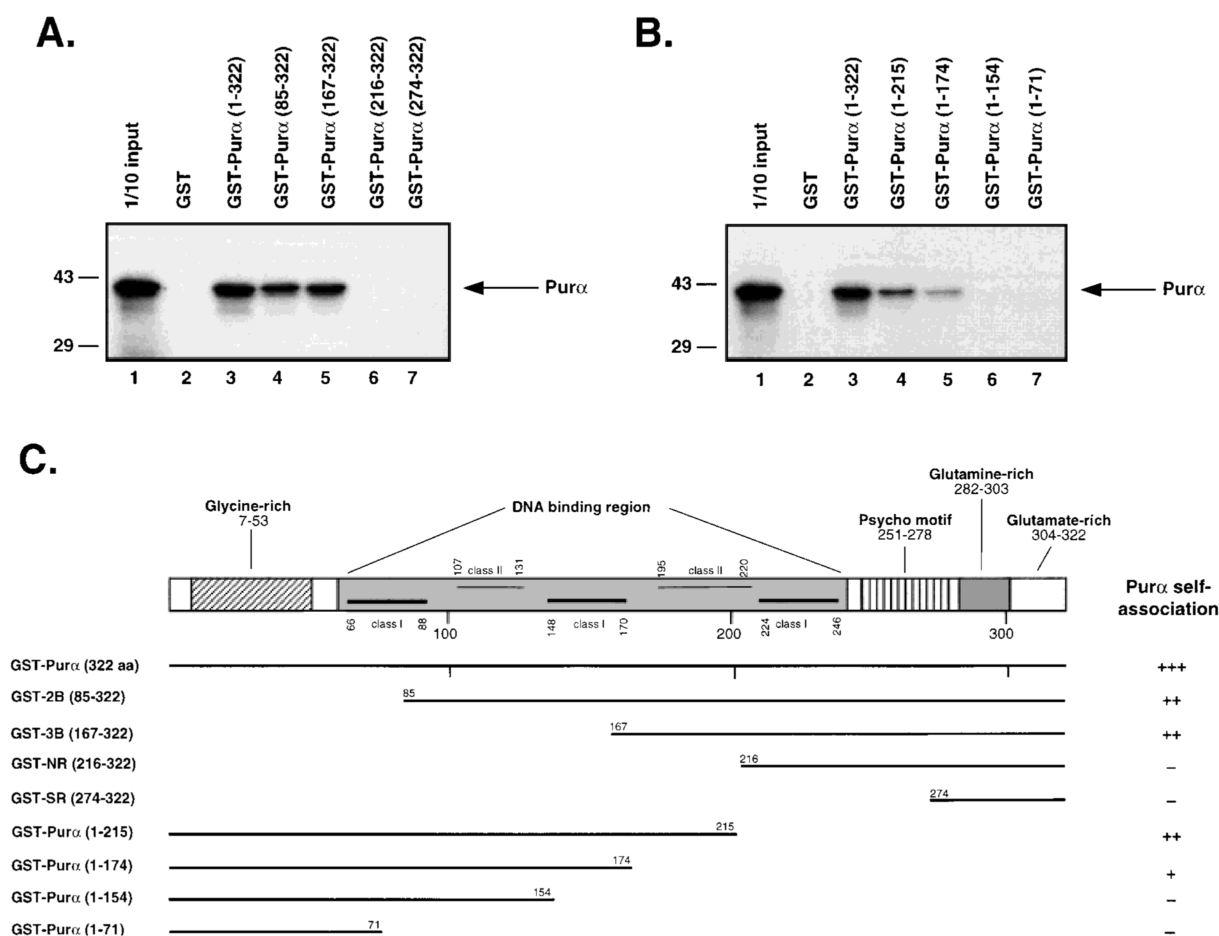

Fig. 3. Localization of the Purα self-association region. [^{35}S]-labeled Purα produced by in vitro transcription-translation was incubated with GST (**A**,**B**, **lane 2**), GST-Purα (A,B, **lane 3**), N-terminal GST-Purα deletion mutants (A, **lanes 4–7**), or C-terminal GST-Purα deletion mutants (B, **lanes 4–7**) immobilized on glutathione-sepharose beads. Bound proteins were fractionated by SDS-PAGE and visualized by autoradiography. The GST fusion proteins used are shown above the lanes with their corresponding amino acid coordinates indicated in parenthesis. **Lane 1** (A,B) contains one-tenth the amount of [^{35}S]-labeled of Purα used in the binding reactions. Arrows indicate the position of Purα, while the positions of molecular mass markers (in kilodaltons) are shown on the left. **C:** Schematic diagram of the Purα deletion mutants used and summary of the self-association results obtained in A and B. Structural motifs are described in the text.

Purα (216–322) and GST-Purα (274–322), were unable to interact with [^{35}S]-labeled Purα (Fig. 3A, lanes 6 and 7). These results sugget that sequences between amino acids 167 and 215 are involved in the self-association of Purα.

To further define the sequences within Purα which are important for its self-association, similar experiments were performed with a series of C-terminal GST-Purα deletion mutants. A carboxyl terminal mutant containing amino acids 1–215, GST-Purα (1–215) retained the ability to interact with [^{35}S]-labeled Purα, albeit to a slightly reduced level (Fig. 3B, lane 4). A carboxy terminal deletion mutant only containing the first 174 amino acids, GST-Purα (1–174) interacted weakly with [^{35}S]-labeled Purα (Fig. 3B, lane 5). Further carboxy terminal deletion mutants, GST-Purα (1–154) and GST-Purα (1–71), were unable to interact with [^{35}S]-labeled Purα (Fig. 3B, lanes 6 and 7). These results indicate that sequences carboxy to amino acid 154 are involved in the self-association of Purα. This is consistent with the results obtained with the amino terminal GST-Purα deletion mutants. Taken together, these experiments demonstrate that the minimal region of Purα which is important for its self-association resides between amino acids 167–215.

The region of Purα important for its self-association, which resides between amino acids 167–215, encompasses the second class II motif (Fig. 3C). While the two class II motifs are not identical, they preserve a number of strictly conserved amino acids at fixed distances along the repeats and a high percentage of conservatively substituted amino acids [Bergemann et al., 1992]. To further investigate the involvement of these sequences in the self-association of Purα, similar GST-pull down experiments as described above were performed in the presence of synthetic peptides derived from various regions of Purα. Peptide A was derived from the first class II repeat (amino acids 100–121) and peptide C was derived from the second class II repeat (amino acids 188–219). A control peptide, peptide B, was derived from the first class I repeat (amino acids 59–80). The similarity between peptides A and C, derived from the first and second class II Purα repeats, respectively, is shown in Figure 4A. GST-pull down experiments were performed with increasing concentrations of each of these peptides. As shown in Figure 4B, inclusion of peptide A in GST-pull down experiments resulted in a diminished ability of Purα to self associate (compare lane 3 to lanes 6 and 7). This occurred in a dose-dependent fashion as inclusion of more peptide resulted in an even more decreased self-association (compare lanes 6 and 7). Likewise, inclusion of peptide C also resulted in a dose-dependent decrease in the self-association of Purα (compare lane 3 and lanes 8 and 9). Inclusion of the control peptide (peptide B) into the reaction mixture did not have any effect on the ability of Purα to self-association (compare lane 3 to lanes 4 and 5). These results demonstrate that inclusion of peptides derived from either class II motif results in the disruption of the self-association of Purα, indicating that sequences located in both class II motif are important in the self-association of Purα. Moreover, this is specific for class II sequences as a peptide derived from the first class I motif does not influence the interaction.

HIV-1 Tat Protein Can Disrupt the Self-Association of Purα

The above experiments demonstrate that the two class II motifs are important in the self-association of Purα. Interestingly, these repeats have been shown to also be important in the interaction between Purα and the human immunodeficiency virus (HIV-1) Tat protein [Krachmarov et al., 1995]. To determine what influence Tat has on the self-association of Purα, GST affinity chromatography assays were performed with a constant amount of [^{35}S]-labeled Purα and an increasing amount of [^{35}S]-labeled Tat protein. As shown in Figure 5, increasing amount of Tat protein disrupted the self association of Purα and resulted in the formation of Purα:Tat complexes.

Reconstitution of Self-Association of Purα With PU-RNA

Since the self-association of Purα is RNA-dependent, RNA should thus be able to reconstitute this association. To examine this possibility, GST affinity chromatography assays were performed in which the endogenous RNA mediating the self-association was first removed and exogenous RNA was subsequently added. In order to determine which of the two components, the GST-Purα fusion protein or the [^{35}S]-labeled Purα, contained the RNA which is important for the self association of Purα, each component was selectively treated with RNase.

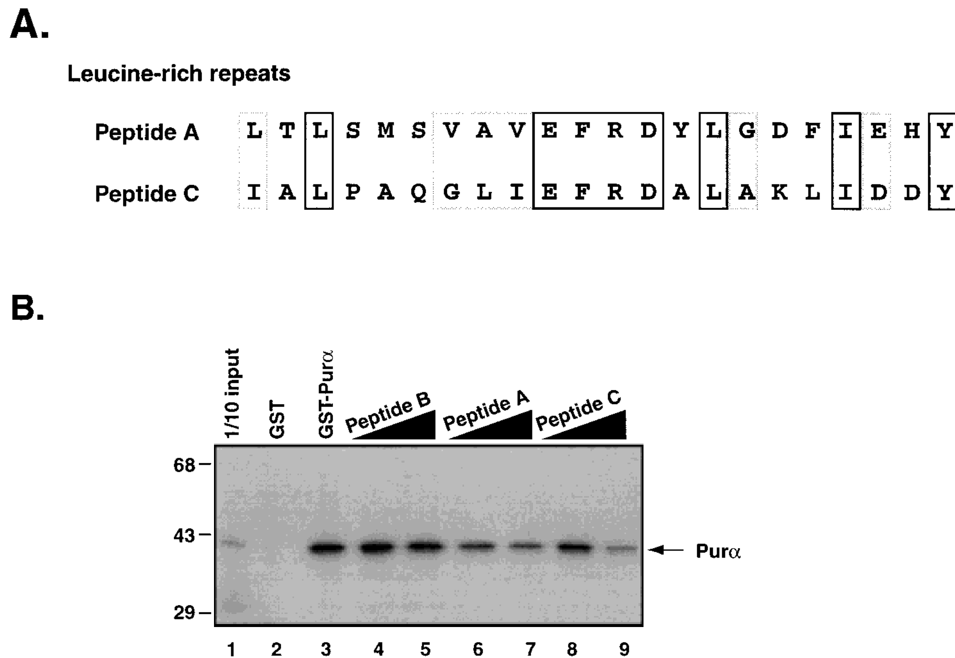

Fig. 4. Leucine rich motifs are involved in the self-association of Purα. **A**: Amino acid sequences of synthetic peptides derived from the class II motifs used. Solid boxes indicate identical amino acid residues and dotted boxes indicate conservative changes. **B**: [^{35}S]-labeled-Purα produced by in vitro transcription-translation was incubated with GST (**lane 2**) or GST-Purα (**lanes 3–8**) immobilized on glutathione-sepharose beads. Experiments were performed in the presence of 0.1µg and 1.0µg of synthetic peptides derived from the first class I Purα repeat (peptide B, lanes 4 and 5), the first class II Purα repeat (peptide A, lanes 6 and 7) or the second class II Purα repeat (peptide C, lane 8 and **lane 9**). Bound proteins were fractionated by SDS-PAGE and visualized by autoradiography. **Lane 1** contains one-tenth the amount of [^{35}S]-labeled Purα used in the binding reaction. Arrow indicates the position of Purα, while the positions of molecular mass markers (in kilodaltons) are shown on the left.

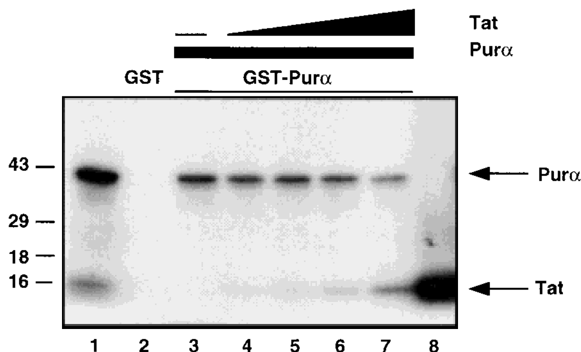

Fig. 5. HIV-1 Tat protein can disrupt the self-association of Purα. [^{35}S]-labeled Purα was incubated with GST **(lane 2)** or GST-Purα (**lanes 3–7**) either in the absence (lane 3) or presence of increasing concentrations (lanes 4–7) of [^{35}S]-labeled HIV-1 Tat protein. **Lane 1** contains one-tenth the amount of [^{35}S]-labeled Purα used in the binding reactions and **lane 8** contains one-fifth of the amount of [^{35}S]-labeled HIV-1 Tat protein used in the binding reaction shown in lane 7. Bound proteins were fractionated by SDS-PAGE and visualized by autoradiography. Arrows indicate the positions of Purα and HIV-1 Tat proteins. The positions of molecular mass markers (in kilodaltons) are shown on the left.

To this end, in vitro translated [^{35}S]-labeled Purα was treated with RNase conjugated to agarose beads. After treatment, the agarose-RNase conjugate was removed from the translation extract by centrifugation. Conversely, GST and GST-Purα sepharose beads were treated with soluble RNase. After treatment, these GST containing sepharose beads were washed extensively to remove any soluble RNase. GST affinity chromatography assays were then performed using various combinations of untreated and RNase-treated components. As shown in Figure 6A, pretreatment of the in vitro translated Purα protein abrogated the self association of Purα (compare lanes 3 and 4) whereas pretreatment of the GST-Purα protein did not affect the association (compare lanes 3 and 5). Not surprisingly, RNase treated [^{35}S]-labeled Purα was unable to interact with RNase-treated GST-Purα (lane 6). These results suggest that the RNA important in the self association of Purα originates from the [^{35}S]-labeled Purα. Thus, subsequent studies were performed with

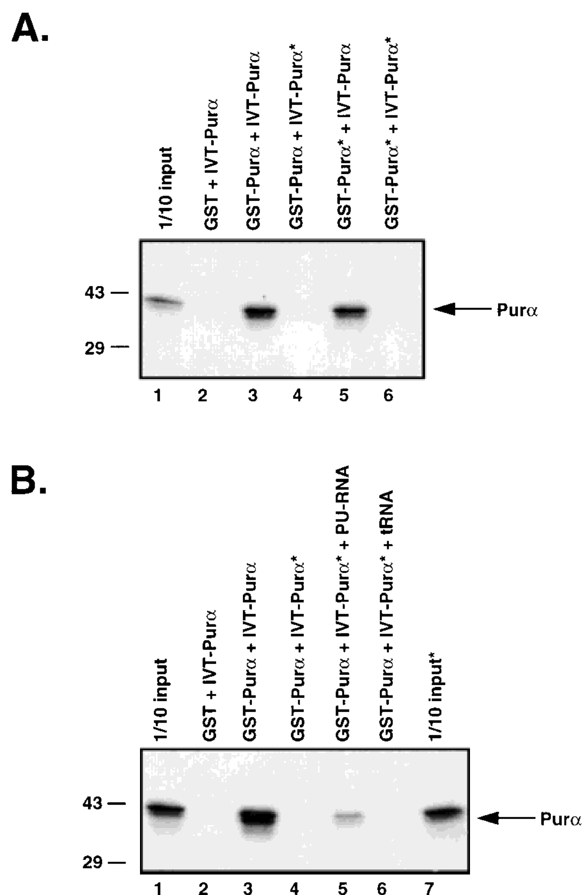

Fig. 6. Purα-associated RNA, PU-RNA, reconstitutes the self-association of Purα. **A**: [^{35}S]-labeled Purα produced by in vitro transcription-translation was incubated with GST **(lane 2)** or GST-Purα **(lanes 3–6)**. Reactions were performed after pretreatment of either [^{35}S]-labeled Purα (lane 4), GST-Purα (lane 5), or both [^{35}S]-labeled Purα and GST-Purα (lane 6) with RNase. **Lane 1** contains one-tenth of the amount used in the binding reactions. Bound proteins were fractionated by SDS-PAGE and visualized by autoradiography. **B**: [^{35}S]-labeled Purα was incubated with GST (**lane 2**) or GST-Purα (**lanes 3–6**). In lanes 4–6, RNase-treated [^{35}S]-labeled Purα was incubated with GST-Purα either in the absence (lane 4) or presence of PU-RNA (lane 5) or tRNA (lane 6). **Lane 1** contains one-tenth of the amount of [^{35}S]-labeled Purα used in the binding reactions shown in lanes 2 and 3 (not treated with RNase) and lane 7 contains one-tenth of the amount of [^{35}S]-labeled Purα used in the binding reactions shown in lanes 4–6 (pretreated with RNase). Bound proteins were fractionated by SDS-PAGE and visualized by autoradiography. Arrows in both panels indicate the position of Purα and asterisks indicate treatment with RNase. The positions of molecular mass markers (in kilodaltons) are shown on the left of each panel.

RNase treated [^{35}S]-labeled Purα and untreated GST-Purα.

In an attempt to reconstitute the self association or Purα, RNase treated in vitro translated Purα was incubated with GST-Purα in the absence and presence of exogenously added RNA. We utilized two species of RNA; one which was recently cloned by virtue of its ability to interact with Purα called PU-RNA [Tretiakova et al., 1998], the other being tRNA. As shown in Figure 6B, the addition of PU-RNA was able to reconstitute the self-association of Purα (compare lanes 4 and 5), whereas the addition of tRNA did not influence the interaction at all (compare lanes 4 and 6). These results not only demonstrate that the self-association of Purα is mediated by RNA, but also suggest that specific RNA molecules, in this case PU-RNA, are involved in the association.

RNA Inhibits the Interaction Between Purα and its DNA Target

Since Purα interacts with RNA [Herault et al., 1995; Tretiakova et al., 1998] and RNA molecules affect the self association of Purα, we next investigated the influence of RNA on another well characterized function of Purα, namely the ability of Purα to bind single-stranded DNA. To this end, band shift assays were performed in the presence of RNase. As shown in Figure 7A, inclusion of RNase into the reaction mixture enhanced the ability of Purα to interact with the probe (compare lanes 4 and 5). Although the intensity of all three complexes was increased, notably, complex B demonstrated the greatest increase. None of these complexes were due to the presence of RNase in the reaction mixture as the incubation of RNase and the probe did not result in the formation of any complex (Fig. 7A, lane 6). These data demonstrate that RNA inhibits the interaction between Purα and its target DNA. In the next series of experiments, we explored the involvement of the oligomerization region in the DNA binding activity of Purα. To this end, band shift assays were performed in the presence of RNase and the various synthetic peptides. As shown in Figure 7B, inclusion of peptide A, corresponding the first class II motif, resulted in a reduction in both higher order Purα complexes, complexes b and c (compare lanes 1 to lanes 2 and 3). Of note, the ability of the monomer form of Purα, complex a, to interact with the probe was unaffected by the addition of peptide A (compare lane 1 to lanes 2 and 3). Inclusion of the control peptide, peptide B, corresponding to the first class I repeat did not influence the formation of any of the nucleoprotein complexes (compare lane 1 to lanes 4 and 5). Interestingly, inclusion of the peptide corresponding to the

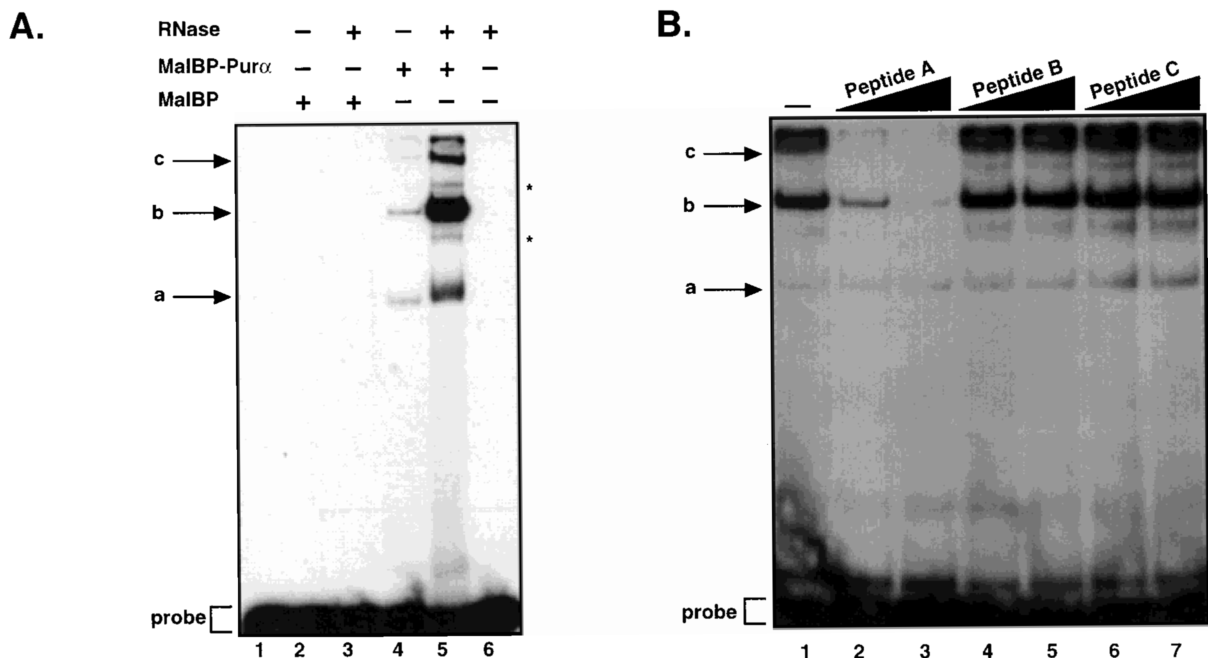

Fig. 7. RNA inhibits the interaction between Purα and its DNA target sequence. **A**: Radiolabeled VG-1 was incubated with 10 ng of MalBP **(lanes 2 and 3)**, or MalBP-Purα **(lanes 4 and 5)** in the absence (lanes 2 and 4) or presence (lanes 3 and 5) of RNase. **Lane 1** contains probe alone (P). Incubation between VG-1 and RNase is shown in **lane 6**. **B:** Radiolabeled VG-1 probe was incubated with 15 ng of MalBP-Purα in the presence of RNase and 100 or 500 ng of the synthetic peptide derived from the first class II Purα repeat (peptide A, **lanes 2 and 3**), the first class I Purα repeat (peptide B, **lanes 4 and 5**) or the second class II Purα repeat (peptide C, **lanes 6 and 7**). The MalBP-Purα/VG-1 nucleoprotein complexes (a, b, and c) formed in the absence of RNase which are enhanced after RNase digestion are indicated by arrows. Asterisks indicate the appearance of new nucleoprotein complexes formed upon addition of RNase to the binding reaction.

second class II motif (peptide C) similarly did not influence the formation of any complex (compare lane 1 to lanes 6 and 7). These results suggest that unlike the self-association of Purα (assayed via GST-pull down experiments which do not contain DNA) which is mediated by both class II motifs, the ability of Purα to form higher order complexes with its target DNA sequence is dependent on the first class II motif only.

DNA Can Specifically Reconstitute the Self-Association of Purα

The band shift assay performed in the presence of RNase enhanced the formation of all complexes, including complex B and C (Fig. 7A) suggesting that DNA in addition to RNA may be able to support the self-association of Purα. To address this question, GST affinity chromatography assays were performed in which the endogenous RNA mediating the self-association was first removed and exogenous DNA was subsequently added. The two DNA molecules utilized in these studies were the same oligonucleotides used above, with VG-1 representing a single stranded oligonucleotide with which Purα can interact (Figs. 1 and 7), and DD-1 representing the complement strand of VG-1 to which Purα cannot interact (Fig. 1). To determine if these single stranded oligonucleotides were able to reconstitute the self-association of Purα, RNase treated in vitro translated Purα was incubated with GST-Purα in the absence and presence of these oligonucleotides. As shown in Figure 8, the addition of VG-1 oligonucleotide was able to reconstitute the self-association of Purα (compare lanes 4 and 5). The addition of DD-1 oligonucleotide resulted in a very slight increase in the self association of Purα, but not to the same extent as that observed with the VG-1 oligonucleotide (Fig. 7, compare lanes 5 and 6). These results demonstrate that the self-association of Purα can not only be reconstituted by RNA, but also by DNA as well. Moreover, the ability of DNA to reconstitute the self-association is specific as a DNA oligonucleotide to which Purα can bind is capable of reconstituting the self-association of Purα whereas a DNA oligonucleotide with which Purα cannot interact is not capable of reconstitution the self-association.

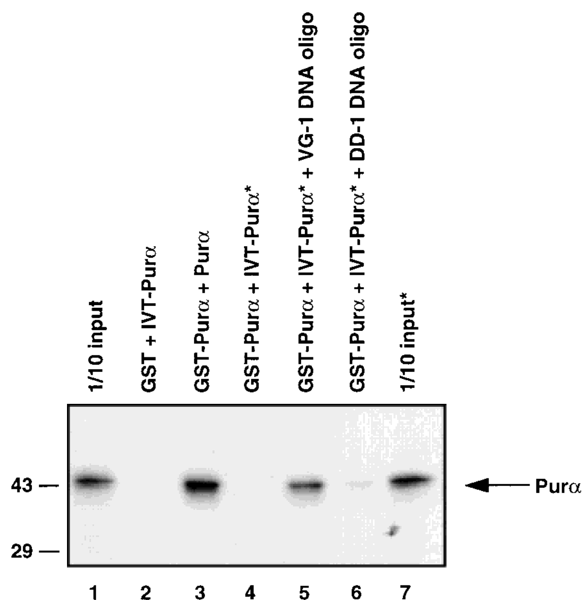

Fig. 8. DNA is capable of specifically reconstituting the self-association of Purα. [^{35}S]-labeled Purα was incubated with GST (lane 2) or GST-Purα (lanes 3–6). In lanes 4–6, RNase-treated [^{35}S]-labeled Purα was incubated with GST-Purα either in the absence **(lane 4)** or presence of the purine-rich VG-1 DNA oligonucleotide **(lane 5)** or the pyrimidine-rich DD-1 DNA oligonucleotide **(lane 6)**. **Lane 1** contains one-tenth of the amount of [^{35}S]-labeled Purα used in the binding reactions shown in **lanes 2 and 3** (not treated with RNase) and **lane 7** contains one-tenth of the amount of [^{35}S]-labeled Purα used in the binding reactions shown in lanes 4–6 (pretreated with RNase). Bound proteins were fractionated by SDS-PAGE and visualized by autoradiography. Arrow indicates the position of Purα and asterisks indicate treatment with RNase. The positions of molecular mass markers (in kilodaltons) are shown on the left.

DISCUSSION

In this paper, we demonstrate that the interaction between Purα and the purine rich strand of the JCV origin of DNA replication results in the formation of multimeric complexes. This is consistent with previous studies demonstrating multimeric complexes between Purα and various PUR elements [Johnson et al., 1995; Krachmarov et al., 1995; Muralidharan et al., 1997]. The ability of Purα to form multimeric complexes in these electrophoretic mobility shift assays lead us to consider if Purα can self-associate in solution in the absence of DNA. Our results demonstrate that Purα does indeed interact in solution in the absence of DNA. This is consistent with another study which notes that Purα is eluted as a 140 kDa protein from a Superose 12 column [Osugi et al., 1996]. Interestingly, while the self-association of Purα can be independent of the presence of DNA, the self-association of Purα is mediated by RNA. This is noteworthy in light of the fact that Purα, in addition to its ability to interact with DNA, has also been shown to interact with RNA [Chepenik et al., 1998; Herault et al., 1995; Tretiakova et al., 1998]. Moreover, Purα has been shown to transactivate the HIV-1 LTR through a TAR RNA-dependent mechanism [Chepenik et al., 1998]. We have recently cloned a Purα associated RNA molecule, termed PU-RNA, which has significant homology to 7SL RNA [Tretiakova et al., 1998]. Interestingly, this PU-RNA inhibits the interaction of immunopurified Purα from mouse brain nuclear extract with a Purα target sequence derived from the myelin basic protein promoter, termed MB1 [Tretiakova et al., 1998]. This is consistent with our results demonstrating that the inclusion of RNase in band shift reactions containing Purα enhances the ability of Purα to interact with its target DNA oligonucleotide (Fig. 7). Another study has demonstrated that small RNA molecules co-purify with a protein which interacts with a putative PUR element upstream of the quail clusterin gene [Herault et al., 1995]. This group has characterized a factor from Rous sarcoma virus-infected quail embryonic fibroblasts, termed the Pur factor, which interacts with their DNA sequence element. Of note, the formation of this PUR complex can be prevented by pretreatment of nuclear extracts with RNase A and Pur binding activity of RNA-depleted nuclear extracts can be restored by addition of these small RNA molecules which co-purify with Pur [Herault et al., 1995]. Although this contrasts with our results presented here, the identity of the Pur factor is yet undescribed. Additional studies, including identification of the Pur factor will be required before these seemingly disparate observations can be reconciled.

We have also localized the region of Purα which is important for its self-association. Our GST-pull down experiments utilizing GST-Purα deletion mutants localize the region of Purα which is important for its self-association to a 49 amino acid region located between residues 167 and 215 (Fig. 3). This region encompasses the second class II motif. To further refine the region of Purα which is involved in its self-association and given the high degree of similarity between the class II motifs, synthetic peptides generated from these regions were utilized in GST-pull down experiments. Interest-

ingly, synthetic peptides derived from either class II motif were able to disrupt the self-association of Purα (Fig. 4) suggesting that both class II motifs are important in the interaction. Perhaps folding and/or other structural constraints of the gross deletion mutants utilized in the GST-pull down assays limited the ability to detect the impact of the first class I motif.

In addition, the disruption of the self-association of Purα by class II peptides is specific as a control peptide derived from the first class I repeat does not influence the interaction. Interestingly, these class II leucine rich motifs are the same region of Purα which binds to the HIV-1 Tat protein [Krachmarov et al., 1995] and HIV-1 Tat is able to disrupt the self-association of Purα (Fig. 5). Recent data demonstrates that the association between Tat and Purα results in the synergistic activation of the HIV-1 LTR [Gallia et al., manuscript submitted]. Given the observation that both the self-association of Purα and the Tat:Purα association are mediated via the same region of Purα (more specifically via class II repeats), this suggests that the association between Tat and Purα, and subsequently the synergistic transcriptional activity between Tat and Purα may be modulated by the self-association of Purα. Or alternatively, one may envision the possibility that Tat may disrupt the cellular role(s) of self-associated Purα.

Purα is one member of the Pur protein family; Purβ is the other characterized member of this family. Alignment of the predicted amino acid sequence of murine Purα and the other known family member, Purβ, reveals that both proteins exhibit the same modular structure with an overall identity of 71% [Kelm et al., 1997]. In particular, the region which we have shown to be important for the self-association of Purα is highly conserved. Aside from a 7 amino acid insertion in Purβ (6 of which are glycine), the region encompassing resides 167–215 of Purα possesses an 86% identity with the same region in Purβ. This raises the possibility of a hetero-association between Purα and Purβ. This is interestingly in light of a prominent structural difference between Purα and Purβ. Purα, unlike Purβ, possesses a C-terminus glutamine-rich sequence. Such glutamine-rich sequences have been associated with transcriptional activation domains [Tjian and Maniatis, 1994]. Although this glutamine-rich domain of Purα has not been shown to be a transcriptional activation domain, several studies have demonstrated that Purα is a transcriptional activator [Chen and Khalili, 1995; Haas et al., 1995; Zambrano et al., 1997]. If Purα and Purβ do in fact associate with one another, the absence of such glutamine sequences within Purβ may be of functional significance with respect to the transcriptional activation of Purα.

Here, we demonstrated that the self-association of Purα is mediated by RNA and is independent of DNA (Fig. 2). Moreover, we have shown that a Purα-associated RNA, PU-RNA, specifically reconstitutes the self-association of Purα (Fig. 6). Interestingly, a DNA molecule is also capable of specifically reconstituting the self-association of this protein (Fig. 8). The observation that both nucleic acids are capable of specifically reconstituting the self-association of Purα, yet only RNA mediates this association is intriguing. This is likely due to the presence of RNA, and not DNA, molecules which are bound of Purα in the extracts used. Nonetheless, the observation that DNA is also competent for reconstituting this interaction may also be important for the regulation of Purα activity, especially during times when single stranded forms of DNA do exist such as during DNA replication and transcription. This is particularly noteworthy in light of the fact that PUR elements are found within origins of eukaryotic DNA replication [Bergemann and Johnson, 1992].

In this report, we demonstrate that a Purα-associated RNA, PU-RNA [Tretiakova et al., 1998], has the capacity to reconstitute the self-association of Purα (Fig. 6). Of note, this PU-RNA, which has significant homology to the 7SL family of small RNAs, does not reconstitute the binding activity to the level observed in untreated extracts (Fig. 6B, compare lanes 3 and 5). Several explanations can account for this observation. In addition to experimental procedures facilitating potentially suboptimal conditions required for the formation of Purα: Purα complexes, perhaps the PU-RNA transcript itself is not the optimal RNA species involved in the interaction. There may, in fact, be a family of small RNA molecules which selectively interact with Purα to regulate its activity. Nonetheless, future studies identifying other Purα associated RNA molecules may help to clarify this issue.

The ability of RNA to modulate the DNA binding capability of Purα is interesting in light

of recent observations focusing on the transcription factor, E2F. E2F is key protein responsible for directing the progression of mammalian cells from the G1 into the S phase of the cell cycle and overexpression of E2F induces quiescent cells to enter the cell cycle. Ishizaki et al. [1996] have isolated several RNA species by an in vitro selection procedure which interact with E2F. Interestingly, these RNAs inhibit the DNA binding capacity of E2F. Moreover, this study demonstrates that an E2F-specific RNA ligand can block the induction of S phase in quiescent cells stimulated by the addition of serum. Future studies examining Purα-associated cellular RNA molecules and their effect on Purα function could provide clues as to the cellular role of Purα.

In addition to demonstrating that RNA inhibits the interaction between Purα and its target DNA, we also demonstrate that peptide A, corresponding to the first class II motif, is able to abrogate the higher order complexes formed between Purα and its target DNA sequence (Fig. 7). Interestingly, the peptide corresponding to the second class II motif, peptide C, does not influence the interaction between Purα and its target DNA sequence. These results suggest that the first class II motif is involved in the formation of higher order complexes between Purα and its target DNA. This is noteworthy in light of our earlier GST-pull down experiments utilizing the same peptides which demonstrated that both class II motifs are involved in the self-association of Purα (Fig. 4). These are two different assays investigating two different properties of Purα. The GST-pull down experiments analyze the self-association of Purα in the absence of DNA (Fig. 2). In this case, both class II motifs contribute to the self-association of Purα. This is in contrast to the band shift assays, which are performed with a DNA probe. In this case, only the first class II motif is involved in facilitating the formation of higher order complexes between Purα and its target DNA. Thus the sequences mediating the interaction between two or more molecules of Purα depends on the presence of Purα's target DNA sequence. Of note, the band-shift assay may not allow the demonstration of the involvement of the second class II motif in the self-association of Purα.

In this report, we demonstrate that Purα can self-associate in solution in the absence of DNA. This self-association, while DNA independent, is mediated by RNA. In addition, we localize the region of Purα which is important in its self-association. Interestingly, this same region of Purα has been shown to interact with the HIV-1 Tat protein [Krachmarov et al., 1995] and here we demonstrate that Tat is able to disrupt the self-association of Purα. Moreover, we demonstrate that PU-RNA, a Purα-associated RNA, can specifically reconstitute the self-association of Purα. Interestingly, a DNA oligonucleotide which interacts with Purα is able to reconstitute the self association of Purα, while a DNA oligonucleotide to which Purα cannot bind does not reconstitute this association. Future studies investigating the relationship between Purα and other proteins and RNA as well as determining the functional cellular form of Purα (i.e. monomer, dimer, or oligomer) will provide clues not only to the functions of Purα, but also will begin to elucidate how this protein is regulated.

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