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RNA-Dependent DNA Binding Activity of the Pur Factor, Potentially Involved in DNA Replication and Gene Transcription

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The PUR element is a polypurine polypyrimidine motif that can stimulate transcription, encountered in the 5′ regions of various genes and in the vicinity of several DNA replication initiation zones. We demonstrate that the PUR complex formation between the purine-rich strand of PUR and nuclear extracts can be prevented by pretreatment of nuclear extracts with RNA-damaging agents such as UV light or RNase A. A biochemical affinity method reveals that small RNA molecules copurify with the Pur factor. Moreover, the PUR binding activity of RNA-depleted nuclear extracts can be restored by addition of phenol-extracted RNAs. This work adds a new member in the emerging class of ribonucleoprotein particles as regulatory factors of the genetic expression.

Ribonucleoprotein factor PUR Transcription Replication

THE identification of DNA binding factors regulating the initiation of gene transcription and of DNA replication is of basic importance in cellular and molecular biology research. The majority of factors known to date are exclusively composed of proteins. Recent observations, however, have established that some gene transcription machineries contain ribonucleic components. RNA molecules have already been found in transcription factors, as free molecules, like the transcription factor TFIIIR (Young et al., 1991), recently identified as the isoleucine tRNA (Dunstan et al., 1994), or in association with proteins (Davis et al., 1989). In addition, the amino acid sequence of several activation domains of eukaryotic transcription factors share striking similarities with some RNA binding proteins. For example, the acidic domain of GCN4 is closely related to that of the hnRNP C proteins (Hope and Struhl, 1986); SP1 and the RNP-CS-containing protein embryonic lethal abnormal visual system (elav) are both endowed with the same glutamine-rich domain (Courey and Tijan, 1988), and similar proline-rich domains are found in PABP and CTF-NF1 (Bohmann et al., 1987). Moreover, ribonucleoproteins have been found associated with several transcription regulation sites (Amero et al., 1993; Takimoto et al., 1993).

The present work extends the possibility of an interaction of a RNA molecule with another factor, binding to a polypurine/polypyrimidine site named PUR. The PUR element was originally identified in the human genomic DNA 5′ flanking the c-*myc* gene, close to the center of a DNA replication initiation zone. Its presence was then enlarged to several other DNA replication origins (Bergemann and Johnson, 1992; DePamphilis, 1993; Vita-Pearlman et al., 1993). The PUR element is also present in the 5′ flanking regions of several genes (Bergemann and Johnson, 1992; Herault et. al., 1992), in the absence of any reported DNA replication origin. When tested in transient expression assays, the PUR element behaves as a *cis* activator of transcription (Herault et al., 1994). The nuclear factor named Pur, which binds specifically to the PUR motif, is thus a novel

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candidate for playing roles in both transcription and in DNA replication, as already known for several transcription factors (Heintz, 1992). The Pur factor displays a marked affinity for the purine-rich single strand of the PUR motif (Bergemann and Johnson, 1992; Herault et al., 1992). The Pur factors present in human and avian nuclear extracts can be UV cross-linked to the purine single strand and then resolved as complexes of apparent molecular weight of 27 kDa (Bergemann and Johnson, 1992; Herault et al., 1994). The single-strand specificity of the Pur factor binding can result from either a helix destabilizing capacity of the Pur factor, or the biophysical propensity of the holopurine/holopyrimidine DNA stretches to adopt non-B DNA structures, as shown for the S1-sensitive element closely related to PUR, present upstream of the CFTR and MUC1 genes and which could also be cross-linked to a molecule of approximately 27 kDa (Hollingsworth et al., 1994). Although of higher molecular weight, a protein present in Hela cells, termed Purα and belonging to a family of related proteins, has been isolated as a Pur binding protein (Bergemann et al., 1992). Interestingly, as for the transcription factors GCN4, SP1, or NF1 previously mentioned, the human Purα protein sequence displays a modular structure typical of transcription factors and possesses an 18 glycine stretch also present in various RNA binding proteins (Bergemann et al., 1992).

The present work is founded on the observation that UV irradiation pretreatment of nuclear extracts impedes the further formation of PUR complexes. This has led us to examine the possibility of an involvement of a nonprotein component in the PUR binding activity. We present here a series of experiments showing that the UV-sensitive material present in the Pur factor is likely to correspond to small RNA molecules. The involvement of RNA factors in the PUR binding reaction is further demonstrated by complementation assays using RNAs purified by phenol extraction of nuclear extracts or bacteria, and RNase A-treated nuclear extracts, providing the protein components.

MATERIALS AND METHODS

Preparation of Nuclear Extracts and Electrophoretic Mobility Shift Assays (EMSA)

Nuclear extract preparation from Rous sarcoma virus (RSV)-infected quail embryonic fibroblasts (QEF) and EMSA were performed as previously described (Herault et al., 1992). When indicated, the nuclear extracts were submitted to a UV-irradiation energy of 2 J/cm^2 in a UV linker (Stratalinker, Appligene) prior to EMSA. The AP-1 gel shift assay was done as described.

Mobility shift assays were performed as described (Herault et al., 1992, 1994), except that the PUR complexes were electrophoresed through a 5.5% polyacrylamide gel (80 : 1, acrylamide : bisacrylamide). For reconstitution experiments, the RNA-depleted extracts were prepared by incubating, for 30 min at 37°C, 100-μl aliquots of the heparin-agarose fraction 14 (Fig. 3) in the presence of 0.3 units of insoluble ribonuclease A attached to agarose beads (Sigma) previously washed twice with the 1 × binding buffer (Herault et al., 1994), or with the oxidized RNase A (RNase A_0 Sigma); the inactive proteinase K was made by an overnight autodigestion of 10 mg/ml solution of proteinase K. In addition, 80 units of RNasin (Promega) were present in all binding reactions using RNA-depleted extracts. The RNA was purified by phenol extraction of 180 μl of fraction 14, and the phenol-extracted fraction of bacterial RNAs soluble in NaCl 1 M after potassium-acetate/ethanol precipitation was prepared as described in Chapeville et al. (1962).

Heparin-Agarose Chromatography of Nuclear Extracts

Nuclear extracts were preequilibrated by dialysis against buffer C (20 mM HEPES, pH 7.9, 1 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM PMSF, 10 mM $ZnCl_2$, 20% glycerol) containing 20 mM KCl, and loaded onto an Econo-pac disposable chromatography column (Bio-Rad), poured with heparin-agarose (IBF) and equilibrated with buffer C in the presence of 20 mM KCl at 4°C. After washing with 4 vol of buffer C, nuclear extracts were eluted in buffer C with increasing KCl concentrations (0.2, 0.35, 0.65, and 1 M KCl).

3′ End Labeling of RNAs

After phenol extraction and ethanol precipitation in the presence of 1 μg glycogen, RNAs were ligated to [^{32}P]Cp (100 μCi, Amersham) by the T4 RNA ligase (20 U) (Biolabs) in 50 mM Tris-Cl$^-$ (pH 7.8), 10 mM $MgCl_2$, 10 mM β-mercaptoethanol, 1 mM ATP, and 1 mM hexamine cobalt chloride, in the presence of 20 U RNasin (Promega) in a final volume of 30 μl at 10°C for 16 h. Aliquots (10 μl) of the ligation reactions were mixed to an equal volume of formamide buffer

and incubated at 90°C for 5 min prior to electrophoresis.

Production of In Vitro-Translated Purα Protein

The 1.1-kb-long Purα full-length cDNA present in the insert of the λgt11 clone λAB6 (Bergemann et al., 1992), kindly provided by Dr. Johnson, was subcloned into the EcoRI site of the pBSK plasmid. Transcription of the cDNA with the T7 RNA polymerase and translation were achieved with the TNT-coupled reticulocyte lysate system (Promega), according to the manufacturer's recommendations.

DNA Affinity Precipitation Assay

Proteins (0.2 μg) from the heparin-agarose fraction 14 were incubated with 1.6 pmol of a 5′ biotinylated oligonucleotide 954 in the same conditions as for EMSA. Streptavidin-coated magnetic beads (20 μg) (Dynabeads, Dynal) were added to the incubation reaction and incubated for 5 min at room temperature. Magnetic beads were pelleted using a magnetic field. The pellets were rinsed four times with binding buffer, and suspended in 10 μl of buffer C.

UV Cross-Linking Analysis

After incubation with the RSV-infected QEF nuclear extracts with 5′ end-labeled oligonucleotide 954, aliquots of DNA–protein mixtures were spotted onto Saran wrap in a UV linker (Stratalinker, Appligene) and exposed at 2 J/cm^2 level of UV irradiation. Aliquots were then removed, mixed to an equal volume of 2 × sample buffer [4.6% sodium dodecylsulfate (SDS), 125 mM Tris-Cl$^-$ (pH 6.8), 20% glycerol] and loaded directly onto a SDS/15% polyacrylamide gel (Laëmmli, 1970). After electrophoresis, the gel was dried onto DEAE paper and analyzed by autoradiography.

RESULTS

Sensitivity of the PUR Complex to RNA-Damaging Agents

UV cross-linking experiments carried out with the PUR probe and nuclear extracts from RSV-infected QEF have revealed an unexpected behavior of the PUR complexes. When irradiated after the binding reaction, cross-linked PUR complexes were readily obtained (Herault et al., 1994). In contrast, when the nuclear extracts were UV irradiated prior to the incubation with the PUR probe, the subsequent formation of the PUR complexes was impeded by remarkably low levels of energy (0.1 J/cm^2) (Fig. 1) as compared to UV cross-linking conditions (2 J/cm^2). In view of a possible deleterious effect of UV irradiation on the nuclear extract, a similar experiment was repeated with another transcription factor present in the same nuclear extract, namely AP-1 (Herault et al., 1992), composed of the proteins c-*fos* and c-*jun*. As shown in Fig. 1 (right), the AP-1 complex forms with a similar efficiency with nuclear extracts, submitted or not to a 0.1 J/cm^2 of UV preirradiation. The different behavior observed between the holoproteic AP-1 factor and the PUR factor suggests that the Pur factor contains a biological component with a high sensitivity to UV light, as expected for nucleic acids. However, the gel-retarded complex is not exclusively composed of nucleic acids because proteinase K treatments prevent its formation (Fig. 2, lane 3). This effect is the consequence of the proteolytic activity of the proteinase K because it is strongly reduced when the same amount of an inactive autodigested proteinase K (K_o) is used (lane 4).

To determine if the UV sensitivity is the consequence of the presence of a UV-labile ribonucleic component, we tested the effect of RNase A on the PUR binding reaction. As shown in Fig. 2 (lanes 7–8), RNase A hinders the PUR complex formation. The potent RNase A inhibitor RNasin blocks this effect when added in the binding reac-

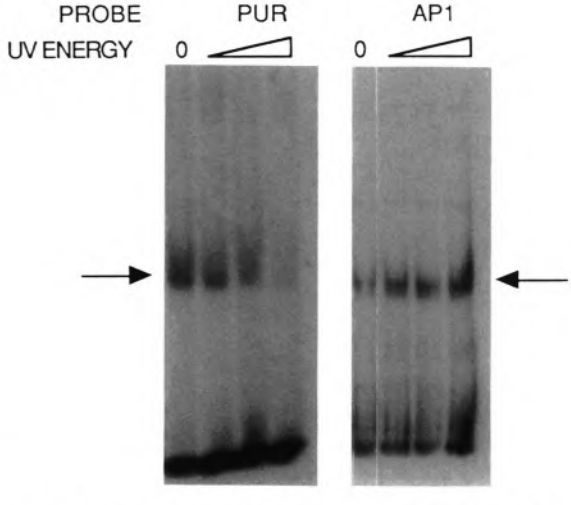

FIG. 1. Comparative sensitivity to UV of PUR and AP-1 complexes. The same nuclear extracts from RSV-infected QEF were used for mobility shift assays with PUR and AP-1 probes. The nuclear extracts were submitted to no (0), or increasing (0.01, 0.05, 0.1 J/cm^2) UV irradiation before PUR or AP-1 oligonucleotide addition.

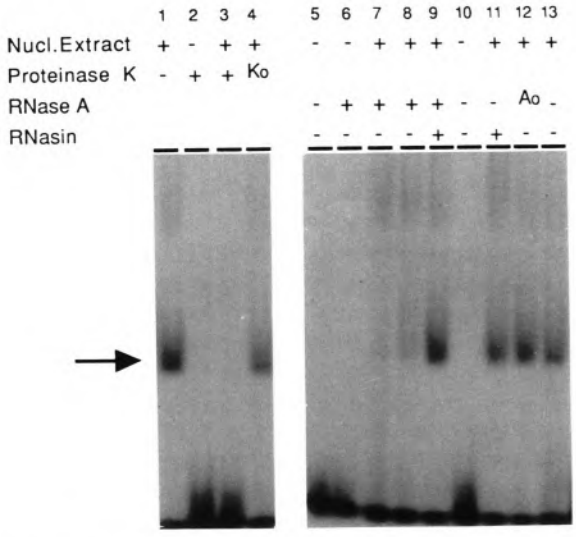

FIG. 2. Proteinase K and RNase A sensitivity of the PUR complex. Mobility shift assays were carried out using the PUR probe and nuclear extracts from RSV-infected QEF, either untreated (lanes 1, 13), or pretreated with proteinase K (lane 3) or RNase A (lanes 6–9). Nuclear extracts were also preincubated in the same conditions in the presence of enzymatically inactive forms of proteinase K (K_0, lane 4) and RNase A (A_0, lane 12). The RNase inhibitor (RNasin) was added (lane 9) prior to incubation with RNase A.

tion prior to RNase A (lane 9), and an oxidized, inactive form of the RNase A, RNase A_0, fails to block the PUR complex formation (lane 12). These two additional observations allow concluding that the RNase A effect on the PUR complex is not the consequence of a mere steric interaction with the RNase A molecule, but depends on its ribonucleolytic activity. Having established that the PUR binding activity is sensitive to RNase A, we sought to verify if RNA molecules are associated with the Pur factors.

Purification of RNA Components in the Pur Factor

Total nuclear extract from RSV-infected QEF was loaded onto a heparin-agarose chromatography column, and was fractionated by stepwise elution with increasing KCl concentrations, as indicated in Fig. 3A. The presence of the Pur factor in the different fractions was tested by EMSA (Fig. 3B), or by UV cross-linking (Fig. 3C) using the PUR probe. Figure 3B and C show that the PUR binding activity is present in fractions 14 and 15, of the 0.2 M KCl step. A strong but nonspecific (not shown) PUR binding activity was also observed from proteins present in the 0.35 M KCl step, migrating to the 50 kDa region (Fig. 3C). The gel-retarded and UV cross-linked PUR complexes could not be distinguished from those previously obtained with the total nuclear extract, suggesting that the expected RNA molecule copurifies with the Pur proteins. Hence, we attempted to isolate the RNA molecules from the partially purified Pur factors. We phenol extracted the total nucleic acid content of an aliquot of the heparin fraction 14, and 3′ end labeled the potential RNA molecules with [^{32}P]Cp. Figure 4 (lane T) shows that the heparin-agarose fraction 14 contains many small RNA molecules, ranging from 10 to more than 200 bases. We therefore sought to characterize the precise RNA molecule involved in the PUR complex by an alternative approach. The

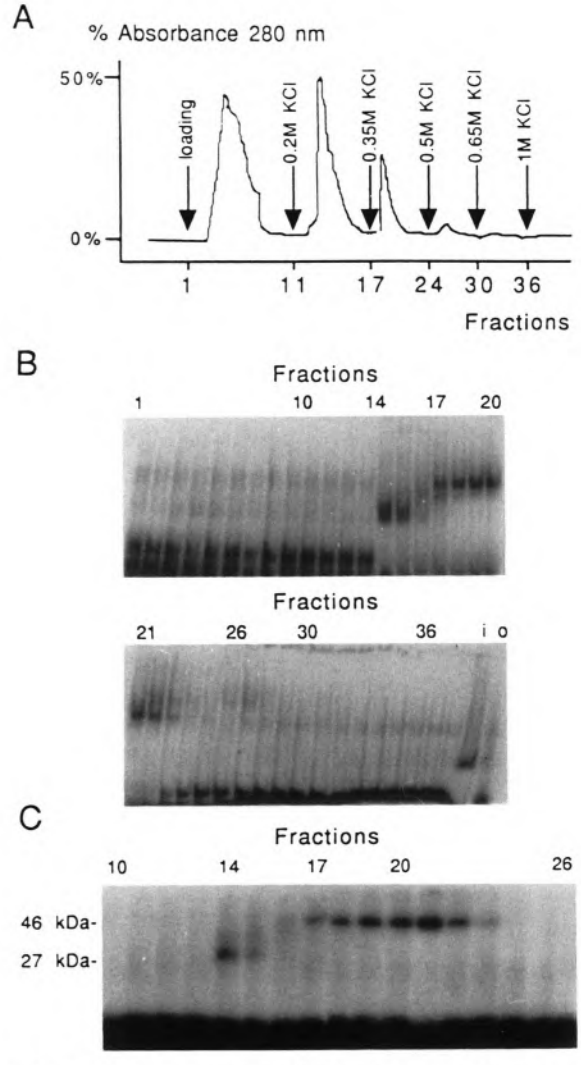

FIG. 3. Heparin-agarose purification of the quail Pur factor. Total nuclear extracts from RSV-infected QEF were loaded onto a heparin-agarose column and eluted with increasing KCl concentrations, as indicated (A). Fractions were then tested for the presence of Pur factors by EMSA (B) and by UV cross-linking analysis (C).

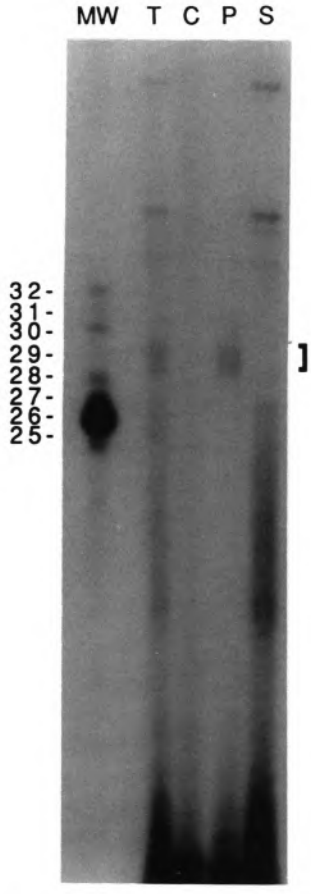

FIG. 4. Characterization of RNAs associated with the Pur factor. The RNA content of the heparin-agarose fraction of the Pur factor was analyzed by 3′ labeling with [^{32}P]Cp, electrophoresis through an 8% polyacrylamide sequencing gel, and visualized by autoradiography. Lane T: total display of RNA molecules obtained by phenol extraction of the fraction containing the Pur factor (fraction 14 of the heparin-agarose column). Lanes P and S correspond to RNAs extracted from the same fraction incubated with a biotinylated PUR oligonucleotide and reacted with streptavidin-coated magnetic beads. Lane P is the Pur factor fraction pelleted with the beads and lane S is the corresponding supernatant. Lane C is a control similar to P, using beads uncoupled to the PUR oligonucleotide. All samples were separated by electrophoresis through an 8% polyacrylamide sequencing gel and were visualized by autoradiography.

prepurified fraction 14 was incubated with a 5′ biotinylated PUR oligonucleotide. The biotinylated PUR complexes were separated from the mixture by using streptavidin-coated magnetic beads in a magnetic field. RNAs were separately prepared from the bead-coupled and supernatant fractions; they were then 3′ end labeled and resolved in a denaturing 8% polyacrylamide gel. As shown in Fig. 4, a radiolabeled band, around 28–29 nucleotides present in the PUR complexes (lane P), was simultaneously removed from the nuclear extract (lane S). After electroelution, this material was resolved in a 20% polyacrylamide denaturing gel, as a doublet of 28 and 29 bases (not shown). Control magnetic beads devoid of the PUR oligonucleotide failed by themselves to precipitate any RNA molecule (lane C).

Functional Reconstitution of the PUR Binding Activity of a RNA-Depleted PUR Fraction With RNAs From Various Origins

To further assess the active role of the RNA components on the formation of the PUR complex, we determined if the addition of purified RNA could restore the PUR binding capacity of an RNA-depleted nuclear extract. On the one hand, RNA-depleted Pur factors were obtained by incubating the heparin-agarose fraction 14 in the presence of RNase A linked to insoluble agarose beads. After extensive digestion, RNase A was eliminated by pelleting the agarose beads. This treatment totally abolishes the PUR binding activity of fraction 14, as shown in Fig. 5, lane 3. Absence of RNase A activity after removal of the agarose beads is controlled by the PUR binding activity of a mixture of RNase A-treated and nontreated aliquots of fraction 14 (lane 4). On the other hand, RNA was purified by phenol extraction of another aliquot of fraction 14 and concentrated by precipitation. As shown in Fig. 5, lane 5, the original PUR complexes can be reobtained by mixing the proteins and RNAs independently purified from fraction 14, if a 20-fold excess of RNA is added, relative to the untreated fraction 14. This experiment clearly demonstrates that RNA and protein components are both required for binding the PUR element, and that their interaction is fully reversible.

The human Pur proteins are at present the best candidates for constituting the protein components of the PUR complex. And yet, the RNA dependence of the PUR binding activity is in apparent contradiction with the isolation of the Pur proteins by affinity screening of a λgt11 expression library with a PUR probe (Bergemann et al., 1992). Indeed, this experiment indicates that the bacterially expressed Purα is able to form PUR complexes in the absence of any eukaryotic RNA molecules. Hence, we tested the potential Pur cofactor activity of bacterial RNAs. A preparation of small RNAs from *E. coli* can restore the PUR binding activity of a RNA-depleted PUR complex with the same efficiency as RNAs present in the Pur fraction of nuclear extracts. This unexpected result reveals that RNA species able to ensure a

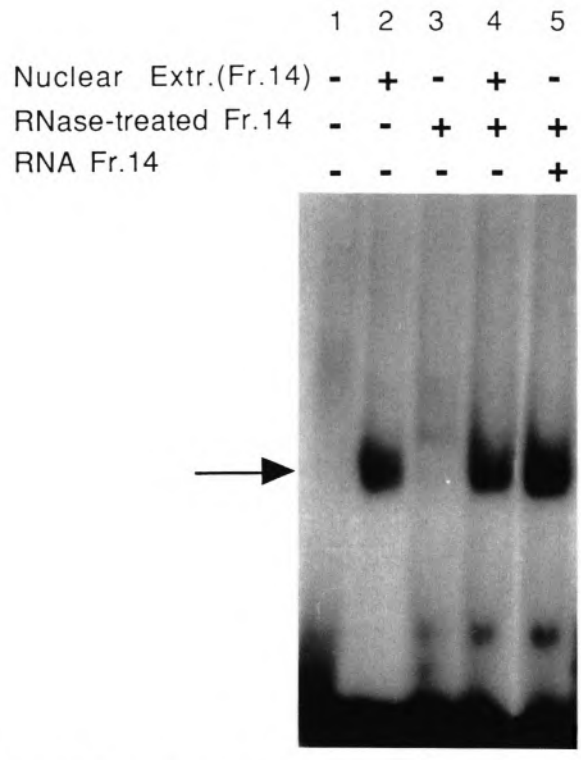

FIG. 5. Reconstitution of the PUR binding activity by RNA complementation. The PUR binding activity of fraction 14 of nuclear extracts was examined by mobility shift assay using a ^{32}P-labeled PUR oligonucleotide. An RNase A-treated fraction 14 was prepared by incubation with RNase A-coupled agarose beads in an aliquot of fraction 14 and subsequent removal of agarose beads by centrifugation (lane 3). RNA was prepared by organic extraction of an other aliquot of the same fraction 14. The complete removal of RNase A was tested by the PUR binding activity of a mixture of RNase A-treated and untreated fractions (lane 4). An excess of RNA corresponding to 20 equivalents of the RNase A-treated fraction was used to reconstitute the Pur factor binding activity (lane 5).

Pur cofactor activity are not restricted to cells containing Pur factors.

RNase A Sensitivity of the PUR Complex Obtained With the In Vitro-Translated Purα Protein

The latter observation, that bacterial RNA is fully able to play a Pur cofactor activity, reinforces the Pur proteins isolated by Bergemann et al. (1992) as possible components of the Pur factors. Hence, we checked for a RNA dependence of the PUR binding activity of the recombinant Purα protein. Figure 7 shows that a 30-min pretreatment of the in vitro-translated Purα protein with RNase A inhibits the PUR binding activity (Fig. 7A, lane 3). We then checked for the presence of 28–29-bases-long RNA molecules in the lysate. Figure 7B shows that the reticulocyte lysate contains multiple small RNA molecules, and among them, a 28–29-bases-long RNA doublet (lane 2) similar to that present in our heparin-agarose fraction 14 (lane 1). This confirms that the RNA molecules involved in the PUR binding activity are in fact widely distributed and occasionally associated with Pur proteins.

DISCUSSION

The present report shows that RNA components are necessary for DNA binding of the Pur factor to the PUR DNA element, originally identified as a DNA replication origin-associated motif (Bergemann and Johnson, 1992) and as a transcription *cis* activator (Herault et al., 1992, 1994). This conclusion is based on the finding that the PUR DNA binding activity is abolished by RNA-damaging procedures of nuclear extracts, like UV irradiation or, more specifically, RNase A diges-

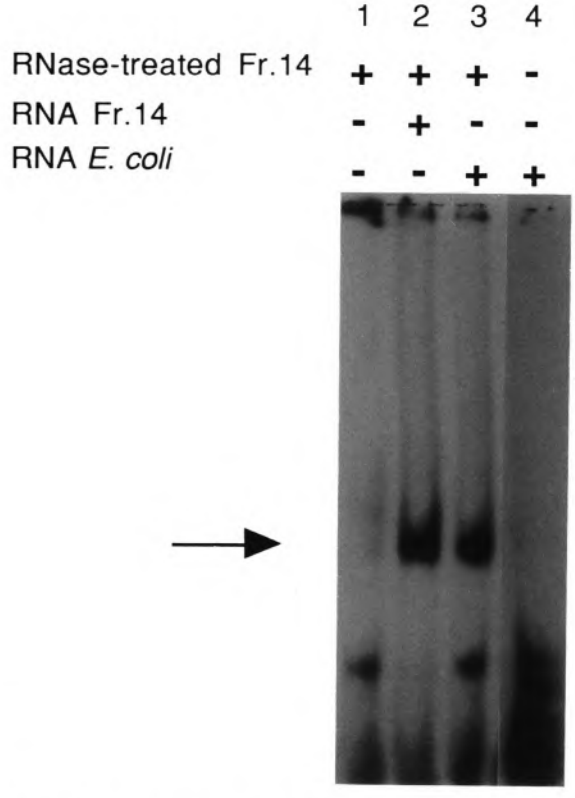

FIG. 6. Reconstitution of the PUR binding activity of a RNA-depleted Pur extract with bacterial RNAs. When added to the RNase A-treated fraction 14 of nuclear extracts devoid of PUR binding activity (lane 1), the tRNA crude fraction of *E. coli* (lane 3) is as efficient as RNAs extracted from fraction 14 (lane 2) for restoring the PUR binding activity. Free bacterial RNAs alone fail to form complexes (lane 4). The position of the PUR complex is identified by an arrow.

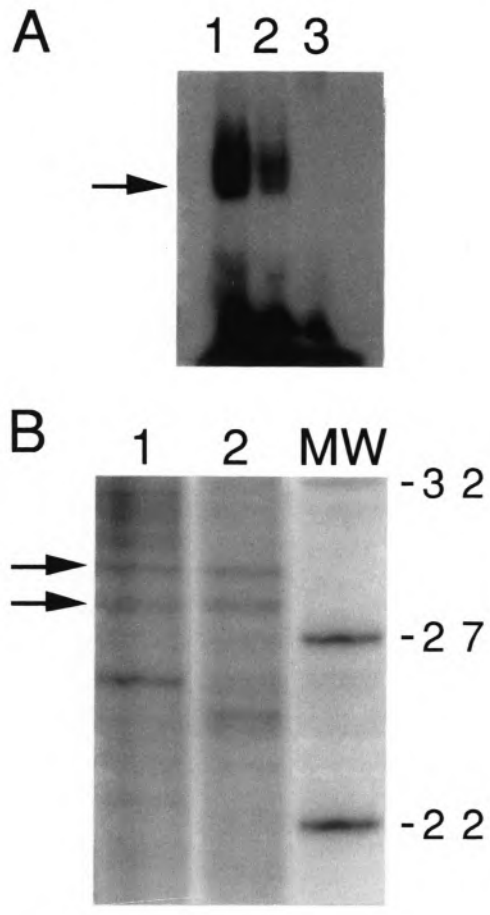

FIG. 7. RNAs interacting with Pur are present in rabbit reticulocyte lysates. (A) Sensitivity to RNase A of the PUR binding activity of the in vitro translated Purα protein. The ability of an in vitro-translated Purα protein to bind ^{32}P-labeled PUR oligonucleotides was examined by mobility shift assay. Binding reactions were done with the reticulocyte lysate immediately after the translation reaction (lane 1), or after an additional 30-min incubation at 37°C in the absence (lane 2) or presence (lane 3) of RNase A. (B) Presence in the rabbit reticulocyte lysate of RNAs similar to the Pur-associated RNAs of fraction 14 of RSV-infected QEF nuclear extracts. Total RNAs were prepared from fraction 14 of nuclear extracts from RSV-infected QEF (lane 1), or the rabbit reticulocyte lysate (lane 2), and 3′ end labeled using the T4 RNA polymerase, and run in parallel in a denaturing 20% polyacrylamide gel, along with 22, 27, and 32 mer radioactive oligonucleotides as molecular weight markers. Identical 28- and 29-bases-long RNAs, similar to those previously found associated with the PUR complexes, are present in both preparations, and are indicated by arrows.

tion, and is further established by the restoration of the PUR binding activity observed after reassociating RNase A-treated and phenol-treated aliquots of the Pur fraction. RNase sensitivity of transcriptional complexes has been documented in other situations. Although they do not provide a unifying view on the function of RNA molecules, these findings have opened new insights on mechanisms leading to finely tuned expression control. In the case of the silkworm RNA polymerase III transcriptional machinery, the factor named TFIIIR is resistant to proteinase K and phenol. It corresponds to a free RNA molecule of the class III transcripts that is believed to assist the assembly of stable preinitiation transcription complexes (Young et al., 1991). It has been recently identified as the isoleucine tRNA (Dunstan et al., 1994), which is thus involved both in translation and in transcription. RNA has also been characterized in the co-antirepressor fraction counteracting the H1 chromatin-mediated repression of RNA polymerase II transcribed genes. In this context, RNA is assumed to function as a negatively charged acceptor for histone H1 (Croston et al., 1992).

The biophysical mechanisms by which a small RNA molecule can interface with binding reactions between a protein and a DNA element are multiple. The ribonucleic component may be required for stabilizing the structural organization of the RNP in an active conformation. This possibility cannot be excluded in the case of PUR complexes, which are stable at KCl concentrations exceeding 0.75 M when obtained with crude nuclear extracts, but dissociate at KCl concentrations over 0.15 M when using in vitro-translated Purα proteins (Herault et al., unpublished data). This situation can reflect the absence of the most convenient Pur cofactor RNA in the rabbit reticulocyte lysate and its replacement by a less efficient ribonucleic molecule.

Alternatively, the RNA molecule may directly interact with the DNA element. This last hypothesis is particularly attractive in view of the dissymetric base composition of the PUR motif. Indeed, holopurine/holopyrimidine DNA stretches, like PUR, are known to display a marked biophysical capacity to accept a third strand (Felsenfeld et al., 1957). Moreover, the stability of triplexes is optimal when composed of two DNA strands and one RNA strand (Roberts and Crothers, 1992). Thus, the presence of a small RNA component in RNP transcription factors would allow the sequence-specific recognition as well as the stable interaction with the DNA *cis* element. It is worth noting that an RNase-sensitive factor has already been reported capable of binding a pyrimidine-rich element (the CT element) present in the P1 promoter of the human c-*myc* gene (Davis et al., 1989). The same element was shown to be able to incorporate an oligodeoxyribonucleotide corresponding to the purine strand of the CT element with an inverted 5′-3′ polarity. This incorporation is effective in vivo, and reduces the transcriptional activity of

the P1 c-*myc* promoter (Postel et al., 1991). One may hypothesize that this artificial stable insertion of an oligonucleotide in the CT element could prevent a natural interaction in vivo with the RNP transcription factor previously described by Davis et al. (1989). The same hypothesis can be proposed for the PUR *cis* element because a stable interaction of the PUR motif with a third strand is also possible (in preparation).

The base sequence determination of the Pur cofactor RNAs would allow us to determine if they can be involved in the sequence recognition step of the PUR DNA element through interactions between nucleic bases. This interaction may not correspond to the classical Watson-Crick hydrogen bonding scheme, because a RNase H treatment does not affect preformed PUR complexes (not shown). We cannot exclude, however, that such an absence of activity of RNase H results from the lack of accessibility of RNAs in the PUR complexes.

However, the wide distribution of RNA molecules able to mediate the PUR complex formation does not support a stringent base sequence requirement, but rather suggests that different RNA motifs are adequate for playing such a role. Moreover, our attempts to determine the base sequence of the radiolabeled RNA fragments visible in Fig. 4 (lane 3), have not yielded a unique sequence pattern (not shown), suggesting that this material is in fact a heterogeneous population of RNA molecules. The molecular cloning of independent RNA species composing this population would be necessary to determine if a common base sequence or composition is present among all the Pur cofactor RNAs. To this end, a functional selection method like the "SELEX" procedure (Tuerk and Gold, 1990) will be adequate to identify the RNA sequences most effective as Pur cofactors.

Finally, though unexpected, the abundance of small RNA molecules, ranging from 10 to 200 bases and surviving the purification processes of protein nuclear extracts, provides good candidates for constituting RNA fractions of RNPs possibly involved in interactions with various DNA elements. The origin of such small RNA molecules, which may be integrated into protein particles, remains puzzling and the determination of their base sequence would allow to elucidate their genomic origin, the class of RNA polymerase involved in their synthesis, and if they are metabolic remnants of larger molecules.

The present work further supports the role of RNPs as transcription factors, by providing the first visualization of RNA molecules copurifying with a transcription factor. This work clearly adds to the idea that, in addition to their roles in the cellular processes of pre-mRNA splicing, polyadenylation, and transfer in the cytoplasm, RNAs can also be involved in the earlier stages of the transcription initiation, as components of DNA binding factors.

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