An A+U-Rich Element RNA-Binding Factor Regulates *c-myc* mRNA Stability In Vitro

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Transient expression of some proto-oncogenes, cytokines, and transcription factors occurs as a cellular response to growth factors, 12-*O*-tetradecanoylphorbol-13-acetate, antigen stimulation, or inflammation. Expression of these genes is mediated in part by the rapid turnover of their mRNAs. A+U-rich elements in the 3' untranslated regions of these mRNAs serve as one recognition signal targeting the mRNAs for rapid degradation. I report the identification of a cytosolic factor that both binds to the proto-oncogene *c-myc* A+U-rich element and specifically destabilizes *c-myc* mRNA in a cell-free mRNA decay system which reconstitutes mRNA decay processes found in cells. Proteinase K treatment of the factor abolishes its *c-myc* mRNA degradation activity without affecting its RNA-binding capacity. Thus, RNA substrate binding and degradation appear to be separable functions. These findings should aid in understanding how the cell selectively targets mRNAs for rapid turnover.

mRNA stability plays an important role in regulating gene expression (9, 10, 34). The rapid turnover of an mRNA ensures that it is maintained at relatively low steady-state levels and that changes in the turnover rate can affect its steady-state level over a short period of time. This type of regulation allows transient alterations in the expression of some cytokines, transcription factors, and the *c-myc* and *c-fos* proto-oncogenes in response to growth factors (7, 37, 46), 12-*O*-tetradecanoylphorbol-13-acetate (23, 37), antigen stimulation (24), or inflammation (45). A common feature of the mRNAs encoded by all of these genes is an A+U-rich element (ARE) in the 3' untranslated region (3'UTR) (14). These AREs serve as one signal targeting the mRNAs for rapid turnover (18, 39, 46), although sequences in the *c-myc* 5'UTR (18, 32, 33) and the *c-fos* coding region (19, 40) can, in some instances, influence mRNA turnover.

Although much is known about the *cis*-acting elements controlling mRNA stability, the *trans*-acting factors remain largely undefined. The development of cell-free mRNA decay systems makes possible the identification, purification, and characterization of these *trans*-acting factors (5, 31, 35, 41). Ross and colleagues developed a system that is based on earlier in vitro translation systems and includes polysomes from the erythroleukemia cell line K562, magnesium, potassium, ATP, GTP, an ATP-regenerating system, and a 130,000 $\times$ *g* postribosomal supernatant (S130) (13, 35). The turnover of polysome-associated mRNA substrates was monitored by nuclease protection assays. This cell-free system reconstitutes in vivo mRNA decay processes, as determined from results of three series of experiments. (i) The rank order of mRNA decay is the same in vitro and in vivo. Both in vitro and in vivo, β - and γ -globin mRNAs are very stable, δ -globin has an intermediate decay rate, and H4 histone and *c-myc* mRNAs are very labile (35). (ii) mRNA degradation pathways are the same in vitro and in vivo. Ross and Kobs (35) observed that histone mRNA was degraded in vitro by an exonucleaselike activity. The first detectable mRNA degradation product lacked 5 nucleotides (nt) from the 3' end, while the second product lacked 12 nt from the 3' end. In subsequent steps, the mRNA was degraded in a 3'-to-5' direction. The same degradation pathway appeared

in cells during degradation of H4 histone mRNA (36). In addition, Brewer and Ross (11, 12) found that *c-myc* mRNA was degraded in vitro by a sequential pathway, in which the A+U-rich sequences of the 3'UTR were cleaved only after most or all of the poly(A) was removed. The remainder of the mRNA was degraded in subsequent steps. This pathway has been observed in vivo for *c-myc* mRNA (42). Thus, the in vitro mRNA decay system reconstitutes degradation pathways found in cells. (iii) The in vitro system reconstitutes regulated mRNA turnover observed in vivo. Translation inhibitors partially stabilize a variety of labile mRNAs, including *c-myc* mRNA, in vivo (2, 15, 25, 29, 43–45, 47). Brewer and Ross (12) found that *c-myc* mRNA was degraded about eightfold more slowly in extracts from cycloheximide-treated cells than in control cells, just as they are in vivo. However, neither globins nor total polysomal poly(A)⁺ mRNAs were affected. Combined, these studies indicate that the cell-free mRNA decay system appears to be a valid model for studying some mRNA decay processes.

The experiments described here were designed to identify and characterize *trans*-acting factors that might regulate the stability of *c-myc* mRNA. Previous experiments indicated that the S130 of K562 cells contains one or more activities which destabilize *c-myc* mRNA in vitro without affecting mRNA metabolism in general (11, 12). My approach was to fractionate the S130 and assay for specific *c-myc* mRNA degradation. Here I present evidence for a factor that selectively binds the *c-myc* ARE and destabilizes the mRNA.

MATERIALS AND METHODS

Construction of molecular clones. To prepare plasmid pSP64H γ (RIHD), a 0.57-kb *EcoRI*-*HindIII* fragment, containing the 3' end of the human γ -globin gene, was cloned into *EcoRI*-*HindIII*-digested pSP64 in the orientation such that the antisense strand was transcribed with use of SP6 RNA polymerase. For plasmid pSP65H γ (RIHD), the 0.57-kb fragment was cloned into *EcoRI*-*HindIII*-digested pSP65 such that the sense strand was transcribed with use of SP6 RNA polymerase. Plasmid pSP64GM(NCORI) was derived

from plasmid pSP64GM+ATG (22) by digestion with *Nco*I and *Hind*III and ligation to produce a plasmid with the granulocyte-macrophage colony-stimulating factor (GM-CSF) 3' UTR containing the ARE in the sense orientation for SP6 transcription. To prepare plasmid pSP64(polyT), the 100-bp *Eco*RI-*Hind*III fragment of plasmid pSD3 (11), encoding 85 nt of poly(A), was inserted into plasmid pSP64 (Promega, Madison, Wis.) in the orientation such that transcription using SP6 RNA polymerase produced 85 nt of poly(U).

Assay of in vitro mRNA decay. mRNA decay reactions (13) were performed in a total volume of 10 μ l at 37°C, using 0.28 A₂₆₀ units of polysomes in 10 mM Tris-HCl (pH 7.5), 100 mM potassium acetate, 5 mM magnesium acetate, 2 mM dithiothreitol, 10 mM creatine phosphate, 1 μ g of creatine phosphokinase, 1 mM ATP, 0.4 mM GTP, 0.1 mM spermine, and 8 U of RNasin, supplemented with sucrose gradient fractions of S130 (described below). RNAs were analyzed by an RNase protection assay as described elsewhere (13).

In vitro transcription and gel mobility shift assay. In vitro transcription reactions were performed with linearized templates and SP6 RNA polymerase (28). [α -³²P]UTP was included for synthesis of radiolabeled RNAs. RNA-binding reaction mixtures contained 5 ng of ³²P-labeled RNA probe and 2 μ l of unconcentrated gradient fractions (10⁶ cell equivalents) in a final volume of 10 μ l containing 10 mM Tris-HCl (pH 7.6), 5 mM magnesium acetate, 100 mM potassium acetate, 2 mM dithiothreitol, 0.1 mM spermine, 8 U of RNasin (Promega), tRNA (0.2 μ g/ μ l), heparin (5 μ g/ μ l), and poly(C) (0.1 μ g/ μ l). The reaction mixtures were incubated on ice for 10 min. Complexes were resolved by electrophoresis through 6% nondenaturing polyacrylamide gels (acrylamide/bisacrylamide ratio of 60:1) in 45 mM Tris-borate (pH 8.3)–1 mM EDTA (21). Gels were prerun for 30 min at 13 V/cm at room temperature prior to sample loading. Gels were then run for 3 h and visualized by autoradiography.

Sucrose gradient fractionation of S130. A 130,000 $\times$ g postribosomal supernatant (S130) was prepared from human erythroleukemia K562 cells by lysis in buffer A (10 mM Tris-HCl [pH 7.6], 1 mM magnesium acetate, 1.5 mM potassium acetate, 2 mM dithiothreitol, 1 μ g each of leupeptin and pepstatin A per ml, 0.1 mM phenylmethylsulfonyl fluoride [PMSF]) as described previously (13). ARE-binding and degradation activities were identified by velocity sedimentation of S130 through a series of continuous sucrose gradients. Five milligrams of S130 protein (5×10^7 cells) was loaded on each of six 5 to 20% (wt/wt) sucrose gradients in buffer A and centrifuged at 39,000 rpm ($260,000 \times g_{\max}$) for 20 h at 4°C in a Beckman SW41 rotor. Sixteen 0.77-ml fractions were collected, dialyzed with buffer A, concentrated in Centricon 100 microconcentrators (Amicon, Danvers, Mass.), and assayed for *c-myc* mRNA decay activity. The active fractions (average 25S with 28S and 18S rRNAs as markers) were pooled and loaded onto a second 5 to 20% (wt/wt) sucrose gradient in buffer A plus 0.5 M potassium acetate to remove loosely bound proteins from the ARE-binding and degradation activity (see reference 19, for example). This gradient was centrifuged as described above, and fractions were assayed for *c-myc* mRNA decay activity. The active fractions (average 7S using bovine serum albumin [BSA] monomer [4.6S] and dimer [6.8S] and urease trimer [10S] and hexamer [15S] as markers) were dialyzed and concentrated. For final analysis of the ARE-binding and degradation activities, the active fractions from the second gradient were loaded onto a third 5 to 20% (wt/wt) sucrose

gradient in buffer A plus 0.5 M potassium acetate and centrifuged, and fractions were collected as described above. The *c-myc* mRNA decay activity of the fractions was rapidly lost upon storage at 4°C, rapid freezing and thawing, or incubation at 37°C (data not shown). Therefore, sucrose gradient sedimentation steps were performed consecutively without freezing of the active fractions, and cell-free mRNA decay reactions were performed immediately.

RESULTS AND DISCUSSION

Identification of *c-myc* A+U-rich RNA-binding and degradation activities. A variety of high-molecular-weight complexes involved in translational regulation of mRNA have been identified by fractionating cell extracts with use of a series of velocity sedimentation steps through continuous sucrose gradients (for example, see references 1, 3, and 16). To identify *trans*-acting regulatory factors for *c-myc* mRNA turnover, S130 from K562 cells was fractionated by using a similar series of velocity sedimentation steps through continuous sucrose gradients as described in Materials and Methods. Two assays were performed with fractions from the final gradient. (i) Mobility-shift assays were performed to identify fractions with *c-myc* ARE-binding activity. The RNA-binding substrate was an SP6-derived ³²P-labeled RNA containing an ARE from the human *c-myc* *Nsi*I-*Ssp*I fragment (positions 5616 to 5812 [6]). After incubation of the ³²P-labeled substrate with gradient fractions, RNA-protein complexes were resolved on a native polyacrylamide gel. (ii) Cell-free mRNA decay reactions were performed to identify fractions with specific degradation activity toward polysome-associated *c-myc* mRNA compared with stable γ -globin mRNA. Degradation of each mRNA was measured by RNase protection assays. Figure 1 shows an analysis of the final sucrose gradient in the series. Maximal ARE-binding activity occurred with fractions 9 and 10 (Fig. 1B). (The specificity of this binding is addressed below.) To determine whether binding activity cosedimented with selective *c-myc* mRNA decay activity, paired fractions were pooled, dialyzed, concentrated, and used in cell-free mRNA decay reaction mixtures containing polysomes. *c-myc* mRNA from unincubated polysomes protected several bands in an RNase P₁+T₁ protection assay (Fig. 1C, 0-min incubation). The bands corresponded to intact *c-myc* mRNA that were polyadenylated at closely linked sites, referred to as poly(A) site 2 (11). Incubation with pooled, concentrated fractions 9 and 10 resulted in 80% degradation of all *c-myc* bands, while other fractions had little effect (Fig. 1C, fraction 9–10). None of the gradient fractions degraded γ -globin mRNA, indicating selective degradation. A decay reaction supplemented with BSA also showed little *c-myc* decay activity, indicating that the decay activity of fractions 9 and 10 was not due to protein concentration (Fig. 1C, BSA, 5-min incubation). Fractions 9 and 10 also correlate with maximal ARE-binding activity. Fractions 9 and 10 corresponded to a sedimentation of 7S. A silver stained sodium dodecyl sulfate (SDS)-polyacrylamide gel of gradient fractions revealed polypeptides with apparent molecular sizes of 37 and 40 kDa (Fig. 1A). I conclude that these polypeptides of the 7S fractions cosediment with both ARE-binding and *c-myc* mRNA degradation activities. The remainder of the experiments were designed to examine the specificity of the RNA-binding activity and whether both polypeptides were part of the RNA-binding complex.

RNA-binding specificity. The gel mobility shift assay was used to examine the specificity of the RNA-binding activity

FIG. 1. Sucrose gradient analysis of *c-myc* ARE-binding and degradation activities. S130 was fractionated by three consecutive sucrose gradients as described in Materials and Methods. Fractions from the final gradient were analyzed for polypeptide profile, ARE RNA-binding activity, and *c-myc* mRNA decay activity. (A) Silver-stained 10% SDS-polyacrylamide gel of fractions from analytical gradient analysis of binding and degradation activities. Each lane contains 5×10^6 cell equivalents of protein. Sedimentation is from right to left. The molecular weights (in thousands) of marker proteins are shown on the left. B and T above lanes 1 and 16 refer to the bottom and top gradient fractions, respectively. (B) Analysis of ARE RNA binding. The ^{32}P -labeled RNA probe was synthesized by *in vitro* transcription of *Ssp*I-digested pMycSD3, using SP6 RNA polymerase. The ARE-containing probe is 196 nt of *c-myc* RNA sequence from the 3'UTR (positions 5616 to 5812 [6]) containing the 140-nt *c-myc* ARE (18). RNA-binding reaction mixtures contained the *c-myc* ARE ^{32}P -labeled RNA substrate and equal cell equivalents of gradient fractions. Positions of the free probe (F) and complexed probe (C) are indicated. (C) *c-myc* mRNA degradation activity. One-half of each gradient fraction was pooled in pairs (fraction 1 with fraction 2, fraction 3 with fraction 4, etc.). Pooled fractions were dialyzed versus buffer A and concentrated to 0.2 ml in Centricon 100 microconcentrators. BSA or $8 \mu\text{l}$ (10^7 cell equivalents) of fractions were added to cell-free decay reaction mixtures, which were incubated for either 0 or 5 min and assayed for *c-myc* and γ -globin mRNA decay activities in RNase protection assays using ^{32}P -labeled probes. The 620-nt ^{32}P -labeled *c-myc* probe was synthesized from *Ssp*I-cleaved pSP65myc(CLARI) (11). This probe protects the 3'-most 190 to 210 nt of *c-myc* mRNA. pA₂ refers to the poly(A) site 2 *c-myc* mRNA, which actually consists of four RNA species with different poly(A) addition sites (11). The 570-nt ^{32}P -labeled γ -globin probe was synthesized from *Eco*RI-cleaved pSP65H γ (RIHD). This probe protects the 3'-most 165 nt of γ -globin mRNA. For quantitation, the percentage of intact *c-myc* mRNA for each decay reaction was determined by densitometric scanning of the autoradiograph and comparison with time zero. Percent degradation is defined as the reciprocal of the percent mRNA remaining. Lanes: BSA, 0 min, uncubated decay reaction supplemented with BSA; BSA, 5 min, decay reaction supplemented with BSA and incubated for 5 min; remaining lanes, decay reactions supplemented with the indicated pooled fractions.

in the 7S fraction. The RNA-binding substrate was the ^{32}P -labeled *c-myc* ARE described above. Incubation of the 7S fraction and ^{32}P -labeled RNA substrate with a specific competitor (unlabeled *c-myc* ARE) resulted in a concentration-dependent reduction in complex formation (Fig. 2A, lanes 3 to 7). Addition of up to 400 ng of nonspecific competitor (γ -globin 3'UTR) had little effect on complex formation (Fig. 2A, lanes 8 to 12). These binding results correlate with the mRNA degradation specificity of the 7S fractions from Fig. 1C.

To further examine RNA-binding specificity, a variety of other RNAs and ribohomopolymers were tested for the ability to compete for binding to the ^{32}P -labeled *c-myc* ARE RNA substrate. The transferrin receptor iron-responsive element (TfR-IRE; Fig. 2B, lane 3), poly(C) (lane 6), and poly(G) (lane 7) were also poor competitors for complex formation. AREs from both *c-myc* (lane 4) and GM-CSF (lane 5) 3'UTRs were effective competitors. Poly(A) (lane 8) and poly(U) (lane 9) appeared to be more effective competitors than either poly(C) or poly(G) (Fig. 2B; compare lanes

FIG. 2. Analysis of RNA binding by mobility shift assay. (A) Inhibition of complex formation by competitor RNA. The RNA-binding reaction mixtures contained a constant amount of 32 P-labeled *c-myc* ARE substrate and 7S fraction and increasing amounts of either unlabeled *c-myc* ARE (*c-myc* positions 5616 to 5812 described in the legend to Fig. 1) or γ -globin 3'UTR competitor RNAs indicated above the lanes. The unlabeled 165-nt γ -globin RNA competitor, containing 80 nt of coding sequence and the 85-nt 3'UTR, was synthesized by *in vitro* transcription of *Sau3AI*-digested plasmid pSP65H(γ HDRI), using SP6 RNA polymerase. RNA-binding reaction mixtures containing the 7S fraction were incubated on ice for 10 min with unlabeled competitor RNAs prior to addition of the 32 P-labeled *c-myc* ARE probe. Lanes: 1, free probe; 2, 7S fraction without competitor RNA; 3 to 12, 7S fraction with increasing amounts of either unlabeled *c-myc* ARE competitor RNA (lanes 3 to 7) or γ -globin 3'UTR competitor RNA (lanes 8 to 12). (B) Specificity of 7S binding to 32 P-labeled *c-myc* ARE substrate. RNA-binding reactions were performed in the presence of various unlabeled competitor RNAs as described in the legend to Fig. 1. Unlabeled competitor RNAs in lanes 3 to 5 were synthesized by *in vitro* transcription of appropriate plasmids, using SP6 RNA polymerase. TfR-IRE RNA was synthesized from *Bam*HI-digested plasmid pTRS-1. The TfR-IRE competitor is 250 nt of 3'UTR sequence containing three stem-loop structures necessary for binding of TfR-IRE-binding protein (20, 30). *c-myc* RNA was synthesized as described for Fig. 1 from plasmid pMycSD3. GM-CSF RNA was synthesized from *Eco*RI-digested plasmid pSP64GM(NCORI) to produce an RNA containing 200 nt of 3'UTR containing the GM-CSF ARE. Lanes: 1, free probe; 2, 7S fraction without competitor; 3 to 10, 7S fraction incubated with 200 ng of either unlabeled competitor RNAs (lanes 3 to 5) or homoribonucleotide polymers (lanes 6 to 9) as indicated above the lanes. (C) Analysis of 7S fraction binding to 32 P-labeled poly(A). RNA-binding reaction mixtures contained 32 P-labeled poly(A) RNA substrate in either the absence (lane 1) or presence (lane 2) of the 7S fraction. The 32 P-labeled poly(A) substrate was synthesized by *in vitro* transcrip-

tion of *Hind*III-digested plasmid pSD3 (11), using SP6 RNA polymerase. The 100-nt RNA substrate contains 85 nt of poly(A). (D) Analysis of 7S fraction binding to 32 P-labeled poly(U). RNA-binding reaction mixtures contained 32 P-labeled poly(U) RNA substrate in either the absence (lane 1) or presence (lane 2) of the 7S fraction. The 32 P-labeled poly(U) substrate was synthesized from *Eco*RI-digested plasmid pSP64(polyT). C, Complexed probe; F, free probe.

8 and 9 with lanes 6 and 7). To test whether the 7S fraction contained either a poly(A)- or poly(U)-binding activity, SP6-derived 32 P-labeled poly(A) and poly(U) were used as substrates in gel mobility shift assays with the 7S fraction. The 32 P-labeled poly(A) substrate did not support complex formation (Fig. 2C), whereas the 32 P-labeled poly(U) substrate did (Fig. 2D). Taken together, the results of the gel mobility shift experiments indicate the following. (i) The 7S fraction contains ARE- and poly(U)-binding activities. It is noteworthy that some labile mRNAs such as thrombospondin mRNA do not contain repeated AUUUA motifs but contain long stretches of uridylate residues (17). (ii) Binding activity to the 3'UTR correlates with mRNA degradation activity, since the 7S factor both binds and degrades *c-myc* mRNA but neither binds nor degrades γ -globin mRNA. (iii) The activity in the 7S fraction binds the 3'UTRs of both *c-myc* and GM-CSF mRNAs. Both mRNAs are normally labile. However, stability of GM-CSF mRNA can be regulated independently of *c-myc* mRNA (24, 38). There is some evidence that GM-CSF mRNA stability might be regulated by an RNase inhibitor (4). Alternatively, sequestering GM-CSF mRNA to membrane-bound polysomes for secretion of the cytokine might remove the mRNA from the mRNA turnover machinery, resulting in stabilization of the mRNA. (For example, see reference 48). (iv) The 7S fraction does not contain poly(A)-binding activity, yet poly(A) appears to compete with 32 P-labeled ARE for binding. It is feasible that poly(A) forms double-stranded regions with the *c-myc* ARE, which contains stretches of 5 to 10 uridylate residues, inhibiting binding to the ARE. I hereafter refer to this activity as ARE/poly(U)-binding/degradation factor (Auf).

Separable RNA-binding and degradation activities of Auf. The mRNA-binding and degradation characteristics of Auf were examined after treatment with protease or RNase. A preparation of Auf was treated with either proteinase K or RNase A. Binding to the 32 P-labeled *c-myc* ARE substrate was not abolished by proteinase K (Fig. 3A, lane 4) but was eliminated by addition of SDS (lane 3). In the absence of Auf, proteinase K alone did not support complex formation (data not shown). These results indicate that the binding activity of Auf is proteinase K resistant under the digestion conditions. Proteinase K treatment of Auf eliminated *c-myc* mRNA degradation activity (Fig. 3B; compare columns 2 and 3), whereas PMSF-inactivated proteinase K had no effect (Fig. 3B, column 4). Since BSA had little effect on *c-myc* degradation *in vitro* (Fig. 3B, column 1), protein concentration was not a determinate for degradation in this experiment. RNase A treatment of Auf had no effect on either complex formation (Fig. 3A, lane 5) or *c-myc* mRNA degradation activity (Fig. 3B, column 5). Control experiments indicated that the RNase treatment was sufficient to degrade small RNAs which cosediment with p37 and p40 (data not shown). In contrast, micrococcal nuclease treatment of S130 inactivated a *c-myc* mRNA decay activity (12). The discrepancy between results with micrococcal nuclease and RNase A could be due to differences in base and

tion of *Hind*III-digested plasmid pSD3 (11), using SP6 RNA polymerase. The 100-nt RNA substrate contains 85 nt of poly(A). (D) Analysis of 7S fraction binding to 32 P-labeled poly(U). RNA-binding reaction mixtures contained 32 P-labeled poly(U) RNA substrate in either the absence (lane 1) or presence (lane 2) of the 7S fraction. The 32 P-labeled poly(U) substrate was synthesized from *Eco*RI-digested plasmid pSP64(polyT). C, Complexed probe; F, free probe.

FIG. 3. Analysis of RNA-binding and mRNA decay activities of Auf following protease or nuclease treatment. (A) RNA-binding activity. Auf protein was treated as indicated below prior to addition to RNA-binding reaction mixtures containing 32 P-labeled *c-myc* ARE substrate RNA. Lanes: 1, free probe; 2, untreated Auf protein; 3, Auf protein with 0.1% SDS added just before addition to RNA-binding reaction mixtures; 4, Auf protein treated with proteinase K (2.5 mg/ml; 34 U/ml; Sigma, St. Louis, Mo.) at 30°C for 30 min, followed by addition of 0.1 mM PMSF; 5, Auf protein treated with RNase A (5 μ g/ml) at 30°C for 30 min, followed by addition of 1 U of INHIBIT-ACE (5 Prime-3 Prime, West Chester, Pa.). (B) *c-myc* mRNA decay activity. In addition to being treated as described above, Auf protein was treated with PMSF-inactivated proteinase K (PK). Either BSA (as a control) or Auf protein treated as described above each column was added to cell-free mRNA decay reaction mixtures and incubated at 37°C for 5 min. *c-myc* mRNA decay was assayed by RNase protection and quantitated as described in the legend to Fig. 1. Three repetitions of this experiment gave comparable results.

structural specificities of micrococcal nuclease and RNase A. Taken together, these results indicate that polypeptides are required for *c-myc* mRNA degradation activity, while the ARE-binding activity is proteinase K resistant. However, RNA binding can occur in the absence of the RNA degradation activity. Separate RNA-binding and effector functions have been observed in other RNA-binding proteins. For example, the human immunodeficiency type 1 Rev protein has an N-terminal arginine-rich RNA-binding domain. Rev proteins with mutations in the C terminus have RNA-binding activity but lack the putative effector functions (mRNA nuclear export or pre-mRNA splicing) (26, 49) and thus have a dominant-negative phenotype.

Polypeptides in the RNA-protein complex. An experiment

FIG. 4. Presence of both p37 and p40 in the 32 P-labeled RNA-protein complex from a gel mobility shift assay. Auf protein was either left untreated or treated with proteinase K as described in the legend to Fig. 3. The 32 P-labeled RNA-protein complexes were resolved on a nondenaturing gel and located by autoradiography. The gel slices were excised and inserted into lanes of a 10% SDS-polyacrylamide gel. Polypeptides were detected by silver staining. Lanes: 1, molecular weight markers (indicated in thousands on the left); 2, Auf protein; 3, gel-purified complex from untreated Auf protein; 4, gel-purified complex from proteinase K-treated Auf protein. The amount of Auf protein loaded in lane 2 was adjusted to give silver staining intensity comparable to that in lane 3.

was performed to determine whether both p37 and p40 were part of the RNA-binding complex in gel mobility shift assays. A preparation of Auf, with and without proteinase K treatment, was used for complex formation. Complexes were resolved on a native gel, the bands were excised, and the polypeptides were resolved on an SDS-polyacrylamide gel and visualized by silver staining. Both p37 and p40 were present in the RNA-protein complex (Fig. 4; compare lanes 2 and 3), suggesting that both p37 and p40 are components of Auf. p37 was also present in RNA-protein complexes from protease-treated Auf preparations (lane 4) and is thus a protease-resistant polypeptide under the digestion conditions. This might account for the protease-resistant RNA-binding activity of Auf. I note that the protease resistance of an intracellular protein such as p37 is not without precedent. For example, Akhayat et al. (1) reported that translationally repressed mRNAs in erythroblasts are associated with a prosomalike particle containing a 21-kDa protein resistant to proteinase K.

I have identified an activity, referred to as Auf, in cytosol which binds ARE-containing RNA substrates and poly(U). Auf contributes to degradation of polysome-bound *c-myc* mRNA in vitro without affecting γ -globin mRNA. *c-myc*

mRNA degradation activity of Auf might require association of the mRNA with polysomes, since it does not degrade ³²P-labeled SP6-derived *c-myc* mRNA in vitro (10a). Auf might be one component of a multicomponent system that targets selected mRNAs for rapid cellular degradation. Malter (27) identified a factor in T cells, AUBF, which bound four repeats of the AUUU motif. Because the cell can discriminate between different mRNAs containing an ARE (24, 38), Auf and AUBF might be members of a family of proteins that regulate ARE-specific mRNA turnover in a cell- or inducer-specific manner.

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