Ubiquitin-dependent mechanism regulates rapid turnover of AU-rich cytokine mRNAs

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An AU rich element (ARE) in the 3′ noncoding region promotes the rapid degradation of mammalian cytokine and proto-oncogene mRNAs, such as tumor necrosis factor-α, granulocyte–macrophage colony-stimulating factor (GM-CSF) and c-fos. Destabilization of ARE-mRNAs involves the association of ARE-binding proteins tristetraprolin or AUF1 and proteasome activity, of which the latter has not been characterized. Here, we show that the stability of a model short-lived mRNA containing the GM-CSF ARE was regulated by the level of ubiquitin-conjugating activity in the cell, which links ARE-mRNA decay to proteasome activity. Increased expression of a cytokine-inducible deubiquitinating protein (DUB) that impairs addition of ubiquitin to proteins fully blocked ARE-mRNA decay, whereas increased expression of a DUB that promotes ubiquitin addition to proteins strongly accelerated ARE-mRNA decay. ARE-mRNA turnover was found to be activated by the ubiquitin-addition reaction and blocked by the ubiquitin-removal reaction. Saturation of the ARE-mRNA decay machinery by high levels of ARE-mRNA, which is well established but not understood, was found to be relieved by increased expression of a DUB that promotes ubiquitin addition to proteins. Finally, inhibition of proteasome activity also blocked accelerated ARE-mRNA decay that is mediated by increased ubiquitin recycling. These results demonstrate that both ubiquitinating activity and proteasome activity are essential for rapid turnover of a model cytokine ARE-mRNA containing the GM-CSF ARE.

Cytoplasmic mRNA levels reflect the outcome of RNA transcription rates, mRNA processing and export rates, and cytoplasmic stability. The cytoplasmic stability of particular mRNAs is subject to strict regulation. In particular, the AU rich element (ARE) acts to strongly destabilize mRNAs when located in the 3′ noncoding regions (NCRs; reviewed in refs. 1, 2–4). The ARE consists of multiple copies of the sequence AUUUA in cytokine, growth factor, and some proto-oncogene 3′NCRs (reviewed in refs. 5 and 6). Insertion of the ARE into the 3′NCR of normally stable mRNAs significantly decreases their half-life (reviewed in refs. 5 and 6). The mechanism by which AREs promote rapid degradation of an mRNA when located in the 3′NCR is not understood. In mammalian cells, there is evidence that mRNA decay involves deadenylation and decapping of the mRNA. Deadenylation is inhibited by poly(A)-binding protein and accelerated by the ARE (7). The ARE has been shown to promote deadenylation of certain mRNAs *in vivo* (8, 9) and to increase decapping activity *in vitro* (10). Thus, the ARE might either stimulate deadenylase activity, promote decapping activity, interfere with poly(A)–tail-poly(A)-binding protein interaction, or interfere with the protective interaction between the cap and the cap-binding translation initiation complex.

A number of proteins bind the ARE and are associated with either stabilization or destabilization of ARE-mRNAs (4). The best characterized ARE-binding protein is HuR (11), which stabilizes certain ARE-mRNAs *in vivo* when it is overexpressed (3, 12–14) and *in vitro* in an ARE-mRNA decay system (15). HuR is a predominantly nuclear protein that undergoes rapid shuttling between the nucleus and cytoplasm and can be found bound to polysomes in the cytoplasm, presumably protecting them from rapid degradation (reviewed in ref. 3). In contrast, two other ARE-binding proteins are associated with promoting destabilization of the mRNA. Binding to the ARE of the protein known as tristetraprolin promotes rapid decay of tumor necrosis factor-α and granulocyte–macrophage colony-stimulating factor (GM-CSF) mRNAs (16–18). The ARE-binding protein known as AUF1 or $HnRNPD_0$ was originally identified as a labile cytoplasmic ARE-mRNA destabilizing activity in an *in vitro* ARE-mRNA decay system (19). AUF1 consists of four isoforms (p37, p40, p42, and p45) that arise by differential splicing of a single transcript (2, 20–22). Binding of AUF1 proteins to the ARE or to polysomes *in vivo*, or in an *in vitro* decay system, is associated with an increased rate of ARE-mRNA degradation (23–28).

Heat shock was shown to inhibit rapid degradation of ARE-mRNAs (23). Heat shock decreases proteasome activity (29, 30), and proteasomes, which degrade the majority of short-lived proteins that are conjugated to multiple ubiquitin proteins, were found to be required for the rapid degradation of ARE-mRNAs (23). Specific inhibition of proteasome activity with the agent MG132 prevented rapid turnover of a reporter GM-CSF ARE-mRNA without altering the stability of non-ARE mRNAs. Proteasomes are sights of proteolysis, however, and are unlikely to be directly responsible for degradation of ARE-mRNAs. Therefore, it is not clear how proteasomes are involved in controlling the fate of ARE-mRNAs. Here, we examined the role of protein polyubiquitination (ubiquitylation) to determine whether it provides the link to proteasome activity in the regulation of ARE-mRNA stability. Our studies support a vital role for ubiquitylation activity in the control of rapid degradation of ARE-mRNAs, and they suggest that the rapid turnover of ARE-mRNAs might be regulated by a group of cytokine-inducible deubiquitinating proteins that mediate the addition and removal of ubiquitin on proteins.

Materials and Methods

Plasmids and Cells. β-galactosidase *(lacZ)* reporter mRNAs are similar to those described (23), except that they use the SV40 early promoter, polyadenylation signal, and a cap-dependent 5′NCR (31). pLACAT contains the GM-CSF ARE in the 3′NCR, which in pLACGC is interspersed with G and C residues, making it nonfunctional (32). pUBPY and its dominant-interfering form were a gift of G. Draetta (European Institute of Oncology, Milan, Italy), pUNP was provided by M. Pagano

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Abbreviations: ARE, AU-rich element; 3′NCR, 3′ noncoding region; GM-CSF, granulocyte–macrophage colony-stimulating factor; UBP, ubiquitin-specific processing proteases; DUB, deubiquitinating protein.

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(New York University, New York), and pGFP was a gift of S. Srivastava (New York University, New York). Cos cells were propagated at 37°C in 7.5% $C0_2$ in DMEM supplemented with 10% (vol/vol) bovine calf serum (HyClone) and 50 μg/ml gentamicin sulfate. Transfection was performed with lipofectamine or lipofectamine plus reagent (GIBCO/BRL) at 70% efficiency by using 1 μg of pLACAT or pLACGC reporter constructs, 1 μg of a green fluorescent protein (GFP), and 5 μg of an empty plasmid carrier DNA per 5×10^6 cells. Additional constructs included 5 μg of pUBPY or pUNP instead of empty plasmid DNA. Cells were treated with 20 μM MG132 or 5 μM actinomycin D for up to 10 h (Calbiochem). Transfection efficiencies were determined by photography and quantitation of the percentage of transfected (fluorescent) cells and then were normalized for studies.

Northern RNA Blot and RNA Half-Life Analysis. Total RNA was isolated from transfected cells by using Trizol reagent. Northern RNA blot analysis was performed by using equal amounts of total RNA, electrophoresed in 1% formaldehyde-agarose gels, transferred to nylon membrane, and hybridized to ^{32}P-labeled probes. mRNA levels were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or cotransfected GFP mRNA. Quantitation used densitometry of autoradiographs or phosphorimage analysis. For RNA half-life analysis, cells were treated with 5 μg/ml actinomycin D for the times indicated; RNA was isolated and examined by Northern blot analysis normalized to GAPDH mRNA. RNA decay profiles were plotted as the $\log_{10}$ relative concentration of RNA vs. time.

Metabolic Labeling of Cells. For analysis of translation rates and protein synthesis, cells were labeled in DMEM lacking methionine for 4 h with 100 μCi (1 Ci = 37 GBq) of *trans*-[^{35}S]methionine per ml. Cell extracts were prepared by using Nonidet P-40 lysis buffer [150 mM NaCI/20 mM Hepes, pH 7.5/2.5 mM EDTA/1% Nonidet P-40/1× protease inhibitor (E-64, Roche Molecular Biochemicals complete)] at 4°C and cleared of debris by centrifugation at $10{,}000 \times g$. Immunoprecipitation analysis was performed with equal amounts of protein using specific antisera or preimmune serum and protein G-Sepharose beads. Samples were analyzed by SDS/PAGE and fluorography. To analyze the rates of total cellular RNA synthesis, cells were labeled with 50 μCi/ml of [^{3}H]uridine for 1 h, as described (33). Cells were harvested, and RNA was extracted with Trizol (GIBCO/BRL); equal amounts of RNA based on A_{260} absorbance were spotted onto glass-fiber filters and dried. Filters were washed three times with cold 5% (vol/vol) trichloroacetic acid (TCA) and 20 mM sodium pyrophosphate, followed by 70% ethanol. Filters were dried and the [^{3}H]uridine incorporation was measured by scintillation counting. All experiments were independently repeated at least three times, and experimental errors were determined. Data represent typical results.

Results

Regulation of Ubiquitin Cycling Regulates Reporter ARE-mRNA Abundance and Stability. Cytokines regulate the expression of a class of deubiquitinating proteins (DUBs) known as ubiquitin-specific processing proteases (UBPs; ref. 34). UBP DUBs can function as negative or positive regulators of the ubiquitin system (34). Certain UBPs recycle the pool of ubiquitin from postdegradation peptide fragments, thereby accelerating the ubiquitin cycle and ubiquitin addition to target proteins (35, 36). The UBP known as UBPY functions in this manner (Fig. 1; ref. 37) to accelerate forward recycling of ubiquitin onto proteins, thereby promoting protein degradation. Conversely, certain UBPs remove polyubiquitin from native or insufficiently denatured proteins, possibly as a safeguard to prevent inappropriate degradation, thereby impairing the ubiquitin cycle (38). The UBP known as UNP functions in this manner (Fig. 1; ref. 39), promoting the reverse ubiquitylation reaction and impairing ubiquitin-mediated protein degradation.

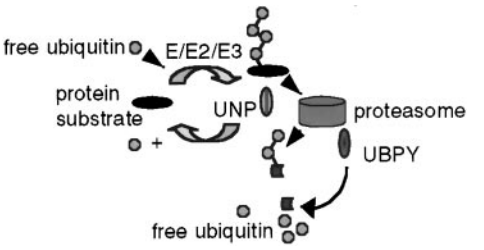

Fig. 1. Schematic representation of the ubiquitin-proteasome cycle and its control by UNP-type and UBPY-type DUBs. Free ubiquitin polypeptides generated by removal of the polyubiquitinated chains from conjugated proteins, or from protein conjugates degraded by the 26S proteasome, are linked to target proteins by the action of E1, E2, and E3 enzymes. Increased expression of a UBPY-type DUB facilitates removal of polyubiquitin from degraded peptides, accelerates the forward ubiquitylation reaction possibly by making ubiquitin more available and thereby promoting protein degradation. Increased expression of a UNP-type DUB promotes the reverse ubiquitylation reaction by prematurely removing polyubiquitin from undegraded proteins, thereby blocking ubiquitin-dependent protein degradation.

To determine whether changes in cellular ubiquitylation activity selectively alters ARE-mRNA stability, cells were transfected with a matched set of β-galactosidase reporter constructs that express mRNAs containing the destabilizing GM-CSF ARE in the 3′NCR or a nonfunctional mutant ARE with G and C residues interspersed within the element (GC-control; ref. 32). A UBPY or UNP expression vector was cotransfected into cells with reporter constructs to positively or negatively alter the ubiquitin addition cycle. Equal amounts of protein extracts were resolved by SDS/gel electrophoresis, transferred to nitrocellulose, and subjected to immunoblot analysis (Fig. 2). The level of reporter β-galactosidase, translation factor eIF4E, which is a stable protein (40), and p53, which is degraded in a ubiquitin-proteasome-dependent manner, were examined by immunoblot analysis. There was no change in steady-state levels of eIF4E, whereas the β-galactosidase reporter encoded by an ARE-mRNA was reduced approximately 12-fold compared with that encoded by the GC-control mRNA (Fig. 2*a*, lanes 2 and 3). β-galactosidase encoded by the ARE-mRNA was almost undetectable in UBPY-transfected cells, whereas that of the GC-

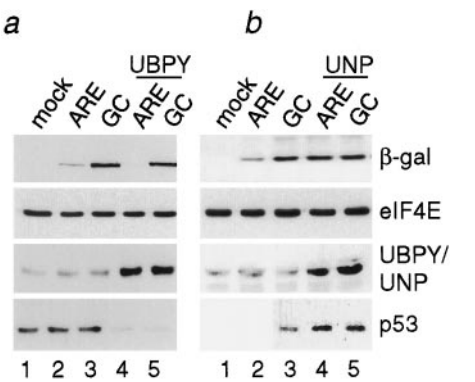

Fig. 2. Effect of UBPY or UNP DUB expression on accumulation of β-galactosidase encoded by GM-CSF, ARE-mRNA, and GC-control reporters. Cos cells were transiently transfected with β-galactosidase ARE- or GC-reporter mRNAs and UBPY or UNP expression vectors or vector alone. Equal amounts of protein extracts were resolved by SDS/gel electrophoresis and immunoblotted with antisera to β-galactosidase, eIF4E, UBPY or UNP, or p53.

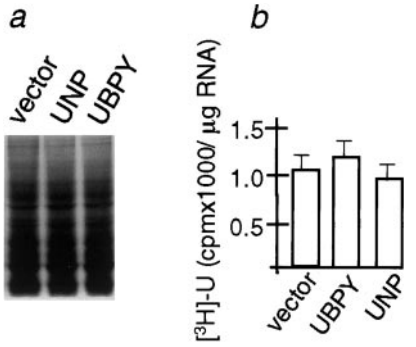

Fig. 3. Effect of UNP or UBPY DUBs on overall translation and transcription activity. (*a*) Cells transfected at 70% efficiency for 40 h were labeled for 4 h with 100 μCi/ml [^{35}S]methionine; equal amounts of protein were resolved by SDS/gel electrophoresis and autoradiographed. (*b*) Cos cells transfected as above were labeled with 50 μCi/ml [^{3}H]uridine for 1 h; incorporation into RNA was assessed by determining the level of radioactivity in equal amounts of TCA precipitable RNA, as described (34). Results represent the average of three independent studies.

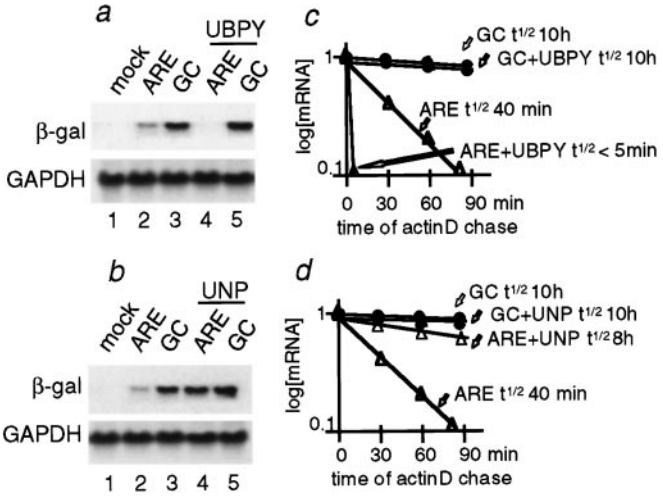

Fig. 4. Effect of increased expression of UBPY or UNP DUBs on ARE-mRNA stability. Cos cells were cotransfected with ARE-mRNA or control GC-mRNA reporter constructs and UBPY (*a, c*) or UNP (*b, d*) expression vectors. RNA was isolated from cells, and equal amounts were resolved by formaldehyde gel-electrophoresis and analyzed by Northern blot hybridization to ^{32}P-labeled β-galactosidase or GAPDH probes. (*c, d*) Cells transfected as above were treated with 5 μg/ml of actinomycin D for up to 10 h to block transcription, RNAs were isolated and subjected to Northern analysis, and levels were quantified by densitometry. The results from two independent experiments were plotted as $\log_{10}$ mRNA concentration (derived by relative densitometry) vs. time of actinomycin D treatment. RNA decay data for the first 90 min of actinomycin D chase are shown. mRNA half-lives were calculated from the slopes of decay curves for the full 10 h of chase.

control was unchanged (Fig. 2*a*). β-galactosidase levels increased to that of the stable GC-control mRNA in UNP-transfected cells (Fig. 2*b*, lanes 4 and 5). p53 levels, which are typically quite low, demonstrated a 5-fold reduction with UBPY overexpression (Fig. 2*a*, lanes 4 and 5) and a moderate elevation with increased UNP expression (Fig. 2*b*, lanes 4 and 5), consistent with the known ubiquitin cycling activities of these DUBs. UBPY and UNP proteins were weakly detectable in nontransfected cells compared with transfected cells.

Before examining the effect of increased expression of UBPY or UNP on ARE-mRNA stability, control studies were conducted to determine whether general cellular translation and transcription rates were altered by DUB overexpression. For translation analysis, cells were transfected with UBPY or UNP expression vectors at 70% efficiency (data not shown) or with a control plasmid and labeled with [^{35}S]methionine for 1 h; equal amounts of protein were resolved by SDS/PAGE and autoradiography. Ectopic overexpression of UBPY or UNP did not detectably alter total cellular translation rates or profiles (Fig. 3*a*). Determination of the specific activity for [^{35}S]methionine incorporation into proteins also was unchanged by increased expression of either protein (data not shown). The effect of UNP- or UBPY-increased expression on total cellular RNA synthesis was ascertained by labeling cells with [^{3}H]uridine for 1 h and by determination of the specific activity of RNA labeling (Fig. 3*b*). Expression of UBPY or UNP did not alter the rate of [^{3}H]uridine incorporation into total RNA. Thus, neither UNP nor UBPY significantly altered the general rates of cellular transcription or translation. Therefore, studies were conducted to determine how control of ubiquitin cycling influences ARE-mRNA stability.

Cells were transfected with ARE- or GC-control β-galactosidase reporter constructs and vectors expressing either UBPY or UNP. Steady-state Northern mRNA analysis demonstrated that ARE-mRNAs accumulated to approximately 12- to 15-fold lower levels compared with stable GC-control mRNAs (Fig. 4*a*, lanes 2 and 3). There was a further 8-fold decrease in the steady-state level of reporter ARE-mRNA in cells cotransfected with UBPY (compare lane 2 to 4), as compared with no change in the level of the GC-control mRNA (lanes 3 and 5). The ARE-mRNA level in the UBPY cotransfected sample was extremely low, but could be detected with prolonged overexposure (data not shown). Cotransfection of ARE-mRNA reporter with the UNP expression vector resulted in a 12-fold increase in ARE-mRNA levels compared with only a slight ($\approx$20%) increase in the level of the long-lived GC-control mRNA (Fig. 4*b*). There was no corresponding change in the level of cellular GAPDH mRNA with expression of UBPY or UNP.

Next, studies determined whether the UBPY-mediated decrease and the UNP-mediated increase in ARE-mRNA abundance is caused by alteration of mRNA stability. Cells were transfected with reporter mRNAs and UBPY or UNP, treated with actinomycin D to block new transcription, and RNA half-life analysis was performed (Fig. 4 *c* and *d*). The GC-control mRNA displayed a cytoplasmic half-life ($t_{1/2}$) of approximately 10 h, compared with 40 min for the matched ARE-mRNA (only time points up to 90 min are shown). Increased expression of UBPY decreased ARE-mRNA stability so substantially that it was difficult to quantify, resulting in a half-life of $<$5 min. These data are consistent with the analysis of ARE-mRNA steady-state levels. Increased UNP expression stabilized ARE-mRNAs to a level approaching that of untreated GC-control mRNAs ($t_{1/2}$ = 8 h), with no affect on the GC-control mRNA. Thus, increased expression of a DUB that promotes the ubiquitylation reaction enhances the rate of ARE-mRNA decay, whereas it is prevented by increased expression of a DUB that down-regulates the ubiquitylation reaction.

Up-Regulation of Ubiquitylating Activity Overcomes the Saturation Block in ARE-mRNA Decay. The ability of cells to rapidly degrade ARE-mRNAs is inhibited by overexpression of mRNAs containing AU-rich motifs (41–43). Because the level of protein ubiquitylation activity is associated with the rate of ARE-mRNA decay, it was determined whether saturation of ARE-mRNA decay is linked to depletion of ubiquitin-conjugating activity. In principle, depletion of ubiquitylation activity might represent the inability to remove a protective ARE-binding protein such as HuR or the inability to degrade a destabilizing binding protein such as AUF1 that might participate in the ARE-mRNA decay reaction. Cells were transfected with 10-fold higher levels of

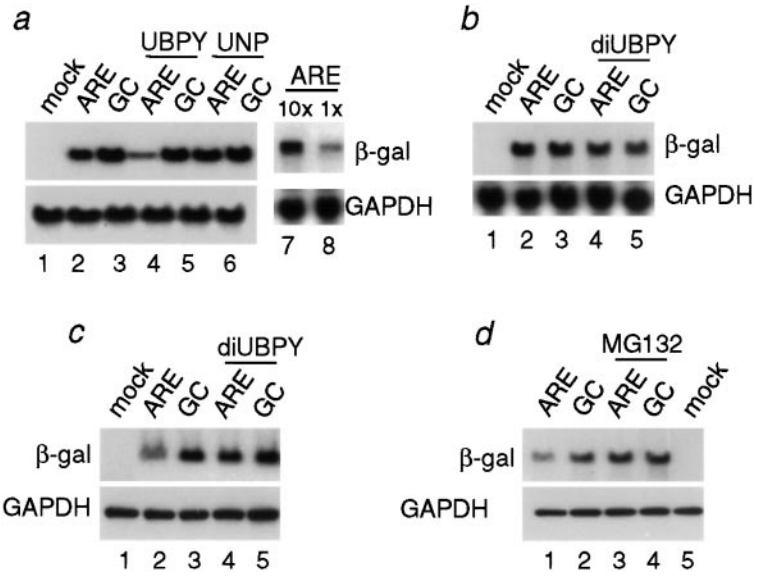

Fig. 5. Inhibition of rapid mRNA decay by overexpression of ARE-mRNA is reversed by overexpression of UBPY. (*a*) Cos cells were transfected with either UBPY or UNP expression vectors and saturating levels of ARE-mRNA or control GC-reporter constructs, which is 10 times the normal transfected amount of plasmid (10 μg of reporter plasmid DNA per 5×10^6 cells). Lanes 7 and 8 compare RNA levels obtained from transfection of 10 μg or 1 μg of reporter plasmid, respectively. (*b*) Cells were transfected with ARE- or GC-reporter constructs at saturating levels as above and a dominant-interfering form of UBPY (diUBPY). (*c*) Subsaturating levels of ARE- or GC-reporter mRNAs (1 μg DNA per 5×10^6 cells) were expressed with diUBPY. (*d*) Cells were transfected with subsaturating levels of ARE- or GC-reporter constructs and wild-type UBPY with or without treatment by 20 mM MG132 to inhibit proteasome function for 10 h before harvest. RNA was isolated, and equal amounts were resolved by Northern blot analysis and hybridized to ^{32}P-labeled probes as shown. Results are typical of at least three independent experiments and were quantified by densitometry.

plasmids expressing ARE- or GC-control mRNAs, a concentration that largely abolishes rapid degradation of the ARE-mRNA (Fig. 5*a*). Northern mRNA analysis showed that the steady-state level of ARE-mRNA reporter was only 1.5-fold lower than the GC-control mRNA when overexpressed (Fig. 5*a*, lanes 3 and 4). Increased expression of UBPY, which promotes ubiquitylation, reversed the block in ARE-mRNA decay during saturation, specifically reducing ARE-mRNA abundance by 10-fold compared with that of the GC-control. Increased expression of UNP, which impairs ubiquitylation, slightly increased the saturating ARE-mRNA levels to that of stable GC-controls (compare lanes 2 to 5 in Fig. 5*a*). There was no change in stable GC-control or endogenous GAPDH mRNA levels with expression of UBPY or UNP. Because increased expression of UBPY or UNP does not affect the transcription rate of the pool of mRNAs, the altered accumulation of ARE-mRNAs represents a change in mRNA stability. The specific requirement for increased ubiquitylation in the rescue of ARE-mRNA decay during saturation was confirmed by overexpression of a dominant-interfering form of UBPY (diUBPY; Fig. 5*b*; ref. 37). The dominant-interfering UBPY failed to restore decreased accumulation of the ARE-mRNA (Fig. 5*b*), consistent with the requirement for enhanced ubiquitylation in the rescue of ARE-mRNA decay during saturation. In addition, in cells expressing subsaturating levels of reporter mRNAs where the steady-state level of ARE-mRNA reporter was 10-fold lower than that of the GC-control, overexpression of diUBPY stabilized the ARE-mRNA by 5-fold (Fig. 5*c*). Thus, two independent lines of evidence, provided by increased expression of UNP or dominant-interfering UBPY, demonstrate that the forward ubiquitylation reaction promotes ARE-mRNA decay.

Next, studies determined whether increased ubiquitylation activity is sufficient to promote selective decay of ARE-mRNAs, or whether functional proteasomes also are required. Cells were transfected with a subsaturating level of ARE- or GC-reporter constructs and a wild-type UBPY expression vector, with or without treatment for 10 h by the proteasome inhibitor MG132 (Fig. 5*d*). Northern blot analysis indicated that MG132 inhibition of proteasomes induced selective stabilization of the reporter ARE-mRNA, which was not overridden by increased expression of UBPY (Fig. 5*d*). These results demonstrate that both ubiquitylating and proteasome activity are required for rapid decay of the GM-CSF reporter ARE-mRNA.

Discussion

We have described studies that investigated the mechanism for control of cytokine ARE-mRNA decay. We previously demonstrated that ARE-mRNA decay is linked to the proteasome network and is blocked by induction of heat-shock protein Hsp70 (23). Heat shock increased interaction of Hsp70 with AUF1 proteins, probably inactivating them, and also decreased the activity of the 20/26S proteasome (29, 30). The role of the ubiquitin cycle, i.e., the addition and removal of ubiquitin from proteins that is involved in targeted proteolysis in proteasomes, was not previously investigated. In this report, therefore, we explored the association between ubiquitylating activity, proteasome activity, and changes in ARE-mRNA stability. We demonstrated that overexpression of deubiquitylating enzymes of the UBP family (reviewed in ref. 34) specifically alters the half-life of a GM-CSF ARE-mRNA reporter. Importantly, increased expression of the DUB UBPY was found to enhance strongly and selectively the rate of ARE-mRNA decay, whereas increased expression of the DUB UNP impaired ARE-mRNA decay. Additionally, UBPY overexpression overcame the phenomenon of ARE-mRNA saturation. Studies have found that high-level expression of an ARE-mRNA can impair rapid degradation directed by the ARE (41–43). Although these data do not determine whether UBPY or a related deubiquitinase is the limiting factor that is saturated by ARE-mRNA overexpression, they do strongly connect the ubiquitin system to regulation of ARE-mRNA decay. Independent confirmation that the ubiquitin system controls GM-CSF ARE-mRNA reporter stability

was obtained by overexpression of a dominant-interfering form of UBPY, which stabilized the normally short-lived ARE-mRNA. Thus, the forward ubiquitylation reaction has been shown to promote rapid decay of a GM-CSF ARE reporter mRNA, whereas the reverse reaction prevents it. Our studies also indicate that ubiquitylation activity is not sufficient to promote ARE-mRNA decay, but in addition requires the function of proteasomes, confirming the role of proteasomes proposed earlier (23).

Although it is not known whether DUBs are normally involved in regulating ARE-mRNA decay, their overexpression in the system used here clearly established the critical involvement of the ubiquitin pathway. However, it is feasible that certain DUBs may normally be involved in regulating ARE-mRNA stability in a cytokine-inducible manner. Analysis of the 3′NCR of DUB mRNAs, particularly those that are growth- or cytokine-regulated, such as DUB-i (44), DUB2 (45), UBPY (37), tre-2 (36, 46), and UBP43 (47), reveals that they all have multiple AUUUA motifs (unpublished results) and, therefore, are likely encoded by unstable mRNAs. Some DUBs also possess ubiquitin targeting sequences (ref. 45 and unpublished results), and are substrates of the ubiquitin-proteasome pathway. Thus, a number of cytokine-inducible DUBs are encoded by ARE-mRNAs, DUBs can be targets of proteasome degradation, and as shown here, DUBs that act on the forward ubiquitylation reaction can accelerate ARE-mRNA decay, whereas DUBs that act on the reverse ubiquitylation reaction can antagonize decay. Shortly after their induction, deubiquitinases DUB-i and DUB-2 are rapidly degraded by the 26S proteasome (45). It is possible that induction of certain deubiquitinases such as UBPY or UNP increase or decrease the accumulation of short-lived mRNAs, including cytokine and proto-oncogene mRNAs, by antagonizing or accelerating proteasome function (which we have shown to be linked to ARE-mRNA decay). Alteration of ubiquitylating or proteasome activity would allow for precise regulation of ARE-mRNA levels during cytokine stimulation and, in addition, alter the degradation of IκB, changing the level of NF-κB available and further impacting on the inflammatory response. Studies now need to determine whether DUBs provide negative and positive autoregulation of cytokine ARE-mRNA levels in response to cytokine stimulation of cells and to identify the protein targets (which are likely ARE-binding proteins) that regulate ARE-mRNA stability in a ubiquitin- and proteasome-dependent manner.

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