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Cellular mutants define a common mRNA degradation pathway targeting cytokine AU-rich elements

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ABSTRACT

To functionally classify AU-rich elements (AREs) from six different cytokine mRNAs, we made use of two previously described HT1080-derived cellular mutants (slowA, slowC) that lack a function required for the rapid degradation of interleukin-3 (IL-3) mRNA. Here we show that the defect is specific for ARE-containing mRNAs, whereas nonsense-mediated decay is intact. Degradation of β-globin reporter transcripts mediated by the AREs of IL-3, GM-CSF, and TNFα, as well as by the structurally different and less potent AREs of IL-2 and IL-6, is impaired in both mutants. All these reporter transcripts are also sensitive to decay induced by ectopic expression of the RNA-binding protein tristetraprolin in the slowC background. Thus, we concluded that the mutants slowA and slowC define a common mRNA degradation pathway that targets cytokine AREs. In NIH3T3 cells, this decay pathway becomes incapacitated by upstream signaling from p38 MAP- or PI3-kinases, which independently stabilize cytokine ARE-containing transcripts. In contrast, c-*fos* ARE-directed mRNA degradation proceeds through a different pathway not affected by these kinases.

Keywords: ARE; mRNA stability; p38 MAPK; PI3-kinase; TTP

INTRODUCTION

The ARE designates a sequence element rich in A and U residues that is located in the 3′ untranslated region (3′ UTR) of certain mRNAs and serves to target the transcripts for rapid degradation. AREs were initially discovered in cytokine transcripts (Caput et al., 1986; Shaw & Kamen, 1986), but they also occur in genes involved in growth control such as the proto-oncogenes c-*myc* (Jones & Cole, 1987), c-*fos* (Wilson & Treismann, 1988), PIM-1 (Wingett et al., 1991), and cyclin D1 (Rimokh et al., 1994), as well as in transcripts not related to cell growth such as cyclooxygenase-2 (Lasa et al., 2000) or urokinase-type plasminogen activator (Nanbu et al., 1994). As a common feature, constitutive expression of most ARE-containing genes is very low, and induction occurs transiently in response to exogenous signals. The ARE has a pivotal role in this process: In resting cells, ARE-directed decay contributes to suppression of mRNA expression, and transient incapacitation of the ARE function allows for rapid mRNA accumulation upon cell activation. Subsequently, decay takes over again and ensures return of the mRNA to basal levels. Under pathological conditions, constitutive stabilization of ARE-containing transcripts has been described for both human malignant cells (Eick et al., 1985; Lebwohl et al., 1994) and experimental tumors (Schuler & Cole, 1988; Nair et al., 1994; Hirsch et al., 1996).

Activation of signal transduction pathways has been shown to stabilize various cytokine mRNAs. Examples include calcium ionophores or TPA, which stabilize IL-3 and GM-CSF transcripts in mast cells and macrophages (Wodnar-Filipowicz & Moroni, 1990; Akashi et al., 1991), or activation of T-cells by anti-CD3 and anti-CD28 antibodies, which stabilizes IL-2, GM-CSF, TNFα, and IFNγ transcripts (Lindsten et al., 1989). The fact that stabilization occurs even in the presence of translation inhibitors suggested that the mechanism involves direct, signal-induced protein modification (Wodnar-Filipowicz & Moroni, 1990). Indeed, transfection of a dominant-negative mutant of the c-*jun* N-terminal protein kinase (JNK) into mast cells precluded ionomycin-induced mRNA stabilization, implying that JNK is involved in

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posttranscriptional induction of IL-3 expression (Ming et al., 1998). The JNK pathway also stabilizes IL-2 mRNA in T-cells (Chen et al., 1998). In HeLa cells it has been shown that activation of the p38 MAPK pathway leads to cytokine mRNA stabilization via MAP kinase-activated protein kinase 2 (Winzen et al., 1999). Recent evidence from our laboratory indicates that IL-3 mRNA can be stabilized in parallel and independently by the p38 MAPK pathway and by the phosphatidylinositol-3-kinase (PI3-K) pathway (Ming et al., 2001). The emerging concept is that exogenous signals, in addition to stimulating transcription and translation, stabilize ARE-containing transcripts via protein phosphorylation that affects, directly or indirectly, ARE-binding proteins.

An ARE-binding protein with an established mRNA stabilizing activity is HuR, a ubiquitously expressed member of the Elav protein family (Fan & Steitz, 1998; Peng et al., 1998), whereas AUF1 (hnRNP D) has been reported to destabilize transcripts in differentiating erythroid cells (Loflin et al., 1999). Under certain circumstances, however, AUF1 may also be involved in mRNA stabilization (Laroia et al., 1999). Tristetraprolin (TTP) has been identified as a negative regulator that promotes degradation of TNFα and GM-CSF mRNA (Carballo et al., 1998, 2000). Recent evidence indicates that mRNA can occur in the cell as circular mRNA–protein structure linked at the 3′ end to polyA/polyA-binding protein and 5′ terminally to components of the translation initiation complex (reviewed in Jacobson & Peltz, 1996; Wilusz et al., 2001). Thus, ARE–protein complexes may function by controlling the access of 3′- or 5′-specific nucleases.

A hallmark of AREs is the pentamer AUUUA that occurs either singly, reiterated, or clustered. This is the criterion for a recent database-derived compilation of AREs (Bakheet et al., 2001). A more empirical classification has taken both sequence features and deadenylation kinetics into consideration (Chen & Shyu, 1995). According to this classification, the AREs of c-*myc* and c-*fos* are prototypes of class I, containing one to three scattered copies of the AUUUA motif within a U-rich context. The GM-CSF, TNFα, and IL-3 AREs are typical class II AREs with a core AUUUA motif cluster (Stoecklin et al., 1994), whereas a third class (e.g., c-*jun*) lacks an AUUUA pentamer (Chen & Shyu, 1994). Interestingly, deadenylation is synchronous with classes I and III, but asynchronous with class II AREs, suggesting that distinct degradation pathways, stabilizing mechanisms, and binding proteins may exist for the different classes or even types of AREs. The fact that TTP-knockout mice displayed selective elevation of TNFα and GM-CSF levels (Taylor et al., 1996; Carballo et al., 2000) supports the idea of independent mRNA turnover pathways.

A novel approach to further understand how AREs function has been pursued in our laboratory by developing cellular mutants that are defective in IL-3 mRNA decay (Stoecklin et al., 2000). Human HT1080 cells stably transfected with a hybrid GFP/IL-3 reporter gene were randomly mutagenized, and high GFP expressing clones were selected by FACsorting and fluorescence microscopy. From these clones, three mutants (slowA, slowB, and slowC) were identified that overexpress the reporter transcript due to a posttranscriptional defect. Reporter mRNA stabilization occurs in *trans*, and the phenotype is recessive as it could be corrected by fusion with wild-type (wt) cells. slowA belongs to a different complementation group than slowB and slowC, but both groups could be reverted by transfection of TTP. Endogenous TTP, however, was found to be wild type and expressed at normal levels in slowA and slowC (Stoecklin et al., 2000). Although the missing function in the mutants remains unknown, experiments are in progress to identify the defect by cDNA transfer. In this paper, we used the mutants slowA and slowC to investigate whether reporter transcripts containing AREs from various cytokines with different sequence features and decay kinetics are processed via the same degradation pathway defined by the mutants. In addition, we examined sensitivity to TTP-induced degradation and tested whether the transcripts can be stabilized by the p38 MAP- and PI3-kinase pathways. Applying these functional criteria allowed us to define a common cytokine mRNA degradation pathway that targets the AREs of IL-3, GM-CSF, TNFα, IL-2, and IL-6, whereas the AREs of IL-4 and c-*fos* are distinct. This pathway becomes incapacitated by p38 MAP- and PI3-kinase signaling.

RESULTS

Defective function is ARE specific

Initial identification of the slowA/C phenotype involved genomic IL-3 constructs and reporter genes containing the entire 3′ UTR of IL-3 (Stoecklin et al., 2000; Fig. 1A, lanes 5–12). It thus could be argued that sequences outside the ARE might be required for the slow decay in the mutants, perhaps by binding an inhibitor. We therefore tested a reporter transcript containing only a 59-nt-long sequence spanning the IL-3 ARE by inserting it immediately after the stop codon of the rabbit β-globin gene (Fig. 3). Upon stable transfection of plasmid puroMXβ-IL3-ARE into the wt and mutant cell lines, decay of the mRNA was monitored by actinomycin D chase experiments. As shown in Figure 1B, the ARE alone was sufficient to trigger rapid mRNA decay in the wt cells (lanes 1–4) with a half-life of 58 $\pm$ 6 min, similar to the decay rate of the GFP-IL3-UTR transcript containing the entire 3′ UTR of IL-3 (Fig. 1A, lanes 1–4), which has a half-life of 63 $\pm$ 3 min (Stoecklin et al., 2000). In the mutants, degradation of β-IL3-ARE mRNA was impaired (Fig. 1B, lanes 5–12), and no difference was seen in comparison to the IL-3 3′ UTR-construct.

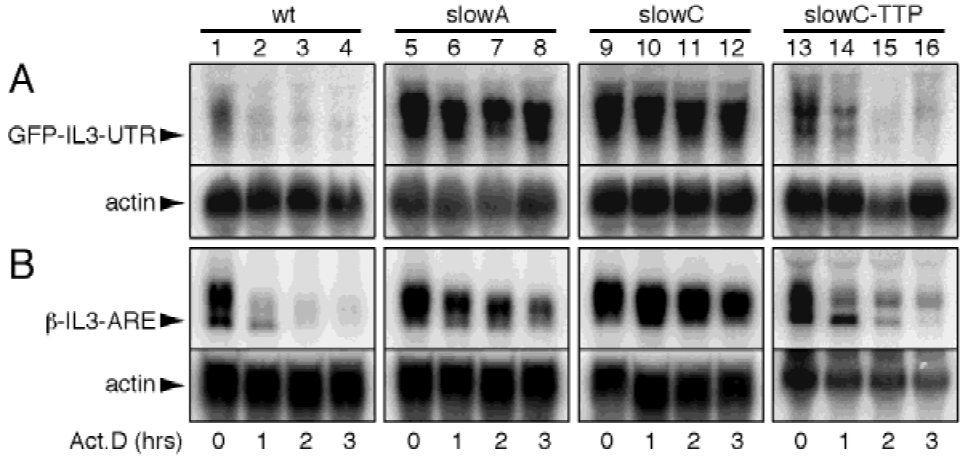


FIGURE 1. mRNA decay mediated by the 3′ UTR and the ARE of IL-3. **A:** wt cells (lanes 1–4), mutant slowA (lanes 5–8), slowC (lanes 9–12), and slowC-TTP (lanes 13–16) stably express the GFP-IL3-UTR reporter construct (Stoecklin et al., 2000). Total RNA was extracted at different time points after actinomycin D treatment and subjected to northern blot analysis using 1.1% agarose/formaldehyde gels. The membranes were hybridized to a radiolabeled SP6 probe against the 3′ UTR of IL-3, and rehybridized to an actin probe for loading control. **B:** The same cell lines were stably transfected with the β-globin reporter construct puroMXβ-IL3-ARE (see Fig. 3). A β-globin probe served to detect the reporter transcript.

We concluded that the decay function lacking in the mutants is restricted to the ARE and requires no outside *cis*-elements in the mRNA.

Inspection of Figure 1B showed that mRNA decay in wt cells was associated with rapid shortening of the transcripts (note the broad signal in lane 1 compared to the lowest part of the band remaining in lane 2). RNase H experiments confirmed that this shortening corresponds to deadenylation of the message (data not shown), which is in agreement with the well-established concept that deadenylation precedes ARE-mediated mRNA degradation (Wilson & Treismann, 1988; Chen & Shyu, 1995). This indicated that failure to degrade IL-3 mRNA in the mutants is coupled to impaired deadenylation.

Nonsense-mediated decay is not affected

Nonsense-mediated decay (NMD) is triggered by a premature stop codon in the mRNA and occurs independently of deadenylation (for review, see Hentze & Kulozik, 1999; Hilleren & Parker, 1999). Because Figure 1 suggested that deadenylation is impaired in the mutants, one would expect NMD not to be affected. To test this prediction, a pair of T-cell receptor β minigene constructs was stably transfected into wt, slowA, and slowC cells. Whereas the control construct (TCRβ-wt) has a single stop codon in the last exon, the mutated construct (TCRβ-PTC) contains an additional, premature stop codon in the third exon (Fig. 2A). TCRβ mRNA from the wt construct was expressed at relatively high levels easily detectable by northern blot analysis, whereas the PTC-containing transcripts were expressed at much lower levels with no differences between wt and mutant cell lines (Fig. 2B). An additional, lower band appearing in the TCRβ-PTC lanes has not been further analyzed, but might represent an alternatively spliced TCRβ mRNA induced by the nonsense mutation. We concluded that NMD indeed is not

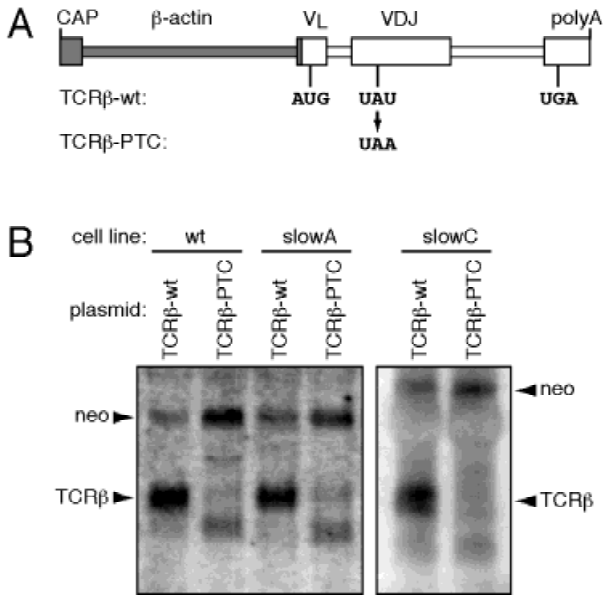


FIGURE 2. Nonsense-mediated mRNA decay. **A:** Schematic representation of the TCRβ reporter gene pre-mRNA (exons: thick bars, introns: thin bars) consisting of a β-actin 5′UTR (in gray) and sequences of a rearranged T-cell receptor β gene (in white). TCRβ-wt contains the normal stop codon in exon 4, whereas TCRβ-PTC has a point mutation in exon 3 that introduces an additional, premature termination codon. **B:** After stable transfection of TCRβ-wt and TCRβ-PTC into wt, slowA, and slowC cells, total RNA was extracted and subjected to northern blot analysis. The membranes were simultaneously hybridized with a probe against TCRβ and, to control for transfection efficiency and loading, with a probe against the neomycin resistance transcript encoded on the same plasmid.

impaired in slowA and slowC, which is in accordance with the observation that the mutants are defective for a deadenylation-dependent mRNA decay mechanism.

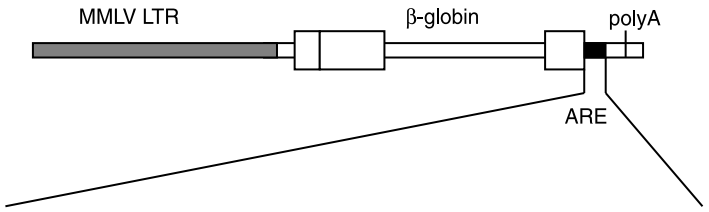
mRNA decay mediated by the AREs of GM-CSF and TNFα

Given the specificity of the slowA/C mutants for the IL-3 ARE (Fig. 1), we now had a tool to functionally classify various cytokine AREs by their ability to trigger mRNA decay within the mutant cellular background. β-globin reporter constructs with ARE sequences from GM-CSF, TNFα, IL-2, IL-4, and IL-6 (see Fig. 3) were stably transfected into wt, slowA, and slowC cells for mRNA decay experiments. We first studied the AREs of GM-CSF and TNFα that share high sequence similarity with the IL-3 element and represent class II AREs (Chen & Shyu, 1995). Decay of the β-GMCSF-ARE transcript was rapid in wt cells with a half-life of 71 $\pm$ 14 min, but impaired in the mutants (Fig. 4A, lanes 1–12).

With TNFα, a short, 34-nt element containing the six core AUUUA motifs (TNFα-ARE$_{34}$, see Fig. 3) was first analyzed. Yet, decay of the β-TNFα-ARE$_{34}$ mRNA was not rapid in the wt cells (Fig. 4B, lanes 1–4), having a half-life of more than 5 h (318 $\pm$ 58 min). However, a longer portion of the TNFα ARE extended by 19 nt at the 5′ end of the AUUUA motif cluster (TNFα-ARE$_{53}$) behaved as the other class II AREs by inducing rapid mRNA decay in wt cells (half-life of 48 $\pm$ 5 min), but not in the mutants (Fig. 4C, lanes 1–12). Taken together, the AREs of IL-3, GM-CSF, and TNFα appeared to trigger mRNA decay by a common pathway, as all three transcripts were stabilized in slowA and slowC. In addition, failure of the short TNFα-ARE$_{34}$ to induce decay in wt cells indicated that sequence elements upstream of the core AUUUA motif cluster are essential.

mRNA decay mediated by the AREs of IL-2, IL-4, and IL-6

Another group of cytokine transcripts including IL-2, IL-4, and IL-6 contain AU-rich sequences that differ considerably in their nucleotide composition from the typical class II AREs as none of them displays clustering of AUUUA pentamers (see Fig. 3). The AREs of IL-4 and IL-6, in fact, have been described as class I-like (Chen & Shyu, 1995), whereas the ARE of IL-2 has so far not been classified. For IL-2, we chose a relatively large fragment within the 5′ portion of the IL-2 3′ UTR, as deletion of this portion has been shown to increase IL-2 mRNA stability (Chen et al., 1998). The 92-nt-long element is particularly U rich and harbors four AUUUA motifs, two of which are overlapping (Fig. 3). In wt cells, β-IL2-ARE reporter mRNA decayed only at a modest rate (Fig. 5A, lanes 1–4) with a half-life of about 4 h



	length (nt)	pentamers[a]	nonamers[b]	mRNA half-life ± SE (minutes)
UUCCAUUAAGGCUAUUUAUUUAUGUAUUUAUGUAUUUAUUUAUUUAUUGCCUUCUGUGA IL3-ARE	59	6	2	58 ± 6[c]
UAAUAUUUAUAUAUUUAUAUUUUUAAAAUAUUUAUUUAUUUAUUUAUUUAA GMCSF-ARE	51	7	3	71 ± 14[c]
UCUAUUUAUAUUUGCACUUAUUAUUUAUUUAUUUAUUUAUUUAUUUAUUUG TNFα-ARE$_{53}$	53	6	5	48 ± 5[c]
AUUAUUUAUUAUUUAUUUAUUAUUUAUUUAUUUA TNFα-ARE$_{34}$	34	6	5	318 ± 58[d]
CCAACACAUAAGGCUCUCUAUUUAUUUAAAUAUUUAACUUUAAUUUAUUUUUGGAUGUA IL2-ARE UUGUUUACUAUCUUUUGUAACUACUAGUCUUCAG	93	4	1	244 ± 54[c]
UUUAACUUAUGAAUUUUUAAUGGUUUUAUUUUAAUAUUUAUAUUUAUAAUUCAUAA IL4-ARE	58	2	0	>6 hrs[e]
AUUUUAAUUAUUUUUAAUUUAUUGAUAAUUUAAAUAAGUAAACUUUAAGUUAAUUUAUG IL6-ARE AUUGAUAUUUAUUAUUUUUA	79	4	0	163 ± 12[c]

FIGURE 3. Schematic representation of the β-globin reporter constructs containing different cytokine AREs (in black). The β-globin gene (in white) is driven by a Murine Moloney Leukemia Virus (MMLV) LTR promoter (in gray). Coding regions are represented as thick bars, noncoding regions as thin bars. AUUUA motifs are boxed and shown in bold in the ARE sequences. The length of the ARE, as well as the number of [a]AUUUA pentamers and [b]UUAUUUAU/AU/A nonamers is indicated on the right side. The half-lives of the corresponding mRNAs, when expressed in wt HT1080 cells, were calculated by quantification of the signal intensities obtained by northern blot analysis from mRNA decay experiments (see Figures 1, 4, and 5). The values for β-globin were normalized to actin, and half-lives were obtained by linear regression. Mean value and standard error calculations are based on [c]$n = 5$ or [d]$n = 4$ repeat experiments. [e]For β-IL4-ARE, a value of 7.8 $\pm$ 2.5 h ($n = 4$) was obtained by extrapolation.

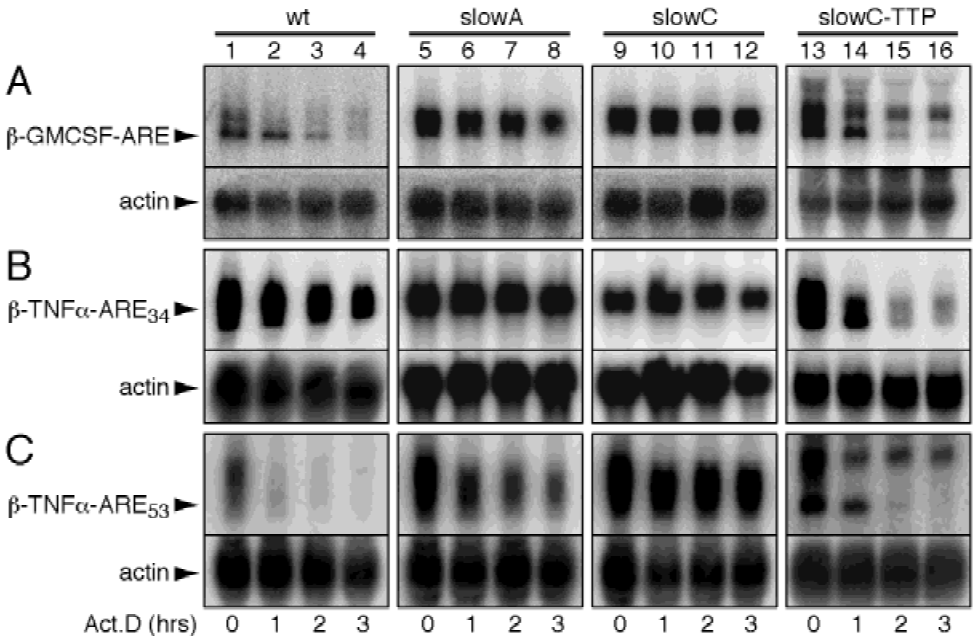


FIGURE 4. mRNA decay mediated by the AREs of GM-CSF and TNFα. **A:** wt cells (lanes 1–4), mutant slowA (lanes 5–8), slowC (lanes 9–12), and slowC-TTP (lanes 13–16) were stably transfected with the β-globin reporter construct puroMXβ-GMCSF-ARE (see Fig. 3). Actinomycin D chase experiments and northern blot analysis was performed as described for Figure 1. **B:** The influence of a short TNFα ARE was studied by transfection of puroMXβ-TNFα-ARE$_{34}$ (see Fig. 3) into the same cell lines. **C:** A longer TNFα ARE was inserted into puroMXβ-TNFα-ARE$_{53}$ (see Fig. 3) and analyzed as above.

(244 $\pm$ 54 min), differing considerably from the class II transcripts. In the mutants, still, β-IL2-ARE mRNA was fully stable (Fig. 5A, lanes 5–12), indicating that the defective function is also required for IL-2 ARE-mediated mRNA degradation.

The IL-4 transcript has a very short 3′ UTR (74 nt up to the poly-A site in the murine transcript) that is mainly composed of a 58-nt-long AU-rich sequence including two AUUUA motifs (Fig. 3). With this fragment inserted into the β-globin reporter gene, hardly any mRNA deg-

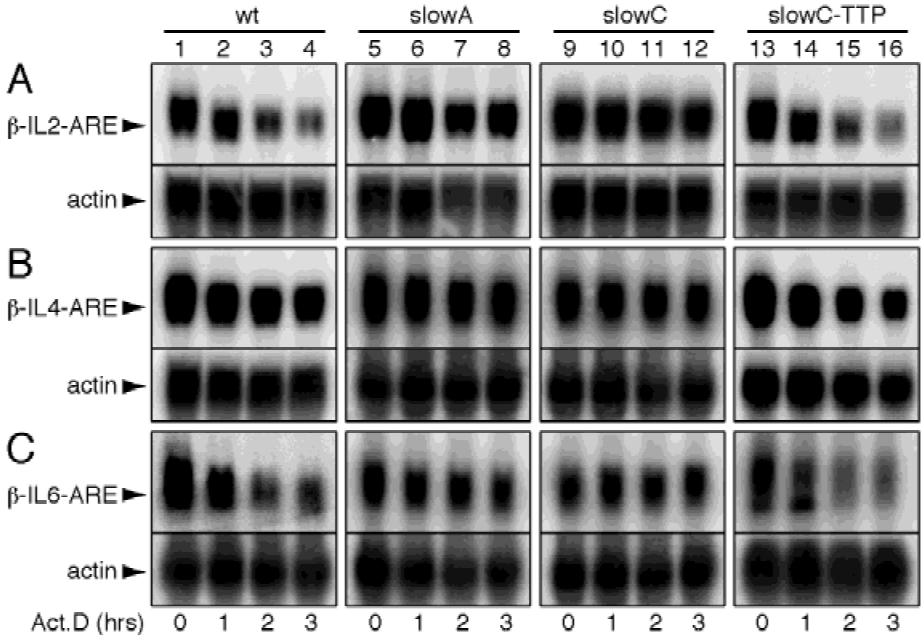


FIGURE 5. mRNA decay mediated by the AREs of IL-2, IL-4, and IL-6. **A:** wt cells (lanes 1–4), mutant slowA (lanes 5–8), slowC (lanes 9–12), and slowC-TTP (lanes 13–16) were stably transfected with the β-globin reporter construct puroMXβ-IL2-ARE (see Fig. 3). Actinomycin D chase experiments and northern blot analysis were performed as described for Figure 1. The same cell lines were transfected with puroMXβ-IL4-ARE (**B**) and puroMXβ-IL6-ARE (**C**; see Fig. 3).

radation could be observed in wt cells (Fig. 5B, lanes 1–4), and the half-life exceeded 6 h. Although the inability of the IL-4 ARE to trigger rapid decay may be cell specific, it is obvious that this element belongs to a functionally different group.

The ARE of IL-6 analyzed was 79 nt in length and contained four AUUUA pentamers, none of which are clustered (Fig. 3). In wt cells, the β-IL-6 ARE transcript degraded fairly rapidly with a half-life of 163 $\pm$ 12 min, whereas in slowA and slowC, again, the mRNA was stable (Fig. 5C, lanes 1–12). These data indicated that the AREs of IL-2 and IL-6, which both contain four, more dispersed AUUUA pentamers, are targeted by the same degradation pathway as the IL-3, GM-CSF, and TNFα AREs. Yet, their potential to recruit the decay machinery seems to be reduced in comparison to the classical class II AREs. We therefore propose to call the IL-2 and IL-6 elements weak class II AREs.

mRNA decay induced by TTP expression

Tristetraprolin is a zinc finger protein that acts as a negative, posttranscriptional regulator of TNFα expression by binding to the ARE and thereby increasing the deadenylation and mRNA turnover rate (Carballo et al., 1998; Lai et al., 1999). TTP has also been shown to destabilize IL-3 transcripts following transfection into slowA and slowC cells, thus reverting the phenotype of the mutants. Because the endogenous TTP alleles appeared to be wt and normally expressed in the mutants (Stoecklin et al., 2000), the role of TTP in correcting defective decay is not understood. Nevertheless, the response to TTP could be used as a second criterion for classifying AREs of different origin. We tested all the constructs studied above in slowC cells stably transfected with TTP (slowC-TTP). Indeed, the decay rates of the β-IL3-ARE, β-GMCSF-ARE, β-TNFα-ARE$_{53}$, β-IL2-ARE, and β-IL6-ARE transcripts were restored to wt levels in slowC-TTP (lanes 13–16 in Figs. 1B, 4A, 4C, 5A, and 5C), whereas the effect was marginal on β-IL4-ARE mRNA (Fig. 4B, lanes 13–16). This was further evidence that the AREs of IL-3, GM-CSF, TNFα, IL-2, and IL-6 are targeted by a common degradation pathway.

An interesting observation was made with the short TNFα-ARE$_{34}$ as its degradation was significantly faster in slowC-TTP than in wt cells (Fig. 4B, lanes 1–4 and 13–16). The corresponding half-lives were 63 $\pm$ 3 min ($n = 3$) in slowC-TTP compared to 318 $\pm$ 58 min in wt cells. Such a difference was not observed with any of the other transcripts where the decay rates in slowC-TTP were similar to those in wt cells. This indicated that the AUUUA motif cluster (TNFα-ARE$_{34}$) is sufficient to target the mRNA for TTP-induced decay, whereas the 5′-extended ARE (TNFα-ARE$_{53}$) is needed for the endogenous decay activity in wt cells.

mRNA stabilization in response to p38 MAPK and PI3-kinase activation

To substantiate our finding that the different cytokine AREs are subjected to the same mRNA decay pathway, we investigated, as a third parameter, the responsiveness of the different AREs to stabilizing signals brought about by two different kinase pathways. These experiments were performed in NIH3T3 cells because in HT1080 cells, transcripts following transient transfection were rather stable (data not shown). As reported elsewhere (Ming et al., 2001), activation of the stress MAP-kinase p38 by expressing a constitutively active form of its upstream activator MEK6 (MEK6DD), as well as expression of a constitutively activated form of the catalytic subunit of PI3-kinase (rCD2-p110) leads to the stabilization of β-globin reporter transcripts containing the 3′ UTR of IL-3. It is thought that these kinases, probably via affecting AU-binding proteins, functionally inactivate the decay machinery. If the cytokine AREs analyzed here are indeed targeted by the same degradation pathway, decay of all the transcripts should be impaired in a coordinated fashion by upstream signals. For this purpose, the β-globin reporter constructs were transiently cotransfected together with vector alone, SRα3-MEK6DD or rCD2-p110.

As shown in Figure 6A, the ARE of IL-3 alone was sufficient to promote rapid decay of the mRNA in NIH3T3 cells and render it susceptible to stabilization by activation of both p38 MAPK and PI3-kinase pathways. The same was observed with the β-GMCSF-ARE and β-TNFα-ARE$_{53}$ transcripts (Fig. 6B,C). Interestingly, decay mediated by the structurally different IL-2 and IL-6 AREs was similarly antagonized by the two kinase pathways (Fig. 6D,F). As already seen in HT1080 cells (Fig. 5B, lanes 1–4), the IL-4 element was unable to target the transcript for rapid decay in NIH3T3 cells (Fig. 6E, lanes 1–3). Because mRNA decay by all the active cytokine AREs (IL-3, GM-CSF, TNFα, IL-2, and IL-6) was abrogated in response to p38 MAPK and PI3-kinase signals, we tested whether a different, class I-type ARE would behave in the same way or not. As shown in Figure 6G, decay of β-c-*fos*-ARE mRNA was hardly affected by cotransfection of either of the activated kinase plasmids. Lack of stabilization was also seen with a β-globin reporter transcript linked to the entire 3′ UTR of c-*fos* (Fig. 6H), indicating that a class I ARE indeed recruits a different mRNA degradation pathway.

DISCUSSION

Mammalian mutants of ARE-mediated mRNA turnover

Genetic analysis in yeast has proven to be a very powerful tool for the dissection of different mRNA degra-

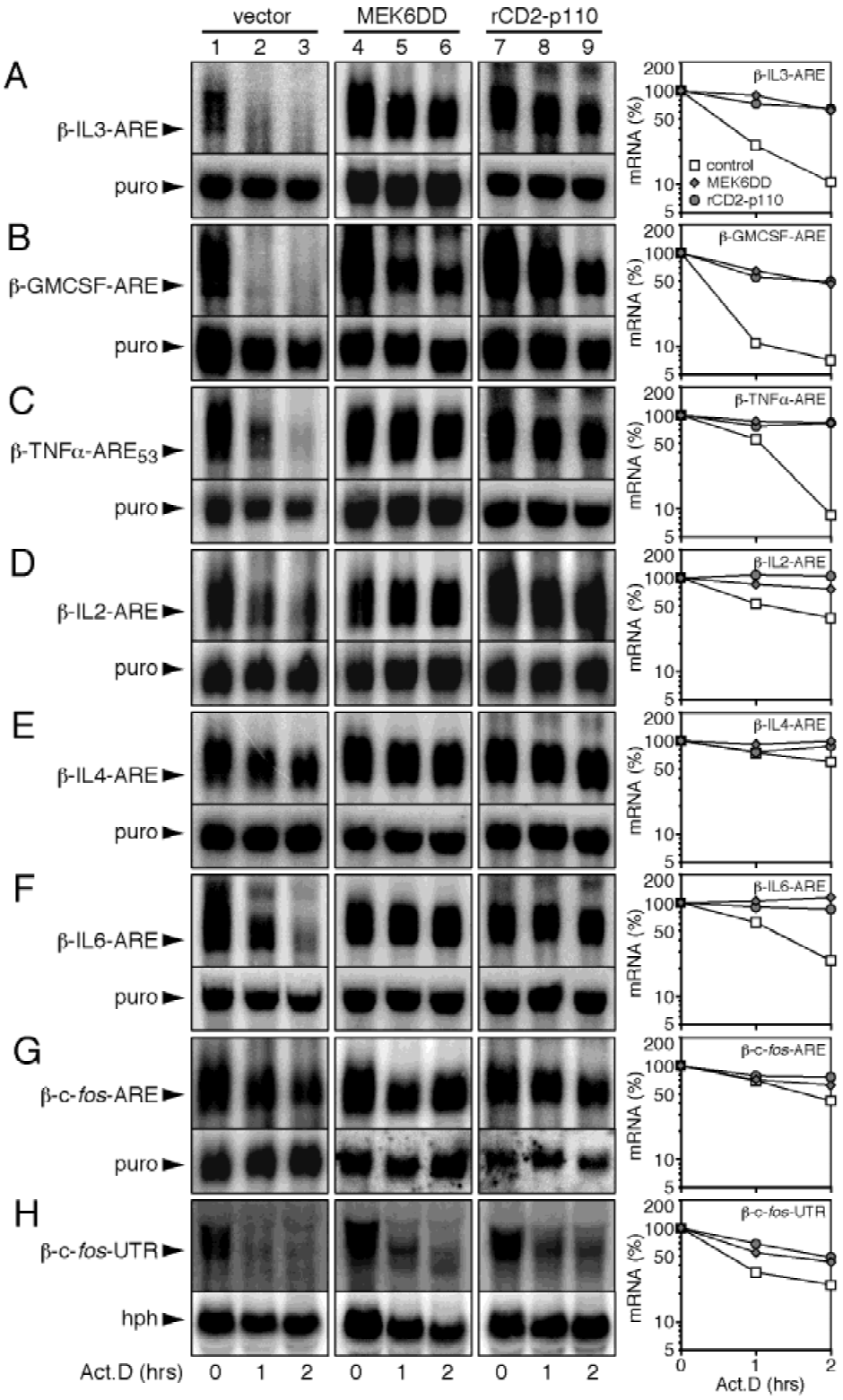


FIGURE 6. mRNA stabilization upon activation of p38 MAPK and PI3-kinase pathways. NIH3T3 B2A2 cells were transiently transfected with empty vector (lanes 1–3), SRα3-MEK6DD, a constitutively active form of MEK6, the upstream activator of p38 MAPK (lanes 4–6), or rCD2-p110, a constitutively activated form of the PI3-kinase catalytic subunit (lanes 7–9). In addition, β-globin reporter constructs were cotransfected as follows: **A:** puroMXβ-IL3-ARE. **B:** puroMXβ-GMCSF-ARE. **C:** puroMXβ-TNFα-ARE$_{53}$. **D:** puroMXβ-IL2-ARE. **E:** puroMXβ-IL4-ARE. **F:** puroMXβ-IL6-ARE. **G:** puroMXβ-c-*fos*-ARE. **H:** MXhβ-c-*fos*-UTR. Actinomycin D chase experiments and northern blot analysis were performed as described for Figure 1, except that puromycin N-acetyl transferase (puro) or hygromycin B phosphotransferase (hph) mRNA, which are encoded on the β-globin reporter plasmids, were detected to control for transfection efficiency and equal loading. The right panel shows quantification of the signal intensities by PhosphoImager. β-globin values were normalized to puro or hph and plotted as a percentage of the initial value against time.

dation pathways (for reviews, see Beelman & Parker, 1995; Jacobson & Peltz, 1996). A similar approach could now be initiated in a mammalian system due to the previous isolation of human HT1080-derived mutant cell lines lacking a function required for rapid degradation of IL-3 mRNA (Stoecklin et al., 2000). In this report, we specified the mutant phenotype by showing that a β-globin reporter transcript containing only the ARE of IL-3 also failed to undergo rapid decay in slowA and slowC (Fig. 1). The defective function thus appeared to specifically target the ARE and did not require any other *cis*-acting element in the mRNA. Ectopic expression of the zinc-finger protein TTP could restore rapid decay, which confirms that TTP destabilizes mRNAs in an ARE-specific manner (Carballo et al., 1998; Lai et al., 1999).

A functional definition of class II AREs

To functionally classify the AREs of different cytokines, we applied various criteria: First, we tested whether mRNA decay mediated by a given ARE depends on the function that is lacking in the mutants slowA and slowC. Second, we analyzed in the mutant background of slowC whether the AREs would respond to TTP-induced decay. As a third criterion, we made use of another system involving transient transfection of NIH3T3 cells and examined whether the same mRNAs could be stabilized in response to p38 MAPK and PI3-kinase activation.

The β-GMCSF-ARE and β-TNFα-ARE$_{53}$ reporter transcripts showed the same behavior as IL-3 mRNA: They were stable in the mutants unless TTP was expressed (Fig. 4A,C), and could be stabilized by coexpression of constitutively activated forms of MEK6 and the PI3-kinase p110 subunit (Fig. 6A–C). Thus, three lines of evidence argued that the AREs of IL-3, GM-CSF, and TNFα are targeted by the same decay pathway. In addition, these three AREs appeared to be functionally equivalent as the corresponding mRNA half-lives in wt HT1080 cells were in the same range (between 48 and 71 min, see Fig. 3). Our data confirm the current classification that groups the AREs of IL-3, GM-CSF, and TNFα as class II based on their sequence similarity and asynchronous deadenylation pattern (Chen & Shyu, 1995; Xu et al., 1997).

Two interesting observations were made with the short version of the TNFα ARE (TNFα-ARE$_{34}$, see Fig. 3). First, this element did not promote rapid mRNA decay in the HT1080 wt background (Fig. 4B, lanes 1–4), which is in contrast to the destabilizing effect of the same element in transiently transfected NIH3T3 cells (Xu et al., 1997). This might simply reflect the fact that decay in NIH3T3 cells is generally faster than in HT1080 cells (compare, e.g., lanes 1–4 of Fig. 6B with lanes 1–4 of Fig. 4A). In any case, extending the element by 19 nt at the 5′ end of the AUUUA motif cluster (TNFα-ARE$_{53}$) greatly increased its potential to induce decay (Fig. 4C, lanes 1–4). This is in accordance with the observation that insertion of a 54-nt-long sequence from the c-*fos* ARE at the 5′ flank of the short TNFα-ARE$_{34}$ could accelerate decay and induce asynchronous deadenylation (Xu et al., 1997). Hence, there seems to be a requirement for 5′-terminal AU-rich sequences flanking the core AUUUA motif cluster. It is noteworthy that a similar AU-rich 5′ portion is also present in the AREs of GM-CSF and IL-3 (Fig. 3).

The second observation was that the β-TNFα-ARE$_{34}$ transcript decayed significantly faster in slowC-TTP than in wt cells (Fig. 4B), whereas the decay rates of all the other transcripts were similar in the two cell lines. It thus appears that the AUUUA motif cluster alone (TNFα-ARE$_{34}$) is sufficient to respond to TTP, whereas the 5′-extended ARE (TNFα-ARE$_{53}$) is required to efficiently recruit the endogenous decay pathway in wt cells. This, in turn, suggests that endogenous decay in wt HT1080 does not involve TTP. As TTP is an inducible protein (Lim et al., 1987; Lai et al., 1990), we propose that cytokine ARE-mediated decay in resting cells is independent of TTP but involves the slowA/C function. Following activation of growth factor and cytokine expression, inducible TTP might ensure return of these mRNAs to basal levels. The notion of a different, constitutive decay activity would be in line with the finding that endogenous TTP is not mutated in slowA or slowC (Stoecklin et al., 2000). This activity is also unlikely to be AUF1, the only other protein known to destabilize class II (and class I) ARE containing mRNAs (DeMaria & Brewer, 1996; Loflin et al., 1999), as p37 AUF1 expression did not restore decay in the mutants (Stoecklin et al., 2000).

IL-2 and IL-6 contain weak class II AREs

Classification of the IL-2, IL-4, and IL-6 AREs by sequence comparison alone is ambiguous. Although members of the cytokine family, these elements lack a large AUUUA motif cluster typical for class II AREs. Having only two (IL-4) or four (IL-2, IL-6) AUUUA pentamers, they are similar to class I AREs that contain one to three dispersed AUUUA pentamers embedded in a rather long, more U-rich sequence. Yet, the β-IL2-ARE and β-IL6-ARE transcripts fulfilled all the criteria of our functional definition: They were stable in both mutants, their degradation was accelerated by TTP (Fig. 5A,C), and stabilization occurred upon p38 MAPK and PI3-kinase activation (Fig. 6D,F). This led us to conclude that ARE-induced degradation of IL-2 and IL-6 mRNA proceeds through a common class II decay pathway. In contrast, the class I c-*myc* and c-*fos* AREs did not trigger decay in the HT1080 wt background (data not shown). Irrespective of whether this is a particularity of this cell line or due to the use of actinomycin D in our decay experiments, it emphasizes the functional differ-

ence between the class I and the IL-2/IL-6 AREs. The mRNA half-lives for the IL-6 and IL-2 AREs (about 3 and 4 h, respectively) were considerably longer compared to the values for the IL-3, GM-CSF, and TNFα AREs (about 1 h). This indicates that the former elements are weaker in recruiting the decay machinery, although it may also mean that their full activity depends on additional factors not expressed in HT1080. The 58-nt-long AU-rich sequence in the 3′ UTR of IL-4, which contains only two AUUUA pentamers, was not sufficient to induce substantial mRNA decay in either of the two cells lines used (Figs. 5B and 6E). If in other cells the ARE of IL-4 is a true destabilizing element, it may utilize a different pathway.

A direct comparison of the ARE sequences in Figure 3 indicated that, although they use the same pathway, the potency of cytokine AREs to recruit the decay machinery is sequence-dependent. In fact, the half-lives correlate very well with the number of AUUUA pentamers. Potent class II elements (IL-3, GM-CSF, and TNFα) contain six to seven AUUUA motifs, most of which are clustered, whereas the weak class II elements (IL-2 and IL-6) harbor four pentamers, and the insufficient IL-4 element contains two AUUUA motifs. In contrast, the number of UUAUUUAU/AU/A nonamers, which has been considered the key motif by other studies (Lagnado et al., 1994; Zubiaga et al., 1995), is not a good predictive value. The IL-6 ARE, for example, does not have a single nonamer, although it induces mRNA decay with a half-life of 3 h approximately. As a rule of thumb, the number of AUUUA motifs gives a fair estimate of the decay potential of a class II ARE provided an AU-rich 5′ flank is present.

The complexity of cytokine mRNA turnover

Although the ARE is certainly a major determinant of mRNA stability, the physiological regulation of cytokine mRNA turnover is more complex and appears to involve additional regulatory *cis*-acting elements. Whereas the AREs of TNFα and IL-2, for example, are sensitive to the mutants defect (Figs. 4C and 5A), the entire 3′ UTRs of these two cytokines still do trigger decay in the slowA/C background (Stoecklin et al., 2000). We thus predict that the IL-2 and TNFα 3′ UTRs contain additional *cis*-elements outside the ARE. Similarly, mRNAs containing the 3′ UTRs of G-CSF, IL-2, and IL-6 were not stabilized by calcium-ionophore to the same extent as transcripts containing an ARE alone (Brown et al., 1996). Furthermore, sequences in the coding region of IL-2 have been implicated in the regulation of mRNA turnover in response to CD28 activation (Ragheb et al., 1999). Although the ARE is sufficient to confer p38- and PI3-kinase mediated stabilization in NIH3T3 cells (Fig. 6) or JNK-mediated stabilization of IL-3 mRNA in mast cells (Ming et al., 1998), stabilization of IL-2 mRNA by JNK in Jurkat cells requires an additional element that has been mapped to the first 22 nt of the 5′ UTR (Chen et al., 1998, 2000). Full description of the different mRNA degradation pathways that in concert regulate cytokine mRNA turnover will require identification of all the important *cis*-elements, the *trans*-acting proteins, and the degrading enzymes. The slowA and slowC mutants should prove useful for the cloning of effector functions driving the common class II decay pathway.

MATERIALS AND METHODS

Plasmid construction

For plasmid puroMXβglobin, the rabbit β-globin gene driven by a retroviral MMLV promoter was excized from plasmid pMXh-β-globin (Ming et al., 1998) as a HindIII-NdeI fragment and ligated blunt-end into the EcoRI site of vector pBABEpuro (Morgenstern & Land, 1990).

To generate the plasmid puroMXβ-IL3-ARE, the ARE of murine IL-3 was amplified by PCR from MXIL3neo (Stoecklin et al., 2000) using primers M1977 (5′-ATGGATCCTTCCATTAAGGC-3′) and M1978 (5′-ATAGATCTTCACAGAAGGC-3′). The amplicon was digested with *Bam*HI and *Bgl*II and ligated into the unique *Bgl*II site of puroMXβglobin, thereby placing the ARE immediately downstream of the β-globin stop codon. Plasmid puroMXβ-IL2-ARE was constructed in similar fashion by amplification of the murine IL-2 ARE from neoMXβglobin-IL2 (Stoecklin et al., 2000) using primer M1966 (5′-ATGGATCCCAACACATAAGGCTCTC-3′) and M1967 (5′-ATAGATCTCTGAAGACTAGTAGTTAC-3′).

Plasmid puroMXβ-GMCSF-ARE was generated by digestion of pTet-BBB+ARE$^{GM\text{-}CSF}$ (kindly provided by A.-B. Shyu) with *Eco*RI and *Bgl*II and insertion of the fragment into the *Eco*RI and *Bgl*II sites of puroMXβglobin. In the same way, plasmids puroMXβ-TNFα-ARE$_{34}$ and puroMXβ-c-fos-ARE were constructed using a *Eco*RI and *Bgl*II fragment of pTet-BBB+ARE$^{TNF\text{-}\alpha}$ and pTet-BBB+ARE$^{c\text{-}fos}$ (kindly provided by A.-B. Shyu).

For plasmid puroMXβ-TNFα-ARE$_{53}$, a longer (53 nt) ARE of murine TNFα was generated using the following two complementary oligonucleotides containing the sequence of interest: M2138 (3′-GATCCTCTATTTATATTTGCACTTATTATTTATTATTTATTTATTATTTATTTATTTGA-5′) and M2139 (3′-GATCTCAAATAAATAAATAATAAATAAATAATAAATAATAAGTGCAAATATAAATAGAG-5′). Twelve picomoles of each oligonucleotide were first phosphorylated at the 5′ terminus by polynucleotide kinase (Promega) in a 40-μL reaction volume. Then, the oligonucleotides were mixed, heated to 95 °C and allowed to cool slowly in a thermal cycler for efficient annealing. After purification (nucleotide removal kit, Qiagen), the annealing product was ligated into the *Bgl*II site of puroMXβglobin. In the same way, plasmids puroMXβ-IL4-ARE and puroMXβ-IL6-ARE were generated using the complementary oligonucleotides M 2126 (5′-GATCCTTTAACTTATGAATTTTTAATGGTTTTATTTTAATATTTATATTTTATAATTCATAAA-3′) M 2127 (5′-GATCTTTATGAATTATAAATATATAAATATTAAAATAAAACCATTAAAAATTCATAAGTTAAAG-3′), and M 2128 (5′-GATCCATTTTAATTATTTTTAATTTATTGATAATTTAAATAAGTAAACTTTAAGTTAATTTATGATTGA

TATTTATTATTTTTAA-3′) M 2129 (5′-GATCTTAAAAATAATAAATATCAATCATAAATTAACTTAAAGTTTACTTATTTAAATTATCAATAAATTAAAAATAATTAAAATG-3′), respectively.

For plasmid Mxhβ-c-*fos*-UTR, the 3′ UTR of c-*fos* (832 nt) was amplified by RT-PCR using primers M1774 (5′-GAGAGCAAGAAGGTGGTCGC-3′) and M1776 (5′-GCAGTCCAGAGAAGGCAAGGC-3′) and blunt-end ligated into the *Sma*I site of pSP73 (Promega). From this plasmid, the *Bam*HI-*Bgl*II fragment was used for replacing the *Bgl*II fragment of Mxh-β-globin-IL3 (Ming et al., 1998) to generate Mxhβ-c-*fos*-UTR.

The T-cell receptor β minigene constructs TCRβ-wt and TCRβ-PTC were derived from constructs A and C (described in Li et al., 1997) by deletion of the 634-bp *Eco*0109I fragment in intron 3.

Cell lines and transfection

The HT1080-derived reporter cell line HT-GFPIL3-wt, referred to as "wt" in this article, as well as its derivative mutants slowA and slowC, have been described previously (Stoecklin et al., 2000). slowC-TTP is a subclone of slowC (referred to as C-TTP-10 in Stoecklin et al., 2000) that ectopically expresses a murine TTP cDNA. HT1080 cells were stably transfected with the β-globin and TCRβ constructs in six-well dishes using 1 μg plasmid and 3 μL of Lipofectamine 2000 (Gibco) according to the manufacturer's protocol. Selection was started 48 h later by addition of puromycin (2 μg/mL) or G418 (1 mg/mL). Cells were maintained in Iscove's modified Dulbecco medium supplemented with 10% fetal calf serum, 50 μM 2-mercaptoethanol, 2 mM glutamine, 100 U of penicillin/mL, and 100 μg of streptomycin/mL. Fresh medium was added to the cells 8–16 h prior to RNA extraction.

NIH3T3 B2A2 cells (Xu et al., 1998), kindly provided by A.-B. Shyu, were transiently transfected with the β-globin reporter constructs together with vector alone, plasmid SRα3-MEK6DD (Ming et al., 1998), or plasmid rCD2-p110 (Reif et al., 1997) generously provided by D.A. Cantrell using the calcium-phosphate method (Shyu et al., 1989). Twelve to 16 h after transfection, the medium was changed to low serum (0.5%) for another 24 h until RNA extraction.

Actinomycin D chase and northern blot analysis

Upon addition of actinomycin D (5 μg/mL), total cytoplasmic RNA was extracted from the cells at various time points and subjected to northern blot analysis as described previously (Stoecklin et al., 2000). A ^{32}P-labeled SP6 probe from the 86 bp *Bgl*II-*Eco*RI fragment of rabbit β-globin served for detection of the β-globin reporter mRNA. Blots were rehybridized with an SP6 probe from a 260-bp fragment of murine β-actin generated by RT-PCR with primers M1171 (5′-ACATCAAAGAGAAGCTGTGC-3′) and M1170 (5′-ACTGTGTTGGCATAGAGGTC-3′) that was cloned into pGEM-T vector (Promega). Northern blots from the TCRβ-wt and TCRβ-PTC transfected cells were hybridized simultaneously with a TCRβ probe from a *Spe*I-*Sal*I fragment of pβ322 (Carter et al., 1996) and a neomycin probe from a *Spe*I-*Sal*I fragment of pMSCVneo (Clontech), both ^{32}P-labeled by random priming (ready-to-go kit, Pharmacia). Efficiency of transient transfections was monitored by hybridization with a random primed probe from a 640-bp fragment of the puromycin N-acetyl transferase gene amplified by PCR from pBABEpuro using primers M2136 (5′-ACAAGCTTCCATGACCGAG) and M2135 (5′-GGGCGGCCGCTCCTTTC-3′).

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REFERENCES

Akashi M, Shaw G, Gross M, Saito M, Koeffler HP. 1991. Role of AUUU sequences in stabilization of granulocyte-macrophage colony-stimulating factor RNA in stimulated cells. *Blood 78*:2005–2012.

Bakheet T, Frevel M, Williams BR, Greer W, Khabar KS. 2001. ARED: Human AU-rich element-containing mRNA database reveals an unexpectedly diverse functional repertoire of encoded proteins. *Nucleic Acids Res 29*:246–254.

Beelman CA, Parker R. 1995. Degradation of mRNA in eukaryotes. *Cell 81*:179–183.

Brown CY, Lagnado CA, Goodall GJ. 1996. A cytokine mRNA-destabilizing element that is structurally and functionally distinct from A+U-rich elements. *Proc Natl Acad Sci USA 93*:13721–13725.

Caput D, Beutler B, Hartog K, Thayer R, Brown-Shimer S, Cerami A. 1986. Identification of a common nucleotide sequence in the 3′-untranslated region of mRNA molecules specifying inflammatory mediators. *Proc Natl Acad Sci USA 83*:1670–1674.

Carballo E, Lai WS, Blackshear PJ. 1998. Feedback inhibition of macrophage tumor necrosis factor-alpha production by tristetraprolin. *Science 281*:1001–1005.

Carballo E, Lai WS, Blackshear PJ. 2000. Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability. *Blood 95*:1891–1899.

Carter MS, Li S, Wilkinson MF. 1996. A splicing-dependent regulatory mechanism that detects translation signals. *EMBO J 15*:5965–5975.

Chen C-YA, Shyu A-B. 1994. Selective degradation of early-response-gene mRNAs: Functional analyses of sequence features of the AU-rich elements. *Mol Cell Biol 14*:8471–8482.

Chen C-YA, Shyu A-B. 1995. AU-rich elements: Characterization and importance in mRNA degradation. *Trends Biochem Sci 20*:465–470.

Chen CY, Del Gatto-Konczak F, Wu Z, Karin M. 1998. Stabilization of interleukin-2 mRNA by the c-Jun NH2-terminal kinase pathway. *Science 280*:1945–1949.

Chen CY, Gherzi R, Andersen JS, Gaietta G, Jurchott K, Royer HD, Mann M, Karin M. 2000. Nucleolin and YB-1 are required for JNK-mediated interleukin-2 mRNA stabilization during T-cell activation. *Genes & Dev 14*:1236–1248.

DeMaria CT, Brewer G. 1996. AUF1 binding affinity to A+U-rich elements correlates with rapid mRNA degradation. *J Biol Chem 271*:12179–12184.

Eick D, Piechaczyk M, Henglein B, Blanchard JM, Traub B, Kofler E, Wiest S, Lenoir GM, Bornkamm GW. 1985. Aberrant c-myc RNAs of Burkitt's lymphoma cells have longer half-lives. *EMBO J 4*:3717–3725.

Fan XC, Steitz JA. 1998. Overexpression of HuR, a nuclear-

cytoplasmic shuttling protein, increases the in vivo stability of ARE-containing mRNAs. *EMBO J 17*:3448–3460.
Hentze MW, Kulozik AE. 1999. A perfect message: RNA surveillance and nonsense-mediated decay. *Cell 96*:307–310.
Hilleren P, Parker R. 1999. mRNA surveillance in eukaryotes: Kinetic proofreading of proper translation termination as assessed by mRNP domain organization? *RNA 5*:711–719.
Hirsch HH, Backenstoss V, Moroni C. 1996. Impaired interleukin-3 mRNA decay in autocrine mast cell tumors after transient calcium ionophore stimulation. *Growth Factors 13*:99–110.
Jacobson A, Peltz SW. 1996. Interrelationships of the pathways of mRNA decay and translation in eukaryotic cells. *Annu Rev Biochem 65*:693–739.
Jones TR, Cole MD. 1987. Rapid cytoplasmic turnover of c-myc mRNA: Requirement of the 3′ untranslated sequences. *Mol Cell Biol 7*:4513–4521.
Lagnado CA, Brown CY, Goodall GJ. 1994. AUUUA is not sufficient to promote poly(A) shortening and degradation of an mRNA: The functional sequence within AU-rich elements may be UUAUU UA(U/A)(U/A). *Mol Cell Biol 14*:7984–7995.
Lai WS, Carballo E, Strum JR, Kennington EA, Phillips RS, Blackshear PJ. 1999. Evidence that tristetraprolin binds to AU-rich elements and promotes the deadenylation and destabilization of tumor necrosis factor alpha mRNA. *Mol Cell Biol 19*:4311–4323.
Lai WS, Stumpo DJ, Blackshear PJ. 1990. Rapid insulin-stimulated accumulation of an mRNA encoding a proline-rich protein. *J Biol Chem 265*:16556–16563.
Laroia G, Cuesta R, Brewer G, Schneider RJ. 1999. Control of mRNA decay by heat shock-ubiquitin-proteasome pathway. *Science 284*:499–502.
Lasa M, Mahtani KR, Finch A, Brewer G, Saklatvala J, Clark AR. 2000. Regulation of cyclooxygenase 2 mRNA stability by the mitogen-activated protein kinase p38 signaling cascade. *Mol Cell Biol 20*:4265–4274.
Lebwohl DE, Muise-Helmericks R, Sepp-Lorenzino L, Serve S, Timaul M, Bol R, Borgen P, Rosen N. 1994. A truncated cyclin D1 gene encodes a stable mRNA in a human breast cancer cell line. *Oncogene 9*:1925–1929.
Li S, Leonard D, Wilkinson MF. 1997. T cell receptor (TCR) mini-gene mRNA expression regulated by nonsense codons: A nuclear-associated translation-like mechanism. *J Exp Med 185*:985–992.
Lim RW, Varnum BC, Herschman HR. 1987. Cloning of tetradecanoyl phorbol ester-induced 'primary response' sequences and their expression in density-arrested Swiss 3T3 cells and a TPA non-proliferative variant. *Oncogene 1*:263–270.
Lindsten T, June CH, Ledbetter JA, Stella G, Thompson GB. 1989. Regulation of lymphokine messenger RNA stability by a surface-mediated T cell activation pathway. *Science 244*:339–343.
Loflin P, Chen CY, Shyu AB. 1999. Unraveling a cytoplasmic role for hnRNP D in the in vivo mRNA destabilization directed by the AU-rich element. *Genes & Dev 13*:1884–1897.
Ming XF, Kaiser M, Moroni C. 1998. c-jun N-terminal kinase is involved in AUUUA-mediated interleukin-3 mRNA turnover in mast cells. *EMBO J 17*:6039–6048.
Ming XF, Stoecklin G, Lu M, Looser R, Moroni C. 2001. Parallel and independent regulation of interleukin-3 mRNA turnover by phosphatidylinositol 3-kinase and p38 mitogen-activated protein kinase. *Mol Cell Biol 21*:5778–5789.
Morgenstern JP, Land H. 1990. Advanced mammalian gene transfer: High titre retroviral vectors with multiple drug selection markers and a complementary helper-free packaging cell line. *Nucleic Acids Res 18*:3587–3596.
Nair AP, Hahn S, Banholzer R, Hirsch HH, Moroni C. 1994. Cyclosporin A inhibits growth of autocrine tumour cell lines by destabilizing interleukin-3 mRNA. *Nature 369*:239–242.
Nanbu R, Menoud PA, Nagamine Y. 1994. Multiple instability-regulating sites in the 3′ untranslated region of the urokinase-type plasminogen activator mRNA. *Mol Cell Biol 14*:4920–4928.
Peng SS, Chen CY, Xu N, Shyu AB. 1998. RNA stabilization by the AU-rich element binding protein, HuR, an ELAV protein. *EMBO J 17*:3461–3470.
Ragheb JA, Deen M, Schwartz RH. 1999. CD28-Mediated regulation of mRNA stability requires sequences within the coding region of the IL-2 mRNA. *J Immunol 163*:120–129.
Reif K, Lucas S, Cantrell D. 1997. A negative role for phosphoinositide 3-kinase in T-cell antigen receptor function. *Curr Biol 7*:285–293.
Rimokh R, Berger F, Bastard C, Klein B, French M, Archimbaud E, Rouault JP, Santa Lucia B, Duret L, Vuillaume M, Coiffier B, Bryon PA, Magaud JP. 1994. Rearrangement of CCND1 (BCL1/PRAD1) 3′ untranslated region in mantle-cell lymphomas and t(11q13)-associated leukemias. *Blood 83*:3689–3696.
Schuler GD, Cole MD. 1988. GM-CSF and oncogene mRNA stabilities are independently regulated in trans in a mouse monocytic tumor. *Cell 55*:1115–1122.
Shaw G, Kamen R. 1986. A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell 46*:659–667.
Shyu A-B, Greenberg ME, Belasco JG. 1989. The c-fos transcript is targeted for rapid decay by two distinct mRNA degradation pathways. *Genes & Dev 3*:60–72.
Stoecklin G, Hahn S, Moroni C. 1994. Functional hierarchy of AUUUA motifs in mediating rapid interleukin-3 mRNA decay. *J Biol Chem 269*:28591–28597.
Stoecklin G, Ming XF, Looser R, Moroni C. 2000. Somatic mRNA turnover mutants implicate tristetraprolin in the interleukin-3 mRNA degradation pathway. *Mol Cell Biol 20*:3753–3763.
Taylor GA, Carballo E, Lee DM, Lai WS, Thompson MJ, Patel DD, Schenkman DI, Gilkeson GS, Broxmeyer HE, Haynes BF, Blackshear PJ. 1996. A pathogenetic role for TNF alpha in the syndrome of cachexia, arthritis, and autoimmunity resulting from tristetraprolin (TTP) deficiency. *Immunity 4*:445–454.
Wilson T, Treismann R. 1988. Removal of poly(A) and consequent degradation of c-fos mRNA facilitated by 3′ AU-rich sequencens. *Nature 336*:396–399.
Wilusz CJ, Wormington M, Peltz SW. 2001. The cap-to-tail guide to mRNA turnover. *Nat Rev Mol Cell Biol 2*:237–246.
Wingett D, Reeves R, Magnuson NS. 1991. Stability changes in pim-1 proto-oncogene mRNA after mitogen stimulation of normal lymphocytes. *J Immunol 147*:3653–3659.
Winzen R, Kracht M, Ritter B, Wilhelm A, Chen CY, Shyu AB, Muller M, Gaestel M, Resch K, Holtmann H. 1999. The p38 MAP kinase pathway signals for cytokine-induced mRNA stabilization via MAP kinase-activated protein kinase 2 and an AU-rich region-targeted mechanism. *EMBO J 18*:4969–4980.
Wodnar-Filipowicz A, Moroni C. 1990. Regulation of interleukin 3 mRNA expression in mast cells occurs at the posttranscriptional level and is mediated by calcium ions. *Proc Natl Acad Sci USA 87*:777–781.
Xu N, Chen CY, Shyu AB. 1997. Modulation of the fate of cytoplasmic mRNA by AU-rich elements: Key sequence features controlling mRNA deadenylation and decay. *Mol Cell Biol 17*:4611–4621.
Xu N, Loflin P, Chen CY, Shyu AB. 1998. A broader role for AU-rich element-mediated mRNA turnover revealed by a new transcriptional pulse strategy. *Nucleic Acids Res 26*:558–565.
Zubiaga AM, Belasco JG, Greenberg ME. 1995. The nonamer UUAUUUAUU is the key AU-rich sequence motif that mediates mRNA degradation. *Mol Cell Biol 15*:2219–2230.

Cellular mutants define a common mRNA degradation pathway targeting cytokine AU-rich elements.

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