Heterogeneity in Control of mRNA Stability by AU-rich Elements*

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AU-rich elements (AREs), located in the 3′-untranslated region of unstable cytokine and chemokine mRNAs, promote rapid decay of otherwise stable mRNAs and may mediate selective mRNA stabilization in response to stimulation with interleukin-1 (IL-1). AREs vary considerably, however, in both size and sequence context. To assess the heterogeneity involved in control of mRNA stability by ARE motifs, human mRNA sequences from IL-1α-stimulated HEK293 cells and T98G cells were screened for either instability or stability using both cDNA (950 ARE containing sequences) and Affymetrix oligonucleotide (U95Av2 GeneChip) array analysis. Although ARE-containing mRNAs exhibited a broad range of stability, IL-1α promoted stability in a subset of mRNAs that were unstable when transcriptionally induced by tumor necrosis factor α. Stabilization of granulocyte/macrophage-colony stimulating factor and IL-8 mRNAs by IL-1α was achieved only after 24 h of stimulation, required ongoing protein synthesis, and depended on the activation of p38 MAPK. In contrast, stabilization of Gro3 mRNA in response to IL-1α was achieved immediately and was insensitive to inhibitors of protein synthesis and p38 MAPK activation. In concert, these findings demonstrate that ARE sequences are functionally heterogeneous; only a subset of unstable mRNAs is sensitive to stabilization by IL-1α. Moreover, IL-1α promotes stabilization of unstable mRNAs through distinct mechanistic pathways that distinguish between specific mRNA sequences.

In the course of an inflammatory response to injury or infection, both resident and infiltrating cells are subject to the action of a diverse collection of stimuli that produce dramatic changes in the pattern of gene expression (1–3). Although much attention has been directed at the role of transcription in the activation of inflammatory gene expression, post-transcriptional events, particularly the stability of specific mRNAs, have also been shown to be important in control of gene expression (4, 5). Indeed many mRNAs encoding inflammatory gene products are inherently unstable, although decay may be controlled selectively in response to extracellular stimulus. The importance of these mechanisms is illustrated in studies of the post-transcriptional control of TNFα mRNA wherein both mRNA stability and translation have been shown to be critical determinants of the magnitude of the inflammatory response (6, 7).

Adenosine uridine-rich elements (AREs) found in the 3′-untranslated regions (3′-UTRs) of many inflammatory cytokines and growth factors are well known to promote rapid mRNA degradation (8, 9). Furthermore, multiple studies have shown that rates of mRNA decay can be modified in response to extracellular stimulation (10–12). For example, IL-1α has been shown to enhance the stability of a variety of cytokine and chemokine mRNAs that otherwise exhibit short half-lives, and this depends, at least in part, upon the presence of ARE motifs in the 3′-UTRs (10–12).

A recent search of human sequence databases has identified over 900 human mRNAs that contain one or more ARE motifs (13), and it seems unlikely that all such mRNAs will exhibit comparable sensitivity to regulatory mechanisms governing their stability. Indeed, studies using mRNAs with defined ARE sequences have demonstrated sequence-specific functional heterogeneity that reflects the differential participation of distinct ARE binding proteins (14–16). To further evaluate the functional heterogeneity of AREs, we determined the sensitivity of a set of ARE-containing mRNAs to IL-1α-mediated stabilization. We also assessed the mechanistic diversity of IL-1α-mediated mRNA stabilization with respect to the pathways through which stimulus and mRNA decay mechanism are coupled.

As a first step, we examined the expression of multiple ARE-containing mRNAs using cDNA and oligonucleotide array analysis. The results demonstrate that, although many IL-1α-inducible, ARE-containing mRNAs are unstable, a subset of the mRNAs are stabilized by the stimulus. This effect is specific for IL-1α, because TNFα induces transcription of many of the same genes but does not lead to stabilization of their mRNAs. Finally, within the set of IL-1α-inducible and stabilized mRNAs, there appear to be multiple intracellular pathways through which the stabilization end point can be achieved.

EXPERIMENTAL PROCEDURES

Reagents—Dulbecco’s modified Eagle’s medium, Dulbecco’s phosphate-buffered saline, antibiotics, glutamine, agarose, guanidine isothiocyanate, and cesium chloride were obtained from PerkinElmer Life Sciences (Rockville, MD). Anhydrous ethanol, Sarkosyl, and formamide were purchased from Fisher Scientific (Pittsburgh, PA). S2000 (1 mg/ml) was purchased from Calbiochem (San Diego, CA). All other reagents were obtained from commercial sources.

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**The abbreviations used are: TNFα, tumor necrosis factor α; ARE, AU-rich element; ActD, actinomycin D; CHX, cycloheximide; UTR, untranslated region; IL-1, interleukin-1; GM-CSF, granulocyte/macrophage-colony stimulating factor; ERR1/2, extracellular signal-regulated kinase 1 and 2; JNK, c-Jun N-terminal kinase.
**TABLE 1**

**Stability of IL-1-inducible mRNAs**

| mRNA | Accession number | Cell type | IL-1[^a] | IL-1/ActD[^b] | ARE[^c] |
|------|------------------|-----------|----------|---------------|---------|
| **Stable mRNAs** | | | | | |
| IL-8 | M28130 | T98G (293) | 604 (31) | 629 (28) | Yes |
| GRO3 | M36821 | T98G (293) | 198 (26) | 191 (24) | Yes |
| Endothelin-1 | J05008 | T98G | 197 | 222 | Yes |
| GRP | X54489 | T98G | 88 | 101 | Yes |
| Exodus | U61497 | T98G (293) | 86 (10) | 154 (14) | Yes |
| ICAM-1 | M24283 | T98G | 58 | 50 | Yes |
| IL-6 | X04430 | T98G | 55 | 53 | Yes |
| VCAM-1 | M72255 | T98G | 42 | 34 | Yes |
| COX-2 | U04636 | T98G | 38 | 41 | Yes |

[^a]: mRNA stability measured in response to IL-1.
[^b]: mRNA stability measured in response to IL-1 and ActD.
[^c]: ARE indicates the presence or absence of a ARE element in the 3' UTR.
were obtained from International Biotechnologies, Inc. (New Haven, CT). Magna nylon transfer membrane was obtained from Micron Separations Inc. (Westboro, MA). Fetal bovine serum was purchased from BioWhittaker (Walkersville, MD). Actinomycin D (ActD) and cycloheximide (CHX) were purchased from Sigma-Aldrich (St. Louis, MO). Recombinant human IL-1β/H9251 and TNFα/H9251 were purchased from R&D Systems (Minneapolis, MN). PerkinElmer Life Sciences (Boston, MA) was the source of [α-32P]dCTP. SB203580 was purchased from Calbiochem (San Diego, CA).

**Cell Culture**—HEK293 cells and T98G glioblastoma cells were obtained from Dr. Xiaoxia Li and Dr. George Stark, respectively (Lerner Research Institute). Both cell lines were maintained in Dulbecco’s mod-

**Figure 1.** **IL-1α but not TNFα stabilizes selective mRNAs.** T98G (A) or HEK293 (B) cells were untreated (NT) or stimulated with either IL-1α (10 ng/ml) or TNFα (10 ng/ml) for 2 h. Some cultures were then treated with ActD (5 μg/ml) alone or with IL-1α (10 ng/ml) for an additional 4 h. Total RNA was prepared for each treatment condition and used to determine the levels of specific mRNAs by Northern hybridization. Similar results were obtained in two separate experiments.

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**Table I—continued**

| mRNA Accession number | Cell type | IL-1α | IL-1α/ActD | ARE |
|-----------------------|-----------|--------|------------|-----|
| SCYB10 X02530         | T98G      | 30     | 33         | Yes |
| NK4 homologous AA631972 | T98G      | 27     | 31         | No  |
| PTX3 M31166           | T98G      | 26     | 26         | Yes |
| My1 X63131            | T98G      | 15     | 18         | No  |
| MN SOD X07834         | T98G      | 14     | 19         | Yes |
| GTP cyclohydrolase U19523 | T98G   | 12     | 13         | Yes |
| B94 M92357            | T98G      | 12     | 13         | No  |
| MCP-2 Y16645          | T98G      | 12     | 12         | Yes |
| Calpain X85030        | T98G      | 11     | 10         | Yes |
| TSG-6 M31165          | T98G      | 11     | 13         | Yes |
| IL-1β M15330          | T98G      | 10     | 10         | Yes |
| MCP-1 M28225          | T98G      | 10     | 12         | No  |
| Fibulin-2 X82494      | T98G      | 9      | 12         | No  |
| HOX11 S38742          | T98G      | 8      | 7          | Yes |
| HSP27 (ISG12) X67325  | T98G      | 7      | 9          | No  |
| ELAM-1 M24736         | 293       | 7      | 8          | Yes |
| PAI-1 NM000602        | T98G      | 6      | 7          | Yes |
| GPP130 U55043         | T98G      | 6      | 5          | Yes |
| Act-2 J04130          | T98G      | 6      | 5          | Yes |
| Tenacin-receptor AL049689 | 293   | 6      | 7          | Yes |
| Gln cyclotransferase U19523 | T98G | 5      | 4          | No  |
| Chromogranin A U06698  | T98G      | 5      | 4          | Yes |

a Increase (-fold) relative to untreated sample.

b NC indicates that no complete 3'-UTR sequence could be found in GenBank™.
ified Eagle’s medium containing 10% fetal bovine serum, penicillin, and streptomycin in humidified 5% CO₂.

Preparation of RNA and Northern Hybridization—Total cellular RNA was extracted by the guanidine thiocyanate-cesium chloride method (17). Northern hybridization analysis was done as previously described (18).

Oligonucleotide Array Experiments—RNA labeling and hybridization were carried out according to the protocol supplied by Affymetrix. In brief, RNA was isolated by the guanidine thiocyanate-cesium chloride method (17), and 10 μg of total RNA per sample was converted to double-stranded cDNA using an oligo(dT) primer containing a T7 polymerase site. The resulting cDNA was then used as template for in vitro transcription with biotinylated CTP and UTP to generate cRNA. 15 μg of fragmented cRNA was hybridized to Affymetrix U95Av2 GeneChips. The arrays were washed and stained according to supplied protocols and scanned using an Affymetrix GeneChip scanner. Raw image data was processed and normalized using Microarray Analysis suite 4.0.

cDNA Array—cDNA array and hybridization were carried out as previously described (19). The ARE cDNA array used in this study was composed of ~950 ARE-containing genes defined in the ARED database (13), 18 genes potentially involved in AU-directed mRNA decay, and 50 housekeeping genes. Briefly, for each array hybridization, RNAs from treated and untreated cells were labeled with Cy3 and Cy5, respectively. The Cy3- and Cy5-labeled cDNAs were pooled and hybridized to the array slide under a coverglass in a CMT hybridization chamber for 16 h. Subsequently, slides were washed and scanned on a GenePix 4000A scanner (Axon). Raw fluorescence data were acquired with the GenePix software and imported into the GeneSpring software version 4.2 for further analysis.

RESULTS

Variable Stability of IL-1-induced mRNAs—To evaluate the functional heterogeneity of ARE-containing mRNAs with respect to instability and stimulus-induced stabilization, the decay characteristics of multiple mRNAs were determined in IL-1α-stimulated cells using both oligonucleotide and cDNA array analyses. HEK293 cells and T98G glioblastoma cells were used to prepare three RNA populations: 1) untreated, 2) stimulated for 2 h with IL-1α, or 3) stimulated for 2 h with IL-1α followed by addition of ActD for an additional 4 h. The three total RNA samples from each cell line were used for preparation of cRNA and subjected to array analysis using either the Affymetrix U95Av2 GeneChips or a custom cDNA array prepared using the sequences defined in the ARED database of ARE-containing mRNAs (13).

The analysis restricted consideration of IL-1α-inducible mRNAs to those showing >5-fold induction after 2 h of stimulation. The IL-1α inducibility of individual mRNAs varied between cell lines (only a subset were identified in both 293 and
T98G cells). These were segregated as either stable (less than 90% decay over 4 h), moderately stable (10–60% decay over 4 h), or unstable (greater than 60% decay over 4 h). The mRNAs identified using these selection criteria are listed in Table I; each classification contains approximately equal numbers of mRNAs. Interestingly, within all three groups the majority of mRNAs contain an ARE (defined as having at least one AUUUA pentamer) within the 3′-UTR. Although some mRNAs in each group did not possess even one AUUUA pentamer, stretches of poly U- or AU-rich regions may also destabilize mRNA (these have been designated class III AREs) (16). Nonetheless, it is apparent that ARE-containing mRNAs exhibit considerable heterogeneity in terms of their rates of decay in the presence of IL-1α ranging from very stable to very unstable. 47 of 60 mRNAs classified as stable or moderately stable contained AREs, and these could be unstable mRNAs that are stabilized in response to IL-1 stimulation. Some of these mRNAs may, however, be inherently stable, and, because decay was not assessed in the absence of IL-1α, we cannot conclude that IL-1α caused a change in their stability.

To confirm the behavior of specific mRNAs seen in array analysis, cultures of HEK293 and T98G cells were stimulated with IL-1α for 2 h, and some cultures were further treated with ActD for an additional 4 h. Total RNA was prepared, and the levels of selected mRNAs were determined by Northern hybridization analysis. In each cell line, we examined two unstable mRNAs and two stable (or moderately stable) mRNAs. The results confirmed the data presented in Table I; the selected mRNAs were strongly induced by IL-1α, and they either decayed rapidly (TNF A20, c-Jun, mannose binding protein) or were stable in the presence of IL-1α (GM-CSF, Gro3, Gro1, IL-8) (Fig. 1, lanes 1–3). To identify unstable mRNAs in which stability was enhanced by IL-1α treatment, we took advantage of a prior observation that, although TNF α and IL-1α can both induce transcription of the mouse KC (CXCL1) chemokine mRNA, only IL-1α treatment promotes its stabilization (20, 21). Each of the mRNAs induced by IL-1α could also be induced in response to TNFα treatment but in this instance, they all exhibited rapid decay (Fig. 1, lanes 4 and 5). It is noteworthy that those mRNAs that are stabilized by IL-1α exhibit markedly greater accumulation in response to IL-1α as compared
with TNFα, whereas those that are unstable in both conditions show comparable expression in response to either stimulus. In cultures treated initially with TNFα to induce detectable levels of each mRNA, the addition of IL-1α along with ActD also resulted in the stabilization of Gro3 and GM-CSF in T98G cells and IL-8 and Gro3 in 293 cells, whereas the other mRNAs, although expressed, were not stabilized (Fig. 1, lane 6). These findings clearly establish that these specific mRNAs are inherently unstable but can be stabilized in response to treatment with IL-1α.

Diversity in Mechanisms for mRNA Stabilization—The preceding findings establish that ARE-containing mRNAs are functionally heterogeneous with respect to their sensitivity to IL-1α-mediated stabilization. To assess whether there exists further heterogeneity within the set of mRNAs that are subject to such stabilization, we compared the stabilization of IL-8, GM-CSF, and Gro3 mRNAs in IL-1α-stimulated T98G cells with respect to their individual dependence on time, protein synthesis, and p38 MAPK activation.

As a first approach, we determined whether enhanced stability of each mRNA is acquired immediately or, rather, only after some stimulation period. T98G cells were stimulated for 1, 2, or 4 h with IL-1α followed by the addition of ActD for a further 2-h period to assess decay (Fig. 2). At 1 h after stimulation both IL-8 and GM-CSF mRNAs decayed rapidly. After 2 h of stimulation, however, both these mRNAs exhibited enhanced stability. In contrast, Gro3 mRNA, although only modestly induced at 1 h, was already stable and exhibited very limited decay at any of the time points.

The time requirement for stabilization of GM-CSF and IL-8 suggested the possibility that synthesis of a new protein might be required as part of the stabilization process induced by IL-1α. To test this, T98G cells were stimulated with IL-1α in the presence or absence of the protein synthesis inhibitor cycloheximide (CHX) for 2 h and washed thoroughly to remove the reversible inhibitor prior to the addition of fresh medium containing ActD. Cultures were harvested at various times afterward, and levels of specific mRNA were determined (Fig. 3). The stabilization of both GM-CSF and IL-8 mRNAs was reduced appreciably when the stimulation was carried out in the presence of CHX. In contrast the stability of Gro3 was largely unaltered in CHX pre-treated cells.

IL-1α can stabilize TNFα-induced mRNAs immediately in the absence of transcription, whereas stabilization of IL-1α-induced GM-CSF and IL-8 mRNAs requires protein synthesis. To resolve this apparent conflict we reasoned that TNFα might provide a partial signal, corresponding to the protein synthesis requirement, which is completed upon the later addition of IL-1α. To evaluate this possibility, the effect of CHX on the ability of IL-1α to stabilize TNFα-induced mRNA was determined. T98G cells were first stimulated with TNFα for 2 h in the presence or absence of CHX. After thorough washing to remove the inhibitor, the cells were treated with IL-1α and ActD, and mRNA levels were determined following further incubation. TNFα-induced Gro3 and GM-CSF mRNAs were both unstable in the absence of IL-1α and were stabilized when IL-1α was added during the decay incubation (Fig. 4). Inclusion of CHX during the 2-h stimulation with TNFα enhanced the accumulation of both mRNAs equivalently but did not alter decay rates as compared with cells stimulated without CHX. In cells treated with CHX and TNF, however, IL-1α did not stabilize GM-CSF mRNA but the ability to stabilize Gro3 mRNA was not affected. These results demonstrate that the stabilization of select mRNAs in response to IL-1α requires protein synthesis and suggest that there are at least two pathways that control ARE-dependent mRNA decay.

IL-1α-induced stabilization of some mRNAs is believed to depend upon activation of the p38 MAPK (10, 22, 23). This has been demonstrated in multiple settings through the use of the specific p38 protein kinase inhibitor SB203580 and by overexpression of constitutively active forms of both MKK6 and MK2 (upstream and downstream kinases in the p38 kinase cascade, respectively). To determine if IL-1α-mediated mRNA stabilization is p38-dependent, we examined the decay of selected mRNAs in IL-1α-treated cells with or without the addition of the kinase inhibitor SB203580. Cultures of T98G cells were stimulated with IL-1α for 2 h prior to the addition of ActD with or without SB203580. Although the stability of GM-CSF was reduced by inhibition of p38 kinase activity, Gro3 mRNA remained relatively stable (Fig. 5A). Gro3 and GM-CSF mRNAs were both unstable when induced by TNFα treatment, and both were stabilized if IL-1α was added at the same time as ActD (Fig. 5B). The ability of IL-1α to stabilize TNFα-induced GM-CSF was blocked by SB203580, whereas the stimulus-induced stabilization of Gro3 mRNA was not altered. This
recently been reported (24) that the time during which p38 kinase modulates mRNA stability has a significant impact on the stabilization of sensitive mRNAs. A similar observation regarding the activity in the post 2-h activation that is required for stabilization is potentially dependent on p38 MAPK activity in the presence of IL-1α alone or with the p38 MAPK inhibitor SB203580 (2 μM) for an additional 2 h. Total RNA was prepared from each culture and used to determine levels of Gro3 and GM-CSF mRNAs by Northern hybridization. Similar results were obtained in three separate experiments. B, cultures of T98G cells were untreated (NT) or stimulated with TNFα (10 ng/ml) for 2 h. Some cultures were subsequently treated with ActD alone or in the presence of IL-1α and/or the p38 MAPK inhibitor SB203580 (2 μM). Total RNA was prepared and used to measure Gro3 and GM-CSF mRNAs by Northern hybridization. Similar results were obtained in three separate experiments.

The importance of AREs in determining the rate of decay of select mRNAs is well established, and the ability of extracellular stimuli to modulate ARE-dependent mRNA turnover is now recognized (8–12, 16). The large number of mRNAs containing such sequences within their 3'-UTRs suggests that there is likely to be substantial heterogeneity in their function (13). The current study was therefore undertaken to assess the scope of this diversity with particular emphasis on sensitivity to IL-1α-mediated stabilization. This has been accomplished by examining the behavior of ARE-containing mRNAs in IL-1α-stimulated cells using a combination of cDNA and oligonucleotide arrays and by assessing the intracellular events associated with the stabilization of a select set of mRNAs. The results demonstrate that there is a broad range of decay rates for ARE-containing mRNAs induced in response to IL-1α. Second, only a subset of unstable ARE-containing mRNAs acquires enhanced stability via the action of IL-1α. Finally, IL-1α treatment appears to utilize at least two separate pathways to achieve stabilization of individual mRNAs. The one or more mechanisms governing acquired stabilization of GM-CSF and IL-8 mRNAs are dependent on time, continuing protein synthesis, and the activation of p38 MAPK. In contrast, the stabilization of Gro3 mRNA occurs immediately and is insensitive to inhibitors of protein synthesis and p38 kinase activity. Because these mechanisms operate differentially on the two different mRNA populations, they are apparently dependent upon functionally distinct regulatory sequences within the mRNAs themselves.

Because the range in decay rates for different ARE-containing mRNAs is quite large, the identification of mRNAs whose stability is subject to modulation in response to IL-1α requires the ability to examine the rate of message decay in the absence of IL-1α. Many of the IL-1α-induced mRNAs are also induced transcriptionally in response to TNFα but are not stable in this circumstance. The ability of IL-1α to stabilize such mRNAs in the presence of the transcriptional inhibitor ActD demonstrates directly the independent stabilization response. Some TNFα-induced mRNAs, however, remain unstable in the pres-
experiments.

Northern hybridization. Similar results were obtained in three separate RNA was prepared and used to determine specific mRNA levels by sensitivity.

for ARE-containing sequences with respect to stimulus requirement for ongoing protein synthesis, because inhibition prevents their stabilization. Although IL-1 of protein synthesis with CHX during this 2-h time period

GM-CSF and IL-8 mRNAs do not exhibit enhanced stability in though Gro3 mRNA appears to be stabilized immediately, both mRNAs can be distinguished, based upon their different temporal requirements for acquisition of enhanced stability. Although Gro3 mRNA appears to be stabilized immediately, both GM-CSF and IL-8 mRNAs do not exhibit enhanced stability in IL-1α-treated T98G cells until nearly 2 h after the initial exposure to IL-1α. This time dependence appears to reflect a requirement for ongoing protein synthesis, because inhibition of protein synthesis with CHX during this 2-h time period prevents their stabilization. Although IL-1α can stimulate mRNA stabilization immediately in the presence of ActD, this only occurs in cells that have been pre-stimulated with TNFα and the ability of TNFα to promote this “priming activity” also exhibits sensitivity to CHX. These findings suggest that IL-1α or TNFα can induce the de novo expression of one or more new gene products that are requisite to the mRNA stabilization mechanism. Alternatively, they may reflect a requirement for the continuous production of one or more short-lived proteins from constitutively expressed mRNAs.

The differential ability of TNFα and IL-1α to modulate stability of the same mRNAs is somewhat surprising in light of the broadly overlapping signaling and biological response profiles exhibited by these two pro-inflammatory stimuli (25). Both agents have in common the activation of multiple protein kinase cascades, including ERK1/2, JNK, and p38. Because p38 MAPK activity has been shown to be necessary for IL-1α-induced mRNA stabilization (10, 22, 23), the inability of TNFα to promote mRNA stability suggests that IL-1α- and TNFα-mediated p38 activation events are not equivalent or that p38 activation is necessary but not sufficient for stabilization.

The requirement for p38 MAPK activation in the IL-1α-induced stabilization of GM-CSF and IL-8 mRNAs, as reported by others (22, 26), is confirmed in T98G cells in the present study through the use of the p38 inhibitor SB203580. Interestingly, IL-1α-mediated stabilization of Gro3 mRNA was relatively insensitive to the p38 inhibitor providing further evidence that there is more than one pathway through which mRNA stability can be affected by this stimulus. Because sensitivity to the p38 inhibitor varies with different mRNAs, the underlying mechanisms apparently depend upon the ability of the decay mechanism(s) to discriminate between the mRNA sequences.

The activation of p38 MAPK that is necessary for stabilization of GM-CSF and IL-8 mRNAs apparently occurs only several hours after the addition of IL-1α. This is indicated by the finding that p38 inhibitors do not alter the accumulation of specific mRNAs during the first 2 h of stimulation but do antagonize the stabilization response after the 2-h time point. Thus the early burst of p38 activation that is well characterized as part of the response to many pro-inflammatory stimuli may not link to downstream effector mechanisms involved in mRNA decay. This is consistent with a recent report suggesting that a quantitatively modest but prolonged activation of p38 in IL-1-stimulated cells is requisite to stabilization of COX-2 mRNA (24). Moreover, this raises the possibility that the difference between TNFα and IL-1α treatments with respect to the stabilization of mRNA resides in differential ability to promote a later activation profile.

Collectively, our findings lead to a hypothesis that there are at least two sequential steps in the IL-1α-activated process leading to stabilization of both GM-CSF and IL-8 mRNAs. The first step appears to be the production, either de novo or from existing mRNAs, of one or more proteins required for subsequent events in the stabilization response to IL-1α. The second step might be a delayed and prolonged activation of p38 MAPK. Indeed, the p38 kinase inhibitor can block IL-1α-induced stabilization of IL-8 mRNA at times ranging from 2 up through 8 h
after initial stimulation of the cells (data not shown). It is appealing to speculate that these two steps are functionally related; the early, protein synthetic event might prepare the cell for the secondary activation of p38 kinase. This could involve an effect on the activation of the kinase itself or the related; the early, protein synthetic event might prepare the ways. Because the function of stimulus-induced alterations in mRNA stability can produce very dramatic changes in the accumulation of target mRNAs and hence their encoded protein products, this diversity of mechanism will be important to dissect in detail and may ultimately lead to important opportunities for precisely tailored interventional manipulation of inflammatory gene expression in human disease.

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