Regulation of Cyclooxygenase 2 mRNA Stability by the Mitogen-Activated Protein Kinase p38 Signaling Cascade

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A tetracycline-regulated reporter system was used to investigate the regulation of cyclooxygenase 2 (Cox-2) mRNA stability by the mitogen-activated protein kinase (MAPK) p38 signaling cascade. The stable \( \beta \)-globin mRNA was rendered unstable by insertion of the 2,500-nucleotide Cox-2 3' untranslated region (3' UTR). The chimeric transcript was stabilized by a constitutively active form of MAPK kinase 6, an activator of p38. This stabilization was blocked by SB203580, an inhibitor of p38, and by two different dominant negative forms of MAPK-activated protein kinase 2 (MAPAPK-2), a kinase lying downstream of p38. Constitutively active MAPAPK-2 was also able to stabilize chimeric \( \beta \)-globin-Cox-2 transcripts. The MAPAPK-2 substrate hsp27 may be involved in stabilization of \( \beta \)-globin-Cox-2 transcripts were partially stabilized by phospho-mimetic mutant forms of hsp27. A short (123-nucleotide) fragment of the Cox-2 3' UTR was necessary and sufficient for the regulation of mRNA stability by the p38 cascade and interacted with a HeLa protein immunologically related to AU-rich element/poly(U) binding factor 1.

Eicosanoids play a critical role in several physiological and pathophysiological processes, including blood clotting, wound healing, kidney function, acute inflammation, and cardiovascular disease (13, 19). The rate-limiting step in eicosanoid synthesis is catalyzed by cyclooxygenase (Cox) enzymes, which are encoded by two distinct genes. The Cox-1 gene is principally homeostatic in function and possesses a typical, GC-rich housekeeping promoter (55). In contrast the Cox-2 gene resembles an early response gene. It is strongly induced by mitogenic and proinflammatory stimuli, superinduced by inhibitors of protein synthesis, and acutely regulated at both mitogenic and proinflammatory stimuli, superinduced by inhibitors of protein synthesis, and acutely regulated at both transcriptional and posttranscriptional levels (17, 38–44). The most abundant (4.6-kb) transcript has a 3' UTR length due to alternative polyadenylation site usage have been described, the two major transcripts being 4.6 and 2.8 kb long (38, 47). The most abundant (4.6-kb) transcript has a 3' UTR of 2,515 nucleotides (nt), containing 22 copies of the pentamer sequence AUUUUU (see Fig. 1). This sequence is found in the 3' UTR of numerous unstable cytokine- and protooncogene-encoding mRNAs and is a prominent feature of AU-rich elements (AREs) which regulate mRNA stability (6–8, 50). Two conserved regions (CR) have been noted within the Cox-2 3' UTR (38). CR1 lies immediately 3' to the translation termination codon and contains six AUUUUA motifs, of which three are overlapping. CR2 lies 1,700 nt 3' to the translation termination codon and contains three dispersed AUUUUA motifs (Fig. 1).

Several proteins have been shown to interact specifically with AU-rich RNA stability determinants, and have been implicated in positive or negative regulation of mRNA stability. For example ARE/poly(U) binding factor 1 (AUF1), a member of the hnRNP D family of RNA binding proteins, is present in a cytosolic fraction which accelerates e-my mRNA decay in vitro (3, 60). Increased AUF1 expression is associated with decreased mRNA stability in vivo (5, 40, 53), and AUF1 overexpression can antagonize mRNA stabilization in vivo (31). Furthermore the immunodepletion of AUF1 from cytoplasmic extracts increases the stability of an ARE-containing transcript in an in vitro degradation assay (4).

The tetracycline-responsive reporter system (20, 59) permits regulatory sequences and pathways to be mapped by performing RNA stability experiments in the absence of toxic transcriptional inhibitors. Reporter mRNAs are transcribed from a tetracycline-responsive promoter in a cell line expressing a chimeric, tetracycline-responsive transcription factor, ITA (20). In the presence of tetracycline, binding of tTA to the
promoter is blocked and transcription is inhibited. Using this system, referred to herein as the TET-off system, we demonstrate that the effects of p38 are mediated by its downstream kinase MAPKAPK-2 and may involve the phosphorylation of the MAPKAPK-2 substrate hsp27. A conserved, 123-nucleotide ARE lying immediately 3' to the translation termination codon (CR1) is necessary and sufficient for stabilization by the p38 pathway and interacts with a HeLa cell protein which is immunologically related to AUF1.

MATERIALS AND METHODS

Plasmids. The MKK6e expression vector was a gift of J. Han. MAPKAPK-2 expression vectors were donated by C. Marshall. Human Cox-2 3' UTR fragments were amplified by reverse transcription-PCR from IL-1-stimulated human gingival fibroblast RNA, using Moloney murine leukemia virus reverse transcriptase and Vent polymerase (New England BioLabs). Human tumor necrosis factor alpha (TNF-α) and c-myc 3' UTR fragments were amplified from genomic DNA using TaqPlus polymerase (Stratagene). A mouse TNF-α 3' UTR fragment was amplified from a mouse TNF-α genomic clone (gift of A. Shakhov), using Vent polymerase. PCR products were cloned into pCR-Blunt (Invitrogen) and then excised with BglII or BamHI and cloned into the BglII site of pTetBBB (gift of Ann-Bin Shyu).

Riboprobe vectors were constructed as follows. A 552-bp HincII-XbaI luciferase fragment was cloned from pGLo3 (Promega) into blue script KS that had been digested with EcoRV and XbaI. A 269-bp SphI-BglII fragment was cloned from a human Cox-2 cDNA construct (gift of D. Fitzgerald) into blue script KS that had been digested with BamHI and Clea. A 269-bp SphI-BglII fragment was cloned from pTetBBB into pBluescript KS that had been digested with BamHI and EcoRV. To construct hsp27 expression vectors, the hsp27 open reading frame was excised from pGEX2-bp27 (gift of S. Lumb), using BamHI, and cloned in frame at the BamHI site of pFlagCMV2 (Sigma-Aldrich). Mutagenesis was performed with the QuikChange Kit (Stratagene). Sequences of oligonucleotides are available on request from the corresponding author. Novel DNA constructs were commercially sequenced by ACGC (London, United Kingdom).

Cell culture and transfection. HeLa-TO cells (Clontech) were maintained in Dulbecco's modified Eagle medium-10% fetal calf serum supplemented with 1.5 g/100 ml sodium bicarbonate. Cells were seeded in six-well plates at a density of 1.5 × 10⁴ cells/well. The following day cells were transfected using Superfect (Qiagen). The amount of total transfected DNA was kept constant within all experiments by addition of appropriate empty expression vectors and/or Blue script plasmid (Stratagene). After 24 h, tetracycline (Sigma) was added at a final concentration of 100 ng/ml, and cells were harvested in guanidine thiocyanate lysis buffer (Ambion) at different intervals, as indicated in each figure legend. Western blots were processed through standard procedures (Qiagen) and stored frozen at −20°C. In some experiments 1 μM SB203580 (Calbiochem) or vehicle control (dimethyl sulfoxide [0.03%]) was added to cells 30 min prior to the Figure. Lysates were passed through shredder columns (Qiagen) and stored at −80°C. Riboprobe template constructs were linearized with the two major sites indicated by larger arrows. Canonical (AATAAA) polyadenylation sites are indicated by arrows with closed triangles heads; non-canonical (ATTAAA) sites are indicated by arrows with Vs for heads. The polyadenylation sites are discussed in the text. ORF, open reading frame. (B) Schematic of 3' UTR-encoding fragments generated by PCR and cloned into the BglII site of pTetBBB. (C) Structure of the reporter construct pTetBBB. Rabbit β-globin exons and introns are shown as closed and open bars. Translation initiation (ATG), translation termination (TGA), and polyadenylation (AATA AA) signals are indicated. The antisense β-globin riboprobe is represented as a dashed arrow. TetOp, tetracycline operator sequences, TATA, minimal cytomegalovirus promoter.

RNA band shift assay. RNA probes were synthesized essentially as described for the ribonuclease protection assay. RNA band shift assays were performed according to the method of Hel et al. (25). The protein extracts were incubated with the indicated RNA probes in a buffer containing 20 ng/ml HEPES (pH 7.6), 3 mM MgCl₂, 40 mM KCl, 2 mM dithiothreitol, and 5% glycerol. Typically, 10 μg of protein was incubated for 20 min with 400,000 to 500,000 cpm of 35S-labeled RNA probe, corresponding to approximately 10 fmol of RNA. RNase T₁ and heparin sulphate were added to finalize concentrations of 50 U/ml and 5 mg/ml, respectively, and the reaction was allowed to continue for a further 20 min on ice. Three microliters of loading buffer (90% glycerol, 0.025% bromophenol blue) was added to the samples which were then resolved by electrophoresis at 4°C on a 0.5 × Tris-borate-EDTA nondenaturing 4% polyacrylamide gel. Gels were subjected to autoradiography and phosphorimaging. In some experiments binding reactions also contained homopolyribonucleotides (Pharmacia) as indicated, or 1 μl of polydymion antisera, HuR (gift of J. Steitz), Jun N-terminal protein kinase (JNK3), and AUF1 antisera were all raised in rabbits.

RESULTS

The Cox-2 3' UTR mediates regulation of mRNA stability by the p38 pathway. The tetracycline-responsive reporter construct pTetBBB (59), contains a rabbit β-globin genomic fragment downstream of the tetracycline operator sequences and a minimal promoter (Fig. 1). Transcriptional activity of this construct was rapidly switched off by addition of 100 ng/ml tetracycline to the culture medium (unpublished data).

A cDNA fragment encoding the Cox-2 3' UTR was inserted at the BglII site of pTetBBB (Fig. 1). The resulting construct (pTetBBB-Cox2.5) was transiently transfected into HeLa-TO cells with a luciferase expression vector and with or without a vector expressing constitutively active MKK6. After 24 h cells were treated with SB203580 or vehicle, and then tetracycline was added and cells were harvested at intervals as indicated in Fig. 2. A ribonuclease protection assay was used to quantify the β-globin–Cox2.5 reporter transcript, Cox-2, luciferase, and GAPDH mRNAs (the last two as controls for transfection efficiency and gel loading). In all such experiments endogenous Cox-2 expression was up-regulated by MKK6, and this was...
up-regulation of luciferase expression by MKK6 was not reversed by SB203580, confirming that the Cox-2 gene is a strong activator of MAPks p42 and p44 but not p38 and strongly induces expression of the Cox-2 gene. Treatment of HeLa cells with phosphor myristate acetate had no impact upon the stability of the β-globin–Cox2.5 chimeric mRNA (unpublished data). The Cox-2 3’ UTR therefore confers instability and p38-dependent stabilization upon a heterologous mRNA.

CR1 of the Cox-2 3’ UTR is necessary and sufficient for the regulation of mRNA stability by the p38 pathway. To determine what proportion of the 2,515-nt Cox-2 3’ UTR is required for p38-mediated regulation of stability, we initially cloned 1.4- and 0.6-kb 3’ UTR fragments into pTetBBB (Fig. 1). These fragments correspond to the 3’ UTRs present within two minor Cox-2 transcripts, which terminate at noncanonical ATT AAA polyadenylation signals (PA1 and PA2 in Fig. 1) (38, 47). The β-globin–Cox1.4 and β-globin–Cox0.6 transcripts behaved similarly to β-globin–Cox2.5 (Fig. 3). Therefore, only the first 600 nt of the Cox-2 3’ UTR is required for the regulation of mRNA stability by the p38 pathway and CR2 is dispensable. This is in agreement with our previous observation that endogenous 4.6- and 2.8-kb Cox-2 transcripts are identically destabilized by SB203580 in actinomycin D chase experiments (12, 44).

The 0.6-kb 3’ UTR fragment was further subdivided into a 0.1-kb fragment which contained six AUUUA repeats (equivalent to CR1) and a 0.5 kb fragment which contained only one AUUUA sequence (Fig. 1). The β-globin–Cox0.5 transcript was stable under all conditions (Fig. 3). In contrast the β-globin–Cox0.1 transcript was unstable and was very strongly stabilized by MKK6. This effect was significantly reversed in the presence of 1 μM SB203580 (Fig. 3). We conclude that CR1 contains all of the sequence elements required to mediate the regulation of mRNA stability by the p38 pathway. Under all p38-activating conditions tested, the β-globin–Cox0.1 transcript was stabilized more strongly than β-globin–Cox0.6 or any of the other chimeric mRNAs. In addition to CR1, the Cox-2 3’ UTR may therefore contain additional determinants of mRNA instability which do not respond to activation of the p38 pathway. The β-globin–Cox0.5 transcript is highly stable, suggesting that additional instability determinants function only in the presence of CR1.

Sequence specificity of p38-regulated mRNA stability. CR1 contains overlapping AUUUA motifs and thus resembles a class II ARE (7). In contrast, class I AREs are characterized by dispersed AUUUA motifs in association with U-rich stretches. To investigate the sequence specificity of p38-mediated regulation of mRNA stability, the MKK6 responsiveness of several control transcripts was tested. These transcripts contained no insert, a class II ARE derived from the TNF-α 3’ UTR, a class I ARE derived from the c-myc 3’ UTR, or CR1 in reverse orientation [β-globin and β-globin–TNF, –myc and –Cox0.1 [as, respectively]. The β-globin–TNF and β-globin–myc transcripts were unstable but unresponsive to MKK6 or SB203580 (Fig. 4); therefore, the regulation of mRNA stability by p38 is reversed by SB203580, confirming that the Cox-2 gene is a target for the p38 pathway in this particular HeLa cell line. Up-regulation of luciferase expression by MKK6 was not reproducible. The antisense β-globin probe spans an intron-exon boundary (Fig. 1), and detects an unspliced pre-mRNA species, which is indicated in Fig. 2A. Following the addition of tetracycline, this pre-mRNA rapidly disappeared, as transcription was inhibited and the pre-mRNA was processed.

Little or no decay of the β-globin transcript was detected over a 6-h tetracycline chase (see Fig. 4 for this and additional controls). In contrast the chimeric β-globin–Cox2.5 transcript decayed with a half-life of approximately 1 h. In the presence of constitutively active MKK6, the half-life of this transcript increased more than twofold. MKK6 coexpression increased the quantity of transcript detected at the start of the tetracycline chase, presumably because the increased transcript stability resulted in a greater mRNA accumulation during the 24 h prior to the addition of tetracycline. The addition of 1 μM SB203580 shortly before the addition of tetracycline reversed the MKK6-dependent stabilization of the chimeric transcript. In spite of very different starting levels, the transcripts decayed with identical half-lives in the absence of MKK6 or in the presence of both MKK6 and SB203580. Therefore, differences in rates of decay do not simply reflect limited capacity within the degradative machinery but are related to the activity of the p38 pathway. In HeLa cells phosphor myristate acetate is a potent activator of MAPks p42 and p44 but not p38 and strongly induces expression of the Cox-2 gene. Treatment of HeLa-TO cells with phosphor myristate acetate had no impact upon the stability of the β-globin–Cox2.5 chimeric mRNA (unpublished data). The Cox-2 3’ UTR therefore confers instability and p38-dependent stabilization upon a heterologous mRNA.
not a general property of AREs. Neither synthesis nor degradation of the highly stable β-globin transcript from the tetracycline-regulated promoter was influenced by MKK6. The CR1 fragment did not confer instability or MKK6 responsiveness when placed in reverse orientation; therefore, it does not possess enhancer-like properties, and transcriptional effects of MKK6 coexpression can be discounted. Further, the regulation of mRNA stability by p38 is not simply a consequence of the high (78%) AU content of CR1, as this AU richness is preserved in β-globin–Cox0.1(as). Finally, all chimeric β-globin transcripts were assessed by Northern blotting and shown to be of the anticipated sizes (data not shown).

**Regulation of mRNA stability is mediated by MAPKAPK-2.** To further dissect the role of the p38 pathway in the regulation of Cox-2 mRNA stability, we used a series of mutants of MAPKAPK-2, a kinase which is phosphorylated and activated by p38 (18, 49). Wild-type MAPKAPK-2 had little or no impact upon the stability of β-globin–Cox0.1 transcripts; however, a constitutively active form (AspX3) (2) strongly stabilized the reporter transcript (Fig. 5A and 5C). Stabilization was also observed with β-globin–Cox2.5 and was not affected by 1 μM SB203580, consistent with the site of action of the drug in the p38 signal transduction cascade (unpublished data).

The stabilization of β-globin–Cox0.1 by MKK6 was blocked by two distinct dominant negative forms of MAPKAPK-2 (1), a nonphosphorylatable mutant (A222/334) and a kinase dead mutant (A207) (Fig. 5B and 5D). We observed a slight but reproducible additive stabilization of the reporter transcript by coexpression of MKK6 and wild-type MAPKAPK-2. Virtually identical results were obtained with β-globin–Cox2.5 (unpublished data). The results described here suggest that the regulation of mRNA stability by the p38 pathway is mediated largely or entirely by the p38 substrate MAPKAPK-2.

**Chimeric β-globin–Cox transcripts are partially stabilized by hsp27 mutants.** The only known substrates of the kinase MAPKAPK-2 are the transcription factors ATF1, CREB, and SRF (24, 26, 54) and the small heat shock protein hsp27 (15, 18, 53). hsp27 is an abundant cytoplasmic protein thought to play a role in cell survival following stress (14, 28, 35). MAPKAPK-2 phosphorylates three serine residues (15, 78, and 82) in human hsp27 and two serine residues (15 and 90) in the rodent homologue hsp25. Phosphorylation is thought to regulate the functional properties of the small heat shock proteins in part by controlling their association into dimers or homopolymers (27, 48). Self-association is primarily regulated by S90 phosphorylation in hamster hsp25. Phosphorylation of

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**FIG. 3.** CR1 is necessary and sufficient for the regulation of mRNA stability by the p38 pathway. Transfections and ribonuclease protection assays were performed as described in the legend to Fig. 2, using the reporter constructs pTetBBB-Cox1.4, -Cox0.6, -Cox0.5, and -Cox0.1. SB, 1 μM SB203580. (A) Representative experiments. Only the β-globin reporter and GAPDH loading control bands are shown. (B) Graphical representation of the experiments shown in panel A. Each transfection was performed at least twice, with qualitatively identical results.
S15 has little or no effect upon self-association but may regulate the interaction of hsp25 and/or hsp27 with other cellular proteins such as actin (27).

To investigate the potential role of hsp27 in the regulation of Cox-2 mRNA stability, we generated mutants in which serine 15, serines 78 and 82, or all three phospho-acceptor serines were mutated to alanine or glutamic acid. The triple glutamate substitution mutant hsp27EEE stabilized β-globin-Cox0.1 mRNA, while the single and double glutamate substitution mutants had less effect (Fig. 6A). Virtually identical results were obtained with β-globin–Cox2.5 (unpublished data). Equal expression of all mutants was confirmed by Western blotting (Fig. 6C).

The phosphorylation of hsp27 may contribute to the stabilization of mRNA by the MAPK p38 pathway, with a potential role for each of the sites in hsp27 phosphorylated by MAPKAPK-2. In this assay system the stabilizing effect of hsp27EEE was weak compared to that of MKK6 (Fig. 6) or MAPKAPK-2 itself (Fig. 5), and the alanine substitution mutants had no dominant negative effect on stabilization by MKK6 (unpublished data). However, the effects of phosphorylation site substitutions in a noncatalytic protein such as hsp27 are difficult to predict, especially within a cell which abundantly expresses the wild-type protein. In order to rule out or to prove more conclusively that hsp27 is involved in the regulation of mRNA stability, it may be necessary to use hsp27 knockout cells. It is possible that an unidentified substrate of MAPKAPK-2 plays a more significant role in this process.

CR1 interacts with AUF1 or an immunologically related protein. In electrophoretic mobility shift assays using a Cox0.1 probe and a HeLa-TO cytoplasmic extract three protein-RNA complexes were observed, with the lowest mobility complex (C3 in Fig. 7) being rather diffuse. No complexes were detected with the Cox0.5 or Cox0.1 antisense probes. All protein interactions with the Cox0.1 probe could be blocked by competition with an excess of unlabeled poly(U) but not with poly(A) (Fig. 7B), poly(C), or poly(G) (unpublished data). Complexes C3 and C4 were common to Cox0.1 and c-myc probes, while complex C5 was detected only with the Cox0.1 probe and complexes C1 and C2 were common to c-myc and TNF-α probes (Fig. 8B).

The ARE-binding proteins AUF1 and HuR are both present in HeLa cells and bind to the c-myc 3′ UTR and to poly(U) RNA (3, 32, 36, 60). Because of the similarities between Cox0.1 and c-myc RNA-protein complexes, we hypothesized that AUF1, HuR, or both proteins might interact with the CR1

FIG. 4. Sequence specificity of p38-regulated mRNA stability. Transfections and ribonuclease protection assays were performed as described in the legend to Fig. 2, using the reporter constructs pTetBBB, pTetBBB-Cox0.1(as), pTetBBB-TNF, and pTetBBB-myc. SB, 1 μM SB203580. (A) Representative experiments. Only the β-globin reporter and GAPDH loading control bands are shown. (B) Graphical representation of the experiments shown in panel A. Each transfection was performed at least twice, with qualitatively identical results.
sequence. Therefore, electrophoretic mobility shift assays were carried out using HeLa-TO cytoplasmic extract and Cox0.1 probe in the presence of antibodies raised against HuR, AUF1, or an irrelevant protein (JNK3) (Fig. 8A). A supershifted complex was detected in the presence of the AUF1 antibody, suggesting that a protein which interacts with the CR1 sequence is identical (or closely related) to AUF1. No supershifted complexes were detected with a nonimmune serum or with HuR or JNK3 antisera. An anti-AUF1 supershifted complex was also detected using the c-myc but not the TNF-α probe (Fig. 8B). The formation of supershifted bands was not accompanied by substantial reduction of RNA-protein complexes C3 to C5. Immunodepletion of AUF1 from the HeLa-TO cytoplasmic extract did not significantly inhibit the formation of complexes C3 to C5 (unpublished data). Therefore, an AUF1-related protein is present in HeLa-TO cells and is able to bind to the CR1 probe but represents only a small proportion of the CR1-binding activity detected in electrophoretic mobility shift assays.

**DISCUSSION**

We previously reported that Cox-2 mRNA stability is regulated by p38 in human monocytes and in HeLa cells (12, 44). Those studies employed pharmacological inhibitors of transcription and of p38, each of which is a potential source of artifacts. The transcriptional inhibitor actinomycin D is cytotoxic, induces nucleocytoplasmic shuttling of several RNA binding proteins (16, 22), and may artificially stabilize some mRNAs (9, 51, 58). At high concentrations SB203580 is able to inhibit some JNK isoforms (10, 56), although at the 1 mM concentration we have used there is little or no effect upon HeLa cell JNK activity (44). At the concentration used, tetracycline has no discernible effects upon HeLa-TO cells other than the regulation of the tetracycline-responsive promoter. The present study, using a tetracycline chase procedure, provides proof of p38-dependent Cox-2 mRNA stabilization, operating through the p38 substrate MAPKAPK-2, and possibly
mediated in part by the phosphorylation of the small heat shock protein hsp27.

The p38-dependent stabilization of mRNA is sequence-specific, since 3’ UTR sequences derived from c-myc or TNFα destabilise the β-globin reporter transcript, but do not confer responsiveness to the p38 pathway. Using a similar system, it has recently been demonstrated that the p38 pathway regulates the stability of reporter transcripts containing IL-6, IL-8, c-fos, and granulocyte-macrophage colony-stimulating factor (GM-CSF) AREs (57). Thus, p38 is able to regulate the stability of a subset of mRNAs containing class I AREs and a subset of mRNAs containing class II AREs. For comparison, several of the relevant ARE sequences are illustrated in Fig. 9.

FIG. 6. Stabilization of a β-globin–Cox chimeric transcript by a mutant of hsp27. (A) HeLa-TO cells were transfected with 200 ng of pGL3c and 20 ng of pTetBBB-Cox0.1, plus 100 ng of MKK6 expression vector or 780 ng of Flag-hsp27 expression vector as indicated. Ribonuclease protection assays were performed as described in the legend to Fig. 2. (B) Graphical representation of the experiment shown in panel A. This experiment was performed three times, with qualitatively identical results. (C) HeLa-TO cells were transfected with 780 ng of pFlagCMV2 (first lane) or Flag-hsp27 expression vector as indicated. After 24 h cells were harvested and Western blotting was performed using an antibody against the Flag epitope.

FIG. 7. The p38-responsive region of the Cox-2 3’ UTR forms several complexes with HeLa-TO cytoplasmic proteins. Complexes C3 to C5 are discussed in the text. FP, free probe. (A) Electrophoretic mobility shift assays were performed using Cox0.1, Cox0.1 antisense, and Cox0.5 RNA probes and 10 µg of HeLa-TO cytoplasmic extract. (B) Electrophoretic mobility shift assays were performed using a Cox0.1 RNA probe and 10 µg of HeLa-TO cytoplasmic extract in the presence of a 0- to 1,000-fold excess (by mass) of homopolyribonucleotides poly(A) or poly(U), as indicated.
define determinants of p38 responsiveness by extending the comparison of p38-sensitive and -insensitive ARE-containing transcripts using the system described here. It will also be interesting to extend the comparison of RNA-protein complexes involving p38-sensitive and p38-insensitive AREs and to determine whether the formation of complex C5 is specific to p38-sensitive AREs.

The most-abundant (4.6-kb) Cox-2 transcript contains a 2,515-nt 3' UTR, while the second-most-abundant (2.8-kb) transcript contains a 603-nt 3' UTR (38, 47). Only 123 nt, immediately 3' to the translation termination codon, are required for the regulation of mRNA stability by p38. Evolutionary conservation of p38-mediated stability regulation is suggested by the high degree of conservation of this region (between mouse and human transcripts: 77% for CR1, 100% for the AUUUA motifs within CR1, and 64% for the entire 3' UTR). The 4.6- and 2.8-kb transcripts are similarly destabilized by inhibition of p38 (12, 44) but are differentially destabilized by the anti-inflammatory glucocorticoid dexamethasone (47), suggesting an involvement of distal sequences (possibly CR2). Regulation of stability by dexamethasone requires glucocorticoid receptor-mediated gene expression and could not be reconstituted using posttranscriptional reporter constructs (37, 46, 47). The p38 and glucocorticoid pathways therefore seem to employ distinct mechanisms and distinct cis-acting sequences to regulate Cox-2 mRNA stability. Thus, both transcriptional and posttranscriptional regulatory elements of the Cox-2 gene have evolved a capacity to respond to diverse extracellular signals.


