3044–3056 *Nucleic Acids Research, 2006, Vol. 34, No. 10*
doi:10.1093/nar/gkl386

Nitric oxide activation of Erk1/2 regulates the stability and translation of mRNA transcripts containing CU-rich elements

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Received March 1, 2006; Accepted May 5, 2006

ABSTRACT

Nitric oxide (NO•) can stabilize mRNA by activating p38 mitogen-activated protein kinase (MAPK). Here, transcript stabilization by NO• was investigated in human THP-1 cells using microarrays. After LPS pre-stimulation, cells were treated with actinomycin D and then exposed to NO• without or with the p38 MAPK inhibitor SB202190 (SB). The decay of 220 mRNAs was affected; most were stabilized by NO•. Unexpectedly, SB often enhanced rather than antagonized transcript stability. NO• activated p38 MAPK and Erk1/2; SB blocked p38 MAPK, but further activated Erk1/2. RT–PCR confirmed that NO• and SB could additively stabilize certain mRNA transcripts, an effect abolished by Erk1/2 inhibition. In affected genes, these responses were associated with CU-rich elements (CURE) in 3′-untranslated regions (3′-UTR). NO• stabilized the mRNA of a CURE-containing reporter gene, while repressing translation. Dominant-negative Mek1, an Erk1/2 inhibitor, abolished this effect. NO• similarly stabilized, but blocked translation of MAP3K7IP2, a natural CURE-containing gene. NO• increased hnRNP translocation to the cytoplasm and binding to CURE. Over-expression of hnRNP K, like NO•, repressed translation of CURE-containing mRNA. These findings define a sequence-specific mechanism of NO•-triggered gene regulation that stabilizes mRNA, but represses translation.

INTRODUCTION

Gene expression in eukaryotic cells is a dynamic process that includes transcription, pre-mRNA splicing, nucleo-cytoplasmic transport, subcellular localization of mRNA and finally transcript translation or degradation. In addition to the many mechanisms that control gene transcription, the importance and complexity of post-transcriptional gene regulation has been increasingly recognized. Recent studies using microarrays have shown that regulation of mRNA stability accounts for about one-half of all changes in mRNA steady-state levels (1,2). Like the role of DNA sequence in regulating transcription, post-transcriptional events, in particular mRNA translation and degradation, have been linked to tightly regulated mechanisms that are dependent on specific *cis*-acting mRNA elements and trans-factors. Important examples of *cis*-acting sequences that control post-transcriptional mRNA regulation include AU-rich elements (ARE) and the less-well characterized differentiation control element (DICE), a CU-rich repetitive motif.

ARE consist of multiple, frequently overlapping copies of the AUUUA motif in the 3′-untranslated region (3′-UTR) of many cytokines, growth factors and proto-oncogenes (3,4). ARE induce rapid shortening of the poly(A) tail followed by exosomal degradation of the mRNA body (5,6). Several ARE-binding trans-factors, such as HuR, tristetraprolin, AUF1 and CUGBP2 have been identified and their various functions have become better understood. By interaction with ARE, HuR stabilizes (7), but tristetraprolin destabilizes mRNA (8,9). AUF1 can either stabilize or destabilize ARE-containing transcripts depending on the relative abundance of different AUF1 isoforms (10). CUGBP2 stabilizes ARE-bearing mRNA but silences its translation (11). DICE, originally described in the 3′-UTR of lipoxygenase mRNA (12), is characterized by repetitive, tandem-like CU-rich sequences with a (C/U)CCAN$_X$ CCC(U/A) (C/U)$_y$ UC(C/U)CC consensus architecture (13,14). DICE binds heterogeneous nuclear ribonucleoprotein (hnRNP) K or E2/E1 to stabilize mRNA (14,15), and to either silence (13,16,17) or drive translation (18). These molecular details of *cis*-acting sequences and trans-factors have provided important tools

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for studying their interactions with major signal transduction networks, such as the stress kinase pathways that regulate mRNA stability and translation.

The connection between p38 mitogen-activated protein kinase (MAPK) signaling and ARE-binding trans-factors has recently been investigated for tristetraprolin. *In vitro* evidence shows that tristetraprolin can be phosphorylated by p38 MAPK, which inhibits its binding to ARE, thereby stabilizing target transcripts (19,20). Alternatively, as shown for IL-3 mRNA, p38 MAPK can also phosphorylate other ARE-stabilizing trans-factors, such as HuR and subsequently antagonize the effects of tristetraprolin (21). To date, the p38 MAPK signaling pathway has been implicated in stabilizing mRNA half-lives of more than 40 ARE genes (22), including cyclooxygenase 2 (23), TNFα (19), IL-3 (21), IL-8 (22,24), vascular endothelial growth factor (25) and p21/Waf1/Cip1 (26). Inhibitors of p38 MAPK or expression of a dominant-negative mutant of p38 MAPK activated protein kinase 2 abolish mRNA stabilization of these genes (19,23,24,27). Likewise, the Erk1/2 signaling pathway has been implicated in the regulation of DICE -containing transcripts. Through phosphorylation of hnRNP K, Erk1/2 increases hnRNP K cytoplasmic accumulation and thereby silences the translation of DICE-containing genes (16).

Nitric oxide ($NO^{\bullet}$) is an important signaling molecule that regulates a wide range of cellular activities including gene expression. It has been demonstrated that $NO^{\bullet}$ regulates transcription through Sp1 (28,29), NF-kB (30), AP-1 (31), Egr-1 (32) and HIF-1 (33). Besides these defined effects on gene transcription, $NO^{\bullet}$ has been further implicated in regulating the mRNA stability of a number of genes including heme oxygenase-1 (34), cytochrome C oxidase (35), flavin-containing monooxygenase (36), transforming growth factor-β3 (37), matrix metalloproteinase-9 (38), IL-8 (24) and p21/Waf1/Cip1 (26). $NO^{\bullet}$ was found to destabilize matrix metalloproteinase-9 mRNA through the cGMP-dependent down-regulation of HuR (38). Conversely, $NO^{\bullet}$ stabilized IL-8 and p21/Waf1/Cip1 mRNA through the cGMP-independent activation of p38 MAPK (24,26). For other genes, the mechanism by which $NO^{\bullet}$ signaling regulates mRNA turnover has not yet been determined.

To more completely characterize transcript stabilization by $NO^{\bullet}$ and to further explore the role of p38 MAPK in these events, we performed a large-scale analysis of mRNA decay using oligonucleotide microarrays in lipopolysaccharide (LPS)-stimulated human THP-1 cells, a monocytic line. In the presence of LPS, a very strong activator of p38 MAPK, $NO^{\bullet}$ was found to increase the half-life of relatively few genes by further engaging this pathway. Unexpectedly, most genes stabilized by $NO^{\bullet}$ were further stabilized by p38 MAPK inhibition. This result prompted a search of UTR databases for *cis*-acting elements that might explain this finding. Downstream experiments were then conducted to define possible mechanisms. $NO^{\bullet}$ was shown to stabilize transcripts while suppressing their translation through DICE-like, CU-rich elements (CURE) in target transcripts. $NO^{\bullet}$ activation of Erk1/2 was required, as was an associated increase in the binding of hnRNP proteins to mRNA. These findings suggest a novel mechanism of $NO^{\bullet}$-mediated post-transcriptional regulation that functions as both a mRNA stabilizer and a translation inhibitor.

MATERIALS AND METHODS

Reagents

Salmonella minnesota Re595 LPS was obtained from List Biologic (Campbell, CA). S-nitrosoglutathione (GSNO), SB202190 (SB) and PD98059 (PD) were purchased from Calbiochem (San Diego, CA). Actinomycin D (ActD), glutathione (GSH), β-mercaptoethanol and dimethyl sulfoxide (DMSO) were from Sigma-Aldrich (St. Louis, MO). DMSO was used to dissolve SB and PD and was similarly added to control cells (final concentration 0.0033%) in experiments that tested these reagents. Rabbit polyclonal antibodies detecting p38 MAPK, phospho-p38 MAPK (Thr180/Tyr182), Erk1/2 and phospho-Erk1/2 (Thr202/Tyr204) were obtained from Cell Signaling Technology, Inc. (Beverly, MA). Goat polyclonal antibodies against hnRNP K and hnRNP E2/E1, normal goat serum and mouse monoclonal antibody against α tubulin were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). MAP3-K7IP2 (MAPK kinase kinase 7 interacting protein 2) antibody was obtained from Affinity BioReagents (Golden, CO).

Microarray experiments

THP-1 cells (2×10^7) were first stimulated with LPS (1 μg/ml) for 4 h to activate the cells and boost transcript levels. After 30 min treatment with ActD (2.5 μg/ml), a transcription inhibitor, in the absence or presence of p38 MAPK inhibitor SB (0.1 μM), cells were then further incubated for 0–180 min with 400 μM of GSNO or GSH control ($N = 4$). Total RNA at different time points (0, 45, 90 and 180 min) was extracted, labeled and hybridized to human U133A microarrays following standard Affymetrix procedures as described previously (26). After staining with streptavidin phycoerythrin (Molecular Probes), microarrays were scanned using Agilent GeneArray Scanner.

Plasmid construction

The plasmid pGL3 containing a firefly luciferase (LUC) reporter gene driven by the SV40 promoter was purchased from Promega (Madsion, WI). Synthetic oligonulceotides containing the consensus CURE sequence (5′-CTTTCT-CCCCCACCCTCTTCTCCCCCTTCCCCCTCCCC-3′; the core sequences are underlined) or its antisense form (5′-GGGGA-GGGGGAAGGGGGAGAAGAGGGTGGGGGAGAAAG-3′) were cloned into pGL3 at the XbaI site residing in the 3′-UTR of LUC, generating the plasmids pGL3/CURE and pGL3/CUREmut, respectively. The plasmid pMEK1-DN encoding a dominant-negative mutant of Mek1 was a gift from Dr Ae-Kyung Yi at University of Tennessee Health Science Center, Memphis, Tennessee (39). The phnRNP-K, plasmid that expresses hnRNP K was kindly provided by Dr Ze'ev Ronai at Mount Sinai School of Medicine, New York (16). The parental plasmids, pUSEamp of pMEK1-DN and pcDNA3 of phnRNP-K, were obtained from Upstate (Charlottesville, VA) and Invitrogen (Carlsbad, CA), respectively.

Cell culture and transfection

THP-1 cells, a human monocytic line obtained from ATCC (Manassas, VA) were maintained in RPMI supplemented

with 10% fetal calf serum (FCS) (Cellgro, Herndon, VA) and 50 μM β-mercaptoethanol as described previously (24). Transfections were performed using Nucleofector® Kit V (amaxa Inc. Gaithersburg, MD) according to the manufacture's instructions. For each transfection, 0.3 μg of pGL3, pGL3/CURE or pGL3/CUREmut and 0.1 μg of pRL-TK were cotransfected into 1.5×10^6 THP-1 cells. In some transfections, 0.2 μg of pMEK1-DN, pUSEamp, phnRNP-K or pcDNA3 were added as indicated. Cells were allowed to recover for 16 h post-transfection in fresh media before exposure to the various conditions tested in each experiment. LUC activities were subsequently measured using the Dual-Luciferase reporter assay system (Promega, Madison, WI) while reporter gene mRNA levels were quantified using real-time RT–PCR (see below). LUC activities and LUC mRNA levels were normalized to Renilla luciferase expressed by co-transfected pRL-TK (Promega, Madison, WI) to adjust transfection efficiency.

Real-time RT–PCR

TaqMan® real-time RT–PCR (ABI, Rockville, MD) was employed to quantify mRNA levels. Gene specific probes and PCR primers for GAPDH, MAP3K7IP2, MRPS18A (mitochondrial ribosomal protein S18A) and TP53BP2 (tumor protein p53 binding protein 2) were purchased from ABI (Foster City, CA). Probes and primers of LUC and Renilla luciferase were designed by us and synthesized through ABI. The sequences were as follows: LUC probe (5′-CATTTCGCAGCCTACCGTGGTGTTC-3′) and primers (5′-AACGTGAATTGCTCAACAGTATGG-3′ and 5′-TTTG-CAACCCCTTTTTGGAA-3′); and Renilla luciferase probe (5′-CCTGATTTGCCCATACCAATAAGGTCTGG-3′) and primers (5′-AGCCAGTAGCGCGGTGTATT-3′ and 5′-TCA-AGTAACCTATAAGAACCATTACCAGATT-3′). The High-capacity cDNA Archive kit (ABI, Foster City, CA) was employed to prepare cDNA from 2 μg of total RNA. Resulting cDNA was used for RT–PCR in triplicate according to the standard ABI protocol. The target mRNA of MAP3K7IP2, MRPS18A and TP53BP2 were normalized to GAPDH. The LUC mRNA were normalized to Renilla luciferase mRNA.

RNA electrophoretic mobility shift assays (REMSA)

Synthetic consensus CURE probe (5′-CUUUCUCCCCC-ACCCUCUUCUCCCCCUUCCCCCUCCCC-3′) and MAP3-K7IP2 CURE (5′-AGACUCCGUCUCUACAGAAGGUUU-UGAA-3′) were labeled with biotin-N4-CTP using Biotin 3′ end Labeling Kit (Pierce, Rockford, IL). Cytoplamic fractions were extracted using Nu-CLEAR™ extraction kit (Sigma–Aldrich. St. Louis, MO). Labeled CURE probe (0.5 pmol) was incubated with cytoplasmic protein (30 μg) in binding buffer [15 mM HEPES (pH 7.4), 10 mM KCl, 5 mM MgCl2, 5% glycerol and 1 mM DTT] for 20 min at room temperature. To prevent nonspecific binding, yeast tRNA (final concentration, 0.1 mg/ml) was added. RNA–protein complexes were separated in 6% polyacrylamide gel with 0.5× Tris/boric acid/EDTA buffer, transfered on to nylon membrane and detected using the LightShiftTM Chemiluminescent EMSA Kit (Pierce, Rockford, IL). In competition experiments, a 100-fold molar excess of unlabeled consensus CURE, or its mutant (5′-CUUUAGAGAAGACACAGAAGAAGAAGAAGACA-CAGACC-3′) or MAP3K7IP2 CURE, were added to the incubation mixture. For antibody supershift assays, 2 μg of specific polyclonal antibody or control goat serum were preincubated with cytoplasmic proteins for 20 min at room temperature prior to addition of labeled CURE probes.

Bioinformatics and data analysis

Affymetrix MAS5 signal values and present call results were stored in the NIHLIMS, a database for storage and retrieval of Affymetrix GeneChip data in use at the NIH. This entire microarray dataset were also submitted to GEO repository (GSE4228). Data were retrieved and analyzed using the MSCL Analyst's Toolbox (http://abs.cit.nih.gov/MSCLtoolbox/) and the JMP statistical software package (SAS, Inc, Cary, NC; http://www.jmp.com). Data were first normalized to the 97th percentile, a value corresponding to the expression level of the 678th most intense probeset on the array. This normalization strategy assumed that the most intense probesets corresponded to mRNA species which were most stable and were generally unaffected by the treatments studied here. Then logarithmically transformed normalized data were subject to linear regression with respect to the four time points studied (0, 45, 90, 180 min following the start of incubation with GSH or GSNO), to estimate a slope corresponding to a first-order decay rate. As expected, the distribution of slope values across probesets showed a long negative tail, corresponding to genes which decayed over the time period studied. The decay slope was calculated for each probeset, for each of the four conditions (GSH, GSNO, SB/GSH and SB/GSNO) using an Analysis of Covariance (ANCOVA), constraining the time 0 expression value to be identical for the pair of conditions without SB and the pair with SB, as necessitated by the design of the experiment. Further, since the experiment was replicated in four distinct batches, a blocked ANCOVA was utilized. A specialized Toolbox script, ANCOVAbatch, was written for this purpose. The analysis results were then used to select genes which decayed, and whose decay rate changed following treatment. The *P*-value for a one-way, four level ANCOVA was calculated and used to compute a false discovery rate (FDR) (40). A total of 238 probesets with the lowest *P*-values were selected, corresponding to a FDR of 10%. Probesets were annotated identifying 220 unique transcripts based on information presented by Affymetrix at the web-site http://www.affymetrix.com/analysis/index.affx as of April 12, 2004.

Mean signal intensities of the 220 identified transcripts across four independent experiments were computed and normalized using the 0 min values for all conditions and time points. These normalized mean signal intensities were hierarchically clustered (Figure 1A) using the complete linkage algorithm in JMP (SAS Institute, Cary, NC). For Figure 1B, mRNA decay slopes for every identified gene in different conditions were hierarchically clustered with an average linkage algorithm and the centered Pearson correlation coefficient as the similarity metric using HCE (Hierarchical Clustering Explorer 2.0 beta, available at http://www.cs.umd.edu/hcil/hce/) (41).

MAP3K7IP2, MRPS18A and TP53BP2 mRNA levels over time were analyzed using a two-way ANOVA (the first factor

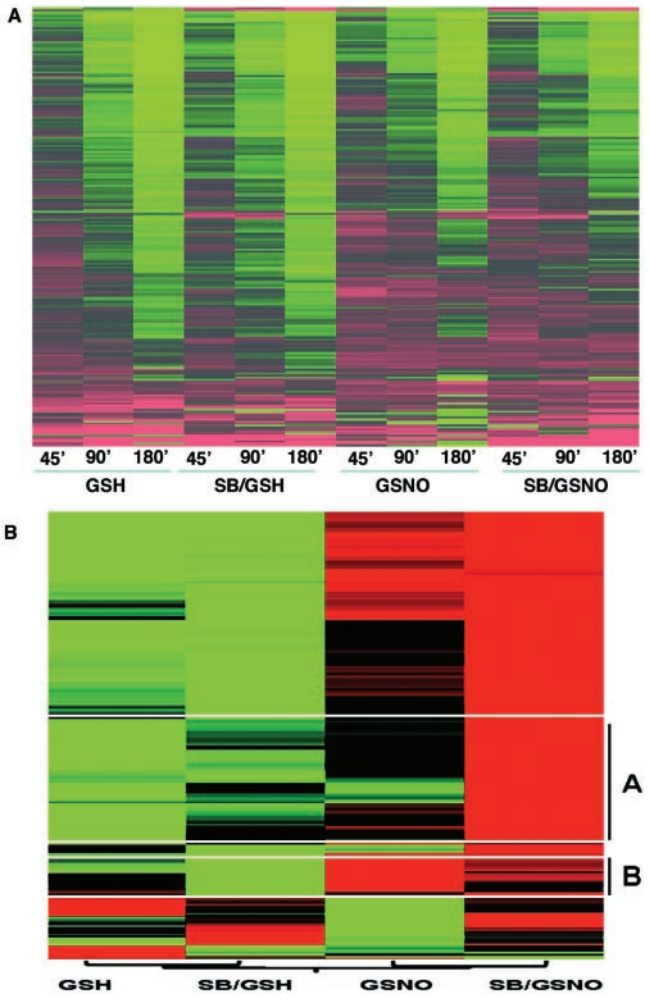

Figure 1. Heat maps; effects of NO• and the p38 MAPK inhibitor SB202190 (SB) on mRNA degradation as determined by microarray. THP-1 cells (2×10^7) were stimulated with LPS (1 μg/ml) for 4 h. After 30 min treatment with ActD (2.5 μg/ml) in the absence or presence of SB (0.1 μM), cells were incubated with GSNO (400 μM) or control GSH (400 μM) for 0–180 min. At the indicated time-points, cells were harvested to extract total RNA for microarray analysis. The half-lives of 220 genes were found to be differentially regulated (see Materials and Methods). (**A**) Hierarchical clustering of normalized mean signal intensities from four independent experiments for all 220 genes at each time point and condition. (**B**) Same results as (A) after conversion of individual time point data into slopes based on a first order mRNA decay model.

was time, the second factor was treatment) followed by *post-hoc* tests. Luciferase mRNA level and activity were analyzed using paired *t*-tests to compare different reporter gene constructs and experimental treatments.

To find putative CURE *cis*-acting elements in 3′-UTR, the mRNA RefSeq for the 220 genes identified by microarray were downloaded from the NCBI nucleotide database (http://www.ncbi.nlm.nih.gov/), and then each sequence was scanned using the UTRScan database (http://www.ba.itb.cnr.it/BIG/UTRScan/) (42) or MacVector™. The ARE-containing transcripts of the 220 genes were found through searching the ARE database (http://rc.kfshrc.edu.sa/ared/) (4). Fisher's exact test for 2×2 contingency tables was used to compare proportions of ARE- and CURE-containing mRNAs between different gene clusters.

RESULTS

NO• regulation of mRNA stability

NO• regulates degradation of IL-8 and p21/Waf1/Cip1 mRNA through the activation of p38 MAPK (24,26). Here, NO• stabilization of mRNA was explored in LPS-stimulated human THP-1 cells using oligonucleotide microarrays (Affymetrx U133A Genechips®). As shown in the Supplementary Table, 220 genes were identified whose mRNA stabilities were significantly regulated by NO•, p38 MAPK inhibitor SB or NO• plus SB. A heat map of expression levels for these 220 genes, arranged by hierarchical clustering (Figure 1A), demonstrated degradation of mRNA over time within each condition (shift from red to green). Also visible in this figure, NO• generally increased overall mRNA stability. Note that the shift from red to green is less pronounced for the NO• and NO•/SB conditions. Unexpectedly, the addition of SB to NO• enhanced rather than antagonized NO•-mediated mRNA stabilization for many of these transcripts, while SB in the absence of NO• had no or only a modest effect. This effect is more obvious in a clustered heat map that condensed expression levels at the four time points into a single mRNA decay rate (Figure 1B). Here, compared to the GSH control without or with SB, GSNO generally stabilized mRNA (shift from green to red). Again, this effect of NO• was further augmented by SB for many of these transcripts, of which 60 genes (cluster A) were also stabilized by SB alone. Antagonism of NO•-mediated effects by SB as would be expected for p38 MAPK stabilized transcripts was seen for only 20 genes (cluster B). The rest of this investigation focused on identifying a mechanism of NO•-mediated mRNA stabilization that could be enhanced by p38 MAPK inhibition.

Activation of p38 and Erk1/2 by NO•

Consistent with our previous findings in THP-1 cells, GSNO activated p38 MAPK in dose-dependent manner (Figure 2A, upper panel) and the p38 inhibitor SB blocked this effect (Figure 2A, lower panel). GSNO also dose-dependently activated Erk1/2 as shown in the upper panel of Figure 2B. Interestingly, this effect of GSNO on Erk1/2 was further enhanced by the p38 MAPK inhibitor SB (Figure 2B, the lower panel). In addition, SB alone dose-dependently activated Erk1/2 in THP-1 cells, though this effect was barely detectable at the dose of SB used here in our microarray experiments (Figure 2C). As expected, neither GSNO nor SB changed total p38 MAPK or total Erk1/2 levels (data not shown).

NO• stabilization of mRNA through Erk1/2

Enhanced NO• activation of Erk1/2 in the presence of SB (Figure 2B) might explain the additive effect of these agents in stabilizing the mRNA transcripts identified by microarray. In order to test whether Erk1/2 activation may be an important signaling pathway involved in NO•-induced mRNA stabilization, we investigated in detail MAP3K7IP2, MRPS18A and TP53BP2, three prototypic genes from cluster A, using real-time RT–PCR. In our microarray experiment, these transcripts were stabilized by NO• and the addition of SB further enhanced this effect. Consistent with these results, RT–PCR (Figure 3A–C; left panels) demonstrated that GSNO

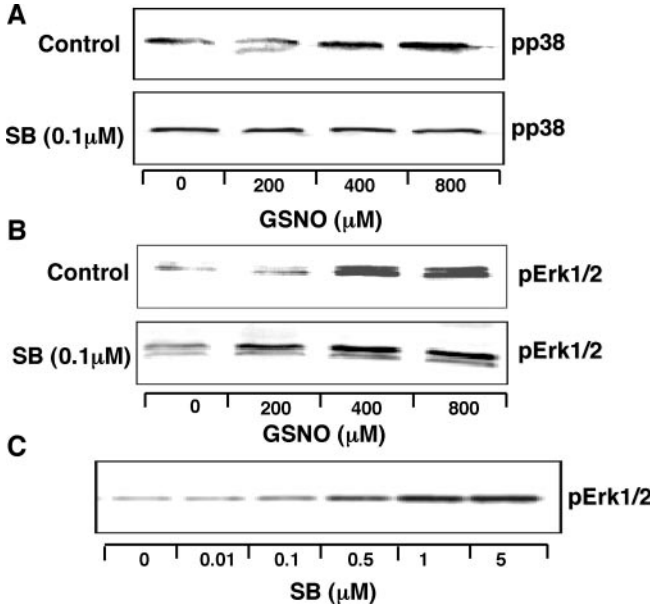

Figure 2. Effects of NO• and the p38 MAPK inhibitor SB202190 (SB) on MAPK phosphorylation. (**A**) NO• increases p38 MAPK phosphorylation, an effect blocked by SB (0.1 μM). (**B**) NO• increases Erk1/2 phosphorylation, an effect enhanced by SB (0.1 μM). (**C**) SB (0–5 μM) alone increases Erk1/2 phosphorylation. THP-1 cells (1×10^7) were stimulated with LPS (1 μg/ml) for 4 h. After 30 min treatment with ActD (2.5 μg/ml) in the absence (control) or presence of SB, cells were incubated without or with GSNO (0–800 μM) for another 30 min, as indicated and then lysed. Each experiment was repeated at least twice with similar results.

significantly stabilized the mRNA of MAP3K7IP2, MRPS18A and TP53BP2 compared with the GSH control ($P < 0.005$ for all); the p38 MAPK inhibitor SB further enhanced the effect of NO• ($P < 0.009$ for all). Treatment with the specific Erk1/2 inhibitor PD prior to GSNO incubation abolished the effects of NO• and SB on the mRNA stability of these three transcripts (Figure 3A–C; right panels). These results suggested that Erk1/2 activation is involved in both the NO•-mediated mRNA stabilization of MAP3K7IP2, MRPS18A and TP53BP2 and the enhancing effects of SB.

Cis-acting elements mediating NO•-MAPK regulation of mRNA stability

3′-UTR elements play pivotal roles in mRNA stabilization (3,5,6). The regulation of mRNA decay by p38 MAPK has been reported to be dependent on ARE within 3′-UTR (19–23,27). We recently found that mRNAs containing ARE are over-represented among NO•-regulated genes in differentiated U937 cells (26). Consistent with these results, 65% (13/20) of transcripts that were NO• stabilized and p38 MAPK inhibitor destabilize (cluster B; Figure 1B and Table 1) contained ARE compared to only 31 of the remaining 200 transcripts (15.5%, $P < 0.001$) identified by microarray. An in depth investigation of ARE functionality in mRNA decay regulated by NO•-MAPK signaling is being conducted in a separate study.

Since the Erk1/2 signaling pathway has been shown to regulate DICE-containing mRNA transcripts (16), we specifically looked for DICE-like sequences in the 3′-UTRs of all 220 genes identified by micorarray using UTRScan database (42). As shown in the Supplementary Table, 64 of 220 genes were found to have identifiable DICE-like sequences, that here, we call CURE to distinguish them from DICE which has multiple CURE repeats. Of these 64 putative CURE-containing genes, 28 were among the 60 genes in cluster A (46.7%, Figure 1B and Table 2) that were additively stabilized by NO• and SB. In contrast, only 36 of the remaining genes (22.5%, $P < 0.001$) similarly contained potential CURE sequences. MAP3K7IP2, MRPS18A and TP53BP2 mRNA, shown here by RT–PCR to be stabilized by NO• through Erk1/2 activation, were among the 28 CURE-containing mRNAs in cluster A. The CURE sequence found within the 3′-UTR of MAP3K7IP2, MRPS18A and TP53BP2 through the UTRScan database is CUCCGUCUCUACAGAAG, UCCCAUCCUCUUCATG and CCAGUCCUCCUGCCAGAAAG, respectively.

NO•-Erk1/2-CURE regulation of mRNA stability and translation using a reporter gene-CURE construct

Since CURE-containing mRNAs were over represented among transcripts that were additively stabilized by NO• and SB, the possible role of CURE in transducing this response was investigated. Inserting a 38 nt consensus CURE, identified from DICE-containing mRNAs (14) or its antisense form into the 3′-UTR of a LUC reporter gene, we generated pGL3/CURE and its mutant pGL3/CUREmut (See Materials and Methods). The responsiveness of these constructs to NO• was then examined after transfection into THP-1 cells. As shown in Figure 4A, wild-type pGL3/CURE, but not pGL3/CUREmut, increased LUC mRNA levels to 180% of control pGL3 values. The NO• donor GSNO further elevated pGL3/CURE mRNA to 280% of control values, but had no effect on pGL3/CUREmut (Figure 4A). NO•-induced LUC mRNA elevations seen with pGL3/CURE were abolished by the Erk1/2 inhibitor PD (Figure 4A; $P < 0.005$). This result suggested that Erk1/2 mediated NO• stabilization of CURE-containing mRNA transcripts.

Despite NO•-induced stabilization of LUC mRNA containing CURE sequence, LUC expression as measured by its activity was significantly decreased by CURE (pGL3/CURE versus pGL3/CUREmut: $P < 0.005$) and further reduced by NO• (GSNO versus GSH for pGL3/CURE: $P < 0.04$; Figure 4A). This inhibitory effect of NO• on LUC translation in pGL3/CURE transfected cells was blocked by the Erk1/2 inhibitor PD ($P < 0.01$; Figure 4A). Although pGL3/CUREmut also demonstrated a slightly lower LUC activity relative to pGL3 control, neither NO• nor PD altered it (Figure 4A). These data suggested that the NO•-Erk1/2 signaling stabilizes mRNA, but represses translation of genes that harbor these DICE-like, CURE sequences. In support of this conclusion, co-transfection of the plasmid pMEK1-DN, encoding a dominant-negative mutant of Mek1, but not its control empty vector pUSEamp, functioned like the Erk1/2 inhibitor PD, abrogating the ability of NO• to stabilize LUC mRNA or repress its translation (Figure 4B) in cells transfected with the pGL3/CURE construct. Mek1 is the upstream kinase that activates Erk1/2 (39).

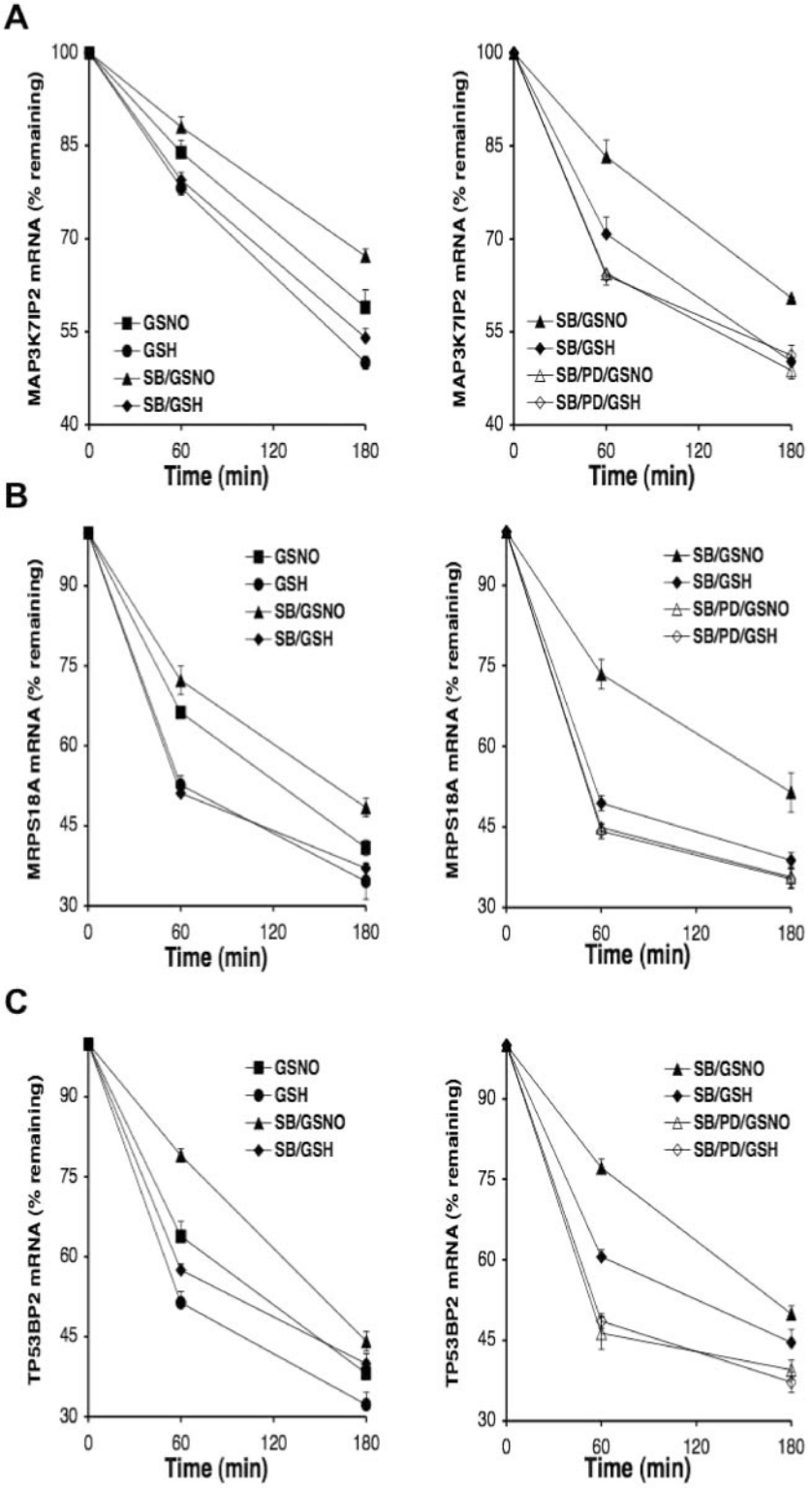

Figure 3. NO• stabilizes (**A**) MAP3K7IP2, (**B**) MRPS18A and (**C**) TP53BP2 mRNA through Erk1/2 as determined by RT–PCR. Left panels show the effects of NO• and the p38 MAPK inhibitor, SB202190 (SB; 0.1 μM), on mRNA degradation. Right panels show the effects of the Erk1/2 inhibitor, PD98059 (PD; 30 μM), on mRNA degradation in the presence of SB. THP-1 cells (2×10^7) were stimulated with LPS (1 μg/ml) for 4 h. After 30 min treatment with transcription inhibitor ActD (2.5 μg/ml) in the absence or presence of indicated MAPK inhibitors, cells were incubated with GSNO (400 μM) or GSH control (400 μM) for 0–180 min. All mRNA levels were quantitated by TaqMan® RT–PCR and normalized to GADPH mRNA. Data, presented as percentage relative to mRNA levels at 0 min, are the mean ± SEM of three independent experiments. The respective mRNA half-lives of MAP3K7IP2, MRPS18A and TP53BP2 were as follows: 179, 98 and 91 min for control GSH; 236, 132 and 121 min for GSNO; 200, 103 and 119 min for SB/GSH; 314, 166 and 155 min for SB/GSNO; 171, 89 and 100 min for SB/PD/GSH; and 160, 90 and 103 min for SB/PD/GSNO.

Table 1. Cluster B: mRNA transcripts stabilized by NO• through p38 MAPK activation

Probe set ID	RefSeq	Symbol	Gene name
201281_at	NM_007002	ADRM1	Adhesion regulating molecule 1
202518_at	NM_001707	BCL7B	B-cell CLL/lymphoma 7B
205780_at	NM_001197	BIK[a]	BCL2-interacting killer (apoptosis-inducing)
205114_s_at	NM_002983	CCL3[a]	Chemokine (C–C motif) ligand 3-like, centromeric
204103_at	NM_002984	CCL4[a]	Chemokine (C–C motif) ligand 4
210046_s_at	NM_002168	IDH2[a]	Isocitrate dehydrogenase 2 (NADP+), mitochondrial
203064_s_at	NM_004514	ILF1	Forkhead box K2
201627_s_at	NM_005542	INSIG1[a]	Insulin induced gene 1
201285_at	NM_013446	MKRN1[a]	Makorin, ring finger protein, 1
208620_at	NM_006196	PCBP1[a]	Poly(rC) binding protein 1
212100_s_at	NM_032311	PDIP46	Polymerase delta interacting protein 3
209533_s_at	NM_004253	PLAA[a]	Phospholipase A2-activating protein
204958_at	NM_004073	PLK3[a]	Polo-like kinase 3 (*Drosophila*)
208361_s_at	NM_001722	POLR3D[a]	Pol III (DNA directed) polypeptide D, 44 kDa
209158_s_at	NM_004228	PSCD2[a]	Pleckstrin-like, Sec7 and coiled-coil domains 2
210573_s_at	NM_006468	RPC62	Pol III (DNA directed) polypeptide C (62 kDa)
58696_at	NM_019037	RRP41	Exosome component 4
213330_s_at	NM_006819	STIP1[a]	Stress-induced-phosphoprotein 1
203112_s_at	NM_005663	WHSC2[a]	Wolf–Hirschhorn syndrome candidate 2
209428_s_at	NM_006782	ZFPL1	Zinc finger protein-like 1

[a]With ARE in 3′-UTR.

Finally, we tested whether NO•-Erk1/2 signaling represses protein expression of a natural CURE-containing mRNA, MAP3K7IP2. This transcript was stabilized by NO• plus SB in our microarray study, a finding confirmed by RT–PCR. Importantly, RT–PCR also demonstrated that PD, an Erk1/2 inhibitor, abolished the effects of NO• and SB on the stability of MAP3K7IP2 mRNA. As shown in Figure 4C, GSNO decreased the expression of MAP3K7IP2 protein; this effect of NO• was enhanced by SB but inhibited by the Erk1/2 inhibitor PD.

Trans-factors associated with the regulation of mRNA stabilization and translation by NO•-Erk1/2-CURE

Trans-factors hnRNP K and hnRNP E2/E1 are major DICE-binding proteins that have recently been implicated in mRNA stabilization (14,15) or translation silencing (13,16,17). To assess the role of these proteins in regulating the stabilization and translation of CURE-containing mRNA by NO•-Erk1/2 signaling, we performed REMSA using two biotin-labeled riboprobes. One probe contained the consensus CURE sequence, and another contained the 3′ MAP3K7IP2 CURE sequence (Figure 5A). In Figure 5A, one major RNA–protein complex was formed by incubating THP-1 cytoplasmic extracts with either of the labeled riboprobes. The specificity of this complex was confirmed by competition in which unlabeled consensus CURE (Figure 5A, lane 5) or MAP3-K7IP2 CURE (Figure 5A, lane 6*) prevented formation of this complex. Mutated consensus CURE (Figure 5A, lanes 6 and 7*) showed no effect on the complex. Antibodies against hnRNP K (Figure 5A, lanes 3 and 3*) and hnRNP E2/E1 (Figure 5A, lanes 4 and 4*), but not control serum (Figure 5A, lane 5*), super-shifted the CURE–protein complex, suggesting that both hnRNP K and E2/E1 proteins are present and specifically bind to CURE mRNA in THP-1 cells. NO• was found to induce the formation of this complex (Figure 5A, lane 2 versus 1 and lane 2* versus 1*) and also to increase the cytoplasmic accumulation of both hnRNP K and hnRNP E2/E1 without changing their overall expression (Figure 5B). Like effects seen on gene regulation, NO•-induced cytoplasmic accumulation of these proteins was enhanced by the p38 MAPK inhibitor SB, but inhibited by the Erk1/2 inhibitor PD (Figure 5B). These results suggest that hnRNP K and hnRNP E2/E1 respond to NO•-Erk1/2 signal transduction by accumulating in the cytoplasm where they differentially regulate the stability and translation of transcripts containing CURE. In support of this concept, cytoplasmic over-expression of hnRNP K was found to repress the translation of LUC linked to a 3′-UTR containing CURE (phnRNP-K/GSH versus pcDNA3/GSH in pGL3/CURE transfected cells; $P < 0.001$) and to mitigate the repressive effect of NO• (Figure 5C).

DISSCUSION

Transcripts containing ARE sites in their 3′-UTR have been shown to be stabilized by activation of p38 MAPK (19,22–24,27). Our previous work demonstrated that NO• stabilizes IL-8 and p21/Waf1/Cip1 mRNA through this mechanism (24,26). Likewise here, NO• was again shown to activate p38 MAPK and to stabilize 20 transcripts that were then destabilized by SB, a p38 MAPK inhibitor. Consistent with our previous findings in differentiated U937 cells (26), ARE-containing mRNAs were over-represented among these NO• up-regulated transcripts; 13 of 20 (65%) have ARE in their 3′-UTR. Three of these, CCL3, CCL4 (chemokine ligand 3 and 4) and PLAA (phospholipase A2-activating protein), were previously reported to be stabilized through p38 MAPK activation by other investigators (22).

However, in human THP-1 cells pre-stimulated with LPS, a strong activator of p38 MAPK, most NO•-stabilized mRNAs had their half-lives further extended by SB, an unexpected finding that we sought to explain. Associated with these microarray results, NO• was found to activate Erk1/2 in addition to p38 MAPK, an effect that was enhanced by SB. This pattern of response and previous reports linking Erk1/2 signaling with post-transcriptional regulatory events

Table 2. Cluster A: mRNA transcripts stabilized by NO• through Erk1/2 activation

Probe set ID	RefSeq	Symbol	Gene name
221492_s_at	NM_022488	APG3[a]	APG3 autophagy 3-like (*Saccharomyces cerevisiae*)
202511_s_at	AK001899	APG5L[b]	APG5 autophagy 5-like (*S.cerevisiae*)
215411_s_at	AL008730	C6orf4	Chromosome 6 open reading frame 4
212711_at	NM_015447	CAMSAP1	Calmodulin regulated spectrin-associated protein 1
205379_at	NM_001236	CBR3	Carbonyl reductase 3
209056_s_at	NM_001253	CDC5L	CDC5 cell division cycle 5-like (*Schizosaccharomyces pombe*)
203721_s_at	NM_016001	CGI-48	CGI-48 protein
203044_at	NM_014918	CHSY1[a,b]	Carbohydrate (chondroitin) synthase 1
212180_at	NM_005207	CRKL[b]	V-crk sarcoma virus CT10 oncogene avian-like
218648_at	NM_022769	CRTC3	CREB regulated transcription coactivator 3
207614_s_at	NM_003592	CUL1[b]	Cullin 1
201371_s_at	NM_003590	CUL3[a]	Cullin 3
202703_at	NM_003584	DUSP11[b]	Dual specificity phosphatase 11
213848_at	NM_001947	DUSP7[b]	Dual specificity phosphatase 7
202776_at	NM_014597	ERBP	Estrogen receptor binding protein
202949_s_at	NM_001450	FHL2	Four and a half LIM domains 2
219083_at	NM_018130	FLJ10539[b]	Hypothetical protein FLJ10539
219933_at	NM_016066	GLRX2	Glutaredoxin 2
217957_at	NM_013242	GTL3[b]	Likely ortholog of mouse gene trap locus 3
219484_at	NM_013320	HCF-2	Host cell factor C2
217965_s_at	NM_013260	HCNGP[b]	Transcriptional regulator protein
218603_at	NM_016217	HECA[a,b]	Headcase homolog (Drosophila)
218946_at	NM_015700	HIRIP5	HIRA interacting protein 5
205526_s_at	NM_007044	KATNA1	Katanin p60 (ATPase-containing) subunit A 1
202417_at	NM_012289	KEAP1[b]	Kelch-like ECH-associated protein 1
203702_s_at	NM_014640	KIAA0173	Tubulin tyrosine ligase-like family, member 4
212846_at	NM_015056	KIAA0179[b]	KIAA0179
203322_at	NM_014913	KIAA0863[b]	KIAA0863 protein
200650_s_at	NM_005566	LDHA[b]	Lactate dehydrogenase A
219631_at	NM_024937	LRP12	Low density lipoprotein-related protein 12
212184_s_at	NM_145342	MAP3K7IP2[b]	MAPK kinase kinase 7 interacting protein 2
202484_s_at	NM_015832	MBD2[b]	Methyl-CpG binding domain protein 2
219348_at	NM_018467	MDS032	Hematopoietic stem cells protein MDS032
219406_at	NM_024097	MGC955[b]	Hypothetical protein MGC955
218385_at	NM_018135	MRPS18A[b]	Mitochondrial ribosomal protein S18A
201829_at	NM_005863	NET1[a]	Neuroepithelial cell transforming gene 1
218889_at	NM_022451	NOC3L[b]	Nucleolar complex associated 3 homolog
204441_s_at	NM_002689	POLA2[b]	Polymerase (DNA-directed), alpha (70 kDa)
207830_s_at	NM_002713	PPP1R8[b]	Protein phosphatase 1, regulatory subunit 8
201934_at	NM_025222	PRO2730[b]	Hypothetical protein PRO2730
203401_at	NM_002765	PRPS2[b]	Phosphoribosyl pyrophosphate synthetase 2
212296_at	NM_005805	PSMD14	Proteasome 26S subunit, non-ATPase, 14
202990_at	NM_002863	PYGL	Phosphorylase, glycogen
200833_s_at	NM_015646	RAP1B	RAP1B, member of RAS oncogene family
218535_s_at	NM_018343	RIOK2[a]	RIO kinase 2 (yeast)
218016_s_at	NM_018119	RPC5	Pol III (DNA directed) polypeptide E (80 kDa)
212018_s_at	NM_015659	RSL1D1	*Homo sapiens* ribosomal L1 domain containing 1
218137_s_at	NM_021940	SMAP1[b]	Stromal membrane-associated protein 1
210053_at	NM_006951	TAF5	TAF5 Pol II, TBP-associated factor, 100 kDa
213301_x_at	NM_015905	TIF1	Transcriptional intermediary factor 1
218118_s_at	NM_006327	TIMM23	Translocase of mitochondrial membrane 23 homolog
202633_at	NM_007027	TOPBP1[a]	Topoisomerase (DNA) II binding protein 1
203120_at	NM_005426	TP53BP2[b]	Tumor protein p53 binding protein, 2
218855_at	NM_016372	TPRA40[b]	Seven transmembrane domain orphan receptor
212544_at	NM_004773	TRIP3	Thyroid hormone receptor interactor 3
202413_s_at	NM_003368	USP1	Ubiquitin specific protease 1
218806_s_at	NM_006113	VAV3[b]	Vav 3 oncogene
210275_s_at	NM_006007	ZNF216	Zinc finger, A20 domain containing 2
209944_at	NM_021188	ZNF410[b]	Zinc finger protein 410
213097_s_at	AI338837	ZRF1	Zuotin related factor 1

[a]With ARE in 3′-UTR.
[b]With CURE in 3′-UTR.

(16,43–45) suggested a candidate mechanism. First, three genes that strongly displayed the characteristics of interest, MAP3K7IP2,MRPS18A and TP53BP2, were chosen to confirm the microarray results and to test for any potential connection with Erk1/2 signaling. By RT–PCR, SB enhanced, while PD, a specific Erk1/2 inhibitor, was shown to block NO• effects on the mRNA stability of MAP3K7IP2, MRPS18A and TP53BP2. NO• activation of both p38 MAPK

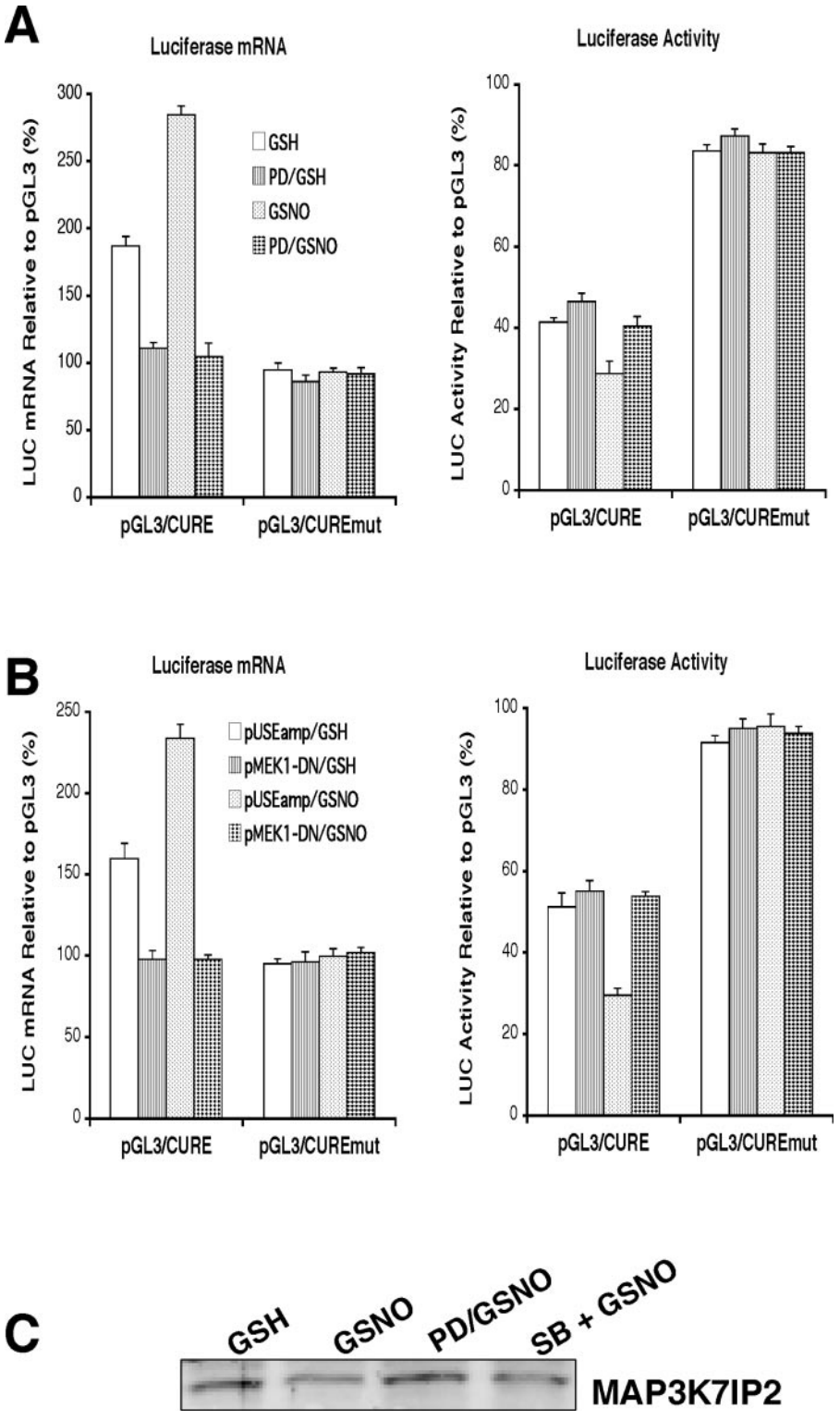

Figure 4. NO• stabilizes CURE-containing mRNA but inhibits its translation through an Erk1/2-dependent mechanism. (**A**) Effect of the Erk1/2 inhibitor, PD98059 (PD; 30 μM), on LUC mRNA levels and LUC activity, respectively. THP-1 cells, transfected with pGL3/CURE, mutant pGL3/CUREmut or control pGL3, were treated with ActD (2.5 μg/ml) for 30 min (for mRNA determinations only) and then incubated with GSH (400 μM) or GSNO (400 μM) for 5 h to measure LUC mRNA by TaqMan® RT–PCR or for 20 h to measure LUC activity. (**B**) Effect of a Mek1 dominant-negative mutant on LUC mRNA levels and LUC activity, respectively. THP-1 cells, co-transfected with pGL3/CURE or mutant pGL3/CUREmut or control pGL3 plus either pUSEamp (empty vector) or pMEK1-DN (dominant-negative Mek1), were similarly treated as in A for measurement of LUC mRNA levels and LUC activity. Data, presented as percentage relative to LUC mRNA level or LUC activity of pGL3, are the mean ± SEM of three to six independent experiments. (**C**) Effect of NO• on the expression of MAP3K7IP2, a naturally-occurring, CURE-containing gene. THP-1 cells (1×10^7) were pretreated with SB (0.1 μM) or PD (30 μM) for 30 min. After 20 h incubation of GSH (400 μM) or GSNO (400 μM), cells were then lysed for western blotting. Each experiment was repeated twice with similar results.

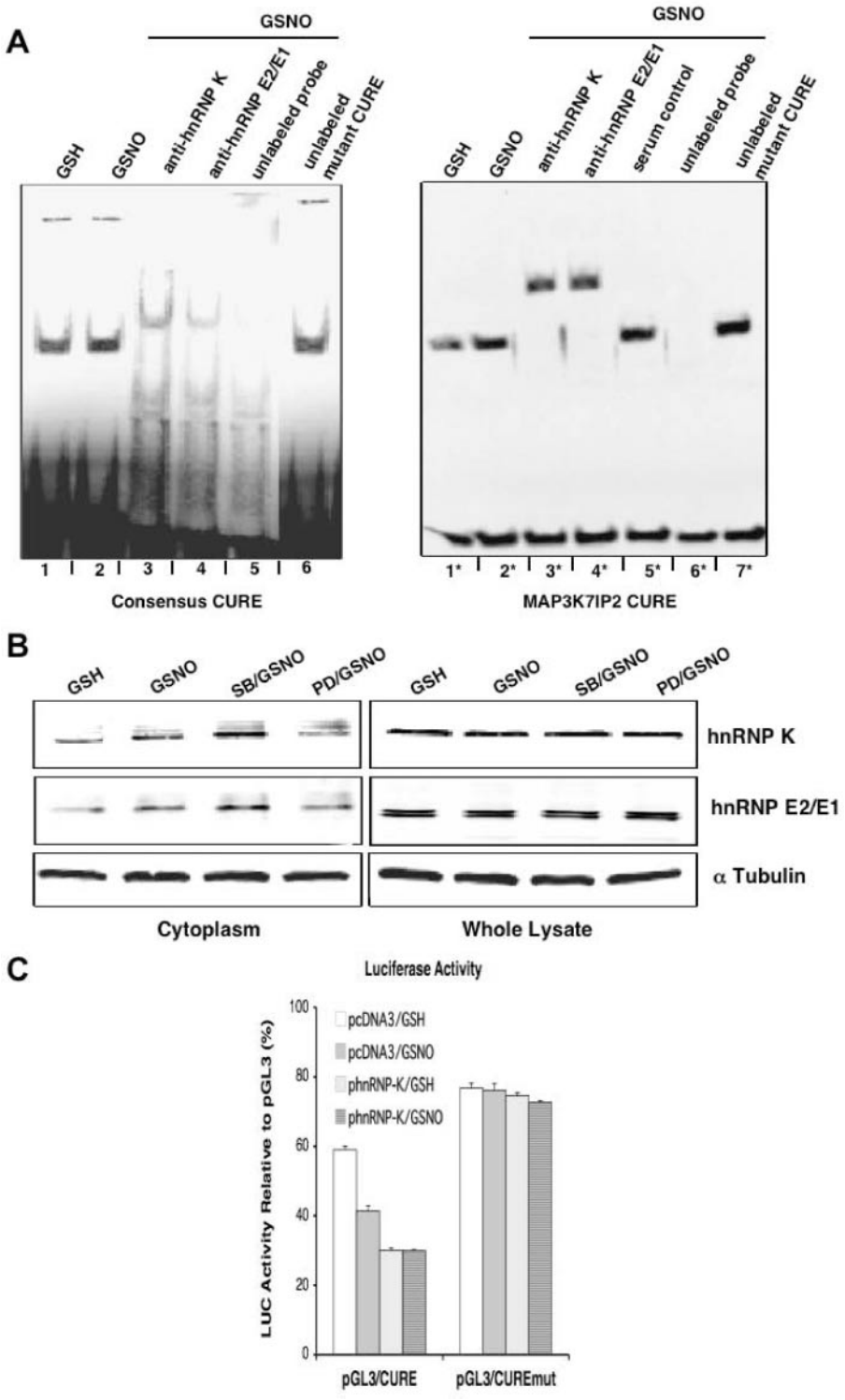

Figure 5. Role of hnRNP K and hnRNP E2/E1 in NO•-Erk1/2-CURE signaling. (**A**) RNA REMSAs with either a consensus (left panel) or MAP3K7IP2 (right panel) CURE riboprobes. GSNO (400 μM) treatment for 3 h increased complex formation compared to control GSH; anti-hnRNP K and anti-hnRNP E2/E1 both super-shift the complex; the unlabeled CURE riboprobes, but not the mutant of consensus CURE (mutant CURE) compete with the labeled CURE riboprobes. (**B**) Translocation of hnRNP K and hnRNP E2/E1 to the cytoplasm by western blotting. GSNO (400 μM) treatment for 3 h increased the presence of hnRNP K and hnRNP E2/E1 in the cytoplasm but not in whole-cell lysates compared to control GSH. This effect was further enhanced by the p38 MAPK inhibitor SB202190 (SB; 0.1 μM), but blocked by the Erk1/2 inhibitor PD98059 (PD; 30 μM). A control protein α tubulin is shown for comparison. Experiments in (A and B) were repeated at least twice with similar results. (**C**) Overexpression of hnRNP K mimicked the effect of NO•, repressing the expression of a chimeric LUC-CURE reporter gene. THP-1 cells were co-transfected with pGL3/CURE, pGL3/CUREmut or control pGL3 and pcDNA3 (empty vector) or phnRNP-K (hnRNP K expression plasmid). After treatment with GSH (400 μM) or GSNO (400 μM) for 20 h, LUC activities were measured. Data, presented as percentage relative to LUC activity with pGL3, are the mean ± SEM of three independent experiments.

and Erk1/2, observed here in THP-1 cells, has also been reported in Jurkat T cells (46,47), but the underlying molecular mechanisms still need to be defined. Notably, previous work has shown that p38 MAPK activation can negatively regulated Erk1/2 in other cell types (48,49). Therefore, the p38 MAPK inhibitor SB potentially may enhance NO• activation of Erk1/2 by blocking this inhibitory effect. Alternatively, SB could activate Erk1/2 through effects on up-stream c-Raf kinase (50), though p38 MAPK modulation of Erk1/2 signaling seems more likely.

In agreement with our data, several groups have demonstrated a major role for Erk1/2 in stabilizing the mRNA of amyloid precursor protein (51), granulocyte–macrophage colony-stimulating factor (44), tristetraprolin (43), nucleolin (52) and transforming growth factor β 1 (45). ARE sites and the RNA-binding proteins HuR and tristetraprolin (44), or a short C/U region and the RNA-binding proteins nucleolin and hnRNP C (51) were variably proposed to mediate these Erk1/2 effects. Of the 60 genes in cluster A that were stabilized by NO• and SB here, only 7 (11.7%) have ARE, but 28 (46.7%) have identifiable CURE in their 3′-UTRs. Of the remaining 160 genes, 37 (23.1%) have ARE, but only 36 (22.5%) have CURE, indicating CUREs but not AREs are over-represented in cluster A. This suggests that CURE may be the main *cis*-element that transduces the mRNA stabilizing effect of Erk1/2. In further support of this, insertion of a consensus CURE sequence, identified from several DICE-containing mRNAs (14), but not its antisense form into 3′-UTR of a LUC reporter gene significantly stabilized its mRNA. NO• further increased the mRNA half-life of this hybrid transcript and the Erk1/2 inhibitor PD or expression of Mek1 dominant-negative mutant both abolished this NO• stabilizing effect.

In concurrent experiments that measured LUC activities instead of mRNA levels, NO•-Erk1/2 signaling was demonstrated to inhibit, rather than enhance, the translation of stabilized, chimeric CURE-containing mRNA. Consistently, NO•-Erk1/2 signaling was further shown to repress protein expression of a natural CURE-containing mRNA, MAP3K7IP2, as measured by western blotting. As discussed earlier, MAP3K7IP2 mRNA was stabilized by NO• through Erk1/2 activation, as demonstrated by RT–PCR. So, NO• activation of Erk1/2 in THP-1 cells stabilized CURE-containing mRNAs but inhibited their translation. These patterns of response, either mRNA stabilization or decreased translation, have been previously reported separately for several DICE-containing genes including α-globin, α-collagen and lipoxygenase (13,14,16,53).

Trans-factors binding to consensus CURE and MAP3K7IP2 CURE were identified by REMSAs performed with cell cytoplasm. NO•-Erk1/2 signaling was shown to increase the cytoplasmic accumulation of hnRNP K and hnRNP E2/E1 and to enhance the formation of a major complex between these proteins and CURE probes. The role of hnRNP K was then confirmed by hnRNP K over-expression which repressed the translation of CURE-containing mRNA, thereby mimicking the effects of NO•. Phosphorylation of hnRNP K by Erk1/2 is known to prompt its cytoplasmic accumulation (16).

Ubiquitously expressed, hnRNP K and hnRNP E2/E1 have triple KH (hnRNP K homology) domains and CURE-binding specificity (54). By binding to CURE, interacting with poly(A)-binding protein, and then blocking deadenylation, these KH domain proteins have been shown to stabilize the mRNA of α-globin (14,54,55), renin (15) and α1 collagen (53). In contrast to their well-characterized ability to stabilize mRNA, hnRNP K and hnRNP E2/E1 have also been shown to repress translation of 15-lipoxygenase (12,13,18) and C/EBPα (17) by blocking 80S ribosome assembly at the AUG initiation site (12,13,17,18). Phorsphorylation of hnRNP K by Erk1/2 increases (16), while phosphorylation by c-Src kinase decreases its repressive effect on 15-lipoxygenase translation (18). Prior data demonstrated that the KH domain complex contributes to the stability of translationally active as well as translationally blocked α-globin mRNA (56,57).

In the present investigation, we have shown that NO• activates Erk1/2 in LPS-stimulated THP-1 cells and subsequently affects hnRNP K and hnRNP E2/E1 stabilization of CURE-containing mRNA transcripts while also suppressing their translation. Although hnRNP K-induced translational repression of 15-lipoxygenase was thought to be dependent on a highly repetitive CU-rich motif previously referred to as DICE (58), the *cis*-element that transduces NO•-Erk1/2 signaling here appears to correspond to a much shorter CURE sequence. CURE is related to a CU-rich consensus sequence (CUUUCU<u>CCCCCA</u>CCCUCUUCU<u>CCCCCUU</u>C-CCCC<u>UCCCC</u>; the underlined nucleotides represent core sequences) that was derived from four DICE-containing mRNAs, namely α-globin, α1 collagen, 15-lipoxygenase and tyrosine hydroxylase (14). However, consistent with the short CURE concept, hnRNP E2/E1-induced C/EBPα repression has been ascribed to a more simple CUCCCCC sequence (17), and the 3′-UTR CURE sequence of MAP3K7IP2 (CUCCGUCUCUACAGAAG), MRPS18A (UCCCAUCCUCUUCATG) and TP53BP2 (CCAGUCCUCCUGCCAGAAAG) described here are also relatively short. Whether NO•-Erk1/2 signaling uniformly stabilizes CURE-containing mRNAs while suppressing their translation cannot be determined from the current study. More likely, under other cellular conditions and through interactions with additional *cis*-acting elements and their binding proteins (such as ARE and HuR or CUGBP2), a variety of responses may be possible.

Accumulating evidence shows that mRNA turnover is linked to translation and these two post-transcriptional processes may be regulated in the same or opposite directions (2,11,24,26,56,57). For rapidly degraded ARE-containing transcripts, including cytokines like IL-8 and cell cycle genes like p21/Waf1/Cip1, mRNA stabilization by NO•-p38 MAPK signaling appears to result in increased protein production (24,26). Conversely, stress-related stabilization of other ARE-containing mRNAs by specific RNA-binding proteins, such as TIAR, HuR and CUGBP2 is associated with translation inhibition (11,59,60). Likewise, a recent microarray study of the endoplasmic reticulum stress response linked global mRNA stabilization to widespread translational repression (2). Adding to these previous reports, our data provide another example of mRNA stabilization coupled to translational inhibition, implicating a CURE *cis*-acting sequence and the RNA-binding proteins hnRNP K and hnRNP E2/E1. NO• regulates this process by activating Erk1/2, which stabilizes mRNA and represses translation by inducing the formation of a mRNA–protein complex. NO•-Erk1/2-

hnRNP-CURE represents a novel mechanism for post-transcriptional regulation that can function as both a mRNA stabilizer and translation inhibitor.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR online.

ACKNOWLEDGEMENTS

This research was supported by the Intramural Research Program of the National Institutes of Health, Clinical Center. Funding to pay the Open Access publication charges for this article was provided by the National Institutes of Health, Clinical Center.

Conflict of interest statement. None declared.

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