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Transcriptional Regulatory Functions of Heterogeneous Nuclear Ribonucleoprotein-U and -A/B in Endotoxin-Mediated Macrophage Expression of Osteopontin

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Osteopontin (OPN) is a highly hydrophilic and negatively charged sialoglycoprotein of ~298 amino acids with diverse regulatory functions, including cell adhesion and migration, tumor growth and metastasis, atherosclerosis, aortic valve calcification, and repair of myocardial injury. OPN is unique as an endogenous negative feedback inhibitor of NO expression. However, the specific cis- and trans-regulatory elements that determine the extent of endotoxin (LPS)- and NO-mediated induction of OPN synthesis are unknown. We have previously shown that LPS-induced S-nitrosylation of heterogeneous nuclear ribonucleoprotein (hnRNP)-A/B inhibits its activity as a constitutive trans-repressor of the OPN transcription by significantly decreasing its DNA binding activity. hnRNPs were originally described as chromatin-associated RNA-binding proteins that form complexes with RNA polymerase II transcripts. The hnRNP family is comprised of >20 proteins that contribute to the complex around nascent pre-mRNA and are thus able to modulate RNA processing. In this subsequent study, again using RAW 264.7 murine macrophages and COS-1 cells, we demonstrate that hnRNP-A/B and hnRNP-U proteins serve antagonist transcriptional regulatory functions for OPN expression in the setting of LPS-stimulated NO synthesis. In the presence of NO, hnRNP-A/B dissociates from its OPN promoter site with subsequent derepression of OPN promoter activity. Subsequently, hnRNP-U binds to the same site to further augment OPN promoter activation. This has not been previously described for the hnRNP proteins. Our results represent a unique transcriptional regulatory mechanism that involves interplay between members of the hnRNP protein family. The Journal of Immunology, 2005, 175: 523–530.
absence of FCS (10%) to induce NO synthesis. In selected instances, the competitive substrate inhibitor of NO synthesis, L-arginine methyl ester (L-NAME; 250 ng/ml) was added. After incubation for 12 h at 37°C in 5% CO₂, the supernatants and cells were harvested for assays.

Plasmids
Expression plasmids for mouse hnRNP-A/B and -U were provided by Dr. Jonathan Dean (Imperial College of Science, Technology and Medicine, London, U.K.) and Dr. Yinon Ben-Neriah (Hebrew University-Hadassah Medical School, Israel), respectively. Both of them are FLAG-fusion plasmids. The OPN reporter plasmid was described previously (3).

Nuclear extract preparation
Cells were washed with PBS and harvested by scraping into cold PBS. The cell pellet obtained by centrifugation was resuspended in buffer containing 10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1.0 mM DTT, and 0.5 mM PMSF; then 10% Nonidet P-40 was added and vortexed briefly. The nuclei were pelleted by centrifugation. The protein concentration was determined by absorbance at 650 nm using protein assay reagent (Bio-Rad).

Western blot analysis
Proteins were separated by 12% SDS-PAGE, and the products were electrotransferred to polyvinylidene difluoride membrane (Amersham Biosciences). The membrane was blocked with 5% skim milk, PBS, 0.05% Tween 20 for 1 h at room temperature. After being washed three times, blocked membranes were incubated with goat polyclonal Ab directed against mouse hnRNP-U, hnRNP-A/B, FLAG-tag, or His-tag (Santa Cruz Biotechnology) for 1 h at room temperature, washed three times in PBS plus 0.05% Tween 20, and incubated with HRP-conjugated secondary Ab for 1 h at room temperature. After an additional three washes, bound peroxidase activity was detected by the ECL detection system (Amersham Biosciences).

Transient transfection and activity assay
DNA transfection of cells was conducted in 12-well plates using Lipofectin (Invitrogen Life Technologies). Briefly, 1 × 10⁶ cells were plated on a 12-well plate and allowed to grow for 24 h before the transfection. Then 2 μg of plasmid DNA diluted in Opti-DMEM and 24 μg of Lipofectin diluted in Opti-DMEM were combined and incubated at room temperature for 20 min. The cells with transfection reagents were incubated for 4 h at 37°C in a CO₂ incubator. Transfection medium was then replaced with 0.6 ml of RIPA buffer (150 mM NaCl, 1 mM EDTA, 0.5 mM DTT, and 0.1% SDS, 100 μg/ml PMSF, and 60 μg/ml aprotinin), then immunoprecipitation was performed using M2-anti-FLAG immunoprecipitation kit (Sigma-Aldrich) or anti-His G Ab (Invitrogen Life Technologies). The purified protein was detected with anti-His G Ab (Invitrogen Life Technologies) or anti-M2-FLAG Ab (Sigma-Aldrich).

Gel shift assays
Gel shift assays were performed using tagged hnRNP-U and -A/B purified from transfected COS-1 cells by immunoprecipitation. In competitive binding assays, unlabeled oligonucleotides were added at 200 M excess. In noncompetitive assays, unlabeled SPI consensus oligonucleotides (Promega) were used. The oligonucleotide (nt-174 to nt-202) used in gel shift was as follows: 5′-GAAAGGATGTATAGCATGCCTAC-3′. Probe was prepared by end labeling the wild-type 29-bp double-stranded oligonucleotides with [γ-32P]ATP (2500 Ci/mmole) using T4 polynucleotide kinase, followed by G-50 column purification. The reactions were resolved on a 6% nondenaturing acrylamide gel in 1× Tris borate-EDTA buffer. All of the oligonucleotides used in the gel shift are HPLC grade.

Purification of transcription factor
The transcription factor was isolated by reacting the biotinylated DNA-protein complex with streptavidin paramagnetic particles (Dynal Biotech). Nuclear proteins were isolated from RAW 264.7 cells, as previously described. Protein concentration of the nuclear extract was determined using the Bio-Rad protein assay system (Amersham Biosciences).

Antisense analysis of hnRNP-U
The sense and antisense oligonucleotides (nt 1903–1921) were designed according to GenBank sequence NM_016805 to block the expression of LPS stimulation and hnRNP-U binding to OPN promoter DNA.

Statistical analysis
The data are expressed as the means ± SEM. Analysis was performed using Student’s t test. Values of p < 0.05 were considered significant.

Results
LPS stimulation and hnRNP-U binding to OPN promoter DNA
We have previously demonstrated that hnRNP-A/B p37 is a constitutive transcriptional repressor that is bound to the OPN promoter in unstimulated control RAW 264.7 macrophages. The core...
binding sequence is AGTTATG, located between nt-183 and nt-196 of the OPN promoter. The sequence specificity was confirmed with in vitro DNA footprinting. Using the biotin-streptavidin DNA affinity technique with the identified DNA binding sequence, hnRNP p37 was purified, isolated, and identified from nuclear extract isolated from unstimulated control RAW 264.7 macrophages. In the setting of LPS-mediated NO synthesis, OPN transcription is enhanced, in part, through S-nitrosylation of hnRNP p37, with associated inhibition of both DNA binding and transcriptional repression (7).

We then sought to determine the potential role of transcriptional activation factors in LPS-dependent enhancement of OPN expression. We again used the biotin-streptavidin DNA affinity technique with the previously identified core binding sequence (Fig. 1). Nuclear protein was isolated from LPS-stimulated RAW 264.7 murine macrophages. One major band was identified. Southwestern blot analysis was performed using radiolabeled tandem double repeat DNA probe (nt-183 to nt-196) containing the described binding sequence; this demonstrated binding to the identified band. Subtraction of the molecular mass of the DNA probe indicates that the molecular mass of the bound protein is ~120 kDa. The band was therefore excised and subjected to protein sequencing. Analysis of two separate tryptic digests of band 1 yielded identical matches with hnRNP-U or scaffold attachment factor-A (NCBI NP058085). In contrast to that found with unstimulated RAW 264.7 cells, LPS stimulation is associated with binding of hnRNP-U rather than hnRNP-A/B to the core binding sequence of AGTTATG in the murine OPN promoter.

Expression of hnRNP-A/B and -U protein in RAW 264.7 macrophages

To determine relative expression of hnRNP-A/B and hnRNP-U protein in RAW 264.7 macrophages, immunoblot analysis was performed in cytoplasmic and nuclear protein fractions (Fig. 2).

LPS (100 ng/ml) was added in the absence of FCS (10%) to induce NO synthesis. In selected instances, the competitive substrate inhibitor of NO synthase L-NAME (250 ng/ml) was added. After incubation for 12 h, cells were harvested for assays. Nuclear and cytoplasmic proteins were separated by 12% SDS-PAGE, and the products were electrotransferred to polyvinylidene difluoride membrane (Amersham Biosciences). The membrane was blocked, washed, and incubated with goat polyclonal Ab directed against mouse hnRNP-U or hnRNP-A/B (Santa Cruz Biotechnology), followed by HRP-conjugated secondary Ab. Bound peroxidase activity was detected by the ECL detection system (Amersham Biosciences). Blot is representative of three experiments.

Effect of hnRNP-A/B and -U on OPN promoter activity

The relative role of hnRNP-A and hnRNP-U in OPN promoter activity was examined by cotransfection in COS-1 cells of hnRNP-A/B, μ-hnRNP-U (with deletion of the DNA binding region), and/or wild-type (wt)-hnRNP-U with the 1.5-kb murine OPN promoter-luciferase reporter construct (Fig. 3A). To aid in deciphering the contributions of NO to the various roles of hnRNP-A/B and -U, 100 μM S-nitroso-N-acetyl-penicillamine (SNAP) was added as an exogenous source of NO, because we have previously determined that S-nitrosylation of hnRNP-A/B decreases DNA binding with dissociation from the OPN promoter and reversal of OPN promoter inhibition. Contributions of the two hnRNPs were normalized to a relative protein expression of 1:1 as determined by immunoblot analysis (data not shown). In control cells, only the OPN promoter-luciferase construct is transfected.

In the presence of hnRNP-A/B, OPN promoter activity was decreased by 80% (p < 0.01 vs unstimulated control cells). When a source of NO was added to cells transfected with hnRNP-A/B, OPN promoter activity increased >5-fold compared with controls (p < 0.01) and >20-fold compared with hnRNP-A/B (p < 0.01). This corroborates our previous findings in which NO-reversed
hnRNP-A/B induction of OPN promoter activity. Transfection of SNAP alone did not alter OPN promoter activity. In the presence of both hnRNP-A/B and wt-hnRNP-U, OPN promoter activity was not statistically different from that of hnRNP-A/B alone. However, when SNAP was also added, promoter activity was enhanced by an additional 3-fold over that noted with hnRNP-A/B + SNAP ($p < 0.01$). In the setting of transfection of $\mu$-hnRNP-U in which the DNA binding sequence is deleted, the effects of wt-hnRNP-U are negated. Finally, when wt-hnRNP-U is added alone without the presence of hnRNP-A/B as a repressor, OPN promoter activity is 13-fold greater than control cells ($p < 0.01$). SNAP does not alter the effects of wt-hnRNP-U or $\mu$-hnRNP-U (data not shown).

**FIGURE 3.** Transient transfection analysis of hnRNP-A/B interactions with the OPN promoter in COS-1 cells. A, The full-length OPN promoter construct was transfected into COS-1 cells. Expression vectors for hnRNP-A/B, wild-type hnRNP-U (wt-hnRNP-U), and/or mutant hnRNP-U with deletion of the DNA-binding domain ($\mu$-hnRNP-U) were cotransfected. SNAP (100 $\mu$M) served as an exogenous source of NO. B, The full-length OPN-Full, OPN-Point, and OPN-Deletion promoter constructs were transfected into COS-1 cells. In OPN-Point, the hnRNP-binding site AGTTATG was mutated to CTGCCGT in the full-length OPN promoter. In OPN-Deletion, the hnRNP site was simply deleted. OPN-Full represents the full-length wt OPN promoter. In COS-1 cells, point mutation or deletion of the hnRNP-A/B-binding site results in OPN promoter activity that is not statistically different from control cells in which the hnRNP expression vectors have not been transfected. In both of these settings with OPN-Point or OPN-Deletion, the effects of hnRNP-A/B, hnRNP-U, and/or SNAP are ablated.

In total, these data suggest that: 1) hnRNP-A/B acts to repress OPN promoter activity; 2) NO reverses hnRNP-A/B repression of OPN promoter activity; 3) in the presence of NO-mediated reversal of hnRNP-A/B repression, hnRNP-U acts as a further transcriptional activator of the OPN promoter; and 4) these antagonistic trans-regulatory activities of hnRNP-A/B and hnRNP-U require the same DNA binding region as the OPN promoter.

Relative in vivo and in vitro DNA binding activity of hnRNP-A/B and hnRNP-U

To determine the relative DNA binding activities of hnRNP-A/B and hnRNP-U, gel shift studies were initially performed with purified FLAG-hnRNP-A/B and/or FLAG-hnRNP-U with a labeled DNA oligonucleotide containing the previously identified binding sequence. Competition studies were also performed. In the first study, FLAG-hnRNP-U binding to DNA was assessed and FLAG-hnRNP-A/B (10-fold diluted) subsequently was added to compete for DNA binding. (Fig. 4A) In this setting, hnRNP-U binding was specific, but exhibited substantially less affinity for the oligonucleotide probe than hnRNP-A/B and was efficiently displaced by hnRNP-A/B. To confirm the identity of the protein bound to the oligonucleotide probe, supershift assays were performed using FLAG-Ab (Fig. 4B). This result further demonstrates hnRNP-U binding to DNA. The converse study was then performed, as depicted in Fig. 4C. hnRNP-A/B bound the DNA probe with great avidity and specificity, and hnRNP-U, even when added in >6-fold excess, did not compete for binding. (Fig. 4C) These results suggest that hnRNP-A/B exhibits greater DNA binding affinity for the labeled oligonucleotide probe than hnRNP-U. These studies were then repeated in the presence of an exogenous source of NO, S-nitrosoglutathione (GSNO). In Fig. 4D, gelshift studies were performed with FLAG-hnRNP-U. In the presence of GSNO and/or DTT, there was no alteration in hnRNP-U DNA binding. We have previously demonstrated that NO inhibits hnRNP-A/B DNA binding. In Fig. 4E, competition DNA binding studies were performed in the presence of an exogenous source of NO. As previously demonstrated, the presence of hnRNP-A/B and hnRNP-U together results only in binding of hnRNP-A/B. However, in the presence of GSNO, binding for both hnRNP-U and hnRNP-A/B is noted. Further addition of the reducing agent, DTT, ablates both the DNA binding of hnRNP-U and the decreased DNA binding of hnRNP-A/B.

To determine whether there is in vivo binding of hnRNP-A/B or hnRNP-U to the segment of interest in the OPN promoter, ChIP assays were performed in unstimulated control, LPS, and LPS + l-NAME-stimulated RAW 264.7 macrophages and FLAG-tagged
hnRNP-A/B or hnRNP-U (Fig. 5). In unstimulated control cells, there is in vivo binding of hnRNP-A/B, but not hnRNP-U. In contrast, in LPS-stimulated cells, only hnRNP-U binding was detected. Finally, in LPS + L-NAME cells, only hnRNP-A/B binding is found. No binding is noted in the presence of irrelevant PCR primer set (data not shown). These in vivo results suggest that hnRNP-U binding to the OPN promoter occurs only in the setting of LPS-mediated NO synthesis. In combination with our previous results, this suggests that S-nitrosylation of hnRNP-A/B with decreased DNA binding allows subsequent binding of hnRNP-U to the same site in the OPN promoter.

**Protein-protein interactions between hnRNP-A/B and hnRNP-U**

To determine potential interaction between hnRNP-A/B and hnRNP-U, immunoprecipitation studies were performed using FLAG-hnRNP-U and HIS-hnRNP-A/B. (Fig. 6A) In the first study, FLAG-hnRNP-U and HIS-hnRNP-A/B were cotransfected into COS-1 cells. FLAG Ab was then used to immunoprecipitate FLAG-tagged protein, and His Ab was used to probe the resulting blot. There was no evidence for interaction in this instance. Cell lysate protein was also probed with His Ab, and His-hnRNP-A/B was found to be adequately expressed. The converse experiment was then performed (Fig. 6B). His Ab was used to immunoprecipitate His-tagged protein, and FLAG Ab was used to probe the resulting blot. There was again no evidence for interaction between the two hnRNP proteins. Again, cell lysate protein probed with FLAG Ab demonstrated adequate expression. Equivalency of protein loading was confirmed by silver staining of the appropriate gels (data not shown).

The potential contribution of NO to protein-protein interactions was also measured. The above studies were repeated in the presence and absence of SNAP (100 μM), an exogenous source of NO (Fig. 6, C and D). FLAG-hnRNP-U and HIS-hnRNP-A/B were cotransfected into COS-1 cells. FLAG Ab was then used to immunoprecipitate FLAG-tagged protein, and His Ab was used to probe the resulting blot. There was no evidence for interaction in this instance. Cell lysate protein was also probed with His Ab and His-hnRNP-A/B was found to be adequately expressed. The converse experiment was then performed (Fig. 6B). His Ab was used to immunoprecipitate His-tagged protein, and FLAG Ab was used to probe the resulting blot. Again, there was no evidence for interaction in the presence or absence of NO.

**Functional relevance of hnRNP-U in OPN expression**

To assess the functional relevance of hnRNP-U in the setting of LPS- and NO-dependent regulation of OPN expression, antisense...
oligonucleotides were designed to inhibit hnRNP-U expression. OPN protein expression in LPS-stimulated RAW cells was measured by immunoblot analysis (Fig. 7A). In normal RAW cells and RAW + sense hnRNP-U, OPN expression was increased by 5-fold in the presence of LPS \((p < 0.02 \text{ vs control})\). In the presence of LPS + L-NAME, NO production was inhibited, and OPN expression was equivalent to that of the control cells. Antisense oligonucleotides were then used to inhibit hnRNP-U expression. In this setting, LPS stimulation is associated with a significantly blunted increase in OPN protein expression \((p < 0.01 \text{ vs control and LPS + L-NAME})\). In antisense-mediated inhibition of hnRNP-U, LPS + L-NAME results in a level of OPN expression that is not different from that of controls. Overall, the presence or absence of antisense hnRNP-U in RAW 264.7 cells did not alter OPN expression in control or LPS + L-NAME cells; only OPN protein expression in the presence of LPS was altered. As we have previously demonstrated, S-nitrosylation of hnRNP-A/B is critical to inhibit its DNA binding and de-repress OPN promoter activity. Control experiments demonstrated that antisense oligonucleotides, but not sense oligonucleotides, directed against hnRNP-U effectively inhibited expression of this protein under all treatment conditions (Fig. 7B) These data demonstrate that hnRNP-U-dependent augmentation of OPN protein expression requires the presence of NO. In combination with results reported above, this suggests that NO must first inhibit hnRNP-A/B DNA binding before hnRNP-U can bind to transactivate the OPN promoter.

**Discussion**

In this study, we use a system of endotoxin-stimulated RAW 264.7 murine macrophage expression of inducible NO synthase (iNOS) to assess the roles of hnRNP-A/B and hnRNP-U in regulating OPN transcription. We have previously shown that LPS-induced S-nitrosylation of hnRNP-A/B inhibits its activity as a constitutive trans-repressor of the OPN transcription by significantly decreasing its DNA binding activity (7). In this subsequent study, we demonstrate that hnRNP-A/B and hnRNP-U proteins serve antagonistic transcriptional regulatory functions in the setting of LPS-mediated OPN synthesis. In the presence of NO, hnRNP-A/B dissociates from its OPN promoter site with subsequent de-repression of OPN promoter activity. Subsequently, hnRNP-U binds to the same site to further augment OPN promoter activation. This has not been previously described for the hnRNP proteins. Our results represent a unique transcriptional regulatory mechanism that involves antagonistic regulatory roles for these two hnRNP proteins.

The exact immunologic roles of OPN are unclear, but it appears to have both inflammatory and anti-inflammatory characteristics (8). OPN is expressed by a variety of inflammatory cells including T cells, macrophages, and NK cells (8, 9). Originally identified as early T cell activation gene-1, expression of OPN is increased inflammatory processes, ranging from infection of macrophages by mycobacteria to atherosclerosis. As a proinflammatory agent, OPN induces chemotaxis and haptotaxis of T cells and macrophages in vitro, functioning as a chemoattractant (9). OPN can also interact directly with the critical cell surface proteins to regulate the expression of matrix metalloproteinases required for invasion through the extracellular matrix (10–12). OPN can also influence differentiation among the Th1 or Th2 phenotypes, by interacting with integrins and/or CD44 to enhance Th1 while also inhibiting Th2 cytokine expression (13, 14). OPN may exert its anti-inflammatory effects by inhibiting NO production. In vitro, OPN down-regulates iNOS and NO production by macrophages and kidney tubule epithelial cells (15, 16). During sepsis, OPN expression is increased in the vasculature, where it attenuates iNOS activity and blocks the production of NO metabolites (17). We, among others, have demonstrated that NO stimulates expression of OPN, which, in turn, inhibits iNOS transcription and reduces NO production, thus establishing an autoregulatory negative feedback loop (3, 18). The immune roles of OPN continue to be elucidated, but clearly, the relationship between OPN and NO production through the OPN-mediated feedback inhibition of iNOS expression represents an intriguing function. In this study, we invoke a transcriptional role for hnRNP in LPS- and NO-dependent OPN expression.

The hnRNPs were originally described as a group of chromatin-associated RNA-binding proteins that form complexes with RNA polymerase II transcripts. The hnRNP family is a collection of >20 proteins that contribute to the complex around nascent pre-mRNA and are thus able to modulate RNA processing (4–6). Members of the group are characterized by their ability to bind to RNA with limited specificity and they are among the most abundant of all of the nuclear proteins. Despite its function in RNA handling, the precise physiological role of hnRNPs has yet to be fully defined and may include transregulatory effects. Recent studies have shown that the hnRNPs DDB, E2BP, and K are able to bind to dsDNA motifs within the complement receptor 2, hepatitis B virus, and c-myc promoters, respectively (19–21). The hnRNPs can also repress viral DNA replication, repress estrogen- and vitamin D-induced transcription, and transcriptionally regulate various genes, including the rat spt 2 gene (4, 22–24). A transcriptional regulatory role for hnRNPs was first determined for hnRNP-K, which has both activator and repressor functions (25). Formation of a complex between hnRNP-K and C/EBP inhibited activation of the α-1-acid glycoprotein gene. Similarly, transcription of the EBV EBNA-1 genes is activated by a heterodimer formed in part by hnRNP-D (26).

hnRNP-U (120 kDa, 806 amino acids) is known as an RNA- and a scaffold/matrix attachment region DNA-binding protein. Approximately 50% of total hnRNP-U is present in the nuclear matrix, and 20% is tightly associated with chromatin, whereas half of hnRNP-U in the remaining 30% soluble fraction is found in the...
hnRNP particles. hnRNP-U is thought to participate in pre-mRNA processing together with other hnRNP proteins and/or to play a role in the higher order organization of chromatin by organizing the genome into topologically independent domains. In addition, hnRNP-U appears to be involved in RNA metabolism because it interacts with other hnRNP complex components and with the carboxyl-terminal domain of RNA polymerase II. Interaction of hnRNP-U with RNA polymerase II, transcription factors such as the glucocorticoid hormone receptor, and the histone acetylase CBP/p300 has indicated a role in the regulation of gene expression, and in X chromosome-specific transcriptional inactivation. In addition, a subpopulation of hnRNP-U is present in hnRNP particles, suggesting that the protein might also be involved in RNA maturation and transport. As demonstrated by Fackelmayer et al. (27–29), the amino-terminal domain of hnRNP-U is composed of two regions, a “spaced leucine motif” (aa 1–45) and a less well defined glutamine-rich region (aa 158–247), both of which are indispensable for DNA binding activity. Our data suggests a role for hnRNP-U as a transcription factor; this has not been previously described. However, as previously discussed, other members of the hnRNP family, such as -A/B, -K, and -D, can function in this context.

In this study, hnRNP-A/B and -U function in opposition with regard to OPN transcription in the setting of macrophage NO synthesis. There have been other situations in which members of a transcription factor family act in opposing fashions. For example, phosphorylated STAT1 homodimers translocate to the nucleus and activate several IFN regulatory factors (IRFs), including, IRF-1 and IRF-2. IRF-1 is strongly inducible by IFN-γ and binds IFN-stimulated responsive elements (ISRE) within promoters, activating transcription. In contrast, IRF-2 is constitutively expressed and acts mostly as a repressor by competing with IRF-1 for the same.

![Image of figure 6](http://www.jimmunol.org/)

**FIGURE 6.** Interaction between hnRNP-A/B and -U proteins. To determine potential interaction between hnRNP-A/B and hnRNP-U, immunoprecipitation studies were performed using FLAG-hnRNP-U and HIS-hnRNP-A/B. FLAG-hnRNP-U and HIS-hnRNP-A/B were cotransfected into COS-1 cells. Immunoblot and Coimmunoprecipitation studies were performed. A, FLAG Ab was used to immunoprecipitate FLAG-tagged protein, and His Ab was used to probe the resulting blot. The blot is representative of three experiments. B, His Ab was used to immunoprecipitate His-tagged protein, and FLAG Ab was used to probe the resulting blot. The blot is representative of three experiments. C, To determine a potential role for NO in protein-protein interactions, SNAP (100 μM) was added as an exogenous source of NO. FLAG Ab was used to immunoprecipitate FLAG-tagged protein, and His Ab was used to probe the resulting blot. CT, unstimulated control cells. The blot is representative of three experiments. D, To determine a potential role for NO in protein-protein interactions, SNAP (100 μM) was added as an exogenous source of NO. His Ab was also used to immunoprecipitate His-tagged protein, and FLAG Ab was used to probe the resulting blot. The blot is representative of three experiments.
The authors have no financial conflict of interest.

Disclosures

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References


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