SUPPLEMENTARY MATERIAL

Oligonucleotides for PCR

Oligonucleotide pairs used for the preparation of PCR templates bearing the T7 RNA polymerase promoter sequence (CCAAGCTTCTAATACGACTCACTATAGGGAGA) were as follows:
For preparing templates corresponding to the p21 mRNA, oligonucleotide pairs used were
(T7)GCCGAAGTCAGTTCCTTGTG and TTCCAGGACTGCAGGCTTC for CR,
(T7)CCAAGAGGAAGCCCTAATCC and CAGGGTATGTACATGAGGAG for 3'A,
(T7)CTCCTCATGTACATACCCTG and CCTCTCATTCAACCGCCTAG for 3'B,
(T7)CTAGGCGGTTGAATGAGAGG and GAAAAGGAGAACACGGGATG for 3'C, and
(T7) CATCCCGTGTTCTCCTTTTC and AAAGTCACTAAGAATCATTTATTG for 3'D.
For preparing templates corresponding to the cyclin D1 mRNA, oligonucleotide pairs used were
(T7)TAGCAGCGAGCAGCAGAGT and CTCAGATGTCCACGTCCCG for CR,
(T7)ACATCTGAGGGCG CCAGG and TTCCGGTGTGAAACATCTAAG for 3'A,
(T7)TCCAGGTTCAACCCACAGCTA and CACCTCCCTTCAACACTTCC for 3'B,
(T7)AGAGTGTGGAGGCTGACGTGT and CCACCATGGCTAAGTGAAGC for 3'C and
(T7)GCTGTTTCACAATACCTCATGC and TGTGAGCTGGCTTCATTGAG for 3'D.
p21 transcripts CR, 3'A, 3'B, 3'C, and 3'D were 632-b, 188-b, 120-b, 124-b, and 1302-b long, respectively.
Cyclin D1 transcripts CR, 3'A, 3'B, 3'C, and 3'D were 1060-b, 1413-b, 925-b, 432-b, and 537-b long, respectively.

Oligonucleotide pairs for the detection of HuR target transcripts, AUF1 target transcripts, or transcripts that were targets of both proteins were as follows:
CAGAACACGGCTCACGCTTAC and CTTGCCCCATCACGACAGAC for cyclin D1;
GGGGAAGGGACACACAAGAAGA and AATGAACTGGGGAGGGATGG for p21;
TGCACCACCAACTGCTTAGC and GGCATGGACTGTGGTCATGAG for GAPDH;
TGGGAACAAGAGGGCATCTG and CCACCACTGCATCAAATTCATG for SDHA;
GCTGGCTGTGTGTTGGCTGA and ACCCGCACTCCTGAGGTGAA for GSK3;
TCTGGCAAACCACGGACAGA and ACGCTTGGTTTCCGAGGATG for MTA1;
CGCACATTGTCCAATCCAGTG and TGTTCCGTGCGAATGAAAACC for PPP1CB;
CATTTGGCAGGGGGAGAATG and CACACCAGGGAGTGCGAATG for TIMP3;

TGCTCGAATTACGGCCACAC and GCCAAAGCTGGATTTGATTGC for CDK7; GGCTGTGGATTTAATGTGGAAATGA and GATAAGGTGTGAAACACTCAGCTTTCT for CXCL5; GCAGCCAAGATGGTGAAGCA and GGAATGTTGGCGTGCATTTG for TXN; AGACTGCATCCGAGCCTTCC and XRCC5 RP TTTGTCTTTGGGGGCCAGAA for XRCC5; GCGCCATCGCCAAGTAAGAA and GCTGGATGCCGTCTAGAGTCCTA for CSP3; GGAAAAGCAGGAAGGAGGAGTGA and GCCTTTCGTTGTCCTTTTTCCA for RAB10; TGTGTTCCTGGCCCACTCTG and CAGCCATCAGCAAGAGTACAGCA for EIF4E; and CCAACCCAAACCATGAGAA and GGTCACACCACAAGTAAAGTCAG for PTMA.

Oligonucleotide pairs for the detection of intron sequences were as follows: ACCGGCTTCTTCCACTGCTC and ACCCCACACACCATCACCAC for cyclin D1 intron 4, and GCCCCTTGCCATCCTTTTAG and AATGCCGGGCCCATATAAAC for p21 intron 2.

Oligonucleotides for Northern blotting

Oligonucleotides used for Northern blot analysis were ACGGTATCTGATCGTCTTCGAACC for 18S rRNA, AGGTTGGCATCGGGGTACGCGCGGCGGATGGTTTCCACTTCGCAGCACAGGAGCTGGTG for cyclin D1 mRNA, and CTGGGCCGAAGAGGCGGCGGCAGGCCTTGCTGCCGCATGGGTTCTG for p21 mRNA.

RNA interference

For HuR RNAi analysis, siRNA duplexes (10 nM, Qiagen) directed against sequences TTCTCCGAACGTG TCACGT (Ctrl. siRNA) or AAGAGGCAATTACCAGTTTCA (HuR siRNA) were transfected using Oligofectamine (Invitrogen).

For AUF1 RNAi analysis, pSILENCER-AUF15 plasmid was generated by insertion of annealed oligonucleotides GATCCCGTTGTAGACTGCACTCTGAttcaagagaTCAGAGTGC AGTCTACAACTTTTTTGGAA and AGCTTTTCCAAAAAAGTTGTAGACTGCACTCTGAtctcttgaaT CAGAGTGCAGTCTACAACGG into the HindIII and BamHI sites of pSILENCER 2.0-U6 (Ambion, Inc.), which target nucleotides 650-670 of AUF1 (NM_031370) with a 9-b loop insertion (lowercase).

Cells were transfected using Lipofectamine 2000 (Invitrogen); control transfections were performed with pSILENCER 2.0-U6.

Antibodies for Western blotting

Primary antibodies were used for the detection of HuR (3A2), β-tubulin, and TFIID (Santa Cruz Biotech), AUF1 and p21 (Upstate Biotech.), cyclin D1 (Cell Signaling), GAPDH and β-actin (Abcam). Antibodies recognizing hrRp4p were a gift from C.-Y. Chen; antibodies recognizing hnRNP A1 and hnRNPC1/C2 were gifts from G. Dreyfuss. Secondary antibodies used were HRP-conjugated anti-mouse and anti-rabbit (Amersham); for AUF1 westerns from IP samples, protein A-HRP was used to avoid masking of the AUF1 bands by the IgG heavy chain band. Protein signals were detected by using ECL-Plus (Amersham).

Cell Fractionation

Cytoplasmic and nuclear fractions were prepared as described (Feng *et al*, 1997) with minor modifications. Briefly, 20 million cells were incubated on ice for 5 min in 750 µl of Cytoplasmic Lysis Buffer containing 20 mM Tris-HCl [pH 7.5], 100 mM KCl, 5 mM $MgCl_2$, 0.3% IGEPAL CA-630, 1000 U/ml of RNaseOUT (Invitrogen), plus inhibitors (1 mg/ml each Aprotinin, Pepstatin, and Leupeptin, and 1 mM PMSF), then centrifuged (10,000 × g, 10 min). After removing the supernatant (cytoplasmic fraction), the pellet was resuspended in 1 ml IP buffer (10 mM Tris-HCl [pH 7.5], 100 mM NaCl, 2.5 mM $MgCl_2$, 0.5 % Triton X-100, 1000 U/ml RNaseOUT, plus inhibitors) and sonicated. The resulting suspension (500 µl) was layered onto a 30% sucrose cushion (500 µl) in IP buffer and centrifuged (5,000 × g, 15 min) to remove chromatin, nucleoli and other insoluble structures; the upper layer (~ 500 µl) was designated as the nuclear extract.

RNP Crosslinking in Intact Cells

Crosslinking with UVC: Two million HeLa cells were washed with PBS and irradiated with 650 J/m^2 UVC for the crosslinking of RNA-protein complexes in intact cells (Mili and Piñol-Roma, 2003). Cells were then lysed with 3 ml of lysis buffer (PBS containing 1% Empigen BB, a detergent that disrupts all non-covalent interactions such as protein-protein and protein-RNA interaction but does not dissociate antigen-antibody interaction). After passing the lysate 10 times through a 25-gauge needle and centrifugation (4°C, 21,000 × *g*, 10 min), the supernatant was divided into two parts and used for IP (1 h,

4°C) with protein A Sepharose beads coated with either IgG or AUF1 antibody. Beads were washed three times with 1 ml lysis buffer and once with 1 ml NT2 buffer. Beads were then incubated (1 h, 30°C) with 100 µl NT2 buffer containing 10 µg RNase A and 10 U RNase T1 to release proteins covalently bound to the RNA. After a brief additional centrifugation, SDS-PAGE sample buffer was added to the supernatant and samples were subjected to SDS-PAGE and western blotting using an anti-HuR antibody.

Crosslinking with formaldehyde: Two million HeLa cells were crosslinked with formaldehyde according to the procedure of Niranjanakumari *et al,* (2002). Briefly, cells were treated with 1% formaldehyde for 10 min at room temperature, whereupon glycine was added to stop the crosslinking reaction. The cells were then washed with PBS, resuspended in 1 ml RIPA buffer, and sonicated. The supernatant obtained after centrifugation was used for IP using either IgG1 or AUF1 antibody for 1 h at room temperature. The beads were washed three times with 1 ml high-stringency RIPA buffer, resuspended in 100 µl elution buffer and kept at 70°C for 45 min to reverse the crosslinks. SDS-PAGE sample buffer was added to the supernatant obtained after reversal of crosslinks and proteins were subjected to SDS-PAGE and Western blotting using an anti-HuR antibody.

Mili S, Pinol-Roma S (2003) LRP130, a pentatricopeptide motif protein with a noncanonical RNA-binding domain, is bound in vivo to mitochondrial and nuclear RNAs. *Mol Cell Biol* **23:** 4972-4982

Niranjanakumari S, Lasda E, Brazas R, Garcia-Blanco MA (2002) Reversible cross-linking combined with immunoprecipitation to study RNA-protein interactions in vivo. *Methods* **26:** 182-190