HuR Interacts with a Large Number of mRNA Ligands in Mouse Hippocampus after Seizure

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Summary

HuR is an ubiquitously expressed AU-rich element (ARE)-binding protein which interacts with and stabilizes early response gene (ERG) mRNAs after cell activation or stress. To date, approximately, 20 mRNAs have been defined as HuR ligands and subject to post-transcriptional gene regulation. Given the discordance between the large number of ERG mRNAs and those few defined as HuR ligands, we applied *in vitro* mRNA selection to isolate previously unknown HuR mRNA ligands from post-seizure mouse hippocampus. Selected mRNAs were converted into cDNA libraries and sequenced. Using this approach, we have identified over 600 novel, putative HuR mRNA ligands. These genes code for a variety of proteins including transcription factors, signaling molecules and kinases, but many are presently novel. Consistent with the means of their selection, several of these HuR ligands are differentially expressed in hippocampus after seizure. These results suggest a rapid means to identify and characterize the diverse repertoire of ligands for regulatory mRNA binding proteins.

Introduction

Early response genes (ERGs) comprise a subset of genes whose activation and expression is triggered by changing environmental conditions. In general, ERGs code for key regulators including transcription factors, kinases, receptors and cytokines. Under resting conditions, these mRNAs are weakly expressed through a combination of transcriptional repression and rapid mRNA decay. Within minutes of cell activation, cascades of ERGs are sequentially expressed, translated and directing alterations in cell physiology. Under these conditions, the decay rate of many ERG mRNAs is attenuated which contributes to their rapid accumulation and pulsatile expression.

Appropriate ERG mRNA decay depends on the interaction between 3'-untranslated region (UTR) AU-rich elements (AREs) and sequence-specific mRNA binding proteins. AREs are structurally diverse with so-called Class I AREs consisting of 1-3 copies of the AUUUA pentamer within a uridine-rich region while Class II AREs contain at least two overlapping copies of the UUAUUUA(U/A)(U/A) nonamer within an uridine-rich region while Class III AREs lack the typical AUUUA elements but possess uridine-rich sequences (1). All three classes of AREs are destabilized in resting cells probably through interaction with the AU-mRNA binding proteins AUF1, TTP and/or KSRP which associate with the exosome degradative machinery (2). After cell activation, ARE mRNAs combine with stabilizing mRNA binding protein(s) which oppose the function of AUF1, TTP and KSRP and prolong mRNA half-life. The best characterized of these binding proteins is HuR, a ubiquitously expressed member of the ELAV-like family of RNA-binding proteins (3). HuR selectively binds to and stabilizes ARE-containing mRNAs including c-fos (4), cyclins A and B (5), vascular endothelial growth factor (VEGF) (6), TNF-α (7), p21 (8) and cyclooxygenase–2 (COX-2) (9) mRNAs. HuR likely associates with ARE-containing mRNAs in the nucleus and protects the bound mRNAs from RNase attack during and after export to the cytoplasm (10).

HuR contains three RNA recognition motifs (RRMs) and a novel shuttling sequence termed the hinge region or HuR Nucleocytoplasmic Shuttling sequence (HNS) (amino acids 190-244) between the second and

third RRMs. The first two RRMs mediate ARE recognition (11) while the third RRM has been implicated in poly(A) tail binding (12) and is required for ARE-mediated mRNA stabilization (10). The HNS domain, containing a nuclear localization signal (NLS) and nuclear export signal (NES), mediates nuclear/cytoplasmic shuttling (13). The HNS of HuR contains a basic sequence (205-RRFGGPVHHQAQRFRF-220) similar to the bipartite nuclear localization signal (NLS) consensus sequence found in nucleoplasmin and HIV-1 Rev (13). HuR migrates across the nuclear membrane via two mRNA export receptors, transportin 2 (Trn2) and chromosome maintenance 1 (CRM1) (14). Heat shock-induced stress disrupts the Trn2/HuR interaction shifting export solely to the CRM1 pathway. The existence of two distinct HuR nuclear export pathways, which are differentially utilized upon cell stress, may provide a rapid means to elevate cytoplasmic HuR levels and thus stabilize ERG mRNAs responsive to HuR.

Given the importance of HuR in regulating a small number of ERG mRNAs, we asked what the full repertoire of HuR ligands might be. We reasoned that ARE containing mRNAs could be selected by HuR affinity chromatogrpahy from a complex mix of total, cellular mRNA and converted to cDNA for identification by sequencing. In order to ensure adequate ERG mRNA representation in the starting material, we employed a rodent epilepsy model. Seizures were induced in mice with pentylenetetrazole (PTZ), which is associated with increased mRNA levels of several early response genes in the hippocampus, such as c-fos, c-jun, NGFI-A (also known as zif/268, Egr-1 and Krox 24) (15) and tissue plasminogen activator (16), as well as the late response genes dynorphin, neuropeptide Y (15) and NeuroD-related factor (17).

Based on sequencing of the affinity selected mRNAs, HuR likely interacts with approximately 600 ligands from post-seizure mouse hippocampus. These mRNAs code for a variety of critical proteins including transcription factors, cytokines, cell surface receptors and signaling molecules with several having been previously defined as ERGs. In order to prove that HuR selection predicts ERG status, 3'-UTR sequencing of the library showed a large number of ARE-containing clones. Considering the rapidly accumulating data regarding

post-transcriptional regulation (PTR) by HuR, many of the clones identified herein are likely ERG mRNAs regulated by instability elements in their 3'-UTRs.

Experimental Procedures

Materials

The pMal protein fusion and purification system was purchased from New England BioLabs (Beverly, MA). The Universal RiboClone cDNA synthesis system and pGEM-T vector were from Promega (Madison, WI) and the ThermalAce DNA polymerase and pcDNA3.1 directional topo expression kit by Invitrogen (Carlsbad, CA). The HotStarTaq DNA polymerase and Omniscript RT were purchased from Qiagen (Valencia, CA). Pentylenetetrazol (PTZ) was from ? (,),protein A sepharose (catalog #9424),RNase T1 and protease inhibitor cocktail were from Sigma (St. Louis, MO), oligo$(dT)_{25}$ magnetic beads from Dynal (Lake Success, NY), oligo(dT)-cellulose from Ambion, Inc. (Austin, TX) and TRI-reagent from Molecular Research Center, Inc. (Cincinnati, OH). The enhanced chemiluminescence (ECL^+) western blotting detection kit and radioisotope {α-^{32}P]dCTP (3000 Ci/mmol) were from Amersham Life Science (Cleveland, OH), the nylon transfer membrane was from Fisher Scientific (Pittsburgh, PA) and the QuikHyb hybridization solution was supplied by Stratagene (La Jolla, CA). NIH/3T3 cells were bought from ATCC (Manassas, VA) and oligos were synthesized by Gibco BRL Life Technologies (Gaithersburg, MD).

pMal/HuR Cloning, Expression and Purification

The plasmid pGEX2T/HuR (3) was a kind gift from Dr. Henry Furneaux (Memorial Sloan-Kettering Cancer Center, New York). The HuR gene was PCR amplified (94^{0}C 1 min, 60^{0}C 1 min, 72^{0}C 1min) with HotStarTaq DNA polymerase per the manufacturer's protocol for 35 cycles. The forward primer 5'-AGTC**GAAGGATTTC**TATGTCTAATGGTTATGAAGACCA-3' incorporated an XmnI site (in bold) into the PCR product and the reverse primer 5'-AGTC**AAGCTT**TTATTTGTGGGACTTGTTGG-3' a HindIII site (in bold). The HuR PCR product was digested with XmnI and HindIII and cloned into the corresponding sites of the pMal-c2 vector.

pMal/HuR was transformed into XL-1 Blue competent cells and grown to A600 of 0.5, induced with IPTG to a final concentration of 0.3 mM, grown an additional 2 hr, and the cell pellet from 1L of cells was resuspended in 50 ml GSA buffer containing 15 mM HEPES (pH 8), 10 mM KCl, 10% glycerol and 1 mM DTT

and frozen overnight at –20^{0}C. The cell suspension was thawed on ice, sonicated at setting 3 for 8-15 sec bursts on ice with a sonic dismembrator 550 from Fisher Scientific (Pittsburgh, PA), centrifuged 9000Xg for 30 min at 4^{0}C. The cleared lysate was applied to 6 ml packed amylose resin previously equilibrated in GSA buffer. Protein binding proceeded for 60 min at 4^{0}C with rotation. The amylose resin was washed 6 times with GSA buffer and MBP/HuR was eluted twice with 10 mL GSA buffer containing 20 mM maltose for 10 min at 4^{0}C. The maltose was removed by ion exchange chromatography over DE52 resin. The MBP/HuR protein was bound to the DE52 in GSA buffer, washed with 6 column volumes of GSA buffer and eluted with GSA buffer containing 0.2 M KCl. Protein concentration was determined by Bradford assay and protein purity by SDS-PAGE. Aliquots of protein were frozen at –80^{0}C.

Seizure Induction and mRNA Isolation

Adult male Haarley C-57, strain 6, mice (7-8 weeks old) were injected with 50 mg/kg body weight pentylenetetrazol (PTZ) (or saline for control animals) and monitored for signs of behavioral seizure activity including altered responsiveness to environmental stimuli, irregular tonic-clonic movements of the extremities and alterations in postural tone. The seizures induced death in 25% of the mice which were discarded. One hour post-PTZ injection, the mice were decapitated and the brains were rapidly removed, partitioned into cortex, hippocampus and hemisphere fractions, and flash frozen in liquid nitrogen. Animal handling and tissue isolation were performed in accordance with NIH and University of Wisconsin-Madison guidelines for experimentation with animals. Frozen hippocampal tissue was homogenized and resuspended in Tri-Reagent at a concentration of approximately 30 mg tissue per 1 mL Tri-Reagent. RNA was prepared according to the manufacturer's procedure with a few modifications. Two additional RNA extractions after the TRI-reagent phase separation were performed, one extraction with an equal volume of water-saturated phenol/chloroform and the other with an equal volume of chloroform. The RNA was precipitated and resuspended in formamide for northern blot analysis or resuspended in water for poly(A) mRNA selection with oligo(dT)-cellulose. The conditions for denaturing formaldehyde-agarose gel electrophoresis, radiolabeled cDNA probe preparation and northern blot analysis have been previously described (18).

HuR Selection of mRNA

50 μg of MBP/HuR protein was bound to 25 μL packed amylose resin equilibrated in GSA buffer for 90 min at 4°C with mixing. The MBP/HuR-amylose resin was washed twice with 1 mL GSA buffer and incubated with 500 ng heat-denatured PTZ-treated mouse hippocampal mRNA and 80 units RNasin in a 1 mL reaction volume for 2 hr at 4°C with mixing. The resin was washed three times with 1 mL cold GSA buffer and bound mRNA was eluted at 37°C for 10 min with 200 μL GSA buffer containing 0.5 M LiCl. MBP/HuR selected mRNA was heat denatured and mixed with 25 uL oligo$(dT)_{25}$ magnetic beads for 1 hr at room temperature with mixing.

cDNA Library Construction, Amplification and Screening

The oligo$(dT)_{25}$ magnetic beads with bound mRNA were washed twice with 50 μL first-strand synthesis buffer. For the first-strand DNA synthesis in a final volume of 25 μL, the oligo$(dT)_{25}$ magnetic beads with bound mRNA were mixed with 15 μL water, 5 μL 5X first-strand buffer, and 40 units RNasin and heated for 5 min at 42°C before the addition of 2.5 μL sodium pyrophosphate 40 mM) and 1.5 μL AMV-RT (20 units/μL). The reaction was incubated at 42°C for 60 min with gently mixing every 10 min to resuspend the magnetic beads. The second-strand DNA synthesis reaction in a final volume of 125 μL contained: 25 μL first-strand reaction, 50 μL second-strand 2.5X buffer, 6.25 μL acetylated BSA (1 mg/mL), 3.13 μL DNA polymerase I (9.2 units/μL), 0.63 μL RNase H (2 units/μL) and 40 μL water. Second-strand synthesis proceeded for 2 hr at 14°C and the beads were resuspended every 10 min. The reaction was heated for 10 min at 70°C, placed on ice, mixed with 4 units of T4 DNA polymerase for 10 min at 37°C to polish the ends and treated with 10 uL of 200 mM EDTA. The oligo$(dT)_{25}$ magnetic beads with bound double-stranded DNA were washed twice with 50 μL T4 DNA ligase buffer and ligated with 5 pmol adaptor in a 30 μL reaction containing 1X T4 DNA ligase buffer plus 0.1 mg/mL BSA and 0.3 units/μL T4 DNA ligase. To prepare the adaptor, the complimentary oligos CACCGGCGGCCGCTCGAGTCTAGA and p-TCTAGACTCGAGCGGCCGCC were annealed (10 nmoles each) in 10 mM Tris pH7.5, 100 mM NaCl, 1 mM EDTA by heating at 65°C for 10 min followed by slow cooling to room temperature. Ligation proceeded at 15°C overnight, was heated at 70°C for 10 min, set on ice, and washed three times with 50 μL PCR buffer. The cDNA library was amplified with ThermalAce DNA polymerase (Invitrogen) to produce a blunt-end PCR product by a two-step PCR process (19). First, the cDNA attached to

the Dynal beads was mixed on ice with 100 ng of the forward primer 5'-CACCGGCGGCCGCTCGAGTCTAGA-3', 2 ng of the reverse primer 5'-GATTAACCCTCACTAAAGGGAT$_{15}$-3', 100 ng of the reverse primer 5'-GATTAACCCTCACTAAAGGGA-3', and PCR buffer and ThermalAce DNA polymerase per the manufacturer's directions, and heated at 95^{0}C for 2 min to release the second strand. The supernatant was transferred to a fresh tube and amplified [30^{0}C 15 min, 40^{0}C 15 min, 72^{0}C 15 min, 95^{0}C 2 min, (95^{0}C 1min, 72^{0}C 5 min, 16 cycles), 72^{0}C 30 min]. Second, 5 μL aliquots of the initial PCR reaction were reamplified with 100 ng of the forward 5'-CACCGGCGGCCGCTCGAGTCTAGA-3' and reverse 5'-GATTAACCCTCACTAAAGGGA-3' primers [95^{0}C 3 min, (95^{0}C 1 min, 72^{0}C 5 min, 16 cycles), 72^{0}C 30 min]. The PCR reactions were combined, extracted with a 50/50 mixture of phenol/chloroform, extracted with chloroform, and precipitated with ethanol in the presence of 50 μg glycogen. The final pellet was resuspended in 50 μL water and 4 μL of the amplified cDNA library was ligated with the pcDNA 3.1 vector per Invitrogen's protocol for the pcDNA3.1 directional topo expression kit and transformed into chemically competent TOP10 *E. coli*. Approximately 1000 colonies resulted from 4 μL of the amplified cDNA library and were screened for inserts by PCR with primers against the vector T7 and BGH sequences and HotStarTaq polymerase [95^{0}C 15 min to lyse and inactivate nucleases, (94^{0}C 1min, 60^{0}C 1 min, 72^{0}C 1min, 30 cycles), 72^{0}C 10 min]. The PCR products were analyzed on 1% agarose gels and ranged in size from several hundred base pair to 4.7 kb. Plasmids were purified from colonies containing inserts with Qiagen's plasmid mini kits (20 μg capacity) and sequenced. Over 1700 clones were sequenced, aligned to the NCBI database by BLAST homology searches (20) and categorized into families based on protein function.

c-fos RT-PCR

Samples were collected during the HuR selection procedure and analyzed for the presence of c-fos mRNA, a seizure-induced positive control. RNA samples [2.5 ng PTZ-treated mouse hippocampal mRNA (0.5% total), 5 μL MBP/HuR-amylose resin flow through (0.5% total), 1 μL MPB/HuR-amylose resin elution (0.5% total) and 1 μL oligo(dT)$_{25}$ flow through (0.5% total)] were reverse transcribed with Omniscript RT and 500 ng oligo(dT) primer per Qiagen's recommendations at 37^{0}C for 60 min. Aliquots (5 μL) of the RT reactions and the two rounds of library amplification (1 μL each) were PCR amplified with the primers 5'-

GGCAGAACCCTTTGATGACTTC-3' and 5'-AGCCCGGAGTACAGGTGACCA-3' designed against the mouse c-fos oncogene (GenBank accession #V00727). Amplification (94°C 1 min, 60°C 1 min, 72°C 30 sec, 35 cycles) produced a 206 bp c-fos fragment.

Amplification of the Full-length Mouse RhoB Gene

Poly(A)-mRNA (27.5 ng) isolated from mouse hippocampal tissue one hour after seizure induction with PTZ was reverse transcribed with Omniscript RT in a 20 ul reaction per the manufacturer's protocol. The RT reaction was diluted five-fold and 1 ul was amplified by PCR (1 min at 94°C, 1 min at 50°C, and 2 min at 72°C for 35 cycles) in a 50 ul reaction with the forward 5'-ATGGCGGCCATCCGCAAGAA-3' and reverse 5'–T_{10}ATAAATGGCATGATCATAGTC-3' primers and Qiagen HotStarTaq DNA Polymerase. The PCR product was gel-purified on a low-melt agarose gel, ligated into the pGEM-T vector and transformed into competent XL-1 Blue *E.coli* cells via electroporation.

Cell Culture

NIH/3T3 Swiss mouse embryo fibroblast adherent cells (ATCC item number CRL-1658) were grown in Dulbecco's Modified Eagle's Medium containing L-glutamine and 4.5 gm/L glucose supplemented with 10% fetal bovine serum and penicillin/streptomycin. Fresh medium was added every 2-3 days and the cells were subcultured with trypsin at 75% confluency. Cells were grown to 60-75% confluency prior to removal of the culture medium, treatment with 60 J/m^2 UV in an UV Stratalinker 2400 (Stratagene, La Jolla, CA), replacement of the culture medium and incubation for the indicated time.

Immunoprecipitations, RT-PCR and Southern Blot Analysis

NIH/3T3 were grown in 6-well dishes, treated +/-UV, cultured for the indicated time, washed with ice-cold DPBS, scraped from tissue culture wells, spun at 2000Xg for 30 sec in a Stratagene picofuge microcentrifuge, resuspended in ice-cold buffer containing 50 mM Tris (pH 8), 100 mM NaCl, 10% glycerol, 1X protease inhibitor cocktail and 5 mM DTT) and frozen at –80°C. The cells were lysed by the addition of NP-40 to 1% final concentration on ice for 30 min with occasional mixing. The lysates were spun at 10,000Xg for 10 min at 4°C. The supernatants were transferred to fresh tubes and frozen at -80°C. The protein concentration of the lysates was quantitatively determined with Bio-Rad protein assay dye reagent per the manufacturer's

recommendations. Lysates (50 μg) were incubated in buffer (50 mM Tris, pH 8, 100 mM NaCl, 10% glycerol, 1X protease inhibitors, 5 mM DTT, and 2 units/μl RNasin) with 7.5 ug HuR/19F12 antibody and mixed at 4^{0}C for 4 hr. For the control immunoprecipitation reactions, rabbit IgG (10 μg) replaced the HuR/19F12 antibody. An equal volume of protein A sepharose (50% slurry in DPBS) was added per immunoprecipitation and mixed overnight at 4^{0}C. The protein A sepharose was pelleted at 5000Xg for 2 min at 4^{0}C, washed with 1ml ice-cold DPBS and resuspended in 1 mL of TRI-Reagent. Total RNA was harvested, resuspended in 10 μL depc-water, denatured for 5 min at 65^{0}C and chilled on ice before reverse transcription with Omniscript RT and a random 9-mer primer in a 20 μL reaction per the manufacturer's recommendations Qiagen (Valencia, CA). The RT reaction (5 μL) was used as template for a 50 μL PCR reaction (1 min at 94^{0}C, 1 min at 60^{0}C, and 30 sec at 72^{0}C for 25 cycles) with RhoB specific primers (forward: 5'–CCTCCGGCAGAGGATCCAGG-3' and reverse: 5'-GTGTGGTCAGAATGCTGTCT-3'). The PCR reactions (10 μL each) were run on 1% agarose gels which were subsequently denatured for 45 min in 0.5 M NaOH and 1.5 M NaCl, rinsed with water, neutralized for 30 min in 1 M Tris-HCl (pH 7.5) and 1.5 M NaCl, washed for 15 min with 20X SSC and transferred to nitrocellulose by vacuum transfer on a VacuGene XL transfer apparatus from Pharmacia (Uppsala, Sweden) for 2 h at 50 mbar in 20X SSC as described by the manufacturer. The membranes were washed for 5 min in 6X SSC, air-dried for 30 min, baked at 65^{0}C for 30 min, hybridized with radiolabeled cDNA probe against the 3'-UTR of RhoB for 3.5 hr at 68^{0}C, and washed twice for 15 min at room temp with 2X SSC (0.3 M sodium chloride, 30 mM sodium citrate), 0.1% SDS followed by a 10 min stringent wash at 50^{o}C with 0.1X SSC, 0.1% SDS. The membranes were exposed to a phosphorimager screen and the signals were quantitated with ImageQuant software from Molecular Dynamics, Inc. (Sunnyvale, CA).

RNA Gel Mobility Shift Assays

The protocol for the binding reactions has been previously described (21). Details regarding the RNA probes and recombinant HuR concentrations are given in the figure legend.

Results

In order to characterize the repertoire of HuR mRNA ligands we have developed an affinity method for the selection of ARE-containing ERG mRNAs from total cellular mRNA. Recombinant HuR bound to a solid support was mixed with total mRNA isolated from mouse hippocampal lysates. After extensive washing, retained mRNAs were eluted and converted into a cDNA library. In order to induce ERG expression, mice were injected with the convulsant pentylenetetrazol (PTZ) which induces tonic-clonic seizures by antagonizing inhibitory GABA receptors (22). One hour post-PTZ injection, total RNA was isolated from hippocampus and analyzed by northern blotting with a cDNA probe against c-fos. This prototypical ERG is rapidly up-regulated by seizure (15). As shown in Figure 1A, there was no detectable c-fos mRNA in control tissue from the hippocampus, cortex or hemispheres, however, ample c-fos mRNA was present in the hippocampus as well as the cortex and hemisphere after PTZ treatment. Therefore, mRNA was isolated by oligo(dT) from these PTZ treated animals and used as starting material for the HuR affinity chromatography.

HuR was expressed as a fusion protein with MBP (42 kD) (Figure 2) and bound to amylose resin which acted as a solid support for the *in vitro* selection of mRNA ligands. Based on Coomassie blue staining, the fusion protein was >90% pure. The ability of the resin to select ARE containing mRNAs was monitored by following the co-isolation of a known amount of full-length, radiolabeled GM-CSF mRNA (23) which was added to the starting mRNA. Thus, the HuR/MBP/amylose resin was incubated with increasing concentrations of heat-denatured hippocampal mRNA (50, 300, 1000 ng) spiked with radiolabeled GM-CSF mRNA, washed, and eluted in 0.5 M KCl. Aliquots from the flow through, washes, elutions and residual MBP/HuR resin were counted in a scintillation counter and the percentage of cpms in the individual fractions plotted against the fraction number (Figure 3). When 50 ng (Figure 3B) or 300 ng hippocampal mRNA (Figure 3C) spiked with radiolabeled GM-CSF mRNA was selected on the MBP/HuR/amylose resin, >72% of GM-CSF mRNA was retained after multiple washes and eluted with 0.5 M salt. When the concentration of cold hippocampal mRNA was increased to 1 μg, 64% of the GM-CSF counts were in the flow-through fraction and 24.4% in the elutions (Figure 3D). Control binding reactions to amylose resin alone (Figure 3A) showed that a very small amount of

radiolabeled GM-CSF mRNA was retained by the column (2.1%) while nearly 90% was present in the flow through. Therefore, the column showed good specificity towards ARE+ mRNAs. The progressively reduced GM-CSF mRNA retention and elution as hippocampal mRNA was increased likely reflects competition for a limited number of HuR molecules. Thus for affinity chromatography, we chose an intermediate amount of input mRNA from post-seizure hippocampus (500 ng) to ensure rare transcripts would not be lost.

After selection, mRNAs were bound to oligo$(dT)_{25}$ magnetic beads for the solid phase synthesis of the cDNA library as described in the materials and methods. The presence of c-fos mRNA was used as a positive control for seizure induction (see Figure 1 and Figure 4, lane 4) and was followed over the course of selection and library construction. Unexpectedly, a significant amount of c-fos mRNA was in the flow through fraction from the HuR/MBP/amylose resin (lane 5), presumably due to saturation of the HuR binding sites with competing endogenous ARE mRNAs. However, c-fos mRNA was present in the high salt amylose resin elution (Figure 4, lane 6) and retained on the oligo(dT) beads (Figure 4, lane 7). Finally, c-fos DNA was detected in both the first (lane 8) and second (lane 9) rounds of library amplification.

The resulting cDNA library was transformed into TOP10 *E. coli* and the clones sequenced, analyzed for homology with the NCBI nonredundant and EST-mouse databases and categorized into families based on protein function. A partial list of clones involved in apoptosis, transcription and cell regulatory events is given in Table 1. A comprehensive list of the isolated HuR ligands is submitted as supplementary data (Tables A-I). Analysis of the 472 cDNAs with homology to the nonredundant NCBI database revealed that 78% contained isolated AUUUA pentamers, canonical AREs and/or uridine-rich stretches (three or more U_4 repeats or at least one U_6 sequence). Thus, these appear to be genuine HuR binding partners. However, given the recent demonstration that HuC interacts with zipcode elements (24), the inclusion of actin and other cytoskeleton mRNAs is also predictable. Of note, we did not isolate any clones previously identified as HuR ligands (25), although we did detect c-fos mRNA in the cDNA library by RT-PCR. The absence of known RNAs bound by HuR in the library is most likely due to a lower level of their expression and a need to sequence more colonies. The current library was constructed after sequencing 1700 clones.

We have isolated clones associated with formation of the actin cytoskeleton and extracellular matrix, including several isoforms of actin, tubulin and myelin basic protein as well as clones for cofilin 1, dynein and profilin. Of note, myelin basic protein is involved in axonal sprouting and has increased mRNA expression in the injured adult mouse central nervous system (26). Clones coding for apoptosis-, calcium or calmodulin-, cell regulatory-, immunoglobulin-, immunosuppressant-, iron homeostasis-, cytokine-, transcription factor-, and replication- related proteins were present in the library and a subset of these clones with known or potential roles as ERGs are listed in Table 1. We have isolated bHLHZip transcription factor BIGMAX beta, inhibitor of DNA binding 3, RB-associated KRAB repressor, TATA box binding protein-associated factor and transcription factor phi AP3 mRNAs. Cytokine-related mRNAs, including those for seven different heat shock proteins, cell regulatory messages like the cyclin-dependent kinases 4 and 5 and three clones related to iron homeostasis (ferritin heavy chain, ferritin light chain 1 and transferrin mRNAs) were also HuR ligands. Another possible ERG, FK506 binding protein 1a (accession number NM_008019)mRNA, was selected by HuR affinity chromatography and contains an extensive 3'-UTR (1132 nucleotides of a 1556 base gene) with type I, II and III AREs.

We also isolated mRNAs coding for kinases, nucleases, phosphatases, proteases, and metabolism- and protein modification-related proteins. The library includes many of the enzymes involved in anaerobic respiration including aldolase, triosephosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase, enolase, pyruvate kinase and lactate dehydrogenase. It is highly plausible that many of these clones are ERGs since hippocampal cells rely heavily on anaerobic respiration to meet growing energy needs during seizure (27-29), hypoxia (30) and depolarization (31). Several of the housekeeping ribosomal protein mRNAs that were selected by HuR affinity chromatography (L4, L9, L10) were also isolated from mitogen-activated PBMC with AUF1 (32). Clones encoding membrane proteins, receptors, membrane trafficking/vesicle transporters, nuclear pore complex proteins, and solute carrier proteins were present in the library, including mRNAs coding for GABA and glutamate transporters as well as two isoforms of the NMDA1 glutamate receptor. HuR bound to mRNAs coding for proteins involved in RNA binding (hnRNP), splicing

(snRNP) and translation (translation elongation factor 1 alpha), with the latter also present in an AUF1 enriched cDNA library (32). Numerous nucleotide binding and Ras-related clones were isolated including RhoB mRNA which is up-regulated in ischemia-damaged neurons (33).

Seizures are associated with neuronal death and we have cloned two distinct mRNAs which code for apoptosis-related BCL2-interacting proteins (BCL2/adenovirus E1B 19 kD-interacting protein 1 and beclin 1 mRNAs), an mRNA involved in apoptosis chromatin condensation induction in the nucleus and RhoB which plays a pivotal role in the cellular apoptotic response to DNA damage. Overall, the HuR ligand library contained many mRNAs possessing high potential for roles in apoptosis, transcription and other cell regulatory events. A comprehensive list of HuR ligands is available upon request.

We have attempted to screen our cDNA library by reverse northern blotting and by RT-PCR in order to measure the differential regulation of specific mRNAs in response to seizure, but our attempts have been unfruitful. French and colleagues have published a report profiling gene expression in the CA1 area of the hippocampus following induction of electroshock-evoked maximal seizure (34). They examined the differential expression of 9000 cDNAs and found only a single gene, nuclear hormone receptor NGF1-B, which is differentially regulated. NGF1-B was up-regulated 2-fold in the CA1 region of the hippocampus and 12-fold in the dentate gyrus. This data suggests that there is not differential regulation of ERGs in area CA1 of the hippocampus and explains our unproductive search for differentially regulated genes using whole hippocampus. Therefore, we have employed an alternative system for the study of stress-induced ERGs to validate the presence of an authentic HuR ligand, RhoB, in our cDNA library.

An mRNA highly homologous to the 3'-UTR of the rat RhoB gene (GenBank accession number NM_022542 (35)) was selected by the affinity chromatography with HuR. The 3'-UTR of the rat RhoB gene comprises two-thirds of the cDNA sequence and contains several AU-rich elements. RhoB, a ras-related GTPase, is a known early-response gene induced by DNA damaging treatments including UVL, v-Fps, EGF and PDGF (35,36). Based on this data, RhoB appeared to be a promising candidate for a post-transcriptionally regulated ERG. Therefore, we cloned the RhoB gene including the entire 3'-untranslated region from seizure-

induced mouse hippocampus tissue. Our putative mouse RhoB fragment was highly homologous with a mouse hippocampal cDNA (GenBank accession number AK013784 (37)). In addition to the published sequence, our fragment contained an additional 89 nucleotides preceding the poly(A) tail. We lacked the first 146 bases of GenBank clone AK013784 of which bases 1-116 contained a 100% match with the terminus of the coding region of the mouse RhoB gene. Therefore, we designed primers against the beginning of the mouse RhoB coding region (GenBank accession number X99963 (38)) and the terminus of the 3'-UTR of our putative mouse RhoB gene and PCR amplified a 1.978 kb sequence which contained the entire RhoB coding sequence (100% match) and its complete 3'-UTR. The 3'-UTR is 1377 nucleotides in length and contains six contains canonical AU-elements (39) at positions 1409 and 1844 and noncanonical AU-elements at positions 1084, 1706, 1926 and 1963 in the cDNA as well as several uridine-rich regions (submitted to GenBank, accession number AF481943). There is 94% homology overall between the mouse and rat RhoB genes and 92% homology between the mouse and a putative human BAC clone human (accession number AC023137 (40)) (Figure 5) with high conservation of the AU-rich elements.

HuR binds to two elements in the RhoB 3'-UTR as shown by RNA gel mobility shift assays in Figure 6. The first binding site occurs in the 355 base fragment containing nucleotides 1342-1696 and the second is found in the 156 base fragment extending from nucleotide 1765-1920. Specific binding to these regions was confirmed by RNA EMSA in the presence of cold competiors. The two fragments which bind to HuR each contain a canonical ARE found at positions 1409 and 1844, respectively. The absence of HuR binding to the $RhoB^{3'\text{-}UTR(1\text{-}1423)}$ radiolabeled RNA suggests that the canonical ARE at position 1409 is not important for HuR binding to $RhoB^{3'\text{-}UTR(1342\text{-}1696)}$, however, this fragment possesses several uridine-rich regions which may mediate binding. HuR binding to the $RhoB^{3'\text{-}UTR(1765\text{-}1920)}$ fragment is presumably mediated by the canonical ARE at position 1844. The 168 base fragment encompassing nucleotides 1653-1820 as well as the 81 base fragment from 1897-1977 which contain noncanonical AREs do not bind to HuR.

To determine if HuR binds to RhoB mRNA in a stress-induced environment, HuR was immunoprecipitated from control versus UV-irradiated NIH/3T3 cell cytoplasmic lysates followed by reverse transcription and PCR

amplification with primers specific for RhoB. As seen in Figure 7, there is a substantial increase in the quantity of RhoB mRNA immunoprecipitated from UV-induced lysates. This data indicates that HuR does bind to RhoB mRNA *in vivo* and that there is increased binding after UV-induced stress.

Discussion

Multiple approaches including SELEX (systematic evolution of ligands by exponential enrichment) (41) and SNAAP (isolation of specific nucleic acids associated with proteins) (42), which involve *in vitro* binding reactions as well as immunoprecipitation of endogenous RNA binding protein/mRNA complexes directly with monoclonal antibody and analysis by microarrays (43), have been employed to discern the protein/mRNA interactions important for the stabilization and translation of mRNAs. We have utilized AUF1 (32) and HuR affinity chromatography to create cDNA libraries of ARE-enriched mRNAs. There are limitations and advantages with each of these techniques. The *in vitro* binding reactions may not be representative of actual *in vivo* protein/mRNA interactions, won't detect binding if ancillary proteins are necessary and involve PCR amplification steps whereas with the immunoprecipitation reactions the epitope bound by the antibody may not be accessible in the mRNA complex or mRNAs which are not directly bound by the protein may be co-immunoprecipitated. Microarrays offer the advantage of rapid analysis but are confined in the number of RNAs spotted on the chip, while the brute-force sequencing approach we have used is time consuming and labor intensive but tenders novel sequences which are not found on arrays. Overall, these techniques will complement each other and contribute to our understanding of the protein/mRNA interactions that control post-transcriptional regulation of ERGs.

We have generated a cDNA library of novel HuR ligands coding for a variety of proteins involved in diverse cell functions. The majority of the clones contained class I, II or III-type AREs. However, 22% of the HuR ligands did not contain any previously identified AREs suggesting there is flexibility in the HuR recognition sequence. Indeed, recent work has shown that HuC interacts with zipcode elements found in cytoskeletal mRNAs subject to directed intracellular transport (24). Bakheet and colleagues have compiled a human ARE-containing mRNA database (ARED) and estimate that 8% of human mRNAs contain functional AREs (44). To date, the regulation of only a small percentage of those mRNAs has been studied. HuR is the most extensively studied ARE-binding protein and yet less than two dozen HuR ligands have been previously described (25).

Thus, the list of over 600 putative HuR ligands from post-seizure hippocampus should facilitate future studies interested in categorizing the full diversity of post-transcriptionally regulated, HuR-dependent genes.

It is likely that the full spectrum of selected mRNAs is dependent on the original cell or tissue source as well as the stress model employed. We have employed a stress model (seizures) to induce ERG mRNAs. Consistent with this, several of the mRNAs we selected by HuR affinity chromatography are differentially regulated in hippocampus by seizure induction, ischemia, glutamate neurotoxicity and aging (Table 2). Different seizure paradigms, including kainate or electroconvulsive shock treatment, have upregulated the expression of clathrin heavy chain (45), cyclin dependent kinase (CDK) 4 (46), CDK5 (47) and VASP/Ena family related (48) mRNAs. We also isolated the housekeeping gene cyclophilin (Table C), a peptide prolyl isomerase, which was downregulated at the mRNA level in rat hippocampus after ECS indicating that seizures can induce widespread differential gene expression (49). The ERGs c-fos, c-jun and zif268 are induced within several minutes after ischemia (50) while five genes have been identified which increase by two-fold within 24 hr of global cerebral ischemia in the rat hippocampus (51). We have cloned the mouse homolog of one of these genes, prosaposin. Glutamate excitotoxcity induced by NMDA treatment increased hippocampal expression of the pip92 mRNA (shares homology with immediate early response 2 mRNA) (52). Aged senescence-accelerated mice demonstrate increased expression of hippocampal cholinergic neurostimulating peptide (HCNP)-related components and their mRNAs (53) and we have isolated the mRNA for HCNP by HuR affinity chromatography. High levels of β-amyloid are found in the brains of Tg2576 mice which overexpress the human amyloid precursor protein carrying the Swedish mutation (APP_{Sw})(K670N; M671L) (54). Three transcripts which were up-regulated in the hippocampus of APP_{Sw} mice were selected by HuR affinity chromatography including the RNA polymerase II subunit 3, ectonucleotide pyrophosphatase/phosphodiesterase 2 and craniofacial development protein I mRNAs.

Becker and colleagues have analyzed changes in hippocampal gene expression from biopsies of humans exhibiting temporal lobe epilepsy by expression array analysis and identified 9 genes which were up-regulated

and 12 which were down-regulated (55). Our HuR-selected cDNA library contains mouse homologs for 2/9 of the up-regulated human genes, calmodulin-dependent phosphatase catalytic subunit and prion protein, as well as 6/12 of the down-regulated genes including cathepsin B proteinase, pyruvate kinase M, neurogranin, superior cervical ganglia-10, calmodulin 1, and rho GDP-dissociation inhibitor 1 mRNAs. Hendriksen and colleagues have identified 92 differentially expressed genes from post-seizure rat hippocampus (56). We have identified several of the corresponding mouse mRNAs in our cDNA library including: 14-3-3 protein gamma-subtype, α-tubulin, ferritin heavy chain, GAPDH, heat shock-like (70 kD) protein, myelin proteolipid protein, and ribosomal proteins L10a and S9 mRNAs (Table 2).

Mossy fiber sprouting is associated with status epilepticus, therefore, genes involved in axonal growth and regeneration would be expected to be upregulated under seizure-induced conditions. We have isolated several mRNAs by HuR affinity chromatography which play a role in the formation of the actin cytoskeleton including isotypes of alpha and beta tubulin and vimentin (Table 2). Alpha-tubulin is upregulated in kainate-induced status epilepticus in rats (57). Transcription of vimentin, which codes for an intermediate filament protein, is induced in focal cortical dysplasia of an epilepsy patient (58) and also in rat brain in response to ischemia, hemorrhage, kainate-induced seizures and hypoglycemia (59).

The identification of a vast array of novel HuR ligands should aid in defining new mRNA instability elements as well as provide a plethora of genes for the study of PTR. Stabilization of ERG mRNAs is often preceded by interactions with ARE-specific mRNA binding proteins and correspondingly we demonstrate that HuR specifically interacts with the ARE-rich 3'-UTR of the GTP binding protein RhoB both *in vitro* and *in vivo*. We have commenced studies on the PTR of RhoB, an early predictor of neuronal death in transient focal ischemia (33). RhoB is an ERG which is transcriptionally activated 3-4 fold within 30 min of UV treatment (36) and mRNA levels are increased in ischemia damaged neurons (33) (Table 2). Analysis of the 3'-UTR of RhoB mRNA reveals multiple AREs suggesting that PTR may also play an important role in controling RhoB mRNA levels. We have demonstrated that RhoB mRNA is progressively stabilized by UVL, an effect which begins

within one hour of exposure and concurrently with HuR translocation from the nucleus to the cytoplasm (Westmark and Malter, manuscript in preparation). *In vitro* mobility shift assays showed that HuR bound to two distinct 3'-UTR fragments of RhoB mRNA (nucleotides1342-1696 and 1765-1920) suggesting a regulatory role for this RNA binding protein. RT-PCR of HuR immunoprecipitations were positive for RhoB mRNA indicating an *in vivo* association. Therefore, RhoB is a bona fide, post-transcriptionally regulated, HuR ligand.

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Footnotes

GenBank Accession Numbers

The mouse RhoB sequence was submitted to GenBank and assigned accession number AF481943. Two recent GenBank submissions are highly homologous with our sequence, BC018275 and BC023334.

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Figure Legends

Figure 1: PTZ up-regulates c-fos mRNA expression in mouse brain. Mice were injected with 50 mg/kg body weight pentylenetetrazol (P) (even numbered lanes) or with saline for control animals (C) (odd numbered lanes) and hippocampus (lanes 1 and 2), cortex (lanes 3 and 4) and hemisphere (lanes 5 and 6) tissue was isolated 1 hour later. RNA was isolated and analyzed as described in the materials and methods. (A) northern blot of the c-fos-specific hybridization signal. (B) ethidium bromide stained agarose gel demonstrating equivalent total RNA loads for control versus PTZ-treated mice.

Figure 2: SDS-PAGE and western blot analysis of MBP/HuR fusion protein. Molecular weight markers (lane 1) and MBP/HuR (lanes 2 and 3) were analyzed by 12% SDS-PAGE followed by either staining with Coomassie blue dye (lane 2) or western blotting with anti-HuR/19F12 monoclonal antibody (1:2000) and staining with ECL+ reagents (lane 3).

Figure 3: Radiolabeled GM-CSF mRNA elutes from MBP/HuR/amylose resin with high salt. Poly(A)-selected mRNA (50 ng Panel A and B, 300 ng Panel C and 1 μg Panel D) from mouse hippocampus was spiked with radiolabeled GM-CSF mRNA and bound to MBP/HuR/amylose resin (Panels B-D) or to resin alone (Panel A) similarly to the HuR selection procedure described in the materials and methods. The MBP/HuR/amylose resin was washed 5X with 1 mL of GSA buffer and bound mRNAs were eluted 2X with 200 μL warm GSA buffer containing 0.5 M KCl. The flow through (column 1), wash (columns 2-6), and elution (columns 7-8) fractions were collected as well as the remaining resin (column 9) and monitored for GM-CSF mRNA by scintillation counting.

Figure 4: c-fos mRNA from PTZ-treated mouse hippocampus binds to the MBP/HuR/amylose resin and is present in the amplified cDNA library. RNA samples were collected from the starting material (lane 4), MBP/HuR/amylose resin flow through (lane 5), MBP/HuR/amylose resin elution (lane 6) and oligo$(dT)_{25}$ flow through (lane 7) and reverse transcribed as described in the materials and methods. Aliquots of the RT reactions, the two rounds of library amplification (lanes 8-9) and c-fos plasmid DNA (lanes 1-3) were PCR amplified and analyzed on a 1% agarose gel in TBE. A 206 base pair PCR product was visualized by ethidium bromide staining.

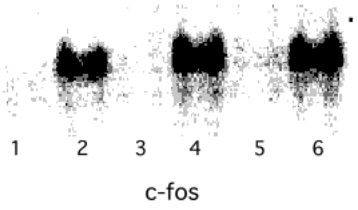
Figure 5: Nucleotide sequence of the mouse RhoB 3'-UTR and homology comparison of mouse, rat, and human RhoB genes. (A) the RhoB gene was PCR amplified from mouse hippocampus and the sequence of the 3'-UTR is shown. The six AU-rich elements are underlined as well as several U-rich stretches. Canonical AREs are found at positions 1406 and 1842) and noncanonical elements at positions 1084, 1706, 1926 and 1963). (B) Clusta1W multiple sequence alignment of mouse, rat and human RhoB genes with MacVector version 7.1 software from Accelrys Inc. (San Diego, CA).

Figure 6: RhoB mRNA deletion constructs and HuR gel mobility shift binding data. RhoB 3'-UTR (bases 592-1978) fragments A (bases 1342-1696), B (bases 1653-1820), C (bases 1765-1920) and D (bases 1897-1977) were PCR amplified and used as templates for transcription reactions with T7 RNA polymerase. RNA probes ($1X10^5$ cpm) were incubated with recombinant MBP/HuR protein, cross-linked, digested with RNase T1 and analyzed by SDS-PAGE. Panel A: Summary of RhoB RNAs represented by starting and final nucleotides and their binding affinity. Fragments are denoted A-D. Panel B: EMSAs with radiolabeled 3'-UTR (lane 1), fragment A (lane 2), fragment B (lane 3), fragment C (lane 4) and fragment D (lanes 5-6) are shown. Lane 6 is a darker exposure of lane 5. Each lane contained 100 ng MBP/HuR protein. Panel C: EMSA s with radiolabeled 3'-UTR and 25 ng MBP/HuR in the presence of unlabeled fragment A (lanes 3-5), unlabeled fragment B (lanes 6-8), unlabeled fragment C (lanes 9-11) and unlabeled 3'-UTR (lanes 12-14). In each set of 3 competitor lanes, 25-, 100- or 500-fold molar excess of cold RNA over probe was used. Panel D: EMSAs with radiolabeled fragment A probe and 20 ng MBP/HuR in the presence of unlabeled fragment A (lanes 3-4), unlabeled fragment B (lanes 5-6), unlabeled fragment C (lanes 7-8), unlabeled fragment D (lanes 9-10) and unlabeled 3'-UTR (lanes 11-12). Competitors were used at 5-fold or 25-fold molar excess over probe. Panel E: EMSAs with radiolabeled fragment C probe and 20 ng MBP/HuR in the presence of cold competitors as described for panel D. All cold competitor RNAs were electrophoresed on ethidium stained agarose gels to ensure they were full-length.

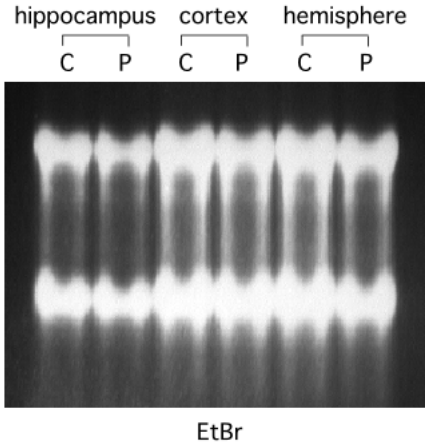
Figure 7: HuR binds to RhoB mRNA *in vivo*. NIH/3T3 control lysates (lanes 1 and 3) versus UV-treated lysates (lanes 2 and 4) were immunoprecipitated with HuR/19F12 monoclonal antibody (lanes 1-2) and rabbit IgG (lanes 3-4). RNA was harvested with Tri-Reagent 3 hr post-UV treatment, reverse transcribed and PCR

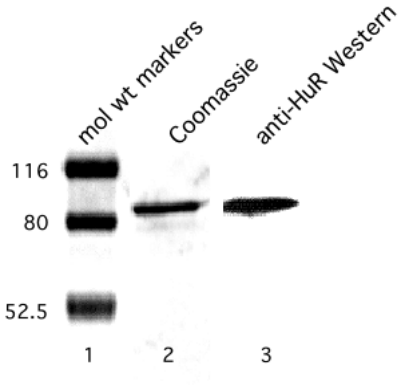
amplified with RhoB-specific primers. (A) ethidium bromide stained agarose gel depicting the 224 bp RhoB PCR product. (B) Southern blot of RhoB-specific hybridization signal.

A



B

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anti-HuR Western
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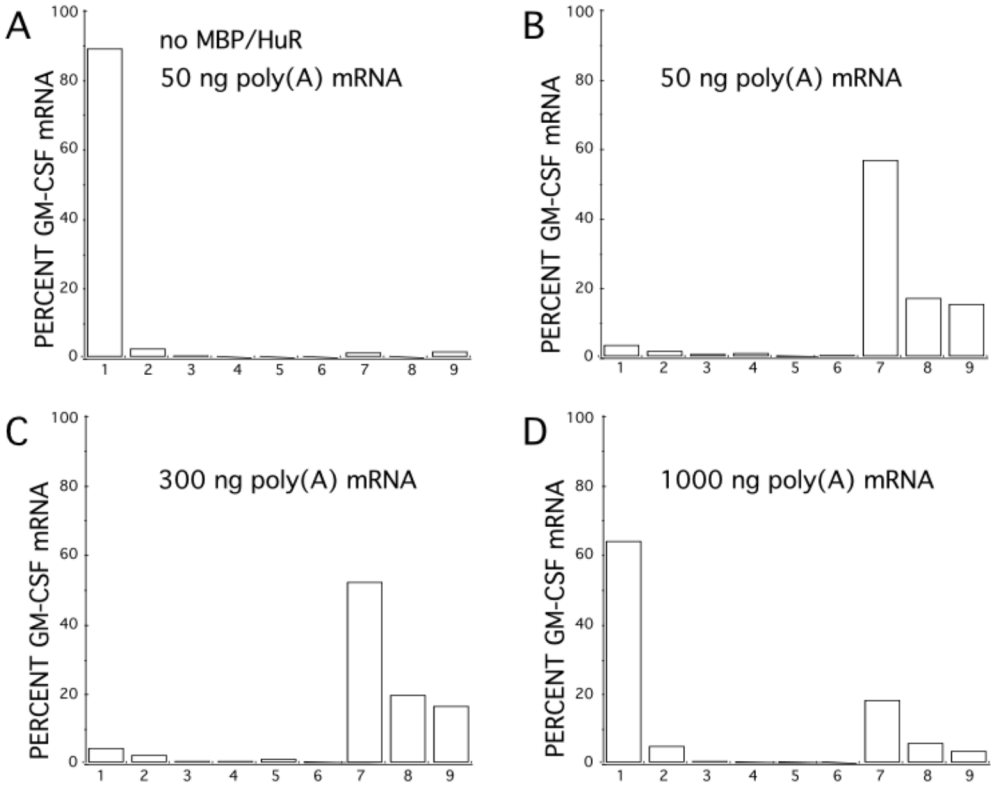
80
52.5
1
2
3


A
no MBP/HuR
50 ng poly(A) mRNA
PERCENT GM-CSF mRNA
B
50 ng poly(A) mRNA
PERCENT GM-CSF mRNA
C
300 ng poly(A) mRNA
PERCENT GM-CSF mRNA
D
1000 ng poly(A) mRNA
PERCENT GM-CSF mRNA
0
20
40
60
80
100
1
2
3
4
5
6
7
8
9

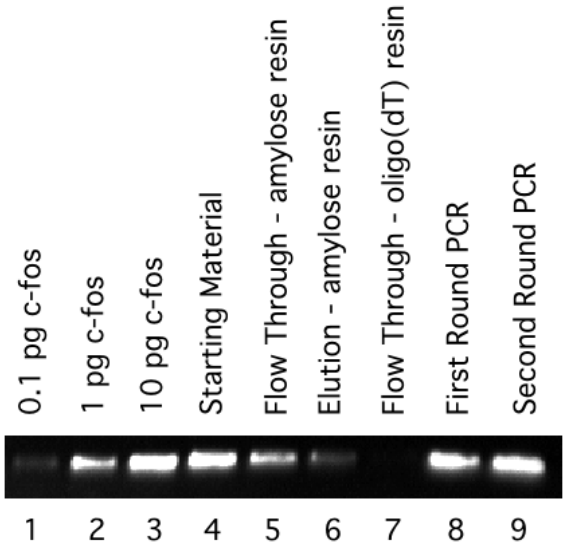
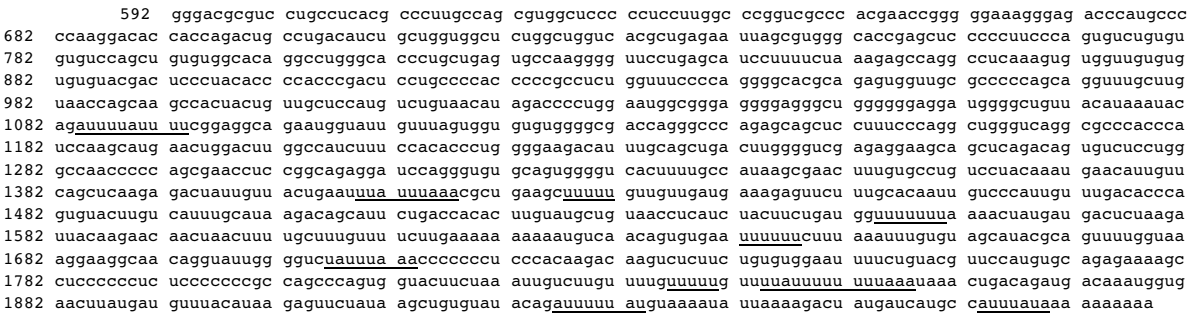

0.1 pg c-fos
1 pg c-fos
10 pg c-fos
Starting Material
Flow Through - amylose resin
Elution - amylose resin
Flow Through - oligo(dT) resin
First Round PCR
Second Round PCR
1
2
3
4
5
6
7
8
9

Figure 5

A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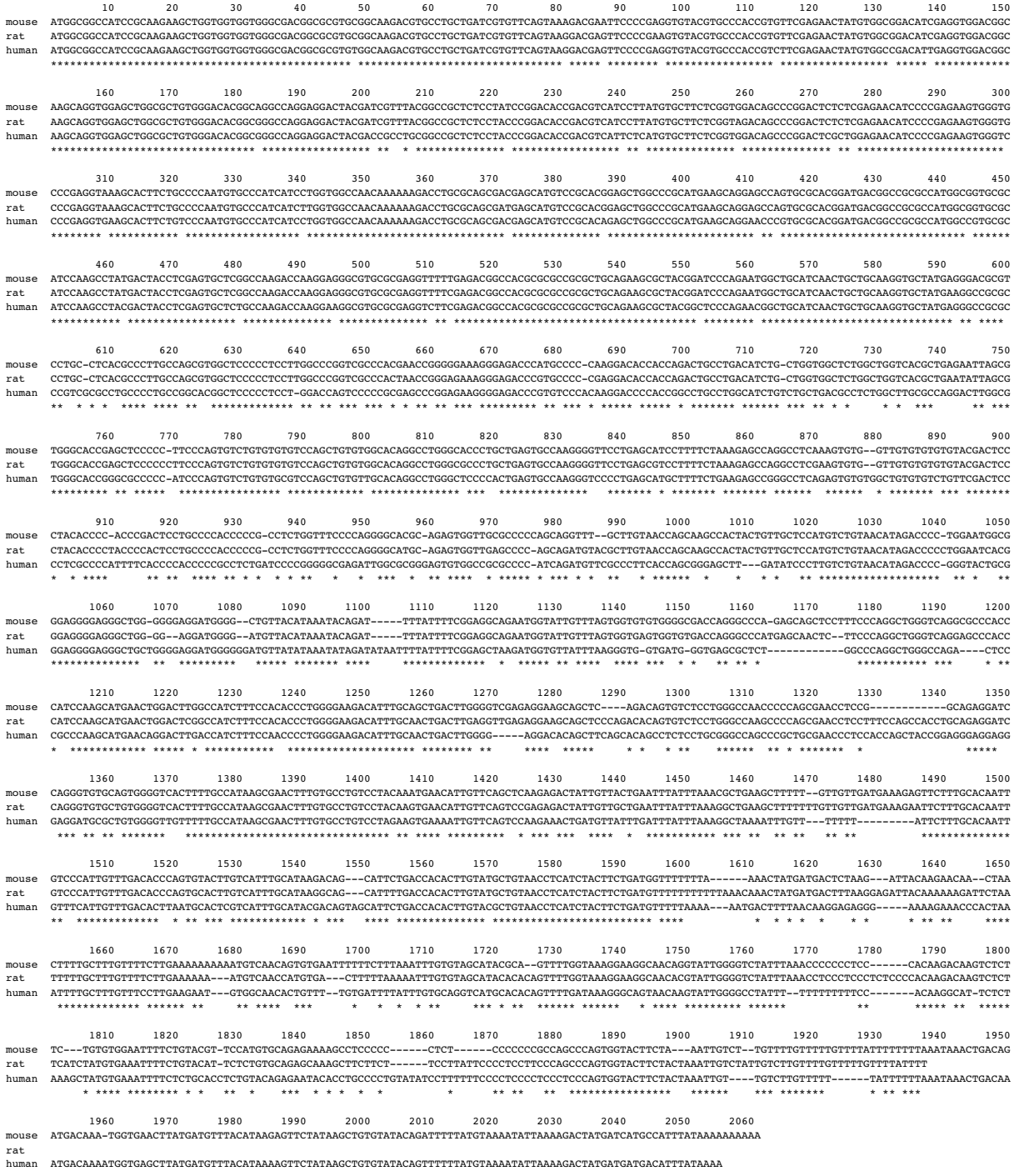
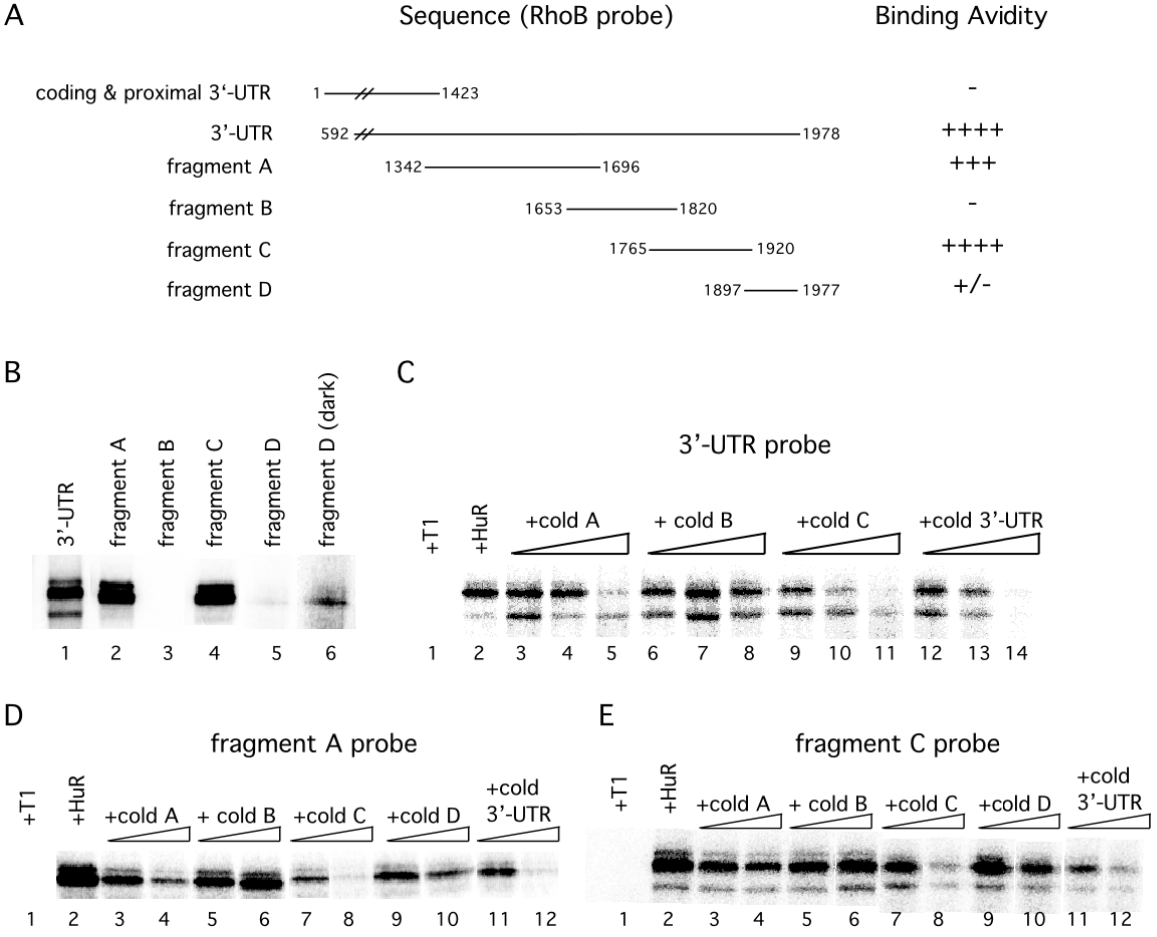
B

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Binding Avidity
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-
3'-UTR
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1978
++++
fragment A
1342
1696
+++
fragment B
1653
1820
-
fragment C
1765
1920
++++
fragment D
1897
1977
+/-
B
3'-UTR
fragment A
fragment B
fragment C
fragment D
fragment D (dark)
1 2 3 4 5 6
C
3'-UTR probe
+T1
+HuR
+cold A
+ cold B
+cold C
+cold 3'-UTR
1 2 3 4 5 6 7 8 9 10 11 12 13 14
D
fragment A probe
+T1
+HuR
+cold A
+ cold B
+cold C
+cold D
+cold 3'-UTR
1 2 3 4 5 6 7 8 9 10 11 12
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+HuR
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+ cold B
+cold C
+cold D
+cold 3'-UTR
1 2 3 4 5 6 7 8 9 10 11 12

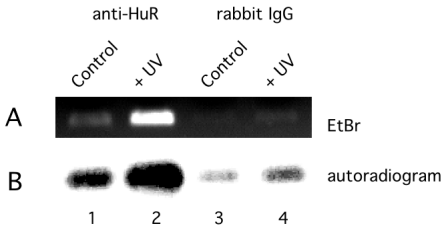
anti-HuR
rabbit IgG
Control
+ UV
Control
+ UV
A
EtBr
B
autoradiogram
1
2
3
4

TABLE 1			
Clone Name	**genus**	**Accession Number**	**ARE class**
apoptotic chromatin condensation inducer in the nucleus mRNA	mouse	NM_019567	2
BCL2/adenovirus E1B 19kDa-interacting protein 1 mRNA*	mouse	XM_128520	1
beclin 1 mRNA	mouse	NM_019584	1
bHLHZip transcription factor BIGMAX beta mRNA	mouse	AF213671	ND
crystallin mRNA	mouse	NM_016669	1
cyclin-dependent kinase 4 mRNA	mouse	BC052694	3
cyclin-dependent kinase 5 mRNA*	mouse	NM_007668	1
DnaJ heat shock protein 40 homolog mRNA*	mouse	NM_178055	3
E4F transcription factor 1 mRNA	mouse	BC031757	3
ferritin heavy chain mRNA	mouse	NM_010239	ND
ferritin light chain 1 mRNA	mouse	BC019840	ND
FK506 binding protein 1a mRNA*	mouse	NM_008019	2
general transcription factor 2B mRNA	mouse	NM_145546	1
heat shock protein mRNA	mouse	BC018378	1
heat shock protein 1, alpha, mRNA	mouse	NM_010480	3
heat shock protein 1, beta, mRNA	mouse	NM_008302	3
heat shock protein 70 binding protein mRNA	mouse	AF338351	ND
heat shock protein 8 mRNA	mouse	BC006722	3
heterogeneous nuclear ribonucleoprotein A/B mRNA*	mouse	NM_010448	1
immediate early response 2 mRNA*	mouse	BC002067	3
inhibitor of DNA binding 3 mRNA*	mouse	NM_008321	3
interferon regulatory factor 3 mRNA	mouse	BC050882	ND
MEK binding partner 1 mRNA*	mouse	AF082526	1
NCK-associated protein 1 mRNA	mouse	NM_016965	3
N-myc downstream regulated 2 mRNA*	mouse	NM_013864	ND
non-POU-domain containing, octamer binding protein, mRNA*	mouse	BC005465	3
nuclear DNA binding protein mRNA*	mouse	NM_020558	1
5', 3'-nucleotidase cytosolic mRNA	mouse	NM_015807	ND
Par-6 (partioning defective 6) homolog alpha mRNA	mouse	BC049593	ND
rhoB gene mRNA*	rat	NM_022542	1
RNA polymerase II polypeptide C mRNA	mouse	BC002023	1
TAF12 RNA polymerase II mRNA*	mouse	NM_025579	1
transferrin mRNA	mouse	NM_133977	ND
* The 3'-UTR comprises 36-84% of the gene sequence.			

TABLE 2

Clone Name	Regulation	System*	Literature Reference
14-3-3 protein gamma-subtype mRNA	down	SE/rat	D17447 (Hendriksen)
alpha-tubulin mRNA	up	SE/rat	V01227 (Hendriksen)
calmodulin 1 mRNA	down	TLE/human	J04046 (Becker)
calmodulin-dependent phosphatase mRNA	up	TLE/human	L14778 (Becker)
cathepsin B mRNA	down	TLE/human	M14221 (Becker)
clathrin heavy chain mRNA	up	KA/rat	(Konopka)
craniofacial development protein I mRNA	down	APP(Sw)/mouse	(Stein)
cyclin-dependent kinase 4 mRNA	up	KA/rat	(Ino)
cyclin-dependent kinase 5 mRNA	up	ECS/mouse	(Chen)
ectonucleotide pyrophosphatase/phosphodiesterase 2 mRNA	up	APP(Sw)/mouse	(Stein)
ferritin heavy chain mRNA	up	SE/rat	U58829 (Hendriksen)
GAPDH mRNA	up	SE/rat	M17701 (Hendriksen)
growth factor inducible immediate early protein (pip92) mRNA	up	NMDA/mouse	(Chung)
HCNP mRNA	up	aging/rat	(Matsukawa)
heat shock-like (70 kD) protein mRNA	down	SE/rat	M11942 (Hendriksen)
myelin proteolipid protein mRNA	up	SE/rat	X62611 (Hendriksen)
neurogranin mRNA	down	TLE/human	Y09689 (Becker)
prion protein mRNA	up	TLE/human	M13667 (Becker)
prosaposin mRNA	up	GCI/rat	(Yokota)
pyruvate kinase M mRNA	down	TLE/human	M23725 (Becker)
RhoB mRNA	up	FI/mouse	(Trapp)
rho GDP-dissociation inhibitor 1 mRNA	down	TLE/human	X69550 (Becker)
ribosomal protein L10a mRNA	up	SE/rat	X93352 (Hendriksen)
ribosomal protein S9 mRNA	up	SE/rat	X66370 (Hendriksen)
RNA polymerase II subunit 3 mRNA	up	APP(Sw)/mouse	(Stein)
superiorcervical ganglia-10 mRNA	down	TLE/human	S82024 (Becker)
VASP/Ena mRNA	up	seizure/rat	(Kato)
vimentin mRNA	up	FCD/human	(Taylor)

*Abbreviations: amyloid precursor protein with Swedish mutation (APP/Sw), electroconvulsive seizure (ECS), focal cortical dysplasia (FCD), focal ischemia (FI), global cerebral ischemia (GCI), kainic acid (KA), N-methyl-D-aspartic acid (NMDA), post-status epilepticus (SE), and temporal lobe epilepsy (TLE).

TABLE A

Actin Cytoskeleton	**genus**	**Accession Number**
actin, gamma 1, mRNA	mouse	BC003337
actin, gamma, cytoplasmic mRNA	mouse	NM_009609
actin, alpha, vascular smooth muscle, mRNA	mouse	NM_007392
actin related protein 2/3 complex, subunit 2, mRNA	human	BC000590
cofilin 1, nonmuscle, mRNA	mouse	NM_007687
double cortin and calcium/calmodulin-dependent PK-like 1 mRNA	mouse	NM_019978
dynein mRNA	mouse	NM_017470
dynein-associated protein RKM23 mRNA	rat	AY026512
Ena-VASP-like isoform mRNA	mouse	AF279662
formin-like mRNA	mouse	NM_019679
HS1 binding protein mRNA	mouse	NM_011826
kinesin-like protein KIF1B mRNA	mouse	AF090190
melanoma X-actin mRNA	mouse	NM_007393
myelin basic protein mRNA	mouse	M15060; M15062; NM_010777
myelin proteolipid protein gene, exon 7	mouse	M37335
myelin proteolipid protein mRNA	mouse	M15442
myosin-binding protein H mRNA	mouse	NM_016749
nuclear distribution gene C homolog mRNA	mouse	NM_010948
plakophilin 4 mRNA	human	XM_002743
profilin mRNA	mouse	NM_011072
prothymosin beta 4 mRNA	mouse	NM_021278
reticulon 3 mRNA	mouse	AF195940
sperm antigen 4 mRNA	rat	AF043345
tropomyosin 3 mRNA	mouse	AF317223
nonmuscle tropomyosin 5 mRNA	mouse	X53753
alpha-tubulin isotype M-alpha-2 mRNA	mouse	M13446
tubulin alpha 1 mRNA	mouse	NM_011653
tubulin alpha 6 mRNA	mouse	BC004745; NM_009448
tubulin beta 4 mRNA	mouse	NM_009451
tubulin beta 5 mRNA	mouse	BC003825
beta-tubulin gene 3'-end	mouse	M28730
similar to tubulin, beta polypeptide, clone mRNA	mouse	BC003475
villin 2 mRNA	mouse	NM_009510
vimentin-bound DNA fragment/ATPase subunit 6	mouse	AJ296978/AF093677
vimentin mRNA	mouse	NM_011701
Extracellular Matrix-Related	**genus**	**Accession Number**
brevican mRNA	mouse	NM_007529
metalloproteinase inhibitor TIMP-2 mRNA	guinea pig	AF127803
SPARC-like 1 mRNA	mouse	BC003759; NM_010097
thrombospondin 2 mRNA	mouse	NM_011581
thrombospondin 4 mRNA	mouse	NM_011582

TABLE B

Apoptosis-Related	**genus**	**Accession Number**	**Cytokine-Related**	**gen**
apoptotic chromatin condensation inducer in the nucleus mRNA	mouse	NM_019567	breast heat shock protein 73 mRNA	mou
BCL2/adenovirus E1B 19kD-interacting protein 1 mRNA	human	XM_003713	complement component 1, q subcompartment, beta polypeptide, mRNA	mou
beclin 1 mRNA	mouse	NM_019584	crystallin mRNA	mou
Calcium or Calmodulin-Related	**genus**	**Accession Number**	HCNPpp mRNA	mou
annexin A7 mRNA	mouse	NM_009674	heat shock protein 70 cognate mRNA	mou
calmodulin mRNA	mouse	NM_009790	heat shock protein 70 binding protein mRNA	mou
calmodulin snythesis mRNA	mouse	M27844	heat shock protein 84 kDa 1 mRNA	mou
calumenin mRNA	mouse	NM_007594	heat shock protein 86 kDa 1 mRNA	mou
neurocalcin mRNA	bovine	D10884	heat shock protein 105 kDa mRNA	mou
neurogranin (PKC substrate, RC3) mRNA	mouse	NM_022029	heat shock protein 105 kDa beta mRNA	mou
nucleobindin mRNA	mouse	NM_008749	heat shock protein DNAJ-like 1 mRNA	mou
synaptic vesicle glycoprotein 2a mRNA	mouse	NM_022030	neuron-specific cortexin mRNA	rat
unc 13 homolog mRNA	mouse	NM_021468	oxidative stress induced mRNA	mou
Cell Regulatory	**genus**	**Accession Number**	rer gene	mou
anaphase-promoting complex subunit 5 mRNA	mouse	NM_021505	similar to T-complex protein 1 mRNA	mou
cell division cycle 2 homolog-like 2 mRNA	mouse	NM_007661	**Transcription Factor-Related**	**gen**
cdc42 gene	mouse	L78075	alpha initiation factor mRNA	rat
non-kinase Cdc42 effector protein SPEC2 mRNA	human	XM_003780	bHLHZip transcription factor BIGMAX beta mRNA	mou
COP9, subunit 7a, mRNA	mouse	NM_012003	chromatin structural protein complex, subunit zeta 1, mRNA	mou
cyclin-dependent kinase 4 mRNA	mouse	NM_009870	CTD-binding SR-like mRNA	rat/l
cyclin-dependent kinase 5 mRNA	mouse	NM_007668	endogenouse retroviral sequence MuERV-L gag, pol, dUTPase genes	mou
growth factor inducible immediate early protein (pip92) gene	mouse	L26490	heterogeneous nuclear ribonucleoprotein A/B mRNA	mou
MEK binding partner 1 mRNA	mouse	AF082526	histone acetylase complex subunit MRG15-1 mRNA	mou
mini chromosome maintenance deficient 2 mRNA	mouse	NM_008564	histone acetylase complex subunit MRG15-2 mRNA	mou
nasal embryonic LHRH factor mRNA	mouse	NM_020276	histone H2a.2-615 gene	mou
neural proliferation, differentiation and control gene 1 mRNA	mouse	BC003320	HOM-TES-103 tumor antigen mRNA	hum
proteasome 26S subunit, non-ATPase, 4 mRNA	mouse	NM_008951	inhibitor of DNA binding 3 mRNA	mou
silent mating type information regulation 2 mRNA	mouse	NM_022433	interferon regulatory factor 3 mRNA	mou
SMC (segregation of mitotic chromosomes 1)-like mRNA	mouse	NM_019710	nucleosome assembly protein 1 mRNA	rat
Tax interaction protein 40 mRNA	mouse	NM_019695	N-myc downstream regulated 2 mRNA	mou
Tax responsive element binding protein 107/ribosomal protein L6 mRNA	mouse	X81987	RB-associated KRAB repressor mRNA	hum
Tousled-like kinase mRNA	mouse	NM_011903	ribosomal protein L10 mRNA	rat
valosin containing protein mRNA	mouse	NM_009503	similar to RNA polymerase II polypeptide C (33 kD) mRNA	mou
Immunoglobulin	**genus**	**Accession Number**	small unique nuclear receptor co-repressor gene	mou
HT7 antigen mRNA/basigin mRNA	mouse	S63813	TATA box binding protein-associated factor mRNA	hum
Immunosuppressant-Related	**genus**	**Accession Number**	TEL protein mRNA	mou
FK506 binding protein 1a mRNA	mouse	NM_008019	transcription factor phi AP3 mRNA	mou
Iron Homeostasis	**genus**	**Accession Number**	transformation/transcription domain-associated factor mRNA	hum
ferritin heavy chain mRNA	mouse	NM_010239	tripartite motif protein TRIM28 beta mRNA	mou
ferritin light chain 1 mRNA	mouse	NM_010240	**Replication**	**gen**
transferrin mRNA	rat	NM_017055	origin of replication 3 homolog mRNA	mou

TABLE C

Kinases	**genus**	**Accession Number**	**Metabolism-Related**	**genus**	**Accession Number**
14-3-3 protein gamma-subtype (Ywhag) mRNA	rat	NM_019376	11 beta-hydroxysteroid dehydrogenase-1 mRNA	mouse	S75207
Similar to neighbor of A-kinase anchoring protein 95 mRNA	mouse	BC003242	acetyl-Coenzyme A dehydrogenase, medium chain, mRNA	mouse	NM_007382
calcium/calmodulin-dependent protein kinase II, beta, mRNA	mouse	NM_007595	adenylosuccinate synthetase 1 mRNA	mouse	NM_007421
creatin kinase brain mRNA	mouse	NM_021273	aldolase 1, A isoform, mRNA	mouse	NM_007438
diacylglycerol kinase 3 (gamma) mRNA	rat	NM_013126	aldoase 3, C isoform, mRNA/zebrin II mRNA	mouse	BC004802; S72537
diacylglycerol kinase 90kDa mRNA	rat	NM_019304	alpha-methylacyl-Coenzyme A racemase mRNA	mouse	NM_008537
double cortin and calcium/calmodulin-dependent protein kinase-like 1 mRNA	mouse	NM_019978	amino levulinate synthase mRNA	mouse	M63245
glycogen synthase kinase 3 alpha mRNA	rat	NM_017344	asparagine synthetase mRNA	mouse	U38940
hexokinase 1 mRNA	mouse	NM_010438	catechol-O-methyltransferase mRNA	mouse	NM_007744
neighbor of A-kinase anchoring protein 95 mRNA	mouse	NM_017476	cytochrome oxidase subunit 1, Ser-tRNA mitochondrial, mRNA	rat	S79304
NIMA-related expressed kinase 6 mRNA	mouse	NM_021606	dihydrolipoamide succinyltransferase mRNA	rat	D90401
nucleoside diphosphate kinase B mRNA	mouse	X68193	enolase 1, alpha non-neuron, mRNA	mouse	BC003934
PKC-gamma mRNA	mouse	L28035	enolase 3, beta muscle, mRNA	mouse	NM_007933
phosphoglycerate kinase pseudogene	mouse	M23962	similar to enolase 1, alpha non-neuron, mRNA	mouse	BC004017
putative lipid kinase mRNA	mouse	AJ401619	fatty acid synthase mRNA	mouse	AF127033
pyruvate kinase 3 mRNA	mouse	NM_011099	flavin containing monooxygenase 5 mRNA	mouse	NM_010232
pyruvate kinase M mRNA	mouse	D38379	glyceraldehyde-3-phosphate dehydrogenase mRNA	mouse	NM_008084
rearranged lck gene encoding lymphocyte-specific protein tyrosine kinase	mouse	M12056	ICR/Swiss glyceraldehyde 3-phosphate dehydrogenase gene	mouse	U09964
serine/arginine-rich protein kinase2 mRNA	mouse	NM_009274	glucosidase alpha acid mRNA	mouse	NM_008064
SFRS protein kinase 2 mRNA	human	XM_004842	glutamate dehydrogenase mRNA	mouse	NM_008133
Tousled-like kinase mRNA	mouse	NM_011903	glutathione peroxidase 4 mRNA	mouse	NM_008162
tyrosine protein kinase mRNA	mouse	L27738	lactate dehydrogenase 1, A chain, mRNA	mouse	NM_010699
Nucleases	**genus**	**Accession Number**	lactate dehydrogenase 2, B chain, mRNA	mouse	NM_008492
5'(3')-deoxyribonucleotidase mRNA	mouse	NM_015807	mitochondrial acetoacetyl-CoA thiolase mRNA	rat	D13921
small fragment nuclease mRNA/CGI-114 mRNA	human	XM_006440	Mpv17 protein gene	mouse	AF038632
Werner's Syndrome protein gene	mouse	AF091216	NADH dehydrogenase (ubiquinone) 1 beta subcompl 9 mRNA	human	NM_005005
Phosphatases			NADH dehydrogenase (ubiquinone) 1 beta subcompl 2 mRNA	human	BC001168
calmodulin-dependent phosphatase catalytic subunit mRNA	mouse	M81483	2-oxoglutarate dehydrogenase E1 component mRNA	mouse	U02971
calmodulin-dependent phosphatase (calcineurin) catalytic subunit mRNA	mouse	J04134	peroxiredoxin 3 mRNA	mouse	NM_007452
protein phosphatase 1a mRNA	mouse	NM_008910	prostaglandin D synthetase mRNA	mouse	AB006361
protein phosphatase 2A 55 kD regulatory subunit alpha mRNA	rat	M83298	pyridoxine 5'-phosphate oxidase mRNA	rat	NM_022601
protein phosphatase 2 (formerly 2a), regulatory subunit A, mRNA	mouse	BC006606/NM_016891	succinate-CoA ligase, GDP-forming, alpha subunit, mRNA	mouse	NM_019879
protein tyrosine phosphatase mRNA	rat	NM_012637	triosephosphate isomerase mRNA	mouse	NM_009415
protein tyrosine phosphatase-IV1b gene	human	L48937	ubiquinol-cytochrome c reductase core protein 1 mRNA	human	XM_003243
protein tyrosine phosphatase mRNA, receptor-type, zeta polypeptide mRNA	rat	NM_013080	**Protein Modification**	**genus**	**Accession Number**
protein tyrosine phosphatase mRNA, receptor-type N, mRNA	mouse	NM_008985	cyclophilin	human	BC003412
protein tyrosine phosphatase mRNA, receptor type, A, mRNA	mouse	NM_008980	ectonucleotide pyrophosphatase/phosphodiesterase 2 mRNA	mouse	BC003264
ser/thr protein phosphatase type 1 alpha mRNA	mouse	U25809	galactose 6-O-sulfotransferase GST-1 mRNA	mouse	AF280087
Proteases	**genus**	**Accession Number**	glutathione S-transferase, mu 1, mRNA	mouse	NM_010358; BC003822
bone morphogenetic protein mRNA	mouse	L24755	glycoprotein specific UDP-glucoronyltransferase mRNA	rat	D88035
carboxypeptidase E mRNA	mouse	U23184	N-myristoyltransferase mRNA	mouse	NM_008707
cathepsin B (carboxypeptidase) mRNA	mouse	NM_007798	peptide N-glycanase mRNA	mouse	NM_021504
protease, serine, 12 neurotrypsin, mRNA	mouse	NM_008939	phosphatidlyserine decarboxylase mRNA	human	NM_014338
signal peptidase complex mRNA	mouse	NM_019951	phosphomannomutase Sec53p homolog mRNA	mouse	AF007267
ubiquitous nuclear protein (Unp) mRNA	mouse	NM_011678	ribophorin I mRNA	rat	NM_013067
ubiquitin-specific protease gene	mouse	AF026469	ribophorin II mRNA	mouse	NM_019642
			sialyltransferase 7 mRNA	mouse	NM_016973

TABLE D

Housekeeping	genus	Accession Number
laminin receptor 1, 67 kDa, ribosomal protein SA, mRNA	mouse	NM_011029
16S ribosomal RNA gene	mouse	AY011146
chimeric 16S ribosomal RNA	mouse	AF089815
mitochondrial ribosomal protein L33 mRNA	mouse	AB049651
ribosomal protein L3 mRNA	mouse	NM_013762
ribosomal protein L4 mRNA	rat	NM_022510
ribosomal protein L6 mRNA/TAX responsive element binding protein 107 mRNA	mouse	NM_011290
ribosomal protein L7a mRNA	mouse	NM_013721
60S ribosomal protein L9 mRNA	mouse	AF260271
ribosomal protein L10 mRNA	rat	X87106
ribosomal protein L10a mRNA	mouse	NM_011287
ribosomal protein, mitochondrial, L14 mRNA	mouse	NM_025302
ribosomal protein L18a mRNA	rat	X14181
ribosomal protein L19 mRNA	mouse	NM_009078
ribosomal protein L27 mRNA	mouse	NM_011289
ribosomal protein L37a mRNA	mouse	NM_009084
ribosomal protein S5 mRNA	mouse	NM_009095, Y12431
ribosomal protein S7 mRNA	mouse	BC002014
30S ribosomal protein S7 homolog mRNA	human	BC000241
ribosomal protein S9 mRNA	rat	X66370
ribosomal protein S18 mRNA	mouse	NM_011296
ribosomal protein S24 mRNA	mouse	X60289
ring finger protein 25 mRNA	mouse	NM_021313
surfeit gene 1 mRNA	mouse	NM_013677
ubiquitin B mRNA	mouse	NM_011664

TABLE E

Membrane Proteins	**genus**	**Accesssion Number**	**Membrane Trafficking/Vesicle Transport**
amyloid beta (A4) precursor-like protein 1 mRNA	mouse	NM_007467	adaptor protein complex AP1 beta 1 subunit mRNA
ANG2 mRNA	human	AF024631	adaptor-related protein complex AP-1 mRNA
brain-specific membrane-anchored protein mRNA	human	XM_012907	adaptor-related protein complex AP-4 mRNA
calsyntenin-1 mRNA	mouse	NM_023051; AJ289016	calumenin mRNA
E25B protein mRNA	mouse	AB030204; AB030203	CDCREL-1 homolog mRNA
glycoprotein 110 mRNA	mouse	NM_019822	CL1BA protein mRNA
GPI anchor attachment protein 1 mRNA	mouse	BC006697	clathrin, heavy polypeptide, mRNA
integral membrane protein mRNA	hamster	U31241	coatomer protein complex, subunit zeta 1, mRNA
integral membrane protein 2 B mRNA	mouse	NM_008410	complexin 1 mRNA
lymphocyte antigen 6 complex mRNA	mouse	NM_011837	densin-180 mRNA
membrane glycoprotein M6 mRNA	mouse	S65735	p65 mRNA
nectin-like protein 1 (Necl1) mRNA	mouse	AF195662	rabaptin-5 gamma mRNA
neuronatin-2 mRNA	mouse	X83569	component of rsec6/8 secretory complex p71 mRNA
neurophilin mRNA	mouse	NM_008737	SEC 24 related gene family member C mRNA
olfactomedin related ER localized protein mRNA	mouse	NM_019498	synaptotagmin 11 mRNA
peroxisomal integral membrane protein mRNA	mouse	AJ006341	syntaxin 1A mRNA
prosaposin mRNA	mouse	NM_011179	syntaxin binding protein 1 mRNA
putative transmembrane protein 2c mRNA	mouse	AF282981	unc 13 homolog mRNA
seven transmembrane domain protein mRNA	human	NM_006326	VSP28 protein mRNA
transmembrane domain protein regulated in adipocytes 40 kD mRNA	mouse	NM_011906	vesicle-associated membrane protein VAMP1 mRNA
transmembrane 4 superfamily member 2 mRNA	mouse	NM_019634	**Nuclear Pore Complex**
unc50 related protein mRNA	rat	U96638	nucleoporin Nup84 mRNA
Receptors or Receptor Binding	**genus**	**Accesssion Number**	Ran-binding protein 2 gene
adenosine A1 receptor mRNA	rat	NM_017155	**mRNAs Coding for Solute Carrier Family or Transporter Proteins**
calcyon mRNA	human	XM_005674	ASC-1 complex subunit P50 mRNA/CGI-18 protein mRNA
centaurin-alpha 2 protein mRNA	rat	NM_020101	FXYD domain-containing ion transport regulator 7 mRNA
cookii corticotropin-releasing hormone-binding protein mRNA	mouse	U33323	GABA transporter mRNA
G10 protein homolog mRNA	rat	AF058791	glucose-6-phosphatase, transport protein 1, mRNA
glucocorticoid receptor mRNA	rat	NM_012576	glutamate transporter mRNA
glutamate receptor, ionotropic, NMDA 1, mRNA	rat	NM_017010	glutamate transporter EAAC1 interacting protein mRNA
glutamate receptor, ionotropic, NMDA1 (zeta 1), mRNA	mouse	NM_008169	mitochondrial carrier homolog 1 isoform a mRNA
imidazole receptor I-1-like protein mRNA	mouse	AF144133	neutral amino acid transporter mRNA
insulin receptor tyrosine kinase 53 kDa subustrate mRNA	hamster	U41899	organic cation transporter mRNA
man 6-P receptor mRNA	mouse	M63286	solute carrier family 7, member 4, mRNA
laminin receptor 1/67 kD, ribosomal protein SA mRNA	mouse	NM_011029	similar to solute carrier family 21, member 11, mRNA
LERK-3 gene	mouse	U92885	Na+-dependent inorganic phosphate cotransporter mRNA, brain specific
low density lipoprotein receptor related protein 1 mRNA	mouse	NM_008512	potasium channel beta 2 subunit mRNA
natriuretic peptide receptor 3 mRNA	mouse	NM_008728	solute carrier (SLC25A18) mRNA
similar to nuclear receptor binding protein mRNA	mouse	BC004756	solute carrier family 1 mRNA
nuclear receptor subfamily 4, group A, member 1 mRNA	mouse	NM_010444	solute carrier family 9, isoform 3 reg 2, mRNA
olfactory receptor gene cluster, OR17 and OR6 genes	mouse	AJ251155	similar to solute carrier family 25 (mitochondrial) member 5 mRNA
platelet derived growth factor receptor, alpha polypeptide, mRNA	mouse	NM_011058	spectrin-like protein (GTRAP41) mRNA
progesterone receptor membrane component mRNA	mouse	NM_016783	synaptic vesicle glycoprotein 2a mRNA
signal sequence receptor, beta, mRNA	human	BC000341; XM_002072	voltage-dependent anion channel 1 mRNA
thyroid hormone receptor mRNA	rat	NM_031134	voltage-dependent anion channel 3 mRNA

TABLE F

RNA Binding	**genus**	**Accession Number**
arsenite inducible RNA associated protein mRNA	mouse	AF224494
heterogeneous nuclear ribonucleoprotein A/B mRNA	mouse	NM_010448
heterogeneous nuclear ribonucleoprotein K mRNA	mouse	NM_025279
unc50 related protein mRNA	rat	U96638
Splicing	**genus**	**Accession Number**
non-POU-domain containing octamer-binding protein mRNA	mouse	NM_023144
pre-mRNA processing 8 protein (DEAH-box RNA helicase) mRNA	mouse	AB047391
small nuclear ribonucleoprotein N (SmN) mRNA	mouse	NM_013670
small nuclear ribonucleoprotein U5-116kD mRNA	mouse	U97079, NM_011431
splicing factor 3a, subunit 1, mRNA	human	XM_009917
Translation	**genus**	**Accession Number**
Clast 4 protein mRNA	mouse	AB031388
eukaryotic translation elongation factor 1, alpha 1, mRNA	mouse	BC005660
eukaryotic translation elongation factor 1 alpha 2 mRNA	mouse	NM_007906
eukaryotic translation initiation factor 3 mRNA	mouse	NM_010123
eukaryotic translaiton initiation factor 3, subunit 2 (beta 36 kD), mRNA	mouse	NM_018799
eukaryotic translation initiation factor 3, subunit 8 (110 kDa), mRNA	mouse	NM_019646
initiation factor eIF-4AI mRNA	mouse	X03039
isoleucine-tRNA synthetase mRNA	human	XM_005496
methionine aminopeptidase mRNA/initiaiton factor2-associated 67 kDa protein	mouse	BC002213
protein synthesis elongation factor Tu (eEF-Tu, eEf-1-alpha) mRNA	mouse	M22432
SPRPN upstream reading frame protein mRNA	mouse	AF101042
transfer RNA-Ser synthetase mRNA	mouse	M74012
translation repressor NAT1 mRNA	mouse	U76112

TABLE G

Nucleotide Binding Proteins	**genus**	**Accession Number**
ATPase H+ transporting lysosomal mRNA	human	BC005876
ATPase, H+ transporting, lysosomal 16 kD, mRNA	mouse	NM_009729
ATPase, H+ transporting, lysomomal, beta 56/58 kDa, isoform 2, mRNA	mouse	NM_007509
ATPase H+, transporting, lysosomal, subunit 1, mRNA	mouse	NM_018794
vacuolar ATPase subunit D mRNA	mouse	AF298810
ATPase, Na+K+ transporting, alpha 1polypeptide subunit, mRNA	rat	NM_012504
ATPase, Na+K+ transporting, alpha 2 polypeptide, mRNA	rat	NM_012505
ATPase, Na+K+ transporting, alpha 3 subunit, mRNA	rat	NM_012506
ATP-binding cassette sub-familiy A member 1 gene	mouse	AF287263
ATP-binding cassette sub-familyA member 2 mRNA	mouse	NM_007379
ATP-binding cassette sub-family G member 1 mRNA	mouse	NM_009593
ATP-binding cassette transporter G1 mRNA	mouse	AF323659
ATP citrate lyase mRNA	rat	NM_016987
ATP synthase, H+ transporting, mitochondrial F0 complex subunit F, mRNA	mouse	NM_016755
ATP synthase, H+ transporting mitochondrial F1 complex, mRNA	mouse	NM_016774
ATP synthase lipid-binding protein P3 precursor mRNA	rat	AF315374
chaperonin subunit 3 (gamma) mRNA	mouse	NM_009836
chaperonin subunit 5 (epsilon) mRNA	mouse	NM_007637
chaperonin subunit 6a (zeta) mRNA	mouse	NM_009838
similar to DEAD/H box polypeptide 15 mRNA	mouse	BC003745
GTP-binding protein homologous to yeast SEC4 mRNA	human	XM_008609; NM_006822
guanine nucleotide binding protein, alpha inhibiting 1, mRNA	rat	NM_013145
guanine nucleotide binding protein related sequence 1 mRNA	mouse	NM_008136
Ras-Related Proteins	**genus**	**Accession Number**
aplysia ras-related homolog N mRNA	mouse	NM_009708
ARL-6 interacting protein 1 (or TBX2 protein) mRNA	mouse	AF223953
cdc42 gene	mouse	L78075
G protein gamma 3 linked gene mRNA	mouse	NM_008144
Gdi-1 mRNA for RhoGDI-1	mouse	AB055070
membrane interacting protein of RGS16 mRNA	mouse	BC003902
PSD-95/SAP90-associated protein-3 mRNA	rat	U67139
RAP1A, member of RAS oncogene family, mRNA	human	XM_001929
Rap2 interacting protein mRNA	mouse	NM_016759
rhoB gene mRNA	rat	NM_022542
sarcoma virus p21 ras protein, complete cds	mouse	M30733
small GTP-binding protein (RAB1B) mRNA	human	NM_030981

TABLE H

Weakly Characterized HuR Ligands	genus	Accession Number	
AD-017 protein mRNA	human	BC001418	mitochondrial DNA
amyloid beta A4 precursor protein-binding, family A, member 3, mRNA	mouse	BC005423	mitochondrial DNA
augmenter of liver regeneration gene	mouse	U40494	mitochondrion
BM88 antigen mRNA	mouse	NM_021316	mouse brain cDNA similar to human Sjogren's Syndrome nuclear autoantigen 1 mF
catenin beta mRNA	mouse	NM_007614	mouse mRNA
cervical cancer 1 protooncogene protein p40 mRNA	human	AF195651	NCU-G1 mRNA for kidney predominant protein
CGI-10 protein mRNA	human	XM_007436	Nedd4 WW domain-binding protein 5 mRNA
chromosome 19 cone CTC-492K19	human	AC010271	neighbor of Cox4 mRNA
CLLL6 protein mRNA	human	AF334405	neural F box protein NFB42 mRNA
craniofacial development protein 1 mRNA	mouse	BC005589	neurochondrin mRNA
cysteine rich protein mRNA (zinc finger protein)	rat	NM_017148	neurochondrin-1 and -2 genes
deleted in polyposis 1 mRNA	mouse	NM_007874	neuronal protein mRNA
DMR-N9 (3'-region) mRNA	mouse	S60312	nuclear localization signal protein absent in velo-cardio-facial patients mRNA
neuronal development-associated protein 7 mRNA	mouse	AF361435	nucleic acid binding protein mRNA
ectodermal-neural cortex (with BTB-like domain) mRNA	human	XM_003863	5'OT-EST gene
ET8 ET putative translation product mRNA	mouse	AF015191	ovarian carcinoma immunoreactive antigen, clone MGC:4962
ethanol induced 6 mRNA	mouse	NM_024166	prion protein mRNA
fused toes mRNA	mouse	NM_010241	prostrate tumor over expressed gene 1 mRNA
ganglioside expressed factor 2 mRNA	rat	NM_022706	rS-Rex-s mRNA
gene trap ROSA 26 antisense mRNA	mouse	NM_008188	rS-Rex-b mRNA
GT12 protein mRNA	mouse	Y13832	15 kDa selenoprotein mRNA
Hepatitus delta antigen-interacting protein A mRNA	human	XM_006503	selenoprotein W, muscle 1, mRNA
hepatoma-derived growth factor mRNA	mouse	NM_008231	serologically defined breast cancer antigen NY-BR-99 mRNA
HSKM-B protein mRNA	human	NM_020197	superiorcervical ganglia, neural specific 10, mRNA
hypothalamus protein HT011 mRNA	human	XM_013066	testis abundant finger protein mRNA
human chromosome 17	human	AC004797	translationally regulated transcript 21 kDa mRNA
human mRNA sequences	human	AB040944	translin-associated factor X mRNA
hypothetical protein, clone I-73, mRNA	mouse	NM_022424	TRIAD2 type 1 mRNA (ring finger protein)
hypothetical protein FLJ10971	human	XM_005855	tripartite motif protein TRIM33 mRNA
hypothetical protein FLJ10782	human	XM_001476	tumor differentially expressed 1 mRNA
hypothetical protein FLJ12707	human	XM_012356	tumor rejection antigen gp96 mRNA
hypothetical protein FLJ20136 mRNA	human	XM_007674	tyrosine 3-monoxy/trypotphan 5-monooxy activator protein eta mRNA
similar to hypothetical protein FLJ20548	human	BC001719	tyrosine 3-monoxy/tryptophan 5-monooxy activator protein theta mRNA
human cDNA FLJ21007	human	AK024660	tyrosine 3-monoxy/tryptophan 5-monooxy activator protein mRNA
hypothetical protein FLJ22386	human	NM_024589	similar to tyrosine 3-monoxy/tryptophan 5-monooxy activator protein mRNA
hypothetical zinc finger protein FLJ22504	human	XM_012934	ubiquilin 2 mRNA
major histocompatibility complex region	mouse	AF110520	zinc finger protein mRNA
major histocompatibility locus class III region	mouse	AF030001	zinc finger protein 239 mRNA

TABLE I

ESTS and Other Uncharacterized Mouse Tissue Sequences				
AA033208	AK005188	AK021110	BE654439	BG292775
AA038067	AK005316	AK021221	BE654980	BG293521
AA073516	AK005372	AK027262	BE852363	BG295960
AA123170	AK005440	AU035340	BE860742	BG342769
AA172344	AK007691	AU067636	BE861817	BG412968
AA182986	AK007745	AU067652	BE862808	NM_021418
AA215069	AK007787	AU079734	BE863310	NM_025448
AA239742	AK007884	AW215767	BE863638	NM_025556
AA250370	AK008070	AW230383	BE865214	NM_025602
AA285772	AK009200	AW322828	BE912184	NM_025934
AA472243	AK009315	AW493786	BE913901	NM_026031
AA516870	AK009511	AW455570	BE944524	NM_026058
AA545264	AK009759	AW456426	BE945942	NM_026437
AA656491	AK009861	AW476440	BE949100	NM_028007
AA682009	AK010175	AW490989	BE951843	W88225
AA870186	AK010484	AW491478	BE981569	
AA982662	AK010642	AW494195	BE985561	
AB030201	AK010815	AW536922	BE994210	
AB041537	AK010925	AW539243	BF016391	
AC083814	AK010949	AW543038	BF021247	
AC78930	AK010995	AW553439	BF102017	
AI118479	AK011229	AW556406	BF121061	
AI154452	AK012047	BB180026	BF135109	
AI316878	AK012163	BB262320	BF142733	
AI390890	AK012224	BB283840	BF143522	
AI430494	AK012472	BB556122	BF160544	
AI464296	AK012958	BB593088	BF164020	
AI466684	AK013308	BC003436	BF178425	
AI528997	AK013519	BC003940	BF181401	
AI510109	AK013554	BC003965	BF228226	
AI549780	AK013556	BC005670	BF321398	
AI592907	AK013661	BE137090	BF323187	
AI877154	AK013768	BE283493	BF383402	
AI840652	AK013852	BE285183	BF468744	
AI882274	AK013971	BE374627	BF585051	
AK002624	AK014384	BE376520	BF578961	
AK002962	AK014435	BE534633	BF719004	
AK003586	AK016053	BE627704	BF784965	
AK003584	AK016504	BE631740	BF785994	
AK003644	AK017688	BE632292	BF787347	
AK003646	AK018036	BE647932	BG062585	
AK003699	AK018184	BE647959	BG066621	
AK003715	AK018202	BE649086	BG069624	
AK004065	AK018339	BE650010	BG073164	
AK004406	AK018493	BE650166	BG073343	
AK004467	AK018693	BE651082	BG147023	
AK004750	AK019321	BE651980	BG176134	
AK004759	AK019698	BE652465	BG244492	
AK004913	AK020444	BE653535	BG246294	
AK005136	AK020972	BE653592	BG247727	