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The Regulation of Glucose Transporter (GLUT1) Expression by the RNA Binding Protein HuR

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Abstract HuR is a ligand for nuclear mRNAs containing adenylate-uridylate-rich (ARE) elements in the 3′-untranslated region. Once bound to the mRNA, HuR is recognized by adapter proteins that then facilitate nuclear export of the complex. In the cytosol, HuR is thought to function to control stability and translation of its ligand message. We have previously demonstrated that HuR is constitutively expressed in the 3T3-L1 cells and shuttles from the nucleus to the cytosol, but remains predominantly nuclear in the preadipocytes and that as the cells differentiate, there is a marked increase in the proportion of HuR in the cytosol at any time. The GLUT1 glucose transporter is also expressed in both preadipocytes and adipocytes and in vitro RNA gel shifts indicate the mRNA is a ligand for HuR. However, HuR complexes containing the GLUT1 mRNA can only be isolated from the terminally differentiated adipocytes. Moreover, position analysis of the GLUT1 mRNA and HuR protein in polysome profiles demonstrates a shift to the most dense region of the gradient for both message and protein with adipocyte differentiation. Consistent with a regulatory role in the control of GLUT1 expression, siRNA-mediated decrease in HuR protein resulted in a decreased expression of GLUT1 protein. These data suggest that HuR contributes to the metabolic function of the adipocyte through mediation of post-transcriptional regulatory events. J. Cell. Biochem. 99: 565–574, 2006.

Key words: HuR; GLUT1; adipocyte; differentiation; polysome profiles; translation

HuR is a widely expressed RNA binding protein belonging to the Hu/ELAV family of mRNA binding proteins [Ma et al., 1996; Lu and Schneider, 2004]. HuR contains a nucleo-cytoplasmic shuttling sequence and functions as an adapter protein in the nuclear export of mRNAs that contain adenylate-uridylate rich elements (AREs) in their 3′ untranslated regions [Ma et al., 1996; Myer et al., 1997; Fan and Steitz, 1998; Levy et al., 1998; Peng et al., 1998]. Once in the cytosol, HuR has been suggested to regulate the stability and translational efficiency of its mRNA ligands [Shaw and Kamen, 1986; Jacobson and Peltz, 1996; Jain et al., 1997; Antic et al., 1999; reviewed in Cherry et al., 2006].

In recent work, we described the constitutive expression of the RNA binding protein HuR in the 3T3-L1 preadipocytes with a modest three-fold increase in protein content as the cells differentiate into adipocytes [Gantt et al., 2005; Cherry et al., 2006]. At confluence in the preadipocyte, the majority of HuR protein is retained in the nucleus. However, on exposure of the cells to the differentiation inducers, there is a rapid formation of a *nuclear* HuR–C/EBPβ complex followed by a translocation of the complex to the cytosol. When HuR expression was reduced using siRNA, the cells retained their preadipocyte morphology, failed to express normal levels of C/EBPβ, and did not differentiate. These observations were consistent with maintenance of HuR protein content

Kira R. Gantt and Joy Cherry contributed equally to this study and are considered co-first authors.

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and the ability of the cells to express C/EBPβ and progress through the differentiation program.

The increased fraction of HuR protein in the cytosol, initiated with the induction of differentiation, is maintained long after C/EBPβ expression has diminished [Gantt et al., 2005]. This suggested to us that in the terminally differentiated adipose cell, HuR is functioning to chaperone other mRNAs to the cytosol as well as playing a role in their translation. Our previous work in which neuronal HuB was ectopically expressed in the 3T3-L1 cells resulted in an altered phenotype as the cells exhibited a more robust differentiation with a 12-fold overexpression of the GLUT1 glucose transporter [Jain et al., 1997]. Analysis by electron microscopy localized the ectopically expressed HuB to the nucleus in the preadipocytes with a transition to the cytosol as the cells began to differentiate; interestingly, cytosolic HuB co-localized with polysomes [Gantt et al., 2003, 2004]. In that study, our hypothesis was that ectopically expressed HuB was functioning for the endogenous Hu protein, HuR. We now examine the ability for HuR to interact with the GLUT1 mRNA and the potential to mediate post-transcriptional regulation of GLUT1 gene expression.

EXPERIMENTAL PROCEDURES

Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco/Invitrogen (Grand Island, NY). Bovine calf serum and fetal calf serum were purchased from Hyclone Laboratories (Logan, UT). The 3T3-L1 cells used in this work were obtained from Howard Green (Harvard University, Boston, MA). The BCA Protein Assay kit, the NE-PER™ Cell Fractionation kit, and HALT™ protease inhibitor mix were from Pierce (Rockford, IL). Reagents for molecular biology were purchased from Invitrogen (Carlsbad, CA). All other chemicals were of reagent grade and purchased from Sigma-Aldrich Biochemical (St Louis, MO). The 3A2 monoclonal antibody directed against HuR and the β-tubulin antibodies were purchased from Santa Cruz Biotechnology, Inc., (Santa Cruz, CA). The mouse anti-Armenian and Syrian hamster IgG1 monoclonal antibody used as an isotype control was obtained from BD Pharmingen (San Diego, CA). The SiGENOME SMARTpool™ reagent and the siCONTROL non-targeting siRNA™ were obtained from Dharmacon (Lafayette, CO), primers for Reverse Transcriptase-PCR and Real-Time PCR were obtained from Invitrogen (Carlsbad, CA).

3T3-L1 Cell Culture

3T3-L1 preadipocytes were cultured, maintained, and differentiated as previously described [Stephens and Pekala, 1992]. For experimentation, preadipocytes were harvested at 2 days post confluence and adipocytes were used at 8 days post induction of differentiation.

RNA Gel Shift Analysis

Gel shift analysis was performed as we have previously described [Gantt et al., 2005]. Molecular weight determinations of RNA binding proteins involved in complex formation with the GLUT1 mRNA were performed as we have previously described [McGowan et al., 1997]. Primers were designed for each potential binding region such that the sense primer contained 20 bases encoding the T7 RNA polymerase start site (5′-gcctaatacgactcactata-3′) at the 5′ end followed by 18 bases complementary to the noncoding strand. The antisense primer contained the final 18 bases of the region. The GLUT1 (Accession #: M23384) binding regions amplified were: Full length GLUT1 3′UTR: 1,660 to 2,560; Δ1:1,820 to 1,950; Binding loop:1,850 to 1,910. Mutations in the binding loop construct were generated using the Stratagene QuickChange® II XL Site-Directed Mutagenesis Kit.

Analysis of Polysomes by Sucrose Density Gradients

Polysome profiles were generated and RNA isolated as described by Long and Pekala [1996].

Real-Time PCR

RNA (0.5 μg) isolated from sucrose gradient fractions or total RNA were subjected to reverse transcription with random primers and reverse transcriptase from the iScript™ cDNA Synthesis kit (c-Rad). When polysome distribution was measured across the entire gradient, individual RNA fractions were processed in this way. Integrity of isolated RNA was determined by ethidium bromide staining of the 28 and 18S rRNA bands on an analytical agarose gel. Quantitative real-time PCR was performed with specific primers designed for each gene with the Beacon Designer tool (Bio-Rad). All

primers were subjected to Blast search to insure specificity and fold analysis to eliminate any primer with potential to form secondary structure. Amplification and detection were done with the iCycler IQ real-time PCR detection system with IQ SYBRgreen Supermix (Bio-Rad). Standard curves were prepared for each target gene and PCR efficiency determined to be in excess of 90% for all primer sets. Threshold temperatures were selected automatically and all amplifications were followed by melt-curve runs. Melt-curve analysis was by plot of the negative first derivative of the fluorescence versus temperature plot with the software assigning the melt temperature. Single melt temperatures were recorded in all cases. To calculate polysomal shifts, the threshold cycle (C_T) for fraction number 3 (no product was detected in fractions 1 and 2) was subtracted from the average C_T of each fraction (ΔC_T). Relative GLUT1 or LDH mRNA levels were calculated as $2^{-\Delta CT}$ and graphically represented as the percentage of GLUT1 or LDH mRNA present in each fraction relative to the total amount of GLUT1 or LDH mRNA (sum of all fractions) as previously described by Dinkova et al. [2005]. Primers used for detection of lactate dehydrogenase (LDH) were: forward—5′-tgcctacgaggtgatcaagctgaa-3′ and reverse—5′-ttatgctctcagccaagtctgcca-3′. Those for GLUT1 were: forward—5′-tcaacgagcatcttcgagaaggca-3′ and reverse—5′-tcgtccagctcgctctacaacaaa-3′. Analyses were performed in triplicate on two separate density gradient separations.

Isolation of Cytosolic and Nuclear Fractions

The Ne-PER™ Cell Fractionation kit was used to isolate cytosolic and nuclear fractions from the 3T3-L1 cells as per manufacturer's (Pierce) instructions with minor modifications as described previously [Gantt et al., 2005].

Immunoprecipitation of mRNP Complexes

The protocol was carried out using RNAse and DNase free conditions as described previously [Gantt et al., 2005]. Detection of GLUT1 mRNA in the immunoprecipitated mRNP complexes by RTPCR was accomplished as previously described using the following primers: Forward 5′-tccctgcagcccaaggatct-3′ and reverse 5′-gcaagtgtctggacagggcg-3′.

Western Blot Analysis

Western blot analysis was performed as previously detailed [Stephens and Pekala, 1992].

SiRNA Treatment of the 3T3-L1 Preadipocytes

Transfection of the cells was performed using Dharmacon siGENOME SMARTpool™ reagent (Cat. #: M-053812-00-0020). A four Si oligo system designed specifically for HuR (Mouse ELAV1, accession number: NM_010485) by Dharmacon was utilized as we have previously described [Gantt et al., 2005]. Briefly, preadipocytes in 12-well plates at approximately 80% confluency were transfected with SiRNA using Lipofectamine 2000 as a carrier according to manufacturer's instructions. Two control transfections were carried out, one with Lipofectamine 2000 alone and the other with Dharmacon siCONTROL non-targeting siRNA™ four oligo pool (SC, cat. # D-001206-13-05). The cells were exposed to the transfection mixture for 6 h at which time the transfection medium was replaced with DMEM supplemented with 10% fetal bovine serum. Twenty-four hours after removal of the transfection media, two monolayers of each treatment group were trypsinized and combined to generate an immediately confluent monolayer, essential for differentiation. Eighteen hours later (48 h after the initial transfection), the cells were exposed to the differentiation protocol. Cells were then analyzed at Day 5 for expression of GLUT1.

RESULTS

Protein Binding to the GLUT1 3′UTR With Respect to Differentiation

In our previous studies, we demonstrated that on attainment of confluency, expression of GLUT1 protein and mRNA decreased to basal levels [Jain et al., 1997]. On induction of differentiation, both transporter mRNA and protein increased (three and fivefold respectively) to homeostatic levels similar to that observed in growing preadipocytes [Jain et al., 1997]. While increased transcription of the *GLUT1* gene could not be detected by nuclear run-on assays [Cornelius et al., 1991; Stephens and Pekala, 1992], the GLUT1 mRNA was observed to be stabilized [Jain et al., 1997]. The half-life in the confluent preadipocytes was

determined to be approximately 42 ± 9 min [Cornelius et al., 1991] while that in the fully differentiated adipocytes was 4.5 ± 1.7 h [Jain et al., 1997]. This suggested that control of GLUT1 expression in the adipocytes was at least in part post transcriptional and may be mediated by RNA binding proteins. Therefore, our first studies involved the use of RNA gel shift analysis to determine if the ARE in the GLUT1 3′UTR was a ligand for HuR, the RNA binding protein demonstrated to be involved in mRNA stabilization. Riboprobes were prepared containing the ARE (bases 1,910 to 1,950; 68.5% A + U content) which we have previously described as the Δ1 region of the GLUT1 3′UTR [Jain et al., 1997]. The Δ1 region is shown schematically in Figure 1A. The analysis was performed using protein derived from whole cell lysates of undifferentiated and fully differentiated 3T3-L1 adipocytes. As shown in Figure 1B, the GLUT1 ARE was a ligand for protein contained in the extracts from both preadipocytes (Lane 2) and fully differentiated adipocytes (Lane 4). The ability of the 3A2 monoclonal anti-HuR antibody to supershift the complex from both phenotypes (Lanes 3 and 5) is consistent with a role for HuR in complex formation with the GLUT1 ARE. We do note that in Lane 3, it appears that not all of the complex was shifted with the antibody. With respect to this observation, we have not ruled out involvement of other proteins in complex formation. Further confirmation of HuR involvement was obtained by isolating cytosolic extracts for the binding assay [Gantt et al., 2005] and UV-crosslinking the mRNA-ribonucleoprotein complex followed by digestion with an RNase A/T1 mixture as we have previously described [McGowan et al., 1997]. The data displayed in Figure 1, Panel C demonstrate that using cytosolic extracts from preadipocytes (Lanes 1–3) or fully differentiated adipocytes (Lanes 4–6) as sources of RNA binding proteins, resulted in the identification of a protein of approx. 36 kDa bound to both the full length probe as well as the Δ1 probe. We note that this observation suggests that binding is not a conformational artifact of using the Δ1 binding domain rather than the full 1,000 bases of the 3′-UTR. This molecular size is consistent with the size of HuR. These data also indicate that when cytosolic extracts are utilized as the source of HuR, there is an approximate fourfold increase in binding as the cells differentiate into adipocytes. This is consistent with our previous data indicating that while HuR is constitutively expressed in the 3T3-L1 cells, there is a modest threefold increase in protein as the cells differentiate and with differentiation, there is an increased residence of HuR in the cytoplasmic compartment [Gantt et al., 2005]. Further studies with deletion and point mutation localized the actual binding site to a uridylate-rich region at the 3′ end of the Δ1 region. Analysis of the sequence by the Fold and Squiggles software (GCG) provided a potential structure based on the most favorable thermodynamic folding pattern of the U-rich region (Fig. 1, Panel D). Alteration of the uridylate residues 1,926 and 1,927 (indicated by arrows in Fig. 1, Panel D) to AC (Lane 2) and CC (Lane 3) resulted in a loss of binding (Fig. 1, Panel E).

Formation of a HuR–GLUT1 mRNA Complex Occurs Only in the Adipocytes

To determine if the GLUT1 message was present in an mRNP complex with HuR, immunoprecipitations were performed on cytosolic extracts prepared from preadipocytes (Day 0) and fully differentiated adipocytes (Day 8). As shown in Figure 2, Panel A, Western blot analysis indicated that HuR protein was detected in the cytosolic fractions of both preadipocytes (PA) and adipocytes (AD). We note that quantification of the HuR protein bands in the immunoprecipitate indicate a threefold increase in immunoprecipitable HuR with respect to differentiation. This is similar to that which we previously reported [Gantt et al., 2005]. Analysis of the mRNA present in the immunoprecipitated mRNP complex shown in Figure 2, Panel B, indicates that prior to addition of the differentiation inducers, HuR could be immunoprecipitated, however, GLUT1 mRNA could not be detected in the complex by RT-PCR analysis. In the fully differentiated adipocytes, RT-PCR analysis confirmed the presence of GLUT1 mRNA in the immunoprecipitated complex. The positive control represents the amplification of GLUT1 mRNA from total RNA isolated from Day 4 adipocytes. The data presented in Figure 2 are consistent with the GLUT1 mRNA serving as a ligand for HuR in a differentiation-specific manner and while both binding partners are present in the preadipocyte, the complex does not form.

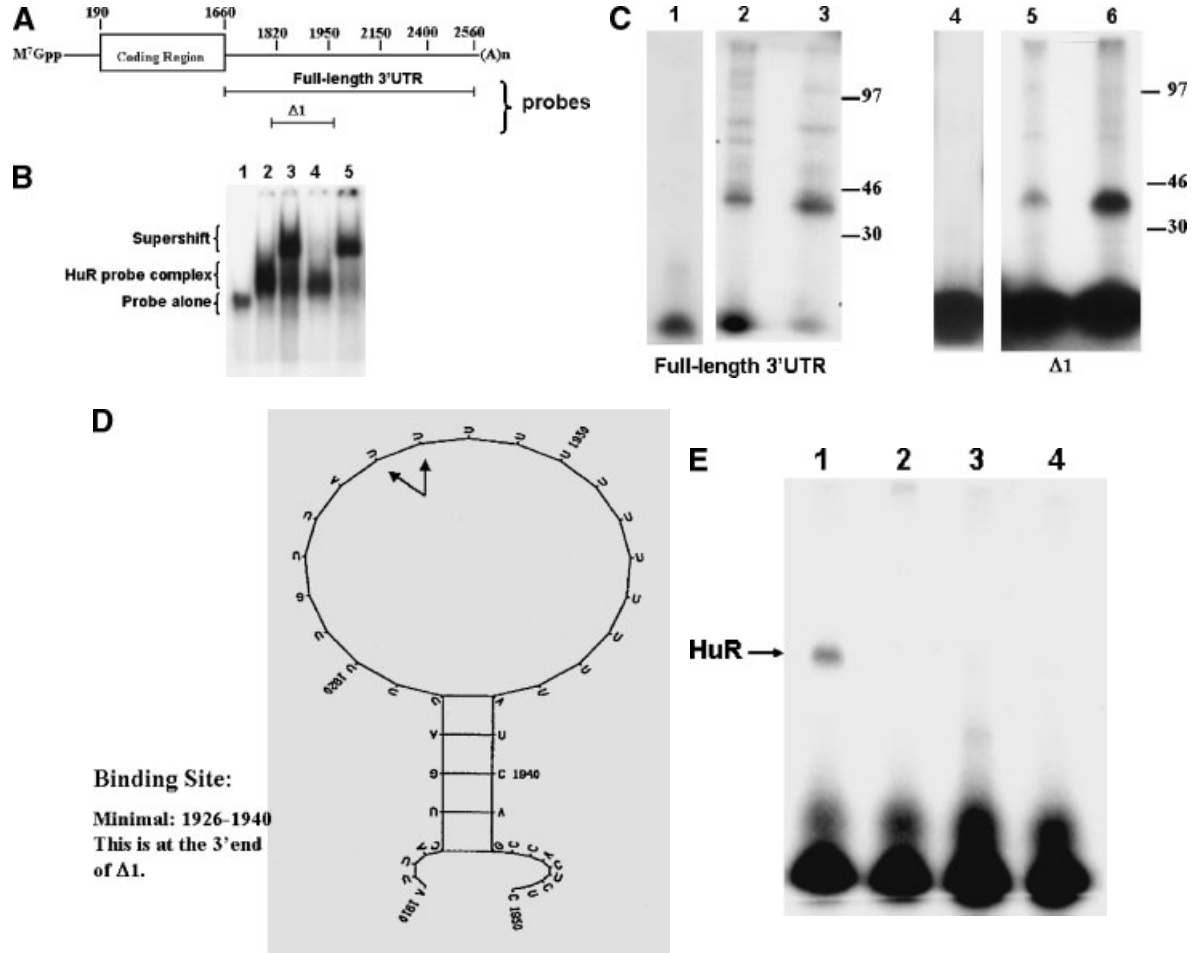

Fig. 1. Identification of HuR as the ligand for the ARE in the GLUT1 3′UTR. **Panel A**. Diagram of GLUT1 mRNA. **Panel B**. Total cellular lysates (20 μg) from either preadipocytes or fully differentiated adipocytes and a radiolabeled probe corresponding to the Δ1 region of the GLUT1 3′UTR were used to perform RNA gel shifts. Lane 1: probe alone; Lane 2: Δ1 probe + preadipocyte extract; Lane 3: Δ1 probe + preadipocyte extract + anti-HuR antibody; Lane 4: Δ1 probe + adipocyte extract; Lane 5: Δ1 probe + adipocyte extract + anti-HuR antibody. **Panel C**. Cytosolic extract (5 μg) prepared as described in Experimental Procedures from preadipocytes (**lanes 1–3**) or fully differentiated adipocytes (**lanes 4–6**), and a probe corresponding to either the full length 3′UTR or the Δ1 region of the 3′UTR were used in a binding assay followed by UV crosslinking to determine the molecular size of the RNA binding protein. **Panel D**. A Fold and Squiggles plot of the HuR binding site characterized by deletion and site-directed mutagenesis. Mutation of the two uridylate residues indicated by the arrows to either adenylate–cytosine or cytosine–cytosine resulted in loss of binding as shown in **Panel E**. Lane 1: Wild type probe + 5 μg adipocyte cytosolic extract; lane 2: Mutant probe uu to ac + extract; lane 3: Mutant probe uu to cc + extract; lane 4: Wild type probe alone. The studies presented in Panels A through E are representative of experiments performed at least three times with similar results.

GLUT1 mRNA and HuR Protein Distribution in Polysome Profiles

Sucrose density gradient analysis was performed to determine if the change in phenotype from preadipocyte to adipocyte altered the distribution of GLUT1 mRNA among ribosomes and polysomes, thereby potentially affecting the rate of translation or half-life of the message. During translation, efficiently initiated mRNAs are found on larger (heavy) polysomes. An increase in the initiation rate relative to elongation results in a shift from light to heavy polysomes [Lodish, 1974]. We reasoned that if HuR was functioning to effect translation, then as the cells acquire the adipocyte phenotype and the GLUT1 mRNA becomes a ligand for HuR, then we should observe a shift in the position of the GLUT1 mRNA from light to heavy polysomes. The analysis was performed on Day 0 preadipocytes and Day 9 fully differentiated adipocytes. Total RNA was isolated from each

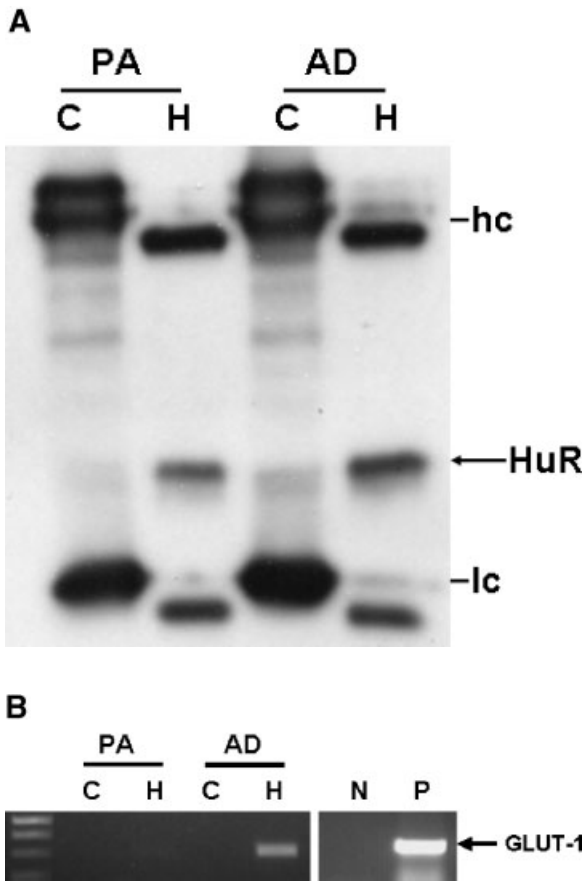

Fig. 2. Formation of cytosolic HuR–GLUT1 mRNA complexes is exclusive to the adipocytes. Cytosolic extracts were prepared as described in Experimental Procedures and immunoprecipitations of HuR containing mRNP performed. **Panel A**: Western blot analysis of cytosolic extracts (100 μg) from both preadipocytes (PA) and adipocytes (AD) confirms presence of HuR in the immunoprecipitate. **H**: Immunoprecipitate performed with the HuR monoclonal antibody. **C**: Immunoprecipitate performed with control (anti-Syrian-Armenian hamster IgG) antibody. The bands at approximately 25 and 50 kDa are the heavy (**hc**) and light (**lc**) immunoglobulin chains from the immunoprecipitations. **Panel B**: Using six identical immunoprecipitates, RNA was extracted and subjected to RT-PCR analysis for the presence of GLUT1 mRNA (arrow indicates GLUT1). Designations and times are as stated in Panel A. **P**: Positive control (total Day 4 adipocyte RNA), **N**: Negative (water) control. These data are representative of four separate cytosolic isolations and immunoprecipitations performed with similar results.

fraction and GLUT1 mRNA content determined by real-time PCR analysis as described in Experimental Procedures. As demonstrated in Figure 3, Panels A through C, relative to the adipocytes, there is more GLUT1 mRNA present in the initiation complexes (fractions 4 through 6). In the polysome region of the profile (fractions 8 through 16), GLUT1 content in the preadipocytes is maximal in fractions 12 to 13, while in the adipocytes there is a distinct shift of the bulk of the GLUT1 mRNA to the most dense fractions of the gradient (fractions 14 through 16). These data as well as those presented in Figure 2, are consistent with HuR mediating an increased efficiency of translation initiation of the GLUT1 mRNA in the adipocytes that leads to the observed shift of the GLUT1 message to the heaviest polysomes. For comparison, we examined the positioning in the gradient of LDH (Fig. 3, Panel D). LDH was selected as this mRNA does not contain a HuR binding site and thus is not expected to be either a ligand for HuR or regulated by the presence of HuR. As shown in Figure 3, Panel D, the bulk of the LDH mRNA localizes to the most dense polysomes in both preadipocytes and adipocytes. Further supporting a role for HuR in the determination of the efficiency by which GLUT1 mRNA is translated.

Finally, in Figure 3, Panel E, the distribution of HuR protein in the polysome profiles is displayed. HuR distribution in the adipocytes is clearly biased toward the most dense fractions of the sucrose gradient, consistent with an association with the heavy polysomes.

Effect of HuR Depletion on GLUT1 Protein Expression

To examine the extent of HuR involvement in the regulation of GLUT1 expression, we used the Dharmacon Smart Pool™ four oligo siRNA (Si) approach, developed in our previous studies to suppress HuR protein content in the 3T3-L1 cells [Gantt et al., 2005]. The study used two controls, our transfection agent, Lipofectamine 2000 (LF) alone and a Dharmacon Non-Targeting™ 4 oligo pool (Sc). Under the conditions described in Experimental Procedures, HuR content remained decreased for at least 5 days. After this time, HuR protein began to increase gradually as the long-term effectiveness of the treatment diminished by Day 10. We have previously documented this phenotype and protocol [Gantt et al., 2005]. Examination of GLUT1 protein expression at Day 5 in HuR-depleted cells is displayed in Figure 4. Densitometric analysis of Western blots of whole cell lysates demonstrate that in this experiment, HuR content was decreased by 80% normalized to control values. When normalized to the positive control, β-tubulin, no change is observed in the GLUT1 content in the two controls, however, a 60% decrease in GLUT1 protein is observed in the HuR-depleted cells.

The data are consistent with a requirement for the maintenance of a HuR–GLUT1 mRNA complex for full expression of the GLUT1 protein.

DISCUSSION

Our previous work described the constitutive expression of the RNA binding protein HuR in

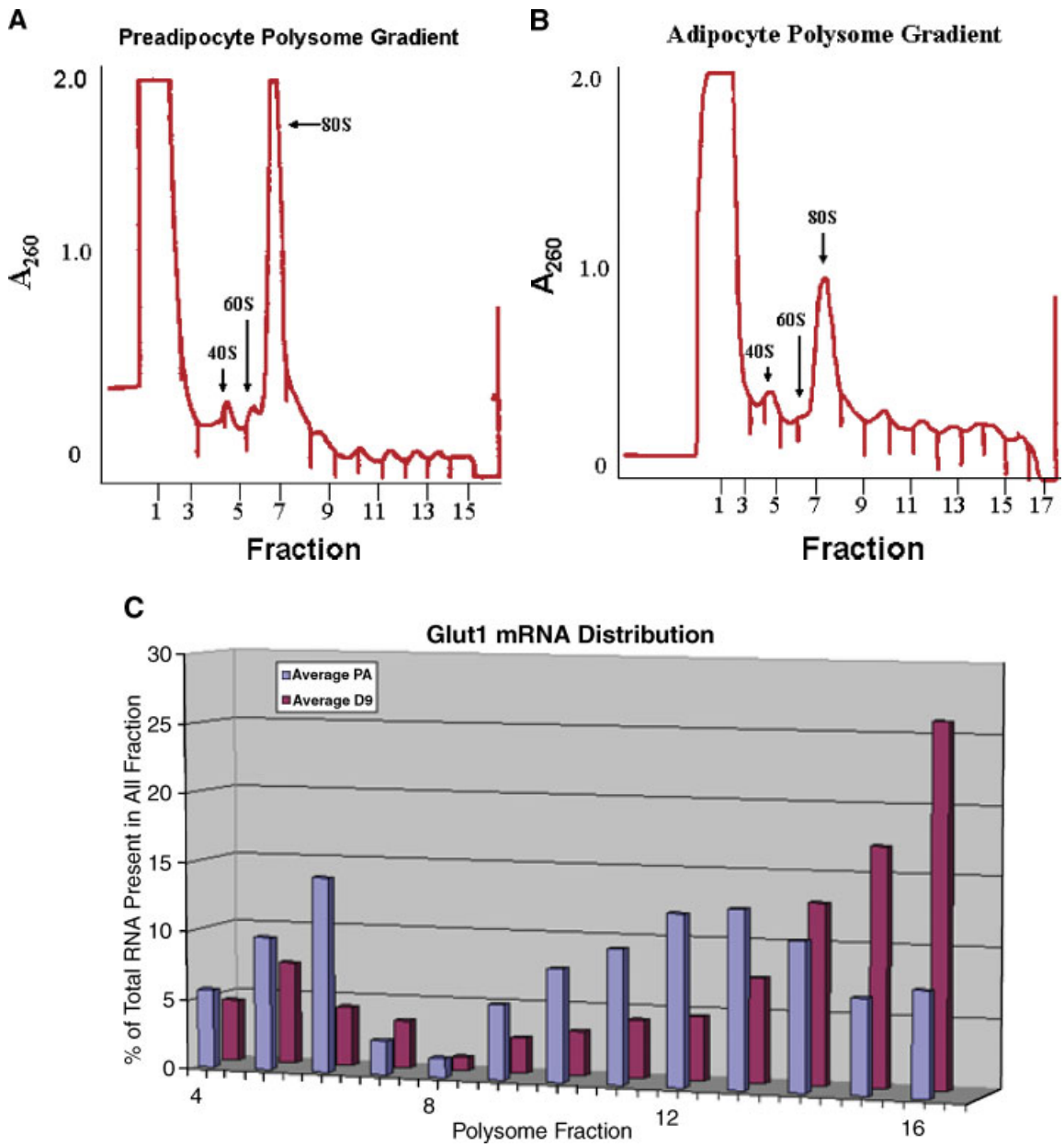

Fig. 3. Polysome profiles and GLUT1 localization. A post mitochondrial supernatant was prepared from preadipocytes (**Panel A**) or adipocytes (**Panel B**) and applied to a 15 to 45% sucrose density gradient, which was centrifuged at 20,000*g* for 2 h. The gradients were fractionated and the absorbance at 260 nm was measured, generating a polysome profile (panel A: preadipocytes, panel B: adipocytes). The tops of the gradients are to the left. RNA from the fractions was isolated and GLUT1 (**Panel C**) or LDH (**Panel D**) mRNA content determined by real-time PCR. Analyses were performed in triplicate on two separate density gradient separations. Relative GLUT1 or LDH mRNA levels were calculated as $2^{-\Delta CT}$ and are graphically represented as the percentage of GLUT1 or LDH mRNA present in each fraction relative to the total amount of GLUT1 or LDH mRNA (sum of all fractions) as previously described by Dinkova et al. [2005]. **Panel E**: Western blot analysis of HuR protein across the sucrose density gradient. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

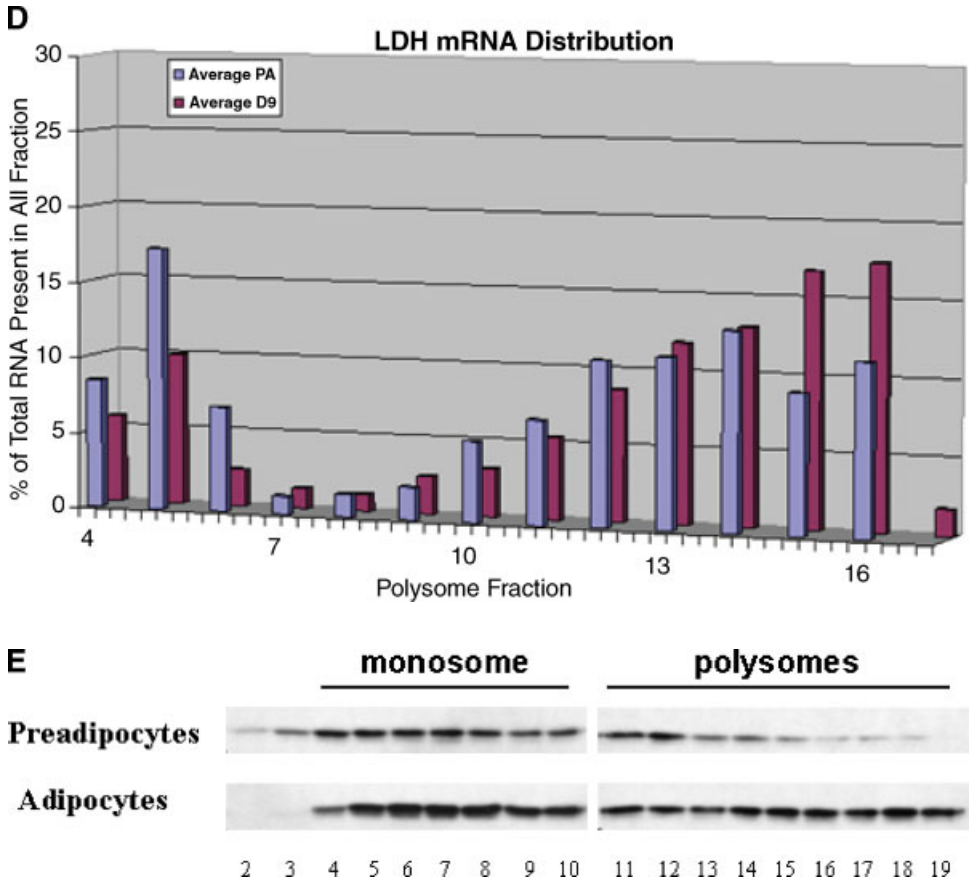

Fig. 3. (*Continued*)

the 3T3-L1 preadipocytes [Gantt et al., 2005]. At confluence in the preadipocyte, the majority of HuR protein was retained in the nucleus. However, on exposure of the cells to the differentiation inducers, there was a rapid formation of a *nuclear* HuR–C/EBPβ complex followed by a translocation of the complex to the cytosol. While these data supported a role for HuR in the induction of differentiation, the establishment of a significant cytosolic presence of HuR in the terminally differentiated adipocytes was consistent with an additional role in the maintenance of the adipocyte phenotype. In the current study, we identify the GLUT1 mRNA as a ligand for HuR in the adipocyte. The HuR binding site was localized to a uridylate-rich region between bases 1,926 and 1,940 in the 3′UTR of the GLUT1 message, the same region defined as a HuB binding site in our previous work [Jain et al., 1997]. In that study, neuronal HuB was ectopically expressed in the 3T3-L1 cells and remained in the nucleus until the cells were induced to differentiate. HuB then moved to the cytosol where it localized to the polysomes [Gantt et al., 2004]. This translocation to the cytosol and localization to the polysomes resulted in a differentiation-specific stabilization of the GLUT1 mRNA and an enhancement of the translation efficiency of the message [Jain et al., 1997]. Those observations led us to propose that ectopically expressed HuB was augmenting the function of an endogenous Hu protein, which was unidentified at that time but we now know to be HuR. The nuclear versus cytosolic distribution of HuR with respect to differentiation is consistent with our ability to immunoprecipitate HuR–GLUT1 mRNA complexes only from adipocytes, an observation consistent with HuR functioning to chaperone the message to the cytosol and manage its translation in the terminally differentiated adipocyte. Moreover, coincident with the differentiation process, the half-life of the GLUT1 mRNA increases sixfold [Cornelius et al., 1991; Jain et al., 1997] consistent with HuR-mediated stabilization.

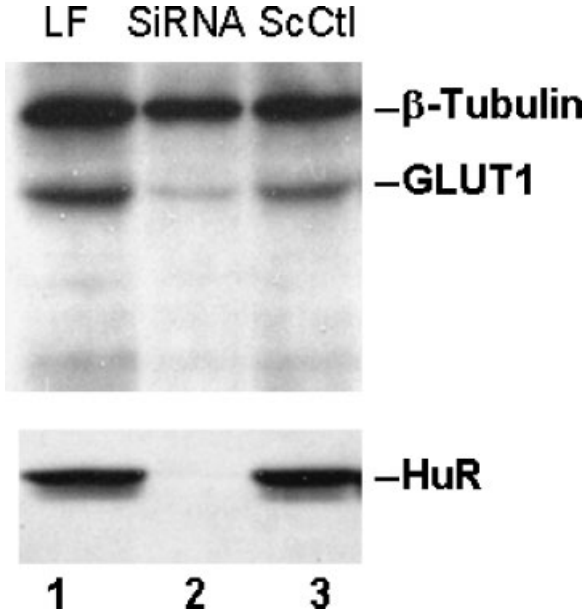

Fig. 4. Depletion of HuR by SiRNA treatment attenuates the expression of GLUT1 protein. Cells were exposed to the transfection protocol as described in Experimental Procedures, confluency established and differentiation induced. At 5 days post induction of differentiation, individual wells corresponding to treatment with Lipofectamine 2000™ alone (LF), the Smart Pool™ siRNA directed against HuR (SiRNA), and the Smart Pool™ four oligo control (ScCtl) were scraped in Western sample buffer, the proteins separated by polyacrylamide gel electrophoresis and Western blots performed. The blots were first probed for GLUT1 and β-Tubulin, then stripped and re-probed for HuR. β-Tubulin, whose protein content does not change over the time course of this experiment, served as a loading/normalization control. The data shown in this figure are representative of an experiment performed three times with identical results.

In the current study, suppression of HuR expression using SiRNA resulted in a markedly decreased expression of GLUT1 protein. Based on the results presented here as well as our previous studies [Gantt et al., 2005], we suggest that the decreased GLUT1 expression results from interference with HuR-mediated: 1. mRNA translocation to the cytosol and 2. Translation initiation and movement of the mRNA to the dense polysomes. In addition, we predict the loss of the stability induced by HuR binding to the message.

Rapid and well-controlled expression of GLUT1 is essential since this transporter is involved not only in the critical process of basal glucose entry into the cell but is vital to maintaining homeostatic metabolic function as the cell responds to a variety of physiological stresses in both insulin-dependent and -independent tissues [Klip et al., 1994; Tsukamoto et al., 1998; Vander Heiden et al., 2001]. We propose that HuR contributes to metabolic function in the adipocyte through the binding stabilization and enhanced translational efficiency of the GLUT1 message.

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