AMP-activated Protein Kinase-regulated Phosphorylation and Acetylation of Importin α1

INVolvement in the Nuclear Import of RNA-Binding Protein HuR

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Nuclear import of HuR, a shuttling RNA-binding protein, is associated with reduced stability of its target mRNAs. Increased function of the AMP-activated protein kinase (AMPK), an enzyme involved in responding to metabolic stress, was recently shown to reduce the cytoplasmic levels of HuR. Here, we provide evidence that importin α1, an adaptor protein involved in nuclear import, contributes to the nuclear import of HuR through two AMPK-modulated mechanisms. First, AMPK triggered the acetylation of importin α1 on Lys88, a process dependent on the acetyl transfer activity of p300. Second, AMPK phosphorylated importin α1 on Ser166. Accordingly, expression of importin α1 proteins bearing K22R or S165A mutations failed to mediate the nuclear import of HuR in intact cells. Our results point to importin α1 as a critical downstream target of AMPK and key mediator of AMPK-triggered HuR nuclear import.

Post-transcriptional mechanisms of gene regulation, including mRNA export, turnover, and translation, are central to the implementation of specific gene expression patterns in mammalian cells. Among the post-transcriptional gene regulatory events, those affecting mRNA stability are emerging as highly effective means of altering mRNA abundance and consequently the levels of protein expressed (1–3). Labile mRNAs can be selectively stabilized or destabilized through the action of particular RNA-binding proteins that recognize specific RNA sequences. One of the major pathways regulating mRNA turnover relies on the presence of U-rich or AU-rich sequences (collectively known as AREs), typically present in the 3′-untranslated regions of transcripts encoding many cytokines, growth factors, and cell cycle regulatory proteins (4, 5). Several RNA-binding proteins that selectively bind to such instability sequences present on many labile mRNAs have been implicated in promoting their decay, including BRF1, AUF1 (hnRNP D), tristetraprolin, NF90, and KSRP (6–11). RNA-binding proteins that recognize AREs and enhance mRNA stability include the Hu proteins: HuR (HuA), which is ubiquitously expressed, as well as HuB, HuC, HuD, primarily expressed in neuronal tissues (12–15).

HuR, like all Hu proteins, contains three classic RNA recognition motifs and binds with high affinity and specificity to AREs in a variety of mRNAs, such as those encoding HSP70, vascular endothelial growth factor, tumor necrosis factor-α, PAI-2, COX-2, p53, p27, p21, cyclin A, cyclin B1, GLUT-1, and c-Fos, and increases their stability, modulates their translation, or performs both functions (15–25). HuR is predominantly (>90%) localized in the nucleus of unstimulated cells. However, its influence on mRNA stabilization and translation has been linked to its cytoplasmic presence, so there has been much interest in identifying the mechanisms regulating its presence in each cellular compartment (16, 26–30). In the nucleus, HuR has been shown to bind proteins SETα, SETβ, pp32, and APRIL (18). SETα, SETβ, and pp32 have been identified as inhibitors of protein phosphatase 2A (31, 32), a serine/threonine phosphatase that becomes activated in response to various stimuli and dephosphorylates several major protein kinases (for review, see Ref. 33). Using cell-permeable peptides, HuR nuclear export was shown to involve the association of HuR with two of its nuclear ligands, pp32 and APRIL, which contain leucine-rich nuclear export signals that are recognized by the export receptor chromosome maintenance region 1 (CRM1).1 Treatment with the CRM1 inhibitor leptomycin B caused the nuclear accumulation of pp32 and APRIL, as well as the increased association of HuR with pp32 and APRIL in the nucleus (34). Alternative pathways of nuclear export have been proposed to involve a shuttling sequence within HuR, the HuR nucleocytoplasmic shuttling sequence (HNS), which bears similarities with the M9 shuttling signal of hnRNP A1, (27, 34),

1 The abbreviations used are: CRM1, chromosome maintenance region 1; AICAR, 5-aminopteridine-4-carboxamide riboside; AMPK, AMP-activated protein kinase; HDAC, histone deacetylase; IP, immunoprecipitation; Kap, karyopherin; NPC, nuclear pore complex; HNS, HuR nucleocytoplasmic shuttling sequence; Trn, transportin; GFP, green fluorescent protein; GST, glutathione S-transferase; DTT, dithiothreitol; Imp, importin; Sbutyr, sodium butyrate; TSA, trichostatin A; HA, hemagglutinin; NLS, nuclear localization sequence.
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but the export factor involved in HNS-dependent transport is currently unknown (35). Although a role for HuR in the export of target mRNAs awaits to be definitively demonstrated, evidence accumulated thus far strongly supports such a function. Therefore, the existence of multiple export pathways for HuR would ensure the rapid and effective export of HuR target mRNAs. Recently, transportin 1 (Trn1; also known as Karyopherin/Kapβ2) as well as the highly similar transportin 2 (Trn2) were shown to participate in the nuclear import of HuR (36). In a series of elegant in vitro studies, the HNS was found to mediate the import of HuR by transportins (35, 36).

In vivo, the levels of cytoplasmic HuR have been shown to be potently regulated by the activity of the AMP-activated protein kinase (AMPK), an enzyme that participates in the cellular response to metabolic stress (37). Believed to function as a “low fuel warning system” of the cell, AMPK activity is strongly elevated by conditions that lower the cytoplasmic ATP/ADP ratio, such as exposure to metabolic poisons like arsenite and azide, depletion of growth factors or glucose, and treatment with the pharmacological agent AICAR (which mimics the effect of AMP) (38). In turn, active AMPK phosphorylates a number of metabolic enzymes causing both a global inhibition of biosynthetic pathways, thus conserving energy, and a global activation of catabolic pathways, thus generating more ATP (reviewed in Ref. 39). AMPK activity is ubiquitous, although different isoforms of its catalytic (α) and regulatory (β and γ) subunits exhibit tissue-specific distribution, as well as preferential localization in different subcellular compartments (e.g. α2 subunits are partly nuclear, whereas α1 subunits are only found in the cytoplasm, etc.). Recently, we found that decreased AMPK activity led to an elevation in cytoplasmic HuR levels; conversely, AMPK activation through interventions such as treatment with AICAR and ectopic expression of a constitutively active isoform of AMPK caused a reduction in cytoplasmic HuR (40). The lowering of cytoplasmic HuR was accompanied by a reduction in complexes of HuR bound to target mRNAs encoding proliferative proteins (cyclins A and B1), a decrease in the stability of such mRNAs, a lessening in the expression of the corresponding protein products, and an ensuing inhibition of cell growth (40).

Given that HuR shuttles between the nucleus and cytoplasm (26, 27, 29, 30) and that AMPK-triggered reductions in cytoplasmic HuR levels profoundly influence the stability of target mRNAs (40), we set out to investigate the mechanisms underlying the AMPK-mediated nuclear import of HuR. In this investigation, we have identified importin α1 as a key cytoplasmic HuR ligand mediating this process. Importin α1 functions as an adapter that associates with importin β, which transports bound cargoes through the nuclear pore complex (NPC). We provide additional evidence in support of a role for AMPK in dually modifying importin α1: AMPK triggered the acetylation of importin α1 through AMPK-stimulated activation of acetylase p300, and AMPK directly phosphorylated importin α1, both in vivo and in vitro. Importantly, overexpression of wild-type full-length importin α1, but not point mutants of importin α1 lacking the phosphorylation and acetylation sites, readily promoted the nuclear import of HuR in intact cells. Together, our data indicate that AMPK induces the acetylation and phosphorylation of importin α1 and strongly suggest that dual modification of importin α1 is required for the nuclear import of HuR.

EXPERIMENTAL PROCEDURES

Cell Culture, Treatment, Transfection, and Infection—Human colorectal carcinoma RKO cells were cultured in minimum essential medium (41), and human embryo kidney fibroblasts 293 cells (ATCC) were cultured in Dulbecco’s modified essential medium, each supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) and antibiotics.

Sodium butyrate, trichostatin A, and AICAR were from Sigma, and calpain inhibitor 1, N-Ac-Leu-Leu-norleucinal (ALLN) was from Calbiochem (La Jolla, CA). Adenoviruses expressing either the control gene GFP (Ad/CyGFP) or a constitutively active isoform of the AMPK α1 subunit (Ad/CAAMPK2) (42) were amplified and titrated in 293 cells using standard methodologies. The infections were carried out in serum-free Dulbecco’s modified Eagle’s medium for 4 h. Infection efficiency of RKO cells was determined by infection with AdGFP at various plaque-forming units/cell and assessment of the percentage of GFP-expressing cells 48 h later. For >90% infection, 100 plaque-forming units/cell was required, in accordance with the low infection rates of RKO cells (41). 100 and 20 plaque-forming units/cell were used in all infections of RKO and 293 cells, respectively. Cytoplasmic, nuclear, and whole cell fractions were prepared as described (24).

Constructs and Recombinant Proteins—All of the purified recombinant proteins were produced in Escherichia coli. His-tagged (C-terminal) importin α1 was purified by nickel-chelate-tetrahydrofuran-acid-affinity chromatography followed by ion exchange and gel filtration chromatography. GST-Importin β and GST-Kapβ2 were purified by glutathione-Sepharose affinity chromatography, cleaved with either Precision protease (Importin β) or Tev protease (Kapβ2), and Importin β and Kapβ2 were further purified by ion exchange and gel filtration chromatography (43).

Bacterial expression constructs were prepared by ligating PCR-amplified inserts into plasmid pBAD-MD20 (Invitrogen). Importin α1 functions in mammalian cells, PCR-amplified full-length and truncated importin α1 cDNA products were inserted into plasmid pcDNA3.1/V5-His-TOPO (Invitrogen). Plasmid pCMV-GFP was used for expression of p300 in cultured cells (Upstate USA Inc., Lake Placid, NY).

For expression in mammalian cells, importin α1 proteins bearing either a lysine-to-arginine mutation on residue 22 (Importin α1[S22R]) or a serine-to-threonine mutation on residue 105 (Importin α1[S105A]) or both mutations (Importin α1[S105A,R22R]) were generated using the QuickChange site-directed mutagenesis kit (Stratagene) and were expressed in plasmid pcDNA3.1/V5-His-TOPO (Invitrogen). All of the constructs were verified by sequencing.

Antibodies and Western Blot Analysis—For Western blotting, whole cells (20 µg), cytoplasmic (40 µg), and nuclear (10 µg) lysates, prepared as described (24), were size-fractionated by SDS-PAGE and transferred onto polyvinylidene difluoride membranes. Monoclonal antibodies recognizing β-tubulin (a control cytoplasmic protein), HDAC1 (a control nuclear protein), HDAC2, HDAC3, HDAC4, c-Myc, and cyclin A, as well as polyclonal antibodies recognizing CRM1 and Nup98, Nup88, and Nup62 were from BD Pharmingen. Monoclonal antibodies recognizing importin α1, α3, α4, α5, and α7 were kindly described (44). Following secondary antibody incubations, signals were detected by enhanced chemiluminescence. Rabbit polyclonal antisera recognizing acetylated importin α1 was a generous gift from Dr. T. Kouzarides (45).

Immunoprecipitation—Whole cell lysates for immunoprecipitation (IP) were prepared by adding 200 µl of IP buffer (10 mM Heps, pH 7.4, 50 mM β-glycerophosphate, 1% Triton X-100, 10% glycerol, 2 mM EDTA, 2 mM EGTA, 10 mM NaF, 1 mM DTT, 1 mM Na3VO4, 20 µM microcystin-LR, 2 µg/ml leupeptin, 2 µg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 1 µM trichostatin A, 20 mM sodium butyrate, 5 µM soybean trypsin inhibitor, and 0.1 mM benzamidine) supplemented with 0.5% SDS. Each sample was passed through a 27.5-gauge needle 20 times and then centrifuged (10 min, 4 °C, 21,000 × g). IP reactions were carried out by using 20 µl of the resulting supernatant, diluting it with 1 ml of IP buffer, and adding 2 µg of the antibodies indicated above. The washes were performed as follows: three times with IP buffer, four times with a high stringency buffer (100 mM Tris-HCl, pH 7.4, 500 mM LiCl, 0.1% Triton X-100, 1 mM DTT, 1 mM NaVO4, 20 µM microcystin-LR, 2 µg/ml leupeptin, 2 µg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 1 µM trichostatin A, 20 mM sodium butyrate, 5 µM soybean trypsin inhibitor and 0.1 mM benzamidine), and then three times with IP buffer.

Pulse Labeling of Endogenous Protein—RKO cells (200,000 cells/6-cm dish) were incubated in methionine- and cysteine-free medium containing 5% dialyzed fetal bovine serum for 30 min and then for an additional 20 min with 900 µCi of L-[35S]methionine and L-[35S]cysteine (NEG-072 ExpresS-S Protein labeling mix; PerkinsElmer Life Sciences) per well and scraped into 500 µl of ice-cold phosphate-buffered...
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Reduced Cytoplasmic HuR Levels after AMPK Activation—Treatment of human colorectal carcinoma RKO cells with the AMP analog AICAR, a strong and highly specific activator of AMPK (38) (Fig. 1A), markedly reduced the levels of cytoplasmic HuR (Fig. 1B). Evidence that the AMPK-imposed reduction in cytoplasmic HuR abundance was not due to increased cytoplasmic degradation of HuR was obtained by using inhibitors of the proteasome and proteases. As shown in Fig. 1C, pretreatment with ALLN (which inhibits the activity of the proteasome, calpain, and cathepsins) failed to prevent the reduction in cytoplasmic HuR levels elicited through treatment with AICAR (Fig. 1C); use of protease inhibitor lactacystin or protease inhibitors ALLM or PD150606 similarly failed to alter the reduction in cytoplasmic HuR levels triggered by AICAR (not shown). In addition, the AMPK-imposed reduction in cytoplasmic HuR abundance was not due to decreased translation, as observed by assessing nascent HuR levels. De novo synthesis of HuR was monitored by pulse labeling (for 20 min) of RKO cells in the presence of L-[35S]methionine and L-[35S]cysteine, followed by detection of the newly synthesized HuR through IP using anti-HuR antibodies (Fig. 1D). These two sets of data lent support to the notion that HuR abundance in the cytoplasm was primarily governed via nucleocytoplasmic transport and not changes in HuR translation or cytoplasmic stability. In keeping with earlier studies demonstrating that increased AMPK activity reduces cytoplasmic HuR levels (40) and studies that HuR shuttles between the nucleus and the cytoplasm (26, 27, 29, 30), these findings supported the existence of an AMPK-triggered effect on nuclear transport of HuR. Given that AMPK-imposed reductions in cytoplasmic HuR levels profoundly impacted on the half-lives of HuR target mRNAs (40), we sought to examine the regulatory mechanisms whereby AMPK regulated the nuclear import of HuR.

Reduction in Cytoplasmic HuR Levels by AMPK Involves Changes in Protein Acetylation—Recently, the acetylase p300 (CBP) was identified as a downstream target of AMPK (47). To study whether changes in protein acetylation were linked to the nuclear import of HuR, RKO cells were treated with two potent inhibitors of HDAC activity, sodium butyrate (SButyr) and trichostatin A (TSA), and cytoplasmic HuR levels were subsequently monitored by Western blotting. As shown in Fig. 2A, cytoplasmic HuR abundance was reduced to ~40 and 20% of the levels seen in untreated RKO populations, respectively, although whole cell HuR levels were essentially unchanged (Fig. 2B, total). As anticipated, both SButyr and TSA strongly lowered histone deacetylase activity (Fig. 2B, top panel), but neither drug was capable of modulating AMPK activity, either alone or in combination (Fig. 2B, bottom panel), revealing that a deacetylase activity was not upstream of AMPK regulatory pathways.

To find out whether regulated protein acetylation was downstream of AMPK in the AICAR-triggered pathway or whether the two pathways (protein acetylation, AMPK activation) were instead contributing independently to the nuclear import of HuR, experiments were carried out using AICAR and HDAC inhibitors simultaneously. To find a greater reduction in cytoplasmic HuR levels with the combined treatment (AICAR together with HDAC inhibitors) than with either of the single treatments would indicate the existence of two distinct, parallel pathways of reducing cytoplasmic HuR, one mediated by

**RESULTS**

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AMPK, the other mediated by acetylases. Instead, as shown in Fig. 2C, treatments with either AICAR alone or SButyr plus TSA were each capable of reducing cytoplasmic HuR levels, but combined treatment with AICAR, SButyr, and TSA did not cause a further reduction in cytoplasmic HuR levels beyond AICAR. Assessment of the levels of \( \beta \)-H9252-actin and \( \beta \)-H9252-tubulin served to monitor equal loading and transfer of cytoplasmic and total protein samples, respectively.

D, RKO cells were treated for 6 h in the presence of the indicated concentrations of AICAR, and then incubated for an additional 20 min in the presence of \( \text{L-[^{35}S]} \)methionine and \( \text{L-[^{35}S]} \)cysteine, whereupon nascent HuR was visualized by IP as described under “Experimental Procedures” and AMPK activity (described in the legend of Fig. 1) was assayed. The data represent the mean of two experiments, each yielding similar results. C, RKO cells were treated for 6 h with 2 mM AICAR, 15 mM SButyr, or 1 \( \mu \)M TSA, alone or in combination, and HuR abundance was assessed in cytoplasmic (40 \( \mu \)g) and whole cell (10 \( \mu \)g) lysates. Relative HuR expression levels were calculated as explained in the legend of Fig. 1; \( \beta \)-actin signals served to normalize for differences in the loading and transfer of protein samples. Ctrl, control.

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those attained by AICAR alone. Together, these observations support the notion that protein acetylation is implicated in the cellular localization of HuR and further suggest that protein acetylation is a downstream event of the AMPK-controlled pathway of HuR localization.

**HuR Associates Specifically with Importin α1**—The above findings prompted a further analysis of components of the nuclear import machinery potentially involved in transporting HuR into the nucleus following AMPK activation. To identify the specific factor(s) that might mediate the import of HuR into the nucleus, we carried out a series of IP reactions coupled with Western blot analyses to investigate potential associations between HuR and other proteins. This survey included a number of transport proteins such as CRM1, NTF2, and members of the karyopherin β, importin β, and importin α families. It also included several negative control proteins, including proteins involved in the structure and function of the nuclear pore complex (such as Ran, Ran-binding protein, and nucleoporins) and nuclear proteins such as HDACs, given the aforementioned evidence that cytoplasmic HuR levels were influenced by altered protein acetylation.

The potential interactions between these proteins and HuR were first assayed by IP using an anti-HuR antibody, followed by Western blotting using antibodies that specifically recognized the proteins of interest (Fig. 3A, left column, Panel). These interactions were further tested by carrying out individual IP reactions with antibodies recognizing each of the proteins listed and then performing Western blot analysis of the IP material to test for HuR presence (Fig. 3A). These studies revealed that importin α, karyopherin β2, and importin α1 were associated with HuR, although importin α appeared to bind less strongly with HuR. Fig. 3B depicts illustrative IP-coupled Western blot analyses performed to obtain the data summarized in Fig. 3A.

Further evidence that importin α1 associated with HuR was obtained by using recombinant proteins in solution binding assays that were visualized by Coomassie Blue staining. Incubations were carried out initially using recombinant His-tagged importin α1 (His-Impa1) immobilized on beads through an antibody recognizing the histidine tag. As shown, both importin β (Impβ) and HuR (expressed and purified as a maltose-binding protein-HuR fusion protein, MBP-HuR), but not MBP alone, were shown to interact with His-Impa1 (Fig. 4A). Conversely, testing of MBP-HuR immobilized on beads through an anti-MBP antibody revealed that recombinant His-Impa1 associated with MBP-HuR (Fig. 4B); Impβ and Kapβ2 were also found to associate with MBP-HuR, revealing that these proteins may also be capable of binding MBP-HuR in vitro, in keeping with previous findings (35, 36). Control incubations using an N-terminal fragment of c-Jun linked to GST showed no binding to MBP-HuR. In each case, control incubations using beads and antibody revealed no unspecific binding (not shown). Importin α1 has been described as an *adaptor* protein that associates with transport receptor protein importin β, thereby delivering its cargo into the nucleus (48, 49). Thus, the following set of experiments sought to examine whether AMPK might regulate importin α1 function and thereby influence HuR import.

**Importin α1 Is Acetylated by p300 in an AMPK-dependent Manner in RKO Cells**—First, we investigated the possibility that AMPK might regulate importin α1 acetylation, an event that has been shown to promote its interaction with importin β (45). In this regard, two recent studies provided important leads: nuclear acetylase p300 is a target of phosphorylation by AMPK (47), and importin α1 is a substrate of acetylation by p300 (45). In agreement with these reports, we found that 293

| Panel | IP: HuR | IP: Panel |
|-------|---------|-----------|
| Importin β | + | +/− |
| Transportin 1/Kap β2 | ++ | ++ |
| Importin α1 | ++ | ++ |
| Importin α3 | − | ND |
| Importin α4 | − | ND |
| Importin α5 | − | ND |
| Importin α7 | − | ND |
| CRM1 | − | ND |
| NTF2 | − | − |
| Ran | − | − |
| Ran-BP1 | − | − |
| Nup-98 | − | − |
| Nup-88 | − | − |
| Nup-62 | − | − |
| HADC1 | − | ND |
| HADC2 | − | ND |
| HADC3 | − | ND |
| HADC4 | − | ND |
| Cyclin A | − | − |
| NF-κB | − | ND |
| p300 acetylase | − | ND |

| HuR | ++++ | ++++ |

**Fig. 3. Interaction of HuR with nuclear proteins and with proteins involved in nucleocytoplasmic transport.** A, whole cell lysates prepared from RKO cells were subjected to two types of analyses. First (IP: HuR; IB: Panel column), HuR was immunoprecipitated along with associated proteins, whereas the presence of nuclear proteins and proteins associated with the nucleocytoplasmic transport system (left column) was detected by Western blot (WB) analysis with the corresponding specific antibodies. Second (IP: Panel; IB: HuR column), IP reactions were performed using a collection of antibodies recognizing each of the proteins listed in the left column, followed by Western blot analysis to detect HuR in the immunoprecipitated material. The details are provided under “Experimental Procedures.” Western blot signals: −, undetectable; +/−, weak; +, moderate; ++, prominent; ++++, very strong; ND, not detected. B, Western blot analysis to illustrate IP-coupled Western blot analyses used to generate the list in A. Two hundred μg of whole cell lysate was used per each immunoprecipitation (IP) reaction, and the immunoprecipitated material was then used to perform Western blotting analysis to detect the presence of the proteins indicated. IP followed by WB analyses were carried out two to five times; representative Western blotting signals are shown. IB, immunoblot.

Based on these pieces of evidence, we set out to examine whether acetylation of importin α1 were dependent on the AMPK activity levels of the cell. To this end, we first prepared cells exhibiting elevated AMPK activity through infection with an adenovirus that expresses a constitutively active isofrom of the α subunit (Ad(CA)AMPK), whereas control populations were prepared by infection using a GFP-expressing adenovirus (AdGFP) (42). Forty-eight h after infection of RKO cells, AMPK activity was 2.8-fold higher in Ad(CA)AMPK-infected cells than cells transfected with a p300-expressing construct (pCMVβ-p300) displayed strongly elevated levels of acetylated importin α1, as well as increased acetylation of histone H4, a known substrate of p300 (50, 51) (Fig. 5A).
in AdGFP-infected control populations (Fig. 5B), in keeping with earlier studies using these adenoviral vectors (40). p300 activity in each infection group was then tested by performing IP of p300 and then using the pelleted material to test acetylase activity (i.e., its ability to add a [14C]acetyl group onto a substrate). As shown, the p300 immunoprecipitate was not capable of acetylating substrate MBP-HuR (or substrate Kap2), but it readily acetylated importin-1 (His-Imp1), in agreement with earlier findings (45); moreover, importin-1 acetylation was enhanced when using material immunoprecipitated from Ad(CA)AMPK-infected cultures (Fig. 5C). Increased phosphorylation of endogenous p300 was seen after IP of p300 from Ad(CA)AMPK-infected cells that had been incubated with 32P (Fig. 5D). Ad(CA)AMPK-infected cells also displayed an increase in the acetylation of histone H4 and importin-1, further demonstrating that elevated AMPK activity was linked to the increased acetylation of p300 targets.

Fig. 4. Importin-1 and HuR interact in vitro. A, 2 μg each of purified recombinant Impβ, MBP-HuR, or MBP were incubated with immobilized His-Imp1 and used in solution binding assays (described under “Experimental Procedures”). B, 2 μg each of purified recombinant Impβ, Kapβ2, His-Imp1, and GST-c-Jun were incubated with immobilized MBP-HuR. All bound and unbound fractions, prepared as described under “Experimental Procedures,” were size-fractionated by 12% SDS-PAGE and detected by Coomassie Blue staining. H.C., heavy chain; MWM, molecular weight marker; *, truncated MBP-HuR product.

Fig. 5. Acetylation of importin-1 by p300 in an AMPK-dependent fashion. A, 24 h after transfection of 293 cells with 20 μg of either the insert-less plasmid pCMVβ (V), or plasmid pCMVβ-p300 (p300), expressing wild-type p300, the abundance of p300, total importin-1, acetylated importin-1, total histone H4, and acetylated histone H4, was assessed by Western blotting using 20 μg of whole cell lysate. Antibodies and procedures are described under “Experimental Procedures.” B, AMPK activity in cells that had been infected with either an adenovirus expressing a constitutively active isoform of the α subunit of AMPK (Ad(CA)AMPK) or control AdGFP adenovirus was calculated as explained in the legend of Fig. 1A. C, top panel, endogenous p300 was immunoprecipitated from RKO cells that had been infected with either Ad(CA)AMPK or AdGFP and then used for in vitro acetylation of 0.5 μg of purified His-importin-1 (His-Impl) or the control protein MBP-HuR. The protein molecular weight markers are indicated. Bottom panel, the presence of p300, His-Impl, and MBP-HuR in the reaction materials was monitored by Western blotting. D, RKO cells infected with either Ad(CA)AMPK or AdGFP were incubated with 32P for 3 h, whereupon phosphorylated p300 was detected by immunoprecipitation of p300, separation of IP material by 12% SDS-PAGE, and visualization using a PhosphorImager. 24 h after infection of 293 cells using either Ad(CA)AMPK or control AdGFP, the abundance of p300, total importin-1, acetylated importin-1, total histone H4, and acetylated histone H4 was assessed by Western blotting using 20 μg of whole cell lysate. The antibodies and procedures are described under “Experimental Procedures.”

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echst staining to visualize nuclei. Representative photographs from
fied His-Imp
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shown in Fig. 7
A
Assessment of the levels of the cytoplasm-specific β-tubulin and nucleus-specific HDAC1 after sequential stripping and reprobing of the filters served to verify the quality and equal loading of the cytoplasmic and nuclear preparations, respectively. Western blotting signals were measured by densitometry scanning; HuR abundance, calculated after normalization against the values of control proteins (either β-tubulin or HDAC1), is presented as the percentage of remaining signals relative to those measured in untreated samples of each lysate group. Shown are represent-
tative Western blotting signals; experiments were performed in triplicate. B, detection of HuR by immunofluorescence in 293 cells 48 h after transfection with either plasmid pCMVβ (V) or plasmid pCMVβ-p300 (p300). Left panels, HuR immunofluorescence; right panels, Hoechst staining to visualize nuclei. Representative photographs from three independent experiments are shown.

Importantly, overexpression of p300 also caused a decrease in cytoplasmic HuR levels, as determined by both Western blotting (Fig. 6, A) and immunofluorescence (Fig. 6, B). These findings strongly support the hypothesis that p300-mediated events influence the subcellular compartmentalization of HuR.

Importin α1 Is Phosphorylated by AMPK in Vitro and in Vivo—Next, we examined whether importin α1 was a target of AMPK phosphorylation. We first obtained active AMPK through infection of RKO cells with Ad(CA)AMPK and then used the immunoprecipitated enzyme in in vitro phosphorylation assays (described under “Experimental Procedures”). As shown in Fig. 7, A, AMPK was capable of phosphorylating purified His-Importin α1 but not control proteins Impβ or MBP-HuR. AMPK was also capable of phosphorylating endogenous importin α1 as well as control IP reactions employing karyopherin β2 or HuR as substrates did not produce phosphorylated bands (Fig. 7B). Finally, evidence supporting the notion that AMPK was capable of phosphorylating importin α1 in vivo was obtained through infection of RKO cells using Ad(CA)AMPK. As shown, importin α1 immunoprecipitated from Ad(CA)AMPK-infected popula-
tions had incorporated considerably more 32P, than control AdGFP-infected populations (Fig. 7C).

Wild-type Importin α1, but Not Phosphorylation-defective Mutants, Can Promote the Nuclear Localization of HuR—Additional studies were aimed at studying the effect of importin α1 mutants on the subcellular localization of HuR. In particular, we sought to map the residue of the importin α1 protein that was phosphorylated by AMPK and investigate the influence of importin α1 phosphorylation on the cytoplasmic abundance of HuR. First, several importin α1 N-terminal deletion mutants (Fig. 8A) were expressed in E. coli as His-tagged proteins. IP of His-tagged products from crude bacterial lysates was followed by Western blotting (Fig. 8B, IP+WB), then kinase assay (Fig. 8B, 32P signal) to determine their suitability as substrates of phosphorylation by AMPK. As shown, full-length

![Figure 6](http://www.jbc.org/content/86/356/3789/F6.F6)

**Fig. 6. Decreased cytoplasmic localization of HuR in cells overexpressing p300.** A, Western blot analysis to assess HuR levels in whole cell (Total), 10 μg), cytoplasmic (Cytopl., 40 μg), and nuclear (10 μg) lysates prepared from 293 cells 48 h after transfection with 20 μg of either plasmid pCMVβ (V) or plasmid pCMVβ-p300 (p300). Assessment of the levels of the cytoplasm-specific β-tubulin and nucleus-specific HDAC1 was done through immunoblotting (Fig. 6, A) and Western blotting (Fig. 6, B). Detection of HuR by immunofluorescence in 293 cells 48 h after transfection with either plasmid pCMVβ (V) or plasmid pCMVβ-p300 (p300). Left panels, HuR immunofluorescence; right panels, Hoechst staining to visualize nuclei. Representative photographs from three independent experiments are shown.

![Figure 7](http://www.jbc.org/content/86/356/3789/F7.F7)

**Fig. 7.** AMPK phosphorylates importin α1 in vivo and in vitro. A, constitutively active AMPK, prepared from 293 cells that had been infected with Ad(CA)AMPK, was prepared by IP using an anti-Myc antibody. The immunoprecipitated material was then used in in vitro phosphorylation assays using purified His-Importin α1 as well as control proteins Impβ and MBP-HuR as substrates. B, endogenous importin α1 and constitutively active AMPK were immunoprecipitated independently, and the immunoprecipitated materials incubated to assess the phosphorylation of importin α1 in vitro. In vitro phosphorylation assays (A and B) were carried out as described under “Experimental Procedures”; the phosphorylated products were visualized through electrophoresis of the kinase reaction mixtures in SDS-containing 12% polyacrylamide gels that were subsequently transferred onto filters and subjected to both PhosphoImager scanning (top panel) and Western blotting (bottom panel). C, in vivo phosphorylation of importin α1. 293 cells infected with either Ad-AMPK(CA) or AdGFP were incubated with 1 μCi/ml 32P, wherein whole cell lysates were immunoprecipitated (IP) and importin α1 abundance and phosphorylation status were analyzed by Western blotting (WB, top panel) and PhosphoImager scanning (bottom panel), respectively.

Wild-type Importin α1, but Not Phosphorylation-defective Mutants, Can Promote the Nuclear Localization of HuR—Additional studies were aimed at studying the effect of importin α1 mutants on the subcellular localization of HuR. In particular, we sought to map the residue of the importin α1 protein that was phosphorylated by AMPK and investigate the influence of importin α1 phosphorylation on the cytoplasmic abundance of HuR. First, several importin α1 N-terminal deletion mutants (Fig. 8A) were expressed in E. coli as His-tagged proteins. IP of His-tagged products from crude bacterial lysates was followed by Western blotting (Fig. 8B, IP+WB), then kinase assay (Fig. 8B, 32P signal) to determine their suitability as substrates of phosphorylation by AMPK. As shown, full-length
importin α1 (Impα1), as well as a deletion construct lacking the first 62 amino acids (Impα1(D62)), were readily phosphorylated by AMPK. However, an importin α1 truncated variant lacking the first 205 amino acids (Impα1(D205)) could not be phosphorylated, indicating that a putative phosphorylation site existed within positions 62 and 205 of the importin α1 protein. The preferred AMPK phosphorylation site, derived from six AMPK target proteins and further refined using synthetic peptides (reviewed in Ref. 39), has been reported to be either a serine or a threonine residue within the following amino acid context: Hyd-Xaa-Bas-Xaa-Xaa-Xaa-Xaa-Hyd (where Hyd (-5) represents amino acids carrying a basic side chain, Leu, Met, Ile, Phe, or Val), and Bas, which can be located at both positions -4 and -3, represents amino acids carrying a basic side chain (Arg > Lys > His)). Although AMPK can phosphorylate synthetic peptides on both threonine and serine residues, all in vitro targets identified thus far are phosphorylated on serine residues. Scanning of the importin α1 protein revealed one such AMPK phosphorylation motif encompassing Ser105 and only one additional motif encompassing Ser279. Initial indications that Ser105 might be a target of AMPK-mediated phosphorylation came from studies in which synthetic peptides were tested. A peptide comprising Ser105 and flanking amino acids (99ARKLLSRKQPP112) was readily phosphorylated by AMPK (Fig. 8C). In fact, phosphorylation levels were greater than 50% of those seen when using the archetypal SAMS peptide. By contrast, peptides encompassing other regions of importin α1 apparently lacking target AMPK phosphorylation sites, such as peptide 71QGTNVNSVDDIVKG185, which comprised Ser77 and surrounding amino acids, and peptide 172ASPHAESEQAVWALG188, which comprised Ser279 and surrounding amino acids, were poor targets of AMPK-mediated phosphorylation (Fig. 8C). An additional demonstration that Ser105 was a target of phosphorylation by AMPK came from the generation and testing of point importin α1 mutants (see Fig. 10).

To test the influence of importin α1 modifications on the cytoplasmic abundance of HuR, we first prepared several importin α1 deletion constructs for analysis in cells. Our initial attempts to carry out import assays in permeabilized cells, the preferred methodology to study nuclear import, were inconclusive; our efforts to modify (phosphorylate and acetylate) bacterially produced importin α1 through post-translational modification reactions rendered a pool of importin α1 in which the extent and stability of these modifications could not be confirmed with certainty and also inevitably generated degradation by-products that yielded false positive results on import assays (not shown). Therefore, we opted for studying this process by investigating the influence of importin α1 overexpression in mammalian cells. The effects of importin α1, expressed either as the full-length wild-type protein or as truncated or point-mutant proteins, were assessed by monitoring cytoplasmic HuR levels by Western blotting and by immunofluorescence. cDNA inserts encoding Impα1, Impα1(D62), and Impα1(D205) (Fig. 8A) were subcloned into mammalian expression vector pcDNA3.1/V5-His-TOPO and expressed as His-
Argued that acetylation at this site is necessary for maximal importin α1-mediated transport of HuR into the nucleus. No changes in either total or nuclear HuR levels were detected in any of the transfection groups (Fig. 9B), as anticipated (24).

The truncated importin α1 mutants lack the importin β-binding domain, which encompasses amino acids 10–41 of importin α1, and were consequently unable to import any substrate into the nucleus. Therefore, further analysis of the influence of phosphorylation and acetylation on the ability of importin α1 to import HuR was carried out by constructing and testing importin α1 proteins carrying specific amino acid mutations: importin α1 mutants lacking Lys22 (Impα1(K22R)), Ser105 (Impα1(S105A)) alone or in combination [Impα1(K22R, S105A)], or Ser179 (Impα1(S179A)) (Fig. 10A). As shown, Impα1(S105A) immunoprecipitated from transfected cells was not radiolabeled, further supporting the notion that Ser105 was phosphorylated in vivo (Fig. 10B). Importantly, only wild-type importin α1 (Impα1), but not Impα1(K22R), Impα1(S105A), or Impα1(K22R,S105A), was capable of promoting a decrease in the cytoplasmic levels of HuR (Fig. 10C). Moreover, in transfected populations overexpressing Lys22 and/or Ser105 importin α1 mutants (Impα1(K22R), Impα1(S105A), or Impα1(K22R, S105A)), AICAR-mediated reduction in cytoplasmic HuR was partly attenuated, further supporting the notion that AMPK-mediated nuclear import of HuR required intact importin α1 function.

Analysis of the subcellular distribution of HuR by immunofluorescence (Fig. 11A) was in precise agreement with the Western blotting data (Fig. 10C). Overexpression of Impα1, but not vector, Impα1(K22R), Impα1(S105A), or Impα1(K22R, S105A), effectively led to an enhanced HuR import, as revealed by the marked reduction in cytoplasmic HuR in these populations. Similar findings were obtained in transient transfections to express the deletion mutants described in Fig. 9 (data not shown). Importantly, only wild-type importin α1 appeared to interact with HuR, as revealed by co-IP analysis using an anti-HuR antibody followed by Western blot analysis of the ectopically expressed (HA-tagged) importin α1 proteins present in the IP material using an anti-HA antibody. As shown in Fig. 11B, wild-type importin α1 appeared to interact with HuR, but no such interactions were detected with any of the point mutants tested. These findings lend support to the notion that wild-type importin α1, but not importin α1 mutants that cannot be phosphorylated or acetylated, are capable of binding HuR.

From the data presented here, a model is proposed whereby AMPK elicits a dual modification of importin α1 (Fig. 12): it phosphorylates importin α1 directly (Ser105) and acetylates importin α1 indirectly, through phosphorylation of p300, which in turn acetylates importin α1 (Lys22). Importin α1 bearing mutations in these modification sites have an impaired ability to promote the nuclear localization of HuR.

**DISCUSSION**

In this study, we sought to investigate the mechanisms whereby AMPK mediated the previously reported nuclear import of HuR (40). Our findings strongly suggest that importin α1 participates in the nuclear import of HuR and further support a direct role for AMPK in modulating this function by eliciting a dual modification of importin α1. Using both *in vivo* and *in vitro* approaches, AMPK was found to directly phosphorylate importin α1 on residue Ser105. In addition, AMPK indirectly caused importin α1 acetylation, an effect that relied on AMPK-mediated phosphorylation of acetylase p300 (47). According, mutated importin α1 proteins lacking the phosphorylation site, the acetylation site, or both sites exhibited an impaired ability to mediate the nuclear import of HuR in intact cells.

![Image](https://via.placeholder.com/150)
Cellular macromolecules are transported between the nucleus and the cytoplasm through the nuclear pore complex (the NPC, a large structure composed of ~30 proteins named nucleoporins), mediated by the action of several families of soluble transport molecules. One such family of transport molecules, represented by NTF2, is involved in the nuclear import of the small GTPase Ran (52, 53), whereas another comprises the TAP/NXF p15/NXT protein dimer, which participates in the nuclear export of mRNA (reviewed in Ref. 54). However, the largest family of transport factors is the family of proteins known as importins/exportins or karyopherins. In mammalian cells, many members of this family have been shown to be involved in nuclear import (such as importin α, karyopherin β2, transportin 2, transportin-SR, importin α1, importin α5, importin α7, and importin α9), whereas others have been shown to mediate nuclear export (like CAS, CRM1, exportin-t, exportin 4, and exportin 5), and yet others have been reported to mediate both import and export (like importin 13) (36, 49, 55).

Members of the importin β family have been most extensively implicated in the “classical” import pathway of cytoplasmic import substrates (cargoes). In addition to receptor molecules such as those comprising the importin β family, adaptor proteins such as those in the importin α family (including importins α1, α3, α4, α5, α6, and α7 in humans (44)) mediate the recognition of nuclear localization signals such as the classical NLS but require importin β to be transported through the NPC. Although some evidence exists that importin α may cross the NPC independently of importin β (56), there are no reports to date that cargo-bound importin α can enter the nucleus freely through the NPC. Our findings further support the view that importin β may be present in complexes that also comprise importin α1 and HuR, given the association of these three proteins in immunoprecipitated material from cell lysates (Fig. 3) and their interaction in solution binding assays (Fig. 4). These interactions suggest that HuR is a nuclear import cargo for the importin α1-importin β pathway. An NLS of HuR had previously been mapped to its HNS, which comprises residues 205–237 (27), and the HNS has been shown to be the NLS for karyopherin β2 and transportin 2 nuclear import pathways (35, 36). The identity of the HuR NLS that binds importin α has not been determined, and no strict monopartite or bipartite NLS sequences have been identified. A candidate bipartite-like NLS, \(205\text{RFFGGVPNHHIQQRFRF}^{220}\), was first suggested by Fan and Steitz (27), but its fusion with pyruvate kinase failed to localize to the nucleus, suggesting that it is likely not an efficient NLS. Another sequence that resembles a monopartite classical NLS, albeit suboptimal, is \(320\text{KTNKSHK}^{326}\), found at the C terminus of HuR. Recently, the repertoire of sequences
that bind in an NLS-like manner to importin α has been expanded to include the MNRRKIAMPKRRMAFK sequence of nucleoporin Nup214, which binds in a different but overlapping site from the classical NLSs (57). Therefore, it is conceivable that importin α binds cargo ligands containing NLS other than the well known mono- and bipartite classical NLSs.

While this work was in progress, Güttinger et al. (36) as well as Rebane et al. (35) reported that Trn1/Kapβ2 and Trn2 can mediate the nuclear import of HuR. Using in vitro import assays, they found that the Trn1/Kapβ2 proteins were able to import HuR, whereas importin β together with importin α5 were not. The Trn/Kapβ2 pathways and the pathway described in the present investigation likely represent multiple parallel and co-existing mechanisms of HuR nuclear import. Supporting this notion are other prominent examples of proteins whose nuclear import occurs via multiple import pathways (58–61). Second, as in the study by Güttinger et al., importin α5 did not appear to bind to HuR, and according to our data, neither did other α importins except importin α1. In this regard, other proteins like RCC1, STAT-1, and STAT-2 are known to be imported via single α importin isoforms (44, 62–64). Finally, it remains to be assessed whether a lack of Kapβ2 in living cells results in a transport defect for HuR in vivo (36). Our findings that overexpression of wild-type importin α1 in living cells enhanced the nuclear translocation of HuR, whereas abolishing phosphorylation and/or acetylation of importin α1 blocked this effect, provide strong evidence that importin α1 is a functionally relevant import factor in vivo. Moreover, our results shed light into the functional significance that two previously reported modifications of importin α1, phosphorylation and acetylation (45, 65), may have in the import of a specific substrate. It is important to note that by preventing importin α1 phosphorylation and/or acetylation in living cells, we were able to reduce, although not completely block, the nuclear translocation of HuR. We therefore propose that the import of HuR, similarly to the previously reported import of RCC1 (66), can be mediated both by a pathway dependent on a specific α importin and by importin α-independent pathway(s).

Importin α has an N-terminal importin β-binding domain that also includes an autoinhibitory region, followed by an NLS-binding domain comprising 10 armadillo repeats that begins at amino acid 70 and a C-terminal domain that is necessary for binding its export factor CAS (48, 67). Our data could provide the basis for a novel regulatory model whereby phosphorylation and/or acetylation of importin α1 may increase its affinity for importin β and also increase its affinity for nuclear import cargoes such as HuR. AMPK triggered the acetylation of residue Lys22, which resides within the importin β-binding domain of importin α1. In the crystal structure of free and autoinhibited mouse importin α, Lys22 is disordered and not observed (68), and it is distant in sequence from the pseudo-NLS autoinhibitory sequence (residues 44–54) in the N-terminal region of importin α. Therefore, acetylation of Lys22 may not affect importin α autoinhibition in the free protein. How-
ever, the side chain of Lys$^{27}$ participates in hydrophobic interactions with the side chain of Trp$^{472}$ of importin $\beta$ (69). Consequently, acetylation of the Lys$^{27}$ side chain, which removes its positive charge and also increases its hydrophobicity, could result in increased affinity between importin $\alpha$ and importin $\beta$. Ser$^{105}$, the importin $\alpha$ residue that is phosphorylated in response to AMPK stimulation, is in the armadillo domain situated at the C terminus of helix H3 of the first armadillo repeat (68, 70). In the crystal structure of mouse importin $\alpha$ bound to the bipartite NLS of nucleoplasmin, the Ser$^{105}$ side chain is near the Lys$^{170}$ side chain of the NLS (71). Phosphorylation of Ser$^{105}$ should increase ionic interactions between Ser$^{105}$ and the NLS, thus increasing the affinity of importin $\alpha$ for its cargo. Taken together, acetylation and phosphorylation of importin $\alpha$ on Lys$^{22}$ and Ser$^{105}$ could result in more efficient formation of importin $\alpha$-importin $\beta$-cargo complexes, respectively, and thus further facilitate nuclear import by this pathway. Taken together, we propose that under basal growth conditions, HuR import into the nucleus is mediated by several transport factors, like karyopherin $\beta$/transportin 1, transportin 2, and importin $\alpha$-importin $\beta$. Upon stimulation of AMPK, importin $\alpha$ is modified by acetylation and phosphorylation, resulting in a further increase in the efficiency of the importin $\alpha$-importin $\beta$ pathway to increase transport of cargoes such as HuR into the nucleus.

It will be important to investigate whether importin $\alpha$-bound HuR is associated with target mRNAs in vivo. The data obtained from in vitro binding assays indicate that it is possible for HuR to bind to importin $\alpha$ without mRNA (Fig. 4). In fact, an intriguing hypothesis that remains to be tested is whether the presence of a target mRNA might actually inhibit the association of HuR with importin $\alpha$. A less plausible possibility, although also worthy of future examination, is that HuR might enter the nucleus bound to a target mRNA, possibly as a means of controlling its stability or preventing its translation. Experiments are underway to test these various scenarios by combining IP and real time reverse transcription-PCR assays to test for the presence of endogenous HuR target mRNAs in importin $\alpha$-HuR complexes. In this regard, IP of importin $\alpha$ under conditions that preserved protein-RNA interactions did not reveal any specific enrichment of HuR target mRNAs in the immunoprecipitated material. These preliminary findings would support the notion that any HuR present in complexes with importin $\alpha$ would likely not be bound to mRNAs.

The findings presented here that AMPK influences importin $\alpha$-mediated events may have potentially important implications beyond the regulation of HuR function. Changes in the subcellular distribution of other importin $\alpha$ cargo molecules, which typically contain classical NLS, should be re-examined in light of a potential role for AMPK in regulating their nucleocytoplasmic transport. In addition, importin $\alpha$ and to a lesser extent importin $\alpha_3$, also appeared to be adequate in vitro targets of AMPK-mediated phosphorylation. This finding was not surprising, given the conservation of sequences around the importin $\alpha$ Ser$^{105}$ (ARKKLSREKQ)$^{100}$, importin $\alpha_3$ (ARKKLSSDRN)$^{104}$, and importin $\alpha_7$ (FRKLLSKEPS)$^{113}$. Although neither importin $\alpha_3$ nor importin $\alpha_7$ appeared to form bona fide complexes with HuR, their target cargoes may likewise be subject to nuclear import in an AMPK-governed fashion. However, the functional consequences of phosphorylation of $\alpha$ importins remains to be examined in greater detail, given a recent report that mutation of the conserved Ser$^{46}$ phosphorylation site of Drosophila importin $\alpha_2$ did not appear to influence its in vivo function in oogenesis (72). Assessment of the influence of importin $\alpha$ mutants on the nuclear import of other $\alpha$ importin cargoes (such as STAT-1, STAT-2, tissue transglutaminase, DNA helicase Q1, the serum- and glucocorticoid-inducible kinase Skg1, p53, lymphoid enhancer factor-1, etc.) was hampered by the need to treat cells with various agents prior to analysis, the lack of availability of suitable antibodies, or the fact that the cargo molecules in question were preferentially imported by importins other than importin $\alpha_1$. Further work is undoubtedly needed to establish whether other importin $\alpha$ cargoes might also be regulated by AMPK in the same fashion as HuR. The functional redundancy of importin $\alpha$ orthologs would make this task a challenging one.

The regulation of AMPK on gene expression has been best studied for the yeast AMPK homolog SNF1. SNF1 phosphorylates various transcription factors that jointly up-regulate the expression of glucose-repressed genes. In mammalian cells, AMPK has been proposed to regulate the expression of genes important for energy conservation at a time of low fuel availability (39). Although the direct effectors of this regulation have not been fully elucidated, a growing number of nuclear proteins, including transcription factors, have been identified as targets of phosphorylation by AMPK (73). In light of the results from this study, we propose that AMPK effects on importin $\alpha_1$, and possibly other importin $\alpha$ proteins, further contribute to altering patterns of expressed genes by regulating the import of proteins that modulate gene expression at multiple levels. Such targets may include NLS-containing transcription factors, as well as other RNA-binding proteins known to undergo nuclear import or nucleocytoplasmic shuttle (74–77). Given the abundance of NLS, both classical and nonclassical (such as M9), in proteins destined for nuclear import, elucidation of the specificity of the cargoes that utilize the AMPK and importin $\alpha$ pathway remains an important matter for future study.

In summary, we have described a novel function for AMPK as regulator of the nuclear localization of HuR, and we propose that such an import process is mediated by importin $\alpha_1$. Our data provide an important specific example of how AMPK may regulate gene expression in mammalian cells, a function of AMPK that had been previously postulated based on the direct effect of the yeast SNF1 homolog on transcription patterns. In mammalian cells, AMPK-driven modifications of importin $\alpha_1$ function may thus dictate the subcellular presence of various NLS-bearing RNA-binding proteins as well as transcription factors and thereby orchestrate transcriptional and post-transcriptional events underlying changes in gene expression patterns.

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AMP-activated Protein Kinase-regulated Phosphorylation and Acetylation of Importin α1: INVOLVEMENT IN THE NUCLEAR IMPORT OF RNA-BINDING PROTEIN HuR

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