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Posttranslational control of HuR function

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Abstract

The RNA-binding protein HuR (human antigen R) associates with numerous transcripts, coding and noncoding, and controls their splicing, localization, stability, and translation. Through its regulation of target transcripts, HuR has been implicated in cellular events including proliferation, senescence, differentiation, apoptosis, and the stress and immune responses. In turn, HuR influences processes such as cancer and inflammation. HuR function is primarily regulated through posttranslational modifications that alter its subcellular localization and its ability to bind target RNAs; such modifications include phosphorylation, methylation, ubiquitination, NEDDylation, and proteolytic cleavage. In this review, we describe the modifications that impact upon HuR function on gene expression programs and disease states.

INTRODUCTION

Posttranslational modifications are covalent changes that occur to almost every protein during or after protein synthesis. Their general role is to modify proteins to alter their stability, structural conformation, localization, or function.[1,2] Posttranslational modifications include the addition or removal of small chemical groups (e.g., phosphate, acetyl, or methyl), carbohydrates, lipids, or other amino acids.[3] Dysregulated posttranslational modifications have been shown to influence disease processes such as cancer, neurodegenerative disorders, and cardiovascular disease.[4–6]

Many RNA-binding proteins (RBPs) are modified posttranslationally, thus influencing the metabolism of target RNAs, including alternative splicing, polyadenylation, messenger RNA (mRNA) transport, turnover, storage, and translation. Some RBPs carry out structural functions (e.g., ribosomal RBPs) or associate with widespread RNA sequences (e.g., poly(A)-binding protein, PABP), while other RBPs bind to select sequences on a distinct subset of transcripts and have regulatory functions on a small group of target RNAs.[7–9] One of the best studied RBPs with regulatory impact upon a select subset of target mRNAs is HuR (human antigen R), also known as HuA and ELAVL1 (embryonic lethal abnormal vision-like 1). In this review, we summarize and discuss the posttranslational modifications of HuR and their impact on HuR function.

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HuR

HuR is the ubiquitous member of a family of RBPs that also includes three primarily neuronal proteins, HuB, HuC, and HuD.[10,11] HuR is best known for binding to a subset of mRNAs and for influencing their stability and/or translation, although it has also been implicated in target pre-mRNA splicing and nuclear export of mRNAs.[12–16] HuR binds transcripts mainly at U- or AU-rich RNA stretches, often located in the 3′-untranslated region (UTR) of target mRNAs.[17,18] HuR targets include many mRNAs that encode proteins involved in cell proliferation, apoptosis, and differentiation.[19–22] Through its impact on the expression of select subsets of proteins, HuR was found to be involved in physiological processes such as adipogenesis, muscle differentiation, and responses to stress and immune agents.[19–23] In addition, HuR been implicated in disease processes, primarily cancer, but also chronic inflammation and pathologies of the cardiovascular, neurological, and muscular systems.[24] Besides coding transcripts, HuR also has the ability to bind to and regulate the functions of microRNAs (miRNAs)[25–27] and long noncoding RNAs (lncRNAs).[27,28]

The function of HuR is regulated in part through control of HuR abundance. HuR transcription is positively controlled via Nuclear Factor (NF)-κB,[29] and HuR levels are enhanced through a positive feedback loop involving HuR binding to the *HuR* (*ELAVL1*) mRNA,[15,30] although other RBPs (e.g., AUF1 and TIA-1) can bind *HuR* mRNA and affect its stability and translation.[30,31] HuR abundance is suppressed by miRNAs miR-519, miR-125, 291b-3p, miR-570-3, and miR-16; these miRNAs target the coding region and 3′UTR of *HuR* mRNA.[31–35] However, most mechanisms affecting HuR function operate at the posttranslational level, as discussed below.

HuR contains three RNA recognition motifs (RRMs) with high homology to those of other Hu proteins.[8] The first two domains (RRM1 and RRM2) form a cleft where RNA binds, while RRM3 is important for stabilizing the RNA–protein complex and for mediating protein–protein interactions. Between RRM2 and RRM3 of HuR is a ‘hinge region’ that has lower homology with other Hu proteins and contains the HuR nuclear shuttling (HNS) domain, important for mobilization of HuR out of and into the nucleus.[36] While HuR is predominantly nuclear in unstimulated cells, it shuttles to the cytoplasm in response to various stimuli, including stress signals and mitogens.[37] The mobilization of HuR through the nucleopore complex is mediated by transportin-1 and -2 (Trn1 and Trn2), as well as the nuclear export factors pp32/PHAP-I, APRIL, and CRM1, which interact with the hinge region and assist with HuR transport into and out of the nucleus.[13,38,39] Given the distinct roles of the different domains of HuR, posttranslational modifications at regions important to interactions with RNAs and with the nucleocytoplasmic shuttling machinery directly influence HuR subcellular localization and RNA-binding activity. Below we review and discuss these modifications.

HuR PHOSPHORYLATION

Owing to its negative charge, addition of a phosphate group results in a change of the protein structure to adjust to the electrostatic effects of the phosphate group with other atoms.[40] Phosphorylation events impact upon HuR function in different ways, mainly by changing HuR protein stability, affinity for binding RNA, and subcellular localization. In general,

phosphorylation at or near the HuR RRMs affects HuR binding to target RNAs; for instance, phosphorylation of serine (S)88 in RRM1, S158 in RRM2, and S100 in the linker region has minimal influence on HuR structure but affect HuR affinity for RNA.[41] In contrast, phosphorylation around the HuR hinge region affects nucleocytoplasmic shuttling, likely by altering the interaction of HuR with the intracellular transport machinery (Figure 1). Other phosphorylation events can affect subsequent posttranslational modifications, as discussed below. For several specific phosphorylations of HuR, the kinases responsible have been identified. In this section, we review the specific kinases that phosphorylate HuR and their impact on HuR function.

Phosphorylation by Cdks

Cyclin-dependent kinases (cdks) are involved mainly in cell cycle progression and cell differentiation.[66] HuR has been shown to change its subcellular localization in a cell cycle-dependent manner. In G1 phase, HuR is predominantly nuclear, but during the S and G2 phases, a substantial fraction of HuR is mobilized to the cytoplasm, where it regulates the stability and translation of target mRNAs.[67,68] Cdks are activated by cyclins with the exception of CDK5, a neuronal cdk that is activated independently of cyclins.[66,69] The first cdk found to phosphorylate HuR was CDK1 (also known as CDC2); phosphorylation of HuR by CDK1 at S202, a residue in the hinge region, was shown to retain HuR in the nucleus.[55,70] S202 phosphorylation most likely occurs during the S and G2 phases, when CDK1 is active, and was proposed to ensure that HuR remained nuclear once mitosis ended.[55] Accordingly, inhibition of CDK1 by pharmacologic agents or by cellular stress enhanced HuR translocation to the cytoplasm.[71] Some upstream regulators of this effect have also been identified. For example, ultraviolet (UV) light activated the stress-response kinase ATR (ataxia telangiectasia and Rad3), an upstream kinase for CDK1. Phosphorylation of CDK1 abolished its interaction with HuR, which was then capable of translocating to the cytoplasm, binding to *p21*/*CDKN1A* mRNA, and enhancing production of the cdk inhibitor p21.[71]

CDK5 was shown to interact with HuR on the centrosomes of glioma cells. This interaction also led to phosphorylation of HuR at S202, in this case preventing HuR binding to *CCNA2* mRNA and causing a reduction in CCNA2 (cyclin A) translation. This change led to cell cycle arrest and defective centrosome duplication, suggesting that HuR may be associated with centrosomes in order to control protein synthesis in proximity to centrosomes.[56]

As the hinge region contained several other potential phosphorylation sites that may regulate nucleocytoplasmic localization of HuR, mutants with nonphosphorylatable residues in this region were also examined.[59] Among them, the HuR serine-to-alanine mutant S242A was found to be more cytoplasmic, regardless of stress conditions, further supporting the idea that phosphorylation at the hinge region influences HuR cytoplasmic translocation.[59]

Phosphorylation by CHK2

The checkpoint kinase 2 (CHK2) is activated upon genotoxic stress and plays a role in cell cycle regulation by phosphorylating CDC25 phosphatases (which in turn inactivates cdks), the tumor suppressor p53 (which stabilizes p53 and triggers growth arrest), and other

proteins important for suppressing proliferation.[59] CHK2 was found to phosphorylate HuR at S88, S100, and threonine (T)118 in response to treatment with hydrogen peroxide (H_2O_2).[44] S88 and threonine (T) 118 are located in RRM1 and RRM2, respectively, while S100 is located in the linker region between RRM1 and RRM2.[44] Phosphorylation of HuR by CHK2 did not affect the subcellular localization of HuR, but instead altered HuR binding to its target transcripts. Following exposure to H_2O_2, HuR dissociated from *SIRT1* mRNA triggering *SIRT1* mRNA decay. Mutation analysis of the three phosphorylation residues revealed that S100 had the greatest effect on HuR–*SIRT1* mRNA dissociation following H_2O_2 treatment.[44]

A subsequent transcriptome-wide analysis of HuR interaction with mRNAs after ionizing radiation (IR) revealed that CHK2 triggered growth arrest and enhanced cell survival by phosphorylating HuR.[45] Upon IR treatment, CHK2 activation led to global dissociation of HuR from its target mRNAs, including mRNAs that encoded proapoptotic and proliferative proteins. Simultaneous mutations of all three residues (S88, S100, and T118) to create a triple nonphosphorylatable mutant and a triple phosphomimetic mutant [HuR(3A) and HuR(3D), respectively] revealed that the nonphosphorylatable mutant HuR (3A) remained bound to target mRNAs after IR, associated with reduced survival, whereas the phosphomimetic mutant HuR(3D) showed reduced interaction with mRNAs constitutively and improved cell survival following exposure to IR.[45] Accordingly, phosphorylation at RRMs generally reduces HuR binding to target mRNAs and influences cell survival.

Although the vast majority of mRNAs dissociated from HuR after it was phosphorylated by CHK2, a few examples indicating an opposite effect have been reported. For instance, polyamines promoted CHK2-mediated HuR phosphorylation and also increased HuR binding to *Myc* mRNA in rat intestinal epithelial cells, although the impact of the three residues was different: compared with binding of WT HuR, mutant S88A showed greater binding, S100A showed less binding, and T118A showed comparable binding to *Myc* mRNA.[49] HuR also bound *Occludin* mRNA better upon phosphorylation by CHK2, leading to increased translation, although only S88 mediated this effect, strengthening the notion that each phosphorylation site may have a distinct function on HuR association with a target mRNA.[46]

CHK2 may regulate additional functions of HuR. Binding of HuR to the splicing factor transformer 2 (*TRA2*) mRNA following sodium arsenite treatment was dependent on phosphorylation of HuR on S88 and T118 by CHK2. This phosphorylation led to an alternative splicing event whereby exon 2 was included and TRA2 isoform 4 was generated, linking CHK2 phosphorylation of HuR to its role in alternative splicing.[47] Finally, CHK2 may protect HuR against proteasomal degradation, as S88A and T118A mutants were more rapidly degraded, whereas phosphomimetic mutants were protected from degradation upon heat shock.[48]

Phosphorylation by MAPK p38

The p38 mitogen-activated protein kinase (MAPK) is involved in the cellular response to DNA damage and to stresses such as UV radiation, oxidative damage, and cytokines.[72] It is also involved in modulating the cell division cycle by regulating G2 arrest induced by IR.[73]

p38 was identified as an additional kinase that phosphorylates HuR at T118 upon IR. This event stabilized *p21* (*CDKN1A*) mRNA, elevating p21 protein expression and triggering cell cycle arrest in the G1 phase. Unlike CHK2, phosphorylation by p38 led to the accumulation of HuR in the cytoplasm and elevated HuR binding to *p21* mRNA.[50]

During the inflammatory response interleukin, (IL)-1β-activated p38 phosphorylated HuR at T118 and enhanced HuR binding to the 3′UTR of *cPLA$_2$α* mRNA, stabilizing and increasing its translation. Similarly, HuR phosphorylation at T118 by p38 upon IL-1β treatment enhanced HuR binding to *COX2* mRNA and increased its stability.[51] These findings indicate that HuR may be a key effector of p38-mediated functions in the inflammatory response. The influence of p38 on HuR is not fully clear, because p38 inhibition was also shown to increase HuR phosphorylation, although the specific phosphorylation sites were not identified.[74]

Phosphorylation by PKC

The protein kinase C (PKC) family consists of nine genes that encode different isozymes with roles in cell proliferation, apoptosis, and migration.[75] Depending on their structures and activation characteristics, they are classified into conventional PKCs (PKCα, PKCβI, PKCβII, and PKCγ), novel PKCs (PKCσ, PKCδ, PKCε, PKCη, and PKCθ), and atypical PKCs (PKCζ and PKCλ).[76] Several target motifs for PKC-dependent phosphorylation exist in HuR and indeed several PKCs have been found to phosphorylate HuR.

Among the potential PKC target residues S158 in RRM2, S221 in the hinge region, and S318 in RRM3 have been studied in detail. PKCα interacts with HuR in the nucleus to phosphorylate S158 and S221, leading to ATP-dependent HuR cytoplasmic translocation. Elevated HuR in the cytoplasm enhances its binding to *COX2*/*PTGS2* mRNA leading to increased mRNA stability, COX2 production, and subsequent increase in prostaglandin E2 synthesis.[52] However, HuR phosphorylation and binding to *COX2* mRNA may be affected differently by other PKCs depending on other factors, including the stimulus and the cell type. For example, angiotensin II treatment increased HuR binding to *COX2* mRNA in human mesangial cells, but here the cytoplasmic shuttling of HuR was dependent on serine phosphorylation by PKCδ.[12]

Phosphorylation of HuR at S318 by PKCδ led to enhanced binding of HuR to targets like *COX2*, *CCNDA2*, and *CCND1* mRNAs, although it did not affect HuR cytoplasmic export. In contrast, HuR phosphorylation at S221 was critical for cytoplasmic translocation but not for binding to RNA.[77] Phosphomimetic mutations at positions S158 and S318 altered HuR-binding preference from AU-rich to U-rich RNA sequences, even in the absence of clear changes in secondary structure.[53] Switches in binding preference may also be observed without changes in HuR phosphorylation status.[78,79] It is possible that phosphorylation at certain sites may induce multimerization of HuR oligomers, in turn changing the affinity of HuR for target RNAs.[80]

The impact of HuR phosphorylation by PKC in carcinogenesis is complex. Although PKCδ functions as a tumor suppressor,[81] its levels are high in colorectal cancer.[82] Indeed, HuR was constitutively phosphorylated at S318 in DLD-1 colon cancer cells and in colon cancer

tissues, and HuR binding to mRNAs was inhibited upon pharmacological or RNAi-mediated PKC inhibition.[64] Migration and invasion were also impaired in cancer cells expressing the nonphosphorylatable HuR mutant S318A and enhanced in cells expressing the phosphomimetic mutant S318D.[64] In breast cancer cells undergoing apoptosis in response to doxorubicin, HuR was phosphorylated at S221 and S318[65]; in prostate cancer cells, PKC-mediated phosphorylation on HuR S318 triggered the cytoplasmic export of HuR, which permitted the recognition of HuR by the E3 ubiquitin ligase βTrCP1 and its subsequent ubiquitin-dependent degradation.[62]

Phosphorylation by Other Kinases

HuR can also be phosphorylated by other kinases such as IkB kinase α (IKKα), extracellular signal-regulated kinase 8 (ERK8), and Janus kinase 3 (JAK3). Phosphorylation of HuR by IKKa at S304 resembled the consequences of S318 phosphorylation by PKCα on HuR binding to βTrCP1 and subsequent ubiquitination and degradation.[62] ERK8, also known as MAPK15, was capable of phosphorylating HuR in response to H_2O_2 treatment, leading to its cytoplasmic accumulation; interestingly, this change led to reduced binding and destabilization of programmed cell death 4 (*PDCD4*) mRNA.[83]

All of the phosphorylated residues on HuR discussed thus far are serine or threonine. However, tyrosine phosphorylation of HuR is emerging as important for regulating HuR function. Upon activation with menadione, JAK3 was recently identified as a kinase that phosphorylates HuR at Y200, Y63, and Y68. However, only HuR phosphorylation at Y200 affected its activity, causing HuR to be excluded from cytoplasmic stress granules upon arsenite treatment and hence reducing its interaction with targets such as *SIRT1* and *VHL* mRNAs.[43] Tyrosine phosphorylation was also involved in the interaction of HuR with nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) in cytoplasmic granules. Although the specific phosphotyrosine residues were not mapped, this modification enhanced the interaction of HuR with CCAAT/enhancer-binding protein β (*C/EBPβ*) mRNA and increased C/EBPβ translation, without affecting HuR subcellular distribution.[84] As mentioned above, HuR phosphorylation by CDK5 led to its localization in the cell's centrosome in glioma cells.[56] Tyrosine phosphorylation by SRC and ABL1 kinases was implicated in HuR accumulation in the pericentriolar matrix, because HuR bearing mutations that precluded phosphorylation at four tyrosines (Y5F, Y95F, Y105F, and Y200F) showed reduced accumulation on the centrosomes and genomic instability.[42]

Other studies indicated HuR phosphorylation without clear identification of the phosphorylation sites. For example, acidic conditions caused a decrease in HuR phosphorylation and increase in HuR binding to phosphoenolpyruvate carboxykinase (*PEPCK*) mRNA leading to enhanced PEPCK expression.[85] Finally, infection of mammalian cells with alphaviruses showed altered phosphorylation status of HuR associated with increased presence of HuR in the cytoplasm.[86]

HuR METHYLATION

Methylation of arginine residues is one of the most frequent posttranslational modifications in mammalian proteins.[87] Protein methylation regulates cellular processes such as signaling,

splicing, protein–protein interactions, nuclear–cytoplasmic transport, and transcription.[87] The enzymes that catalyze protein methylation, known as protein arginine methyltransferases (PRMTs), transfer methyl groups from *S*-adenosylmethionine to specific arginine residues of a target protein generating monomethylated and dimethylated arginine.[88]

A member of the family of PRMTs is the protein coactivator-associated arginine methyltransferase 1 (CARM1/PRMT4), originally discovered as a methyltransferase implicated in transcription activation by methylating histone H3 and p300/CBP.[89,90] Radioactive sequencing and analysis of specific HuR arginine-to-lysine mutant peptides indicated that R217 is a key target of CARM1. HuR methylation at R217 increased in lipopolysaccharide-stimulated macrophages,[57] and in human cervical carcinoma HeLa cells, CARM1-mediated protein methylation enhanced HuR function, promoting the stabilization of *CCNA2*, *CCNB1*, *FOS*, and *SIRT1* mRNAs.[91] Another study showed that methylation by CARM1 could strengthen the association of HuR with *SIRT1* 3′UTR during the differentiation of human embryonic stem cells (hESCs).[92] HuR methylated at R217 also correlated significantly with cytoplasmic HuR staining in non-small cell lung carcinoma and methyl(R217)HuR was a prognostic marker.[93]

UBIQUITINATION

Proteins that are targeted for proteasomal degradation are marked with a chain of ubiquitins that is recognized by the proteasome. This process requires three enzymes: E1, E2, and E3. The E1 ubiquitin-activating enzyme transfers ubiquitin to E2, the ubiquitin-conjugating enzyme, which in turn interacts with an E3 ubiquitin ligase that transfers the ubiquitin to a substrate protein.[94]

Inhibition of proteasome activity was found to elevate HuR in human diploid fibroblasts.[95] In HeLa cells, HuR was found to be ubiquitinated and degraded upon heat shock, but phosphorylation by CHK2 enhanced resistance of HuR against proteasomal degradation.[48] Recently, the tumor suppressor esophageal cancer-related gene 2 (ECRG2) was shown to increase in response to DNA damage, in turn favoring ubiquitination of HuR at K182 and HuR degradation.[54] In the same report, lowering HuR led to reduced expression of the inhibitor of apoptosis XIAP (encoded by the HuR target *XIAP* mRNA), prompting the authors to propose that the tumor suppressive actions of ECRG2 were associated with the suppression of HuR and XIAP levels.[54] Glycolytic stress in prostate cancer cells triggered the translocation of HuR to the cytoplasm, where it was targeted by the ubiquitin E3 ligase βTrCP1 for degradation.[62] In this metabolic stress model, phosphorylation by PKCδ and IKKα contributed to the recognition of HuR by βTrCP1.[62] Finally, some instances of HuR ubiquitination do not affect its stability and instead influence its RNA-binding activity. For example, ubiquitination of HuR with a short atypical K29 chain attached to HuR Lys 313 and Lys 326 (K313/K326) signals HuR to dissociate from *p21* (*CDKN1A*), *MKP1* (*DUSP1*), and *SIRT1* mRNAs.[63] Taken together, dynamic ubiquitination and deubiquitination of HuR serve as a mechanism for rapidly switching HuR activity and abundance in response to cellular stresses.

NEDDylation

Neural precursor cell-expressed developmentally downregulated 8 (NEDD8) is a ubiquitin-like protein that is covalently conjugated to proteins. Originally, the cullin subunits of E3 ubiquitin ligases were identified as the only neddylation targets, as this modification was required for their activation and efficient conjugation to substrates.[96] However, NEDD8 modification has been found in other proteins and plays important roles in biological processes such as cell cycle control and embryogenesis.[97] Murine double minute 2 (Mdm2) was characterized as an E3 NEDD8 ligase for p53 and later for HuR.[60] HuR can be neddylated at lysine residues K283, K313, and K326, and modifications at K313 and K326 are involved in HuR protein stability by increasing their nuclear localization. As a consequence, inhibition of the neddylation process may sensitize cancer cells to the chemotherapy drug carboplatin, potentially by regulating HuR levels in the cytoplasm.[61] The impact of HuR neddylation on HuR binding to target mRNAs remains to be investigated.

PROTEOLYTIC CLEAVAGE

Upon certain apoptotic stresses, HuR translocation to the cytoplasm leads to its proteolytic cleavage at aspartate (D)226 by caspases 3 and 7, and the generation of two smaller active HuR products, HuR-CP1 and HuR-CP2. Both cytoplasmic translocation and proteolytic cleavage require association of HuR with the apoptosome activator pp32/PHAP-I. A noncleavable mutant, HuR D226A, maintains its association with pp32/PHAP-I and delays apoptotic cell death.[58] While cleavage by caspase-3 and caspase-7 plays a crucial role in apoptotic cell death, cleavage by caspase-8/caspase-3 is triggered by protein kinase R (PKR) and plays a role in myogenesis.[98] During early myogenesis, the nuclear import of HuR is mediated by the import factor transportin 2 (TRN2).[99] When myoblasts are differentiated to myotubes, HuR is cleaved to generate HuR-CP1, which is exported by TRN2 leading to HuR accumulation in the cytoplasm, a step that is important in promoting myogenic gene expression programs.[100] In sum, proteolytic HuR cleavage by caspases plays roles in vital cellular processes such as apoptosis and myogenesis. HuR cleavage products may influence the posttranscriptional fate of target mRNAs, but the entire spectrum of actions resulting from HuR cleavage remains to be studied.

CONCLUDING REMARKS AND PERSPECTIVES

Posttranslational modification of HuR is involved in important biological processes including carcinogenesis, apoptosis, myogenesis, cell division, and cell response to stress agents. HuR function is regulated via changes in its subcellular localization, affinity for RNA, abundance, and cleavage, which in turn influence HuR’s ability to affect the fate of target mRNAs. Thus, posttranslational modifications enable HuR to elicit quick changes in gene expression programs.

Several studies have reported HuR phosphorylation by different kinases with various impacts on gene expression and cell fates. PKCs, cdks (CDK1 and CDK5), and CHK2 are major kinases that phosphorylate HuR and modulate cell proliferation and survival. Phosphorylation by CDK1, CDK5, and PKCs changes the subcellular distribution of HuR and subsequent binding to target transcripts. Phosphorylation by CHK2 under DNA-damage

conditions influences HuR binding to target mRNAs without altering its subcellular localization. Although phosphorylation at or near RRMs generally affects HuR binding to target RNAs, HuR phosphorylation at the same residue by different kinases may have distinct outcomes; for example, phosphorylation at T118 by CHK2 influences HuR binding to mRNAs, affecting their stability,[48] translation,[45] and alternative splicing,[47] while T118 phosphorylation by p38 causes HuR to accumulate in the cytoplasm and stabilize *p21* mRNA.[50] Additionally, phosphorylation events that affect HuR subcellular localization in a similar manner may have distinct outcomes on HuR binding to target RNAs. For example, HuR phosphorylation by PKC increases HuR in the cytoplasm and enhances its interaction with *COX2* mRNA,[52] while HuR phosphorylation by p38 also elevates cytoplasmic HuR levels, promoting HuR binding to *p21* and *cPLA2α* mRNAs as well.[50,51] These examples suggest that the posttranslational modifications of HuR cannot be interpreted in isolation and instead may involve other factors like the particular stimulus (type, magnitude, and duration), the cellular environment, and perhaps other concurrent posttranslational modifications of HuR. Regarding the latter possibility, several posttranslational modifications appear to coordinate to elicit a specific outcome; for instance, ubiquitination and deubiquitination can alter HuR levels and activity dynamically depending on the stimulus and the cellular response. While PKC and IKKα phosphorylation of HuR at S318 and S304 leads to ubiquitin-mediated degradation of HuR, CHK2-mediated phosphorylation at S88 and S100 can block it.[48] Interestingly, NEDDylation stabilizes HuR, even though NEDD8 is closely related to ubiquitin.

Despite accumulating information on the posttranslational modifications that affect HuR function, there are still many open questions. How do they impact upon the affinity of HuR for target RNAs? How do they modify the interaction of HuR with other proteins? How do posttranslational modifications jointly regulate HuR abundance, subcellular localization, and activity? What signaling pathways regulate the effectors of HuR posttranslational modification? What are the functions of HuR cleavage products in different cell compartments? What processes and proteins (e.g., phosphatases, demethylases, and deubiquitinating enzymes) reverse these posttranslational modifications? Understanding the impact of HuR posttranslational modifications on HuR function in different physiologic and pathologic settings will highlight novel targets for therapeutic intervention.

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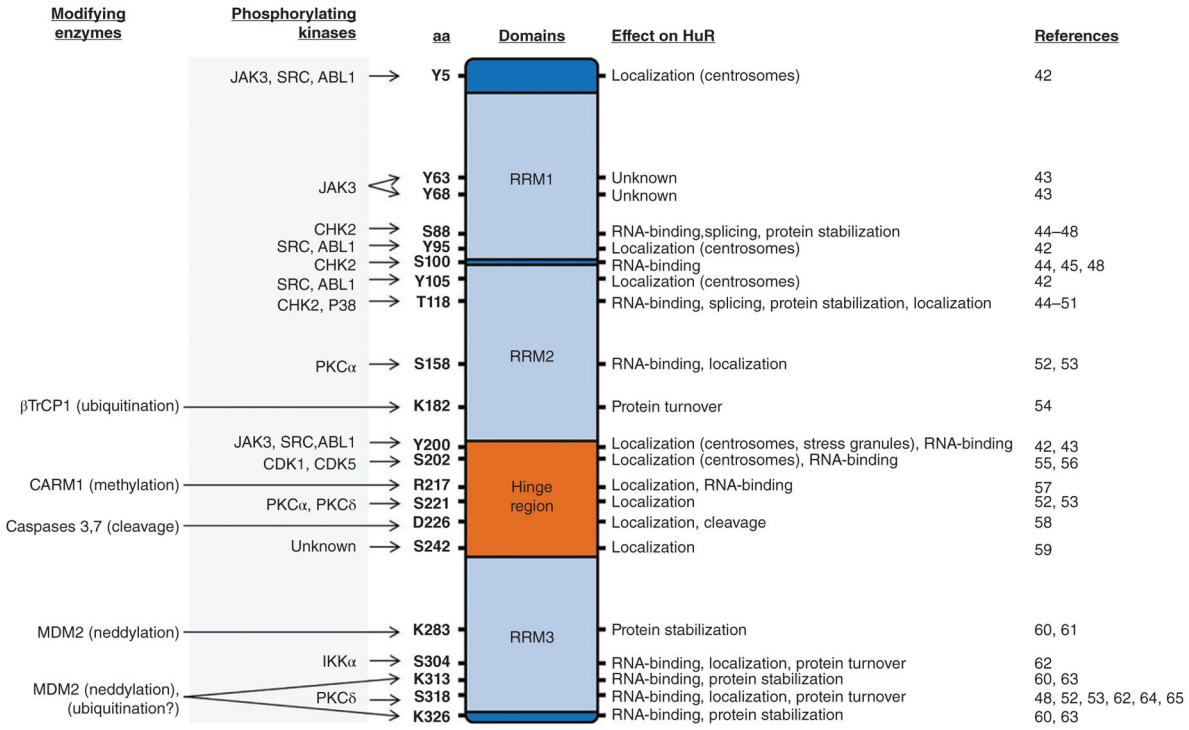

FIGURE 1.

Map of posttranslational modifications of HuR (human antigen R). Structure of HuR, depicting the three RNA recognition motifs (RRMs) and the central hinge region. The modified amino acids are indicated. The modifying enzymes, including the kinases (gray shading), are indicated on the left. The impact of each modification on HuR localization and function is indicated on the right. References citing the relevant studies are listed.