Biochimica et Biophysica Acta 1813 (2011) 1663–1667

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

HuR and myogenesis: Being in the right place at the right time ☆

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ARTICLE INFO

Article history:
Received 27 October 2010
Received in revised form 20 January 2011
Accepted 28 January 2011
Available online 20 February 2011

Keywords:
HuR
Myogenesis
Transportin 2
Caspase
Myogenic regulatory factor

ABSTRACT

The process of muscle cell differentiation into myotubes, termed myogenesis, depends on a complex coordination of myogenic factors, many of which are regulated post-transcriptionally. HuR, an mRNA-binding protein, is responsible for regulating the expression of several such myogenic factors by stabilizing their mRNAs. The critical role for HuR in myogenesis also involves the nucleocytoplasmic shuttling ability of this protein. Indeed, in order to perform its stabilizing functions, HuR must accumulate in the cytoplasm. This requires its dissociation from the import factor Transportin 2 (TRN2) which is actually caused by the cleavage of a portion of cytoplasmic HuR. In this review, we describe the roles of HuR during myogenesis, and the mechanisms regulating its cytoplasmic accumulation. This article is part of a Special Issue entitled: Regulation of Signaling and Cellular Fate through Modulation of Nuclear Protein Import.

1. Post-transcriptional regulation of muscle cell differentiation

During mammalian development, mono-nucleated muscle cells, myoblasts, must fuse to form multi-nucleated myotubes and then myofibers. This process is called myogenesis, and it is also crucial for muscle repair and growth [1]. Given the clear importance of this physiological event, mammals have evolved a tight regulatory system to govern the onset of myogenesis. A variety of factors contribute to this process, termed myogenic regulatory factors (MRFs), and their expression has been clearly demonstrated to be tightly regulated during the myogenic process [1–7]. In particular, many of the mRNAs encoding for these MRFs contain destabilizing elements in their 3′ untranslated region (3′UTR). These include A/U repeats as well as G/U rich elements, called A/U-rich elements (AREs) and GREs, respectively [8–11]. GRE- and ARE-containing mRNAs are susceptible to enhanced decay, such as ARE-Mediated Decay (AMD) for ARE-containing mRNAs, and are characterized by having short half-lives that govern their protein expression levels [8,12,13]. This allows a tight control over the time at which they are expressed, enabling the specific activation of physiological processes such as myogenesis.

Certain proteins have been shown to specifically bind these elements. For example, a class of RNA-binding proteins, called ARE-binding proteins (AUBP), is capable of recognizing ARE-containing mRNAs. These AUBPs, however, can either assist in the decay process, or can actually stabilize these messages, thus allowing their expression [12–15]. To date, KSRP (KH-domain Splicing Regulatory Protein) and HuR are the only AUBPs that have been shown to modulate the expression of certain MRFs. KSRP has been shown to bind and destabilize the mRNAs of *p21* and *myogenin*, important MRFs [16]. During muscle development however, KSRP is phosphorylated, which leads to a loss of association with its pro-myogenic targets. Other destabilizing factors have also been linked to myogenesis in an ARE-independent manner. CUGBP1 was recently identified to bind GREs and was implicated in the instability linked to these elements [8]. Other RNA-decay systems, such as nonsense-mediated decay (NMD) and Staufen1-mediated decay (SMD), are also involved in the regulation of myogenesis, having been recognized to influence the stability of *myogenin* mRNA [17–19]. While the implication of KSRP, CUGBP1 and Staufen1 seems to be important for proper regulation of myogenesis, very little is known about the molecular mechanisms regulating their actions during this process.

HuR, however, is a well-characterized stabilizing AUBP, the regulation of which is better understood in myogenesis. HuR is a ubiquitously expressed protein of the ELAV-1 family (embryonic lethal abnormal vision in *Drosophila*) which transports ARE-containing messages from the nucleus to the cytoplasm [12,13,20–23]. Once in the cytoplasm, HuR regulates mRNA expression by either stabilizing messages, or influencing their translation. While HuR has an abundance of mRNA targets involved in various cellular processes including cell-cycle regulation and stress-response, several myogenic factors have been identified to be regulated by HuR. These

Abbreviations: TRN2, Transportin 2; ARE, A/U-rich element; AUBP, ARE-binding protein; AMD, ARE-mediated decay; MRF, myogenic regulatory factor; CP, cleavage product; AP, antennepedia; GST, glutathione-*S*-transferase; IP, immunoprecipitation; IF, immunofluorescence; SEM, standard error of the mean

☆ This article is part of a Special Issue entitled: Regulation of Signaling and Cellular Fate through Modulation of Nuclear Protein Import.

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doi:10.1016/j.bbamcr.2011.01.036

include myogenin, MyoD and p21 [9,10], which are stabilized by HuR. Myogenesis also requires that the post-transcriptional regulation of certain mRNAs by HuR be interrupted. This was recently demonstrated when it was shown that the mRNA level and stability of the cell-cycle control factor Ccnd1/Cyclin D1 is reduced during myogenesis [24]. This result was not particularly surprising, since muscle cell differentiation requires that myoblasts undergo cell-cycle arrest [1]. Gherzi et al. demonstrated that the HuR-stabilizing complex loses association from *Ccnd1* mRNA during the differentiation of murine myoblast cells (C2C12), causing the destabilization of this mRNA and a reduction in its protein expression. Thus, beyond promoting the expression of pro-myogenic MRFs, HuR also contributes to halting unwanted processes. Together, these observations have clearly established a role for HuR in myogenesis, further supported by the fact that an absence of HuR prevents this process [9–11,25]. Therefore, this review focuses on the HuR-mediated control of myogenesis, with particular emphasis on how HuR localization impacts this important process.

2. Cytoplasmic HuR is crucial for myogenesis

Characterization of how HuR regulates myogenesis led to the observation that the cytoplasmic localization of HuR is required in order for it to execute its pro-myogenic function [9,10]. The ability of HuR to traffic between the nuclear and cytoplasmic compartments is mediated by the HNS (HuR Nucleocytoplasmic Shuttling) motif. This motif modulates the localization of HuR through interactions with protein partners. Generally, the stabilizing function of HuR has been shown to correlate with its cytoplasmic localization [11,12,14,15]. Indeed, if HuR is sequestered to the nucleus, known mRNA targets of HuR have been shown to have a reduced half-life [26]. This was recently demonstrated during inflammation, where the phosphorylation of HuR and its cytoplasmic localization were reduced by interleukin-19 (IL-19). The phosphorylation status of HuR was previously reported to regulate the localization of this mRNA-binding protein. Several phosphorylation sites for HuR have been identified, and they do not all have the same influence on HuR localization. PKCα phosphorylation at S158 and S221 causes HuR to accumulate in the cytoplasm [27], whereas Cdk1 phosphorylation of HuR at S202 causes it to be retained in the nucleus [28]. In the study by Cuneo et al., PKCα activity was inhibited by IL-19, preventing HuR from localizing to the cytoplasm, and thus reducing its mRNA stabilizing effect. While these studies have been useful in understanding the general mechanisms regulating HuR localization, they have not specifically looked at the mechanisms behind the shuttling abilities of HuR during myogenesis.

The localization of HuR has been shown to involve both CRM1-dependent and -independent pathways [21–23,29]. Through binding to proteins containing a Nuclear Export Signal (NES), HuR, via CRM1, can move to the cytoplasm. These protein partners include pp32/PHAPI and APRIL/PHAPII [21,29]. Also responsible for nuclear import of HuR are Transportin 1 (TRN1) and Transportin 2 (TRN2) proteins [30,31]. Interference with these pathways has allowed the characterization of HuR localization in different cell systems [12,13,20–23,29].

During myogenesis, one intriguing observation was the specific involvement of TRN2 in regulating the cytoplasmic accumulation of HuR [11]. Indeed, the siRNA-mediated knockdown of TRN2 enhances the cytoplasmic accumulation of HuR, thus enhancing muscle cell differentiation. These findings were supported when the short, cell-permeable antennapedia peptide (AP, which allows the uptake of proteins with a >90% efficiency) was conjugated to the nucleocytoplasmic shuttling domain of HuR (HNS) and was added to myoblast cells undergoing muscle differentiation [10]. This AP–HNS fusion peptide proved to be capable of competing with HuR for binding to TRN2, thus preventing the import of HuR into the nucleus. The consequence of this, the cytoplasmic accumulation of HuR, enhanced myogenesis [11].

In this study, we also discovered that the inhibition of the TRN2/HuR interaction is reflective of the cellular mechanisms which occur during differentiation. As myogenesis progresses, the HuR and TRN2 interaction is lost, which correlates with the cytoplasmic accumulation of HuR, and an increase in the stability of HuR pro-myogenic mRNA targets *myogenin* and *MyoD* [11]. It was only recently, however, that the cellular mechanism responsible for this interruption in HuR/TRN2 was deciphered [25], described below.

3. Implication of caspase-mediated cleavage products of HuR in muscle fiber formation

3.1. HuR cleavage regulates its cytoplasmic accumulation

During myogenesis, it has been shown that proteolytic enzymes, called caspases, are activated [32,33]. While caspases are best known for their role in apoptotic cell death [34,35], data from several groups have indicated that during muscle cell differentiation they exercise an essential non-apoptotic function leading to muscle fiber formation [32,33]. HuR was previously shown to be cleaved by caspases-3 and -7 during apoptotic cell death [36,37], and so it was not completely surprising to find that during myogenesis, when caspase-3 is activated, HuR cleavage also occurs [25]. This cleavage produces two HuR cleavage products (24 kDa HuR-CP1 and 8 kDa HuR-CP2) and occurs at Asp 226 of HuR, dividing the HNS between the two fragments. Of the 3 RNA Recognition Motifs (RRMs) of HuR, the first two (RRM1 and RRM2) are contained in HuR-CP1, while the C-terminal RRM3 is a part of HuR-CP2 [36].

In stress-response, where cleavage was first discovered, we noted that the cleavage products have different abilities to interact with the protein partners of HuR. Specifically, HuR-CP2, but not -CP1, was found to associate with pp32/PHAPI [36]. This was not particularly surprising given that the interaction between HuR and pp32/PHAPI was previously shown to involve the HNS and 3rd RNA-recognition motif (RRM3) of HuR [29].

The association of TRN2 with HuR is also mediated by the HNS. Since the majority of the HNS is retained in HuR-CP1, we assessed the ability for HuR-CP1 to bind with TRN2, in C2C12 cells. Importantly, HuR-CP1 not only bound to TRN2, but was even able to compete off HuR-binding to TRN2 in an *in vitro* binding assay [25]. This is in contrast to HuR-CP2, which does not bind TRN2.

These observations raised the possibility that the generation of HuR-CP1, which we also identified to occur *in vivo* in a mouse muscle-regeneration model [25], could be responsible for the interruption of HuR/TRN2 binding that was previously reported to occur during muscle cell differentiation [11]. Overexpression of HuR-CP1 triggered the cytoplasmic accumulation of full-length HuR, as normally seen in myogenesis, and also caused an increase in the stability of *myogenin* mRNA [25]. This positive effect of HuR-CP1 on myogenesis was seen to be indirect, however, dependent on the presence of full-length HuR. Indeed, in the absence of full-length HuR, HuR-CP1 was unable to induce differentiation, and also failed to rescue the mRNA level of *myogenin*, which full-length HuR managed to achieve. These results have thus shown that through its cleavage, HuR localization is auto-regulated. By having HuR-CP1 bind TRN2 and thus block nuclear re-import of full-length HuR, cytoplasmic accumulation of this RNA-stabilizing factor occurs, which results in an increase in MRF mRNA stability and production.

3.2. Non-cleavable HuR inhibits myogenesis

In order to determine the exact cleavage site of HuR, a non-cleavable HuR point mutant was generated (HuR-D226A) [36]. This mutant has been used to better understand the importance and physiological significance of HuR cleavage, and as such, was used to