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UneCLIPsing HuR nuclear function

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Abstract

The RNA-binding protein HuR, while known to stabilize cytoplasmic mRNAs, is largely nuclear. In this issue of *Molecular Cell*, Mukherjee et al. (2011) and Lebedeva et al. (2011) identify transcriptome-wide HuR-RNA interactions using PAR-CLIP, unveiling HuR's nuclear role in pre-mRNA processing.

HuR associates with mRNAs bearing U/AU-rich sequences, typically present in 3'-untranslated regions (UTRs) (Lopez de Silanes et al., 2004). Although HuR is predominantly nuclear, its post-transcriptional influence is linked to its translocation to the cytoplasm, where it stabilizes and/or modulates the translation of numerous target mRNAs (Hinman and Lou, 2008). Over the past 15 years, the identification of dozens of HuR target mRNAs has implicated HuR in important cellular processes (for example, proliferation, apoptosis, differentiation) and in pathologic states (for example, cancer, inflammation) (Hinman and Lou, 2008; Abdelmohsen and Gorospe, 2010). However, several key aspects of HuR biology remained unanswered; namely, the complete set of RNAs controlled by HuR (including pre-mRNAs and noncoding RNAs), the functions of HuR in the nucleus, and the influence of microRNAs/RISC on the post-transcriptional fate of HuR targets.

Two articles in this issue, Mukherjee et al. (2011) and Lebedeva et al. (2011), provide valuable insight into these questions. Using the state-of-the-art method PAR-CLIP [photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation (Hafner et al., 2010)], they identified specific short RNA segments, of approximately 25 nucleotides, bound to HuR. RIP-chip [ribonucleoprotein (RNP) immunoprecipitation followed by microarray analysis] was used to identify more stable native HuR-mRNA targets (Mukherjee et al., 2001). Side-by-side microarray and RNA-seq analyses (Mukherjee et al., 2001; Levedeva et al., 2001) measured global HuR-dependent changes in total RNA, while pSILAC analysis (Levedeva et al. 2001) assessed new translation. Through these approaches, the groups elucidated *bona fide* HuR-regulated RNAs, reaching remarkably similar conclusions.

The most significant revelation from these reports is that one-third of HuR-RNA associations occur at pre-mRNA introns many in the proximity of 3' splice sites; with the remaining approximately two-thirds present at 3'UTRs. The intronic RNA-HuR interactions partially answer the long-unresolved question of HuR's high nuclear abundance when its documented actions on mRNA metabolism, including both mRNA stability and translation,

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were cytoplasmic. Although HuR was reported to participate in the splicing of the *FAS* pre-mRNA, which encodes an apoptosis-promoting receptor (Izquierdo, 2010), its nuclear localization was loosely thought to represent a means of storing nuclear mRNAs not yet ready for export, perhaps to avoid their premature degradation or translation. Besides demonstrating that nuclear HuR interacts with introns, both groups make a compelling case for HuR's role in splicing, as illustrated for several randomly chosen mRNAs (*ZNF207*, *GANAB, PTBP2* and *DST*). These studies pave the way for the systematic testing of: HuR's role in splicing; its influence on exon usage; whether splicing co-factors are involved; and, whether HuR-modulated splicing contributes to HuR-regulated responses.

HuR's interaction with pre-mRNAs marks the beginning of a proposed *continuum* of post-transcriptional influence (Keene, 1999). These new studies demonstrate that HuR binds nascent pre-mRNA, co-transcriptionally or soon after transcription, and assists with its splicing and nuclear processing. Subsequently, HuR likely helps to export mature target mRNAs to the cytoplasm, a function that remains poorly characterized. In transit through the cytoplasm, HuR performs its best-known, mRNA-stabilizing function – perhaps including periods of storage in cytoplasmic domains like stress granules, where HuR accumulates following some types of cellular damage. Eventually, HuR ensures the timely engagement of the mRNA with ribosomes for translation (Figure 1). Although Levedeva et al. (2011) report that HuR-elicited changes in translation generally mirrored HuR-dependent changes in mRNA abundance, HuR has been shown to modulate the translation of some target mRNAs without affecting their half-lives. Further studies using pSILAC are therefore warranted, since this proteomics method is ideally suited to detect *en masse* HuR-effected changes in nascent translation.

Besides this coupled regulation, both groups recognize an additional layer of HuR influence on gene expression as it modulates proteins that control transcription, splicing, mRNA maturation, turnover, and translation. In addition, HuR appears to favor the processing of at least one microRNA, miR-7, which resides in the last intron of the *HNRNPK* gene (Levedeva et al., 2011). With emerging functional interactions between HuR and microRNAs/RISC, both competitive and cooperative (Kim et al., 2009; Bhattacharyya et al., 2006), it is significant that both groups identified specific, transcriptome-wide microRNA sites in the vicinity of HuR sites. Although this finding suggests that some microRNA functions may depend on proximal HuR binding, more comprehensive identification of HuR's noncoding RNA targets and the functional interconnections between noncoding RNA and HuR are warranted.

The methodologies used in these reports deserve special mention. Thus far, PAR-CLIP has been the most enlightened approach to studying HuR-RNA associations, showing that HuR interacts with heterogeneous, extensively U-rich RNAs, and that it can bind most target transcripts at numerous sites. Moreover, the side-by-side comparison of PAR-CLIP targets and RIP-chip targets has helped to reconcile a controversy in the RNP field, fueled by concerns that RNPs may reassociate after lysis and thus crosslinking might be essential to detect authentic target mRNAs. Although PAR-CLIP identified several-fold more HuR target transcripts than RIP-chip, there was extensive overlap of targets: approximately 70% of RNAs identified by RIP-chip had PAR-CLIP hits (usually many hits) and were more extensively downregulated by HuR silencing. Some non-overlapping transcripts were identified because RIP-chip enriched in certain low-abundance mRNAs. Accordingly, one might picture the dynamic remodeling of HuR RNPs as HuR 'shopping' for RNA, with both transient and stable interactions detected by PAR-CLIP and stably interacting RNAs detected by RIP-chip. While all crosslinking methods have sequence biases, PAR-CLIP is superior in that it identifies the sites of RNA interaction with a lesser degree of preference; RIP-chip and RIP-seq analyses have no known biases, but only identify whole transcripts,

leaving binding sites to motif prediction algorithms. Whether to study RNPs with one approach or the other will depend on the biological question at hand, however, the combination seems powerful.

In sum, most previous work on HuR has firmly established its cytoplasmic function in stabilizing or modulating the translation of many mRNAs (Abdelmohsen and Gorospe, 2010; Keene, 1999). These cytoplasmic activities eclipsed HuR's nuclear function for almost two decades, despite HuR's prominent nuclear presence. The PAR-CLIP analyses by Mukherjee et al. (2011) and Lebedeva et al. (2011) have unveiled a previously unrecognized role for HuR in pre-mRNA processing. Nuclear HuR functions will now undoubtedly share the spotlight with cytoplasmic HuR. We expect that HuR's important physiologic and pathologic roles will be best understood in a richer context, wherein the cytoplasmic fates of HuR target mRNAs are influenced by their earlier nuclear history.

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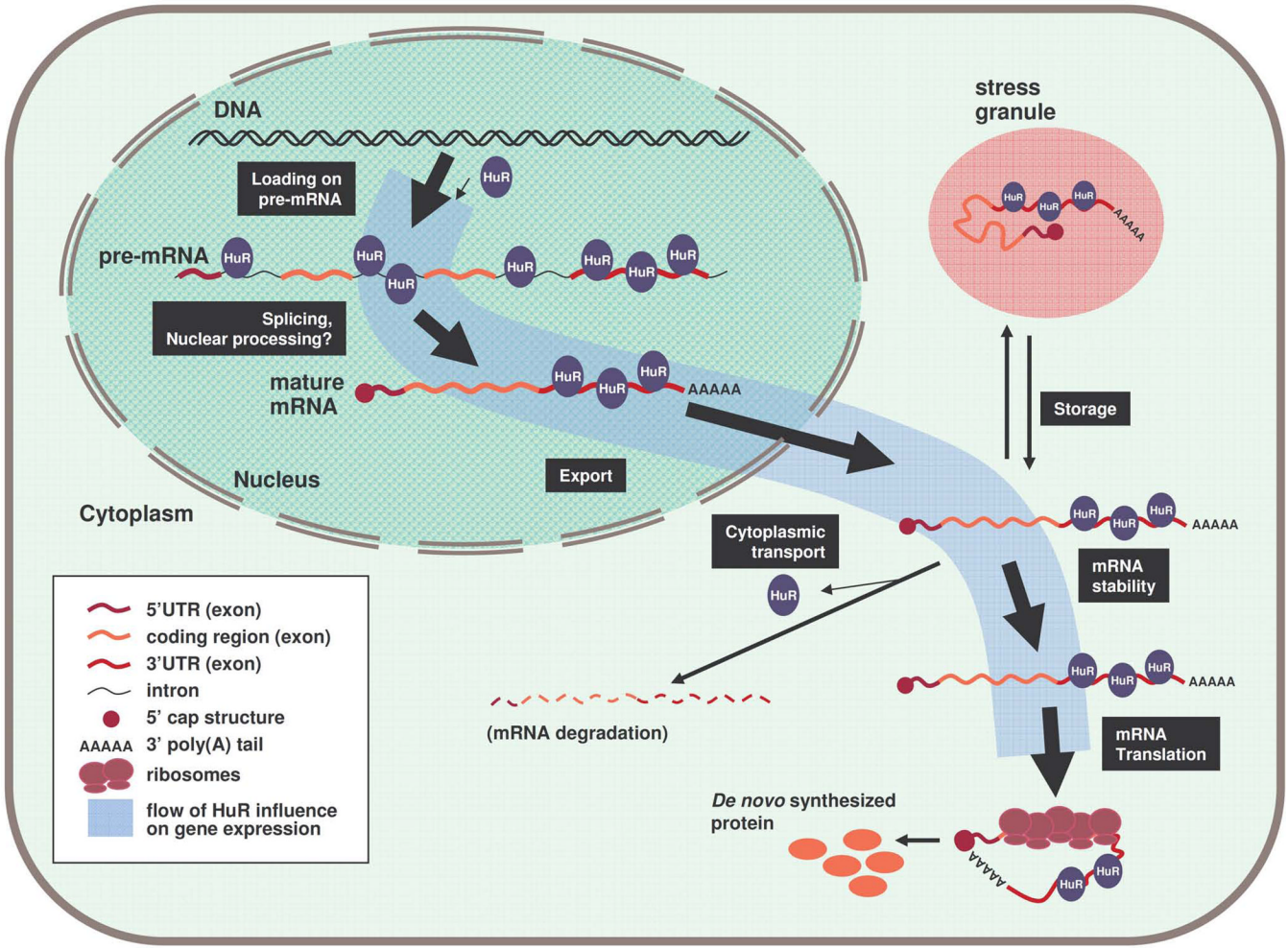

Figure 1. Flow of HuR influence on target gene expression

As uncovered by the studies of Mukherjee et al. (2011) and Lebedeva et al. (2011) in this issue of *Molecular Cell*, the influence of HuR upon target transcripts begins in the nucleus, where HuR associates with the pre-mRNA introns (thin black lines) and affects splicing and likely other nuclear processing events (although these functions are unconfirmed, as shown by the '?'). After assisting with export of the mature mRNA out of the nucleus, HuR's impact continues in the cytoplasm, where it stabilizes mRNAs, may help with transient mRNA storage (as in stress granules), and modulates the recruitment of the mRNA with the translation machinery (polysomes).