Ribonucleoprotein dynamics connects mRNA networks with drug mechanisms

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Beyond transcription, gene expression is potently controlled through processes that affect pre-mRNA splicing and maturation, as well as mRNA transport, turnover, storage, and translation. These events are tightly regulated by two main classes of trans-binding factors, RNA-binding proteins (RBPs) and non-coding RNAs (ncRNAs) (Moore, 2005; Mattick and Makunin, 2006). RBPs bind to target transcripts and influence virtually all facets of post-transcriptional gene expression; ncRNAs, particularly microRNAs (miRNAs), mainly lower the translation and stability of target mRNAs (Keene, 2007; Bartel, 2009). As mRNAs transit through the cell, their association with RBPs and miRNAs determines their recruitment to cellular sites such as processing bodies (PBs), stress granules (SGs), polysomes, the exosome, and the RNA-induced silencing complex (RISC), each specialized in distinct aspects of mRNA metabolism (Filipowicz et al, 2008). In this manner, ribonucleoprotein (RNP) interactions directly affect the types, location, and abundance of expressed proteins.

In a report published in Molecular Systems Biology, Mukherjee et al (2009) use quantitative RNA dynamics to present a fundamentally new approach to study global RNP networks. The study bridges two key and reciprocal concepts in post-transcriptional gene regulation. The first is that each individual mRNA is capable of associating with numerous RBPs. Several studies have shown that RBPs can interact combinatorially on a single mRNA and thereby alter its post-transcriptional fate and ultimately the levels of expressed protein; for example, an RBP can compete or cooperate with another RBP for binding to a shared target mRNA, while miRNAs can promote or prevent the action of RBPs on a given mRNA (e.g., Lal et al, 2004; George and Tenenbaum, 2006; Filipowicz et al, 2008). The second, that each RBP can interact with multiple mRNAs and jointly coordinate their post-transcriptional outcome; as described in the post-transcriptional regulatory operon (PTRO) model proposed by Keene and Tenenbaum (2002). The PTRO model further postulates that such mRNA subsets encode proteins that are functionally related, such that an RBP can synchronize the production of multiple proteins in specific metabolic or response pathways.

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In a report published in Molecular Systems Biology, Mukherjee et al (2009) use quantitative RNA dynamics to present a fundamentally new approach to study global RNP networks. The study bridges two key and reciprocal concepts in post-transcriptional gene regulation. The first is that each individual mRNA is capable of associating with numerous RBPs. Several studies have shown that RBPs can interact combinatorially on a single mRNA and thereby alter its post-transcriptional fate and ultimately the levels of expressed protein; for example, an RBP can compete or cooperate with another RBP for binding to a shared target mRNA, while miRNAs can promote or prevent the action of RBPs on a given mRNA (e.g., Lal et al, 2004; George and Tenenbaum, 2006; Filipowicz et al, 2008). The second, that each RBP can interact with multiple mRNAs and jointly coordinate their post-transcriptional outcome; as described in the post-transcriptional regulatory operon (PTRO) model proposed by Keene and Tenenbaum (2002). The PTRO model further postulates that such mRNA subsets encode proteins that are functionally related, such that an RBP can synchronize the production of multiple proteins in specific metabolic or response pathways.

The past decade has witnessed immense progress in the analysis of RNP interactions. The vast majority of studies have focused on ‘single RBP-single mRNA’ associations, some of them further tracking changes in RNP composition in response to particular stimuli. A minority of studies have assessed more complex RNPs, comprising ‘single RBP-multiple mRNAs’; by RNP immunoprecipitation followed by microarray analysis of enriched mRNAs (the RIP chip method), RBPs were found to associate with hundreds of mRNAs, many of them also targets of other RBPs. However, the value of RIP chip analyses was hampered by its ‘static’ nature: it provided a useful ‘snapshot’ of RNP interactions at a specific point in time, but no information regarding previous or subsequent associations. Accordingly, it has not yet been possible to integrate RNP complexes within networks of interaction with other trans-binding factors or biological processes, nor has it been possible to predict if they might be effectors of specific compounds.

The new report by Mukherjee et al overcomes some of these barriers. By studying changes in RNP associations over time, they have gained unique new insight into the biology of RNP interactions. Their study focuses on the RNA-binding protein HuR within the context of Tcell activation, fine choices for this proof-of-principle study, as many HuR target mRNAs are known and several HuR-regulated processes (such as T-cell activation) have been described. Statistical modeling of HuR RIP chip experiments at different times after activating Jurkat cells with phytohemagglutinin and phorbol ester led to several important discoveries. Among the first observations, the mRNA subsets bound to HuR followed different interaction patterns, with mRNA subsets associating progressively more after activation, others progressively less, yet others transiently increasing or decreasing their association. These results are significant because they provide the first systematic demonstration that the dissociation of an RBP from an mRNA is at least as prominent an event as increased association. They
also indicate that the binding patterns are strongly affected by features intrinsic to the mRNA itself, not only by the binding properties of the RBP. This finding further suggests that specific signature RNA motifs may exist among target mRNAs which become dissociated from an RBP under specific conditions, distinct from signature motifs in target mRNAs which become associated with the same RBP.

Additional analysis revealed that many HuR-associated mRNAs did not show increased abundance in activated Jurkat cells. This discordance is important, as it further supports the notion that HuR is not only an mRNA-stabilizing RBP—HuR’s first-identified and best-known function (Brennan and Steitz, 2001)—but also can have other post-transcriptional influences, including translational regulation, as reported for several target mRNAs (Hinman and Lou, 2008), and possibly also subcellular transport or storage. There was also an important concordance between the pathways that HuR was reported to modulate in the Mukherjee report (e.g., cell proliferation, Wnt signaling, T-cell activation) and the pathways that HuR target mRNAs were predicted to affect. By comparing HuR target mRNAs and the targets of other RBPs, the authors show that many mRNAs are amply shared with some RBPs (e.g., TTP) but not with others (e.g. TIAR). Similarly, numerous miRNAs were also predicted to interact with HuR target mRNAs. Adding complexity to this rich network of interactions, HuR target transcripts included several mRNAs that encoded RBPs, in keeping with earlier evidence that a handful of RBPs bound combinatorially to one another’s mRNAs (Pullmann et al., 2007).

One of the most promising aspects of this approach is that the dynamic HuR-mRNA associations can be scanned against profiles of the Connectivity Map (CMAP), a large collection of mRNA expression profiles collected from cell lines perturbed by hundreds of bioactive small molecules (Lamb et al., 2006). By correlating HuR query signatures with specific CMAP profiles (Figure 1), Mukherjee et al. (2009) identify several drugs that are predicted to modulate HuR function, including previously known compounds, such as inhibitors of histone deacetylases. The discovery and validation of resveratrol as a novel inhibitor of HuR function shows that HuR is an effector of resveratrol action and demonstrates the power of this methodology. Intriguingly, resveratrol—an anti-inflammatory COX-2 inhibitor—has been suggested to exert an anti-aging effect, possibly by activating sirtuin-1 (Lagouge et al., 2006), which is a known HuR target mRNA (Abdelmohsen et al., 2007).

The field of post-transcriptional regulation of gene expression is ripe to study similar RNP dynamics for hundreds of other RBPs. It can thus be anticipated that the approach described by Mukherjee et al. (2009) will be extended to achieve the pressing goal of identifying chemical modulators of other RBPs and potentially ncRNAs. Such analyses are bound to uncover other exciting mRNA networks and implicate other regulatory factors, metabolic pathways, and biological processes. To help organize the information obtained from these studies, it is also vital to develop methodologies to identify systematically the trans-binding factors—RBPs and ncRNAs—that interact with a specific mRNA; to maximize the information obtained, such tagged mRNAs should also be tracked in a dynamic fashion. Other critical knowledge will come from live microscopic imaging of RNP complexes, so that their composition can be studied together with their subcellular localization and the conditions of individual cells. Though still rudimentary, the technologies and computational resources to study these processes are improving rapidly. The information gained from studying RNP interaction networks will be highly complex, yet organized in PTROs—befitting of the versatile post-transcriptional processes that ensure protein production in the proper concentration, subcellular locale, developmental stage, and triggering stimuli, to ensure that homeostasis is maintained in complex cell-environment interactions.

**Conflict of interest**

The authors declare that they have no conflict of interest.

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