In this review we describe and highlight the complex processes involved in the post-transcriptional regulation of mRNAs in shaping the functional outcomes of the cardiac transcriptome. We feature recent advancements in the field and discuss a paradigm-shifting role for mRNA metabolism in cardiac function that has led to a greater understanding of cardiac biology. We describe the key players in and the functional impact of post-transcriptional regulation. This includes a description of key RNA binding proteins involved in the modification, splicing, and turnover of mRNA within the context of cardiac development and disease. Finally, we discuss the current challenges and future directions in this exciting area of research, which has far-reaching implications for new insights in cardiac biology and for the development of novel therapies to treat heart disease.
mRNA METABOLISM IN CARDIAC DEVELOPMENT AND DISEASE: LIFE AFTER TRANSCRIPTION

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Gao C, Wang Y. mRNA Metabolism in Cardiac Development and Disease: Life After Transcription. *Physiol Rev* 100: 673–694, 2020. First published November 21, 2019; doi:10.1152/physrev.00007.2019.—The central dogma of molecular biology illustrates the importance of mRNAs as critical mediators between genetic information encoded at the DNA level and proteomes/metabolomes that determine the diverse functional outcome at the cellular and organ levels. Although the total number of protein-producing (coding) genes in the mammalian genome is ~20,000, it is evident that the intricate processes of cardiac development and the highly regulated physiological regulation in the normal heart, as well as the complex manifestation of pathological remodeling in a diseased heart, would require a much higher degree of complexity at the transcriptome level and beyond. Indeed, in addition to an extensive regulatory scheme implemented at the level of transcription, the complexity of transcript processing following transcription is dramatically increased. RNA processing includes post-transcriptional modification, alternative splicing, editing and transportation, ribosomal loading, and degradation. While transcriptional control of cardiac genes has been a major focus of investigation in recent decades, a great deal of progress has recently been made in our understanding of how post-transcriptional regulation of mRNA contributes to transcriptome complexity. In this review, we highlight some of the key molecular processes and major players in RNA maturation and post-transcriptional regulation. In addition, we provide an update to the recent progress made in the discovery of RNA processing regulators implicated in cardiac development and disease. While post-transcriptional modulation is a complex and challenging problem to study, recent technological advancements are paving the way for a new era of exciting discoveries and potential clinical translation in the context of cardiac biology and heart disease. 

I. INTRODUCTION

In sync with transcriptional synthesis by the RNA polymerase II complex, the nascent mRNA transcripts are immediately processed by several highly integrated RNA modification steps, including the classic 5'-capping, intron splicing, and 3'-polyadenylation to yield matured mRNA transcripts. The processed mRNAs are subsequently transported out of the nucleus into the cytoplasm where they will be either loaded onto ribosomes for polypeptide synthesis or shuttled to other discrete compartments, such as the stress granules or P-bodies, for sequestration and/or degradation (101, 153, 162). Although much of the core machinery involved in the modification, splicing, and degradation of RNA are highly conserved, each step of these post-transcriptional processes can be modulated by developmental cues, cellular signaling, or environmental conditions in a cell type- and tissue-specific manner, and this is accomplished by a large number of RNA binding proteins (22, 33,
Furthermore, post-transcriptional modifications and editing are emerging as additional reprogramming signals that can potentially affect the functionality of RNA molecules (15, 53, 63, 139, 210, 247). Consequently, a majority of protein coding genes in humans can produce multiple mRNA species encoding different protein products with potentially different regulatory properties that alter their intracellular localization, translational capacities, and turnover rates (FIGURE 1). Therefore, the finite number of protein coding genes in mammalian genome can contribute, through alternative splicing and distinct modifications, to many orders of magnitude greater complexity to a cell’s transcriptome that leads to diverse and specific external conditions. Thus it is not difficult to appreciate that the highly regulated processes of mRNA maturation and disposal (i.e., mRNA metabolism) should have critical importance in a wide array of physiological and disease settings.

Post-transcriptional modulation is an integral part of the transcriptome programming during cardiogenic processes in developing embryos. The emerging genetic and molecular evidence implicates post-transcriptional regulation of mRNA as an important underlying mechanism to physiological adaptation or pathological maladaptation in the heart (17, 72, 86, 110, 159). Matching the complexity of the post-transcriptional RNA modulatory processes, the complexity of the world of RNA-binding proteins (numbering ~1,500) encoded by the human genome is manifest by the large number of this class of protein (42, 136). They work in a highly coordinated and regulated manner to accomplish each of the metabolic activities during the life cycle of RNA transcripts. Despite such multilayered complexities, post-transcriptional regulation of mRNA is still very much understudied compared with the intense research efforts afforded to gene expression in the heart. The lack of progress is also due to the technical challenges uniquely associated with the experimental manipulation of RNA. However, recent advancement in RNA-focused molecular approaches has now provided new opportunities to extend our knowledge regarding the scope of RNA metabolic dynamics in cardiac development and disease. In this review and based on recent discoveries in model organisms, we provide an overview of the major players in the key processes of RNA metabolism, and will discuss their role in cotranscriptional processing and maturation to targeted translation and degradation. Then, we highlight some of the recent findings implicating specific RNA post-transcriptional regulators in cardiac development and diseases (FIGURE 1). Finally, we discuss the key gaps in our knowledge, and the main challenges in RNA studies, as well as potential opportunities for future progress offered by recent advancements in RNA-focused technologies. This review will remain confined to protein-coding RNAs since the topics of the noncoding RNAs, particularly the microRNAs and the long-non-coding RNAs, have been well covered in recent excellent reviews by Beermann et al. (16) and others (128, 191, 209).

### A. mRNA Maturation Process

Like all eukaryotes, mammalian mRNAs are generally transcribed by the RNA polymerase II complex (PolII). The nascent mRNA molecules are capped at the 5’ end with a m7G structure to prevent degradation and facilitate nuclear export and ribosomal loading. The emerging intronic sequences in the pre-mRNAs are efficiently removed by the RNA polymerase II complex (PolII). The nascent mRNA molecules are capped at the 5’ end with a m7G structure to prevent degradation and facilitate nuclear export and ribosomal loading. The emerging intronic sequences in the pre-mRNAs are efficiently removed by the
RNA spliceosome while the PolII continues RNA synthesis along the DNA template (184). At the end of transcription, RNA cleavage occurs, and the polyadenylated tail is then added to the 3’ end after its release from the PolII complex (FIGURE 2).

It remains enigmatic how sequential RNA splicing events are accurately and efficiently performed along the multi-exon RNA molecules, particularly when the exon/intron junctions appear to be interchangeable. One solution to resolve this issue is to couple RNA splicing with RNA polymerase-mediated RNA elongation in a highly synchronized manner (FIGURES 2 AND 3) (12, 17, 27, 85, 86, 120, 178, 184). As soon as the nascent RNA molecule is synthesized and emerges from the PolII complex, the spliceosome begins to assemble on the mRNA template (85), starting with the recruitment of U1/U2 small-nuclear ribonucleoproteins (snRNPs) to demarcate the 5’ and the 3’ splicing site (SS) at the exon/intron junctions. The subsequent recruitment and maturation of the splicing machinery (by stepwise recruitment of U2, U5, and U6 snRNPs and other factors) lead to a two-step reaction that yields two spliced exons and a lariat intron RNA (FIGURE 4).

Following proper cleavage and polyadenylation at the 3’ end, the fully processed mRNAs are recognized by a highly conserved multicomponent transcription/export complex (TREX) (50, 84), which interacts with the hyperphosphorylated PolII COOH-terminal domain (CTD) (77), the exon-junction complex (EJC) (69), the 5’-cap binding complex (176, 201, 238), and the polyA binding protein (PABP) (194). Through additional interaction with nuclear RNA export factor 1 (NXF1), the bulk of mRNAs are transported out of the nucleus into the cytosol through the nuclear pore complex (NPC) (95, 174). As illustrated in FIGURES 2 AND 3, the entire maturation process of mRNA is highly integrated and synchronized to ensure efficient and accurate excision of intronic sequences for the ultimate and timely transport of mature mRNAs out of the nucleus. Mis-capped or misspliced products are quickly and efficiently degraded by extensive mRNA quality control mechanisms (56, 106, 169, 213, 237, 238). As a consequence, under normal conditions the intron-containing pre-mRNAs are often detected at very low levels relative to the mature mRNAs.

Compared with mRNAs and perhaps a majority of the long-noncoding RNAs, the maturation processes for several other species of noncoding RNAs and histone mRNAs are different and highly specialized. For example, the small nucleolar RNAs (snoRNAs) guided modifications of spliceosomal small nuclear RNA (snRNAs) involve pseudouridylation and 2’-O-methylation, which are restricted to the Cajal body (8, 143). In contrast, histone mRNAs do not require splicing, but they need specialized 3’ modification involving stem-loop-dependent trimming and uridylation instead of polyadenylation. These specialized post-transcriptional modifications of histone mRNAs are carried out in histone locus bodies (HLB) (26, 143) where transcription from the histone locus and post-transcriptional processing are integrated in the same physical location. Finally, ribosomal RNA (rRNA) synthesis, processing (including
snoRNA guided pseudouridylation and 2′-O-methylation), and ribosome assembly are carried out in the nucleolus (96, 202). In contrast, the maturation and export of primary microRNA (miRNA) requires a dedicated trimming machinery involving Drosha, with exportin 5 facilitating miRNA export (230) (FIGURE 3).

Among the mRNA maturation processes, alternative splicing, alternative initiation, and alternative polyadenylation contribute the most to transcriptomic variation (9, 12, 17, 156). In addition to canonic mRNA spliceosome core subunits (U1-U6 RNPs) (FIGURE 4), a large number of RNA binding proteins serve as RNA splicing regulators. They serve as trans-acting regulators interacting with cis-regulatory elements (RNA sequence motifs) to manipulate spliceosome activities on the targeted intron/exon junction (FIGURE 5). These alternative RNA splicing events can significantly enrich the overall diversity of the mRNA species generated from a single gene through a combination of intron retention, exon skipping/insertion, alternative 5′ or

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**FIGURE 3.** Localized RNA biogenesis and post-transcriptional processing. Specialized RNA processing centers are illustrated, including the nucleolus, rRNA synthesis, snoRNA modification, and ribosome assembly. HLB, histone locus body; NPC, nuclear pore complex.

**FIGURE 4.** Overview of RNA splicing process. A prototypic splicing event from pre-mRNA to spliced exons and lariat intron RNAs. BPS, branch point site.
3’ splicing site utilization, and mutually exclusive exon utilization (FIGURE 5). Among the more than 1,500 known RNA binding proteins (RBPs) in the human genome, only a very small number of them are identified as RNA splicing regulators and remain functionally uncharacterized. According to SpliceAid F, a database cataloguing human splicing factors and their binding sites (74), a total of 71 human trans-acting splicing regulatory proteins are known, including 13 serine/arginine-rich proteins, 27 heterogeneous nuclear ribonucleoprotein (hnRNP) proteins, and 31 additional proteins from other protein families (ELAV, KHDRBS, CUGBP, Nova, and RBFox). Considering that the majority of human mRNAs have multiple RNA species due to alternative splicing, it would be very surprising if the number of RNA splicing regulators remains at the current number. As the main mediators of RNA splicing regulation, these RBPs are subject to additional layers of regulation, which often gives rise to cell type specificity. These additional layers of RBP regulation include differential expression, post-translational modification, localization (nuclear retention vs. exclusion), and alternative splicing of RBPs (120, 177, 204). Similar to transcription factors, RBPs often have multifunctional roles in gene expression, as in the case of RBFox2, which serves as regulator of both mRNA splicing and splicing site utilization, and mutually exclusive exon utilization (FIGURE 5).

Since mRNA maturation has such a major impact on the functional output of our genome, it is not surprising that genetic defects or population variants resulting in aberrant RNA splicing can lead to many diseases including cancer and neural diseases (22, 27, 66). We present greater detail of mRNA splicing as a focal point of cardiac development and pathological remodeling later in this review.

B. mRNA Localization, Ribosomal Loading, and Translation

When the processed mRNAs are transported outside of the nucleus, they are generally destined for one of two fates (FIGURE 6). One is cap-dependent translation after the mRNA is loaded onto ribosomes, and the second is degradation. With respect to translation, certain mRNA transcripts are targeted to specific cellular compartments to generate cellular polarity and/or molecular gradients (6, 148, 203). This is particularly prevalent to maternal mRNA species in the developing embryos (89, 196). In addition, mRNAs encoding endoplasmic reticulum (ER) targeted proteins (for transmembrane or secreted proteins) will be anchored on ER membrane through specific receptor complexes interacting with the membrane targeting recognition signal in the nascent peptides (44, 183, 188). Similarly, targeted translation has been observed for mitochondrial-targeted proteins (75, 122). Under stress conditions, such as starvation, mRNAs with stalled translation are sequestered in a structure called stress granules where translation is suppressed, and where the mRNAs remain intact while being decorated with 40S ribosome subunit and other RNA
processing proteins as protection against conditions that may adversely affect the integrity of the RNA molecules (5, 51, 81, 82, 193) (FIGURE 3). With respect to RNA degradation, the misprocessed or mistranscribed mRNAs are targeted delivered to P-bodies where the nonsense-mediated degradation (NMD) machinery triggers decapping and deadenylation before undergoing exosome-mediated degradation (166) (FIGURE 6). Beyond the degradation of mutant RNA molecules as a quality control mechanism, NMD is also involved in the targeted degradation of specific mRNA transcripts in a highly regulated and precise manner (56, 107, 135).

The selectivity and efficiency of ribosomal loading and translational initiation are contributed by the interactions between eIF-4E (for m7GpppG cap recognition) and translation initiation complexes (eIF-4F) (7, 65). This process is modulated by sequence and structural features in the 5′- (65) or the 3′-untranslated regions (UTR) of mRNAs. Different from the housekeeping genes, which in theory have steady translational activities, a subset of stress-inducible genes have their mRNAs blocked from translation by interacting with translation-suppressing RBPs, such as HuR (31, 187, 218). Upon stimulation, the RBP interactions suppressing translation are removed and the abundance of these mRNAs can be increased as a consequence of higher transcriptional activity, or the abundance of the encoded protein can be increased as a consequence of increased ribosomal loading. The examples include p38 mitogen-activated protein kinase (MAPK)-mediated induction of inflammatory genes such as IL-1β and TNF-α (1, 138, 168). Another group of specialized mRNAs are ribosomal protein encoding mRNAs, which are highly abundant transcripts, but the translation of these transcripts is generally basally suppressed by LARP1 (60). These mRNAs have a shared 5′-terminal oligopyrimidine motif in their 5′-UTR, also referred as TOP mRNAs. Upon nutrient and growth signaling stimulation, mTORC1-mediated signaling leads to translational derepression by LARP1 on the TOP mRNAs (172). mTORC1-mediated phosphorylation of eIF4E-binding
proteins (4E-BPs) and the ribosomal S6 kinases (S6Ks) leads to a robust induction of translational initiation efficiency (60). Thus stabilizing mRNAs through a 3′-UTR-mediated RNA-protein interaction or coordinated ribosomal loading can dictate the functional activities of specific mRNAs under different conditions.

C. mRNA Degradation and Stability

Misspliced mRNAs or mRNA transcripts carrying premature termination codon (PTC) mutations are efficiently removed by NMD (107) (FIGURES 3 AND 6). This is an important step for translation-dependent mRNA quality control since aberrant production of truncated proteins can lead to disastrous outcomes. There are two highly conserved schemes that contribute to the efficiency and target specificity of NMD (93, 102, 106), both involving a RNA-dependent helicase and the ATPase UPF1. One is EJC-dependent, involving the recognition by UPF1 of the abnormal (>50 nucleotides) distances between EJC and translation termination complex (TTC). Another is EJC-independent, where UPF1 recognizes the extra-long distances (>1,000 nucleotides) between TTC and the polyA structure (FIGURE 6). These common PTC features allow UPF1 to efficiently direct them to RNA degradation machinery within the P-bodies (5, 134, 239).

Other than clearance of “rogue” mRNA species due to missplicing or premature nonsense mutations, normal turnover of mRNAs occurs in and outside the P-bodies. Different mRNAs exhibit very broad spectrum of turnover rates due to the differential regulation of their stabilities through a myriad of RBP interactions (51, 147, 166). However, RNA degradation often shares similar molecular machinery in 5′-decapping by the Dcp1/Dcp2, and 3′-deadenylation by the CCR4/POP2/NOT complex. The resulting unprotected mRNAs will be degraded quickly by exonucleases such as Xrn1 (134). NMD is also employed for targeted mRNA degradation via specific interactions of RBPs (121).

It is important to recognize that the translational activity of an mRNA molecule must be highly orchestrated with its stability. When premature termination is detected by NMD, ribosomes will be released from the affected mRNAs followed by 5′-decapping and 3′-deadenylation, and quick degradation by the RNA exosomes (157, 187). However, aberrant expression of mutant proteins due to NMD escape is associated with diseased conditions (56, 106).

In summary, mRNAs undergo an elaborate maturation process following PolII-mediated synthesis by 5′ and 3′ modification and alternative splicing. The mature mRNAs that are destined for translation or degradation are transported to specific compartments in the cytosol by dedicated molecular pathways. RNA processing is highly integrated and coupled with RBPs, which serve as the molecular links and entry points for external signaling modulations (FIGURES 3 AND 6).

II. mRNA POST-TRANSCRIPTIONAL MODIFICATIONS

Other than the above discussed 5′ capping and 3′ polyadenylation, recent studies have revealed a pervasive level of post-transcriptional modification in the mRNA transcriptome. In fact, more than 100 chemical moieties have been identified on the RNA molecules, and this undoubtedly contributes to an explosive increase in transcriptome complexity (43, 63, 161, 170, 182, 200). This generally describes the area of research that aims to elucidate the role that transcriptional modifications play in transcript fate, and is now referred to as the field of epi-transcriptomics. As a field of research, epi-transcriptomics is in its infancy, with only a few types of RNA modifications having been studied for their functional significance.

A. Post-transcriptional Modification in mRNA Metabolism

For mRNAs, two types of post-transcriptional modifications are relatively well studied. One is adenosine methylation at the nitrogen-6 position (m6A) and another is uridylation at the 3′-end. Although these modifications have long been observed, the biological function and molecular players have just begun to emerge from recent studies. With the use of mi-CLIP seq, a high-throughput method for identifying m6A modifications in eukaryotic RNA, the full scale of m6A modification was profiled for the human transcriptome, and distinct modification patterns emerged (126). For mRNA m6A modification, the homeostatic level is accomplished by the balance between the epigenetic writers (m6A methyltransferases), including METTL3 and METTL14 and WTP, and the epigenetic erasers (N6A demethylase), including FTO and ALKBH5. The modified mRNAs are recognized by the epigenetic readers, consisting of YTHDF1–3 family members, which bind to the m6A modified mRNAs (FIGURE 7). The m6A modification sites are highly enriched at the 5′-translation start site (TSS) and the 3′-UTR. In general, m6A modification promotes mRNA translation and stability. Modification of these sites can influence many steps of mRNA metabolism, including CAP-independent translation, nuclear retention, alternative splicing, P-body targeting, translational modulation, and mRNA degradation (FIGURE 7) (2, 10, 25, 29, 43, 57, 63, 158, 161, 170, 182, 200). Despite a broad impact on mRNA metabolism, many of the molecular details involving m6A-dependent regulation of RNA remain unknown (29). On the other hand, uridylation at the 3′-tail of mRNA is another form of post-transcriptional modification that is carried out by a family of terminal uridylyl transferases (TUTases) (36, 119, 247). The consensus is that adding the
uridylation to polyadenylated mRNAs promotes exonuclease digestion of stalled RNA molecules; thus this type of modification effectively leads to “gene silencing” by targeted degradation of the mRNA (36, 49, 143, 151, 247).

B. Functional Impact of mRNA Modification in Cardiac Hypertrophy and Dysfunction

There is increasing evidence that m6A mRNA modification is an important epitranscriptomic feature for normal gene regulation during development and disease, including cancer and neurological disorders (46, 63, 200). Early studies of m6A writers showed Mettl3 knockout in mice caused complete abrogation and m6A modification of RNA in developing embryos, and led to early lethality due to adverse changes in mRNA stability for key molecules involved in the regulation of pluripotency.

In a pair of recent studies, the functional impact of m6A modification is investigated in the context of heart failure. Both studies found that diseased hearts, resulting from different pathological etiologies, including pressure-overload and ischemic injury, are characterized by changes in m6A modification, and these changes can be classified as molecular hallmarks of heart disease (54, 140). In the study reported by Dorn et al. (54), genetic inactivation of the main m6A writer Mettl3 blocks the development of pathological hypertrophy while activation of Mettl3 expression is sufficient to induce hypertrophic remodeling in mouse hearts. On the other hand, Mathiyalagan et al. (140) observed a loss of the m6A eraser FTO is associated with the pathogenesis of heart failure, and genetic induction or silencing of FTO expression leads to either cardioprotection or deterioration of cardiac function, respectively. These two studies provide complementary evidence that m6A modification is dynamically regulated in diseased heart, and elevated m6A level is a pathological contributor to the onset of heart failure. At a mechanistic level, the authors of these two reports find that m6A modification affects the expression levels of the cardiac mRNAs encoding hypertrophy-related signaling molecules and contractile proteins, as a result of modulating the stabilities and translation capacities of these mRNAs. Thus targeted manipulations of m6A modification via its writer or eraser may have a major impact on the pathological outcome in the stressed hearts (FIGURE 7).

Despite these exciting new findings, the molecular basis of m6A-mediated modulation of cardiac mRNA turnover or translation is largely unknown. Nevertheless, other forms of mRNA modifications and their potential contribution to the complexity and function of cardiac transcriptome still need to be explored. Epirtranscriptomics is proving to be a new and exciting area of research in cardiac biology and medicine and is opening new possibilities for understanding the molecular factors underpinning heart function and disease.

III. RNA SPLICING REGULATION

Compared with epitranscriptomics, the field of RNA splicing is well established. Constitutive splicing is performed by the core spliceosome, which includes the ubiquitous ribonucleoprotein complex (FIGURE 3). In contrast, alternative splicing is regulated by specific cis-regulatory elements located in the pre-mRNA and the trans-acting factors with dynamic and tissue-specific expression patterns. These alternative splicing events can generate a variety of splicing isoforms from a single gene. (FIGURE 5). To date, several cardiac specific splicing regulator families have been identified and characterized (FIGURE 8). However, the scope of targeted mRNA splicing by each splicing regulator in the heart is not fully established. Although the proteins of each family recognize unique cis-regulatory elements, each mRNA may be regulated by multiple splicing factors. While splicing is well described, the regulation of alternative splicing remains only superficially characterized, and what we do know is primarily based on the dynamic expression pat-
tern of the specific splicing factors. Moreover, the molecular details of the regulation of alternative splicing in heart remains a very active area of research.

A. RBFox Family

The RBFox family of proteins (RBFox1, RBFox2, and RBFox3) constitutes a collection of highly conserved, sequence-specific RNA binding proteins. They share a single high-affinity RNA recognition motif (RRM) domain that binds to the cis-regulatory element containing a consensus sequence motif of (U)GCAUG located in the pre-mRNA intronic, exonal, and 3′-UTR regions. Among the three family members, both RBFox1 and RBFox2 showed high expression in the heart but with distinct dynamic patterns (47, 155). The expression of RBFox1 is low in the embryonic heart but upregulated in the matured postnatal heart, while the RBFox2 expression is high in the embryonic heart but downregulated postnatally (67). RBFox1 is highly conserved and has been shown to play critical roles in multiple species. For example, mutation of RBFox1 in Caenorhabditis elegans affects RNA splicing patterns of the nematode’s body wall muscle (108), inactivation of RBFox1 in zebrafish causes developmental defects in the heart (61, 64), and RBFox1 cardiac specific conditional knockout mice show exacerbated heart failure upon stress (67). In addition to RBFox1, RBFox2 also plays important roles during cardiac pathogenesis. Both RBFox1 and RBFox2 showed a decreased expression in response to pressure overload in mouse heart (67, 225), while RBFox2 is elevated in hearts affected with diabetic cardiomyopathy (160). Ablation of RBFox2 in a cardiac specific manner led to a lethal cardiac phenotype in mice that is associated with a global shift in alternative splicing pattern, and is similar to the changes observed in the pressure-overloaded failing hearts (225). Interestingly, besides splicing regulation, RBFox2 also has a direct role in epigenetic regulation through the direct interaction with the polycomb repressing complex 2 (PRC2) (226). Indeed, Hu et al. (90) reported recently that RBFox2 regulates cardiac function through transcriptional suppression of miR-34a expression and its target Junctophilin 2. Therefore, many of the RNA splicing regulators may “moonlight” in other steps of gene regulation, providing more regulatory links with other transcriptional or post-transcriptional processes.

Although both RBFox1 and RBFox2 have been demonstrated to be critical in cardiac pathological remodeling in vivo, RBFox1 and RBFox2 appear to target a different set of alternative splicing events (67, 217, 225). This is likely the result of differential interacting partners for RBFox1 and RBFox2. However, the detailed molecular basis of their target specificity and the interacting partners responsible for the functional outcome in heart remain to be determined. More importantly, RBFox1 and RBFox2 undergo extensive alternative splicing themselves, yielding different isoforms which may affect their molecular function and target specificities. Therefore, loss of RBFox1 and RBFox2 expression and misregulation of these proteins may contribute significantly to the pathogenesis of heart failure.

B. RBM Family

Another class of well-characterized splicing regulators in the heart is the RNA Binding Motif (RBM) family of proteins. In particular, RBM20 features two RNA binding zinc finger (ZnF) domains: one is the RNA-recognition motif (RRM) and another is the arginine/serine-rich domain (224). Mutations in the human Rbm20 gene are linked to dilated cardiomyopathy (224, 228). RBM20 has been suggested to be the main splicing regulator for TTN mRNA, which contains 363 exons and encodes the TITIN protein. In the normal adult heart, TTN mRNA undergoes extensive exon skipping within the regions corresponding to the middle immunoglobulin (Ig) and the proline-glutamate-valine-lysine-abundant (PEVK) domains, but inactivation of RBM20 abolishes these alternative splicing events (125).
RBM20 is enriched in nuclear speckles where TTN pre-mRNAs are processed. Although mutations in the Rbm20 gene leads to dilated cardiomyopathy, reducing the RBM20 activity improves cardiac diastolic dysfunction and cardiac atrophy, possibly due to the increasing lengths of the TITIN isoforms as well as other downstream splicing targets regulated by RBM20 (87).

RBM24, another key member of the RBM family of proteins, shows high expression in human and mouse hearts. Interestingly, the expression of RBM24 can also regulate cardiac gene expression, sarcomeric assembly, and cardiac contractility in zebrafish (173). RBM24 expression is induced during the differentiation of human embryonic stem cells into cardiomyocytes. With the use of an inducible RBM24 expression system, it was demonstrated that RBM24 can also regulate a large number of alternative splicing events during mouse embryonic stem cell differentiation (242). RBM38, a homolog of RBM24, can also function in mRNA splicing and stability, but is dispensable for pressure overload-induced cardiac remodeling (205). Finally, RBM10 mutations may be causal of the talipes equinovarus, atrial septal defect, Robin sequence, and persistent left superior vena cava (TARP) syndrome (77a). The expression of RBM10 is reduced during H9C2 myoblasts differentiation (131). At the mechanistic level, RBM10 seems to regulate alternative exon skipping as well as 3’-UTR-dependent processing during cardiac hypertrophic response (145, 219). Overall, the RBM family of genes plays an important role in the genetics of cardiomyopathy.

C. CUGBP Family

Members of the CUG binding protein (CUGBP) family of proteins are also highly expressed in heart, including CUGBP1, also known as CUGBP CUGElav-like family member 1 (CELF1), and CUGBP2, also known as CELF2. Both CUGBP1 and 2 are present in the nucleus and cytoplasm, with high expression in the embryonic heart but downregulated postnatally (111). With the use of CLIP-Seq analysis, it was found that the CUGBP1 binding motifs are highly conserved across different tissues, including heart, muscle, and myoblasts (214). CUGBP1 is also implicated in embryonic heart development (48, 98). In the Cugbp1 knockout mouse model, Giudice et al. (73) observed a significantly lower body weight, which was associated with compromised cardiac function. With the use of RNA-seq analysis, it was further shown that a total of 45 splicing events are affected after Cugbp1 depletion. Among them, 69% contain at least one Cugbp1-CLIP tag adjacent to the alternatively spliced exon, implicating them as direct targets of splicing modulation by Cugbp1 (73). In myotonic dystrophy (MD), accumulation of CUG triplet nucleotides due to mutational expansion leads to CUGBP1 sequestration, CUGBP1 induction, and aberrant RNA splicing (117, 197, 198, 215). CUGBP1 is induced in MD muscle via protein kinase C (PKC)-dependent phosphorylation and protein stabilization (109). Overexpression of CUGBP1 is sufficient to confer the MD phenotype to skeletal and cardiac muscle (103, 221). These findings highlight the importance of CUGBP1 in the phenotypic manifestation of MD. It is now important to determine if and how the CUGBP family of genes contributes to the overall transcriptome reprogramming in the common forms of heart failure.

D. MBNL Family

The human muscleblind-like splicing regulator (MBNL) genes include MBNL1, MBNL2, and MBNL3 and are homologous to the Drosophila gene muscleblind. Like CUGBP1, the recruitment of human MBNL proteins to the CUG expansions in mRNAs is also implicated in the pathogenesis of MD (52, 144). In the heart, the expression of the MBNL1 protein is upregulated during cardiac development (98). In addition to binding to the CUG expansion-containing mRNAs, MBNL1 can also regulate alternative splicing of cardiac sodium channels (such as SCN5A), which potentially contributes to cardiac conduction block and cardiac arrhythmia (62). Reducing MBNL1/CUGBP1-mediated CUG RNA foci via gene targeting or small molecule-based approaches represents a potential therapeutic approach to treat MD (103, 132).

E. SR Family

Members of the serine/arginine-rich (SR) gene family are important components of the generic splicing machinery and are characterized by their ability to interact simultaneously with RNA and other proteins (185). Some of the SR proteins are among the earliest proteins that were demonstrated to have an important role in cardiac-specific alternative splicing regulation. The ablation of the SR protein SC35 (or SRSF2) during early cardiogenesis has no detectable effect on embryonic cardiac development. However, SC35 knockout in adult mice developed significant cardiac hypertrophy associated with chamber dilation (53). Although it is unclear what the main downstream targets of SC35 are in the heart, evidence suggests that the expression of SC35 can alter the pre-mRNA splicing of atrial natriuretic factor (58). In contrast to SC35, ASF1/SF2 (or SRSF1) is critical during embryonic cardiac development as ablation of ASF1/SF2 in cardiomyocytes leads to a hypercontractile phenotype due to a defect in postnatal splicing switch for numerous cardiac genes, including cardiac troponin T and calmodulin kinase-IIβ (231).

F. hnRNP Family

The hnRNP family encompasses a large number of RNA binding proteins that contribute to multiple aspects of alternative splicing. The functions of hnRNPs are different
Alternative mRNA splicing generates multiple mRNA species from one gene. The changes in the exon composition of the final mRNAs can lead to either subtle or disparate changes in the functional properties of the encoded proteins (91). Some striking changes were demonstrated for the protein isoforms encoded by different splice variants of an ion channel gene, where localization to the cytoplasm or the mitochondrial membrane was dependent on the splice variant encoding the particular ion channel isoform (189, 233). In addition, some dramatic changes, even those resulting in a protein isoform with functional opposition to the primary protein, have been reported (e.g., Bcl-xS and Bcl-xL) (11, 21). Indeed, a majority of human mRNAs are subjected to alternative RNA splicing, and the splicing variants help to define the cellular identity and differentiation status in a significant way (12). It is conceivable that a majority of cardiac genes are subjected to alternative splicing, and this has a high likelihood of affecting cardiac physiology and pathology. While there is a myriad of genes regulated by alternative splicing, herein we will only discuss the well-characterized role of alternative splicing in isoform switching observed with the sarcomeric proteins, which are critical during cardiac development and disease.

A. Alternative mRNA Splicing in Cardiac Development

At a global level, using a large-scale splicing microarray combined with reverse transcriptase-polymerase chain reaction, Kalsotra et al. (98) were the first to identify a large number of altered alternative splicing events during cardiac development. These alternative splicing events are highly conserved between mouse and chicken. Among these alternative splicing events, a subset of them are coordinated at specific time points during mouse heart development, and this supports the notion that mRNA splicing during cardiac development is highly regulated. With the use of computational analysis, it was further observed that both the GCAUG (RBFox binding motif) and the GUGUG (CUGBP binding motif) elements are enriched in the intronic regions of the alternative spliced exons in the heart, suggesting these RNA binding proteins coordinately regulate at least a subset of alternative splicing events during mouse cardiac development (98). Global mRNA splicing shifts are also observed in human hearts during development, again supporting the conservation of RNA splicing regulation in heart (111, 204, 216).

One of the earliest documented alternative splicing events in the heart occurs with the cardiac troponin T (or TNNT2) gene (41). The embryonic isoform of cardiac troponin T contains exon 5, and the adult isoform lacks exon 5, which contains 10 highly acidic amino acids, and is the result of both cis-regulatory elements and trans-acting factors (39, 40). Another example of cardiac specific alternative splicing is the titin (or TTN) gene. At both transcript and protein levels, the N2BA isoform of titin (3.7 MDa) is the dominant isoform expressed in the perinatal heart. However, the N2BA isoform is rapidly replaced by a smaller N2B isoform (3.0 MDa) due to mRNA splicing (163, 223). This alternative splicing event is critical during postnatal heart development as force measurements show that titin isoform switching greatly increases myofibrillar passive tension (28).

B. Alternative mRNA Splicing in Cardiac Diseases

During cardiac pathological remodeling in both human patients and mouse heart failure models, alternative splicing...
of mRNAs is dramatically changed at a global scale (204). Using an exon array, Kong et al. (104) compared the left ventricle RNA from both control and ischemic cardiomyopathy patients and identified a significant decrease in RNA splicing efficiency in the ischemic heart. Among the differentially spliced genes, a large set of sarcomere genes are shown to be altered in the diseased human heart. Motif analysis further demonstrated that MBNL and hnRNP F binding motifs are enriched in the sequences flanking the differentially spliced exons. Similar to humans, in mice with pressure overload-induced failing hearts, Lee et al. (116) identified a large set of differentially spliced genes using whole transcriptome RNA-seq. In this study, a total of 1,087 genes were identified to be differentially spliced due to either alternative transcription start site utilization or alternative splicing. As the heart progresses into decompensated heart failure following long-term pressure overload, a larger number of alternative splicing events are identified, showing a correlation between alternative splicing and progression of heart failure (116). Among the differentially spliced genes, many have a known role in cardiac regulation and function, including Rgs12, Rabgap1, and Hdac7 (116). In addition to ischemic and dilated cardiomyopathy, diabetic cardiomyopathy also shows a global change of alternative splicing. Using a mouse type 1 diabetic cardiomyopathy model established with streptozotocin treatment, Verma et al. (206) identified a total of 967 alternative splicing events in diabetic mouse hearts. Many of these alternatively spliced genes are involved in cardiac development and RNA metabolism (206). Interestingly, the alternative splicing observed in diseased heart also appears to be part of the phenomenon of “fetal gene reprogramming,” which occurs in diseased hearts (e.g., dilated cardiomyopathy and diabetic cardiomyopathy) and is characterized by an alternative splicing pattern typically observed during embryonic development (67, 206).

Among the differentially spliced genes in heart failure, sarcomere genes are perhaps the best characterized group. Similar to cardiac development, titin is also subjected to extensive alternative splicing regulation in cardiomyopathy. The titin PEVK segment (named for its enriched Pro-Glu-Val-Lys amino acids) is regulated by RBM20-mediated splicing of exon 8. Mutations in RBM20 cause dilated cardiomyopathy and aberrant titin alternative splicing (80, 125). Interestingly, a mutation in the glutamate-rich region of RBM20 (E913K/+)) caused massive inclusion of titin exons coding for the spring region, leading to a dramatic shift from the less compliant N2B towards the highly compliant N2BA isoforms (18). In addition to RBM20, RBM24 has also been shown to regulate alternative splicing of sarcomere genes, including titin. Using a reporter construct, Liu et al. (127) demonstrated that expression of RBM24 is sufficient to promote the exon 13 inclusion of titin. hnRNPU deletion also changes titin alternative splicing, although it is unclear whether hnRNPU directly regulates titin splicing in diseased hearts (234).

Tropomyosins are a family of actin-binding proteins that are expressed in both muscle and non-muscle cells. They are important regulators for actin filament function (78, 88) and can form rod-shaped homodimer or heterodimer structures arranged as a coiled coil. In humans, there are four conserved genes (TPM1, TPM2, TPM3, and TPM4) encoding four different tropomyosin protein isoforms, TPM1, TPM2, TPM3, and TPM4 (236). Each of these genes can also produce multiple transcript variants and protein isoforms through alternative promoter usage, alternative splicing, and alternative polyadenylation (220). For example, the TPM1 gene is subject to tissue-specific alternative splicing, generating TPM1k and TPM1a that are highly expressed in the heart. In human failing hearts, TPM1k appears to be upregulated (99, 175). To examine the impact of elevated TPM1k levels on heart function, Rajan et al. (175) overexpressed TPM1k in the mouse heart, and this led to systolic and diastolic dysfunction. Thus alternative splicing of tropomyosin could potentially contribute to cardiac disease progression. TPM1 splicing is regulated by RBM20 and PTB, although it is unclear whether they are directly responsible for the TPM1a and TPM1k isoform-switch during cardiac development and diseases (80, 235). Troponin T (TnT or TNNT2) links the troponin complex to tropomyosin and is also subject to alternative splicing in the heart. In humans, there are at least four isoforms of cardiac TnT (cTnT) that result from the alternative splicing of exon 4 and 5 (133). The splicing regulator responsible for the inclusion of exon 5 into cTnT is SRp55. Inactivation of SRp55 suppresses exon 5 inclusion into cTnT, while overexpression of SRp55 facilitates its inclusion as shown by minigene reporter analysis (133, 134). Similarly, Mbnl can also recognize its binding motif within intron 4 of the cTnT transcript, an interaction that mediates exon 5 splicing (222). In addition to exon 5, exon 7 of cTnT also plays an important role in cardiac function. This was demonstrated in transgenic mice overexpressing a mutant cTnT isoform lacking exon 7 that caused a significant decrease in cardiac function (59). Nevertheless, it is not clear which factors are the responsible trans-acting regulator(s) of exon 7 splicing in the heart.

During heart disease progression, apoptotic genes also undergo extensive alternative splicing. For example, the Bcl-x gene can generate two isoforms as a result of alternative splicing. The short protein Bcl-xS is pro-apoptotic, while the large protein Bcl-xL is anti-apoptotic (21). The alternative splicing of Bcl-x can be regulated by both protein arginine methyltransferase 2 (PRMT2) and polypyrimidine tract-binding protein 1 (PTBP1) (19, 208). In the failing heart, the expression of Bcl-xL mRNA is significantly increased after ventricular assist device implantation (14). In the failing heart, the expression of Bcl-xL mRNA is significantly increased after ventricular assist device implantation (14). This increase leads to an imbalance in the ratio of Bcl-xL to Bcl-xS that leads to mitochondrial stress and cell apoptosis, which may, in part, contribute to an increased risk of heart failure (181).
V. mRNA TRANSLATION AND DEGRADATION IN HEART

The proteome of a cell largely defines its unique functions, with different proteomic profiles specifying the myriad of cell types in the animal kingdom, and it is the maintenance of this proteome that characterizes the life of most cells in the human body. However, acute stress causes a transient cellular response, which is strongly dependent on the temporal and dynamic expression of key proteins (23). Long-term or chronic exposure to insults often leads to irreversible changes in the proteome that causes an altered and persistent dysfunctional phenotype in affected cells and tissues. In the heart, it is commonly recognized that the rate of protein synthesis in mature cardiomyocytes is relatively low, but is dramatically increased during cardiac hypertrophy (68, 124). While translation of mRNA into protein occurs at the translation machinery (i.e., ribosomes), RNA binding proteins, which bind to and shuttle the newly synthesized mRNAs to the ribosomes, present another level for the regulation of mRNA translation. In fact, a lot of the aforementioned RNA binding proteins are able to translocate between the nucleus and cytosol, or undergo alternative splicing to generate both nuclear and cytosolic isoforms. These properties suggest that RNA binding proteins can regulate both alternative splicing and other processes of RNA metabolism, including mRNA translation and stability regulation (FIGURE 9).

A. RBPs in Cardiac Regulation of mRNA Translation and Decay

1. RBFox family

In addition to the canonical activity in mRNA splicing, RBFox1 also undergoes alternative splicing, which generates two splicing isoforms, each located either in the nucleus or the cytosol (114, 115). While the function of the nuclear RBFox1 in alternative splicing has been well characterized, emerging evidence also suggests that the cytosolic RBFox1 could play important roles in regulating mRNA stability. In a recent study, Ray et al. (180) demonstrated that the RBFox1 binding motif is present in the 3’-UTR of various RNA transcripts across different tissues. In the neuron, double inactivation of both RBFox1 and RBFox3 caused significant changes in mRNA abundance for a large number of mRNA transcripts, an effect that was rescued by reintroducing the cytosolic isoform of RBFox1, but not the nuclear isoform. With the use of the iCLIP-Seq method, cytosolic RBFox1 is found to directly interact with 3’-UTRs and has the effect of increasing mRNA stability. One of the possible mechanisms in the cytosolic RBFox1-mediated mRNA regulation may be due to competition with miRNA-mediated regulation of mRNA stability and translation. Conceptually, this competitive model is plausible given that there may be overlap between the binding motifs for each molecule within the 3’-UTR (114, 212). Similar to RBFox1, a recent study shows that RBFox2 is associated with cytoplasmic stress granules, a translational silencing machinery assembled in response to cellular stress (164) (see FIGURE 3). In a study performed by Park et al. (164), a subset of cell cycle-related genes including Rb1 are molecular targets of RBFox2 in stress granule-mediated translational suppression, leading to changes in cell proliferation. In the heart, Verma et al. (207) demonstrated a change in the subcellular localization of RBFox2 in individuals affected with hypoplastic left heart syndrome (HLHS), towards a more cytoplasmic distribution. A portion of the differentially expressed genes in the HLHS ventricles may be RBFox1/2 downstream targets, given that there is a significant enrichment in the differentially expressed gene list for transcripts harboring RBFox binding sites in the 3’-UTR (207). How-

![FIGURE 9](image-url). Summary of cardiac mRNA translation and degradation control with representative regulators. Translation and stability of cardiac mRNAs are regulated by protective RBPs (RNA binding proteins) versus pathogenic RBPs based on functional analysis.
ever, the detailed mechanism underlying RBFox2-mediated gene expression in the heart remains unclear. In addition to directly regulating mRNA stability and translation, RB-Fox2 could also have an indirect impact on downstream mRNA abundance through miRNA synthesis. For example, Chen et al. (34) suggest that RBFox proteins can regulate miRNA biogenesis by binding to their precursors and regulating the miRNA processor Dicer.

2. CUGBP protein family

CUGBP1 (also known as CUGBP Elav-like family member 1, CELF1) is also a multifunctional RNA-binding protein regulating pre-mRNA alternative splicing, translation, and mRNA stability (48). CUGBP1 appears to localize to both the nucleus and cytosol. In the cytosol, CUGBP1, but not CUGBP2, can specifically bind to the GU-rich element (GRE) in the 3'-UTR region and promote mRNA degradation (118, 179, 211). In addition to 3'-UTR, CUGBP1 can also interact with mRNA 5'-UTR and regulate mRNA translation for genes such as p21 (94, 199). In the heart, CUGBP1 binds to the 3'-UTR of Cx43, a major cardiac gap junction protein containing UG-rich motifs, and downregulates Cx43 mRNA by interacting with a 3'- to 5'-exoribonuclease, RRP6, to promote Cx43 mRNA degradation. Depletion of CUGBP1 CUGBP1 in the infarcted heart preserves Cx43 mRNA levels and attenuates contractile dysfunction (30). Another example of CUGBP1-mediated mRNA degradation is the heme-oxygenase 1 (HO-1) gene. CUGBP1 downregulates HO-1 mRNA levels through interacting with the conserved GU-rich elements in the HO-1 3'-UTR, and this promotes HO-1 mRNA degradation in myoblasts (130). In addition, CUGBP1 expression is downregulated by carbon monoxide in H9C2 cells and highly induced in hypertrophic cardiomyopathy hearts. Therefore, it may serve as an important player in the positive regulatory circuit for carbon monoxide and heme clearance in the heart. Interestingly, in contrast to CUGBP1, the CUGBP2 gene seems to have the opposite effect on mRNA stability compared with CUGBP1. For example, CUGBP2 can bind to the A/U-rich sequence in the 3'-UTR of cyclooxygenase-2 (COX-2) and stabilizes the COX-2 mRNA while inhibiting its translation (149, 152). This appears to be an important mechanism in the paradox observed for COX-2 in cancer cells, where an abundance of COX-2 induced mRNA is contrasted with a relatively low abundance of COX-2 protein. Another downstream target of CUGBP2 is Mcl-1 mRNA, which encodes an anti-apoptotic protein. Expression of CUGBP2 reduces the expression of Mcl-1 protein by interacting with the Mcl-1 mRNA 3'-UTR and destabilizing it, effectively blocking the Mcl-1 mRNA from interacting with the translation machinery (195). In short, CUGBP1 and 2 are both able to regulate mRNA stability and protein translation, but their full spectrum of downstream targets in cardiac tissue is yet to be fully established.

3. Heterogeneous nuclear ribonucleoproteins

HuR (also known as ELAVL1) plays a key role in many aspects of RNA metabolism, including mRNA alternative splicing and mRNA decay. HuR recognizes and interacts with adenylate-uridylate-rich elements (ARE) within the 3'-UTR of the target mRNA (13, 20, 141). In cardiomyocytes, one established downstream target for the HuR is transcription factor Mef2c. HuR protein increases Mef2c mRNA expression by protecting its mRNA from degradation (244). A similar mechanism is also attributed to NADPH oxidase-catalytic subunit 1 (NOX-1) in vascular smooth muscle cells and cardiac sodium channel gene SCN5A in cardiomyocytes. HuR blockade reduces NOX-1 mRNA stability, and binding of HuR to SCN5A mRNA protects it from decay (4, 245). SCN5A is also a splicing target of MBNL in promoting cardiac arrhythmia associated with myotonic dystrophy (62). Therefore, in addition to splicing regulation, HuR can coordinate with other RBPs to regulate gene expression and cellular physiology in cardiac tissue by modulating target mRNA splicing and stability.

4. Other RBPs

A) QUAKING. The quaking homolog KH domain RNA binding (QKI) family of RNA binding proteins is important in heart physiology (50). Studies by de Bruin et al. (50) demonstrated that QKI5 protects cardiomyocytes from apoptosis by reducing FoxO1 expression. This regulation plays an important role in obesity as a result of ER stress activation. Both mRNA and protein levels of the FoxO1 gene are markedly reduced by QKI5 in the ob/ob mouse hearts through QKI5-mediated mRNA degradation (79).

B) POLY(C)-BINDING PROTEINS. Poly(C)-binding proteins (PCBPs) are a family of RNA binding proteins that bind to single-stranded poly C sequence. The PCBP family includes PCBP1–4 and are expressed across different tissues with multiple roles in mRNA metabolism (137). Among them, PCBP2 has been demonstrated to be downregulated in human failing hearts and mouse hypertrophic hearts and is an anti-hypertrophic factor that can protect cardiomyocytes from angiotensin II-induced hypertrophy (243). In cultured cardiomyocytes, one of the downstream targets of PCBP2 is GPR56. Inactivation of PCBP2 reduced the decay rate of GPR56 mRNA, which is induced during cardiac hypertrophy (243).

B. Functional Impact of mRNA Translation and Decay in Cardiac Development

During cardiac development and cardiomyocyte differentiation, Cnot3, a key regulator of the de-adenylation complex, is critical for cardiomyocyte proliferation in vitro, an effect that was demonstrated using human embryonic stem cell-derived cardiomyocytes (hiCM). Cnot3 is predomi-
nantly found in the cytoplasm. Under actinomycin D treatment, depletion of Cnot3 significantly reduces the degradation of cyclin D kinase inhibitor 1A (CDKI) mRNA, which encodes the cell cycle inhibitor p21. This observation provides a potential mechanism for the regulation of cardiomyocyte proliferation (246).

The tumor suppressor 53 (TP53 or p53) protein plays a critical role in cardiac development and is a master regulator of stress-induced gene expression. Post-transcriptional regulation of p53 is mediated by RBM24 via direct binding to the p53 3'-UTR (241). In vitro, RBM24 knockdown significantly increases p53 protein levels. It is believed that this regulation is a consequence of the interaction between RBM24 and the 5'-cap-binding protein eIF4E, an interaction that may lead to dissociation of eIF4E from the p53 mRNA 5'-cap and p53 mRNA translation suppression. Deficiency of RBM24 in vivo causes endocardial cushion defect, and this is associated with elevated p53 levels, an effect that was rescued by p53 deletion (241).

C. Functional Impact of mRNA Translation and Decay in Heart Diseases

Using a genome-wide approach, Park et al. (165) compared mRNA isoforms between mouse hearts with transverse aortic constriction (TAC)-induced hypertrophy and “healthy” hearts (i.e., sham operated). The investigators identified a global shift towards an abundance of mRNA isoforms with shorter 3'-UTRs in 1 wk TAC hearts, compared with healthy hearts. Because mRNAs with short 3'-UTRs are generally considered to be more stable than mRNAs with longer 3'-UTRs, the finding in TAC hearts suggests that the heart’s response to TAC-induced hypertrophy leads to a stabilization of some/most mRNA species, a feature that may be characteristic of cardiac hypertrophy. In parallel, Park et al. (165) compared miRNA expression and did not find increased activity for any miRNA against their respective mRNA targets, suggesting that miRNA activity is generally inhibited in cardiac hypertrophy. Taken together, the authors concluded that hypertrophic hearts tend to have more stable mRNA transcripts compared with healthy hearts (165). However, because the observation was made at an early stage of hypertrophic remodeling, it remains to be demonstrated if such a shift in the profile of mRNA isoforms is functionally compensatory or detrimental.

In addition to a shift towards an abundance of stable mRNA transcripts during hypertrophy, the translation of mRNAs is also regulated in hypertrophic hearts. Poly(A) binding protein C1 (PABPC1) is a ubiquitously expressed mRNA binding protein that facilitates translation while also protecting the mRNA from exonucleases (38, 97, 123). In a recent study, Chorghade et al. (35) demonstrated that PABPC1 protein levels are regulated post-transcriptionally and found that shortening of the PABPC1 mRNA poly(A) tail leads to reduced polysome association and lower translation activity. During cardiac development, the PABPC1 tail is silenced. Interestingly, hypertrophic cardiomyocytes stimulated by both endurance exercise and heart disease can restore the PABPC1 poly(A) tail length and protein expression. This restoration of PABPC1 is important and necessary for global protein translation in cardiomyocytes and for their physiological growth (35).

In addition to extensive alternative splicing regulation, titin is also subject to translation regulation through the 5'-UTR region of its mRNA. The 5'-UTR of titin is highly conserved among human, mouse, and rat and contains a stem-loop structure and two upstream AUG codons (uAUGs) converging on a shared in-frame stop codon. Using a luciferase reporter construct containing the 5'-UTR sequence of titin, Cadar et al. (24) showed that the translation efficiency mediated by this 5'-UTR sequence was reduced in cardiomyocytes unless both uAUG sequences were mutated. Furthermore, treatment of cardiomyocytes with doxorubicin-induced stress reduced titin 5'-UTR-mediated translation suppression in cardiomyocytes (24). However, the physiological significance of such regulation remains to be demonstrated.

VI. CHALLENGES AND OPPORTUNITIES FOR mRNA METABOLISM STUDIES IN HEART

Despite the significant progress in the past decade, major gaps remain in our understanding of mRNA metabolism, as well as its relevance to cardiac biology and medicine. The enormous complexity found in the processes that encompass mRNA metabolism and the technical challenges of experimentally manipulating RNA molecules present significant barriers to elucidating the role of RNA metabolism in cardiac biology and disease. Several key questions remain to be addressed.

1) What are the molecular players in mRNA metabolic regulation in the heart? There are thousands of known RBPs in the human genome, but only a small fraction of them are fully characterized and validated (70). RBP and the current CLIP-seq and iCLIP-seq approaches suffer from specificity and signal-noise issues (126, 229). Most of the interactions can only be assessed by in vitro biochemical assays because of the
technical difficulties of using in situ methods when the conditions are more physiologically relevant. Therefore, identifying the molecular basis for many of the mechanisms regulating mRNA metabolism remains just beyond our reach.

2) What is the functional status of mRNA in living cells? mRNA metabolic regulation involves a multitude of highly dynamic processes in mRNA processing, modification, transportation, and anchoring (142, 192). Most of our current analysis of mRNA function relies on global and bulk assessment of mRNA expression levels, stability, and ribosomal loading, with poor temporal and special resolution. A real-time approach to investigating the life cycle of mRNA molecules, during synthesis, modification, translation, and degradation, will offer tremendous insight into the underlying mechanisms involved in mRNA metabolic regulation in health and disease.

3) What are the emerging frontiers in mRNA regulation? Clearly, mRNA modification (63, 146), editing (190, 201, 232), and compartmented function will open new areas for exploration. Beyond m6A modification, other forms of post-transcriptional modifications and interactions with other noncoding RNAs in subcellular compartments and organelles will shed new light on the ever-expanding universe of mRNA metabolism.

Current technological advancements and the advent of large data-based systems approaches have offered many exciting opportunities for rapid progress in the field. Molecular imaging techniques have finally shed some light on the mRNA life cycle at the resolution of a single molecule (113, 167). Large-scale gene editing tools and whole transcriptome sequencing make it feasible to perform a comprehensive analysis for all RBP proteins in the human genome (37, 100). An unbiased systems approach may uncover key regulators in the RBP network and establish novel links (83). These powerful tools (142) will help us better understand the intricate regulatory network underlying mRNA metabolism that may lead to new discoveries critical for developing novel diagnostic and therapeutic approaches for the clinical treatment of heart disease.

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