

# UCLA

## UCLA Previously Published Works

### Title

Transcriptome Complexity in Cardiac Development and Diseases

### Permalink

<https://escholarship.org/uc/item/3jb5x5r4>

### Journal

Circulation Journal, 78(5)

### ISSN

1346-9843

### Authors

Gao, Chen  
Wang, Yibin

### Publication Date

2014

### DOI

10.1253/circj.cj-14-0412

Peer reviewed



Published in final edited form as:  
*Circ J.* 2014 ; 78(5): 1038–1047.

## Transcriptome Complexity in Cardiac Development and Diseases:

### An Expanding Universe Between Genome and Phenome

Chen Gao, PhD and Yibin Wang, PhD

Departments of Anesthesiology, Physiology and Medicine, Molecular Biology Institute, David Geffen School of Medicine at University of California at Los Angeles, Los Angeles. CA, USA

### Abstract

With the advancement of transcriptome profiling by micro-arrays and high-throughput RNA-sequencing, transcriptome complexity and its dynamics are revealed at different levels in cardiovascular development and diseases. In this review, we will highlight the recent progress in our knowledge of cardiovascular transcriptome complexity contributed by RNA splicing, RNA editing and noncoding RNAs. The emerging importance of many of these previously under-explored aspects of gene regulation in cardiovascular development and pathology will be discussed.

### Keywords

Cardiovascular diseases; Genes; Molecular biology; Signal transduction

---

The mature heart develops through complex cellular differentiation involving morphological and functional changes during the embryonic and postnatal periods. Each step of this process, from lineage commitment to morphogenesis, is marked by distinct changes in gene expression profiles in both cardiomyocyte and non-myocyte components.<sup>1</sup> Earlier studies have revealed an elaborate transcriptional regulatory network driven by tissue-specific and temporally coordinated expression of transcription factors.<sup>2,3</sup> The importance of cardiac gene regulation is underscored by the critical contribution of transcriptional dysfunction to both congenital heart diseases and the pathogenesis of heart failure. In fact, many of the same transcriptional regulators involved in cardiac development also have important roles in cardiac hypertrophy, pathological remodeling and heart failure. Therefore, understanding cardiac transcriptome dynamics and regulation has been a major focus of research in the field of cardiac biology and cardiovascular medicine. However, much of our current knowledge of cardiac gene regulation is based on dynamic changes in the composition and expression level of mRNA. With recent advancement of RNA-sequencing technology, our view of transcriptome complexity has been expanded dramatically. Novel mRNA transcripts derived from alternative splicing and editing, and previously unrecognized species of

---

Yibin Wang, Ph D, Division of Molecular Medicine, Departments of Anesthesiology, Physiology and Medicine, Cardiovascular Research Laboratory, Molecular Biology Institute, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA. yibinwang@mednet.ucla.edu.

noncoding RNAs have emerged as dominant features of the mammalian transcriptome, reshaping the fundamental concept of gene regulation, genome structure and genetic/epigenetic contribution to development and diseases. This review will highlight some of the recent progress and the new concepts emerging from studies of cardiac transcriptome regulation, with particular focus on RNA splicing and editing, as well as noncoding RNAs. Considering the fact that microRNA function and regulation have been discussed extensively by several excellent reviews,<sup>4-6</sup> we will not cover those issues except when it intercepts with the topics covered by this review.

## Alternative RNA Splicing in Transcriptome Regulation

### Regulatory Machinery of RNA Splicing

All multi-exon genes require coordinated splicing to generate mature RNA transcripts. Multiple mRNA species can be generated from a single gene through alternative RNA splicing events. Extensive profiling has revealed that alternative splicing is a major contributor to mRNA complexity in the mammalian transcriptome, affecting more than 94% of human transcripts.<sup>7-9</sup> At the global transcriptome level, alternative RNA splicing is a highly regulated process associated with physiological and pathological conditions,<sup>10</sup> including embryonic stem cell (ESC) differentiation and cancer development.<sup>11-14</sup> However, our knowledge of RNA splicing regulation and its role in cardiac development and diseases remains very limited, especially compared with the wealth of information about transcriptional regulation.

The constitutive RNA splicing event excises the intronic sequences according to pre-demarcated exon/intron boundaries. An alternative RNA splicing event, however, can use alternative 5' or 3' splice sites, leading to specific exon skipping or inclusion or intron retention.<sup>7,15,16</sup> Although a basic spliceosome is responsible for constitutive RNA splicing, additional *trans*-acting factors and *cis*-acting sequence motifs are responsible for enhancing or repressing the alternative splicing events.<sup>9</sup> The RBP superfamily includes serine/arginine-rich (SR) proteins, neuro-oncological ventral antigen (Nova) proteins, heterogeneous nuclear ribonucleoproteins (hnRNPs) and RBFOX proteins.<sup>17-19</sup> The tissue-specific and signal-regulated expression or activities of the RBPs are the key to coordinated mRNA splicing events<sup>20</sup> (Figure 1). More importantly, the RNA splicing machinery is an integral part of gene regulation, and its function has been implicated beyond RNA splicing events to effects on RNA transcription, quality control, transportation and other post-transcriptional processes.<sup>21-25</sup> However, very limited information is available on how these splicing factors are regulated in response to developmental or pathological cues to achieve coordinated RNA splicing in the heart.

### RNA Splicing Regulation in Cardiac Development and Diseases

Salomonis et al revealed that alternative RNA splicing is important for calcium signaling and cardiomyocyte differentiation from progenitor cells,<sup>26,27</sup> In another study, cross-talk between the microRNA regulatory network and alternative splicing is demonstrated to define transcriptome maturation during postnatal cardiac development in the mouse.<sup>28</sup> However, a comprehensive analysis of mRNA splicing in the heart during development has

not been reported. Therefore, it would be interesting to perform RNA splicing analysis at different stages of cardiac development, a task becoming increasingly feasible with more sensitive high-throughput RNA-sequencing capabilities and more sophisticated bioinformatics tools.<sup>29–31</sup>

A number of cardiac-enriched RNA splicing regulators, including muscle-blind-like protein 1 (MBNL1), RBFOX2, CUG-BP1 and CUG-BP2, are highly expressed during early fetal heart development but decreased postnatally. In contrast, the cardiac expression of RBFOX2 is significantly induced only after birth.<sup>32–33</sup> On the other hand, the change in the expression of CUGBP1 and CUGBP2 is directly regulated by miR-23a/b during cardiac development and this contributes to a significant number of developmentally associated splicing events in the heart.<sup>28</sup>

In addition to cardiomyocyte differentiation and development, the alternative splicing profiles in the heart are also tightly associated with the pathogenesis of heart failure. Global alternative splicing profiling has been done in the diseased heart, including cardiac hypertrophy and heart failure.<sup>34,35</sup> For example, an earlier study compared pressure overload-induced cardiac hypertrophy and heart failure in the mouse heart using deep RNA-sequencing and revealed a global change of alternative splicing in the failing murine heart.<sup>35</sup> More recently, Ames et al and other groups also identified a significant number of alternative splicing events during cardiac hypertrophy in rats.<sup>34,36</sup> For splicing regulators, it is suggested that the expression of PTB and ASF/SF2 is altered in the pressure overload-induced hypertrophic heart.<sup>37</sup> The transcriptome signature and RNA splicing events have also been profiled in human heart disease.<sup>38,39</sup> Based on a gene expression profiling analysis, a total of 17 splicing factors were found to be upregulated in the human failing heart, including RBM25, QKI, hnRNPA1 and Tra2a.<sup>40</sup>

Genetic inactivation of ASF/SF2 in cardiomyocytes causes a hypercontractile phenotype, in part because of aberrant splicing of the Ca<sup>2+</sup>/calmodulin-dependent kinase II  $\alpha$  (CaMKII $\alpha$ ) transcript.<sup>41</sup> Cardiac-specific ablation of SC35 also causes dilated cardiomyopathy, associated with mis-regulated splicing of cardiac ryanodine receptor 2 (RyR2).<sup>42</sup> Disruption of the MBNL1 gene in the mouse also leads to muscle, eye and splicing abnormalities mimicking the phenotype of myotonic dystrophy.<sup>43</sup> Lastly, as a key regulator for alternative splicing of Titin mRNA, RBM20-deficient rats develop dilated cardiomyopathy.<sup>44,45</sup> Therefore, tissue-specific and coordinated RNA splicing events carried out by a well-orchestrated RNA splicing regulatory network are critical to normal cardiac development and physiology.

### **Stress Signaling in Cardiac RNA Splicing Regulation**

Emerging evidence suggests that the RNA splicing machinery is also a common target of pathological signaling. In response to pressure overload, the expression level of dual-specificity tyrosine-phosphorylated and regulated kinase 1A (Dyrk1A) is significantly upregulated, which represses the expression of the splicing factor ASF and downstream alternative splicing of CaMKII $\delta$ .<sup>46</sup> In addition, angiotensin II (AngII) can also affect the expression of another splicing regulator (68-kDa Src) associated during mitosis (SAM68) in the heart.<sup>47</sup> SAM68, in turn, regulates the alternative splicing of both mTOR and SMN and

plays a key role in spinal muscular atrophy.<sup>48,49</sup> In a recent report by el Mabrouk et al,<sup>47</sup> AngII was shown to stimulate the binding of SAM68 to a poly-U region through a PI3K-dependent pathway. Lastly, splicing factor hnRNP A1 forms a molecular complex with activated p38 *in vivo*, which is important for the proper intracellular localization of this splicing factor.<sup>50</sup>

Among the different stress-signaling molecules, the best characterized regulator for RNA splicing is protein kinase C (PKC). Its regulatory role in alternative splicing was first demonstrated for the alternative splicing of Bcl-x pre-mRNA, which generates 3 distinct isoforms, Bcl-xL and Bcl-xs.<sup>51</sup> Sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase1 (SERCA1) splicing is also regulated by PKC.<sup>52</sup> A potential underlying mechanism is through PKC-mediated modulation of the activity of an RNA splicing regulator, SC35. In the postnatal rat heart, nucleus-localized PKC can also phosphorylate and activate SC35, which is important for cardiac morphogenesis.<sup>53,54</sup> As demonstrated by Cataldi et al, hypoxia in the adult heart can modulate the phosphorylation level of SC35 through a PKC-dependent pathway that contributes to the hypoxia-induced expression of vascular endothelial growth factor (VEGF),<sup>55</sup> thus providing a RNA splicing dependent mechanism for hypoxia-induced neoangiogenesis (Figure 2). A recent study has demonstrated that in diabetic cardiomyopathy, a significant number of alternative splicing events switch back to an embryonic pattern. This phenotype is potentially regulated by PKC $\alpha/\beta$  via phosphorylating and up-regulating of 2 key cardiac splicing regulators: CELF1 and RBFOX2.<sup>56</sup> All together, it appears that the RNA splicing machinery is an important downstream target of stress signaling in the heart. Considering the molecular complexity of the stress-signaling network and the RNA splicing machinery, there must be more interactions between alternative RNA splicing and stress signaling waiting to be explored.

### Molecular Targets of Alternative RNA Splicing in the Heart

Alternative RNA splicing affects many genes in the heart. One example is SCN5A, which encodes the Na-channel  $\alpha$ -subunit,<sup>57,58</sup> whereby its splicing variants are associated with heart failure, arrhythmia and Brugada syndrome in humans.<sup>40,59–61</sup> Its splicing is regulated at least in part by RBM25/LUC7L3.<sup>40</sup> Another cardiac gene subjected to alternative splicing is KCNQ1 which generates a truncated isoform with a dominant-negative function associated with cardiac arrhythmias in both human and rat hearts.<sup>62,63</sup> In addition, the aberrant splicing isoforms of cardiac troponin T (cTnT) has been identified in dilated cardiomyopathy.<sup>64</sup> Both *in vitro* and *in vivo* studies have revealed that cardiac-enriched splicing regulators, including CUGBP1, CUGBP2 and MBNL,<sup>65–67</sup> are responsible for cTnT alternative splicing at different exons. Titin has also been demonstrated to be regulated by alternative splicing during both cardiac development and disease. The inclusion level of the PEVK domain and the alternatively spliced IG domains increases in mature cardiomyocytes, generating the N2B-containing Titin isoform that is critical for cardiomyocytes' passive tension regulation. During early development or heart failure, however, the dominant isoform of Titin switches to the N2BA isoform.<sup>68,69</sup> This alternative splicing switch is a highly conserved splicing event across different species.<sup>45,68,70</sup> A recent study further demonstrated that the splicing regulator RBM20 is responsible for regulating

Titin alternative splicing, and a mutation of RBM20 is associated with human dilated cardiomyopathy.<sup>45,71</sup>

The Bnip3L/Nix gene also produces an alternative splicing variant: sNix, which is localized in both the cytoplasm and the nucleus. On stimulation of tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), sNix rapidly translocates to the nucleus where it activates cardiac gene expression through the nuclear factor  $\kappa$ -B (NF- $\kappa$ B) pathway.<sup>72</sup> Finally, transcription co-regulator PGC-1 $\alpha$  is also subjected to alternative splicing regulation to generate a splicing variant: PGC-1 $\alpha$ 4. Instead of targeting classical PGC-1 $\alpha$  downstream targets, this splicing variant induces expression of insulin-like growth factor and can further stimulate muscle hypertrophy both in vitro and in vivo.<sup>73</sup>

In summary, RNA splicing is a prevailing molecular event in cardiac transcriptome programming and reprogramming during development and pathogenesis. It is an emerging area of research with the advances in high-throughput whole transcriptome profiling. The importance of RNA splicing in cardiac development and diseases is beginning to be recognized. However, our understanding of the molecular nature of the RNA splicing machinery in the heart, the regulatory mechanisms during development and pathogenesis, the molecular targets of RNA splicing as well as their functional impact remains very primitive at this time and need to be further explored in the future.

## RNA Editing in Cardiac Development and Disease

### RNA Editing Machinery

RNA editing refers to post-transcriptional sequence alterations in mature transcripts different from their genomic sequences.<sup>74</sup> The types of RNA editing identified so far include nucleotide insertions, deletions and exchanges. RNA editing can result in amino acid substitutions, alternative splicing and changes in gene expression levels, leading to an expansion of transcriptome complexity.<sup>18,75</sup> RNA editing has been suggested to play important roles during multiple biological processes and diseases, including cancer and the immune response.<sup>76</sup> By targeting RNA editing, novel therapeutic strategies can be formulated to treat genetic disorders.<sup>77,78</sup> With newly developed high-throughput sequencing method and bioinformatics tools, RNA editing is now recognized as a significant phenomena in transcriptome programming during tissue regeneration, including of the heart. Currently, at least 2 major proteins are implicated in RNA editing for the mammalian transcriptome. The ADARs (adenosine deaminases) can convert adenosines to inosines in double-stranded or specific structured regions of RNA.<sup>79,80</sup> Another involves deaminating cytidine to uridine, which is carried out by the APOBEC family of cytidine deaminases.<sup>81–84</sup> At least 2 members of the APBEC family, APOBEC1 and APOBEC2, have C (cytidine) to U (uridine) editing capability. Although APOBEC1 is ubiquitously expressed among multiple tissues, APOBEC2 is a cardiac- and skeletal muscle-specific RNA editing enzyme.<sup>85</sup>

### RNA Editing of Cardiac Genes

The mRNA expression level of ADAR1 is significantly increased in the developing heart and limbs, correlating with increased proliferation and cellular remodeling.<sup>86</sup> In a parallel

study, RNA editing events were found to be significantly increased in children with cyanotic congenital heart disease and it is suggested that A-to-I RNA editing in the MED 13 gene was significantly higher among the cyanotic patients and the RNA level of ADAR2 was reduced.<sup>87</sup> Furthermore, both the expression level and alternative splicing of ADARs are tightly regulated in the heart. ADAR2 is the key enzyme responsible for Q/R site editing in the GluR-B transcript, and ADAR2 itself is regulated by alternative splicing. Recently, a novel alternative splicing variant including part of the ADAR2 intron 7 was found to be highly expressed in heart and skeletal muscle, but not in the brain. The inclusion of this novel exon generates stop codons that potentially can affect the total ADAR2 expression.<sup>88</sup>

One of the key substrates for APOBEC 1 is the translational repressor, NAT1. APOBEC1 deaminates specific cytidine bases in NAT1 to uridine, changing a codon for glutamine into a premature stop codon. In the embryonic heart, NAT1 expression level is high in both atrial and ventricular myocytes based on immunohistochemical staining analysis; however, western blotting showed that the different isoforms of NAT1 generated by RNA editing have distinct expression levels during cardiac development.<sup>89</sup> Interestingly, during cardiac hypertrophy, the mRNA level of NAT1 is significantly increased without an associated increase at the total protein level. However, western blot analysis confirms the presence of different protein species because of RNA editing, providing a possible link between NAT1 RNA editing regulation and cardiac hypertrophy.<sup>90</sup> Another important substrate for APOBEC1 is apolipoprotein B (ApoB). ApoB mRNA editing involves converting a single C into U at the codon of glutamine 2153, resulting in an in-frame stop codon (UAA) and a truncated protein. The RNA editing of ApoB mRNA is tissue-specific and developmentally regulated.<sup>91</sup> In the heart, it is suggested that the regulatory role of APOBEC1 in ApoB editing involves another chaperone regulator, Bcl2-associated athanogene-4 (BAG-4). BAG-4 is predominantly expressed in the heart and brain, and by interacting with both Hsp90 and APOBEC1. BAG-4 functions as a negative regulator for APOBEC1-mediated RNA editing by shuttling APOBEC1 from the nucleus to the cytoplasm.<sup>92</sup> ApoB RNA editing could also be regulated by PKC.<sup>93</sup> Further, it is suggested that, at least in vitro, increasing the extracellular calcium concentration or depleting SR calcium stores is sufficient to change the level of mRNA editing of ApoB.<sup>94</sup> Lastly, the cardiac-enriched splicing regulator, CUGBP2, can also regulate ApoB mRNA editing. According to Anant et al,<sup>95</sup> CUGBP2 forms a complex with APOBEC1 together with another factor, ACF, CUGBP2 binds to the AU-rich sequence located upstream of the edited cytidine in ApoB RNA and inhibits the RNA editing process.

In summary, RNA editing is a newly recognized phenomena in cardiac transcriptome programming and reprogramming. Much of the data so far offers only a correlative indication of its relevance in cardiac development and pathological process. APOBEC1 knockout mice have an abnormal lipoprotein profile because of abnormal editing of ApoB RNA, and APOBEC2 knockout leads to muscle-type switch and myopathy.<sup>96,97</sup> While genetic evidence for other RNA editing enzymes is still lacking, the functional significance of RNA editing in cardiac development and diseases remains to be established.

## Long Noncoding RNA in Cardiac Development and Disease

### Genesis of Long Noncoding RNA

Although the definition of long noncoding RNA (lncRNA) continues to evolve, the generally accepted hallmarks include: (1) longer than 200 nucleotides, (2) no functional open reading frames, and (3) poor conservation at the sequence level.<sup>98</sup> Although the concept of lncRNA was established decades ago, few studies have explored their functional significance beyond X chromosome inactivation and imprinting based on the early discovery of Xist and H19.<sup>99,100</sup> With deep RNA-sequencing and recently developed lncRNA-sensitive microarray technologies, much more information is now available about the expression profiles and biochemical properties of the lncRNAs.<sup>101,102</sup> The identification, annotation and predication of lncRNAs are further facilitated by recent developments in bioinformatics analysis tools,<sup>103</sup> including lncRNAMap and NONCODEv4.<sup>104–106</sup> In contrast to the previous notion that low copies of lncRNA may represent “transcriptional noise” because of aberrant transcriptional initiation,<sup>107</sup> it is increasingly recognized that lncRNAs actually are expressed in a developmental and cell type-specific manner.<sup>108,109</sup> The lncRNAs species are generally classified into 4 categories depending on their relationship to neighboring coding transcripts, including (a) overlapping with annotated gene bodies with transcription initiating from either exons or introns from the sense or (b) antisense strands, (c) lying within the cis-regulatory regions of genes, and (d) lying in the intergenic regions.<sup>110,111</sup> Although lncRNAs are widely detected, it remains controversial to what extent their expressions are functional in development and human diseases.

### lncRNAs in Cardiac Development

The expression of lncRNAs shows distinct profiles in different tissues (eg, brown adipose tissue vs. skeletal muscle)<sup>112</sup> and in different cell types (eg, erythroblasts, megakaryocytes and megakaryocyte-erythroid precursors).<sup>113</sup> Although the sequence conservation of lncRNAs is relatively low compared with mRNAs and miRNAs, multiple evidence suggests that lncRNAs can also be highly conserved at the functional region. In addition to mammals, lncRNAs are also widely expressed and tightly regulated in zebrafish,<sup>114,115</sup> pigs and tetrapods,<sup>116–118</sup> suggesting a potentially highly conserved function among some lncRNA species. The critical role of lncRNAs in development was reported for neurogenesis, involving a large screening approach demonstrating that inactivation of lncRNAs can block human ESC differentiation into mature neurons.<sup>119</sup>

In the heart, microarray and deep RNA-sequencing analyses have revealed a large number of lncRNAs with expression profiles associated with both cardiac development and disease<sup>120–122</sup> (Table). In a recent study,<sup>121</sup> a total of 1,237 lncRNAs were found to have different expression levels during development, with a particular potential effect on developmental processes, metabolic processes and mTOR signaling pathways, according to gene ontology (GO) analysis of neighboring mRNAs. The first lncRNA identified to play a critical role in cardiovascular lineage commitment was Braveheart (Bvht).<sup>123–126</sup> This 590-nt transcript was identified during screening of lncRNAs potentially important for ESC cardiac lineage commitment. Another lncRNA that also controls lineage commitment and cell differentiation in cardiomyocytes is Fendrr.<sup>127,128</sup> As more lncRNAs are discovered in



the heart, their functional role in cardiac development will be expanded beyond gene expression to morphogenesis and growth regulation.

### LncRNAs in Cardiovascular Diseases

The expression profiles of lncRNAs have been demonstrated to be dynamically regulated under different disease conditions.<sup>129–131</sup> In a study by Song et al of human ventricular septal defects (VSD), more than 1,500 lncRNAs showed altered expression associated with the disease state.<sup>132</sup> Moreover, changes in lncRNA expression are significantly associated with the expression of their neighboring genes, suggesting a possible cis-regulatory relationship between lncRNAs and coding mRNAs. A more recent study comparing ischemic and dilated human failing heart samples pre- and post-LVAD (left ventricular assisted device) therapy with non-failing human heart samples using deep RNA-sequencing analysis also revealed a significant number of lncRNAs with dysregulated expression in the diseased hearts and some of these lncRNAs were normalized in post-LVAD hearts.<sup>133</sup> More interestingly, the lncRNA expression profiles in the human failing hearts were found to be a more significant indicator for the different etiologies than the mRNA profiles, suggesting a potential contribution to the different etiologies of heart failure. In addition, lncRNAs detected in the plasma of a mouse heart failure model were also significantly associated with heart failure, and thus may serve as independent biomarkers of the diseases.<sup>120</sup>

Taken together, the dynamic expression of lncRNAs during cardiac development and disease suggests a potential link between them and cardiac pathology (Table). Beyond these correlative analyses, however, the direct contribution of lncRNAs to cardiovascular diseases is only beginning to be revealed.

### Molecular Mechanisms of LncRNA-Mediated Regulation

As lncRNA species proliferate, the variations in the underlying mechanisms have also expanded to involve almost every aspect of gene regulation. By binding directly to the promoter region of coding genes, or facilitating transcription factor or miR binding, the lncRNAs are able to activate/silence expression in both a *cis* and *trans* manner.<sup>134–137</sup>

The lncRNAs can function as a functional sponge for miRNAs to interfere global gene expression.<sup>138</sup> A recently identified lncRNA, cardiac hypertrophy related factor (CHRF),<sup>139</sup> is one of the lncRNAs showing expression changes in AngII-treated NRVM. This 1843-nt lncRNA plays a critical role in the cardiomyocytes' hypertrophic response by interacting directly with miR-489, which in turn regulates Myd88 expression, leading to the activation of NF- $\kappa$ B pathway.

Finally, lncRNAs can act as a scaffold for the histone modification complex, antagonizing the localization and regulatory function of the SWI/SNF chromatin-modifying complex or directly interacting with the polycomb repressive complex 2 (PRC2) and the TrxG/MII complex in order to modify the status of histone methylation and acetylation.<sup>127,140,141</sup> This is one of the most important mechanisms for lncRNA regulation of cell differentiation.<sup>140</sup> In the heart, the lncRNA, Bvht, acts as a molecular scaffold<sup>140,142</sup> by interacting with SUZ12, a core subunit of PRC2, and together the complex regulates the promoter activity of genes

critical for the cardiac lineage, including *MesP1*, *Gata6*, *Hand1* and *Nkx2.5* by changing the histone lysine methylation status. In contrast, *Fendrr* regulates downstream target expression either in *-cis* by binding to *Foxf1* promoter or in *-trans* by binding to the *Pitx2* promoter.

The lncRNAs are a newly recognized transcriptome component with an emerging significance in development and diseases.<sup>143,144</sup> Studies of lncRNAs in the heart have just begun and many questions about lncRNA function, regulation and underlying mechanisms are yet to be addressed (Figure 3). More investigations are needed to demonstrate the diagnostic and therapeutic value of lncRNAs in heart diseases.

## Small Nucleolar RNAs and Cardiovascular Disease

During RNA metabolism, another noncoding RNA species present in the transcriptome is the small nucleolar RNAs (snoRNAs). These are a family of noncoding small RNAs playing important roles in guiding the modification of other noncoding RNAs, including ribosomal, small nuclear and transfer RNAs.<sup>145–147</sup> Although originally considered as a biological byproducts of alternative splicing, the dynamic expression profiles of snoRNAs suggest they could have functional effects in disease, including cancer.<sup>148,149</sup>

The expression of snoRNAs associated with cardiac diseases has been demonstrated at the whole transcriptome level. By comparing right ventricular tissue samples from 16 infants with nonsyndromic tetralogy of Fallot (TOF) and 8 normal samples, more than 100 snoRNA expression profiles were identified to be significantly changed. Interestingly, it should be noted that the expression profiles of snoRNAs in the infants with TOF resembled the expression profiles observed in the fetal myocardium.<sup>150</sup> To date, this is the first evidence of a global change in snoRNA expression associated with cardiovascular disease. As regulation of snoRNA expression in the heart is still poorly studied and the molecular mechanisms for snoRNA functions in cardiovascular diseases remain elusive. snoRNAs in the cardiovascular system should be an interesting area for future investigations.

## Conclusions

Gene regulation has been a central issue in modern molecular biology, and understanding the underlying mechanisms and functional roles is at the core of cardiac research, both in fundamental understanding for cardiac development and in translational investigation of disease mechanisms. With the rapid expansion of transcriptome complexity, the functional paths from genome to phenome have become even more complicated. The emerging roles of RNA splicing, RNA editing, lncRNAs and snoRNAs in the heart add additional intricacy to the regulatory network of cardiac gene expression, and also reveal more ways of potential perturbation in response to pathological stressors (Figure 4). Although much of the work remains to be accomplished, current progress has demonstrated the potential of these new insights of cardiac transcriptome regulation for diagnostic and therapeutic applications. Revealing and understanding cardiac transcriptome complexity should be an important goal for future efforts.

## References

1. Miquerol L, Kelly RG. Organogenesis of the vertebrate heart. *Wiley Interdiscip Rev Dev Biol.* 2013; 2:17–29. [PubMed: 23799628]
2. Bruneau BG. Signaling and transcriptional networks in heart development and regeneration. *Cold Spring Harb Perspect Biol.* 2013; 5:a008292.10.1101/cshperspect.a008292 [PubMed: 23457256]
3. Rana MS, Christoffels VM, Moorman AF. A molecular and genetic outline of cardiac morphogenesis. *Acta Physiol (Oxf).* 2013; 207:588–615. [PubMed: 23297764]
4. Condorelli G, Latronico MV, Cavarretta E. microRNAs in cardiovascular diseases: Current knowledge and the road ahead. *J Am Coll Cardiol.* 2014 Feb 26.10.1016/j.jacc.2014.01.050.
5. Boettger T, Braun T. A new level of complexity: The role of microRNAs in cardiovascular development. *Circ Res.* 2012; 110:1000–1013. [PubMed: 22461364]
6. Da Costa Martins PA, De Windt LJ. MicroRNAs in control of cardiac hypertrophy. *Cardiovasc Res.* 2012; 93:563–572. [PubMed: 22266752]
7. Irimia M, Blencowe BJ. Alternative splicing: Decoding an expansive regulatory layer. *Curr Opin Cell Biol.* 2012; 24:323–332. [PubMed: 22465326]
8. Hallegger M, Llorian M, Smith CW. Alternative splicing: Global insights. *FEBS J.* 2010; 277:856–866. [PubMed: 20082635]
9. Witten JT, Ule J. Understanding splicing regulation through RNA splicing maps. *Trends Genet.* 2011; 27:89–97. [PubMed: 21232811]
10. Kalsotra A, Cooper TA. Functional consequences of developmentally regulated alternative splicing. *Nat Rev Genet.* 2011; 12:715–729. [PubMed: 21921927]
11. David CJ, Manley JL. Alternative pre-mRNA splicing regulation in cancer: pathways and programs unhinged. *Genes Dev.* 2010; 24:2343–2364. [PubMed: 21041405]
12. Ohta S, Nishida E, Yamanaka S, Yamamoto T. Global splicing pattern reversion during somatic cell reprogramming. *Cell Rep.* 2013; 5:357–366. [PubMed: 24139801]
13. Venables JP, Lapasset L, Gadea G, Fort P, Klinck R, Irimia M, et al. MBNL1 and RBFOX2 cooperate to establish a splicing programme involved in pluripotent stem cell differentiation. *Nat Commun.* 2013; 4:2480. [PubMed: 24048253]
14. Choudhury R, Roy SG, Tsai YS, Tripathy A, Graves LM, Wang Z. The splicing activator DAZAP1 integrates splicing control into MEK/Erk-regulated cell proliferation and migration. *Nat Commun.* 2014; 5:3078. [PubMed: 24452013]
15. McManus CJ, Graveley BR. RNA structure and the mechanisms of alternative splicing. *Curr Opin Genet Dev.* 2011; 21:373–379. [PubMed: 21530232]
16. Chen M, Manley JL. Mechanisms of alternative splicing regulation: Insights from molecular and genomics approaches. *Nat Rev Mol Cell Biol.* 2009; 10:741–754. [PubMed: 19773805]
17. Han SP, Tang YH, Smith R. Functional diversity of the hnRNPs: Past, present and perspectives. *Biochem J.* 2010; 430:379–392. [PubMed: 20795951]
18. Licatalosi DD, Darnell RB. Applications of next-generation sequencing RNA processing and its regulation: Global insights into biological networks. *Nat Rev Genet.* 2010; 11:75–87. [PubMed: 20019688]
19. Shepard PJ, Hertel KJ. The SR protein family. *Genome Biol.* 2009; 10:242. [PubMed: 19857271]
20. Xiao X, Lee JH. Systems analysis of alternative splicing and its regulation. *Wiley Interdiscip Rev Syst Biol Med.* 2010; 2:550–565. [PubMed: 20836047]
21. Mathieu O, Bouche N. Interplay between chromatin and RNA processing. *Curr Opin Plant Biol.* 2014; 18C:60–65. [PubMed: 24631845]
22. Zlotorynski E. Transcription: Splicing keeps RNA polymerase II in check. *Nat Rev Mol Cell Biol.* 2014; 15:222. [PubMed: 24622615]
23. Yap K, Makeyev EV. Regulation of gene expression in mammalian nervous system through alternative pre-mRNA splicing coupled with RNA quality control mechanisms. *Mol Cell Neurosci.* 2013; 56:420–428. [PubMed: 23357783]

24. Takemura R, Takeiwa T, Taniguchi I, McCloskey A, Ohno M. Multiple factors in the early splicing complex are involved in the nuclear retention of pre-mRNAs in mammalian cells. *Genes Cells*. 2011; 16:1035–1049. [PubMed: 21929696]
25. Escudero-Paunetto L, Li L, Hernandez FP, Sandri-Goldin RM. SR proteins SRp20 and 9G8 contribute to efficient export of herpes simplex virus 1 mRNAs. *Virology*. 2010; 401:155–164. [PubMed: 20227104]
26. Salomonis N, Nelson B, Vranizan K, Pico AR, Hanspers K, Kuchinsky A, et al. Alternative splicing in the differentiation of human embryonic stem cells into cardiac precursors. *PLoS Comput Biol*. 2009; 5:e1000553.10.1371/journal.pcbi.1000553.
27. Salomonis N, Schlieve CR, Pereira L, Wahlquist C, Colas A, Zambon AC, et al. Alternative splicing regulates mouse embryonic stem cell pluripotency and differentiation. *Proc Natl Acad Sci USA*. 2010; 107:10514–10519. [PubMed: 20498046]
28. Kalsotra A, Wang K, Li PF, Cooper TA. MicroRNAs coordinate an alternative splicing network during mouse postnatal heart development. *Genes Dev*. 2010; 24:653–658. [PubMed: 20299448]
29. Vitting-Seerup K, Porse BT, Sandelin A, Waage J. spliceR: An R package for classification of alternative splicing and prediction of coding potential from RNA-seq data. *BMC Bioinformatics*. 2014; 15:81. [PubMed: 24655717]
30. Gatto A, Torroja-Fungairino C, Mazzarotto F, Cook SA, Barton PJ, Sanchez-Cabo F, et al. FineSplice, enhanced splice junction detection and quantification: A novel pipeline based on the assessment of diverse RNA-Seq alignment solutions. *Nucleic Acids Res*. 2014 Feb 25.10.1093/nar/gku166.
31. Boley N, Stoiber MH, Booth BW, Wan KH, Hoskins RA, Bickel PJ, et al. Genome-guided transcript assembly by integrative analysis of RNA sequence data. *Nat Biotechnol*. 2014; 32:341–346. [PubMed: 24633242]
32. Kalsotra A, Xiao X, Ward AJ, Castle JC, Johnson JM, Burge CB, et al. A postnatal switch of CELF and MBNL proteins reprograms alternative splicing in the developing heart. *Proc Natl Acad Sci USA*. 2008; 105:20333–20338. [PubMed: 19075228]
33. Ladd AN, Stenberg MG, Swanson MS, Cooper TA. Dynamic balance between activation and repression regulates pre-mRNA alternative splicing during heart development. *Dev Dyn*. 2005; 233:783–793. [PubMed: 15830352]
34. Ames EG, Lawson MJ, Mackey AJ, Holmes JW. Sequencing of mRNA identifies re-expression of fetal splice variants in cardiac hypertrophy. *J Mol Cell Cardiol*. 2013; 62:99–107. [PubMed: 23688780]
35. Lee JH, Gao C, Peng G, Greer C, Ren S, Wang Y, et al. Analysis of transcriptome complexity through RNA sequencing in normal and failing murine hearts. *Circ Res*. 2011; 109:1332–1341. [PubMed: 22034492]
36. Park JY, Li W, Zheng D, Zhai P, Zhao Y, Matsuda T, et al. Comparative analysis of mRNA isoform expression in cardiac hypertrophy and development reveals multiple post-transcriptional regulatory modules. *PLoS One*. 2011; 6:e22391.10.1371/journal.pone.0022391
37. Kim T, Kim JO, Oh JG, Hong SE, Kim do H. Pressure-overload cardiac hypertrophy is associated with distinct alternative splicing due to altered expression of splicing factors. *Mol Cells*. 2014; 37:81–87. [PubMed: 24552714]
38. Joehanes R, Ying S, Huan T, Johnson AD, Raghavachari N, Wang R, et al. Gene expression signatures of coronary heart disease. *Arterioscler Thromb Vasc Biol*. 2013; 33:1418–1426. [PubMed: 23539218]
39. Ricci M, Xu Y, Hammond HL, Willoughby DA, Nathanson L, Rodriguez MM, et al. Myocardial alternative RNA splicing and gene expression profiling in early stage hypoplastic left heart syndrome. *PLoS One*. 2012; 7:e29784.10.1371/journal.pone.0029784 [PubMed: 22299024]
40. Gao G, Xie A, Huang SC, Zhou A, Zhang J, Herman AM, et al. Role of RBM25/LUC7L3 in abnormal cardiac sodium channel splicing regulation in human heart failure. *Circulation*. 2011; 124:1124–1131. [PubMed: 21859973]
41. Xu X, Yang D, Ding JH, Wang W, Chu PH, Dalton ND, et al. ASF/SF2-regulated CaMKII $\delta$  alternative splicing temporally reprograms excitation-contraction coupling in cardiac muscle. *Cell*. 2005; 120:59–72. [PubMed: 15652482]

42. Ding JH, Xu X, Yang D, Chu PH, Dalton ND, Ye Z, et al. Dilated cardiomyopathy caused by tissue-specific ablation of SC35 in the heart. *EMBO J*. 2004; 23:885–896. [PubMed: 14963485]
43. Kanadia RN, Johnstone KA, Mankodi A, Lungu C, Thornton CA, Esson D, et al. A muscleblind knockout model for myotonic dystrophy. *Science*. 2003; 302:1978–1980. [PubMed: 14671308]
44. Guo W, Pleitner JM, Saupé KW, Greaser ML. Pathophysiological defects and transcriptional profiling in the RBM20<sup>-/-</sup> rat model. *PLoS One*. 2013; 8:e84281.10.1371/journal.pone.0084281 [PubMed: 24367651]
45. Guo W, Schafer S, Greaser ML, Radke MH, Liss M, Govindarajan T, et al. RBM20, a gene for hereditary cardiomyopathy, regulates titin splicing. *Nat Med*. 2012; 18:766–773. [PubMed: 22466703]
46. Yao JSH, Lu XC, Gu QQ, Zhu JH. Metoprolol attenuates pressure overload-induced myocardial hypertrophy through modulating Dryk1A-ASF-CaMKII $\delta$  signaling pathways. *Zhonghua Xin Xue Guan Bing Za Zhi*. 2013; 41:1029–1033. [PubMed: 24524606]
47. El Mabrouk M, Diep QN, Benkirane K, Touyz RM, Schiffrin EL. SAM68: A downstream target of angiotensin II signaling in vascular smooth muscle cells in genetic hypertension. *Am J Physiol Heart Circ Physiol*. 2004; 286:H1954–H1962. [PubMed: 14693677]
48. Huot ME, Vogel G, Zabarauskas A, Ngo CT, Coulombe-Huntington J, Majewski J, et al. The Sam68 STAR RNA-binding protein regulates mTOR alternative splicing during adipogenesis. *Mol Cell*. 2012; 46:187–199. [PubMed: 22424772]
49. Pedrotti S, Bielli P, Paronetto MP, Ciccocanti F, Fimia GM, Stamm S, et al. The splicing regulator Sam68 binds to a novel exonic splicing silencer and functions in SMN2 alternative splicing in spinal muscular atrophy. *EMBO J*. 2010; 29:1235–1247. [PubMed: 20186123]
50. Shimada N, Rios I, Moran H, Sayers B, Hubbard K. p38 MAP kinase-dependent regulation of the expression level and subcellular distribution of heterogeneous nuclear ribonucleoprotein A1 and its involvement in cellular senescence in normal human fibroblasts. *RNA Biol*. 2009; 6:293–304. [PubMed: 19430204]
51. Revil T, Toutant J, Shkreta L, Garneau D, Cloutier P, Chabot B. Protein kinase C-dependent control of Bcl-x alternative splicing. *Mol Cell Biol*. 2007; 27:8431–8441. [PubMed: 17923691]
52. Zhao Y, Koebis M, Suo S, Ohno S, Ishiura S. Regulation of the alternative splicing of sarcoplasmic reticulum Ca(2+)-ATPase 1 (SERCA1) by phorbol 12-myristate 13-acetate (PMA) via a PKC pathway. *Biochem Biophys Res Commun*. 2012; 423:212–217. [PubMed: 22609207]
53. Zara S, Bosco D, Di Giulio C, Antonucci A, Cataldi A. Protein kinase Calpha early activates splicing factor SC-35 during postnatal rat heart development. *J Biol Regul Homeost Agents*. 2009; 23:45–54. [PubMed: 19321046]
54. Zara SFM, Rapino M, Zago M, Orsini G, Mazzotti G, Cataldi A, Teti G. pPKC $\delta$  activates SC35 splicing factor during H9c2 myoblastic differentiation. *Histol Histopathol*. 2011; 26:59–69. [PubMed: 21117027]
55. Cataldi A, Zingariello M, Rapino M, Zara S, Daniele F, Di Giulio C, et al. Effect of hypoxia and aging on PKC delta-mediated SC-35 phosphorylation in rat myocardial tissue. *Anat Rec (Hoboken)*. 2009; 292:1135–1142. [PubMed: 19645017]
56. Verma SK, Deshmukh V, Liu P, Nutter CA, Espejo R, Hung ML, et al. Reactivation of fetal splicing programs in diabetic hearts is mediated by protein kinase C signaling. *J Biol Chem*. 2013; 288:35372–35386. [PubMed: 24151077]
57. van Stuijvenberg L, Yildirim C, Kok BG, van Veen TA, Varro A, Winckels SK, et al. Alternative promoter usage and splicing of the human SCN5A gene contribute to transcript heterogeneity. *DNA Cell Biol*. 2010; 29:577–587. [PubMed: 20618077]
58. Murphy LL, Moon-Grady AJ, Cuneo BF, Wakai RT, Yu S, Kunic JD, et al. Developmentally regulated SCN5A splice variant potentiates dysfunction of a novel mutation associated with severe fetal arrhythmia. *Heart Rhythm*. 2012; 9:590–597. [PubMed: 22064211]
59. Gao G, Dudley SC Jr. SCN5A splicing variants and the possibility of predicting heart failure-associated arrhythmia. *Expert Rev Cardiovasc Ther*. 2013; 11:117–119. [PubMed: 23405830]
60. Wahbi K, Algalarrondo V, Becane HM, Fressart V, Beldjord C, Azibi K, et al. Brugada syndrome and abnormal splicing of SCN5A in myotonic dystrophy type 1. *Arch Cardiovasc Dis*. 2013; 106:635–643. [PubMed: 24140416]

61. Gao G, Dudley SC Jr. RBM25/LUC7L3 function in cardiac sodium channel splicing regulation of human heart failure. *Trends Cardiovasc Med*. 2013; 23:5–8. [PubMed: 22939879]
62. Yamada Y, Chen X, Kobayashi T, Kamada Y, Nagashima M, Tsutsuura M, et al. A truncated splice variant of KCNQ1 cloned from rat heart. *Biochem Biophys Res Commun*. 2002; 294:199–204. [PubMed: 12051693]
63. Mohammad-Panah R, Demolombe S, Neyroud N, Guicheney P, Kyndt F, van den Hoff M, et al. Mutations in a dominant-negative isoform correlate with phenotype in inherited cardiac arrhythmias. *Am J Hum Genet*. 1999; 64:1015–1023. [PubMed: 10090886]
64. Biesiadecki BJ, Elder BD, Yu ZB, Jin JP. Cardiac troponin T variants produced by aberrant splicing of multiple exons in animals with high instances of dilated cardiomyopathy. *J Biol Chem*. 2002; 277:50275–50285. [PubMed: 12377784]
65. Warf MB, Berglund JA. MBNL binds similar RNA structures in the CUG repeats of myotonic dystrophy and its pre-mRNA substrate cardiac troponin T. *RNA*. 2007; 13:2238–2251. [PubMed: 17942744]
66. Ho TH, Bundman D, Armstrong DL, Cooper TA. Transgenic mice expressing CUG-BP1 reproduce splicing mis-regulation observed in myotonic dystrophy. *Hum Mol Genet*. 2005; 14:1539–1547. [PubMed: 15843400]
67. Goo YH, Cooper TA. CUGBP2 directly interacts with U217S snRNP components and promotes U2 snRNA binding to cardiac troponin T pre-mRNA. *Nucleic Acids Res*. 2009; 37:4275–4286. [PubMed: 19443441]
68. Warren CM, Krzesinski PR, Campbell KS, Moss RL, Greaser ML. Titin isoform changes in rat myocardium during development. *Mech Dev*. 2004; 121:1301–1312. [PubMed: 15454261]
69. Guo W, Bharmal SJ, Esbona K, Greaser ML. Titin diversity--alternative splicing gone wild. *J Biomed Biotechnol*. 2010 Mar 21.10.1155/2010/753675.
70. Nelson OL, Robbins CT, Wu Y, Granzier H. Titin isoform switching is a major cardiac adaptive response in hibernating grizzly bears. *Am J Physiol Heart Circ Physiol*. 2008; 295:H366–H371. [PubMed: 18502907]
71. Li S, Guo W, Dewey CN, Greaser ML. Rbm20 regulates titin alternative splicing as a splicing repressor. *Nucleic Acids Res*. 2013; 41:2659–2672. [PubMed: 23307558]
72. Chen Y, Decker KF, Zheng D, Matkovich SJ, Jia L, Dorn GW 2nd. A nucleus-targeted alternately spliced Nix/Bnip3L protein isoform modifies nuclear factor kappaB (NFkappaB)-mediated cardiac transcription. *J Biol Chem*. 2013; 288:15455–15465. [PubMed: 23603904]
73. Ruas JL, White JP, Rao RR, Kleiner S, Brannan KT, Harrison BC, et al. A PGC-1 $\alpha$  isoform induced by resistance training regulates skeletal muscle hypertrophy. *Cell*. 2012; 151:1319–1331. [PubMed: 23217713]
74. Chateigner-Boutin AL, Small I. Organellar RNA editing. *Wiley Interdiscip Rev RNA*. 2011; 2:493–506. [PubMed: 21957039]
75. Farajollahi S, Maas S. Molecular diversity through RNA editing: A balancing act. *Trends Genet*. 2010; 26:221–230. [PubMed: 20395010]
76. Galeano F, Tomaselli S, Locatelli F, Gallo A. A-to-I RNA editing: The “ADAR” side of human cancer. *Semin Cell Dev Biol*. 2012; 23:244–250. [PubMed: 21930228]
77. Decher N, Netter MF, Streit AK. Putative impact of RNA editing on drug discovery. *Chem Biol Drug Des*. 2013; 81:13–21. [PubMed: 23253127]
78. Athanasiadis A. Zalpha-domains: At the intersection between RNA editing and innate immunity. *Semin Cell Dev Biol*. 2012; 23:275–280. [PubMed: 22085847]
79. Pullirsch D, Jantsch MF. Proteome diversification by adenosine to inosine RNA editing. *RNA Biol*. 2010; 7:205–212. [PubMed: 20200492]
80. Nishikura K. Functions and regulation of RNA editing by ADAR deaminases. *Annu Rev Biochem*. 2010; 79:321–349. [PubMed: 20192758]
81. Conticello SG. The AID/APOBEC family of nucleic acid mutators. *Genome Biol*. 2008; 9:229. [PubMed: 18598372]
82. Conticello SG, Thomas CJ, Petersen-Mahrt SK, Neuberger MS. Evolution of the AID/APOBEC family of polynucleotide (deoxy) cytidine deaminases. *Mol Biol Evol*. 2005; 22:367–377. [PubMed: 15496550]

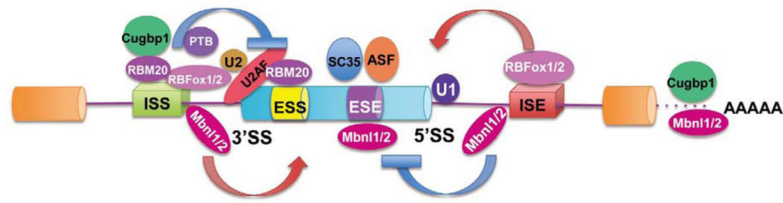
83. Roberts SA, Lawrence MS, Klimczak LJ, Grimm SA, Fargo D, Stojanov P, et al. An APOBEC cytidine deaminase mutagenesis pattern is widespread in human cancers. *Nat Genet.* 2013; 45:970–976. [PubMed: 23852170]
84. Smith HC, Bennett RP, Kizilyer A, McDougall WM, Prohaska KM. Functions and regulation of the APOBEC family of proteins. *Semin Cell Dev Biol.* 2012; 23:258–268. [PubMed: 22001110]
85. Liao W, Hong SH, Chan BH, Rudolph FB, Clark SC, Chan L. APOBEC-2, a cardiac- and skeletal muscle specific member of the cytidine deaminase supergene family. *Biochem Biophys Res Commun.* 1999; 260:398–404. [PubMed: 10403781]
86. Witman NM, Behm M, Ohman M, Morrison JI. ADAR-related activation of adenosine-to-inosine RNA editing during regeneration. *Stem Cells Dev.* 2013; 22:2254–2267. [PubMed: 23534823]
87. Borik S, Simon AJ, Nevo-Caspi Y, Mishali D, Amariglio N, Rechavi G, et al. Increased RNA editing in children with cyanotic congenital heart disease. *Intensive Care Med.* 2011; 37:1664–1671. [PubMed: 21720910]
88. Agranat L, Sperling J, Sperling R. A novel tissue-specific alternatively spliced form of the A-to-I RNA editing enzyme ADAR2. *RNA Biol.* 2010; 7:253–262. [PubMed: 20215858]
89. Pak BJ, Pang SC. Developmental regulation of the translational repressor NAT1 during cardiac development. *J Mol Cell Cardiol.* 1999; 31:1717–1724. [PubMed: 10471355]
90. Sangaralingham SJ, Pak BJ, Tse MY, Angelis E, Adams MA, Smallegange C, et al. Expression of the translational repressor NAT1 in experimental models of cardiac hypertrophy. *Mol Cell Biochem.* 2003; 245:183–190. [PubMed: 12708758]
91. Funahashi T, Giannoni F, DePaoli AM, Skarosi SF, Davidson NO. Tissue-specific, developmental and nutritional regulation of the gene encoding the catalytic subunit of the rat apolipoprotein B mRNA editing enzyme: Functional role in the modulation of apoB mRNA editing. *J Lipid Res.* 1995; 36:414–428. [PubMed: 7775854]
92. Lau PP, Chan L. Involvement of a chaperone regulator, Bcl2-associated athanogene-4, in apolipoprotein B mRNA editing. *J Biol Chem.* 2003; 278:52988–52996. [PubMed: 14559896]
93. Chen ZG, Eggerman TL, Patterson AP. Phosphorylation is a regulatory mechanism in apolipoprotein B mRNA editing. *Biochem J.* 2001; 357:661–672. [PubMed: 11463337]
94. Chen Z, Eggerman TL, Potosky D, Arborati M, Patterson AP. Calcium increases apolipoprotein B mRNA editing. *Biochem Biophys Res Commun.* 2000; 277:221–227. [PubMed: 11027667]
95. Anant S, Henderson JO, Mukhopadhyay D, Navaratnam N, Kennedy S, Min J, et al. Novel role for RNA-binding protein CUGBP2 in mammalian RNA editing: CUGBP2 modulates C to U editing of apolipoprotein B mRNA by interacting with apobec-1 and ACF, the apobec-1 complementation factor. *J Biol Chem.* 2001; 276:47338–47351. [PubMed: 11577082]
96. Sato Y, Probst HC, Tatsumi R, Ikeuchi Y, Neuberger MS, Rada C. Deficiency in APOBEC2 leads to a shift in muscle fiber type, diminished body mass, and myopathy. *J Biol Chem.* 2010; 285:7111–7118. [PubMed: 20022958]
97. Nakamuta M, Chang BH, Zsigmond E, Kobayashi K, Lei H, Ishida BY, et al. Complete phenotypic characterization of apobec-1 knockout mice with a wild-type genetic background and a human apolipoprotein B transgenic background, and restoration of apolipoprotein B mRNA editing by somatic gene transfer of Apobec-1. *J Biol Chem.* 1996; 271:25981–25988. [PubMed: 8824235]
98. Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, et al. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. *Nature.* 2009; 458:223–227. [PubMed: 19182780]
99. Penny GD, Kay GF, Sheardown SA, Rastan S, Brockdorff N. Requirement for Xist in X chromosome inactivation. *Nature.* 1996; 379:131–137. [PubMed: 8538762]
100. Leighton PA, Ingram RS, Eggenschwiler J, Efstratiadis A, Tilghman SM. Disruption of imprinting caused by deletion of the H19 gene region in mice. *Nature.* 1995; 375:34–39. [PubMed: 7536897]
101. Huang W, Long N, Khatib H. Genome-wide identification and initial characterization of bovine long non-coding RNAs from EST data. *Anim Genet.* 2012; 43:674–682. [PubMed: 22497321]
102. Pang KC, Dinger ME, Mercer TR, Malquori L, Grimmond SM, Chen W, et al. Genome-wide identification of long noncoding RNAs in CD8+ T cells. *J Immunol.* 2009; 182:7738–7748. [PubMed: 19494298]

103. Liu MX, Chen X, Chen G, Cui QH, Yan GY. A computational framework to infer human disease-associated long noncoding RNAs. *PLoS One*. 2014; 9:e84408.10.1371/journal.pone.0084408 [PubMed: 24392133]
104. Chan WL, Huang HD, Chang JG. IncRNAMap: A map of putative regulatory functions in the long non-coding transcriptome. *Comput Biol Chem*. 2014 Jan 23.10.1016/j.compbiolchem.2014.01.003.
105. Yang X, Gao L, Guo X, Shi X, Wu H, Song F, et al. A network based method for analysis of lncRNA-disease associations and prediction of lncRNAs implicated in diseases. *PLoS One*. 2014; 9:e87797.10.1371/journal.pone.0087797 [PubMed: 24498199]
106. Xie C, Yuan J, Li H, Li M, Zhao G, Bu D, et al. NONCODEv4: Exploring the world of long non-coding RNA genes. *Nucleic Acids Res*. 2014; 42:D98–D103. [PubMed: 24285305]
107. Louro R, Smirnova AS, Verjovski-Almeida S. Long intronic non-coding RNA transcription: Expression noise or expression choice? *Genomics*. 2009; 93:291–298. [PubMed: 19071207]
108. Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. *Cell*. 2009; 136:629–641. [PubMed: 19239885]
109. Dong R, Jia D, Xue P, Cui X, Li K, Zheng S, et al. Genome-wide analysis of long noncoding RNA (lncRNA) expression in hepatoblastoma tissues. *PLoS One*. 2014; 9:e85599.10.1371/journal.pone.0085599 [PubMed: 24465615]
110. Yang L, Froberg JE, Lee JT. Long noncoding RNAs: Fresh perspectives into the RNA world. *Trends Biochem Sci*. 2014; 39:35–43. [PubMed: 24290031]
111. Guttman M, Rinn JL. Modular regulatory principles of large non-coding RNAs. *Nature*. 2012; 482:339–346. [PubMed: 22337053]
112. Zhang J, Cui X, Shen Y, Pang L, Zhang A, Fu Z, et al. Distinct expression profiles of lncRNAs between brown adipose tissue and skeletal muscle. *Biochem Biophys Res Commun*. 2014; 443:1028–1034. [PubMed: 24365148]
113. Paralkar VR, Mishra T, Luan J, Yao Y, Kossenkov AV, Anderson SM, et al. Lineage and species-specific long noncoding RNAs during erythro-megakaryocytic development. *Blood*. 2014; 123:1927–1937. [PubMed: 24497530]
114. Pauli A, Valen E, Lin MF, Garber M, Vastenhouw NL, Levin JZ, et al. Systematic identification of long noncoding RNAs expressed during zebrafish embryogenesis. *Genome Res*. 2012; 22:577–591. [PubMed: 22110045]
115. Ulitsky I, Shkumatava A, Jan CH, Sive H, Bartel DP. Conserved function of lincRNAs in vertebrate embryonic development despite rapid sequence evolution. *Cell*. 2011; 147:1537–1550. [PubMed: 22196729]
116. Necusulea A, Soumillon M, Wamefors M, Liechti A, Daish T, Zeller U, et al. The evolution of lncRNA repertoires and expression patterns in tetrapods. *Nature*. 2014; 505:635–640. [PubMed: 24463510]
117. Du ZQ, Easley CJ, Onteru SK, Madsen O, Groenen MA, Ross JW, et al. Identification of species-specific novel transcripts in pig reproductive tissues using RNA-seq. *Anim Genet*. 2014; 45:198–204. [PubMed: 24450499]
118. Kaushik K, Leonard VE, Kv S, Lalwani MK, Jalali S, Patowary A, et al. Dynamic expression of long non-coding RNAs (lncRNAs) in adult zebrafish. *PLoS One*. 2013; 8:e83616.10.1371/journal.pone.0083616 [PubMed: 24391796]
119. Ng SY, Johnson R, Stanton LW. Human long non-coding RNAs promote pluripotency and neuronal differentiation by association with chromatin modifiers and transcription factors. *EMBO J*. 2012; 31:522–533. [PubMed: 22193719]
120. Li D, Chen G, Yang J, Fan X, Gong Y, Xu G, et al. Transcriptome analysis reveals distinct patterns of long noncoding RNAs in heart and plasma of mice with heart failure. *PLoS One*. 2013; 8:e77938.10.1371/journal.pone.0077938 [PubMed: 24205036]
121. Zhu JG, Shen YH, Liu HL, Liu M, Shen YQ, Kong XQ, et al. Long noncoding RNAs expression profile of the developing mouse heart. *J Cell Biochem*. 2014; 115:910–918. [PubMed: 24375461]
122. Korostowski L, Sedlak N, Engel N. The Kcnq1 long non-coding RNA affects chromatin conformation and expression of Kcnq1, but does not regulate its imprinting in the developing heart. *PLoS Genet*. 2012; 8:e1002956.10.1371/journal.pgen.1002956. [PubMed: 23028363]



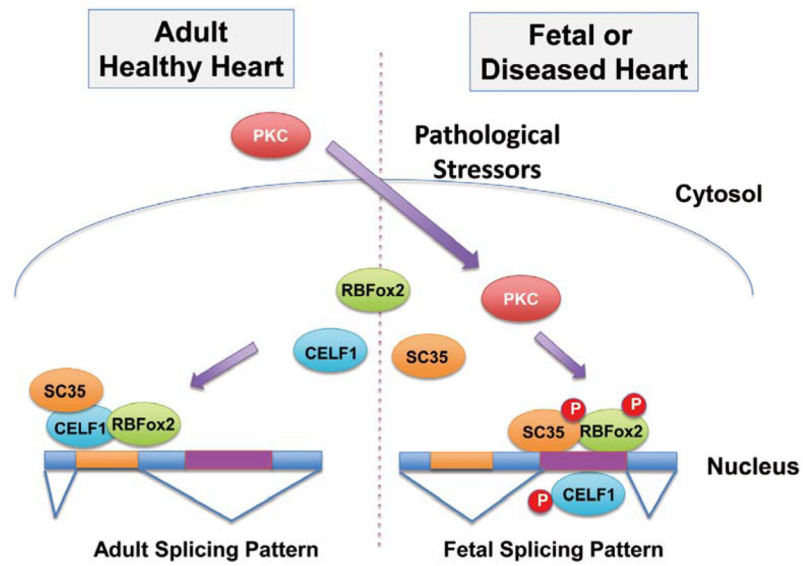
123. Hambridge JA, Turner A, Baker AL. BraveHeart begins: Pilot results of group cognitive behaviour therapy for depression and anxiety in cardiac patients. *Aust NZ J Psychiatry*. 2009; 43:1171–1177.
124. Klattenhoff CA, Scheuermann JC, Surface LE, Bradley RK, Fields PA, Steinhauser ML, et al. Braveheart, a long noncoding RNA required for cardiovascular lineage commitment. *Cell*. 2013; 152:570–583. [PubMed: 23352431]
125. Srivastava D, Cordes Metzler KR. Fending for a Braveheart. *EMBO J*. 2013; 32:1211–1213. [PubMed: 23524853]
126. Kataoka M, Huang ZP, Wang DZ. Build a braveheart: The missing linc (RNA). *Circ Res*. 2013; 112:1532–1534. [PubMed: 23743224]
127. Grote P, Herrmann BG. The long non-coding RNA Fendrr links epigenetic control mechanisms to gene regulatory networks in mammalian embryogenesis. *RNA Biology*. 2013; 10:1579–1585. [PubMed: 24036695]
128. Grote P, Wittler L, Hendrix D, Koch F, Wahrlich S, Beisaw A, et al. The tissue-specific lncRNA Fendrr is an essential regulator of heart and body wall development in the mouse. *Dev Cell*. 2013; 24:206–214. [PubMed: 23369715]
129. Prensner JR, Chen W, Iyer MK, Cao Q, Ma T, Han S, et al. PCAT-1, a long noncoding RNA, regulates BRCA2 and controls homologous recombination in cancer. *Cancer Res*. 2014; 74:1651–1660. [PubMed: 24473064]
130. Yang F, Zhang H, Mei Y, Wu M. Reciprocal regulation of HIF-1 alpha and lincRNA-p21 modulates the Warburg effect. *Mol Cell*. 2014; 53:88–100. [PubMed: 24316222]
131. Chen FJ, Sun M, Li SQ, Wu QQ, Ji L, Liu ZL, et al. Upregulation of the long non-coding RNA HOTAIR promotes esophageal squamous cell carcinoma metastasis and poor prognosis. *Mol Carcinog*. 2013; 52:908–915. [PubMed: 24151120]
132. Song G, Shen Y, Zhu J, Liu H, Liu M, Shen YQ, et al. Integrated analysis of dysregulated lncRNA expression in fetal cardiac tissues with ventricular septal defect. *PLoS One*. 2013; 8:e77492.10.1371/journal.pone.0077492 [PubMed: 24147006]
133. Yang KC, Yamada KA, Patel AY, Topkara VK, George I, Cheema FH, et al. Deep RNA sequencing reveals dynamic regulation of myocardial noncoding RNAs in failing human heart and remodeling with mechanical circulatory support. *Circulation*. 2014; 129:1009–1021. [PubMed: 24429688]
134. Vance KW, Sansom SN, Lee S, Chalei V, Kong L, Cooper SE, et al. The long non-coding RNA Paupar regulates the expression of both local and distal genes. *EMBO J*. 2014; 33:296–311. [PubMed: 24488179]
135. Ng SY, Bogu GK, Soh BS, Stanton LW. The long noncoding RNA RMST interacts with SOX2 to regulate neurogenesis. *Mol Cell*. 2013; 51:349–359. [PubMed: 23932716]
136. Zhao X, Tang Z, Zhang H, Atianjoh FE, Zhao JY, Liang L, et al. A long noncoding RNA contributes to neuropathic pain by silencing Kcna2 in primary afferent neurons. *Nat Neurosci*. 2013; 16:1024–1031. [PubMed: 23792947]
137. Tripathi V, Shen Z, Chakraborty A, Giri S, Freier SM, Wu X, et al. Long noncoding RNA MALAT1 controls cell cycle progression by regulating the expression of oncogenic transcription factor B-MYB. *PLoS Genet*. 2013; 9:e1003368.10.1371/journal.pgen.1003368 [PubMed: 23555285]
138. Kallen AN, Zhou XB, Xu J, Qiao C, Ma J, Yan L, et al. The imprinted H19 lncRNA antagonizes let-7 microRNAs. *Mol Cell*. 2013; 52:101–112. [PubMed: 24055342]
139. Wang K, Liu F, Zhou LY, Long B, Yuan SM, Wang Y, et al. A Long noncoding RNA, CHRF regulates cardiac hypertrophy by targeting miR-489. *Circ Res*. 2014 Feb 20.10.1161/circres-saha.114.302476.
140. Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, et al. Long noncoding RNA as modular scaffold of histone modification complexes. *Science*. 2010; 329:689–693. [PubMed: 20616235]
141. Prensner JR, Iyer MK, Sahu A, Asangani IA, Cao Q, Patel L, et al. The long noncoding RNA SCHLAP1 promotes aggressive prostate cancer and antagonizes the SWI/SNF complex. *Nat Genet*. 2013; 45:1392–1398. [PubMed: 24076601]

142. Guttman M, Donaghey J, Carey BW, Garber M, Grenier JK, Munson G, et al. lincRNAs act in the circuitry controlling pluripotency and differentiation. *Nature*. 2011; 477:295–300. [PubMed: 21874018]
143. Maeda N, Kasukawa T, Oyama R, Gough J, Frith M, Engstrom PG, et al. Transcript annotation in FANTOM3: Mouse gene catalog based on physical cDNAs. *PLoS Genet*. 2006; 2:e62.10.1371/journal.pgen.0020062 [PubMed: 16683036]
144. Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, et al. Landscape of transcription in human cells. *Nature*. 2012; 489:101–108. [PubMed: 22955620]
145. Decatur WA, Fournier MJ. rRNA modifications and ribosome function. *Trends Biochem Sci*. 2002; 27:344–351. [PubMed: 12114023]
146. Darzacq X, Jady BE, Verheggen C, Kiss AM, Bertrand E, Kiss T. Cajal body-specific small nuclear RNAs: A novel class of 2'-O-methylation and pseudouridylation guide RNAs. *EMBO J*. 2002; 21:2746–2756. [PubMed: 12032087]
147. Clouet d'Orval B, Bortolin ML, Gaspin C, Bachellerie JP. Box C/D RNA guides for the ribose methylation of archaeal tRNAs: The tRNA Trp intron guides the formation of two ribose-methylated nucleosides in the mature tRNA Trp. *Nucleic Acids Res*. 2001; 29:4518–4529. [PubMed: 11713301]
148. Ronchetti D, Mosca L, Cutrona G, Tuana G, Gentile M, Fabris S, et al. Small nucleolar RNAs as new biomarkers in chronic lymphocytic leukemia. *BMC Med Genomics*. 2013; 6:27. [PubMed: 24004562]
149. Su H, Xu T, Ganapathy S, Shadfan M, Long M, Huang THM, et al. Elevated snoRNA biogenesis is essential in breast cancer. *Oncogene*. 2013; 33:1348–1358. [PubMed: 23542174]
150. O'Brien JE Jr, Kibiryeva N, Zhou XG, Marshall JA, Lofland GK, Artman M, et al. Noncoding RNA expression in myocardium from infants with tetralogy of Fallot. *Circ Cardiovasc Genet*. 2012; 5:279–286. [PubMed: 22528145]

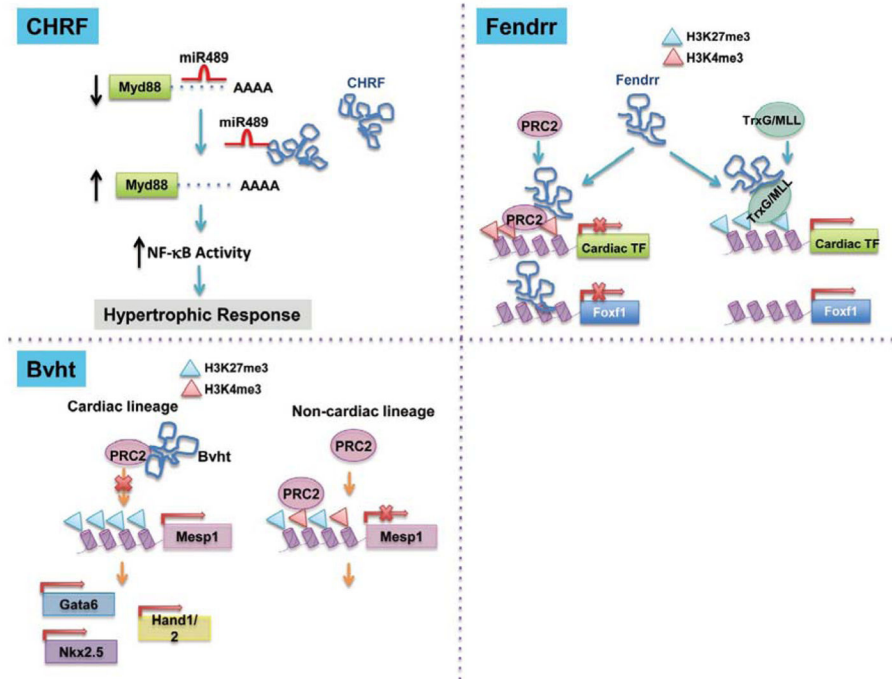


**Figure 1.**

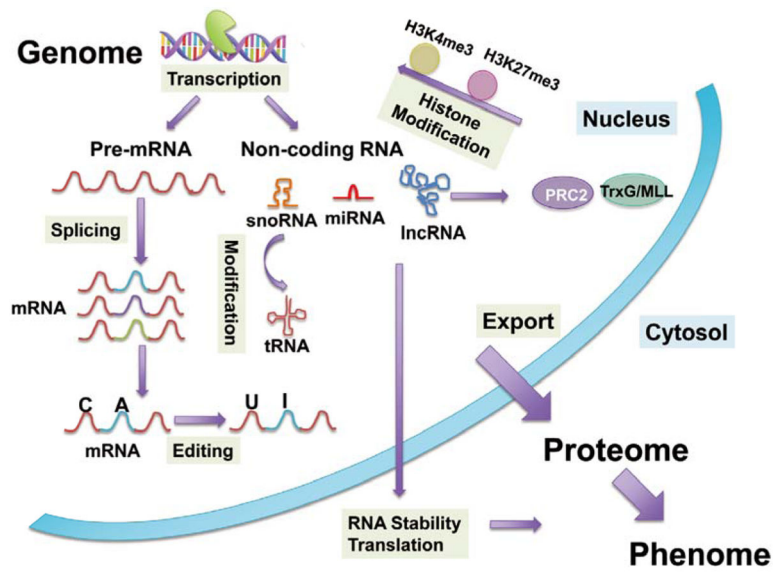
Illustration of key splicing regulators in heart. A representative alternatively spliced exon with ISS (intron splicing silence), ESS (exon splicing silence), ESE (exon splicing enhancer) and ISE (intron splicing enhancer) is shown. *Trans-acting* splicing regulators that have been implicated in cardiac gene regulation include: CUGBP1/2, MBNL1/2, RBFox-1/2, RBM20, PTB, SC35, ASF. Constitutive RNA splicing factors illustrated include U1/2, U2AF. Arrows indicate enhancing alternative splicing, and block indicates repressing alternative splicing of the neighboring exon.



**Figure 2.** PKC-mediated regulation of alternative splicing in cardiac development and diseases. PKC, protein kinase C.



**Figure 3.**  
Molecular mechanisms of known long noncoding RNA functions in the heart.



**Figure 4.** Transcriptome programming contributed by RNA splicing, editing and noncoding RNA-mediated gene regulation.

**Table**

List of LncRNAs implicated in Cardiovascular Development and Diseases

Category	lncRNAs	Reference
Upregulated lncRNAs during cardiac development	Bvht	Klattenhoff et al <sup>124</sup>
	Kcnq1	Korostowski et al <sup>122</sup>
	DT903035	Zhu et al <sup>121</sup>
	BC049716	
	AK085135	
	AK013988	
	Uc008hzy.1	
	Uc008mey.1	
	MM9LINC RNA EXON10678 NR_029457	
Downregulated lncRNAs during cardiac development	Fendrr	Grote et al <sup>127,128</sup>
	AK045554	Zhu et al <sup>121</sup>
	AK050713	
	Uc007xf.1	
	BC024929	
	AK008015	
	AK019733 Gm16133	
Upregulated lncRNAs in diseased heart	CHRF	Wang et al <sup>139</sup>
	N339730	Song et al <sup>132</sup>
	N408065	Yang et al <sup>133</sup>
	KCONS_0000467	Liu et al <sup>103</sup>
	N406465	
Downregulated lncRNAs in diseased heart	N383233	Song et al <sup>132</sup>
	N407211	Yang et al <sup>133</sup>
	N339159	Liu et al <sup>103</sup>

lncRNA, long noncoding RNA.