The precise regulation of gene expression patterns is paramount to cardiovascular development, homeostasis, and stress response. Critical transcription factors responsible for driving cardiac differentiation, chamber identity, and patterning are relatively well established because of decades of investigations. More recent studies have revealed how large-scale transcriptional and epigenetic changes promote lineage transitions during embryonic cardiogenesis, postnatal cardiac maturation, and regeneration. Although transcription factors, chromatin modifiers, signaling molecules, and microRNAs (miRNAs) are known to participate in these processes, accumulating evidence indicates that a new class of noncoding RNAs called long noncoding RNAs (lncRNAs) represent an additional layer of regulation to coordinate cardiac transcriptional programs.

Dynamic transcriptional and epigenetic changes drive cardiac cell fate transitions and maladaptive tissue remodeling in cardiovascular diseases (CVDs). In response to stress, cardiac cells undergo considerable changes, such as hypertrophic growth without division, re-expression of fetal genes, and metabolic and energetic state changes, leading to pathological remodeling and hypertrophy of the myocardium. For example, cardiac injury can induce multiple signaling cascades that converge in the nucleus to activate various transcription factors and chromatin regulators, including NFAT, GATA, MEF2, and histone deacetylases (HDACs), that drive the expression...
because of an increasing number of cRNA (or lincRNA), enhancer RNA (eRNA), and circular lncRNA, antisense lncRNA, intronic lncRNA, intergenic lncRNA, and their impact on gene expression, lncRNAs are roughly categorized as sense lncRNAs, antisense lncRNAs that may or may not be alternatively spliced, polyadenylated, or 5′-capped,16–18 there are various types of lncRNAs, which can be further divided into subcategories and annotated accordingly. On the basis of their genomic organization and structure, lncRNAs are roughly categorized as sense lncRNA, antisense lncRNA, intronic lncRNA, intergenic lncRNA (or lincRNA), enhancer RNA (eRNA), and circular RNA (circRNA).19,20 Because of an increasing number of genome-wide sequencing and functional studies, the exact categorization of lncRNAs is becoming more complex.20–22

LncRNAs have limited coding potential, as indicated by their lack of protein domains or significant open reading frames.17 To date, thousands of lncRNAs have been identified across vertebrates.17,23 Because the field of cDNA biology continues to evolve, the catalog of lncRNAs is growing and revealing their complex nature. However, only a small percentage of recently identified lncRNAs have been functionally characterized in detail. These studies revealed that lncRNAs are diverse in terms of their cellular expression patterns, subcellular localization, evolutionary conservation, and mechanisms of action.23 Most lncRNAs display tissue-specific expression patterns similar to master transcription factors,18 and regulate diverse processes including epigenetic and transcriptional regulation,24–26 nuclear organization,27 X-inactivation,28 embryogenesis and development,29–31 differentiation,32–34 proliferation/cell cycle progression,35,36 and metabolism17 among many others. Mutations in lncRNAs or dysregulation of lncRNA expression has also been increasingly associated with many human diseases, such as neurodegeneration, cancer, and heart disease.38 Furthermore, some lncRNAs are implicated as signal relay molecules, where they may be activated by environmental and stress stimuli as well as chemokine signaling to orchestrate downstream epigenetic and transcriptional responses.39,40

LncRNAs can regulate gene expression through multiple mechanisms. They can function in cis to modulate expression of a neighboring gene and in trans to impact gene expression across chromosomes.23,25,27,41,42 For instance, a class of lncRNAs with apparent enhancer-like activity, termed eRNAs, are synthesized at enhancers that activate neighboring genes in cis via DNA looping.43–46 Some lncRNAs also recruit chromatin modifiers and transcription factors to genomic sites in trans to impact transcription.36,45–47 Other lncRNAs have been reported to function as miRNA sponges, titrating the availability of these small transcripts away from their sites of action.41,44,49 Furthermore, lncRNAs can influence mRNA splicing, translation or degradation by binding to mRNAs or protein components of RNP complexes.27,50–52 Thus, lncRNAs have functions in a diverse array of transcriptional and post-transcriptional gene regulatory processes. Although the recent discovery of lncRNAs has identified previously unrecognized pathways for regulating gene programs, only a handful of lncRNAs have been studied in detail. Therefore, systematic studies to dissect the functions of lncRNAs in specific contexts are essential to determine the full biological impact of these regulators.

**LncRNAs in Transcriptional and Epigenetic Regulation of Cardiovascular Development**

Concerted differentiation and organization of different cardiovascular cell types, such as endothelial cells, smooth muscle cells (SMCs), and cardiomyocytes is necessary to form the mature heart. During heart development, pluripotent stem cells progressively differentiate into mesodermal and cardiac precursors in response to signaling cues, followed by
maturation and terminal differentiation. Disruption in the transcriptional networks that drive cardiac development and physiological homeostasis can lead to congenital heart diseases as well as chronic cardiac problems during adulthood. The studies discussed below emphasize how lncRNAs represent an important layer of transcriptional regulation during cardiovascular development (Figure 1).

Dynamic lncRNA Expression During Normal Heart Development
Recent genome-wide transcriptional profiling studies provide evidence that lncRNAs are probably critical components of cardiac transcription networks. In a study investigating how global gene expression patterns and chromatin structure change during cardiac commitment, Wamstad et al identified a large number of dynamically expressed lncRNAs. This study used an in vitro directed differentiation scheme to profile the transcriptome of cells undergoing cardiomyocyte differentiation at critical transition stages, namely embryonic stem cells (ESCs), mesodermal cells, cardiac progenitor cells, and cardiomyocytes, and identified nearly 200 lncRNAs expressed in a stage-specific manner. Further analyses revealed a correlation between lncRNAs and neighboring gene expression, suggesting that at least some of these transcripts may carry out cis-regulatory functions during cardiomyocyte differentiation. Another study, which used a similar directed differentiation of the pluripotent P19 embryonic carcinoma cell line, identified 40 differentially expressed lncRNAs during myocyte formation in vitro. The identification of a large cohort of dynamically expressed lncRNAs during the transition of stem cells to cardiomyocytes has prompted further investigations into the roles of lncRNAs in cardiogenesis.

Although in vitro cardiac differentiation models are informative and have provided crucial insights into this process, in vivo characterization of lncRNA expression and functions is necessary to understand the physiological functions of these transcripts. To this end, the transcriptomes of whole hearts from E11.5, E14.5, and E18.5, time points representing critical developmental transitions, were profiled to identify temporally expressed lncRNAs in the developing fetal mouse heart. Several hundred lncRNAs displayed distinct expression changes between the embryonic time points, providing a preliminary list of potentially important lncRNAs in early cardiac development. Whether there are overlapping lncRNAs among these studies remains to be explored.

Beyond early embryonic development, little is known about the expression profiles of lncRNAs in adult hearts. Matkovich et al carefully analyzed the expression of all annotated lncRNAs in adult mouse hearts to determine cardiac-specific lncRNAs. This analysis revealed 321 cardiac-expressed lncRNAs, 117 of which are enriched at least 3-fold in expression in the heart compared with liver or skin. Furthermore, about half of these lncRNAs exhibited differential expression profiles in developing embryonic hearts at E13.5 compared with adult hearts. Follow-up experiments suggested that a subgroup of cardiac-enriched lncRNAs probably modulate the expression of important neighboring developmental genes.

Figure 1. Long noncoding RNAs in the transcriptional circuitry of cardiovascular development. A. Many long noncoding RNAs (lncRNAs) are important for the development of different cell types in the cardiovascular system. Specific examples important for developmental transitions, such as Braveheart and Fendrr, as well as formation of cardiovascular cell types, such as Sencr and Malat1 are depicted. B. Transcriptional profiling studies have identified many lncRNAs that are differentially expressed during cardiac development. Many of these transcripts still await validation and further characterization (illustration credit: Ben Smith).
and highlight the possibility that at least some of these lncRNAs directly impact key cardiac transcriptional and signaling pathways. Collectively, these studies provide a plethora of uncharacterized cardiac lncRNAs with possible functions in cardiomyocyte differentiation, growth, and homeostasis that will serve as a resource for downstream functional studies.

**LncRNAs in Early Cardiac Differentiation**

Recent studies highlight the importance of lncRNAs in cardiovascular lineage commitment. For example, Braveheart (Bvht) is expressed in the early stages of embryonic heart development as well as in the postnatal heart in mouse.\(^7\) RNAi-mediated depletion of Bvht in mouse ESCs inhibits proper cardiomyocyte differentiation in vitro and impairs the formation of beating cardiomyocytes. Bvht seems to act upstream of MesP1 to induce its expression and its downstream target genes that include core cardiac transcription factors, such as Hand1, Hand2, Gata4, Gata6, Tbx2, and Nkx-2.5.\(^1,5\) MESP1 is a master transcription factor expressed in the earliest cardiac progenitor cells during development, is critical for mesoderm patterning, and is essential in the specification of all cell types of the heart, namely cardiomyocytes, SMCs, and endothelial cells.\(^6,6\) Although Bvht is necessary for promoting early cardiac gene expression programs in vitro, its precise mode of action is unknown. Moreover, whether Bvht is also required for early heart development in vivo and if it has a conserved counterpart in humans has yet to be determined.

Grote et al\(^4\) reported that targeted homozygous deletion of the lncRNA Fendrr, generated by replacing the first exon with strong transcriptional termination sequences in mouse, results in embryonic lethality around E13 as a consequence of impaired body wall development and myocardial dysfunc-

tion, consistent with its enrichment in lateral plate mesoderm. Through interactions with histone modifying complexes, Fendrr was found to orchestrate epigenetic modifications of their overlapping counterpart, SENCR, which does not seem to disrupt cardiac transcriptional programs.\(^6,6\) Early inactivation of Ezh2 in mouse hearts also results in reduced cardiomyocyte proliferation.\(^6\) Moreover, inhibition of PRC2 components results in deregulation of early fetal genes, such as Six1, and disrupts cardiac transcriptional programs.\(^5,6\)

Discovery of Bvht and Fendrr lncRNAs opened up a new avenue in our understanding of epigenetic control of cardiogenesis. In addition, the surprisingly robust functions exhibited by Bvht and Fendrr support a major role for lncRNAs in cardiovascular development. Future in vivo studies to identify additional lncRNAs during cardiac development will deepen our understanding of the biological and therapeutic potential of these diverse transcripts.

**Roles in Vascular Development**

Formation and functionality of vascular endothelial cells is important for cardiovascular development and health. Several lncRNAs are implicated as regulators of vascular and endothelial cell biology. Transcriptional profiling of human coronary artery SMCs identified many previously unannotated lncRNAs expressed in these cells.\(^5,6\) Further characterization led to the identification of a vascular cell–enriched lncRNA termed smooth muscle and endothelial cell–enriched migration/differentiation-associated lncRNAs (SENCR). SENCr is transcribed as 2 isoforms and in the antisense direction from within the first intron of Friend of Leukemia virus integration 1 (FLI1) gene that codes for a key transcription factor controlling endothelial and blood cell formation.\(^6\) Interestingly, expression of SENCr coincides with FLI1 in different tissues, suggesting a common upstream transcriptional regulator. Although some antisense lncRNAs control the expression of their overlapping counterpart, SENCr does not seem to simply regulate FLI1 transcription in cis. Instead, SENCr is localized predominantly in the cytoplasm. Interestingly, siRNA-mediated knockdown of the lncRNA results in deregulation of SMC contractile genes. Namely, MYOCID, a critical transcriptional regulator of SMC-sigature is downregulated in SENCr-depleted human coronary artery smooth muscle cells.\(^6\) Further studies to understand how a cytoplasmic IncRNA influences gene expression are necessary to fully understand SENCr’s mechanisms of action.

Another study identified lncRNAs highly expressed in human umbilical vein endothelial cells, human cardiac microvascular endothelial cells, human lung microvascular endothelial cells, human coronary artery endothelial cells, and the human aorta endothelial cells.\(^6\) The authors focused on 5 transcripts that displayed significant sequence conservation between mouse and human, namely LINRC00493, metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), maternal expressed 3, taurine upregulated gene 1 (TUG1), and LINRC00657. In particular, MALAT1 displayed a predominantly nuclear expression pattern and was highly responsive to hypoxia in human umbilical vein endothelial cells. MALAT1 was originally identified in different cancers and seems to interact with PRC2 components to modulate cellular proliferation by epigenetic regulation of target genes.\(^2,7\) Loss of MALAT1
results in a switch from a proliferative to a promigratory state of endothelial cells in vitro and significantly impairs endothelial cell proliferation, vessel growth, and vascularization in vivo in mice. Thus, this lncRNA may modulate cell cycle regulatory gene programs to control vascular/endothelial functions and angiogenesis.69,72 Although Malat1 knockout mice do not display gross developmental abnormalities under normal growth conditions,73,74 it is possible that Malat1 lncRNA plays important roles during cardiovascular development or homeostasis under stress conditions. Future experiments to tease out MALAT1 function under pathological stress will surely yield insights into the regulatory roles of this conserved lncRNA.

**LncRNAs and Transcriptional Regulation in CVDs**

The potential functions of lncRNAs in the control of mature cardiovascular homeostasis and adaptation to stress remained relatively unexplored until recently. During the past few years, studies have begun to identify lncRNAs that are differentially expressed during cardiac maturation and pathological conditions in the adult. A cumulative look at these reports, described below, reveals an emerging theme that lncRNAs are dynamically regulated in response to cardiac stress and disease (Figure 2). Thus, these transcripts may represent important regulators of heart homeostasis as well as novel disease biomarkers or targets for therapeutic intervention. Although only at the beginning stages, the identification of disease-associated lncRNAs can also serve as a useful framework to establish direct links to biological outcomes.

**Ventricular Septal Defect**

Ventricular septal defect (VSD) is the most prominent form of congenital heart disease leading to severe cardiac abnormalities.75 In an effort to determine novel regulatory molecules that may contribute to the development and pathophysiology of VSD, a recent study investigated whether the expression of lncRNAs is perturbed in human VSD.76 Using lncRNA-specific microarrays, >1000 lncRNAs were aberrantly expressed in VSD hearts from 17- to 20-week-old fetuses. Bioinformatic characterization of these transcripts showed that these expressed during cardiac maturation and pathological conditions in the adult. A cumulative look at these reports, described below, reveals an emerging theme that lncRNAs are dynamically regulated in response to cardiac stress and disease (Figure 2). Thus, these transcripts may represent important regulators of heart homeostasis as well as novel disease biomarkers or targets for therapeutic intervention. Although only at the beginning stages, the identification of disease-associated lncRNAs can also serve as a useful framework to establish direct links to biological outcomes.

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IncRNAs reside near protein-coding genes associated with functions in organogenesis, metabolism, cellular differentiation, heart growth, and cell fate commitment. Given their proximity to tissue-specific genes, it is possible that many of these IncRNAs overlap transcriptional enhancers that regulate specific gene programs during development.

Heart Failure

Complex and disease-specific changes in gene expression contribute to pathological reprogramming that leads to the onset of heart failure (HF). Recent work identified IncRNAs differentially expressed in various HF models in both mice and humans. One study analyzed global gene expression changes in left ventricles from mice 1 week (hypertrophied tissue) and 8 weeks (tissue undergoing HF) post pressure overload induced by transaortic constriction as well as in sham-operated controls. Fifteen IncRNAs were found to be differentially expressed between hypertrophied versus control ventricles, whereas 135 showed changes in expression in HF samples. In a separate report using a genetic HF model in mouse, expression profiles of Pdk1 knockout mouse hearts were compared with those of wild-type Pdk1-harboring animals using microarrays. In this model, the mice develop severe HF around postnatal day 40 and die between 5 and 11 weeks of age. Several thousand IncRNAs were induced or repressed in Pdk1-deficient hearts that have undergone HF.

IncRNAs also seem to be highly regulated in human models of HF. In a recent study, samples from left ventricles of patients with severe HF were collected before and after left ventricular assist device implantation in addition to those of nonfailing hearts as controls. This analysis identified 679 differentially expressed IncRNAs in ischemic-origin HF hearts and 570 in nonischemic-origin myocardium. Among these transcripts, the levels of ≈10% of IncRNAs from each group were significantly reversed in response to left ventricular assist device support, providing a candidate set for further investigation. The authors also observed a strong positive correlation between the expression of IncRNAs and their neighboring genes, suggesting a possible cis-regulatory transcriptional mechanism in the failing human myocardium.

Intriguing new evidence suggests that IncRNAs also act as circulatory signaling molecules and possible biomarkers of cardiac disease. Li et al identified ≈1000 differentially expressed IncRNAs by profiling cardiac tissue, whole blood, and plasma of mice in an isoproterenol-induced HF model. Surprisingly, more than twice as many IncRNAs displayed differential expression in whole blood and plasma of HF mice compared with healthy animals. Only 10% of these transcripts were shared between cardiac samples and blood samples, suggesting different pathological responses to HF in the heart and the blood. Interestingly, the procardiovascular commitment IncRNA Bvht (annotated as uc008fch.1, Online Table III in this report) seemed to be significantly downregulated in the plasma of HF mice, suggesting that Bvht may mediate reactivation of the fetal gene program observed during HF.

In a subsequent study, Kumarswamy et al measured IncRNA expression in the plasma of patients at early (left ventricular remodeling) and late (HF) response to myocardial infarction (MI). These data revealed 7 mitochondrial-transcribed IncRNAs that were consistently and differentially expressed during left ventricular remodeling. One of these IncRNAs, named long intergenic noncoding RNA predicting cardiac remodeling (LIPCAR), displayed the most robust upregulation in both early left ventricular remodeling and during chronic HF after MI. Follow-up with nearly 300 patients revealed that elevation of LIPCAR levels in patients with HF may be significantly associated with a higher risk of mortality because of future cardiovascular events. The identification of specific IncRNAs that act as circulating factors and as potential prognostic biomarkers of HF suggests that these transcripts have the potential to convey pathological response signals throughout the body and contribute to global homeostatic changes during CVD.

Ischemia/Reperfusion Injury and Hypertrophy

The idea that IncRNAs are stress responsive transcriptional modulators of maladaptive cardiac remodeling is also supported by recent reports demonstrating dynamic IncRNA expression after acute cardiac injury. Transcriptional profiling of adult mouse hearts subjected to anterior descending artery ligation-induced infarction relative to sham-operated control hearts revealed a large number of both annotated and previously unannotated IncRNAs with putative functions in key remodeling processes post-MI. The majority of IncRNAs differentially expressed between MI and sham tissues was highly heart-specific, suggesting specialized cardiac functions. Similar to previous studies, these IncRNAs generally reside near cardiac-specific genes that carry out biological processes involved in heart development, function, and transcriptional control. In an effort to understand their transcriptional functions, the authors used publicly available chromatin immunoprecipitation sequencing data to analyze the chromatin states around the transcription start site proximal regions of differentially expressed IncRNAs. Interestingly, novel heart-specific IncRNAs were enriched for active enhancer marks such as H3K4me1 and H3K27ac suggesting that these transcripts may function as enhancer RNAs. Finally, the authors reported possible conserved candidates in human hearts, some of which are differentially modulated in human cardiac diseases. In agreement with this report’s findings, a separate study found changes in the expression of numerous IncRNAs in the infarct zone shortly after ischemia/reperfusion injury inflicted in a similar manner in mice. Experimental validation of the function and mechanism of IncRNAs discovered in these reports is necessary to address the question of whether these IncRNAs regulate the adaptive response to cardiac injury.

Myocardial Fibrosis

Cardiac stress leads to remodeling of cardiac tissue, which involves overpopulation of fibroblasts that result in excessive extracellular matrix formation and fibrotic tissue production within the myocardium. To address the question of whether IncRNAs have specific roles in cardiac fibroblast homeostasis and myocardial fibrosis, Jiang and Ning used isolated adult rat fibroblasts that were treated with angiotensin II (Ang II), a peptide hormone that induces cardiac fibrosis. Transcriptional changes were measured in response to Ang II treatment, revealing ≈300 IncRNAs that displayed >2-fold
change in expression in rat fibroblasts. Although functional follow-up experiments were missing from this study, identification of significantly altered lncRNA profiles specifically in fibroblasts suggests that these transcripts participate in coordinated and differential responses of the cardiovascular system to orchestrate distinct cell type–specific outcomes.

**Aortic Valve Calcification**

A genetic defect that leads to the formation of 2 instead of 3 aortic valve leaflets (bicuspid aortic valve) poses a major risk factor for developing aortic valve calcification. Bicuspid aortic valve leaflets experience increased biomechanical stretch, which is thought to exacerbate aortic calcification. The lncRNA, Hox transcript antisense RNA (HOTAIR), seems to be involved in the molecular response to elevated mechanical stress in human aortic valve interstitial cells in individuals with bicuspid aortic valve. HOTAIR normally regulates HOX gene transcription through recruitment of PRC2 to target sites. In addition, HOTAIR expression is elevated in numerous tumors and it functions to alter chromatin states to promote metastatic progression. Carrion et al discovered that HOTAIR is downregulated in aortic valve interstitial cells exposed to biomechanical stretch. Interestingly, HOTAIR levels were also lower in human bicuspid aortic valve leaflets, which naturally undergo higher levels of mechanical stress. siRNA-mediated knockdown of HOTAIR in aortic valve interstitial cells resulted in transcriptional induction of calcification genes and pathways, suggesting that HOTAIR participates in the biomechanical stress response in aortic valve cells and may normally restrain calcification gene programs. Additional experiments indicated that Wnt/β-catenin signaling probably controls HOTAIR and downstream gene expression in response to mechanical strain. This work suggests that HOTAIR is an important player in aortic valve disease and may represent an exciting therapeutic target in the search for aortic calcification therapies.

Looking forward, although the studies described above identified thousands of lncRNAs that are differentially expressed in various cardiovascular pathologies, the authenticity of these transcripts requires extensive validation before they can be considered true regulators of cardiac pathogenesis. One of the issues of lncRNA expression screens that use disease models could be the difficulty of correcting for changes in cell types or quantities in diseased tissue compared with healthy tissue, thereby introducing a high rate of false-positive or negative findings. Moreover, microarray studies are subject to higher rates of false positives because of technical issues with hybridization platforms. Comparing datasets and identifying the common transcripts most robustly changed across similar studies and disease models will probably help highlight biologically relevant lncRNAs, allowing for mechanistic characterization of the high confidence lncRNAs with putative functions in cardiac homeostasis and disease.

**Genetic Risk Factors**

Evidence from genome-wide association studies demonstrates that a large proportion of genetic variation in complex human diseases lies in noncoding regions of the genome. Genome-wide association studies and transcriptional profiling experiments in humans have been instrumental in revealing links between lncRNA mutations and disease traits, including genetic risk factors for CVD. In fact, one of the strongest genetic susceptibility loci for coronary artery disease maps to a region at the 3′ end of a lncRNA named antisense noncoding RNA in the INK4 locus (ANRIL). Homozygosity for the risk haplotype gives ≥20% of the population a 2-fold increased risk of having MI. ANRIL can be alternatively spliced to produce different tissue-specific variants. Patients harboring the risk haplotype show elevated ANRIL expression in peripheral blood cells and atherosclerotic plaques. suggesting that ANRIL somehow participates in plaque formation. ANRIL associates with PRC1 and PRC2 to confer changes in histone methylation to regulate neighboring tumor suppressor gene expression, such as INK4, in cis, yet whether this mechanism is relevant to cardiovascular functions of ANRIL is not known. One of the variants within the coronary artery susceptibility locus lies in an enhancer that controls the binding of STAT1 transcription factor and alters ANRIL expression. Thus, it is possible that ANRIL acts downstream of STAT1 during coronary artery disease because STAT1 regulates an inflammatory pathway associated with the pathogenesis of atherosclerosis.

MI-associated transcript (MIAT)—also referred to as Gomafu or RNCR2) also seems to be a risk factor for MI. A variant in exon 5 correlates with higher levels of MIAT, yet whether this directly leads to effects on coronary artery disease has not been determined. MIAT is highly conserved in mammals, forms nuclear structures in neurons, is highly expressed in the central nervous system, has roles in retinal cell development, and controls Oct4 and Nanog transcription to contribute to the regulation of mouse ESC pluripotency and differentiation. More recently, MIAT depletion was found to lead to a reduction in diabetes mellitus–induced retinal microvascular dysfunction in vivo in rats, and inhibition of endothelial cell proliferation, migration, and tube formation in vitro. Further analyses suggested that MIAT forms a feedback regulatory loop with VEGF and miR-150-5p to modulate endothelial cell function. Although a direct molecular mechanism linking MIAT and MI susceptibility is still lacking, accumulating evidence suggests that MIAT may control transcriptional programs that govern the fidelity of vascular–endothelial cell development and function.

Steroid receptor RNA activator (SRA) is a complex gene, which can give rise to both protein-coding and noncoding transcripts. SNPs in the SRA1 gene are associated with human dilated cardiomyopathy and SRA depletion results in contractile dysfunction in the ventricles of zebrafish, suggesting that this gene is important in cardiac development and function. Noncoding SRA transcripts display tissue-specific expression patterns and are abundant in the liver, skeletal muscle, and heart. SRA RNAs participate in the regulation of muscle myogenic differentiation as cofactors of SRA proteins and other factors, and modulate the expression of MyoD and the myogenic program. The mechanism underlying the susceptibility conferred by variation in the SRA locus and the exact function of SRA lncRNA in cardiovascular physiology is not yet elucidated. Collectively, these studies suggest that
further analysis of large-scale genome-wide association studies will be critical to identify additional IncRNA loci and will help elucidate the roles of IncRNAs as potential risk factors for CVD.

**Cardiac IncRNA Mechanisms of Action**

Although IncRNAs carry out important roles in biology, only a handful of these transcripts have been studied in mechanistic detail. We provide a few examples below that demonstrate how IncRNAs regulate transcriptional and epigenomic programs to control cardiac development and disease (Figure 3).

**Interactions With Histone Modifying Complexes**

Some IncRNAs can modulate transcription by interacting with epigenetic- and transcriptional-regulatory complexes, such as PRC2, LSD1, CBX2, MLL, and others.\(^{113}\) Both *Bvht* and *Fendrr* seem to interact with PRC2 to regulate target gene expression during cardiac development.\(^ {46,47}\) *Bvht* was shown to interact with the catalytic SUZ12 component of PRC2 by in vitro RNA pulldown or RNA-immunoprecipitation experiments in both ESCs and in vitro differentiated cardiac progenitor cells.\(^ {46}\) *Bvht* depletion resulted in an elevation of SUZ12 as well H3K4me3 levels at the promoters of cardiac control genes, including *MesP1, Gata6, Hand1, Hand2,* and *Nkx2.5.* These data suggest that *Bvht* interacts with at least some components of the PRC2 complex to modulate its localization and activity at target gene promoters. Similarly, *Fendrr* was shown to coprecipitate with PRC2 components EZH2 and SUZ12 in mouse embryos.\(^ {46}\) Depletion of *Fendrr* resulted in a decreased association of EZH2 and SUZ12 with the promoters of lateral mesoderm control genes such as *Pitx2* and *Foxf1,* and led to a robust loss of the repressive H3K27me3 mark catalyzed by PRC2 at these genes. These findings suggest that *Fendrr* regulates lineage commitment through recruiting PRC2 to key lateral mesoderm genes.\(^ {46}\) However, more detailed molecular analyses are necessary to confirm the reported associations between these cardiac IncRNAs and PRC2, given the recent studies showing that PRC2 can nonspecifically interact with many types of RNAs.\(^ {114}\)

Besides PRC2, *Fendrr* IncRNA has been shown to associate with WDR5, a component of the histone methyltransferase complex trithorax/mixed lineage leukemia (TrxG/MLL), and suggested to modulate its histone modifying activity in the developing embryo.\(^ {46,115}\) In contrast to PRC2, the TrxG/MLL complex deposits activating H3K4me3 marks to regulate mesoderm differentiation genes programs. Follow-up studies into the temporal and spatial nature of these interactions as well as determining the domains of *Fendrr* that mediate its differential association with these chromatin modifiers will provide novel insights into its mechanism of action.

As IncRNAs may act in cis or trans to modulate gene expression, can be transcribed antisense to other genes, expressed from genomic loci that harbor regulatory elements controlling nearby gene transcription, it is often challenging to make conclusions about RNA-dependent mechanisms of action using genomic alterations or using RNAi-mediated depletion.\(^ {117}\) Especially in the case of transacting IncRNAs, most convincing evidence for RNA-dependent IncRNA function comes from loss-of-function studies, followed by complementation approaches where the IncRNA is reintroduced to rescue the phenotype.\(^ {117}\) Although this approach has been successfully demonstrated for *Fendrr,*\(^ {46}\) it will also be important to generate an in vivo knockout model of *Bvht* that can be rescued by overexpressing the transcript.

**Interactions With Chromatin Remodelers**

Several IncRNAs interact with BRG1, a subunit of the Brg1/Brm-associated factor (BAF), an ATP-dependent chromatin remodeling complex. BAF regulates gene expression during cardiac growth, differentiation, and hypertrophy in mice as well as vascular SMC response to thoracic aortic aneurysms.\(^ {118–120}\)
During embryonic heart development, BRG1 partners with an HDAC and poly ADP-ribose polymerase (PARP) to control gene expression programs that drive fetal cardiac differentiation.118 Interestingly, reactivation of the BRG1/HDAC/PARP complex under cardiac stress in the adult induces transcriptional reprogramming and a pathological switch from Myh6 to Myh7 expression, thereby leading to cardiac hypertrophy in mouse.118 A group of alternatively spliced lncRNA transcripts, collectively named Mhrt, are expressed from the Myh7 locus in the heart and regulate cardiac stress response and cardiomyopathies.119 Conversely, Mhrt expression is repressed by the BRG1/HDAC/PARP complex in response to pathological stress. In addition, Mhrt lncRNAs specifically associate with the BRG1 helicase domain to sequester BRG1 from its genomic targets, and inhibit chromatin remodeling. Remarkably, inhibiting stress-induced Mhrt repression seems to protect animals against the hypertrophic response. The authors suggest that Mhrt carries out similar functions in humans and represent novel therapeutic targets.119 Whether human MHRT transcripts induce chromatin remodeling through interacting with BRG1 during human cardiac hypertrophy or whether other mechanisms can explain this phenotype requires further investigation.

Effects on Chromatin Conformation and Enhancer Activity

The imprinted lncRNA, named Kcnq-overlapping lncRNA 1 (Kcnq1ot1), is a specific cis regulator of the Kcnq1 gene,121 which encodes a voltage-gated potassium channel required for repolarization of the cardiac action potential. Mutations in Kcnq1 perturb proper conduction in the heart, leading to congenital long QT syndrome, a cardiac disorder that manifests in varying severities of cardiac symptoms, including serious arrhythmias.122 Biallelic expression of Kcnq1ot1 later in embryogenesis and in the postnatal heart impacts the transcription of Kcnq1,121 whereas deletion of the noncoding transcript leads to elevated Kcnq1 levels in the heart. Interestingly, Kcnq1ot1 seems to regulate higher order chromatin interactions between an enhancer and Kcnq1. Chromatin conformation capture assays revealed that loss of function of Kcnq1ot1 results in aberrant interactions between the Kcnq1 promoter and additional sites. Thus, Kcnq1ot1 may be critical for restricting cell type expression of this important potassium ion channel in developing hearts.122 This finding adds to the diverse chromatin regulatory mechanisms mediated by lncRNAs to control key cardiac genes and transcription factors.

Regulation of Cardiovascular miRNA Function

Some lncRNAs act as molecular sponges to sequester miRNAs and titrate miRNA-regulated functions to control post-transcriptional gene silencing. Several examples of this mechanism in different systems include linc-MD1 that sponges miR-133 and miR-135 to regulate the expression of MAML1 and myocyte enhancer factor-2C during the muscle differentiation process;46 the imprinted lncRNA H19, which regulates let-7 functions in muscle development;123 lncRNA linc-RoR that interacts with miRNAs to control ESC pluripotency;49; and a network of lncRNAs that interact with miRNA targets to regulate oncogenesis during gastric cancer.124 Interestingly, recent reports suggest that at least some circular RNAs act as strong miRNA sponges.125–127

Recent studies highlight additional functional connections between lncRNAs and miRNAs to modulate cardiac development and disease. For example, an lncRNA named cardiac hypertrophy–related factor (CHRF) titrates the levels of a miRNA involved in cardiac hypertrophy response in cardiomyocytes.128 In this work, the authors set out to identify miRNAs whose expression changed in response to Ang II–induced hypertrophy in mouse cardiomyocytes. In particular, miR-489 resulted in increased hypertrophy of cardiomyocytes when inhibited by antagonims, synthetic miRNA inhibitors, and reduced hypertrophic responses on enforced expression both in vitro and in vivo. To investigate how this antihypertrophic miRNA is regulated, the authors examined lncRNAs whose levels were altered in response to Ang II treatment. One lncRNA, AK048451 (CHRF), displayed elevated expression on Ang II. Endogenous knockdown of CHRF via siRNAs led to elevated levels of miR-489 suggesting CHRF may inhibit its expression. Further reporter and overexpression experiments demonstrated that CHRF counteracts target gene activation by miR-489, particularly myeloid differentiation primary response gene 88 (Myd88), leading the authors to suggest that CHRF acts as a sponge to regulate miR-489 levels and activity during maladaptive hypertrophy.128 It remains to be determined whether these functions of CHRF are conserved in humans and whether a similar mechanism of action is at work during human heart hypertrophy and failure.

Recent evidence also suggests that lncRNAs participate in the regulation of cardiac mitochondrial function and cardiomyocyte apoptosis through miRNAs.129 In this study, the authors discovered that a mitochondrial inner membrane protein, prohibitin 2 (PHB2), regulates mitochondrial fission and apoptosis in mouse cardiomyocytes in response to anoxia or ischemia. PHB2 protein levels declined in cardiomyocytes during anoxia or ischemia, and overexpression of PHB2 attenuated mitochondrial fission and apoptosis during anoxia in vitro or after ischemia/reperfusion in vivo. miR-539 exhibited elevated expression in response to anoxia and directly inhibited PHB2 levels in cardiac mitochondria after MI. The authors subsequently discovered that an lncRNA they named cardiac apoptosis–related lncRNA (CARL) can directly interact with and modulate the function of miR-539 in the cardiomyocyte cytoplasm. Overall, results from this study are consistent with a model in which the CARL lncRNA responds to anoxia or ischemia by titrating the levels of its downstream target miR-539, which itself alters PHB2 levels to increase mitochondrial fission and apoptosis in cardiomyocytes. Future studies to determine the mechanism by which CARL regulates mir-539 as well as additional downstream targets and physiological/pathological effects of CARL will provide important insights into mitochondrial dynamics in cardiomyocytes. In addition, further in vivo studies to investigate the CARL/miR-539 pathway as a therapeutic target to ameliorate MI pathologies will be informative.

Although these studies are certainly of interest and suggest new modes of lncRNA action to regulate gene expression
programs, the role of lncRNAs as miRNA sponges remains controversial because in many cases the level of lncRNA expression as well as the number of miRNA sites within these transcripts could be insufficient to effectively titrate miRNAs from their targets.\(^{23}\) Thus, future studies to carefully dissect their functions in this process will continue to shed light on the diverse roles of lncRNAs in regulating biological outcomes.

Conclusions, Challenges, and Future Directions

**Identifying Functional Cardiac lncRNAs**

LncRNA biology in cardiovascular development and disease is an exciting and rapidly evolving field. The studies summarized in this review provide compelling evidence that lncRNAs play global roles in regulating gene networks during normal and pathological heart development and provide a strong foundation for detailed functional investigations (Figure 4). Although the abundance and diversity of lncRNA transcripts differentially expressed in various contexts opens up possibilities for the discovery of lncRNA networks and unknown pathways, it also poses a great challenge for honing in on the most relevant transcripts for mechanistic characterization. Comparison of transcriptional profiling datasets from different studies and determination of the overlapping lncRNAs may be one strategy to get at the most biologically relevant cardiac lncRNAs. In addition, the candidate lncRNAs can be prioritized for functional characterization based on (1) tissue/cell type–specific expression, (2) robust, dynamic response to developmental, environmental, or stress-cues, and (3) sequence, positional (promoter and syntenic), or structural conservation. Some lncRNAs can remodel their local epigenetic landscape and regulate the expression of nearby developmental or physiological factors, such as transcription factors.\(^{24-26,130}\) Therefore, analyzing the expression patterns and identities of lncRNA-neighboring genes may be one way to highlight functional lncRNAs. To identify lncRNAs that probably modulate epigenetic and transcriptional activities in the cardiovascular system, examining their subcellular localization and focusing on nuclear lncRNAs may be another helpful strategy. Finally, it is of utmost importance to confirm that the transcripts annotated as noncoding RNA are indeed nonprotein coding. A recent report illustrated that putative lncRNAs currently annotated in pre-existing databases may actually code for small peptides.\(^{131}\) In this study, a skeletal muscle–specific transcript annotated as a lncRNA (LINC00948 in humans and 2310015B20Rik in mice) was discovered bioinformatically. Subsequent examination of the evolutionary conservation of the transcript as well as experimental validation revealed that a short nucleotide open reading frame that encodes a highly conserved micropeptide exists in this lncRNA. Therefore, a combination of bioinformatics (eg, analyzing putative open reading frames) and experimental methods (eg, in vitro translation) to confirm lack of coding potential should be used when candidate lncRNAs are selected for functional studies.

Although the strategies mentioned here can help prioritize lncRNAs for functional investigations, they may at the same time introduce bias and restrict identification of novel lncRNAs. The current focus on conserved lncRNAs or annotated lncRNAs represent some of these biases and could potentially result in a neglect of the majority of novel lncRNAs. Therefore, designing unbiased, high-throughput phenotypic screens to identify functional lncRNAs may facilitate discovery of novel transcripts in cardiac biology.

**Conservation**

The fact that most lncRNAs seem to be highly species-specific and lack clear sequence conservation signatures\(^{23}\) raises the question of how much of the findings from murine models can

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**Figure 4.** Future directions for the study of long noncoding RNAs (lncRNAs) in cardiovascular biology. There still remain many unanswered questions about the exact functions and molecular mechanisms of lncRNAs that govern diverse aspects of cardiac physiology. Some of the outstanding questions and promising areas of research are outlined in the schematic. CM indicates cardiomyocyte (illustration credit: Ben Smith).
be extended to humans. Although lncRNA sequences are less conserved than protein-coding genes, being generally restricted to short-patches of similar sequences,132,133 they display high conservation compared to intronic or intergenic regions134,135 and a high level of splice-site homology among placental mammals.136 Many vertebrate lncRNAs are found at syntenic genomic sites and display remarkable promoter similarity, suggesting some functional conservation.10,132 In addition, these positionally conserved lncRNAs tend to reside nearby key developmental regulators and exhibit conserved, cell type restricted expression patterns.137,138 Therefore, synteny represents one benchmark when determining orthologous lncRNAs between vertebrate models and humans. Because RNAs often fold into complex secondary or tertiary structures, conservatism at the level of functional domains and base pairing, not necessarily primary sequence, may underpin orthologous functions.132 Besides the cited articles in this section, a recent study that compared the transcriptomes of 17 species and comprehensively determined features of lncRNA conservation provides an excellent resource to inspect interestingmurine and human cardiac lncRNAs with conserved functions.133

Another consideration is that intrinsic cross-species variation exists between small rodent and human cardiovascular physiologies, such as differences in heart rate and cardiomyocyte nucleation status.139 Ultimately, it is likely that many lncRNAs are not conserved and regulate the species-specific aspects of cardiac physiology. It is estimated that ≈20% of human lincRNAs are not expressed or conserved beyond the chimpanzee lineage.137 Therefore, assessing the pros and cons of studying conserved versus nonconserved lncRNAs can be challenging, however, studying lncRNA function in any context promises to reveal general modes of action that can be extrapolated across species.

**Dissection of Biological lncRNA Functions**

To better understand the physiological roles of lncRNAs during development and CVD, in vivo knockdown or knock-out experiments carry utmost importance. Given that the lncRNA field is still evolving and there are unanswered questions about their conservation and mechanisms, several important considerations should be taken into account before delving into functional lncRNA experiments and drawing conclusions from new discoveries. For example, a recent editorial on LIPCAR raised the issue that LIPCAR is likely transcribed from both nuclear and mitochondrial DNA,140 suggesting that the conclusions from Kumarswamy et al141 may have been incomplete or misleading. Thus, it is crucial to validate major findings by careful analysis and multiple complementary approaches.

With the introduction of effective genome editing technologies such as CRISPR/Cas9,141 generating and characterizing large numbers of lncRNA mutants has never been more feasible. However, to overcome artificial phenotypes caused by off-target effects of CRISPR, multiple knockout or knock-in lines should be generated to ensure the fidelity of genome-editing. Similarly, siRNA knockdown studies may have off-target effects. Thus, findings from siRNA experiments can be confirmed by using antisense oligonucleotides.142 For loss-of-function studies, where the lncRNA is trans-acting, exogenous expression to rescue the phenotype may provide confirmation that the effect is lncRNA-specific.117 For cases where a lncRNA acts in cis or that transcription through the locus is important for regulation, CRISPR–mediated repression/activation could be used to inhibit/induce transcription and validate the effect without disrupting the genomic architecture of the locus.142 Additional considerations for investigating lncRNA functions is comprehensively reviewed by Bassett et al.117

Detailed experimental interrogations into mechanisms of action of lncRNAs are necessary to understand the regulatory potential of these transcripts. LncRNAs may harbor conserved structural motifs that can mediate RNA–RNA, RNA–DNA, or RNA–protein interactions24 among other functions. Recent studies have begun to make headway in determining lncRNA secondary structures and elucidating their modes of action.143-145 To gain a deeper understanding of how lncRNAs may achieve transcriptional specificity, it will be important to examine their genomic localization, RNA or protein partners, and structure–function relationships. Specific tools to probe lncRNA mechanisms is extensively discussed in Chu et al.146

Do lncRNAs modulate cardiac cell reprogramming, transdifferentiation, regeneration, response to injury, and proliferation? miRNAs have been shown to be involved in cardiac aging147,148. Do lncRNAs similarly play a part in the aging process? Addressing these questions among others has the potential to identify specific lncRNAs that can be targeted for cardiac regenerative therapies. Overall, carefully weighing the pros and cons of experimental approaches and erring on the side of conservative interpretation when dissecting the functions of lncRNAs will facilitate the discovery process. Despite these challenges, exploring the relatively uncharted territory of cardiovascular lncRNAs has great potential to advance our understanding of cardiac biology and disease.

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**References**


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