Cardiac development is anchored on an intricate program of gene regulation and coordination, associated with critical timing and cell–cell interactions. Rather than a single master regulatory process, as originally envisioned to reside in a transcriptional complex or protein signaling cascade, cardiac development is likely regulated by a network of coordinated gene expressions, critically timed, and calibrated. Noncoding RNAs (ncRNA) are now seen as key new players in this regulatory network, and the road map is only beginning to be constructed.

Long Noncoding RNAs in the Heart
The Regulatory Roadmap of Cardiovascular Development and Disease
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Cardiovascular diseases often recapitulate cardiac development when the cardiovascular systems sustain stress or injury. Searches for disease-associated genes often ended up in the noncoding intergenic regions of the genome where ncRNAs are often expressed (eg, 9p21 chromosomal region for atherosclerosis). Thus, the role of ncRNAs in the pathogenesis of diseases has assumed an increasing importance, even though the full mechanistic understanding is yet to evolve.

Noncoding RNAs (ncRNA) are RNA transcripts longer than 200 nucleotides expressed by the genome, but do not themselves code proteins. They are transcribed across the genome, including the intergenic regions as potentially overlapping sense and antisense transcripts that can flank protein-coding genes. Coordinated activities of lncRNAs likely play a major role in the regulatory networks of organ development, normal organ function, and disease pathogenesis.

Until recently, ncRNAs were considered generic regulators of cell function, controlling the basic pathways of mRNA splicing and protein translation. However, in the past 10 years large scale community projects such as ENCODE (Encyclopaedia of DNA elements) or FANTOM (Functional Annotation of the Mammalian Genome) have taught us that ncRNAs outnumber protein-coding genes by nearly 40:1 and play integrated and coregulated lncRNAs that are functionally involved in cardiac developmental gene programs. Coordinated activities of lncRNAs likely play a major role in the regulatory networks of organ development, normal organ function, and disease pathogenesis.

In their findings suggest that lncRNAs are intricately involved in regulating cardiac development and set the stage for further elucidation of these pathways.

Beyond direct regulation of the protein-coding genome, He et al revealed that cardiac lncRNAs may act in concert with epigenetic histone modifications during development. They find that the adult heart is enriched in lncRNAs in proximity to promoter regions with chromatin modifier histone H3K methytransferases, similar to what had been reported previously. There were also interactions with other chromatin modifiers such as polycomb repressive complex when compared with the fetal heart. Moreover, the coexpressed lncRNA–mRNA pairs often share promoter regions, suggesting that coordinated activation of lncRNAs and their protein-coding partners could provide a feedback relationship where lncRNAs represses genes no longer needed during phases of...
development (Figure). Furthermore, many of the lncRNAs expressed in adult and fetal cardiac tissue contain binding sites for transcription factors, such as Activating Transcription Factor (ATF) and RAR Related Orphan Receptor (RORα), which may be differentially activated during development or disease. Although these mechanisms need to be validated experimentally, this study elegantly highlights how lncRNAs can act as a nexus of control of cardiac development and could thus be further probed for their contribution to cardiac dysfunction.

Unlike protein-coding genes and miRNAs, lncRNAs are often poorly conserved between species and are highly tissue specific. This study represents the first in-depth analysis of lncRNAs in cardiac development in humans. Although this approach naturally represented a snapshot of lncRNA profiles in time, and prevented a thorough analysis of lncRNAs expression at each stage of cardiac development or identification of contributing cell types to the same degree as what can be accomplished in mice, these data provide a unique resource for the future study of lncRNAs that may functionally contribute

### Table. Select Examples of Different Types of Long Noncoding RNA (lncRNA) in Cardiovascular Development and Disease

| IncRNA Type          | Examples  | Potential Function                                                                 |
|----------------------|-----------|------------------------------------------------------------------------------------|
| Enhancer IncRNAs     | HOTTIP    | Binds genes such as WDR5 and transcription initiator H3K4me3 to activate genes     |
| lincRNA              | Braveheart| Acts upstream of MesP1 gene for cardiac lineage specification                       |
| lincRNA              | MIAT      | RNA Splicing, also a potent susceptibility locus for myocardial infarction          |
| Natural antisense RNA| β-MHC antisense | Regulates isoform switching between α- and β-MHC                              |
| Natural antisense RNA| ANRIL     | Scaffold for polycomb repressive complexes that regulates CDKN2A/B, the most potent genetic locus for coronary atherosclerosis |
| uaRNA                | DMPK-3′UTR| Induction of Nkx2.5 leading to myotonic muscular dystrophy                         |

ANRIL indicates antisense noncoding RNA in the INK4 locus (or CDKN2B-AS); DMPK, dystrophia myotonia protein kinase; HOTTIP, HOXA transcript at the distal tip; lincRNA, large intervening or intergenic noncoding RNA; MHC, myosin heavy chain; MIAT, myocardial infarct associated; uaRNA, 3′UTR–associated RNA transcript; and UTR, untranslated region.

Figure. Long noncoding RNAs (IncRNA) are differentially activated at the fetal and adult stages of cardiac development. Possible mechanisms by which IncRNAs can modulate gene expression include mRNA decay, mRNA stabilization, or microRNA (miRNA) sponge during the transition from fetal to adult heart. Epigenetic changes promote the expression of IncRNAs (H3K4me1 as enhancers and H3K4me3 as promoters) and their coordinate mRNA. In the fetal heart, IncRNAs associate more with genes involved in development and programming, whereas in the adult heart IncRNAs associate more with genes involved in disease and dysfunction.

![Image](image-url)
to human disease. To further support this, the authors used data integrated from single nucleotide polymorphism (SNP) databases to interrogate whether transcription factor–binding sites for adult and fetal lncRNAs could be influenced by variation in the genetic sequence between individuals. They suggest that SNPs present in transcription factor–binding sites of lncRNAs may result in their altered expression during development. Although they did not test their hypotheses experimentally, their data demonstrate the additional layer of potential complex regulation when assigning function to ncRNAs as causative factors in cardiovascular disease.

Many important limitations exist within this study, but do represent important opportunities for further exploration. First, the authors focus on lncRNAs that are coexpressed with protein-coding mRNAs, but do not actually verify whether these transcripts lead to a functional protein. There exists the possibility that although coexpressed, an lncRNA may in fact target its protein-coding partner to prevent its translation. The specific targets of these lncRNAs were not investigated and were beyond the scope of this study, yet will undoubtedly allow us to further understand how lncRNAs function in regulating development and disease. Finally, the study restricted its analysis to coding regions that were in close proximity to promoter regions and lncRNAs, and may have thus overlooked important relationships that are distal to protein-coding regions. As the tools to investigate trans noncoding elements improve, so too will the understanding of how fetal versus adult lncRNAs may be contributing to cardiac physiology and ultimately disease.

Nevertheless, this study provides a valuable resource for the in-depth investigation of lncRNAs in human cardiovascular health and disease. The investigators are particularly lauded for posting and sharing their data on Heart Development Associated lncRNA Database (http://210.42.113.162/Heart/index.php) to encourage collaboration among other interested investigators of the scientific community. This will help to further experimental explorations of how RNAs specifically regulate human heart development and shed light on the pathogenesis of disease. Working as a community to coordinate and unravel the program represents a new way to move science forward for the benefit of understanding ourselves, our patients, and society at large.

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Disclosures
None.

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