Noncoding RNAs in Cardiovascular Biology and Disease

Priyatansh Gurha, Ali J. Marian

The genome continues to fascinate the enthusiasts, and the captivation seems unabating because of the continuous stream of new discoveries. What was once considered a junk DNA has now emerged to contain a large number of important regulatory elements. As an example of such discoveries is the recent finding that the genome contains ≈6200 enhancer elements that are operational in the human fetal and adult hearts. Genes occupy only ≈1.5% and coding exons only ≈1% of the genome, which make up only ≈60 million nucleotides of the 6.4 billion nucleotides (3.2 billion base pairs) in the genome. Yet, ≈5% of the human genome has undergone purifying selection and hence are likely functional. It is intriguing that about two third of the evolutionary constrained genomic elements are located in introns and the intergenic regions, suggestive of their regulatory roles in the genome. These conserved regions are enriched in loci that have been found to be associated with clinical phenotypes in the genome-wide association studies. The initial findings of the ENCODE (Encyclopedia of DNA Elements) project, although preliminary, illustrate the presence of numerous regulatory elements in the genome, including the enhancers.

The discoveries largely made possible by the recent advances in the high-throughput RNA sequencing point to enormous RNA splicing diversity and the plethora of alternative splicing variants. Approximately 95% of the multiexon genes undergo alternative splicing, resulting in ≈100,000 abundant splice variants in various tissues. Moreover, it seems that almost all genomic regions in 1 form or shape are transcribed, which seems perplexing as only ≈1% of the genome codes for proteins, as has been understood to date. Only recently we have started to appreciate the diverse biological functions of these nonprotein coding transcripts, which are referred to as noncoding RNAs (ncRNAs). And yet, it is mesmerizing to learn that ncRNAs might indeed contain small open reading frames and encode functional peptides. The long ncRNA (IncRNA) pncr003:2L in Drosophila encodes two 28 and 29 amino acid peptides sarcolamban A and B because of their structural and functional similarities to sarcolipin and phospholamban, regulators of Ca\(^{2+}\) uptake by SERCA2 and cardiac function. Thus, in a sense, the term ncRNAs might be a misnomer for some ncRNAs, because they might code for small peptides. If this discovery turns out to be a common feature of the IncRNAs, the discovery has the potential to change the landscape of genomic biology and medicine dramatically.

The rRNA, tRNA, snRNA, and snoRNA were among the first ncRNAs to be identified and characterized to have a role in mRNA translation and RNA processing events such as nucleotide modification and splicing. The discovery of Lin-4, the first microRNA (miRNA), in Caenorhabditis elegans by Ambros and colleagues in 1993 ushered in a new era and was soon followed by identification of new classes of ncRNA, primarily based on whole transcriptome sequencing (Table 1). These discoveries expanded the function of ncRNAs as fine regulators of various biological processes. It is now evident that ncRNAs mediate both post-transcriptional and transcriptional gene regulation, predominantly through RNA-guided (dependent) mechanisms. Moreover, recent data also suggest that miRNAs have autoregulatory functions. The current classification of ncRNAs is based primarily on transcript size as small ncRNAs (<200 nucleotides) and IncRNAs (>200 nucleotides). Small ncRNAs consist of several diverse arrays of RNAs ranging in size from 17 to 200 nucleotides that follow distinct path for their biogenesis and function (Table 1). A notable member in the class of ncRNAs is miRNAs, which are ≈22 nucleotides long and orchestrate post-transcriptional gene regulation (as RNA protein complex)
miRNAs, first discovered in worms (Lin-4) 20 years ago, are now known to have a role in many biological processes through base pairing to their target RNAs and either degradation of the mRNA or inhibition of its translation. miRNAs are generally produced as RNA polymerase II–transcribed primary transcript, namely pri-miRNA. The biogenesis of pri-miRNA transcript occurs either through the canonical pathway involving DROSHA and DICER (RNase type III enzyme) or through various noncanonical pathways that are DROSHA-and even DICER-independent.13–15 Likewise, recent data show that miRNAs could be produced from snoRNA, tRNA, or Y-RNA, as intermediate products.16–18

miRNAs in vivo.24–34 These discoveries have established the

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<th>Table 1. Classification and Function of Noncoding RNAs</th>
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<tr>
<td>RNA</td>
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<tr>
<td>Small noncoding RNA</td>
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<td>miRNAs</td>
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<td>tRNA-derived small RNAs</td>
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<td>snoRNA-derived RNAs</td>
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<td>Y-RNA–derived RNA</td>
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<td>Transcription start site–associated RNA</td>
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<td>Termi-associated small RNA</td>
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<td>lRNA</td>
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<td>Y-RNA</td>
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<td>Long noncoding RNA</td>
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<td>snRNA</td>
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<td>snoRNA</td>
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<td>l1 RNA</td>
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<td>Telomerase RNA component</td>
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<td>Promoter-associated long noncoding RNA</td>
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<td>Enhancer RNA (eRNA)</td>
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<td>Unidirectional eRNA</td>
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<td>Bidirectional eRNA</td>
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<td>Long intervening noncoding RNAs</td>
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<td>Clustered regularly interspaced short palindromic repeat RNA</td>
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<td>tmRNA</td>
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The importance of miRNAs in cardiac development, biology, and physiology was initially demonstrated by cardiac deletion of Dicer1 at different stages of development.20,21 Conditional deletion of Dicer during development (E8.5) could lead to embryonic lethality, whereas deletion at early age (3 weeks) could lead to cardiac arrhythmia. Deletion at older age (8 weeks) could lead to heart failure with ventricular enlargement, fibrosis, and cardiac myocyte disarray.22,23 Several expression-based studies in human diseased hearts and animal models, along with gain- and loss-of-function studies in mice, have shown pathogenic and protective functions of miRNAs in vivo.24–34
importance of miRNAs in regulating cardiovascular development, hypertrophy, contractility, fibrosis, apoptosis, valve formation, and gene expression in the heart.\textsuperscript{28,30,35,38-40} Recently, miRNA-24 has been shown to be a finetuner of the excitation/contraction coupling through regulation of junctophilin-2 at the dyad in heart.\textsuperscript{42,50} In addition to myocardial biology, miRNAs have a prominent role in vascular biology and are known to modulate gene expression and cell fate in smooth muscle and endothelial cells.\textsuperscript{36,37,39,43,51–55} For example, miRNA-143/145 cluster is involved in the regulation of contractile smooth muscle cell phenotype through targeting KLF4, ELK1, and CAMKII δ.\textsuperscript{56} Similarly, miRNA-17–92 cluster and miRNA-126 regulate angiogenesis and endothelial cell function.\textsuperscript{57} In addition, age-related downregulation of extracellular matrix proteins has been associated with increased expression of miRNA-29 in aneurysmal aortic dilatation.\textsuperscript{58,59} miRNAs also regulate mitochondrial function, metabolism, and cholesterol biosynthesis. miRNA-10b regulates reverse cholesterol transport, partly through interaction with gut microbiota.\textsuperscript{42,50,61}

A remarkable discovery with notable implications is the discovery of cardiac miRNA 208-a regulating systemic energy homeostasis and body weight through targeting MED13 subunit of the mediator complex, which controls transcription by nuclear hormone receptors.\textsuperscript{52,62} The ability of miRNAs to determine pluripotency, lineage commitment, reprogramming, proliferation, and differentiation has set forth the potential use of miRNAs in cardiac repair and regeneration.\textsuperscript{64,66} New areas of research in cardiac regeneration have now focused on the use of miRNAs for direct and indirect in vivo reprogramming. A combination of miRNAs-1, -133, -208, and -499 has been used in the proof-of-concept for in vivo and in vitro direct reprogramming of cardiac fibroblasts to cardiac myocytes.\textsuperscript{65,69} Recently, miRNA-15 and miRNA-17–92 families have been identified as regulators of cardiac myocyte mitotic arrest and proliferation.\textsuperscript{65,70} Similarly, miRNA-590-3p and miRNA-199-3p were identified through high-throughput screening and shown to regulate neonatal cardiac myocyte proliferation, as indicated by DNA synthesis and increased cytokinesis.\textsuperscript{71,72} The fields of miRNAs have now moved from expression-based studies to define the role of subsets of miRNAs and their targetomes.\textsuperscript{73} Not surprisingly, miRNAs are now considered as therapeutic reagents to promote cardiac myocyte re-entry into the cell cycle and improve cardiac function in the diseased heart.\textsuperscript{73–77} Finally, in addition to the resident miRNAs, circulating miRNAs are also emerging as potential biomarkers and extracellular communicators, which target recipient cells and potentially regulate translation in host cells.\textsuperscript{78}

miRNAs represent just 1 class of small ncRNAs. The characterization of additional small ncRNAs, namely piRNAs, spliRNAs, tRNAs, along with their associated proteins in the cardiovascular system, is expected to open new avenues for gene regulation/modulation. Such discoveries are expected to offer new dimensions to the exciting world of gene regulation and small RNA biology in the cardiovascular system.

The advent of high-throughput sequencing coupled with mass spectrometry and bioinformatics techniques has led to the discovery of IncRNAs and hence expanded the field of ncRNAs dramatically. IncRNAs range in size from 200 bp to a hundred kilobases. Computational analysis identified ≈7000 IncRNAs in the human genome.\textsuperscript{79} The NONCODE database has catalogued 73327 IncRNAs from various organisms, including 33 788 from humans (http://159.226.118.44/NONCODERv3/index.htm). IncRNAs have been classified into multiple groups based on their genomic location, such as intergenic/intervening RNAs (lincRNA) and intronic or exonic IncRNAs. IncRNAs are transcribed by RNA polymerase II, and most of them undergo alternative splicing, 5′-capping, and polyadenylation. They could also serve as a template for transcription of small ncRNAs.\textsuperscript{80} Mature IncRNAs are thought to have low protein coding potential, because they lack known protein coding domains or open reading frames, display random codon usage, and have no significant bias toward silent nucleotide substitutions. Furthermore, IncRNAs are under less selective pressure and, therefore, show less sequence conservation.\textsuperscript{81,82} However, as discussed earlier, at least a subgroup of IncRNAs also might code for small peptides. The IncRNA pncr003:2 L encodes sarcomablan A and B, which are small peptides involved in regulating Ca\textsuperscript{2+} uptake and cardiac contractility.\textsuperscript{67,83} Discoveries on the coding potential of IncRNAs, which suggest a dual functional role for IncRNAs, if ubiquitous rather than a rare event of nature, have the potential to shift the paradigms in molecular biology and medicine.

Mechanistically, IncRNAs might function either in cis or trans to modulate the expression of their target genes by using a wide range of molecular mechanisms, such as serving as a scaffold for recruitment of chromatin modifiers or transcription factors, or as decoys for protein sequestration and mRNA sponges to activate or silence genes.\textsuperscript{82,84–87} Furthermore, IncRNAs have also been reported to influence mRNA splicing, translation, and turnover.\textsuperscript{88–90} In the heart,
a few lncRNAs have been implicated in regulating cardiac lineage commitment and cardiac development, which underscore the possibility that lncRNAs represent new modes of developmental regulation (Table 2). The lncRNA Brackearth regulates expression of cardiovascular genes through targeting the mesoderm posterior I transcription factor.93 It also binds to the PRC2 complex and influences epigenetic regulation of gene expression.94 The lncRNA Fendrr (FOXF1 adjacent noncoding developmental regulatory RNA) also binds to PRC2 and TRXG/MLL complexes and regulates transcription factors involved in cardiac mesoderm differentiation.93 Another class of lncRNAs that is complementary to other endogenous RNAs is called natural antisense transcripts (NATs). They can be transcribed in cis from opposing DNA strands at the same genomic locus (cis-NATs) or in trans from separate loci (trans-NATs). These RNAs regulate corresponding sense mRNAs by transcriptional silencing, imprinting, splicing, or editing. Two of the better-characterized NATs with role in cardiac pathophysiology are the myosin heavy chain antisense RNA transcript (mhs-NAT) and the antisense ncRNA CDKN2B-AS (ANRIL).94–96 Whereas the former is coregulated with myosin heavy chain genes during neonatal development,97 the latter is an inflammation-responsive lncRNA that targets cell cycle regulator CDKN2B and hence regulates smooth muscle cell proliferation and senescence.98 In view of these recent developments, one might speculate that almost all processes of cardiac pathology are governed by ncRNAs. Whereas miRNAs have emerged as the major tweakers and nudgers of the genome management,99 it remains to be seen whether lncRNAs, similar to their smaller-sized counterparts, are more of finetuners or major regulators of the genome. Genomic discoveries are expected to continue to fascinate enthusiasts in the years to come.

Disclosures

None.

References


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