Long Noncoding RNAs in Heart Disease

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Abstract

Advances in research technology with systematic and unbiased measurements of transcriptional activity revealed the surprising fact of pervasive transcription of mammalian genomes. However, most of these transcripts are not obviously protein coding nor do they reveal easily inferable biological relevance and thus have been termed "noncoding". This universe of noncoding RNAs with diverse and versatile families such as transfer RNAs (tRNA), ribosomal RNAs (rRNA), micro-RNAs (miRNA) small nucleolar RNAs (snoRNA) has fuelled an entire

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new branch of research and already challenged major dogmas in molecular biology. Among the diverse classes of noncoding RNAs, long noncoding RNAs (lncRNA) have emerged as major regulators of transcription, nucleolar organization, and chromatin-modifying complexes. The goal of this chapter is to present the state of research of lncRNAs in the context of heart disease and heart failure.

Abbreviations

ChIP-Seq	Chromatin immunoprecipitation sequencing
eRNA	Enhancer-associated RNA
lincRNA	Long intergenic/intervening noncoding RNA
lncRNA	Long noncoding RNA
ncRNA	Noncoding RNA
PARP	Poly ADP ribose polymerase
RPKM	Reads per kilobase per million mapped reads
TAC	Transverse aortic constriction
UTR	Untranslated region

13.1 Introduction

Heart failure is among the most prevalent causes for morbidity and mortality in the Western world with as many as 1–2 % of the adult population afflicted (McMurray et al. 2012). Five-year survival is as a low as 50 %, numbers that underscore the severity of this condition (Go et al. 2014; Wong et al. 2014). Heart failure develops if the cardiac muscle is unable to comply with the oxygen demands of the organism due to either a decrease in systolic function (heart failure with reduced ejection fraction) and/or an increase in ventricular-filling pressures (heart failure with preserved ejection fraction), both leading to a decrease in cardiac output and to clinical symptoms such as dyspnea, edema, and sudden cardiac death (Hill and Olson 2008).

Heart failure is a dynamic process and the consequence of an intricate network of cardiac remodeling processes, including cardiac physiological and pathological hypertrophy, atrophy, and dilatation (Frey et al. 2004; Burchfield et al. 2013; Lyon et al. 2015; van Berlo et al. 2013). A mechanistic understanding of cardiac signaling cascades and structural cellular components, including receptors and kinases, sarcomeric "hubs," such as the z-disk, transcription factors, and miRNAs, has helped to advance current and future medical therapy, for example, medications modulating the renin–angiotensin–aldosterone axis (Frank et al. 2006; Packer et al. 2015; Olson 2014).

Elucidating the genomic sequence of humans has promised to deliver the blueprint for combatting disease in a causal fashion, directly aimed at its molecular roots. Nevertheless, the promises of genomic medicine to develop novel therapies

have not been fulfilled yet, but helped to elucidate further levels of complexity in the regulation of cellular processes. Research efforts have now turned from individual genomic sequences toward sequencing thousands of individuals to unravel genomic variation (Lander et al. 2001; 1000 Genomes Project Consortium et al. 2012).

Furthermore, the efforts of research consortiums, such as ENCODE, FANTOM, or NIH Roadmap Epigenomics to catalog and analyze regulatory and functional elements of different genomes and their epigenetic states are thus only the beginning and leave the research community with vast amounts of data of a complex regulatory landscape that now need individual characterization and further refinement (ENCODE Project Consortium 2012; Forrest et al. 2014; Kundaje et al. 2015).

Epigenetics is classically defined as the field of genetic research that analyses the phenotypic changes imposed by mechanisms, which do not depend on changes of the initial genomic sequence (Waddington 1942; Berger et al. 2009).

Epigenetics has recently contributed substantially to the deciphering of many key-regulating factors of transcriptional and translational activity and to our understanding of a variety of pathologic conditions, especially in cardiovascular and cancer research (Backs and Olson 2006; Chang and Bruneau 2012; Weichenhan and Plass 2013).

Advances in research technology with systematic and unbiased measurements of transcriptional activity revealed the surprising fact of pervasive transcription of mammalian genomes (Carninci et al. 2005). However, most of these transcripts are not obviously protein coding nor do they reveal easily inferable biological relevance and thus have been termed "noncoding". This universe of noncoding RNAs with diverse and versatile families such as transfer RNAs (tRNA), ribosomal RNAs (rRNA), micro-RNAs (miRNA) small nucleolar RNAs (snoRNA) has fuelled an entire new branch of research and already challenged major dogmas in molecular biology (Cech and Steitz 2014). Among the diverse classes of noncoding RNAs, long noncoding RNAs (lncRNA) have emerged as major regulators of transcription, nucleolar organization, and chromatin-modifying complexes. The main goal of this chapter is to present the state of research of lncRNAs in the context of heart disease and heart failure.

13.2 Pervasive Transcription and the Characterization of lncRNAs

The history of research in noncoding RNAs can be traced back to the 1950s with the discovery of rRNAs and tRNAs, followed by snRNAs and antisense transcription in the 1980s and 1990s. Apart from individual lncRNAs such as H19 and Xist, it was not before the technological advancements of the years after 2000, especially tiling microarrays and next-generation sequencing, together with sophisticated immunoprecipitation approaches, that a systematic understanding of the RNA landscape emerged (Willingham and Gingeras 2006).

Pioneering research was conducted using tiling microarrays of human chromosomal transcription that allowed analysis of transcription at windows of only a

couple of nucleotides. Thereby it became evident that large parts of the human genome are pervasively transcribed and a high number of these new transcripts have very low or no potential at all to encode for proteins. Sequence analysis of mouse-noncoding transcription revealed a lesser degree of conservation on average compared to 5′- or 3′-UTRs. Conversely, the putative promoter regions of ncRNAs were more conserved than those of protein-coding genes. Noncoding RNAs were arbitrarily divided into short- and long-noncoding RNAs at a length of 200 nucleotides. Long-noncoding transcripts show specific expression patterns in cellular compartments with partly restricted expression to the nucleus or cytosol and high degrees of tissue-specific expression. They tend to be lower expressed, compared to protein-coding transcripts (Kapranov et al. 2002; Rinn et al. 2003; Bertone et al. 2004; Cawley et al. 2004; Kampa et al. 2004; Cheng et al. 2005; Carninci et al. 2005; Kapranov et al. 2007).

The research conducted by the ENCODE-consortium added further understanding to the class of long noncoding RNAs. The combination of RNA-sequencing maps of chromatin regulatory marks delineated that in fact 62.1 % and 74.7 % of the human genome give rise to processed transcripts (Djebali et al. 2012).

An in-depth analysis of all human coding and noncoding sequences exemplified a variety of important characteristics of long noncoding RNAs: The GENCODE analysis identified 14,880 human lncRNA transcripts, 9,518 of them from intergenic and 5,362 from intragenic transcriptional origin. Transcriptional start sites of long noncoding RNAs are comparable to protein-coding genes with respect to active histone marks (H3K4me2, H3K4me3, H3K9ac, H3K27ac), but show a higher level of marks associated with both silencing (H3K27me3) and activity (H3K36me3). LncRNAs are transcribed via polymerase II-dependent mechanisms and most of them are post-transcriptionally modified via 5′-capping and 3′-polyadenylation, although other modifications of lncRNAs, such as methylation at N(6)-methyladenosine, have been described (Ponting et al. 2009; Zhang et al. 2014; Fu et al. 2014).

There is a high degree of correlation between expression of long noncoding RNA transcripts and neighboring protein-coding genes, both in *cis* and in *trans*, but generally more pronounced in *cis*. This observation of expression correlation holds also true for long noncoding RNAs that are intertwined with protein-coding genes and is partly attributed to transcriptional coregulation mechanisms (Derrien et al. 2012; Orom et al. 2010; Kim et al. 2010).

Classification of long noncoding RNAs correspond to genomic organization and association with DNA-regulatory elements, this is summarized in Table 13.1 and exemplified in Fig. 13.1.

Following these initial results, several groups reported catalogs of lncRNAs and refinements of systematic analyses, confirming this initially reported characteristics of lncRNAs. Complementary approaches using correlation of expression data with histone marks of activated protein and ribosome profiling further advanced our understanding, especially considering biological relevance and coding potential (Guttman et al. 2009; Mikkelsen et al. 2007; Guttman et al. 2013).

Table 13.1 Classification of long noncoding RNAs

Genomic localization	Intergenic	
	Genic	Exonic
		Intronic
		Overlapping
Genomic strand	Sense	
	Antisense	
Direction of transcription	Sense	
	Antisense	
	Bidirectional	
Association with DNA regulatory elements	Promoter associated	
	Enhancer associated	

(Modified after Derrien et al., Genome Res 2012)

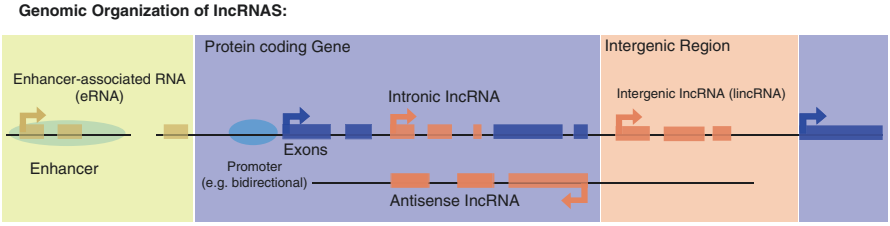

Fig. 13.1 Exemplifies the genomic organization of long noncoding RNAs, together with different regulatory elements e.g. promoter and enhancer regions and protein coding genes

13.3 Evolutionary Conservation of Long Noncoding RNAs

Strong evolutionary conservation is generally viewed as an indicator of biological significance, as evolution can be seen as nature's experiment of function on the scale of millions of years. Thereby, understanding evolutionary conservation may aid understanding relevance. In the case of long noncoding RNAs, this has become increasingly more difficult. The combination of comparably lower transcriptional activity and interspecies sequence variation in noncoding regions led to the initial notion that transcription at noncoding loci is genomic "junk", leading to "transcriptional noise" without any biological significance (Ponjavic et al. 2007).

Two systematic analyses of lncRNA ancestry in 6 different mammals and 11 tetrapod species identified families of homologous lncRNAs at different evolutionary stages. The highest degree of conservation and sequence constraints was found in older lncRNA families. However, newer families showed evidence of purification selection, especially at the promotor level. As a class, lncRNA are considered to be able to evolve fast and to show strong tissue specificity (Necsulea et al. 2014; Washietl et al. 2014). Hezroni et al., directly compared transcriptomes from 17 different species using a novel computational pipeline to identify lncRNAs, sequence

homology, orthologous and syntenic sequences. Their results confirmed several previous findings about long noncoding RNAs, but additionally led to the discovery that conserved lncRNAs are biased towards sequence conservation at their 5′ regions. Furthermore, exoneration of transposable elements plays a pivotal role in the evolutionary development of lncRNA sequences (Hezroni et al. 2015).

Diederichs summarizes the current research findings and distinguishes four different entities of long noncoding RNA conservation: sequence, structure, function, and expression from syntenic loci (Diederichs 2014).

These results led to interesting conclusions: First, lack of sequence similarity does not necessarily exclude evolutionary conservation and thus high biological relevance. Second, understanding of a long noncoding RNA can only be achieved in its genomic context and with understanding of the associated regulatory elements. Evolutionary conservation will be especially relevant to translate findings from research in cardiovascular model systems to humans, as morphology and physiology of the heart as an organ changed profoundly over the course of evolution, with obvious significance for human disease, that is, the low regenerative potential of the mammalian heart.

13.4 Long Noncoding RNAs in Cardiovascular Research: Bench

Research in mouse models of heart embryonic development and models mimicking human pathology have led to important discoveries and characterized lncRNAs as potent epigenetic regulators of cardiac gene expression and chromatin modification:

13.4.1 Polycomb, Trithorax, and Noncoding RNAs: Fendrr and Braveheart

Polycomb and Trithorax Group proteins are central modulators of epigenetic gene regulation, development and differentiation and are associated with cardiac development and disease (Wang 2012). Proteins of the TrxG/MLL complex catalyze H3K4 methylation and act toward the activation of transcription (Schuettengruber et al. 2011). Proteins of the PRC2 complex act as a methyltransferases and catalyze methylation of H3K27, thereby repressing gene expression (Di Croce and Helin 2013). It has been shown that components of both the PRC2 and the TrxG/MLL complexes are able to interact with long noncoding RNAs (lncRNAs) and this interaction might constitute an important regulatory mechanism (Khalil et al. 2009; Wang and Chang 2011; Brockdorff 2013). RNA-immunoprecipitation of PRC2 components combined with RNA-sequencing (RIP-Seq) in mouse embryonic stem cells led to discovery of more than 9000 different RNAs that bind to PRC2 (Zhao et al. 2010). This is mediated through specific binding mechanisms, as well as promiscuous binding of RNAs. This led to ongoing controversy regarding the

prevailing mechanism and possible experimental artifacts (Davidovich et al. 2013). However, new data confirmed both specific and promiscuous RNA binding *in vitro* (Davidovich et al. 2015). Polycomb and Trithorax proteins act antagonistically through DNA regulatory responsive elements. Interestingly, also Polycomb/Trithorax response elements show transcriptional activity and can switch their respective function.

Recently, important roles for long noncoding RNAs in mediating regulatory effects via PRC2/TrxG in the heart have been elucidated: Long noncoding RNA "Fetal-lethal noncoding developmental regulatory RNA" (Fendrr) was identified from RNA-Seq and ChIP-Seq analysis from early somite stage mouse embryos. Fendrr is divergently transcribed upstream of the Foxf1-locus and predominantly expressed in lateral plate mesoderm. Knockdown of Fendrr leads to embryonic lethality around E13.75, accompanied by cardiac hypoplasia and omphalocoele. Fendrr is predominantly expressed in EOMES-positive cells of the early cardiac mesoderm lineage. Knockdown of Fendrr leads to increase of H3K4-promoter trimethylation of key cardiac developmental transcription factors Gata6 and Nkx2-5 and increased their expression at E8.5. At later stages, Gata6, Foxf1, together with Irx3 and Pitx2 show higher expression in mutant embryos. Fendrr mutant embryonic stem cells show reduced promoter occupancy of Ezh2 and Suz12 at several potential target genes of Fendrr. Fendrr directly binds PRC components Ezh2 and Suz12 and TrxG/MLL component Wdr5. No binding could be observed for the parts of PRC1, suggesting specificity of discrimination between different histone-modifying complexes. Furthermore, a sequence domain of Fendrr is able to bind double-stranded Foxf1 and Pitx2 promoter regions. Taken together, Fendrr links PRC2 to its target promotors, thereby increasing PRC2 occupancy and methylation of H3K27, which subsequently leads to the modulation of cardiac developmental gene expression (Grote and Herrmann 2013; Grote et al. 2013).

Expression analysis of different mouse embryonic stem cells and the adult heart led to identification of the long noncoding RNA "Braveheart" (Bvht). Although knockdown of Bvht did not impede differentiation of embryonic stem cells toward all three lineages, differentiation of embryoid bodies toward cardiac myocytes was severely impaired. Transcriptome analysis of such depleted cells revealed failed activation of key cardiac transcription factors (among them MesP1, Hand1/2, Nkx2-5, Tbx20) and transcripts for epithelial–mesenchymal transition (Snai, Twist). A significant overlap between Bvht-depletion and MesP1-regulated genes was observed. Forced expression of MesP1 in Bvht-depleted embryonic stem cells was able to rescue this phenotype, that is, permitting differentiation to the cardiac lineage. Therefore, the authors concluded Bvht to function upstream of MesP1. Mechanistically, Bvht interacts with Suz12 as part of PRC2. Furthermore, knockdown of Bvht in neonatal cardiac myocytes leads to disruption of myofibrillar ultrastructure and reduced expression of cardiac structural proteins. Taken together, the authors reveal Bvht as a novel key regulator of cardiac development (Klattenhoff et al. 2013).

13.4.2 Antisense Transcription and Chromatin Remodeling at the Myosin Locus

The switch of cardiac myosin isoform expression between adult Myh6 (α-myosin) and fetal Myh7 (β-myosin) is a hallmark of cardiac hypertrophy and heart failure (Miyata et al. 2000). A role for antisense transcription in regulating myosin expression was described earlier; nevertheless, an understanding of downstream mechanism has been lacking (Haddad et al. 2003). Antisense transcription and alternative splicing from exonic and intergenic regions of the Myh7-locus give rise to a cluster of cardiac specific long noncoding RNAs, named "Myhrt". The most abundant isoform, Mhrt770, was characterized as a potent inhibitor of pathologic cardiac hypertrophy. Cardiac-specific overexpression of Mhrt is able to alleviate early and late cardiac hypertrophy in mice after TAC surgery. Mechanistically, Mhrt770 binds Brg1, a member of the SWI/SNF ATP-dependent chromatin-remodeling complex to form a feedback circuit. Brg1 interacts with several HDACs and PARPs to form a complex on Myh6 and Myh7 promoters for antithetic regulation (Hang et al. 2010). Mhrt770 prevents Brg1 to bind to its target genomic region at the promoters of Myh6, Myh7, and Opn. These findings could be translated to human cardiomyopathies: Expression of MHRT is reduced in hearts from patients with cardiomyopathies (Han et al. 2014) (Fig. 13.2).

13.4.3 Fetal Cardiac Enhancer-Associated Long Noncoding RNAs

Enhancers are defined as "intergenic or intragenic regulatory sequences that can activate gene expression […]" (Orom and Shiekhattar 2013). Enhancers harbor transcriptional activity and can even give rise to transcription of lncRNAs (Kim et al. 2010). These enhancer-associated long noncoding RNAs themselves can provide an enhancer-like function in regulating transcriptional activity (Orom et al. 2010). Furthermore, they can be distinguished as a class from promoter-associated lncRNAs with distinct characteristics concerning tissue specificity and expression levels, as well as evolutionary conservation. (Marques et al. 2013) Recently, analyses of differential lncRNA expression at fetal cardiac enhancers during cardiac progenitor cell differentiation, after TAC surgery and LAD ligation were able to identify many dynamically transcribed lncRNAs. These were correlated with transcriptional activity at neighboring genes. Knockdown of two selected cardiac-enhancer-associated lncRNAs led to the suppression of gene expression of the corresponding genomic locus (Ounzain et al. 2014b).

13.4.4 Interactions Between miRNAs and lncRNAs

Long noncoding RNAs have also been characterized for their interactions with microRNAs via miRNA-binding sequence domains. Thereby, they serve as competing endogenous RNAs (ceRNAs) and bind miRNAs (as "sponges"), which reduces

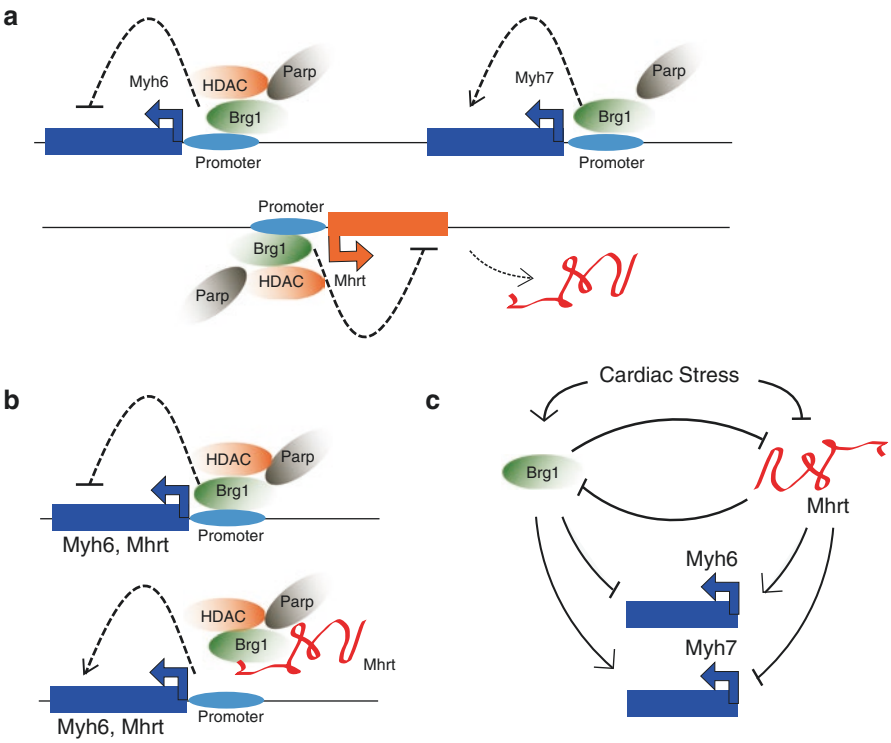

Fig. 13.2 LncRNA Mhrt modulates DNA binding of Brg1–chromatin repressor complex in cardiac hypertrophy: **(a)** During conditions of cardiac stress, chromatin repressor complex Brg1-Parp-Hdac suppresses Myh6 and activates Myh7 transcription, promoting the myosin isoform switch. LncRNA Myhrt is an antisense-lncRNA, overlapping parts of the Myh7 locus and also transcriptionally repressed by the Brg1 complex, **(b)** The Brg1–Parp–Hdac complex interacts with the Myh6 promoter to repress the transcription of Myh6 and Mhrt. Mhrt binds the Brg1-helicase domain and thereby impedes Brg1 to recognize its chromatin targets, **(c)** Pathological stress activates the Brg1–Hdac–Parp chromatin repressor complex to inhibit Mhrt transcription in the heart. Brg1 and Mhrt form a negative feedback loop. Brg1 is a mediator of cardiac hypertrophy and functions as a repressor of Myh6 and activator of Myh7. Mhrt inhibits Brg chromatin repressor complexes to recognize their targets and thereby leads to a normalization of Myh6/Myh7-ratio, *Parp* poly (ADP ribose) polymerase, *HDAC* histone deacetylase, *Myh6* alpha-myosin heavy-chain, *Myh7* beta-myosin heavy-chain, *Mhrt* myosin heavy-chain-associated RNA transcripts

target miRNA levels and alleviates its downstream effect (Salmena et al. 2011; Tay et al. 2014). The relevance of this interaction has been established in several examples for skeletal muscle transcriptional regulation and recently also in the heart (Cesana et al. 2011; Dey et al. 2014).

Long noncoding RNA AK048451, named "cardiac hypertrophy-related factor" (CHRF), is significantly induced after the treatment of cardiac myocytes with angiotensin-II and harbors a binding site for miR-489, with high interspecies sequence conservation. Knockdown and overexpression of CHFR modulates

expression levels of miR-489, followed by impaired or exaggerated hypertrophic response via change of sequestration of miR-489 and changed expression of downstream target Myd88 (Wang et al. 2014a).

Prohibitin subunit 2 (Phb2) is downregulated in cardiac myocytes after exposure to anoxia. Overexpression of Phb2 reduces mitochondrial fission, apoptosis, and size of myocardial infarction in mice. Vice versa, the knockdown increases mitochondrial fission and infarct size. Phb2 is specifically regulated though microRNA miR-539 via binding at the 3′ UTR. Downregulation of miR-539 inhibited both mitochondrial fission and apoptosis induced by anoxia. These effects were abrogated by the knockdown of Phb2, therefore indicating a functional link of miR-539 and downstream Phb2. Using bioinformatics, lncRNA AK017121 (named "CARL") was identified, that is able to specifically bind miR-539 and, by modulating its expression, CARL exert functional effects on apoptosis and mitochondrial fission in cardiac myocytes (Wang et al. 2014b).

Autophagy is a key stress response in cardiac myocytes, aimed at preventing cell death at the expense of digesting short-term dispensable proteins and/or organelles. Atg7 encodes the E1 enzyme in the autophagosome and is involved in the regulation of autophagy and cell death. MicroRNA miR-188-3p is able to specifically bind to the 3′-UTR of Atg7 and thereby exerts an inhibitory effect on autophagic response of cardiac myocytes. LncRNA AK079427 (named "AFP") is able to specifically bind miR-188-3p and thereby interferes with this signaling cascade. AFP participates in the autophagic regulation both in cardiac myocytes and in an in vivo model of ischemia-reperfusion injury (Wang et al. 2015).

13.4.5 LncRNAs, Short Open Reading Frames and Small Peptides

Attributing the coding potential of a transcript is usually achieved using a combination of several bioinformatics approaches, combining measures of evolutionary conservation of amino acid sequences known protein domains and other data (e.g., from ribosome sequencing, mass spectroscopy). This is especially difficult for short transcripts and is prone to error. Short open reading frames can be defined by their size ranging from 2 to 100 codons. Several transcripts originally attributed as noncoding were found to be coding after experimental validation, some of them being translated to micropeptides with important regulatory functions (Andrews and Rothnagel 2014): ELABELA is a 32 amino acid protein that is highly conserved between species. Experimental deletion in zebrafish leads to cardiac dysplasia of varying severity and even aplasia of the heart, due to impaired endodermal differentiation and possibly signaling as a novel apelin-receptor ligand (Chng et al. 2013).

LncRNA gene RNA 003 in 2 L (pncr003:2 L) in fruit flies was detected to harbor two evolutionary conserved small open reading frames of 28 and 29 amino acids length. Both transcripts are strongly expressed in somatic muscles and the postembryonic heart. Overexpression and knockdown modulate calcium transients and induce cardiac arrhythmia. Both peptides show homology to sarcolipin and

phospholamban and were therefore named sarcolamban, as strong phylogenetic evidence points toward a shared ancestral peptide (Magny et al. 2013).

Myoregulin is a conserved micropeptide encoded by a skeletal muscle-specific transcript, also originally attributed as a lncRNA. Myoregulin is a direct interaction partner of the calcium pump SERCA and inhibits calcium transport activity into the sarcoplasmatic reticulum. Genetic knockdown improves skeletal muscle exercise performance in mice (Anderson et al. 2015).

13.4.6 Signatures of Long Noncoding RNAs in Mouse Models of Cardiac Development and Disease

Several research groups reported differential ncRNA expression data from high-throughput assays from different models of cardiac development and disease, such as pressure overload due to transverse aortic constriction or coronary artery ligation to induce myocardial infarction (Tarnavski 2009). We summarized the most significant findings in Table 13.2 and Fig. 13.3.

13.5 Long Noncoding RNAs in Cardiovascular Research: Bedside

Low evolutionary conservation of lncRNAs makes translation of research results from experimental models difficult. Nevertheless, some groups already reported a role for lncRNAs in human myocardial infarction, heart failure and of course, sequence variation in noncoding regions.

13.5.1 Heart Failure

Mitochondrial lncRNA LIPCAR was identified in serum of patients post myocardial infarction and characterized as a biomarker for heart failure. Initially, LIPCAR-plasma levels decrease after myocardial infarction but show strong induction in patients who develop pathologic left ventricular (LV) remodeling in the future. Furthermore, it is also induced in patients suffering of heart failure from non-ischemic etiology and expression levels strongly and independently correlate with mortality, with incremental diagnostic value compared to a model comprised of classic risk factors (Kumarswamy et al. 2014).

Analyses of human LV samples from ischemic and nonischemic cardiomyopathies before and after implantation of a mechanical assist device (LVAD) compared to control hearts using RNA-sequencing identified signatures of lncRNAs that are able to distinguish between etiologies of heart failure and discriminate patients between pre- and post-LVAD state. Furthermore, correlation analysis of lncRNA and neighboring mRNA expression revealed a significant prevalence of cis-regulation of lncRNAs and neighboring protein-coding genomic loci (Yang et al. 2014).

Table 13.2 High-throughput screening experiments in cardiac disease models

Authors	Species	Model	Assay	Key findings
Lee et al. (2011)	Mouse	TAC	RNA-Seq	15 and 135 differentially regulated lncRNAs between hypertrophy and control and failing and control, respectively. Strong induction of lncRNA H19. LncRNAs show a higher degree of tissue specific expression compared to protein coding genes
Li et al. (2013)	Mouse	Isoproteronol	Microarray	Identification of 32 lncRNAs with the patterns of regulation in plasma, heart tissue, and peripheral blood cells that might be used as potential biomarkers for heart failure
Liu et al. (2014)	Mouse	Ischemia reperfusion (LAD ligation)	Microarray	Identification of 151 differentially regulated lncRNAs
Matkovich et al. (2014)	Mouse	Embryonic heart E14.5, adult heart, TAC	RNA-Seq	Cardiac hypertrophy does not lead to the activation of embryonic expressed lncRNAs. Larger extend of differentially regulated lncRNAs between embryonic and adult conditions than between adult and hypertrophic/failing state
Ounzain et al. (2014a)	Mouse	LAD ligation	RNA-Seq	Identification of differentially regulated lncRNAs in a LAD-ligation mouse model, correlation with chromatin-state maps (strongest correlation of lncRNAs with chromatin marks of enhancer activity) and validation of evolutionary conserved lncRNAs in human-diseased tissues
Ounzain et al. (2014b)	Mouse	Embryonic stem cell differentiation, LAD ligation, TAC	RNA-Seq	Identification and characterization of foetal cardiac enhancer-associated lncRNAs. These are differentially regulated in the models of ischemia and pressure overload, knockdown of eRNAs regulates the transcription of associated target genomic locus
Zangrando et al. (2014)	Mouse	LAD ligation	Microarray	30 differentially regulated lncRNAs, most robustly induced are Miat1 and Miat2
Zhu et al. (2013)	Mouse	Embryonic hearts E11.5, E14.5, E18.5	Microarray	1237 differentially regulated long noncoding RNAs

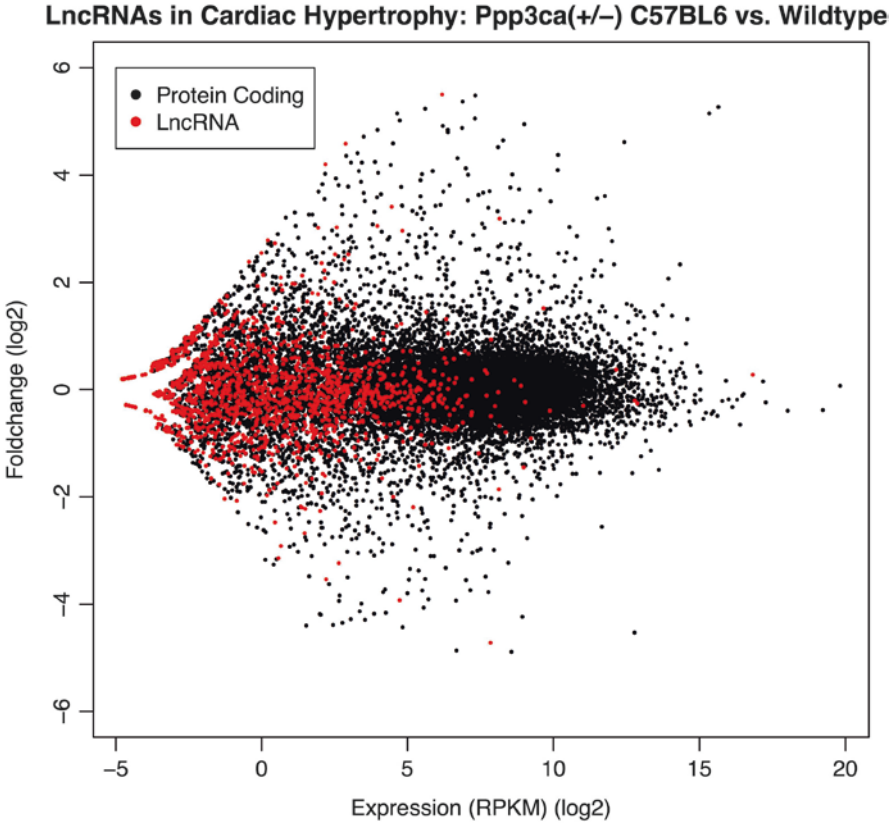

Fig. 13.3 LncRNA expression levels are lower compared to protein coding transcripts and show a lower degree of differential regulation. RNA-Seq data from Calcineurin-transgenic mouse hearts and controls (unpublished data Kühl/Frey)

13.5.2 Myocardial Infarction

Vausort et al. measured expression levels of five long noncoding RNAs with known relevance in the cardiovascular system in peripheral blood lymphocytes of 414 patients with acute coronary syndromes and 86 control patients. They selected antisense hypoxia-inducible factor 1α (aHIF), ANRIL within the 9p21-risk locus for coronary heart disease, KCNQ1-overlapping transcript 1 (KCNQ1OT1) that regulates the imprinting of KCNQ1 sodium channel, myocardial infarction-associated transcript (MIAT), and metastasis-associated lung adenocarcinoma transcript 1 (MALAT), one of the most abundantly expressed lncRNAs. In ST-elevation-myocardial infarction, the expression of ANRIL, KCNQ1OT1, MIAT, and MALAT was reduced, compared to expression levels in non-ST-elevation-myocardial infarction. The authors later used univariate and multivariate analysis to identify several associations between ejection fraction, age, diabetes mellitus, and hypertension and

expression values of lncRNAs. The measurement of ANRIL and KCNQ10T1 added significant discriminatory value in identifying patients at risk of developing LV dysfunction using multivariate analysis (Vausort et al. 2014).

13.6 Human Noncoding Variation and Heart Disease

The understanding of human genomic variation and mutations in noncoding regions and the effect on pathogenesis of human disease is incomplete. Data from the ENCODE and NIH Roadmap Epigenomics Project provide a resource to link genomic sequence, transcriptional activity, and chromatin state (Bernstein et al. 2010).

An analysis of data from the "1000 genomes project" aimed to characterize variations in noncoding regions of the human genome. Transcription factor-binding sites and noncoding RNAs were less constrained for SNPs compared to protein-coding sequences. Per contra, noncoding RNAs showed a higher degree of evolutionary constraint for insertions and deletions. A higher degree of purifying selection in a lncRNA correlated with higher levels of transcriptional expression (Mu et al. 2011).

The human genomic region 9p21.3 is among the most thoroughly studied susceptibility loci for several entities of cardiovascular disease and has been characterized in different populations (Roberts 2014; Johnson et al. 2013). Of note, this region contains the protein-coding genes CDKN2A and CDKN2B and the long noncoding RNA ANRIL ("antisense non-coding RNA in the INK4 locus"), which is strongly associated with several major SNPs of cardiac disease (Pasmant et al. 2011).

Ishii et al. conducted a case–control genetic association study and identified a new susceptibility locus for myocardial infarction located on chromosome 22q12.1 and a novel long noncoding RNA, named MIAT ("myocardial infarction-associated transcript") and characterized the impact of several SNPs that changed MIAT expression and binding to nuclear proteins (Ishii et al. 2006).

A large genomewide association trial of dilated cardiomyopathy in more than 4,100 cases and 7,600 controls reported several single nucleotide polymorphisms (SNPs) on chromosome 6p21. Highest significance after replication was attributed to a SNP that mapped to a noncoding gene, HCG22 (Meder et al. 2014).

A recent systematic analysis of published genomewide association studies found that 93% of disease and trait-associated variants lie within noncoding sequences, which comprises noncoding DNA-regulatory regions such as enhancers, promoters, DNAse hypersensitivity sites but also lncRNAs (Maurano et al. 2012).

13.7 Summary

Research into long noncoding RNAs has led to important discoveries in cardiac physiology and pathophysiology. Characterizing lncRNAs sparked the discovery of intricate links between transcription, chromatin modification, and organization that will be translated to an improved understanding of cardiac pathophysiology and disease. Hopefully, this also will lead to new targets and novel therapies. For

example, targeting antisense transcripts instead of the coding transcript allows modifying transcriptional activity at a genomic locus without changing the locus itself. As expression of many lncRNA is restricted to a specific tissue, targeting via systemically delivered gene therapy potentially causes fewer systemic off-target effects.

Looking at the growing body of research, several gaps remain. First, the wealth of long noncoding RNA sequences so far lacks clear annotation and is divided into a variety of databases. A unifying nomenclature has recently been proposed for human long noncoding RNA but needs to be introduced into all fields of lncRNA research (St Laurent et al. 2015; Wright 2014). The quality and depth of chromatin maps from human and mouse heart that allowed a more detailed understanding of cardiac lncRNAs is still not available for rat and zebrafish, two relevant species for cardiovascular research. Also, for an understanding of lncRNAs in the heart, we need to replicate the findings from ENCODE that did not include cells from the heart. So far, most results were obtained in heterogeneous cardiac cell populations. Thus, delineating the transcriptome of individual populations, such as cardiac myocytes, fibroblasts, smooth muscle, and endothelial cells, will be another important goal. Second, we will need to readjust our knowledge about mutations in noncoding regions of the genome and will have to include them for a deeper understanding of hereditary factors and mutations in cardiac disease, such as hypertrophic cardiomyopathies. Third, the individual characterization and delineation of long noncoding RNA mechanisms will need translation to the bedside. Apart from a use as biomarkers – and in that case, lncRNAs have to prove their added value compared to classic biomarkers or miRNA signatures, we will need to develop novel approaches to target and modulate cellular lncRNA expression levels.

Conflict of Interest The authors declare that they have no conflict of interest.

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