CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Elsevier - Publisher Connector

Biochimica et Biophysica Acta 1833 (2013) 923–933

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Small and long non-coding RNAs in cardiac homeostasis and regeneration ☆

Samir Ounzain, Stefania Crippa, Thierry Pedrazzini *

Experimental Cardiology Unit, Department of Medicine, University of Lausanne Medical School, Lausanne, Switzerland

ARTICLE INFO

Article history:
Received 2 July 2012
Received in revised form 10 August 2012
Accepted 11 August 2012
Available online 23 August 2012

Keywords:
Non-coding RNA
miRNA
lncRNA
Heart failure
Regeneration, stem cell
Cardiac progenitor cell

ABSTRACT

Cardiovascular diseases and in particular heart failure are major causes of morbidity and mortality in the Western world. Recently, the notion of promoting cardiac regeneration as a means to replace lost cardiomyocytes in the damaged heart has engendered considerable research interest. These studies envisage the utilization of both endogenous and exogenous cellular populations, which undergo highly specialized cell fate transitions to promote cardiomyocyte replenishment. Such transitions are under the control of regenerative gene regulatory networks, which are enacted by the integrated execution of specific transcriptional programs. In this context, it is emerging that the non-coding portion of the genome is dynamically transcribed generating thousands of regulatory small and long non-coding RNAs, which are central orchestrators of these networks. In this review, we discuss more particularly the biological roles of two classes of regulatory non-coding RNAs, i.e. microRNAs and long non-coding RNAs, with a particular emphasis on their known and putative roles in cardiac homeostasis and regeneration. Indeed, manipulating non-coding RNA-mediated regulatory networks could provide keys to unlock the dormant potential of the mammalian heart to regenerate. This should ultimately improve the effectiveness of current regenerative strategies and discover new avenues for repair. This article is part of a Special Issue entitled: Cardiomyocyte Biology: Cardiac Pathways of Differentiation, Metabolism and Contraction.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Coronary artery disease is the most frequent cardiovascular disorder, which typically leads to acute myocardial infarction and ultimately heart failure. Heart failure is one of the major causes of morbidity and mortality worldwide. According to the latest report from the American Heart Association, one in nine death certificates in the United States in 2008 mentioned heart failure as the cause of death [1]. Despite the constant development of new drugs and device-based therapies, no approach currently exists to reverse the loss of functional myocardium. The only option for end-stage heart failure remains heart transplantation. However, organ transplant will not be able to meet the increasing demand due to the relative scarcity of donor hearts [2]. Heart failure is thus evolving into a global epidemic for which medicine has no viable solution.

One area that has engendered considerable interest over the last decade is the premise of promoting cardiac regeneration in the mammalian heart [3,4]. Several ways can be envisaged to induce *de novo* replenishment of lost cardiac cells post injury. Indeed, despite the post-mitotic nature of differentiated cardiomyocytes, recent studies provided evidence for a mitotic activity in the adult myocyte population under normal conditions and during the adaptation to stress [5–7]. In addition, resident cardiac progenitor cells, sharing properties with other adult stem cells, and demonstrating a capacity to differentiate into functional cardiomyocytes *in vitro* and *in vivo* have been identified in the heart [3,4]. Altogether, this suggested that myocytes could be replaced through a process involving cell replication and differentiation. Therefore, two main strategies are currently evaluated. First, pathways that are important for the mobilization, activation and differentiation of resident cardiac stem cells are being identified. These pathways will represent attractive therapeutic targets for stimulating cardiac regeneration *in situ*. Second, cell replacement therapy can be envisaged. In this case, cardiac precursor cells are transplanted in the damaged heart to induce healing.

2. Cardiac regeneration: many avenues for repair

Cardiac regeneration in adulthood is observed in lower vertebrates such as certain amphibians and fish [8,9]. For example, zebrafish are able to regenerate their cardiac muscle after injuries such as cryoinjury [10–12], ventricular resection [9] or genetic ablation of cardiomyocytes [13]. Lineage tracing experiments revealed that dedifferentiated cardiomyocytes are the major source of the newly generating cardiac muscle. In response to injury, pre-existing mature cardiomyocytes undergo a process of sarcomeric disassembly,

☆ This article is part of a Special Issue entitled: Cardiomyocyte Biology: Cardiac Pathways of Differentiation, Metabolism and Contraction.

* Corresponding author at: Experimental Cardiology Unit, Department of Medicine, University of Lausanne Medical School, Rue du Bugnon 27, 1011 Lausanne, Switzerland. Tel.: +41 21 314 0765; fax: +41 21 314 5978.
E-mail address: thierry.pedrazzini@chuv.ch (T. Pedrazzini).

0167-4889/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbamcr.2012.08.010

dedifferentiation, reentry into the cell cycle and proliferation to replenish the lost myocardium [14,15]. This process is associated with minimal fibrosis. In addition to zebrafish, it has recently been demonstrated that early during the post-natal period, mice also exhibit a comparable regenerative capacity [16]. This regenerative plasticity is strictly confined to the first week after birth; a period in which cardiomyocytes have not terminally exited the cell cycle. Once the cardiomyocytes completed terminal differentiation, the myocardium undergoes a maladaptive reparative process associated with fibrosis following injury [16]. These findings do, however, support the notion of a dormant regenerative capacity in mammals, which could potentially be reactivated in the adult heart.

Alternatively, one might want to target endogenous cardiac stem cells in the adult heart. For instance, using high-throughput screening, we have identified the Notch pathway as activated in the hypertrophic and failing adult hearts. This pathway is known to be implicated in cardiac development and in the regeneration of adult self-renewing organs, suggesting that it could play a similar role in the adult heart [17]. Briefly, we have shown that Notch prevents cardiogenic differentiation and favors cardiac precursor expansion [18]. Furthermore, our recent data suggest that the Notch pathway is pivotal in a complex network of interactions between cardiomyocytes and mesenchymal cells, including multipotent stromal cells. Through regulating fibrogenesis and cardiogenesis, Notch is able to switch cardiac tissue repair mechanisms from a profibrotic default pathway to a procardiogenic pathway. Altogether, our results suggest that the Notch pathway represents a unique therapeutic target that can be manipulated to improve the cardiac response to stress and regenerate the damaged myocardium via mobilization of cardiac precursor cells [19].

As an alternative to inducing regeneration *in situ*, the utilization of exogenous sources of stem cells for cellular therapy constitutes a major avenue under investigation (Fig. 1) [3,4]. The field can be conceptually organized into working with adult stem cells or with embryonic stem cells (and analogous stem cells such as induced pluripotent stem cells). The first cell type to attract clinical interest was bone marrow-derived cells [20]. Subsequent follow-up studies suggested, however, that improvements in ventricular function upon cellular administration were likely to be as a consequence of beneficial paracrine signaling [21,22]. Nevertheless, it cannot be rule out that the beneficial effects of transplanted cells reflect a recruitment of resident cardiac progenitors that contribute in turn to myocardial repair [23]. Therefore, interest began to shift from bone marrow-derived cells when several investigators reported the isolation of resident cardiac progenitor cells within the postnatal heart. Cardiac progenitor cells cannot be easily recognized in cardiac tissues since no truly specific markers are currently available. Therefore, surrogate markers, which are expressed on the surface of hematopoietic stem cells, such as the stem cell antigen (Sca)-1 or the stem cell factor receptor c-kit, are used to identify cardiac progenitor cells in the mouse heart [20,24,25]. Although these preliminary results are encouraging, adult stem cells maintain inherent drawbacks. Arguably their most serious limitation is their lack of ability to be easily expanded to produce homogenous cardiac progenitor populations. In principle, this limitation does not apply to embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs). ESCs and iPSCs can be expanded indefinitely while retaining robust capacity to differentiate into almost all cell types. Additionally, patient-specific iPSCs represent a convenient source of autologous precursors. *In vivo*, ESC- and iPSC-derived cardiomyocytes are able to engraft within the heart, and form islands of nascent, proliferating myocardium [26–30]. However, although the cardiogenic potential of ESCs and iPSCs is without doubt, their pluripotency and unique origins pose different barriers to their utilization in cellular therapy. Indeed, their specification to the cardiogenic lineage and differentiation into mature cardiomyocytes need to be tightly controlled not to produce unwanted cell types. Therefore, a clear understanding of the differentiation steps leading to cardiomyocyte production is a prerequisite to the use of stem cells in cell therapy.

Finally, a novel approach to cardiac regenerative medicine that has recently attracted excitement is the direct reprogramming of somatic cells into cardiovascular cells. Conceptually this is not a new idea and seminal work fifteen years ago demonstrated the ability of a single master regulatory transcription factor, MYOD1, to convert different cell types into skeletal muscle cells [31]. This work was then expanded upon when it was shown that the introduction of three core cardiac regulatory transcription factors, namely GATA4, Mef2C and Tbx5, was able to reprogram fibroblasts into cardiomyocyte-like cells [32,33]. These findings raised the possibility of reprogramming scar-forming fibroblasts into cardiomyocytes within the infarcted zone itself. Two groups of investigators have now confirmed this possibility using retroviruses to deliver the core-cardiac transcription factors directly to the injured heart [34,35]. Both studies demonstrated that one month after treatment, reprogrammed cardiomyocyte-like cells contributed to up to 35% of all cardiomyocytes in the infarct border zone. Treated mice exhibited significantly improved cardiac function in support of a regenerative response [34,35].

3. Non-coding RNAs in gene regulatory networks

Although the discussed regenerative avenues have distinct characteristics, they all fundamentally depend on overlapping cellular and biological processes. Indeed, these regenerative processes are under the control of complex gene regulatory networks, which rely on core cardiac transcription factors (e.g.: Mesp1, Nkx2.5, Mef2c, GATA4) [36]. These transcription factors interact in a combinatorial manner at target *cis*-regulatory modules to elicit specific temporal and spatial gene expression programs. This integrated modulation of protein coding gene expression is ultimately responsible for cellular fate, phenotype and behavior (Fig. 2). However, the notion of the gene regulatory networks being primarily protein-based regulatory systems has been somewhat premature. A number of recent studies have convincingly demonstrated that non-coding RNA networks participate to the control of gene expression. Non-coding RNAs (ncRNAs) appear to control every aspect of gene regulatory network activity, including transcriptional control, post-transcriptional gene regulation and epigenetic targeting [37–41].

From an evolutionary perspective, the repertoire of protein coding genes has remained relatively static when compared to the non-coding portion of the genome, which has markedly increased with evolution. Indeed, a number of recent high throughput transcriptomic screens have revealed that eukaryotic genomes transcribe up to 90% of their genomes [42,43]. Most of the transcripts derived from the non-coding portion of the genome are expressed dynamically during development and in response to environmental stimuli and stress, suggesting that some ncRNAs carry regulatory functions [40,44–48]. Therefore, ncRNAs can be classified into infrastructural and regulatory ncRNAs. Infrastructural ncRNAs tend to be more constitutively expressed and include transfer, ribosomal, small nuclear and small nucleolar RNAs. Regulatory non-coding RNAs include the small microRNAs (miRNAs), piwi-interacting RNAs (piRNAs), small interfering RNAs (siRNAs) and the larger long non-coding RNAs (lncRNAs) [49]. Within the lncRNAs, also exists a novel class of enhancer derived RNAs (eRNAs or edRNAs) [50]. In this review, we will focus on highlighting the characteristics and biological roles of two classes of regulatory non-coding RNAs, i.e. miRNAs and lncRNAs, with a particular emphasis on their known and putative roles in cardiac homeostasis and regeneration.

4. Gene silencing by miRNAs

The first miRNAs, lin-4 and let-7, were identified two decades ago in *Caenorhabditis elegans* [51,52]. Since then, approximately 20,000 miRNAs have been identified in the genome of animals, plants and viruses, representing about 1 to 2% of the genome. Among the large

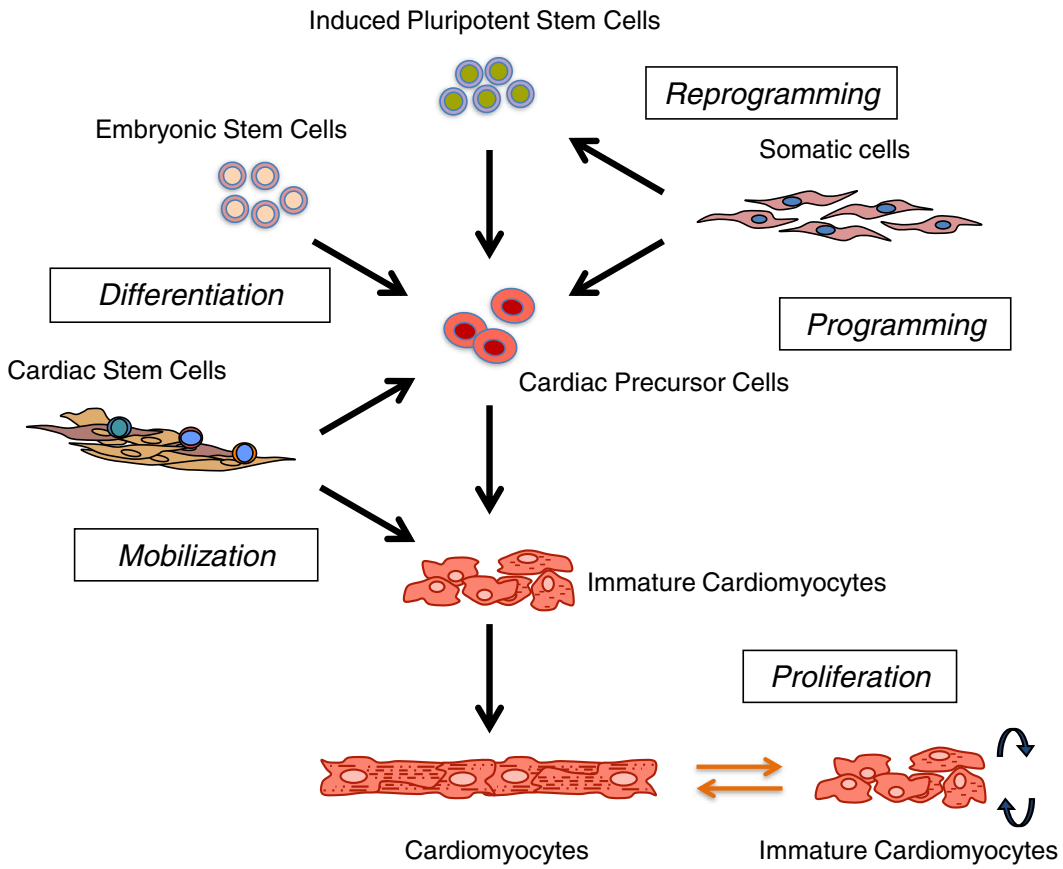

Fig. 1. Cardiac regenerative strategies. Cardiac regeneration can be induced by reactivating cell division of pre-existing cardiomyocytes and/or new cardiomyocytes can be generated via the mobilization and differentiation of resident cardiac stem cells and precursor cells. Alternatively, exogenous stem cell populations such as embryonic stem cells and induced pluripotent stem cells can be differentiated ex vivo and transplanted. Somatic cell populations, e.g. cardiac fibroblasts, can also be directly programmed into cardiac precursor or myocytes.

class of non-coding RNAs, miRNAs are characterized by a short length of about 22 nucleotides. These small RNAs regulate messenger RNA (mRNA) expression post-transcriptionally by degradation or translational repression. Sequence complementary between miRNAs and their target mRNAs is the principle that mediates repression. In plants, perfect pairing leads to mRNA degradation whereas animal miRNAs use both transcript degradation and repression of gene translation, which requires mismatch bulges between the miRNA and its target [53]. However, base pairing between the seed sequence, i.e. nucleotides 2 to 7 at the 5′ end of the miRNA, and the 3′ untranslated region of the target gene is the basic requirement for efficient inhibition.

MiRNAs are transcribed by RNA polymerase II into a primary transcript, the pri-miRNA, which is processed by the RNase Drosha into a 60- to 70-nucleotide precursor named pre-miRNA. The pre-miRNA is then exported into the cytoplasm by exportin-5, where it is further processed by Dicer, another RNase, into the 22-nucleotide miRNA duplex. The strand with the weakest base pair at its 5′ end is incorporated into the RNA-induced silencing complex (RISC) to repress target gene expression. The other strand is released and degraded. However, both strands can be loaded in to the RISC when they show similar stability, increasing the number of putative target genes associated with a single miRNA gene [54]. Although the mechanisms of repression are still unclear, it seems that the protein components of the RISC execute mRNA silencing. Argonaute proteins recruit the repression machinery, promoting translation inhibition, deadenylation and decapping [55,56]. Additionally, the protein GW182 directs the RISC to cytoplasmic mRNA processing foci (or P bodies), which are enriched in enzymes involved in degradation [57,58]. Finally, miRNAs are released by the RISC, and degraded by microRNases.

Computational analyses predict that approximately half of the proteins could be targets of miRNAs [59]. Interestingly, many miRNAs target more than one protein and many proteins can be the target of several miRNAs. Despite this broad network of interactions, data demonstrate that miRNA-mediated inhibition usually results in a modest reduction of protein expression. Therefore, miRNAs are viewed mainly as modulators to reinforce specific pathways during key biological processes. Typically, miRNAs could play important roles to maintain specific function in cells subjected to stressful conditions. Moreover, miRNA networks have been shown to be part of positive and negative feedback loops, which are crucial in cell fate decision during development. Importantly, although it is expected that expression of miRNAs and their putative targets being negatively correlated, high throughput profiling also predicts positive correlations between miRNAs and their specific target proteins. These findings have implications that are discussed below.

5. Role of miRNAs in cardiac homeostasis

Many studies have revealed a central role for miRNAs as core regulators of gene expression during cardiac disease, with the integration of miRNAs into the regulatory circuitry of the heart providing regulatory interactions to control cardiac gene expression. Several recent reviews have discussed the importance of miRNAs in the normal adaption of the heart to biomechanical stress [60,61]. The prototypic miRNAs that are modulated in animal model of cardiac hypertrophy and failure as well in the heart of cardiac patients are miR-1 and miR-133 [62,63]. MiR-1 appears to be

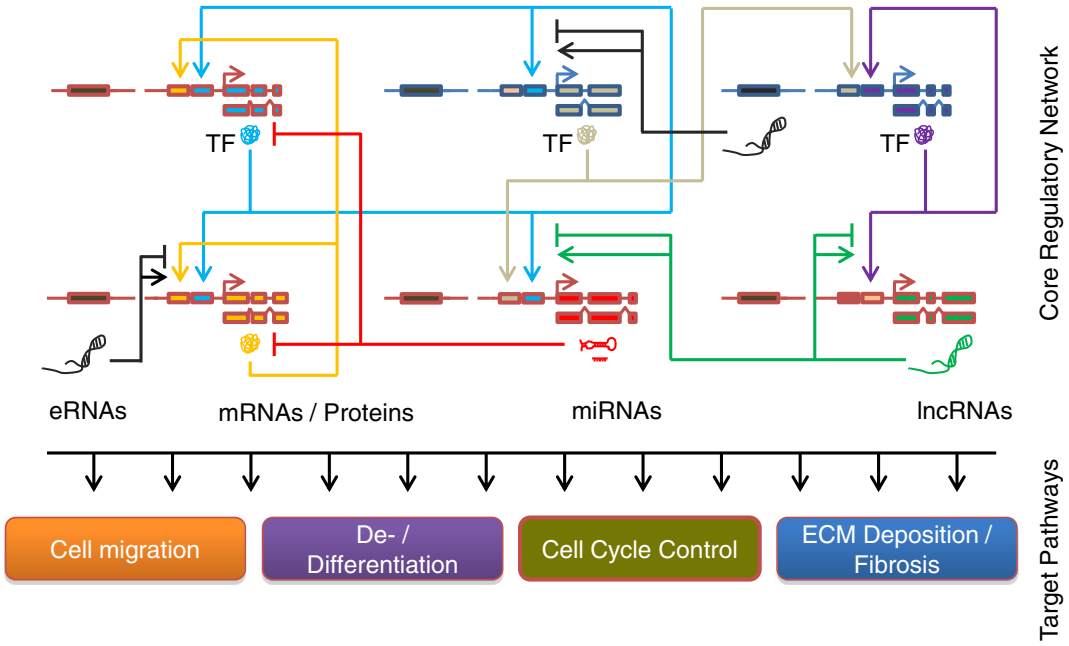

Fig. 2. Cardiac gene regulatory networks. Cardiac regenerative biological pathways are under control of highly integrated gene regulatory networks. These consist of the core regulatory transcription factors (proteins), which interact combinatorially to drive expression of downstream gene expression programs. An interleaved network of small (miRNAs) and long non-coding RNAs (lncRNAs, eRNAs) interact to specify and modulate the activity these networks, ensuring correct temporo-spatial execution of genetic programs. miRNAs: microRNAs; lncRNAs: long non-coding RNAs; eRNA: enhancer RNAs; mRNA: messenger RNAs.

downregulated in the diseased heart, and overexpression attenuates cardiomyocytes hypertrophy, possibly by targeting calmodulin and Mef2a [64]. Similarly, cardiac miR-133 expression is decreased under stress. This miRNA is thought to reduce the extent of cardiac hypertrophy by targeting the RhoA pathway [65]. In contrast, miR-23 and miR-195 are upregulated in several forms of cardiac hypertrophy [66]. MiR-23a promotes cardiomyocyte hypertrophy via downregulation of the muscle-specific RING-finger protein 1, an anti-hypertrophic protein [67]. In addition, miR-195 appears to induce apoptosis by inhibiting the anti-apoptotic factor Bcl-2 [68]. In the damaged heart, a family of miRNA, namely miR-208a, miR-208b and miR-499, controls the switch from α- to β-myosin heavy chain (MHC) expression (encoded by the *Myh6* and *Myh7* gene respectively). Interestingly, the intronic sequences of the *Myh6* gene contain miR-208a, which targets the T3 receptor associated protein-1, a transcriptional repressor of *Myh7* expression. Inhibition of miR-208a using anti-miR-208a inhibitor in Dahl hypertensive rats prevents pathological myosin switching and cardiac remodeling while improving cardiac function [69]. Similarly, miR-208b and miR-499 are embedded in intronic sequences of the *Myh7* and the closely related isoform *Myh7b*. Transcriptional repressors of *Myh7* and *Myh7b*, i.e. Sox6, Purβ and Sp3, are preferential targets of these miRNAs, which thereby reinforce slow skeletal muscle gene expression [70,71].

In the stressed myocardium, fibroblasts differentiate into myofibroblasts in response to cytokines and growth factors such as TGF-β and its downstream target CTGF, and are responsible for fibrosis production. Several miRNAs have been described to regulate the development of cardiac fibrosis, in particular miR-21, miR-29 and miR-30. For instance, miR-21 targets sprouty homology 1, a negative regulator of the ERK pathway in cardiac fibroblasts, and thereby facilitates FGF-2-dependent fibroblast proliferation [72]. As expected, inhibition of miR-21 after antagomir delivery attenuated cardiac hypertrophy in the transaortic constriction (TAC) model [73]. Although the importance of miR-21 was recently challenged in experiments using miR-21 knockout mice [74], the role of miR-21 in fibrosis was further supported by independent studies [75]. Moreover, miRNAs belonging to the miR-29 family are mainly expressed in cardiac fibroblasts in which they target matrix proteins. Since these miRNAs are downregulated in the heart in response to stress, this downregulation promote extracellular matrix deposition [66,76]. Similarly, miR30 has been found to target CTGF [77]. Therefore, the picture emerging is that miR-21, miR-29 and miR-30 are required to balance extracellular matrix turnover during cardiac pathological remodeling. When the expression of these three miRNAs is decreased, the repression of pro-fibrotic genes is relieved and enhanced matrix synthesis is observed.

The importance of miRNAs in angiogenesis and endothelial cell function was recently revealed using conditional *Dicer* knockouts. Endothelial-specific deletion typically resulted in a reduced angiogenic response to exogenous VEGF [78,79]. Specific miRNAs such as miR-126, the miR-17–92 cluster and the miR-23–27–24 cluster have been shown subsequently to regulate endothelial cell function by targeting multiple growth factors and their cognate receptors [80,81]. For instance, miR-126 is encoded by the EGF-like-domain multiple 7 gene, which is highly expressed in endothelial cells. This miRNA blocks the expression of a negative regulator of growth factor-induced angiogenesis, and exerts a pro-angiogenic action by enhancing the VEGF and FGF cascades [82]. Mice lacking miR-126 expression exhibit an increased mortality after myocardial infarction due to defective cardiac neovascularization [83]. In contrast, the miR-17–92 cluster inhibits angiogenesis and endothelial cell migration [81,84]. Accordingly, treatment targeting miR-92a leads to enhanced blood vessel growth in the border zone of the infarct and improves cardiac performance. Similarly, inhibition of miR-24 in the heart reduces the infarct size by preventing endothelial cell apoptosis [85,86]. Finally, miR-21 was also shown to exert anti-angiogenic function via inhibition of endothelial cell proliferation and migration [87].

6. Role of miRNAs in cardiac regeneration

The regulatory circuits that are operational during development are thought to be reactivated as regenerative networks in the damaged heart. Many of them implicate miRNAs. The prominent role of miRNAs in heart development has been highlighted in Dicer-null mice. These mutants demonstrate developmental arrest, in part because of cardiac

defects, suggesting that blockade of the miRNA-mediated silencing machinery affects cardiomorphogenesis [88]. MiR-1 and miR-133 are good examples of the importance of miRNAs in cardiac development. In the mouse, miR-133a1, miR-133a2, and miR-133b are clustered with miR-1–2, miR-1–1 and miR-206, [89,90]. Then, miR-133 negatively controls cardiomyocyte proliferation by targeting cyclinD2 and SRF. On the other hand, miR-1 promotes cardiac precursor differentiation into functional cardiomyocytes by inhibiting the expression of Hand2, a basic-loop-helix transcription factor involved in ventricle myocyte expansion [91–94]. During development, the bone morphogenetic protein (BMP) signaling pathway controls cardiac progenitor differentiation in the secondary heart field. Interestingly, BMP2 and 4 directly regulates the miR-17–92 cluster. In turn, these miRNAs downregulate *Isl1* and *Tbx1* expression, thereby promoting myocardial differentiation and formation of the outflow tract [95]. In the mouse, the miR-15/miR-195 family has been shown to regulate withdrawal of cardiomyocytes from the cell cycle after birth. In particular, miR-195 inhibits the expression of cell cycle genes around the first week of age [96]. Accordingly, overexpression of miR-195 in the embryonic heart is associated with ventricular hypoplasia and septal defects, whereas knockdown of miR-15 family by anti-miRNAs inhibitors is associated with an increased number of mitotic cardiomyocytes [96].

Unlike the mammalian heart, certain fish retain a robust capacity for regeneration in the adult life. This capacity is a consequence of the differential utilization of regenerative genetic circuits, many of which are absent in the adult mammalian heart. To systematically characterize these regulatory circuits, we have generated global gene and miRNA expression profiles in the poorly regenerating mouse heart after myocardial infarction and the regenerating zebrafish heart following ventricular apex resection. We have developed a novel integrated bioinformatic approach to identify differentially regulated miRNA dependant genetic programs in the mouse and zebrafish injury models. Many well characterized miRNA networks implicated in cardiac fibrosis (miR-133, -29, -30, -21, -208, -499) and hypertrophy (miR-1, -133, -208) were differentially modulated in the two species, highlighting the fundamentally different response of the mouse and zebrafish to cardiac injury at the miRNA level. Additionally, this global characterization has allowed us to identify novel miRNA regulatory circuits potentially involved in critical biological pathways. These pathways are implicated in the regenerative response, including extracellular matrix deposition, cell cycle control, cytokinesis and sarcomeric disassembly (unpublished data).

Several types of progenitor cells have been studied for their ability to differentiate into cardiomyocytes. In particular, ESCs have substantial potential to generate large numbers of functional cardiomyocytes [3,97]. In ESCs, a tight control of gene expression is required to maintain stemness on one hand and to induce specification towards the appropriate cell type. In this context, miRNAs have been highlighted as integral part of gene networks that regulate self-renewal, pluripotency and specification in ESCs. For instance, the miR-290 and miR-302 families in mice, and miR-371 and miR-302 families in humans are the most expressed miRNAs in undifferentiated ESCs. These miRNAs directly repress key inhibitors of the cell cycle to control G1 to S transition, and thereby self-renewal. Interestingly, expression of these particular miRNAs is directly regulated by Oct4, Nanog, Sox2 and c-Myc [98–100]. Since miRNAs regulate pluripotency factors in ESCs, they have tested for their capacity to generate iPSCs [101]. Overexpression of miR-291-3p, miR-294 and miR-295 together with the transcription factors Oct4, Sox2 and Klf4 facilitates iPSC production [101]. Silencing of let-7 (an anti-stemness miRNA) has been shown to increase the reprogramming efficiency of murine fibroblasts [102]. Somatic cell reprogramming by miRNAs has also been recently reported [103]. Overexpression of miR-302 and -367, in the presence of a histone deacetylase inhibitor, induces the formation of iPSCs from embryonic mouse fibroblasts. Somatic cells can even be reprogrammed to pluripotent stem cells by direct delivery of mature double-stranded miRNAs (miR-200c, miR-302s, and miR-369s) [104].

Two fundamental steps, necessary to trigger ESC differentiation, are reduced proliferation and cell lineage commitment. These phenotypic changes are associated with upregulation of specific miRNAs. Among those, the "anti-stemness" miRNA let-7 represses G1/S and G2/M cycle progression by targeting CDK6, CDC25A and CCND2, and activates cell differentiation by inhibiting pluripotency factor expression [102]. Moreover, several miRNAs, namely miR-21 miR-134, miR-296, and miR-470, target Oct4, Nanog and Sox2 [105]. Lineage specification is driven by the combination of specific transcription factors and by miRNAs that reinforce the differentiation program. For instance, miR-1 and miR-133 have been reported to play important roles in cardiac progenitor cell differentiation. Specifically, miR-1 and miR-133 suppress neural differentiation in ESCs. In particular, the Notch ligand Delta-like-1 is repressed by miR-1. The Notch pathway controls a binary cell fate decision between the mesoderm and the neuroectoderm lineages in ESCs [106]. Downregulation of the Notch pathway facilitates specification to the mesodermal and cardiogenic lineages. Similarly, miR-145 and miR-143 cooperatively targeted a network of transcription factors, including Klf4, myocardin and Elk-1 to repress proliferation and promote differentiation of smooth muscle cells [107,108].

As mentioned above, CPCs have been identified in the adult mammalian heart. However, the regenerative potential of resident CPCs is limited, in part because of their poor capacity to be expanded upon injury and meet the high demand to replenish lost cardiomyocytes. In particular, CPCs isolated from adult cardiac biopsies demonstrated reduced proliferation as compared to neonatal CPCs. In adult CPCs, expression of specific miRNAs (miR-1, -103, -130a, -130b, -185, -200b, -208b and -486), targeting proteins implicated in cell proliferation, contributes to limit precursor expansion [109]. MiRNAs appear to be also involved in CPC differentiation. Interestingly, miR-1 has been suggested to promote cardiac specification whereas miR-133 represses cardiac markers and increases proliferation [89]. This function is reminiscent to that observed during heart development. Another miRNA important for differentiation in CPCs is miR-499. Although barely detectable in undifferentiated precursors, it is strongly induced in postmitotic cardiomyocytes. In human CPCs, miR-499 enhances cardiogenesis by repressing Sox6 and Rod1. Importantly, CPCs overexpressing miR-499 have increased potential to regenerate the damaged myocardium in an animal model of myocardial infarction [110]. It is noteworthy that the cardiogenic potential of miR-499 is not restricted to CPCs. Indeed, overexpression of miR-499 in bone marrow mesenchymal stem cells increased the expression of cardiac-specific genes, such as Nkx2.5, GATA4 and Mef2C [111].

Finally, one of the major hurdles to achieve long-term improvement of heart function through stem cell therapies is the low survival rate of the injected stem cells in the hostile environment of the damaged myocardium. A miRNA cocktail has recently been shown to increase viability of transplanted cells. Cardiac progenitors overexpressing miR-21, miR-24 and miR-221 survive significantly longer after transplantation in the infarcted heart and, thus, induce better recovery. Bioinformatic analysis identified many pro-apoptotic genes as targets of these miRNAs. In particular, Bim (also named Bcl2l11), a potent activator of the apoptotic pathway, is a common target of all three miRNAs [112]. Altogether, these studies demonstrated the critical role of miRNAs in guiding stem cell differentiation and survival.

7. Long non-coding RNAs (lncRNAs)

In addition to the small ncRNAs (miRNAs), several studies utilizing high-throughput genomic screens have demonstrated that mammalian genomes produce thousands of long transcripts that have no significant protein coding potential [42,50,113–115]. These transcripts are collectively known as long (or large) non-coding RNAs (lncRNAs) because these RNA molecules are more than 200 nucleotides long. They are typically Polymerase II transcribed, 5′-capped, alternatively spliced and polyadenylated. Despite these shared characteristics,

lncRNAs are typically less conserved (at least at the exonic level) and less expressed compared to mRNAs. However, they are extremely tissue and cell specific with their expression, about 10-fold more specific versus mRNA [113]. On the basis of their genomic properties, lncRNAs have been tentatively classified. For example, one recognizes sense or antisense lncRNAs that overlap known protein coding genes, intronic lncRNAs that are encoded within introns of protein coding genes, and long intergenic ncRNAs that are encoded completely within the intergenic genomic space between protein coding genes [116]. Current studies have shown that lncRNAs represent key modulators of cell behavior. In addition, they appear dysregulated in various human diseases, including cardiovascular disorders [117,118]. Several recent reviews have discussed the roles of lncRNAs (Fig. 3) [119–121]. Putative functions in the context of cardiac homeostasis and regeneration are described below.

8. LncRNAs as molecular guides and structural scaffolds

Recent studies have contributed to understand how the ubiquitously expressed chromatin-modifying protein complexes, which lack DNA-binding domains, can recognize their target genomic loci. LncRNAs could guide chromatin-modifying complexes to their required genomic destination [115,122]. Once targeted, these ubiquitously expressed protein complexes can elicit their regulatory effects in a gene and cell specific manner [41,122]. The exact mechanisms via which this guidance occurs are unknown. However, lncRNAs could serve as docking stations for complex recruitment [123]. In addition, lncRNAs have been shown to be structural components of ribonucleoprotein complexes. These include those containing splice factors and transcription factors [124]. Although a detailed understanding of how lncRNAs encode such scaffold functionality is currently lacking, it is likely that lncRNAs contain structural modules that allow distinct interactions with protein-binding partners [125].

9. LncRNAs function as molecular decoys

Growth arrest specific 5 (Gas5) represents a good example of a lncRNA acting as decoy for proteins. Gas5 functions as a *cis*-binding competitor, directly interacting with the DNA-binding domain of the glucocorticoid receptor, thereby preventing binding of this nuclear receptor to target gene *cis*-elements. Therefore, Gas5 serves as a repressor of glucocorticoid receptor signaling. This decoy effect could lead to significant changes in gene expression controlled by specific transcription factors [126]. PANDA is another lncRNA that modulates cell death by binding to the transcription factor NF-YA, therefore titrating it away from chromatin and impacting upon NF-YA dependant regulatory functions [127].

In addition to functioning as a decoy for regulatory proteins, lncRNAs can also serve as RNA-binding decoys or competitive endogenous RNAs (ceRNAs) for small non-coding miRNAs [128,129]. Indeed, recent studies have shown that mammalian lncRNAs can regulate gene expression programs post-transcriptionally by serving as *bona fide* targets for miRNAs. One particular lncRNA, linc-MD1, was shown to act as a 'sponge' for two miRNAs, which regulate the gene regulatory network governing skeletal muscle differentiation [128]. Interestingly, the concept of ceRNA networks provides an attractive explanation for positive correlations between miRNAs and their target proteins, which are predicted from high throughput screens. Since miRNAs have been shown to be important regulators of cardiac specification and differentiation, we suspect many cardiac-specific ceRNA networks exist. As a proof-of principle, we have used a high throughput approach to identify lncRNAs that are differentially expressed in differentiating human CPCs. Several hundred lncRNAs were found modulated in differentiating cells. We also identified miRNA response elements (MREs) in these lncRNAs. Differentially regulated lncRNAs were significantly enriched for specific MREs corresponding to miRNAs with well characterized regulatory roles in cardiac specification and differentiation (Unpublished data).

10. Regulation of gene expression by lncRNAs

Gene regulation is a complex process that requires many *cis*-binding factors and chromatin remodeling co-factors [119]. LncRNAs could promote both gene expression and repression via interaction with various classes of epigenetic modifying protein complexes [119–121,130,131]. One of the first epigenetic phenomena demonstrated to utilize lncRNAs was genomic imprinting [132]. Imprinted genes are typically master regulators in development, and many lncRNAs are expressed adjacently to imprinted genes [133]. An example of one such lncRNA is *Air*, which is expressed from the paternal allele, interacts with a histone methyltransferase, and guides this protein to the genome to repress imprinted genes [134]. In addition, another lncRNA, HOTAIR, regulates the expression of human HOXD genes [135]. Interestingly, HOTAIR serves as molecular scaffold allowing the formation of the polycomb repressive complex 2 (PRC2) and the LSD1/CoREST regulatory complex at their endogenous genomic target loci. This lncRNA-dependent targeting of chromatin modifying complexes is common with many other human and mouse lncRNAs able to regulate gene expression *in trans* [136].

Within the human genome, a very important cell-cycle modulating genomic locus spans approximately 42-kbs on human chromosome 9p21. This region contains three very important tumor suppressors, p15 (INK4b), p16 (INK4a) and p14 (ARF) [137,138]. Regulation of this locus is mediated by PRC2 and PRC1 to maintain a transcriptionally repressed heterochromatin state. ANRIL, a multi-exonic lncRNA expressed antisense to p15 and p16, binds both to PRC1 and -2, targeting these complexes to this cell-cycle control locus [137,138]. Deletion or knock-down of ANRIL results in a significant upregulation of p15 and p16, with concomitant decrease in cell-cycle activity. Moreover, genome-wide association studies have recently implicated ANRIL in abdominal aortic aneurysm and coronary artery disease [117,118]. In this context, PRC2 has recently been shown to be an important epigenetic modulator during cardiac development and in the postnatal heart [139–141]. Specifically, inactivating Ezh2, the catalytic component of PRC2, in cardiac progenitors in the developing heart, led to a number of cardiac defects. In particular, mutant heart was characterized by myocyte hypoplasia, resulting in an atrophied compact myocardium [139–141]. Arguably and most importantly for the decreased proliferation observed in PRC2 deficient hearts, is the up-regulation of the cell cycle inhibitors p16 and p15. Assuming that lncRNAs are important for targeting PRC2 at specific loci in the genome, identifying cardiac-specific lncRNAs could provide fundamental insights into how the cardiac epigenome is programmed.

11. Regulation of alternative splicing by lncRNAs

Alternative splicing of pre-mRNAs represents one of the primary contributing mechanisms for proteomic complexity by generating several protein products with non-overlapping functions from a single mRNA. LncRNAs are emerging as important modulators of alternative splicing. For instance, the lncRNA MALAT1 localizes to nuclear speckles, which are known to be enriched with many alternative splicing-modulating proteins [142]. Upon MALAT1 knock-down, specific subsets of mRNAs appear to be spliced in different patterns. Considering the importance of context-specific alternative splicing in biological processes associated to the cardiac response to stress, lncRNAs regulating splicing could prove to be interesting therapeutic targets. This, however, will require the global identification of cardiac lncRNAs associated with splicing factors. RNA co-immunoprecipitation followed by high throughput deep sequencing (RIP-Seq) on proteins known to be involved in cardiac alternative splicing should lead to the identification of such lncRNAs.

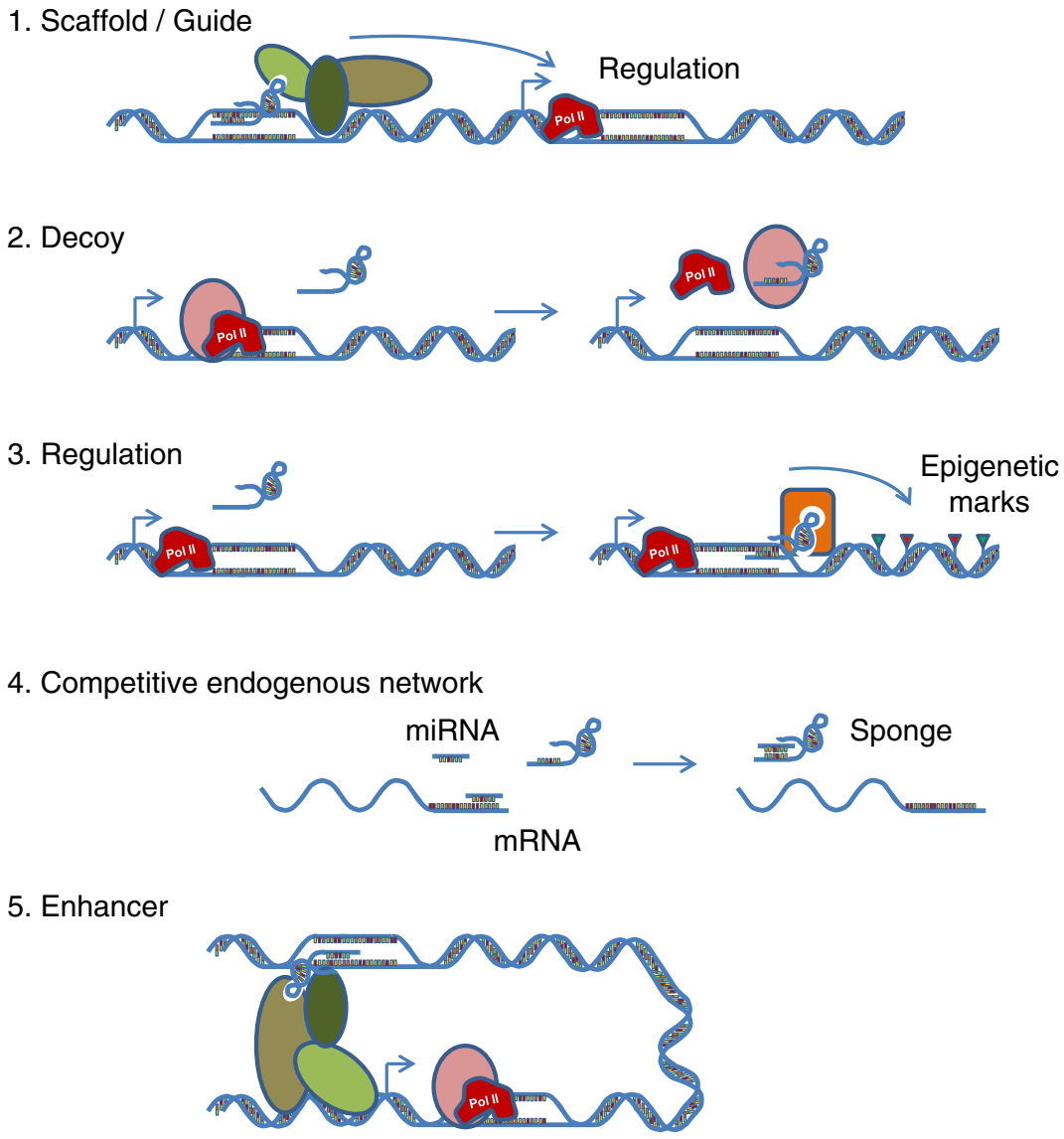

Fig. 3. Long non-coding RNA mechanisms of action. (1) LncRNAs can act as molecular guides and scaffolds to target/interact with protein complexes. (2) LncRNAs can act as molecular decoys for proteins, including DNA-binding transcription factors. (3) LncRNAs can modulate the epigenome through guiding chromatin-modifying complexes to target genomic loci. (4) LncRNAs can act as endogenous sponges for miRNAs. (5) Enhancer-derived lncRNAs can facilitate enhancer activity via promoting chromatin looping and enrichment of remodeling complexes.

12. Regulation of cellular reprogramming by lncRNAs

The regulatory power of lncRNAs has been illustrated in a recent study, which described the global roles of lncRNAs in modulating pluripotency and lineage determination circuits in ESCs. Interestingly, transcription factors important in pluripotency such as Oct4 and Nanog were found to bind lncRNA promoters [143]. To further elucidate the roles of lncRNAs in ESCs in a more systematic manner, lncRNAs were globally knocked down using a shRNAi high-throughput screen [144]. This study demonstrated that 26 lncRNAs are required for maintaining the pluripotent state in ESCs. Moreover, reprogramming of somatic cells into iPSCs is one of the most exciting avenues in regenerative medicine. One study identified lncRNAs able to efficiently reprogram fibroblasts into iPSCs. The authors characterized lincRNA-RoR, which was significantly upregulated during iPSC generation [145]. Knocking-down the lncRNA itself severely impaired the ability of fibroblasts to be reprogrammed to iPSCs. Conversely, overexpressing lncRNA-RoR increased the efficiency of iPSC-colony formation [145]. One could, therefore, envisage a situation, in which comparable cardiac lncRNAs might be used to promote direct reprogramming of cardiac fibroblast into cardiomyocytes.

13. Regulation of enhancer activity by lncRNAs

Enhancers are the key information processing units within gene regulatory networks [146,147]. Chromatin immunoprecipitation followed by sequencing (ChIP-Seq) analysis of enhancer associated chromatin marks have recently demonstrated that many enhancer regions generate lncRNAs [45,47,50,148]. These can take the form of classical lncRNAs, which are polyadenylated, capped and spliced, or non-polyadenylated bi-directionally low copy transcripts. Importantly, some enhancer-associated lncRNAs were shown to be required for the transcriptional activation of proximal genes [47,50]. For instance, HOTTIP has recently been shown to function as an enhancer

associated lncRNA. HOTTIP directly interacts with the WDR5 protein, bringing it into spatial proximity via an enhancer-promoter chromatin loop, promoting transcriptional activation. Knocking-down this lncRNA, without changing the *cis*-sequence of the enhancer, abolishes the ability of the enhancer to activate its downstream HOXA genes [149].

Cardiac-specific developmental enhancers, potentially generating enhancer-derived lncRNAs, could constitute cardiac-enriched regulatory lncRNAs and ideal regenerative molecular targets. We have recently attempted to test this assumption through the utilization of a previously developed cardiac enhancer screen utilizing cardiac-specific p300 enrichments as readout of enhancer activity [150–152]. This identified hundreds of *bona fide* cardiac developmental enhancers, many of which were putative regulators of important cardiac transcription factors and structural proteins. We selected several of these enhancers and found that all generated lncRNAs. Furthermore, expression of these transcripts was dynamically regulated during cardiac development, and the kinetics correlates with specific morphogenetic transitions. More importantly, the expression of enhancer-derived RNAs (edRNAs) preceded induction of their predicted target genes. These developmental edRNAs were also expressed in cardiac progenitors derived from mouse ESCs. Many of these enhancers have orthologous sequences in human, which are functionally conserved and transcribed. These human enhancers were expressed in both fetal human hearts and more importantly from isolated human CPCs. We anticipate that it should be possible to modulate the activity of cardiac gene regulatory network, and the regenerative biological processes they control, through manipulation of these cardiac edRNAs (Ounzain et al. Submitted).

14. Future directions and conclusion

Since many miRNAs have been implicated in the development of cardiac hypertrophy and fibrosis and in the control of neovascularization during the adaptation of the heart to damage, the pathways that are regulated by miRNAs represent attractive therapeutic targets. Moreover, new evidence supports a role for miRNAs in the induction of reprogramming, cell renewal and differentiation. In addition, the potential of lncRNAs for the understanding of processes associated with cardiac homeostasis and regeneration, and therefore identifying novel strategies for regenerative therapy, is clearly significant. Arguably, the most important initial step will be a comprehensive systematic genome-wide lncRNA annotation in cardiac tissues and cells. This profiling can be carried out using lncRNA microarrays or more desirably high-throughput RNA-sequencing. LncRNAs are remarkably cell-specific and likely expressed solely in particular contexts. Ideally, to aid in functionally characterizing lncRNAs, RNA-seq screens should be integrated with small RNA-seq (miRNA profiling), protein coding gene expression profiling (mRNAs) and ChIP-Seq analysis. In particular, integration of miRNA and lncRNA expression profiles would allow the identification of ceRNA networks. Once lncRNAs have been identified, functional manipulations need to be executed. These can include gain-of-function and loss-of-function approaches. A number of genomic technologies have been adapted and developed for molecular dissection of lncRNA regulatory functions. RNA-immunoprecipitation followed by sequencing (RIP-Seq) [153] and direct cross-linking of RNA-protein (CLIP-Seq) [154] interactions in vivo are highly promising strategies for the identification cardiac epigenomic targeting lncRNAs. These methods utilize a protein centric view. However, once lncRNAs are identified one can take advantage of the recently developed RNA-centric approach, i.e. chromatin isolation by RNA-purification (ChIRP) [123]. Based on the key role the epigenome plays in dictating cell-fate transitions, lncRNA-dependent epigenetic targeting therapies could also represent a promising approach for modulating regeneration-associated processes. Over the recent years, it has become clear that short and long ncRNAs are important orchestrators of gene regulatory networks. Identification and functional characterization of ncRNAs involved in cardiac regeneration promise to open up a treasure trove of new therapeutic targets for inducing efficient cardiac regeneration in the diseased heart.

Acknowledgement

We are grateful to the Swiss National Science Foundation for their support (National Research Program 63 “Stem Cells and Regenerative Medicine”; Grant no: 406340-128129).

References

[1] V.L. Roger, A.S. Go, D.M. Lloyd-Jones, E.J. Benjamin, J.D. Berry, W.B. Borden, D.M. Bravata, S. Dai, E.S. Ford, C.S. Fox, H.J. Fullerton, C. Gillespie, S.M. Hailpern, J.A. Heit, V.J. Howard, B.M. Kissela, S.J. Kittner, D.T. Lackland, J.H. Lichtman, L.D. Lisabeth, D.M. Makuc, G.M. Marcus, A. Marelli, D.B. Matchar, C.S. Moy, D. Mozaffarian, M.E. Mussolino, G. Nichol, N.P. Paynter, E.Z. Soliman, P.D. Sorlie, N. Sotoodehnia, T.N. Turan, S.S. Virani, N.D. Wong, D. Woo, M.B. Turner, C. American Heart Association Statistics, S. Stroke Statistics, Heart disease and stroke statistics–2012 update: a report from the American Heart Association, Circulation 125 (2012) e2–e220.
[2] J. Stehlik, L.B. Edwards, A.Y. Kucheryavaya, C. Benden, J.D. Christie, F. Dobbels, R. Kirk, A.O. Rahmel, M.I. Hertz, The Registry of the International Society for Heart and Lung Transplantation: Twenty-eighth Adult Heart Transplant Report—2011, J. Heart Lung Transplant. 30 (2011) 1078–1094.
[3] R. Passier, L.W. van Laake, C.L. Mummery, Stem-cell-based therapy and lessons from the heart, Nature 453 (2008) 322–329.
[4] V.F. Segers, R.T. Lee, Stem-cell therapy for cardiac disease, Nature 451 (2008) 937–942.
[5] A.P. Beltrami, K. Urbanek, J. Kajstura, S.M. Yan, N. Finato, R. Bussani, B. Nadal-Ginard, F. Silvestri, A. Leri, C.A. Beltrami, P. Anversa, Evidence that human cardiac myocytes divide after myocardial infarction, N. Engl. J. Med. 344 (2001) 1750–1757.
[6] J. Kajstura, K. Urbanek, S. Perl, T. Hosoda, H. Zheng, B. Ogorek, J. Ferreira-Martins, P. Goichberg, C. Rondon-Clavo, F. Sanada, D. D'Amario, M. Rota, F. Del Monte, D. Orlic, J. Tisdale, A. Leri, P. Anversa, Cardiomyogenesis in the adult human heart, Circ. Res. 107 (2010) 305–315.
[7] O. Bergmann, R.D. Bhardwaj, S. Bernard, S. Zdunek, F. Barnabe-Heider, S. Walsh, J. Zupicich, K. Alkass, B.A. Buchholz, H. Druid, S. Jovinge, J. Frisen, Evidence for cardiomyocyte renewal in humans, Science 324 (2009) 98–102.
[8] J.O. Oberpriller, J.C. Oberpriller, Response of the adult newt ventricle to injury, J. Exp. Zool. 187 (1974) 249–253.
[9] K.D. Poss, L.G. Wilson, M.T. Keating, Heart regeneration in zebrafish, Science 298 (2002) 2188–2190.
[10] F. Chablais, J. Veit, G. Rainer, A. Jazwinska, The zebrafish heart regenerates after cryoinjury-induced myocardial infarction, BMC Dev. Biol. 11 (2011) 21.
[11] J.M. Gonzalez-Rosa, V. Martin, M. Peralta, M. Torres, N. Mercader, Extensive scar formation and regression during heart regeneration after cryoinjury in zebrafish, Development 138 (2011) 1663–1674.
[12] K. Schnabel, C.C. Wu, T. Kurth, G. Weidinger, Regeneration of cryoinjury induced necrotic heart lesions in zebrafish is associated with epicardial activation and cardiomyocyte proliferation, PLoS One 6 (2011) e18503.
[13] J. Wang, D. Panakova, K. Kikuchi, J.E. Holdway, M. Gemberling, J.S. Burris, S.P. Singh, A.L. Dickson, Y.F. Lin, M.K. Sabeh, A.A. Werdich, D. Yelon, C.A. Macrae, K.D. Poss, The regenerative capacity of zebrafish reverses cardiac failure caused by genetic cardiomyocyte depletion, Development 138 (2011) 3421–3430.
[14] C. Jopling, E. Sleep, M. Raya, M. Marti, A. Raya, J.C. Izpisua Belmonte, Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation, Nature 464 (2010) 606–609.
[15] K. Kikuchi, J.E. Holdway, A.A. Werdich, R.M. Anderson, Y. Fang, G.F. Egnaczyk, T. Evans, C.A. Macrae, D.Y. Stainier, K.D. Poss, Primary contribution to zebrafish heart regeneration by gata4(+) cardiomyocytes, Nature 464 (2010) 601–605.
[16] E.R. Porrello, A.I. Mahmoud, E. Simpson, J.A. Hill, J.A. Richardson, E.N. Olson, H.A. Sadek, Transient regenerative potential of the neonatal mouse heart, Science 331 (2011) 1078–1080.
[17] M. Nemir, T. Pedrazzini, Functional role of Notch signaling in the developing and postnatal heart, J. Mol. Cell. Cardiol. 45 (2008) 495–504.
[18] A. Croquelois, A.A. Domenighetti, M. Nemir, M. Lepore, N. Rosenblatt-Velin, F. Radtke, T. Pedrazzini, Control of the adaptive response of the heart to stress via the Notch1 receptor pathway, J. Exp. Med. 205 (2008) 3173–3185.
[19] M. Nemir, M. Metrich, I. Plaisance, M. Lepore, S. Cruchet, C. Berthonneche, A. Sarre, F. Radtke, T. Pedrazzini, The Notch Pathway Controls Fibrotic and Regenerative Repair in the Adult Heart, Eur. Heart J. (in press).
[20] D. Orlic, J. Kajstura, S. Chimenti, I. Jakoniuk, S.M. Anderson, B. Li, J. Pickel, R. McKay, B. Nadal-Ginard, D.M. Bodine, A. Leri, P. Anversa, Bone marrow cells regenerate infarcted myocardium, Nature 410 (2001) 701–705.
[21] L.B. Balsam, A.J. Wagers, J.L. Christensen, T. Kofidis, I.L. Weissman, R.C. Robbins, Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium, Nature 428 (2004) 668–673.
[22] C.E. Murry, M.H. Soonpaa, H. Reinecke, H. Nakajima, H.O. Nakajima, M. Rubart, K.B. Pasumarthi, J.I. Virag, S.H. Bartelmez, V. Poppa, G. Bradford, J.D. Dowell,

D.A. Williams, L.J. Field, Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts, Nature 428 (2004) 664–668.
[23] K.E. Hatzistergos, H. Quevedo, B.N. Oskouei, Q. Hu, G.S. Feigenbaum, I.S. Margitich, R. Mazhari, A.J. Boyle, J.P. Zambrano, J.E. Rodriguez, R. Dulce, P.M. Pattany, D. Valdes, C. Revilla, A.W. Heldman, I. McNiece, J.M. Hare, Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation, Circ. Res. 107 (2010) 913–922.
[24] N. Rosenblatt-Velin, M.G. Lepore, C. Cartoni, F. Beermann, T. Pedrazzini, FGF-2 controls the differentiation of resident cardiac precursors into functional cardiomyocytes, J. Clin. Invest. 115 (2005) 1724–1733.
[25] H. Oh, S.B. Bradfute, T.D. Gallardo, T. Nakamura, V. Gaussin, Y. Mishina, J. Pocius, L.H. Michael, R.R. Behringer, D.J. Garry, M.L. Entman, M.D. Schneider, Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 12313–12318.
[26] J.Q. He, Y. Ma, Y. Lee, J.A. Thomson, T.J. Kamp, Human embryonic stem cells develop into multiple types of cardiac myocytes: action potential characterization, Circ. Res. 93 (2003) 32–39.
[27] I. Kehat, D. Kenyagin-Karsenti, M. Snir, H. Segev, M. Amit, A. Gepstein, E. Livne, O. Binah, J. Itskovitz-Eldor, L. Gepstein, Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes, J. Clin. Invest. 108 (2001) 407–414.
[28] J. Zhang, G.F. Wilson, A.G. Soerens, C.H. Koonce, J. Yu, S.P. Palecek, J.A. Thomson, T.J. Kamp, Functional cardiomyocytes derived from human induced pluripotent stem cells, Circ. Res. 104 (2009) e30–41.
[29] W.Z. Zhu, L.F. Santana, M.A. Laflamme, Local control of excitation–contraction coupling in human embryonic stem cell-derived cardiomyocytes, PLoS One 4 (2009) e5407.
[30] L. Zwi, O. Caspi, G. Arbel, I. Huber, A. Gepstein, I.H. Park, L. Gepstein, Cardiomyocyte differentiation of human induced pluripotent stem cells, Circulation 120 (2009) 1513–1523.
[31] C.E. Murry, M.A. Kay, T. Bartosek, S.D. Hauschka, S.M. Schwartz, Muscle differentiation during repair of myocardial necrosis in rats via gene transfer with MyoD, J. Clin. Invest. 98 (1996) 2209–2217.
[32] J.A. Efe, S. Hilcove, J. Kim, H. Zhou, K. Ouyang, G. Wang, J. Chen, S. Ding, Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy, Nat. Cell Biol. 13 (2011) 215–222.
[33] M. Ieda, J.D. Fu, P. Delgado-Olguin, V. Vedantham, Y. Hayashi, B.G. Bruneau, D. Srivastava, Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors, Cell 142 (2010) 375–386.
[34] K. Song, Y.J. Nam, X. Luo, X. Qi, W. Tan, G.N. Huang, A. Acharya, C.L. Smith, M.D. Tallquist, E.G. Neilson, J.A. Hill, R. Bassel-Duby, E.N. Olson, Heart repair by reprogramming non-myocytes with cardiac transcription factors, Nature 485 (2012) 599–604.
[35] L. Qian, Y. Huang, C.I. Spencer, A. Foley, V. Vedantham, L. Liu, S.J. Conway, J.D. Fu, D. Srivastava, In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes, Nature 485 (2012) 593–598.
[36] E.N. Olson, Gene regulatory networks in the evolution and development of the heart, Science 313 (2006) 1922–1927.
[37] M.S. Ebert, P.A. Sharp, Roles for microRNAs in conferring robustness to biological processes, Cell 149 (2012) 515–524.
[38] J.T. Mendell, E.N. Olson, MicroRNAs in stress signaling and human disease, Cell 148 (2012) 1172–1187.
[39] J.S. Mattick, The double life of RNA, Biochimie 93 (2011) viii–ix.
[40] M.E. Dinger, P.P. Amaral, T.R. Mercer, K.C. Pang, S.J. Bruce, B.B. Gardiner, M.E. Askarian-Amiri, K. Ru, G. Solda, C. Simons, S.M. Sunkin, M.L. Crowe, S.M. Grimmond, A.C. Perkins, J.S. Mattick, Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation, Genome Res. 18 (2008) 1433–1445.
[41] J.L. Rinn, H.Y. Chang, Genome regulation by long noncoding RNAs, Annu. Rev. Biochem. 81 (2012) 145–166.
[42] P. Carninci, T. Kasukawa, S. Katayama, J. Gough, M.C. Frith, N. Maeda, R. Oyama, T. Ravasi, B. Lenhard, C. Wells, R. Kodzius, K. Shimokawa, V.B. Bajic, S.E. Brenner, S. Batalov, A.R. Forrest, M. Zavolan, M.J. Davis, L.G. Wilming, V. Aidinis, J.E. Allen, A. Ambesi-Impiombato, R. Apweiler, R.N. Aturaliya, T.L. Bailey, M. Bansal, L. Baxter, K.W. Beisel, T. Bersano, H. Bono, A.M. Chalk, K.P. Chiu, V. Choudhary, A. Christoffels, D.R. Clutterbuck, M.L. Crowe, E. Dalla, B.P. Dalrymple, B. de Bono, G. Della Gatta, D. di Bernardo, T. Down, P. Engstrom, M. Fagiolini, G. Faulkner, C.F. Fletcher, T. Fukushima, M. Furuno, S. Futaki, M. Gariboldi, P. Georgii-Hemming, T.R. Gingeras, T. Gojobori, R.E. Green, S. Gustincich, M. Harbers, Y. Hayashi, T.K. Hensch, N. Hirokawa, D. Hill, L. Huminiecki, M. Iacono, K. Ikeo, A. Iwama, T. Ishikawa, M. Jakt, A. Kanapin, M. Katoh, Y. Kawasawa, J. Kelso, H. Kitamura, H. Kitano, G. Kollias, S.P. Krishnan, A. Kruger, S.K. Kummerfeld, I.V. Kurochkin, L.F. Lareau, D. Lazarevic, L. Lipovich, J. Liu, S. Liuni, S. McWilliam, M. Madan Babu, M. Madera, L. Marchionni, H. Matsuda, S. Matsuzawa, H. Miki, F. Mignone, S. Miyake, K. Morris, S. Mottagui-Tabar, N. Mulder, N. Nakano, H. Nakauchi, P. Ng, R. Nilsson, S. Nishiguchi, S. Nishikawa, F. Nori, O. Ohara, Y. Okazaki, V. Orlando, K.C. Pang, W.J. Pavan, G. Pavesi, G. Pesole, N. Petrovsky, S. Piazza, J. Reed, J.F. Reid, B.Z. Ring, M. Ringwald, B. Rost, Y. Ruan, S.L. Salzberg, A. Sandelin, C. Schneider, C. Schonbach, K. Sekiguchi, C.A. Semple, S. Seno, L. Sessa, Y. Sheng, Y. Shibata, H. Shimada, K. Shimada, D. Silva, B. Sinclair, S. Sperling, E. Stupka, K. Sugiura, R. Sultana, Y. Takenaka, K. Taki, K. Tammoja, S.L. Tan, S. Tang, M.S. Taylor, J. Tegner, S.A. Teichmann, H.R. Ueda, E. van Nimwegen, R. Verardo, C.L. Wei, K. Yagi, H. Yamanishi, E. Zabarovsky, S. Zhu, A. Zimmer, W. Hide, C. Bult, S.M. Grimmond, R.D. Teasdale, E.T. Liu, V. Brusic, J. Quackenbush, C. Wahlestedt, J.S. Mattick, D.A. Hume, C. Kai, D. Sasaki, Y. Tomaru, S. Fukuda, M. Kanamori-Katayama, M. Suzuki, J. Aoki, T. Arakawa, J. Iida, K. Imamura, M. Itoh, T. Kato, H. Kawaji, N. Kawagashira, T. Kawashima, M. Kojima, S. Kondo, H. Konno, K. Nakano, N. Ninomiya, T. Nishio, M. Okada, C. Plessy, K. Shibata, T. Shiraki, S. Suzuki, M. Tagami, K. Waki, A. Watahiki, Y. Okamura-Oho, H. Suzuki, J. Kawai, Y. Hayashizaki, F. Consortium, R.G.E.R. Group, G. Genome Science, The transcriptional landscape of the mammalian genome, Science (2005) 1559–1563.
[43] E.P. Consortium, The ENCODE (ENCyclopedia Of DNA Elements) Project, Science 306 (2004) 636–640.
[44] S. Blackshaw, S. Harpavat, J. Trimarchi, L. Cai, H. Huang, W.P. Kuo, G. Weber, K. Lee, R.E. Fraioli, S.H. Cho, R. Yung, E. Asch, L. Ohno-Machado, W.H. Wong, C.L. Cepko, Genomic analysis of mouse retinal development, PLoS Biol. 2 (2004) E247.
[45] F. De Santa, I. Barozzi, F. Mietton, S. Ghisletti, S. Polletti, B.K. Tusi, H. Muller, J. Ragoussis, C.L. Wei, G. Natoli, A large fraction of extragenic RNA pol II transcription sites overlap enhancers, PLoS Biol. 8 (2010) e1000384.
[46] M.A. Faghihi, F. Modarresi, A.M. Khalil, D.E. Wood, B.G. Sahagan, T.E. Morgan, C.E. Finch, G. St Laurent III, P.J. Kenny, C. Wahlestedt, Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of beta-secretase, Nat. Med. 14 (2008) 723–730.
[47] T.K. Kim, M. Hemberg, J.M. Gray, A.M. Costa, D.M. Bear, J. Wu, D.A. Harmin, M. Laptewicz, K. Barbara-Haley, S. Kuersten, E. Markenscoff-Papadimitriou, D. Kuhl, H. Bito, P.F. Worley, G. Kreiman, M.E. Greenberg, Widespread transcription at neuronal activity-regulated enhancers, Nature 465 (2010) 182–187.
[48] J.L. Rinn, M. Kertesz, J.K. Wang, S.L. Squazzo, X. Xu, S.A. Brugmann, L.H. Goodnough, J.A. Helms, P.J. Farnham, E. Segal, H.Y. Chang, Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs, Cell 129 (2007) 1311–1323.
[49] M. Esteller, Non-coding RNAs in human disease, nature reviews, Genetics 12 (2011) 861–874.
[50] U.A. Orom, T. Derrien, M. Beringer, K. Gumireddy, A. Gardini, G. Bussotti, F. Lai, M. Zytnicki, C. Notredame, Q. Huang, R. Guigo, R. Shiekhattar, Long noncoding RNAs with enhancer-like function in human cells, Cell 143 (2010) 46–58.
[51] R.C. Lee, R.L. Feinbaum, V. Ambros, The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14, Cell 75 (1993) 843–854.
[52] B. Wightman, I. Ha, G. Ruvkun, Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*, Cell 75 (1993) 855–862.
[53] J. Brennecke, A. Stark, R.B. Russell, S.M. Cohen, Principles of microRNA-target recognition, PLoS Biol. 3 (2005) e85.
[54] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[55] G.L. Sen, H.M. Blau, Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies, Nat. Cell Biol. 7 (2005) 633–636.
[56] J. Liu, M.A. Valencia-Sanchez, G.J. Hannon, R. Parker, MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies, Nat. Cell Biol. 7 (2005) 719–723.
[57] U. Sheth, R. Parker, Targeting of aberrant mRNAs to cytoplasmic processing bodies, Cell 125 (2006) 1095–1109.
[58] J. Rehwinkel, I. Letunic, J. Raes, P. Bork, E. Izaurralde, Nonsense-mediated mRNA decay factors act in concert to regulate common mRNA targets, RNA 11 (2005) 1530–1544.
[59] R.C. Friedman, K.K. Farh, C.B. Burge, D.P. Bartel, Most mammalian mRNAs are conserved targets of microRNAs, Genome Res. 19 (2009) 92–105.
[60] E.M. Small, R.J. Frost, E.N. Olson, MicroRNAs add a new dimension to cardiovascular disease, Circulation 121 (2010) 1022–1032.
[61] G.W. Dorn II, MicroRNAs in cardiac disease, Transl. Res. 157 (2011) 226–235.
[62] S.J. Matkovich, W. Wang, Y. Tu, W.H. Eschenbacher, L.E. Dorn, G. Condorelli, A. Diwan, J.M. Nerbonne, G.W. Dorn II, MicroRNA-133a protects against myocardial fibrosis and modulates electrical repolarization without affecting hypertrophy in pressure-overloaded adult hearts, Circ. Res. 106 (2010) 166–175.
[63] S. Ikeda, S.W. Kong, J. Lu, E. Bisping, H. Zhang, P.D. Allen, T.R. Golub, B. Pieske, W.T. Pu, Altered microRNA expression in human heart disease, Physiol. Genomics 31 (2007) 367–373.
[64] S. Ikeda, A. He, S.W. Kong, J. Lu, R. Bejar, N. Bodyak, K.H. Lee, Q. Ma, P.M. Kang, T.R. Golub, W.T. Pu, MicroRNA-1 negatively regulates expression of the hypertrophy-associated calmodulin and Mef2a genes, Mol. Cell. Biol. 29 (2009) 2193–2204.
[65] A. Care, D. Catalucci, F. Felicetti, D. Bonci, A. Addario, P. Gallo, M.L. Bang, P. Segnalini, Y. Gu, N.D. Dalton, L. Elia, M.V. Latronico, M. Hoydal, C. Autore, M.A. Russo, G.W. Dorn II, O. Ellingsen, P. Ruiz-Lozano, K.L. Peterson, C.M. Croce, C. Peschle, G. Condorelli, MicroRNA-133 controls cardiac hypertrophy, Nat. Med. 13 (2007) 613–618.
[66] E. van Rooij, L.B. Sutherland, N. Liu, A.H. Williams, J. McAnally, R.D. Gerard, J.A. Richardson, E.N. Olson, A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 18255–18260.
[67] Z. Lin, I. Murtaza, K. Wang, J. Jiao, J. Gao, P.F. Li, miR-23a functions downstream of NFATc3 to regulate cardiac hypertrophy, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 12103–12108.
[68] J.F. He, Y.M. Luo, X.H. Wan, D. Jiang, Biogenesis of MiRNA-195 and its role in biogenesis, the cell cycle, and apoptosis, J. Biochem. Mol. Toxicol. 25 (2011) 404–408.
[69] R.L. Montgomery, T.G. Hullinger, H.M. Semus, B.A. Dickinson, A.G. Seto, J.M. Lynch, C. Stack, P.A. Latimer, E.N. Olson, E. van Rooij, Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure, Circulation 124 (2011) 1537–1547.
[70] E. van Rooij, D. Quiat, B.A. Johnson, L.B. Sutherland, X. Qi, J.A. Richardson, R.J. Kelm Jr., E.N. Olson, A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance, Dev. Cell 17 (2009) 662–673.

[71] E. van Rooij, L.B. Sutherland, X. Qi, J.A. Richardson, J. Hill, E.N. Olson, Control of stress-dependent cardiac growth and gene expression by a microRNA, Science 316 (2007) 575–579.
[72] T. Thum, C. Gross, J. Fiedler, T. Fischer, S. Kissler, M. Bussen, P. Galuppo, S. Just, W. Rottbauer, S. Frantz, M. Castoldi, J. Soutschek, V. Koteliansky, A. Rosenwald, M.A. Basson, J.D. Licht, J.T. Pena, S.H. Rouhanifard, M.U. Muckenthaler, T. Tuschl, G.R. Martin, J. Bauersachs, S. Engelhardt, MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts, Nature 456 (2008) 980–984.
[73] T. Thum, N. Chau, B. Bhat, S.K. Gupta, P.S. Linsley, J. Bauersachs, S. Engelhardt, Comparison of different miR-21 inhibitor chemistries in a cardiac disease model, J. Clin. Invest. 121 (2011) 461–462 (author reply 462–463).
[74] D.M. Patrick, R.L. Montgomery, X. Qi, S. Obad, S. Kauppinen, J.A. Hill, E. van Rooij, E.N. Olson, Stress-dependent cardiac remodeling occurs in the absence of microRNA-21 in mice, J. Clin. Invest. 120 (2010) 3912–3916.
[75] A. Zarjou, S. Yang, E. Abraham, A. Agarwal, G. Liu, Identification of a microRNA signature in renal fibrosis: role of miR-21, Am. J. Physiol. Renal Physiol. 301 (2011) F793–F801.
[76] E. van Rooij, L.B. Sutherland, J.E. Thatcher, J.M. DiMaio, R.H. Naseem, W.S. Marshall, J.A. Hill, E.N. Olson, Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 13027–13032.
[77] R.F. Duisters, A.J. Tijsen, B. Schroen, J.J. Leenders, V. Lentink, I. van der Made, V. Herias, R.E. van Leeuwen, M.W. Schellings, P. Barenbrug, J.G. Maessen, S. Heymans, Y.M. Pinto, E.E. Creemers, miR-133 and miR-30 regulate connective tissue growth factor: implications for a role of microRNAs in myocardial matrix remodeling, Circ. Res. 104 (2009) 170–178 (176 pp. following 178).
[78] Y. Suarez, C. Fernandez-Hernando, J.S. Pober, W.C. Sessa, Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells, Circ. Res. 100 (2007) 1164–1173.
[79] A. Kuehbacher, C. Urbich, A.M. Zeiher, S. Dimmeler, Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis, Circ. Res. 101 (2007) 59–68.
[80] C. Urbich, D. Kaluza, T. Fromel, A. Knau, K. Bennewitz, R.A. Boon, A. Bonauer, C. Doebele, J.N. Boeckel, E. Hergenreider, A.M. Zeiher, J. Kroll, I. Fleming, S. Dimmeler, MicroRNA-27a/b controls endothelial cell repulsion and angiogenesis by targeting semaphorin 6A, Blood 119 (2012) 1607–1616.
[81] C. Doebele, A. Bonauer, A. Fischer, A. Scholz, Y. Reiss, C. Urbich, W.K. Hofmann, A.M. Zeiher, S. Dimmeler, Members of the microRNA-17–92 cluster exhibit a cell-intrinsic antiangiogenic function in endothelial cells, Blood 115 (2010) 4944–4950.
[82] J.E. Fish, M.M. Santoro, S.U. Morton, S. Yu, R.F. Yeh, J.D. Wythe, K.N. Ivey, B.G. Bruneau, D.Y. Stainier, D. Srivastava, miR-126 regulates angiogenic signaling and vascular integrity, Dev. Cell 15 (2008) 272–284.
[83] S. Wang, A.B. Aurora, B.A. Johnson, X. Qi, J. McAnally, J.A. Hill, J.A. Richardson, R. Bassel-Duby, E.N. Olson, The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis, Dev. Cell 15 (2008) 261–271.
[84] A. Bonauer, G. Carmona, M. Iwasaki, M. Mione, M. Koyanagi, A. Fischer, J. Burchfield, H. Fox, C. Doebele, K. Ohtani, E. Chavakis, M. Potente, M. Tjwa, C. Urbich, A.M. Zeiher, S. Dimmeler, MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice, Science 324 (2009) 1710–1713.
[85] L. Qian, L.W. Van Laake, Y. Huang, S. Liu, M.F. Wendland, D. Srivastava, miR-24 inhibits apoptosis and represses Bim in mouse cardiomyocytes, J. Exp. Med. 208 (2011) 549–560.
[86] Q. Zhou, R. Gallagher, R. Ufret-Vincenty, X. Li, E.N. Olson, S. Wang, Regulation of angiogenesis and choroidal neovascularization by members of microRNA-23 27 24 clusters, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 8287–8292.
[87] C. Sabatel, A.M. Cornet, S.P. Tabruyn, L. Malvaux, K. Castermans, J.A. Martial, I. Struman, Sprouty1, a new target of the angiostatic agent 16K prolactin, negatively regulates angiogenesis, Mol. Cancer 9 (2010) 231.
[88] E. Bernstein, S.Y. Kim, M.A. Carmell, E.P. Murchison, H. Alcorn, M.Z. Li, A.A. Mills, S.J. Elledge, K.V. Anderson, G.J. Hannon, Dicer is essential for mouse development, Nat. Genet. 35 (2003) 215–217.
[89] J.F. Chen, E.M. Mandel, J.M. Thomson, Q. Wu, T.E. Callis, S.M. Hammond, F.L. Conlon, D.Z. Wang, The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation, Nat. Genet. 38 (2006) 228–233.
[90] P.K. Rao, R.M. Kumar, M. Farkhondeh, S. Baskerville, H.F. Lodish, Myogenic factors that regulate expression of muscle-specific microRNAs, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 8721–8726.
[91] K.R. Cordes, D. Srivastava, MicroRNA regulation of cardiovascular development, Circ. Res. 104 (2009) 724–732.
[92] N. Liu, S. Bezprozvannaya, A.H. Williams, X. Qi, J.A. Richardson, R. Bassel-Duby, E.N. Olson, microRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart, Genes Dev. 22 (2008) 3242–3254.
[93] Y. Zhao, E. Samal, D. Srivastava, Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis, Nature 436 (2005) 214–220.
[94] Y. Zhao, D. Srivastava, A developmental view of microRNA function, Trends Biochem. Sci. 32 (2007) 189–197.
[95] J. Wang, S.B. Greene, M. Bonilla-Claudio, Y. Tao, J. Zhang, Y. Bai, Z. Huang, B.L. Black, F. Wang, J.F. Martin, Bmp signaling regulates myocardial differentiation from cardiac progenitors through a MicroRNA-mediated mechanism, Dev. Cell 19 (2010) 903–912.
[96] E.R. Porrello, B.A. Johnson, A.B. Aurora, E. Simpson, Y.J. Nam, S.J. Matkovich, G.W. Dorn II, E. van Rooij, E.N. Olson, MiR-15 family regulates postnatal mitotic arrest of cardiomyocytes, Circ. Res. 109 (2011) 670–679.
[97] C.E. Murry, G. Keller, Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development, Cell 132 (2008) 661–680.
[98] Z. Lichner, E. Pall, A. Kerekes, E. Pallinger, P. Maraghechi, Z. Bosze, E. Gocza, The miR-290–295 cluster promotes pluripotency maintenance by regulating cell cycle phase distribution in mouse embryonic stem cells, Differentiation 81 (2011) 11–24.
[99] L. Sinkkonen, T. Hugenschmidt, P. Berninger, D. Gaidatzis, F. Mohn, C.G. Artus-Revel, M. Zavolan, P. Svoboda, W. Filipowicz, MicroRNAs control de novo DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells, Nat. Struct. Mol. Biol. 15 (2008) 259–267.
[100] B. Stadler, I. Ivanovska, K. Mehta, S. Song, A. Nelson, Y. Tan, J. Mathieu, C. Darby, C.A. Blau, C. Ware, G. Peters, D.G. Miller, L. Shen, M.A. Cleary, H. Ruohola-Baker, Characterization of microRNAs involved in embryonic stem cell states, Stem Cells Dev. 19 (2010) 935–950.
[101] R.L. Judson, J.E. Babiarz, M. Venere, R. Blelloch, Embryonic stem cell-specific microRNAs promote induced pluripotency, Nat. Biotechnol. 27 (2009) 459–461.
[102] C. Melton, R.L. Judson, R. Blelloch, Opposing microRNA families regulate self-renewal in mouse embryonic stem cells, Nature 463 (2010) 621–626.
[103] F. Anokye-Danso, C.M. Trivedi, D. Juhr, M. Gupta, Z. Cui, Y. Tian, Y. Zhang, W. Yang, P.J. Gruber, J.A. Epstein, E.E. Morrisey, Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency, Cell Stem Cell 8 (2011) 376–388.
[104] N. Miyoshi, H. Ishii, H. Nagano, N. Haraguchi, D.L. Dewi, Y. Kano, S. Nishikawa, M. Tanemura, K. Mimori, F. Tanaka, T. Saito, J. Nishimura, I. Takemasa, T. Mizushima, M. Ikeda, H. Yamamoto, M. Sekimoto, Y. Doki, M. Mori, Reprogramming of mouse and human cells to pluripotency using mature microRNAs, Cell Stem Cell 8 (2011) 633–638.
[105] Y. Tay, J. Zhang, A.M. Thomson, B. Lim, I. Rigoutsos, MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation, Nature 455 (2008) 1124–1128.
[106] M. Nemir, A. Croquelois, T. Pedrazzini, F. Radtke, Induction of cardiogenesis in embryonic stem cells via downregulation of Notch1 signaling, Circ. Res. 98 (2006) 1471–1478.
[107] K.R. Cordes, N.T. Sheehy, M.P. White, E.C. Berry, S.U. Morton, A.N. Muth, T.H. Lee, J.M. Miano, K.N. Ivey, D. Srivastava, miR-145 and miR-143 regulate smooth muscle cell fate and plasticity, Nature 460 (2009) 705–710.
[108] N. Xu, T. Papagiannakopoulos, G. Pan, J.A. Thomson, K.S. Kosik, MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells, Cell 137 (2009) 647–658.
[109] P. Sirish, J.E. Lopez, N. Li, A. Wong, V. Timofeyev, J.N. Young, M. Majdi, R.A. Li, H.S. Chen, N. Chiamvimonvat, MicroRNA profiling predicts a variance in the proliferative potential of cardiac progenitor cells derived from neonatal and adult murine hearts, J. Mol. Cell. Cardiol. 52 (2012) 264–272.
[110] T. Hosoda, H. Zheng, M. Cabral-da-Silva, F. Sanada, N. Ide-Iwata, B. Ogorek, J. Ferreira-Martins, C. Arranto, D. D'Amario, F. del Monte, K. Urbanek, D.A. D'Alessandro, R.E. Michler, P. Anversa, M. Rota, J. Kajstura, A. Leri, Human cardiac stem cell differentiation is regulated by a mircrine mechanism, Circulation 123 (2011) 1287–1296.
[111] L.L. Zhang, J.J. Liu, F. Liu, W.H. Liu, Y.S. Wang, B. Zhu, B. Yu, MiR-499 induces cardiac differentiation of rat mesenchymal stem cells through wnt/beta-catenin signaling pathway, Biochem. Biophys. Res. Commun. 420 (2012) 875–881.
[112] S. Hu, M. Huang, P.K. Nguyen, Y. Gong, Z. Li, F. Jia, F. Lan, J. Liu, D. Nag, R.C. Robbins, J.C. Wu, Novel microRNA prosurvival cocktail for improving engraftment and function of cardiac progenitor cell transplantation, Circulation 124 (2011) S27–S34.
[113] M.N. Cabili, C. Trapnell, L. Goff, M. Koziol, B. Tazon-Vega, A. Regev, J.L. Rinn, Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses, Genes Dev. 25 (2011) 1915–1927.
[114] M. Guttman, I. Amit, M. Garber, C. French, M.F. Lin, D. Feldser, M. Huarte, O. Zuk, B.W. Carey, J.P. Cassady, M.N. Cabili, R. Jaenisch, T.S. Mikkelsen, T. Jacks, N. Hacohen, B.E. Bernstein, M. Kellis, A. Regev, J.L. Rinn, E.S. Lander, Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals, Nature 458 (2009) 223–227.
[115] A.M. Khalil, M. Guttman, M. Huarte, M. Garber, A. Raj, D. Rivea Morales, K. Thomas, A. Presser, B.E. Bernstein, A. van Oudenaarden, A. Regev, E.S. Lander, J.L. Rinn, Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 11667–11672.
[116] T. Derrien, R. Guigo, R. Johnson, The long non-coding RNAs: A new (p)layer in the "dark matter", Front. Genet. 2 (2011) 107.
[117] O. Harismendy, D. Notani, X. Song, N.G. Rahim, B. Tanasa, N. Heintzman, B. Ren, X.D. Fu, E.J. Topol, M.G. Rosenfeld, K.A. Frazer, 9p21 DNA variants associated with coronary artery disease impair interferon-gamma signalling response, Nature 470 (2011) 264–268.
[118] I. Hinterseher, G. Tromp, H. Kuivaniemi, Genes and abdominal aortic aneurysm, Ann. Vasc. Surg. 25 (2011) 388–412.
[119] K.C. Wang, H.Y. Chang, Molecular mechanisms of long noncoding RNAs, Mol. Cell 43 (2011) 904–914.
[120] T.R. Mercer, M.E. Dinger, J.S. Mattick, Long non-coding RNAs: insights into functions, Nat. Rev. Genet. 10 (2009) 155–159.
[121] J.S. Mattick, P.P. Amaral, M.E. Dinger, T.R. Mercer, M.F. Mehler, RNA regulation of epigenetic processes, BioEssays 31 (2009) 51–59.
[122] M.J. Koziol, J.L. Rinn, RNA traffic control of chromatin complexes, Curr. Opin. Genet. Dev. 20 (2010) 142–148.

[123] C. Chu, K. Qu, F.L. Zhong, S.E. Artandi, H.Y. Chang, Genomic maps of long noncoding RNA occupancy reveal principles of RNA-chromatin interactions, Mol. Cell 44 (2011) 667–678.
[124] A.M. Khalil, J.L. Rinn, RNA–protein interactions in human health and disease, Semin. Cell Dev. Biol. 22 (2011) 359–365.
[125] M. Guttman, J.L. Rinn, Modular regulatory principles of large non-coding RNAs, Nature 482 (2012) 339–346.
[126] T. Kino, D.E. Hurt, T. Ichijo, N. Nader, G.P. Chrousos, Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor, Sci. Signal. 3 (2010) ra8.
[127] T. Hung, Y. Wang, M.F. Lin, A.K. Koegel, Y. Kotake, G.D. Grant, H.M. Horlings, N. Shah, C. Umbricht, P. Wang, Y. Wang, B. Kong, A. Langerod, A.L. Borresen-Dale, S.K. Kim, M. van de Vijver, S. Sukumar, M.L. Whitfield, M. Kellis, Y. Xiong, D.J. Wong, H.Y. Chang, Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters, Nat. Genet. 43 (2011) 621–629.
[128] M. Cesana, D. Cacchiarelli, I. Legnini, T. Santini, O. Sthandier, M. Chinappi, A. Tramontano, I. Bozzoni, A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA, Cell 147 (2011) 358–369.
[129] L. Salmena, L. Poliseno, Y. Tay, L. Kats, P.P. Pandolfi, A ceRNA hypothesis: the Rosetta stone of a hidden RNA language? Cell 146 (2011) 353–358.
[130] T. Nagano, P. Fraser, No-nonsense functions for long noncoding RNAs, Cell 145 (2011) 178–181.
[131] M.B. Clark, J.S. Mattick, Long noncoding RNAs in cell biology, Semin. Cell Dev. Biol. 22 (2011) 366–376.
[132] W. Reik, A. Murrell, Genomic imprinting. Silence across the border, Nature 405 (2000) 408–409.
[133] F. Mohammad, T. Mondal, C. Kanduri, Epigenetics of imprinted long noncoding RNAs, Epigenetics 4 (2009) 277–286.
[134] T. Nagano, J.A. Mitchell, L.A. Sanz, F.M. Pauler, A.C. Ferguson-Smith, R. Feil, P. Fraser, The air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin, Science 322 (2008) 1717–1720.
[135] R.A. Gupta, N. Shah, K.C. Wang, J. Kim, H.M. Horlings, D.J. Wong, M.C. Tsai, T. Hung, P. Argani, J.L. Rinn, Y. Wang, P. Brzoska, B. Kong, R. Li, R.B. West, M.J. van de Vijver, S. Sukumar, H.Y. Chang, Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis, Nature 464 (2010) 1071–1076.
[136] M.C. Tsai, O. Manor, Y. Wan, N. Mosammaparast, J.K. Wang, F. Lan, Y. Shi, E. Segal, H.Y. Chang, Long noncoding RNA as modular scaffold of histone modification complexes, Science 329 (2010) 689–693.
[137] Y. Kotake, T. Nakagawa, K. Kitagawa, S. Suzuki, N. Liu, M. Kitagawa, Y. Xiong, Long non-coding RNA ANRIL is required for the PRC2 recruitment to and silencing of p15(INK4B) tumor suppressor gene, Oncogene 30 (2011) 1956–1962.
[138] K.L. Yap, S. Li, A.M. Munoz-Cabello, S. Raguz, L. Zeng, S. Mujtaba, J. Gil, M.J. Walsh, M.M. Zhou, Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a, Mol. Cell 38 (2010) 662–674.
[139] A. He, Q. Ma, J. Cao, A. von Gise, P. Zhou, H. Xie, B. Zhang, M. Hsing, D.C. Christodoulou, P. Cahan, G.Q. Daley, S.W. Kong, S.H. Orkin, C.E. Seidman, J.G. Seidman, W.T. Pu, Polycomb repressive complex 2 regulates normal development of the mouse heart, Circ. Res. 110 (2012) 406–415.
[140] P. Delgado-Olguin, Y. Huang, X. Li, D. Christodoulou, C.E. Seidman, J.G. Seidman, A. Tarakhovsky, B.G. Bruneau, Epigenetic repression of cardiac progenitor gene expression by Ezh2 is required for postnatal cardiac homeostasis, Nat. Genet. 44 (2012) 343–347.
[141] L. Chen, Y. Ma, E.Y. Kim, W. Yu, R.J. Schwartz, L. Qian, J. Wang, Conditional ablation of Ezh2 in murine hearts reveals its essential roles in endocardial cushion formation, cardiomyocyte proliferation and survival, PLoS One 7 (2012) e31005.
[142] V. Tripathi, J.D. Ellis, Z. Shen, D.Y. Song, Q. Pan, A.T. Watt, S.M. Freier, C.F. Bennett, A. Sharma, P.A. Bubulya, B.J. Blencowe, S.G. Prasanth, K.V. Prasanth, The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation, Mol. Cell 39 (2010) 925–938.
[143] J. Sheik Mohamed, P.M. Gaughwin, B. Lim, P. Robson, L. Lipovich, Conserved long noncoding RNAs transcriptionally regulated by Oct4 and Nanog modulate pluripotency in mouse embryonic stem cells, RNA 16 (2010) 324–337.
[144] M. Guttman, J. Donaghey, B.W. Carey, M. Garber, J.K. Grenier, G. Munson, G. Young, A.B. Lucas, R. Ach, L. Bruhn, X. Yang, I. Amit, A. Meissner, A. Regev, J.L. Rinn, D.E. Root, E.S. Lander, lincRNAs act in the circuitry controlling pluripotency and differentiation, Nature 477 (2011) 295–300.
[145] S. Loewer, M.N. Cabili, M. Guttman, Y.H. Loh, K. Thomas, I.H. Park, M. Garber, M. Curran, T. Onder, S. Agarwal, P.D. Manos, S. Datta, E.S. Lander, T.M. Schlaeger, G.Q. Daley, J.L. Rinn, Large intergenic non-coding RNA-RoR modulates reprogramming of human induced pluripotent stem cells, Nat. Genet. 42 (2010) 1113–1117.
[146] M. Levine, Transcriptional enhancers in animal development and evolution, Curr. Biol. 20 (2010) R754–R763.
[147] C.T. Ong, V.G. Corces, Enhancer function: new insights into the regulation of tissue-specific gene expression, Nat. Rev. Genet. 12 (2011) 283–293.
[148] U.A. Orom, T. Derrien, R. Guigo, R. Shiekhattar, Long noncoding RNAs as enhancers of gene expression, Cold Spring Harb. Symp. Quant. Biol. 75 (2010) 325–331.
[149] K.C. Wang, Y.W. Yang, B. Liu, A. Sanyal, R. Corces-Zimmerman, Y. Chen, B.R. Lajoie, A. Protacio, R.A. Flynn, R.A. Gupta, J. Wysocka, M. Lei, J. Dekker, J.A. Helms, H.Y. Chang, A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression, Nature 472 (2011) 120–124.
[150] A. Visel, M.J. Blow, Z. Li, T. Zhang, J.A. Akiyama, A. Holt, I. Plajzer-Frick, M. Shoukry, C. Wright, F. Chen, V. Afzal, B. Ren, E.M. Rubin, L.A. Pennacchio, ChIP-seq accurately predicts tissue-specific activity of enhancers, Nature 457 (2009) 854–858.
[151] D. May, M.J. Blow, T. Kaplan, D.J. McCulley, B.C. Jensen, J.A. Akiyama, A. Holt, I. Plajzer-Frick, M. Shoukry, C. Wright, V. Afzal, P.C. Simpson, E.M. Rubin, B.L. Black, J. Bristow, L.A. Pennacchio, A. Visel, Large-scale discovery of enhancers from human heart tissue, Nat. Genet. 44 (2012) 89–93.
[152] M.J. Blow, D.J. McCulley, Z. Li, T. Zhang, J.A. Akiyama, A. Holt, I. Plajzer-Frick, M. Shoukry, C. Wright, F. Chen, V. Afzal, J. Bristow, B. Ren, B.L. Black, E.M. Rubin, A. Visel, L.A. Pennacchio, ChIP-Seq identification of weakly conserved heart enhancers, Nat. Genet. 42 (2010) 806–810.
[153] J. Zhao, T.K. Ohsumi, J.T. Kung, Y. Ogawa, D.J. Grau, K. Sarma, J.J. Song, R.E. Kingston, M. Borowsky, J.T. Lee, Genome-wide identification of polycomb-associated RNAs by RIP-seq, Mol. Cell 40 (2010) 939–953.
[154] S.W. Chi, J.B. Zang, A. Mele, R.B. Darnell, Argonaute HITS-CLIP decodes microRNA–mRNA interaction maps, Nature 460 (2009) 479–486.