Non-coding RNA Research 3 (2018) 1–11

Contents lists available at ScienceDirect

Non-coding RNA Research

journal homepage: http://www.keaipublishing.com/NCRNA

Circular RNAs in the cardiovascular system

Clarissa P.C. Gomes [a, 1], Antonio Salgado-Somoza [a, 1], Esther E. Creemers [b], Christoph Dieterich [c], Mitja Lustrek [d], Yvan Devaux [a, *], On behalf of the Cardiolinc™ network[2]

[a] Cardiovascular Research Unit, Luxembourg Institute of Health, Luxembourg, Luxembourg
[b] Experimental Cardiology, Academic Medical Center, Amsterdam, The Netherlands
[c] German Center for Cardiovascular Research, University Hospital Heidelberg, Heidelberg, Germany
[d] Department of Intelligent Systems, Jozef Stefan Institute, Ljubljana, Slovenia

ARTICLE INFO

Article history:
Received 31 October 2017
Received in revised form
16 January 2018
Accepted 22 February 2018
Available online 25 February 2018

Keywords:
Non-coding RNAs
Circular RNAs
Cardiovascular system
Cardiovascular disease
Artificial intelligence
Biomarker

ABSTRACT

Until recently considered as rare, circular RNAs (circRNAs) are emerging as important regulators of gene expression. They are ubiquitously expressed and represent a novel branch of the family of non-coding RNAs. Recent investigations showed that circRNAs are regulated in the cardiovascular system and participate in its physiological and pathological development. In this review article, we will provide an overview of the role of circRNAs in cardiovascular health and disease. After a description of the biogenesis of circRNAs, we will summarize what is known of the expression, regulation and function of circRNAs in the cardiovascular system. We will then address some technical aspects of circRNAs research, discussing how artificial intelligence may aid in circRNAs research. Finally, the potential of circRNAs as biomarkers of cardiovascular disease will be addressed and directions for future research will be proposed.

1. Introduction

February 2001 marked the completion of the sequencing of the human genome [1,2]. Eleven years later, researchers from the Encode Project Consortium revealed that, while 80% of our genome is transcribed, only 2% of expressed RNAs encode proteins [3]. This landmark discovery implied that the vast majority of our genes are transcribed into non-protein coding RNAs, more simply named non-coding RNAs (ncRNAs). Multiple categories of ncRNAs have been characterized and classified. The most widely used classification is based on the size of ncRNAs, the shorter ones (<200 nucleotides) being classified as small ncRNAs, while those exceeding 200 nucleotides are grouped as long non-coding RNAs (lncRNAs) (Fig. 1). Inside the lncRNAs family, both linear and circular forms of RNAs can be found. Although circular RNAs (circRNAs) were previously thought to be rare as compared to linear messenger RNAs, recent work showed that circRNAs can be relatively abundant and be the predominant transcripts of hundreds of genes [4]. These circular molecules are ubiquitously expressed in human and more than 30,000 different circRNAs have been identified so far [5]. They are regulated in several disease states and contribute to disease development and progression. The role of circRNAs in the cardiovascular system in only emerging.

Cardiovascular (CV) disease is still the main cause of death and disabilities worldwide [6,7]. While research has made enormous progress in terms of healthcare and treatment of CV disease, the use of RNAs for its treatment and diagnosis is facing technical challenges that slow down their clinical application. Recent developments revealed the potential of microRNAs (miRNAs) for the management of CV disease patients [8,9]. LncRNAs have also emerged as potential diagnostic and therapeutic targets of CV disease [10,11]. Yet, the knowledge of the role of circRNAs in the diseased heart and their value as CV disease markers and

Abbreviations: circRNAs, circular RNAs; CRISPR, clustered regularly interspaced short palindromic repeats; CV, cardiovascular; DCM, dilated cardiomyopathy; EMT, epithelial-mesenchymal transition; lncRNAs, long non-coding RNAs; miRNAs, microRNAs; ncRNAs, non-coding RNAs; RNA-seq, RNA sequencing; RPAD, RNase R treatment followed by polyadenylation and poly(A)+ RNA depletion; RT-qPCR, reverse transcription quantitative polymerase chain reaction.

* Corresponding author. Luxembourg Institute of Health, Cardiovascular Research Unit, 84, Val Fleuri, L-1526, Luxembourg, Luxembourg.
E-mail address: yvan.devaux@lih.lu (Y. Devaux).
[1] These authors contributed equally to the work.
[2] www.cardiolinc.org.

https://doi.org/10.1016/j.ncrna.2018.02.002

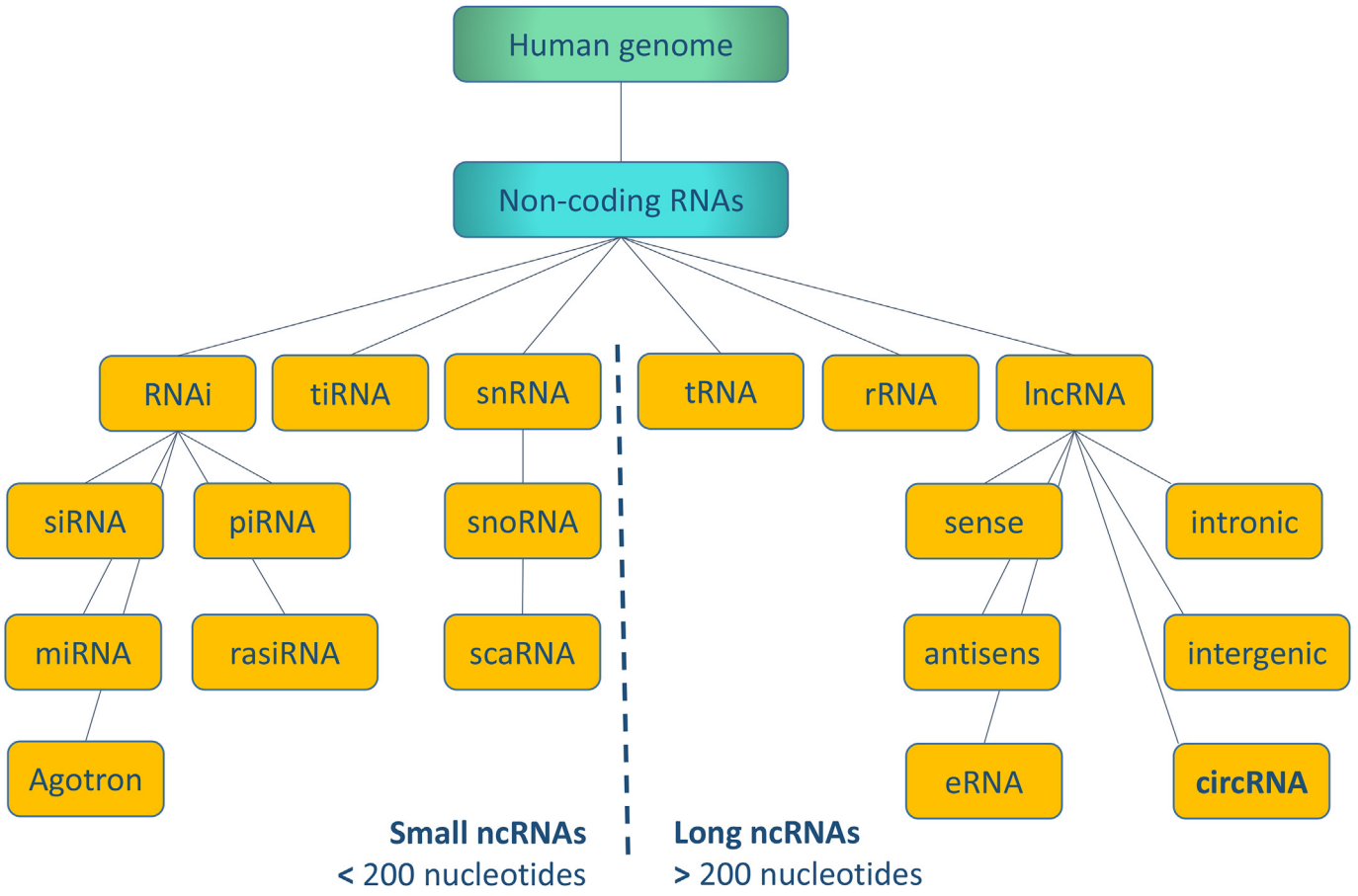

Fig. 1. The non-coding RNA family. Non-coding RNAs are classified depending on their size with an arbitrary cut-off of 200 nucleotides. Abbreviations: ncRNAs: noncoding RNAs; lncRNA: long noncoding RNA; eRNA: enhancer RNA; circRNA: circular RNA; rRNA: ribosomal RNA; snRNA: small nuclear RNA; scaRNA: small cajal body-specific RNA; snoRNA: small nucleolar RNA; tiRNA: tRNA-derived stress-induced small RNA; tRNA: transfer RNA; RNAi: RNA interference; siRNA: small interfering RNA; piRNA: piwi-interacting RNA; miRNA: microRNA; rasiRNA: repeat associated small interfering RNA.

therapeutic targets are still in their infancy.

This review article is part of a Special Issue on "Non-coding RNAs in Cardiovascular Health and Disease" and will focus on circRNAs in the CV system. After a description of the mechanisms leading to circRNA biogenesis, we will gather the knowledge of the expression, regulation and function of circRNAs in the CV system. We will then address some methodological aspects of circRNAs research and we will provide some considerations of the potential of artificial intelligence to aid in circRNAs research. The value of circRNAs as biomarkers of CV disease will be delineated and we will finally propose directions for future research.

2. Biogenesis of circRNAs

CircRNAs are produced by the spliceosome machinery by direct back-splicing of exons or introns within pre-mRNA (Fig. 2) [12] or by exon skipping [13]. As a result of a direct back-splicing event, an exon is not associated with an adjacent downstream exon, as seen in linear splicing, but with an upstream exon or intron. This gives rise to circular RNA molecules harboring exons or introns that are out-of-order from the genomic context [14,15]. Exon skipping involves a lariat that undergoes internal splicing, removing the intron and generating a circRNA. Both proposed mechanisms involve the spliceosome machinery, but the mechanism by which the spliceosome selects only certain exons to circularize is largely unknown. It is however known that many circRNAs in mammals are processed from exons with long flanking introns, containing reverse complementary sequences [16,17]. These sequences can be inverted repeats, such as very abundant Alu elements (~300 base pairs repeats), or non-repetitive complementary sequences [16]. The common feature is that the intronic sequences base-pair to one another, thereby bringing the splice sites into close proximity of each other, allowing the spliceosome to perform a back-splicing reaction. An alternative mechanism to bridge flanking introns is through interaction with RNA binding proteins that bind cis-regulatory regions in these introns.

Among the hundreds of RNA binding proteins characterized so far [18], the first one identified as a regulator of circRNA production was Muscleblind (MBL), a zinc finger protein which is known to regulate alternative splicing in muscle [14] and for which dysregulation can lead to pathologies, particularly myotonic dystrophies (reviewed in Ref. [19]). Interestingly, MBL regulates circRNA production from its own pre-mRNA. Numerous MBL-binding sites are present in the introns flanking the back-spliced junction of *circMBL*, which turned out to be essential for the biogenesis of *circMBL*. This raises the possibility of a sophisticated control mechanism for MBL protein levels. When MBL protein levels are high, it decreases the production of its own mRNA by promoting *circMBL* production. The circRNA can subsequently sponge out the excess MBL protein by binding to it [14] (more details about this function in the next section).

The second RNA binding protein shown to regulate circRNA production by pre-mRNA binding was Quaking (QKI) [20], a splicing factor that has been implicated in diseases including ataxia,

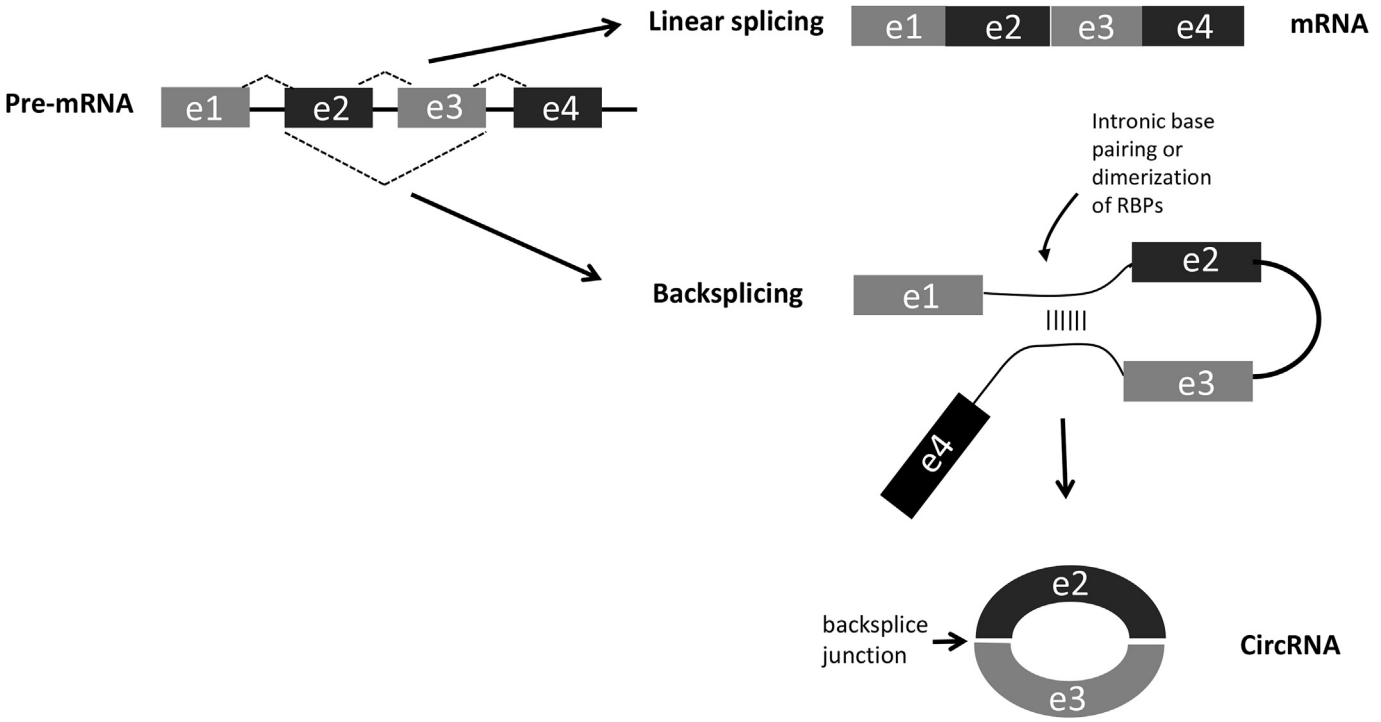

Fig. 2. Biogenesis of circRNAs. The spliceosome machinery, which normally catalyses linear splicing of pre-mRNA, can also perform a back-splicing reaction between two exons, resulting in the formation of a circRNA. Back-splicing uses the same canonical splicing machinery and canonical splice sites as needed for linear splicing. Mechanistically, back-splicing requires that the donor and acceptor site of the back-spliced exons are brought in close proximity to each other, which can be accomplished by direct RNA base-pairing of reverse complementary sequences in the introns flanking the back-spliced exons, or by the interaction of RNA binding proteins (RBPs) that dock on these flanking introns.

schizophrenia, and glioblastoma [21]. The insertion of synthetic QKI-binding sites into introns was found to be sufficient to produce circRNAs. Mechanistically it was shown that QKI binds to two flanking introns and subsequently dimerizes to bridge the circularizing exons. During epithelial-mesenchymal transition (EMT) it was shown that about one-third of the circRNAs were regulated by QKI [20]. Whether QKI is also required for circRNA formation in the CV system is currently unknown.

Among the post-transcriptional modifications that increase molecular diversity and can lead to alternative splicing and changes in gene expression levels, called RNA editing, the prevailing modification in higher eukaryotes is the single base substitution of adenosine to inosine mediated by the adenosine deaminase acting on RNA (ADAR) enzyme family [22,23]. A recent study revealed that the RNA-editing enzyme ADAR1 functions as an antagonist of circRNA production [24,25]. Circularized exons are normally between long introns, often termed bracketing introns. It was found that introns bracketing circRNAs are enriched for RNA editing events and that knockdown of ADAR1 significantly enhanced circRNA expression in HEK293 cells. Repression of RNA circularization is probably not a general function of ADAR1, as only a few circRNAs were affected by ADAR1 knockdown [25]. RNA Binding Motif protein 20 (RBM20) is yet another splicing factor that has recently been implicated in circRNA production, specifically from the titin gene (*TTN*) [26].

3. Function of circRNAs in the CV system

In the following lines, we will summarize the current knowledge of the roles of circRNAs in CV pathophysiology, focusing on their mechanisms of action and interaction with partner molecules. A schematic representation of the different functions of circRNAs is provided in Fig. 3.

3.1. circRNAs: coding or non-coding?

First, we would like to discuss the "non-coding RNA" feature of circRNAs. While circRNAs are widely considered as lacking protein-coding potential, recent reports questioned this concept. Translation of circRNAs (Fig. 3a) was suggested by ribosome footprinting data in several animal models (from flies to murine ones), which revealed that a number of circRNAs were consistently found to be associated with translating ribosomes [27]. In another study in murine and human myoblasts, *cZNF609* (also known as myocardial infarction-associated circRNA – *MICRA* [28]) was reported to contain an open reading frame allowing for protein production after overexpression [29]. However, the protein-coding potential of circRNAs in naïve conditions may differ from artificial up-regulation and thus their capability to encode proteins is still a matter of debate.

3.2. Expression level

As discussed in section 2, splicing events can determine the expression levels of circRNAs (Fig. 3b). Thus, the balance between circular and linear isoforms originated from the same hosting gene is modified by general splicing factors and the regulation of back splicing mechanisms [14].

3.3. Sponge for miRNAs

More attention has been brought to the ability of circRNAs to bind miRNAs (Fig. 3c), ~22 nucleotide ncRNAs that regulate transcription [8,9]. The circRNA antisense of the cerebellar degeneration-related protein 1 (*Cdr1as*, also known as *ciRS-7*) harbours more than 60 potential binding sites for miR-7 [30,31]. The sequestration of miR-7 by *Cdr1as*, a process termed "sponge

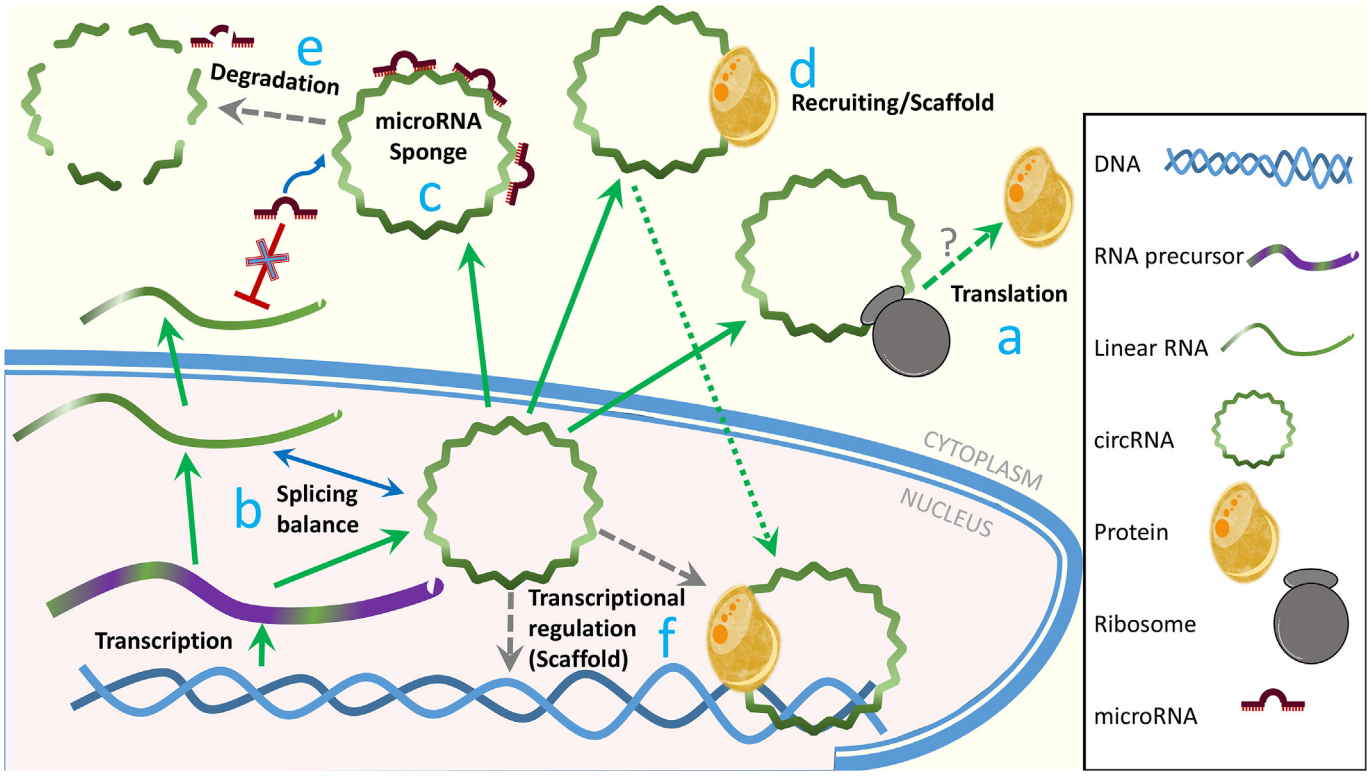

Fig. 3. Functions of circRNAs. a) In the presence of internal ribosomal enter sites and a suitable open reading frame, circRNAs could be translated into proteins. b) Generation of circRNAs by back-splicing of the precursor RNA can lead to a regulation of the linear/circular ratio of the hosting gene. c) CircRNAs can sponge microRNAs to act as a cytoplasmic reservoir of microRNAs. The scavenging of microRNAs removes the repression of target RNAs leading to an increase in their translation (if mRNAs) or activity (lncRNAs). d) CircRNAs can act as a scaffold for cytoplasmic proteins, retain certain transcription factors in the cytoplasm or serve as a vehicle for the transport of these molecules. e) CircRNAs could undergo degradation after being targeted by microRNAs. f) CircRNAs could function as a scaffold for transcription factors leading them to specific locations of the genome or directly interact with the DNA to regulate transcription.

effect", relates to insulin production and secretion by beta cells [32]. Furthermore, *Cdr1as* is elevated after myocardial infarction in mice and its effect on apoptosis seems to be regulated via miR-7 [33]. Also in mice, the heart-related circRNA (*Hrcr*) scavenges the pro-hypertrophic miR-223 and thus blocks the isoproterenol-induced cardiomyocyte hypertrophy [34]. Although miR-223 is up-regulated in human failing hearts [34], whether it is sequestered by *HRCR* in human is still unknown.

In serum samples from patients with carotid artery dissection, the ratio between the circRNA *circR-284* and miR-221 is altered due to an increase of *circR-284* and a decrease of miR-221 [35]. In thoracic aortic dissection patients, an increase of matrix metalloproteinase 9 expression is observed and bioinformatics tools predicted that *hsa_circ_101283* could contribute to it by binding three miRNAs (mir-320a, miR-583-5p and miR-138-5p) [36]. In human aortic smooth muscle cells, *circACTA2* regulates alpha-actin expression by interacting with miR-548f-5p [37]. In mouse cardiac fibroblasts, *circRNA_000203* increases collagen production by binding miR-26b-5p [38]. The mitochondrial fission and apoptosis-related circRNA (*MFACR*) sponges miR-652-3p, upregulating its target, MTP18, consequently regulating mitochondrial fission and cardiomyocyte apoptosis in mice [39]. Finally, *cZNF609* appears to regulate vascular endothelial dysfunction by sponging miR-615-5p [40]. The therapeutic potential of these findings remains to be demonstrated.

3.4. Scaffold

CircRNAs could also behave as scaffolds, either drive other elements in the cell towards their targets or block them to prevent their action (Fig. 3d). As an example of the latter, *circ-Foxo3* sequesters proteins like Id1, E2f1, Fak, and Hif1a in the cytoplasm of cardiomyocytes and cardiac fibroblasts, hence avoiding their translocation to the nucleus and contributing to the ageing of those cells [41]. In a mouse cell line, *circ-Foxo3* showed an affinity for cell cycle-related proteins such as Cdk2 and p21, and to a lesser extent Cdk6, p16 and p27, also suggesting a role of this circRNA in cell proliferation [42].

3.5. Other putative functions of circRNAs

The miRNA-sponge function of circRNAs described before is typically presumed for cytoplasmic circRNAs. Of note, although *cZNF292* is cytoplasmic, a miRNA-sponge effect could not be demonstrated using high-throughput sequencing of RNA isolated by cross-linking and immunoprecipitation [43]. In addition to the sequestration of miRNAs by circRNAs, it can be foreseen that miRNAs could bind circRNAs to induce their degradation (Fig. 3e), similar to the down-regulation of linear RNAs. However, this needs further investigation.

Some RNA molecules are able to target genomic DNA in a sequence-specific manner. This characteristic is the basis of the gene editing mechanism named Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), where a small guide RNA places the CRISPR associated protein (Cas9) at a specific genomic location [44]. Together with their scaffold ability, one could speculate that circRNAs can act as transcription regulators, either alone or in combination with other RNA or protein partners (Fig. 3f). Therefore, circRNAs could target specific DNA sequences and

modulate their transcription, as it was previously described for lncRNAs [10,45].

4. Expression and regulation of circRNAs in the CV system

Although circRNAs have been described at least as early as 1976 as viroids [46] and then in the 1990s in eukaryotes [47,48], interest in their function and application has been recently renewed [30,49–51]. RNA sequencing data of non-polyadenylated transcripts allowed the realization that circRNA expression is widespread [16,24,30,50–52]. Studies revealed that at least 14% of expressed genes in human fibroblasts yield over 25,000 circRNAs [51].

CircRNAs are abundant and conserved in eukaryotes, have been detected in different developmental stages and cell types, and their expression can also be highly cell-type dependent [30,50,51,53]. These observations together with their specific changes in pathological states (reviewed in Ref. [54]) suggest their expression is a regulated process [4]. Investigations of circRNA expression during human development revealed dynamic changes [55,56], particularly a trend in upregulation [56]. Interestingly, the most expressed gene during foetal development was the circular isoform of *NCX1* in the heart, a calcium transport gene essential for cardiac development. In this study, several other circRNAs were more expressed than their linear mRNAs [56]. This observation is consistent with other reports that indicate that the circRNA expression is not necessarily correlated with the expression of its linear mRNA from which it derives, varying according to the cell type [25,51,53,56].

Two contributions towards a better understanding of the potential role of circRNAs in the CV system were the characterization of these molecules' expression in both developing and adult hearts [57,58]. One study detected over 9,000 candidate circRNAs for each species using RNA sequencing (RNA-seq) of heart tissue from humans (failing and non-failing), mice (sham or after transverse aortic constriction) and rats (neonatal and adult) [57]. The other study identified also by RNA-seq over 15,000 circRNAs in humans and over 3,000 in mice. The group analysed tissue from failing and non-failing hearts from both species and from human embryonic stem cell-derived cardiomyocytes across different stages of differentiation [58]. The most expressed circRNAs, which were more abundant than their linear counterparts, were produced from critical cardiac genes, such as *TTN*, ryanodine receptor 2 (*RYR2* – encoding a protein found primarily in cardiac muscle and involved in calcium release from the cytoplasm), and dystrophin (*DMD* – encoding a component of the dystrophin-glycoprotein complex which links the cytoskeleton to the extracellular matrix and which genetic alterations cause Duchenne muscular dystrophy) [58].

The titin gene encodes titin (TTN), a giant protein involved in muscle contraction. RBM20, involved in circRNA biogenesis, is expressed in the heart and skeletal muscle, where it regulates alternative splicing of multiple genes, including *TTN* [59]. Mutations in the *RBM20* gene lead to a severe and early onset dilated cardiomyopathy (DCM), and aberrant splicing of *TTN* is believed to be the main reason why *RBM20* mutation carriers develop DCM [59–61]. However, a recent study showed that RBM20 is also required for the production of a large set of circRNAs that arise from the *TTN* gene [26]. The authors profiled circRNAs of human left ventricle samples and control from hypertrophic or dilated cardiomyopathy patients, finding 826 back-splice junctions in common between the groups, where 80 were from the titin transcript. The RBM20-binding sites were found to be enriched in the introns flanking the titin back-splice junctions. These RBM20-dependent TTN circRNAs specifically arise from a region within *TTN* transcript: the so-called I-band, which is known to undergo extensive alternative splicing. Interestingly, in the hearts of Rbm20 knockout mice, and also in human *RBM20* mutation carriers, circRNAs from *TTN*'s I-band were completely absent, while at the same time, the corresponding exons were abundantly included in the linear *TTN* transcript. This implies a mechanism in which exons that are spliced out of the *TTN* pre-mRNA can serve as a substrate for the production of circRNAs [26]. In conclusion, this study shows that *TTN* circRNA production is intimately connected to alternative splicing of *TTN*, and this is in line with other studies demonstrating that the more an exon is circularized, the less it is represented in the linearly processed mRNA [14,62].

A microarray study contributed to the identification of 63 differentially expressed circRNAs from 1,163 circRNA candidates in murine tissues from normal and failing hearts due to myocardial infarction [63]. Twelve of these differentially expressed circRNAs were validated by quantitative PCR, including *Cdr1as*. The authors observed an upregulation in failing compared to normal hearts, a result consistent with another report showing that *Cdr1as* was overexpressed in mice hearts with increased infarct size and cardiomyocytes under hypoxia. Additionally, *Cdr1as* acted as a sponge to miR-7a in myocardial cells, suppressing its activity [33].

Few other circRNAs have been reported correlated with cardiovascular disease. In an analysis of plasma from myocardial infarction patients, the circRNA *circRNA_081881* was downregulated more than ten-fold compared to plasma from healthy volunteers and was predicted to bind miR-548, which targets the heart-protective gene *PPARγ* [64]. The circRNA produced from the forkhead family of transcription factors Foxo3, *circ-Foxo3*, was shown to promote cardiac senescence in hearts of aged patients and mice, where it was highly expressed. Silencing of the circRNA inhibited senescence of mouse embryonic fibroblasts inhibited senescence suppressing cardiomyopathy symptoms [41].

As diabetes mellitus is a prime risk factor for cardiovascular disease and cardiac fibrosis is one of the main pathologies of diabetic cardiomyopathies, studies investigating the role of circRNAs in such conditions are emerging. A report with diabetic mice hearts and Angiotensin II-induced cardiac fibrosis investigated the modulation of *circRNA_000203* on fibrosis-associated genes expression. This circRNA was found to be upregulated compared to controls and to interact with the anti-fibrotic miR-26b-5p, enhancing the proliferation of cardiac fibroblast [65]. High levels of another *circRNA_010567* were found in diabetic mice myocardium and cardiac fibroblasts, negatively regulating miR-141, and thus upregulating its target, TGF-β1. These three molecules play a role in regulating the pathogenesis of myocardial fibrosis in the studied model [66].

In vascular endothelial cells, computational analysis of RNA-seq data resulted in the identification of over 7,000 circRNAs, among which *cZNF292* was shown to be highly expressed and up-regulated under hypoxic stress [43]. Importantly, *cZNF292* was not a mere byproduct of RNA splicing but was functionally involved in angiogenic sprouting [43].

5. Methodological aspects of circRNAs research

Most circRNAs escaped identification for decades because they are not easily separated from other RNAs by size (as miRNAs can be), they do not have polyadenylation or free 3′ or 5′ ends, and they have exons arranged in a different order (back-splice), thus being considered as artefacts [12]. However, technological advances in RNA analyses and bioinformatic tools enabled circRNAs to be appreciated as an important type of ncRNAs and potential regulators of gene expression.

The events that give rise to circRNAs specify certain properties of these molecules, such as the presence of a non-linear splice junction, the absence of a polyA tail and the resistance to exonuclease

treatment, which distinguish them from linear mRNAs. These characteristics can be used to detect novel circRNAs.

5.1. Detection of back-splicing events

Back-splicing junctions can be readily detected from ribosomal RNA (rRNA) depleted whole transcriptome sequencing data as opposed to conventional polyA + sequencing data (Fig. 4). The detection of circRNAs is a multi-stage bioinformatics process. Briefly, short sequencing reads, which are indicative of back-splicing events and, hence, do not align co-linearly to their genomic locus of origin, are identified in rRNA-depleted whole transcriptome sequencing data. Depending on tissue and condition, this will lead to a list of potential back-splicing junction candidates, which may represent hundreds to thousands of potential circRNAs. The quality of these predictions is typically assessed by their absence of polyA sequencing data (i.e. false positives) and by their relative enrichment in RNase R treated over mock-treated rRNA-depleted whole transcriptome sequencing data (Fig. 4) [67]. An alternative RNase R-based approach, which is generally applicable for all species where no rRNA removal strategy exists, has been proposed and termed RPAD (RNase R treatment followed by polyadenylation and poly(A)+ RNA depletion) [68]. Briefly, total RNA is digested by RNase R and remaining linear RNAs receive a polyA tail in a second step, which is then used to deplete them from the RNA pool. This method is more efficient to eliminate linear RNAs than treatment with RNase R alone, facilitating the detection of novel circRNAs [68].

A number of software tools exist for circRNA recognition through the detection of back-splicing junctions (Table 1). A comparison of the benefits and drawbacks of some of these tools has been recently reported [69]. However, publicly available downstream analysis tools for the reconstruction of circRNA species and additional in-depth analyses are rare.

For example, Circtools (https://github.com/dieterich-lab/circtools), a modular, Python/R-based framework for circRNA-related tools that unifies several essential functions in a one-step software solution, aims to address the issue of circRNA detection and quantification. Circtools' base modules cover detection of circRNAs [67], a beta-binomial model for testing relative abundance changes of circular vs. linear RNA species from the same host gene (CircTest), and to fully reconstruct the exon-intron structure of circRNAs from long sequencing reads (FUCHS) [70]. This framework was used to identify circRNAs and host gene dynamics in cardiac development and disease [55].

5.2. Alternative splicing analysis

Oftentimes, several alternative circRNAs emerge from the same gene locus. Similar to alternative linear splicing, circRNAs could share the same back-splicing site, yet differ in their internal exon composition, that is, by alternative exon skipping. Moreover, alternative back-splicing may occur within the same host gene by using alternative splice site acceptors or donors. Two software packages are specifically designed for resolving this question, namely FUCHS [70] and CIRI-AS [71].

5.3. Assessment of circRNAs by RT-qPCR

Often used to validate RNA-seq data, reverse transcription quantitative polymerase chain reaction (RT-qPCR) has been adapted to specifically assess the expression of circRNAs. Primer design is a necessary first step in validating and quantitating circRNAs. Having considered alternative circRNA splicing, one

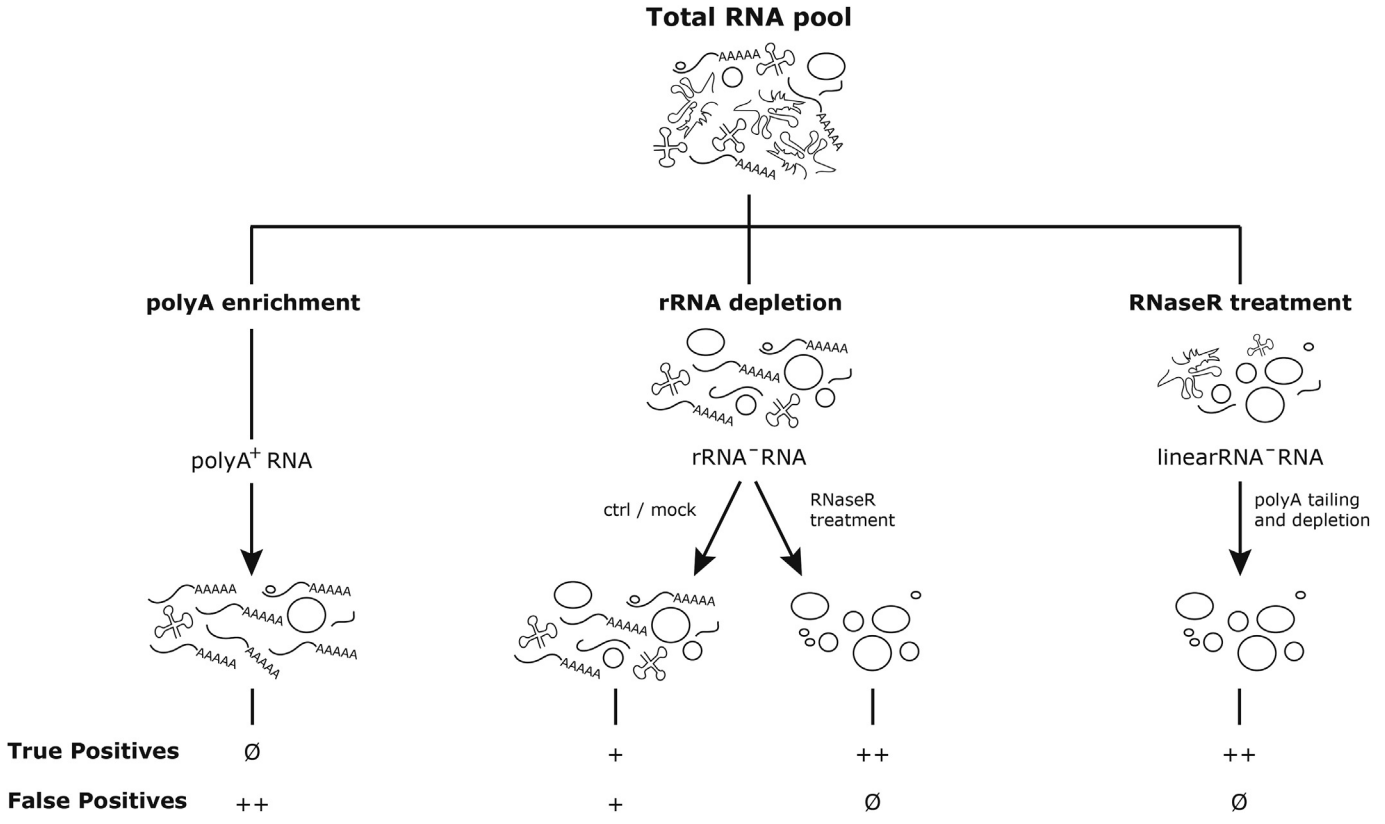

Fig. 4. Different sequencing strategies to detect back-splicing junctions and to recover the internal structure of circular RNAs.

Table 1
Prediction tools for the discovery of circRNAs from RNA sequencing (RNA-seq) datasets.

Tool name	Website	Description	Aligner	Reference
Acfs	https://github.com/arthuryxt/acfs	Identifies back-splicing junctions from RNA-seq data	BWA-MEM	[84]
circExplorer	http://yanglab.github.io/CIRCexplorer/	Identifies junction reads from back-spliced exons and intron lariats	TopHat Fusion	[16]
circRNA_finder	https://github.com/bioxfu/circRNAFinder	Pipeline to find circRNAs from RNA-seq data	STAR	[85]
CIRI2	https://sourceforge.net/projects/ciri/files/CIRI2/	Detects back-splicing junction reads using efficient maximum likelihood estimation based on multiple seed matching	BWA-MEM	[86]
DCC	https://github.com/dieterich-lab/	Detects back-splicing junctions from RNA-seq data and applies filters to integrate data across replicate sets	STAR	[67]
find_circ	https://github.com/marvin-jens/find_circ	Detects back-spliced sequencing reads from RNA-seq data	bowtie2	[30]
KNIFE	https://github.com/lindaszabo/KNIFE	Statistically-based splicing detection for circular and linear isoforms from RNA-Seq data	bowtie and bowtie2	[56]
MapeSplice	http://www.netlab.uky.edu/p/bioinfo/MapSplice2	Maps RNA-seq data to reference genome to discover splice junctions	bowtie	[87]
NCLScan	https://github.com/TreesLab/NCLscan	Pipeline to identify intra and intergenic non-colinear transcripts (including circRNA) from paired-end RNA-seq data	BWA	[88]
PTESFinder	https://sourceforge.net/projects/ptesfinder-v1/	Pipeline for identifying post-transcriptional exon shuffling events from high-throughput RNA-seq data	Bowtie	[89]
Sailfish-cir	https://github.com/zerodel/sailfish-cir	Estimates the relative abundance of circRNAs from RNA-seq data	RapMap	[90]
Segemehl	http://www.bioinf.uni-leipzig.de/Software/segemehl/	Maps short sequencer reads to reference genomes, detecting mismatches, insertions and deletions	Segemehl	[91]
SUPeR-seq	https://www.illumina.com/science/sequencing-method-explorer/kits-and-arrays/super-seq.html	Method to sequence both polyadenylated and non-polyadenylated RNAs from individual cells	bowtie2	[52]
UROBORUS	https://github.com/WGLab/UROBORUS	Pipeline to identify circRNA from total RNA-seq data without RNase R treatment	TopHat Bowtie	[92]

needs to keep in mind that sequences flanking a particular back-splicing junction may represent one or more circRNAs. A comparison to the expression of the host gene could become a complex endeavour too if multiple isoforms exist. Herein, Circtools supports the experimentalist by its primer design module.

6. Artificial intelligence for circRNA research

Artificial intelligence methods, particularly machine learning, are increasingly used to analyse biological data. The large amount of experimental data generated by high-throughput methods makes this both possible and necessary, since machine learning requires a lot of training data, but is also often the most effective approach for finding interesting hidden relationships. So far there have been few applications of artificial intelligence to circRNAs since they have not been researched as intensely as other molecules such as linear RNAs or proteins. However, we expect new utilisations of artificial intelligence methods to arise following the continuous improvement of the knowledge related to the role of circRNAs in biology and disease. Both existing and potential applications of artificial intelligence to circRNA are discussed in this chapter.

6.1. CircRNA recognition

The first problem that requires sophisticated computational methods is circRNA recognition as discussed in section 5. This is usually tackled with heuristic methods gathered in Table 1. These tools take RNA-seq data as input and use two main approaches to recognize circRNAs: one that is based on detecting back-splices in RNA-seq data, and another that requires genomic information (explained in Ref. [12]).

With increasing numbers of experimentally verified circRNAs, it is becoming feasible to develop machine-learning methods to recognize them. A method called PredcircRNA, as well as the web tool WebCircRNA that implements a version of this method distinguishes circRNAs from other lncRNAs [72,73]. The study describing it started with a dataset of 14,084 circRNAs and 19,722 linear lncRNAs. From these, 188 features were extracted: graph describing RNA structure and sequence, conservation score, nucleotide composition, and other features such as single nucleotide polymorphism density at miRNA binding sites. Each group of features was transformed via a Gaussian kernel, which was fused using lp-norm multiple kernel learning to build a model to recognize circRNAs. The evaluation showed an accuracy of around 80% (depending on the dataset). For a general overview of machine learning from RNA-seq data (of which PredcircRNA is an example), refer to [74].

6.2. Binding site recognition

A possible application of artificial intelligence to circRNAs is to recognize miRNAs or protein binding sites [75]. Here again, to our knowledge, no applications of artificial intelligence to this problem

Table 2
Databases to search for a potential function of circRNAs.

Database name	Website	Description	Reference
circBase	http://circbase.org/	Merged and unified public datasets of circRNAs	[93]
circ2Traits	http://gyanxet-beta.com/circdb/	Disease-circRNA associations according to a network of predicted interactions between disease-associated miRNAs and protein coding, long non-coding and circRNA genes, as well as the disease-and traits-associated variations in circRNA loci	[94]
CircNet	http://circnet.mbc.nctu.edu.tw/	Tissue-specific circRNA expression profiles and circRNA-miRNA-gene regulatory networks	[95]
starBase	http://starbase.sysu.edu.cn/mirCircRNA.php	Predicted miRNA-circRNA interactions overlapped with CLIP-Seq data	[96]

CLIP: cross-linking immunoprecipitation.

Table 3
Bioinformatic tools to evaluate the protein-coding potential of circRNAs.

Tool name	Website	Aim	Reference
CircPro	http://bis.zju.edu.cn/CircPro/	Predicts the protein-coding potential of circRNAs and discovers junction reads from Ribo-Seq data	[97]
circRNADB	http://202.195.183.4:8000/circrnadb/circRNADb.php	A comprehensive database for human circRNAs with protein-coding annotations (IRES, ORF, protein domains)	[5]
CPAT	http://lilab.research.bcm.edu/cpat/	Imputes the probability of an RNA to encode a peptide	[98]
IRESite	http://iresite.org/	Aligns validated IRES to circRNA sequences	[99]
ORF Finder	https://www.ncbi.nlm.nih.gov/orffinder/	Searches for open reading frames in DNA sequences	
Pfam	http://pfam.xfam.org/	Collects protein families with sequence alignments	[100]
PhyloCSF	https://github.com/mlin/PhyloCSF/wiki/	Determines whether a nucleotide sequence represents a protein-coding region	[101]

CAPT: coding-potential assessment tool; IRES: internal ribosomal entry site; ORF: open reading frame.

has been reported but computational methods have been used to recognize RNA-binding proteins and binding sites in other contexts [76]. These methods largely rely on machine learning from protein sequence and structure. It is so far still unclear to what degree they apply to circRNA and whether they can be used to recognize binding to circular as opposed to linear RNAs.

Unlike most work on RNA binding, which deals with proteins and their binding sites, one framework detects binding sites on RNA. The novelty of this approach is that it uses not only the primary RNA sequence and secondary structure but also tertiary structure [77]. In addition, instead of relying on traditional machine learning algorithms, the framework uses deep learning, an approach that builds deep artificial neural networks and has recently proven superior to traditional machine learning for many problems. It seems likely that such an approach could be used to recognize binding sites on circRNAs as well.

7. Biomarker value of circRNAs

Any newly discovered type of RNA molecules has been evaluated for its biomarker potential. Bioinformatics and computational biology approaches based on the concepts of systems biology allowed the discovery of messenger RNAs [78], miRNAs [8,9,79] and lncRNAs [10,11,45] as biomarkers of CV disease. The blood transcriptome has been revealed as a rich reservoir of potential biomarkers of CV diseases [80]. Yet, the biomarker value of circRNAs for CV disease has only recently been suggested. A study in patients with acute myocardial infarction identified the circRNA MICRA which showed an association with the development of heart failure in the few months following the event [28]. This association was validated in two independent patient cohorts. Importantly, MICRA provided a significant incremental value to predict heart failure development over existing risk factors and biomarkers [28,81]. Even if, so far, no other circRNA has been reported to have a diagnostic or a prognostic value for CV disease, it is expected that the resistance of circRNAs to exonuclease degradation confers them with an interesting biomarker potential that needs to be further explored.

8. Considerations for future work

The knowledge of the role of circRNAs in CV health and disease is still scarce and more research is needed to reach a better knowledge of their potential as therapeutic targets and biomarkers of CV disease. The following considerations may be pondered when designing future research projects.

- *Discovery of circRNAs.* Different prediction algorithms available for free download from several databases have been developed for the discovery of circRNAs from RNA-seq datasets (Table 1). Use of RNase R-digested total RNA as input for RNA-seq allows targeting circRNAs for increased specificity and sensitivity of detection.
- *Technical aspects of circRNAs detection.* Since circRNAs are produced as an alternative of linear RNA transcripts, it is important to ensure that what is thought of as a circRNA is effectively in a circular form. This verification can be achieved using RNase R digestion and divergent primers or probes spanning the junction site in RT-qPCR experiments.
- *Biogenesis of circRNAs.* A thorough characterization of the mechanisms responsible for circRNAs biogenesis will help to understand their regulation in disease states and may lead to the identification of novel therapeutic targets.
- *Role of RNA binding proteins in circRNA biogenesis.* Since there are hundreds of RNA binding proteins known, an important challenge for the future will be to identify those proteins specifically involved in circRNA biogenesis.
- *Function of circRNAs.* Several databases are available to search for a potential function of circRNAs (Table 2). One of the most interesting, since it links circRNAs to disease states, might be the Circ2Traits database.
- *Mechanisms of action.* Apart from their presumed miRNA sponge effect, the mechanisms of action of circRNAs are only partly understood and deserve further molecular studies.
- *Protein-coding potential.* Further investigations are required to clarify the potential of circRNAs to encode proteins, as recently suggested in several reports [27,29,82,83]. Some bioinformatics tools have been developed to evaluate the protein-coding potential of circRNAs (Table 3), which may be helpful for future research.
- *Artificial intelligence.* As research on circRNAs advance, we envision that new solutions based on artificial intelligence methods will help to identify circRNAs, their interacting molecules, their biological function, and their role in disease.
- *Biomarker value of circRNAs.* The potential of circRNAs to be used as biomarkers is highly related to their relative stability in body fluids due to resistance to exonucleases. However, as any novel biomarker, circRNAs will have to satisfy stringent criteria to be clinically useful: reliable detection, automatable detection, robust predictive value (specificity and sensitivity), incremental value to existing markers, extensive validation in independent and large patient cohorts, and cost-effectiveness.

Acknowledgements

C. P. C. Gomes is funded by the Eurostars E! 9686 MIPROG project. A. S. S. is funded by the Ministry of Higher Education and Research and the National Research Fund of Luxembourg (grant # C14/BM/8225223). Y. Devaux is supported by the National Research Fund (grant # INTER/EUROSTARS/15/10282117) and the Ministry of Higher Education and Research of Luxembourg.

References

[1] E.S. Lander, L.M. Linton, B. Birren, C. Nusbaum, M.C. Zody, J. Baldwin, K. Devon, K. Dewar, M. Doyle, W. FitzHugh, R. Funke, D. Gage, K. Harris, A. Heaford, J. Howland, L. Kann, J. Lehoczky, R. LeVine, P. McEwan, K. McKernan, J. Meldrim, J.P. Mesirov, C. Miranda, W. Morris, J. Naylor, C. Raymond, M. Rosetti, R. Santos, A. Sheridan, C. Sougnez, N. Stange-Thomann, N. Stojanovic, A. Subramanian, D. Wyman, J. Rogers, J. Sulston, R. Ainscough, S. Beck, D. Bentley, J. Burton, C. Clee, N. Carter, A. Coulson, R. Deadman, P. Deloukas, A. Dunham, I. Dunham, R. Durbin, L. French, D. Grafham, S. Gregory, T. Hubbard, S. Humphray, A. Hunt, M. Jones, C. Lloyd, A. McMurray, L. Matthews, S. Mercer, S. Milne, J.C. Mullikin, A. Mungall, R. Plumb, M. Ross, R. Shownkeen, S. Sims, R.H. Waterston, R.K. Wilson, L.W. Hillier, J.D. McPherson, M.A. Marra, E.R. Mardis, L.A. Fulton, A.T. Chinwalla, K.H. Pepin, W.R. Gish, S.L. Chissoe, M.C. Wendl, K.D. Delehaunty, T.L. Miner, A. Delehaunty, J.B. Kramer, L.L. Cook, R.S. Fulton, D.L. Johnson, P.J. Minx, S.W. Clifton, T. Hawkins, E. Branscomb, P. Predki, P. Richardson, S. Wenning, T. Slezak, N. Doggett, J.F. Cheng, A. Olsen, S. Lucas, C. Elkin, E. Uberbacher, M. Frazier, R.A. Gibbs, D.M. Muzny, S.E. Scherer, J.B. Bouck, E.J. Sodergren, K.C. Worley, C.M. Rives, J.H. Gorrell, M.L. Metzker, S.L. Naylor, R.S. Kucherlapati, D.L. Nelson, G.M. Weinstock, Y. Sakaki, A. Fujiyama, M. Hattori, T. Yada, A. Toyoda, T. Itoh, C. Kawagoe, H. Watanabe, Y. Totoki, T. Taylor, J. Weissenbach, R. Heilig, W. Saurin, F. Artiguenave, P. Brottier, T. Bruls, E. Pelletier, C. Robert, P. Wincker, D.R. Smith, L. Doucette-Stamm, M. Rubenfield, K. Weinstock, H.M. Lee, J. Dubois, A. Rosenthal, M. Platzer, G. Nyakatura, S. Taudien, A. Rump, H. Yang, J. Yu, J. Wang, G. Huang, J. Gu, L. Hood, L. Rowen, A. Madan, S. Qin, R.W. Davis, N.A. Federspiel, A.P. Abola, M.J. Proctor, R.M. Myers, J. Schmutz, M. Dickson, J. Grimwood, D.R. Cox, M.V. Olson, R. Kaul, C. Raymond, N. Shimizu, K. Kawasaki, S. Minoshima, G.A. Evans, M. Athanasiou, R. Schultz, B.A. Roe, F. Chen, H. Pan, J. Ramser, H. Lehrach, R. Reinhardt, W.R. McCombie, M. de la Bastide, N. Dedhia, H. Blocker, K. Hornischer, G. Nordsiek, R. Agarwala, L. Aravind, J.A. Bailey, A. Bateman, S. Batzoglou, E. Birney, P. Bork, D.G. Brown, C.B. Burge, L. Cerutti, H.C. Chen, D. Church, M. Clamp, R.R. Copley, T. Doerks, S.R. Eddy, E.E. Eichler, T.S. Furey, J. Galagan, J.G. Gilbert, C. Harmon, Y. Hayashizaki, D. Haussler, H. Hermjakob, K. Hokamp, W. Jang, L.S. Johnson, T.A. Jones, S. Kasif, A. Kaspryzk, S. Kennedy, W.J. Kent, P. Kitts, E.V. Koonin, I. Korf, D. Kulp, D. Lancet, T.M. Lowe, A. McLysaght, T. Mikkelsen, J.V. Moran, N. Mulder, V.J. Pollara, C.P. Ponting, G. Schuler, J. Schultz, G. Slater, A.F. Smit, E. Stupka, J. Szustakowski, D. Thierry-Mieg, J. Thierry-Mieg, L. Wagner, J. Wallis, R. Wheeler, A. Williams, Y.I. Wolf, K.H. Wolfe, S.P. Yang, R.F. Yeh, F. Collins, M.S. Guyer, J. Peterson, A. Felsenfeld, K.A. Wetterstrand, A. Patrinos, M.J. Morgan, P. de Jong, J.J. Catanese, K. Osoegawa, H. Shizuya, S. Choi, Y.J. Chen, Initial sequencing and analysis of the human genome, Nature 409 (6822) (2001) 860–921.

[2] J.C. Venter, M.D. Adams, E.W. Myers, P.W. Li, R.J. Mural, G.G. Sutton, H.O. Smith, M. Yandell, C.A. Evans, R.A. Holt, J.D. Gocayne, P. Amanatides, R.M. Ballew, D.H. Huson, J.R. Wortman, Q. Zhang, C.D. Kodira, X.H. Zheng, L. Chen, M. Skupski, G. Subramanian, P.D. Thomas, J. Zhang, G.L. Gabor Miklos, C. Nelson, S. Broder, A.G. Clark, J. Nadeau, V.A. McKusick, N. Zinder, A.J. Levine, R.J. Roberts, M. Simon, C. Slayman, M. Hunkapiller, R. Bolanos, A. Delcher, I. Dew, D. Fasulo, M. Flanigan, L. Florea, A. Halpern, S. Hannenhalli, S. Kravitz, S. Levy, C. Mobarry, K. Reinert, K. Remington, J. Abu-Threideh, E. Beasley, K. Biddick, V. Bonazzi, R. Brandon, M. Cargill, I. Chandramouliswaran, R. Charlab, K. Chaturvedi, Z. Deng, V. Di Francesco, P. Dunn, K. Eilbeck, C. Evangelista, A.E. Gabrielian, W. Gan, W. Ge, F. Gong, Z. Gu, P. Guan, T.J. Heiman, M.E. Higgins, R.R. Ji, Z. Ke, K.A. Ketchum, Z. Lai, Y. Lei, Z. Li, J. Li, Y. Liang, X. Lin, F. Lu, G.V. Merkulov, N. Milshina, H.M. Moore, A.K. Naik, V.A. Narayan, B. Neelam, D. Nusskern, D.B. Rusch, S. Salzberg, W. Shao, B. Shue, J. Sun, Z. Wang, A. Wang, X. Wang, J. Wang, M. Wei, R. Wides, C. Xiao, C. Yan, A. Yao, J. Ye, M. Zhan, W. Zhang, H. Zhang, Q. Zhao, L. Zheng, F. Zhong, W. Zhong, S. Zhu, S. Zhao, D. Gilbert, S. Baumhueter, G. Spier, C. Carter, A. Cravchik, T. Woodage, F. Ali, H. An, A. Awe, D. Baldwin, H. Baden, M. Barnstead, I. Barrow, K. Beeson, D. Busam, A. Carver, A. Center, M.L. Cheng, L. Curry, S. Danaher, L. Davenport, R. Desilets, S. Dietz, K. Dodson, L. Doup, S. Ferriera, N. Garg, A. Gluecksmann, B. Hart, J. Haynes, C. Haynes, C. Heiner, S. Hladun, D. Hostin, J. Houck, T. Howland, C. Ibegwam, J. Johnson, F. Kalush, L. Kline, S. Koduru, A. Love, F. Mann, D. May, S. McCawley, T. McIntosh, I. McMullen, M. Moy, L. Moy, B. Murphy, K. Nelson, C. Pfannkoch, E. Pratts, V. Puri, H. Qureshi, M. Reardon, R. Rodriguez, Y.H. Rogers, D. Romblad, B. Ruhfel, R. Scott, C. Sitter, M. Smallwood, E. Stewart, R. Strong, E. Suh, R. Thomas, N.N. Tint, S. Tse, C. Vech, G. Wang, J. Wetter, S. Williams, M. Williams, S. Windsor, E. Winn-Deen, K. Wolfe, J. Zaveri, K. Zaveri, J.F. Abril, R. Guigo, M.J. Campbell, K.V. Sjolander, B. Karlak, A. Kejariwal, H. Mi, B. Lazareva, T. Hatton, A. Narechania, K. Diemer, A. Muruganujan, N. Guo, S. Sato, V. Bafna, S. Istrail, R. Lippert, R. Schwartz, B. Walenz, S. Yooseph, D. Allen, A. Basu, J. Baxendale, L. Blick, M. Caminha, J. Carnes-Stine, P. Caulk, Y.H. Chiang, M. Coyne, C. Dahlke, A. Mays, M. Dombroski, M. Donnelly, D. Ely, S. Esparham, C. Fosler, H. Gire, S. Glanowski, K. Glasser, A. Glodek, M. Gorokhov, K. Graham, B. Gropman, M. Harris, J. Heil, S. Henderson, J. Hoover, D. Jennings, C. Jordan, J. Jordan, J. Kasha, L. Kagan, C. Kraft, A. Levitsky, M. Lewis, X. Liu, J. Lopez, D. Ma, W. Majoros, J. McDaniel, S. Murphy, M. Newman, T. Nguyen, N. Nguyen, M. Nodell, S. Pan, J. Peck, M. Peterson, W. Rowe, R. Sanders, J. Scott, M. Simpson, T. Smith, A. Sprague, T. Stockwell, R. Turner, E. Venter, M. Wang, M. Wen, D. Wu, M. Wu, A. Xia, A. Zandieh, X. Zhu, The sequence of the human genome, Science 291 (5507) (2001) 1304–1351.

[3] E.P. Consortium, An integrated encyclopedia of DNA elements in the human genome, Nature 489 (7414) (2012) 57–74.

[4] S.P. Barrett, J. Salzman, Circular RNAs: analysis, expression and potential functions, Development 143 (11) (2016) 1838–1847.

[5] X. Chen, P. Han, T. Zhou, X. Guo, X. Song, Y. Li, circRNADb: a comprehensive database for human circular RNAs with protein-coding annotations, Sci. Rep. 6 (2016) 34985.

[6] E.J. Benjamin, M.J. Blaha, S.E. Chiuve, M. Cushman, S.R. Das, R. Deo, S.D. de Ferranti, J. Floyd, M. Fornage, C. Gillespie, C.R. Isasi, M.C. Jimenez, L.C. Jordan, S.E. Judd, D. Lackland, J.H. Lichtman, L. Lisabeth, S. Liu, C.T. Longenecker, R.H. Mackey, K. Matsushita, D. Mozaffarian, M.E. Mussolino, K. Nasir, R.W. Neumar, L. Palaniappan, D.K. Pandey, R.R. Thiagarajan, M.J. Reeves, M. Ritchey, C.J. Rodriguez, G.A. Roth, W.D. Rosamond, C. Sasson, A. Towfighi, C.W. Tsao, M.B. Turner, S.S. Virani, J.H. Voeks, J.Z. Willey, J.T. Wilkins, J.H. Wu, H.M. Alger, S.S. Wong, P. Muntner, C. American Heart Association Statistics, S. Stroke Statistics, Heart Disease and Stroke Statistics-2017 Update, A report from the american heart association, Circulation 135 (10) (2017) e146–e603.

[7] N. Townsend, L. Wilson, P. Bhatnagar, K. Wickramasinghe, M. Rayner, M. Nichols, Cardiovascular disease in Europe: epidemiological update 2016, Eur. Heart J. 37 (42) (2016) 3232–3245.

[8] J. Beermann, M.T. Piccoli, J. Viereck, T. Thum, Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches, Physiol. Rev. 96 (4) (2016) 1297–1325.

[9] E. Goretti, D.R. Wagner, Y. Devaux, miRNAs as biomarkers of myocardial infarction: a step forward towards personalized medicine? Trends Mol. Med. 20 (12) (2014) 716–725.

[10] C.P.C. Gomes, H. Spencer, K.L. Ford, L.Y.M. Michel, A.H. Baker, C. Emanueli, J.L. Balligand, Y. Devaux, n. Cardiolinc, The function and therapeutic potential of long non-coding RNAs in cardiovascular development and disease, Mol. Ther. Nucleic Acids 8 (2017) 494–507.

[11] S. Greco, A. Salgado Somoza, Y. Devaux, F. Martelli, Long noncoding RNAs and cardiac disease, Antioxidants Redox Signal. (August 2017) (ahead of print). https://doi.org/10.1089/ars.2017.7126.

[12] W.R. Jeck, N.E. Sharpless, Detecting and characterizing circular RNAs, Nat. Biotechnol. 32 (5) (2014) 453–461.

[13] S. Petkovic, S. Muller, RNA circularization strategies in vivo and in vitro, Nucleic Acids Res. 43 (4) (2015) 2454–2465.

[14] R. Ashwal-Fluss, M. Meyer, N.R. Pamudurti, A. Ivanov, O. Bartok, M. Hanan, N. Evantal, S. Memczak, N. Rajewsky, S. Kadener, circRNA biogenesis competes with pre-mRNA splicing, Mol. Cell 56 (1) (2014) 55–66.

[15] S. Starke, I. Jost, O. Rossbach, T. Schneider, S. Schreiner, L.H. Hung, A. Bindereif, Exon circularization requires canonical splice signals, Cell Rep. 10 (1) (2015) 103–111.

[16] X.O. Zhang, H.B. Wang, Y. Zhang, X. Lu, L.L. Chen, L. Yang, Complementary sequence-mediated exon circularization, Cell 159 (1) (2014) 134–147.

[17] D. Liang, J.E. Wilusz, Short intronic repeat sequences facilitate circular RNA production, Gene Dev. 28 (20) (2014) 2233–2247.

[18] A. Castello, B. Fischer, K. Eichelbaum, R. Horos, B.M. Beckmann, C. Strein, N.E. Davey, D.T. Humphreys, T. Preiss, L.M. Steinmetz, J. Krijgsveld, M.W. Hentze, Insights into RNA biology from an atlas of mammalian mRNA-binding proteins, Cell 149 (6) (2012) 1393–1406.

[19] P. Konieczny, E. Stepniak-Konieczna, K. Sobczak, MBNL proteins and their target RNAs, interaction and splicing regulation, Nucleic Acids Res. 42 (17) (2014) 10873–10887.

[20] S.J. Conn, K.A. Pillman, J. Toubia, V.M. Conn, M. Salmanidis, C.A. Phillips, S. Roslan, A.W. Schreiber, P.A. Gregory, G.J. Goodall, The RNA binding protein quaking regulates formation of circRNAs, Cell 160 (6) (2015) 1125–1134.

[21] C.A. Chenard, S. Richard, New implications for the QUAKING RNA binding protein in human disease, J. Neurosci. Res. 86 (2) (2008) 233–242.

[22] J.M. Gott, R.B. Emeson, Functions and mechanisms of RNA editing, Annu. Rev. Genet. 34 (2000) 499–531.

[23] K. Nishikura, Functions and regulation of RNA editing by ADAR deaminases, Annu. Rev. Biochem. 79 (2010) 321–349.

[24] A. Ivanov, S. Memczak, E. Wyler, F. Torti, H.T. Porath, M.R. Orejuela, M. Piechotta, E.Y. Levanon, M. Landthaler, C. Dieterich, N. Rajewsky, Analysis of intron sequences reveals hallmarks of circular RNA biogenesis in animals, Cell Rep. 10 (2) (2015) 170–177.

[25] A. Rybak-Wolf, C. Stottmeister, P. Glazar, M. Jens, N. Pino, S. Giusti, M. Hanan, M. Behm, O. Bartok, R. Ashwal-Fluss, M. Herzog, L. Schreyer, P. Papavasileiou, A. Ivanov, M. Ohman, D. Refojo, S. Kadener, N. Rajewsky, Circular RNAs in the mammalian brain are highly abundant, conserved, and dynamically expressed, Mol. Cell 58 (5) (2015) 870–885.

[26] M.A. Khan, Y.J. Reckman, S. Aufiero, M.M. van den Hoogenhof, I. van der Made, A. Beqqali, D.R. Koolbergen, T.B. Rasmussen, J. van der Velden, E.E. Creemers, Y.M. Pinto, RBM20 regulates circular RNA production from the titin gene, Circ. Res. 119 (9) (2016) 996–1003.

[27] N.R. Pamudurti, O. Bartok, M. Jens, R. Ashwal-Fluss, C. Stottmeister, L. Ruhe, M. Hanan, E. Wyler, D. Perez-Hernandez, E. Ramberger, S. Shenzis, M. Samson, G. Dittmar, M. Landthaler, M. Chekulaeva, N. Rajewsky, S. Kadener, Translation of CircRNAs, Mol. Cell 66 (1) (2017) 9–21 e7.

[28] M. Vausort, A. Salgado-Somoza, L. Zhang, P. Leszek, M. Scholz, A. Teren, R. Burkhardt, J. Thiery, D.R. Wagner, Y. Devaux, Myocardial infarction-

associated circular RNA predicting left ventricular dysfunction, J. Am. Coll. Cardiol. 68 (11) (2016) 1247–1248.
[29] I. Legnini, G. Di Timoteo, F. Rossi, M. Morlando, F. Briganti, O. Sthandier, A. Fatica, T. Santini, A. Andronache, M. Wade, P. Laneve, N. Rajewsky, I. Bozzoni, Circ-ZNF609 is a circular RNA that can Be translated and functions in myogenesis, Mol. Cell 66 (1) (2017) 22–37 e9.
[30] S. Memczak, M. Jens, A. Elefsinioti, F. Torti, J. Krueger, A. Rybak, L. Maier, S.D. Mackowiak, L.H. Gregersen, M. Munschauer, A. Loewer, U. Ziebold, M. Landthaler, C. Kocks, F. le Noble, N. Rajewsky, Circular RNAs are a large class of animal RNAs with regulatory potency, Nature 495 (7441) (2013) 333–338.
[31] T.B. Hansen, T.I. Jensen, B.H. Clausen, J.B. Bramsen, B. Finsen, C.K. Damgaard, J. Kjems, Natural RNA circles function as efficient microRNA sponges, Nature 495 (7441) (2013) 384–388.
[32] H. Xu, S. Guo, W. Li, P. Yu, The circular RNA Cdr1as, via miR-7 and its targets, regulates insulin transcription and secretion in islet cells, Sci. Rep. 5 (2015) 12453.
[33] H.H. Geng, R. Li, Y.M. Su, J. Xiao, M. Pan, X.X. Cai, X.P. Ji, The circular RNA Cdr1as promotes myocardial infarction by mediating the regulation of miR-7a on its target genes expression, PLoS One 11 (3) (2016) e0151753.
[34] K. Wang, B. Long, F. Liu, J.X. Wang, C.Y. Liu, B. Zhao, L.Y. Zhou, T. Sun, M. Wang, T. Yu, Y. Gong, J. Liu, Y.H. Dong, N. Li, P.F. Li, A circular RNA protects the heart from pathological hypertrophy and heart failure by targeting miR-223, Eur. Heart J. 37 (33) (2016) 2602–2611.
[35] H.A. Bazan, S.A. Hatfield, A. Brug, A.J. Brooks, D.J. Lightell Jr., T.C. Woods, Carotid plaque rupture is accompanied by an increase in the ratio of serum circR-284 to miR-221 levels, Circ. Cardiovasc. Genet. 10 (4) (2017).
[36] M. Zou, C. Huang, X. Li, X. He, Y. Chen, W. Liao, Y. Liao, J. Sun, Z. Liu, L. Zhong, J. Bin, Circular RNA expression profile and potential function of hsa_circRNA_101238 in human thoracic aortic dissection, Oncotarget 8 (47) (2017).
[37] Y. Sun, Z. Yang, B. Zheng, X.H. Zhang, M.L. Zhang, X.S. Zhao, H.Y. Zhao, T. Suzuki, J.K. Wen, A novel regulatory mechanism of smooth muscle alpha-actin expression by NRG-1/circACTA2/miR-548f-5p axis, Circ. Res. 121 (6) (2017) 628–635.
[38] W.H. Tang, S.L. Hazen, The gut microbiome and its role in cardiovascular diseases, Circulation 135 (11) (2017) 1008–1010.
[39] K. Wang, T.Y. Gan, N. Li, C.Y. Liu, L.Y. Zhou, J.N. Gao, C. Chen, K.W. Yan, M. Ponnusamy, Y.H. Zhang, P.F. Li, Circular RNA mediates cardiomyocyte death via miRNA-dependent upregulation of MTP18 expression, Cell Death Differ. 24 (6) (2017) 1111–1120.
[40] C. Liu, M.D. Yao, C.P. Li, K. Shan, H. Yang, J.J. Wang, B. Liu, X.M. Li, J. Yao, Q. Jiang, B. Yan, Silencing of circular RNA-ZNF609 ameliorates vascular endothelial dysfunction, Theranostics 7 (11) (2017) 2863–2877.
[41] W.W. Du, W. Yang, Y. Chen, Z.K. Wu, F.S. Foster, Z. Yang, X. Li, B.B. Yang, Foxo3 circular RNA promotes cardiac senescence by modulating multiple factors associated with stress and senescence responses, Eur. Heart J. 38 (18) (2017) 1402–1412.
[42] W.W. Du, W. Yang, E. Liu, Z. Yang, P. Dhaliwal, B.B. Yang, Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2, Nucleic Acids Res. 44 (6) (2016) 2846–2858.
[43] J.N. Boeckel, N. Jae, A.W. Heumuller, W. Chen, R.A. Boon, K. Stellos, A.M. Zeiher, D. John, S. Uchida, S. Dimmeler, Identification and characterization of hypoxia-regulated endothelial circular RNA, Circ. Res. 117 (10) (2015) 884–890.
[44] T. Doetschman, T. Georgieva, Gene editing with CRISPR/cas9 RNA-directed nuclease, Circ. Res. 120 (5) (2017) 876–894.
[45] Y. Devaux, J. Zangrando, B. Schroen, E.E. Creemers, T. Pedrazzini, C.P. Chang, G.W. Dorn 2nd, T. Thum, S. Heymans, n. Cardiolinc, Long noncoding RNAs in cardiac development and ageing, Nat. Rev. Cardiol. 12 (7) (2015) 415–425.
[46] H.L. Sanger, G. Klotz, D. Riesner, H.J. Gross, A.K. Kleinschmidt, Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures, Proc. Natl. Acad. Sci. U.S.A. 73 (11) (1976) 3852–3856.
[47] C. Cocquerelle, B. Mascrez, D. Hetuin, B. Bailleul, Mis-splicing yields circular RNA molecules, Faseb. J. : Official Publ. Fed. Fed. Am. Soc. Exp. Biol. 7 (1) (1993) 155–160.
[48] J.M. Nigro, K.R. Cho, E.R. Fearon, S.E. Kern, J.M. Ruppert, J.D. Oliner, K.W. Kinzler, B. Vogelstein, Scrambled exons, Cell 64 (3) (1991) 607–613.
[49] T.B. Hansen, E.D. Wiklund, J.B. Bramsen, S.B. Villadsen, A.L. Statham, S.J. Clark, J. Kjems, miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA, EMBO J. 30 (21) (2011) 4414–4422.
[50] J. Salzman, C. Gawad, P.L. Wang, N. Lacayo, P.O. Brown, Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types, PLoS One 7 (2) (2012) e30733.
[51] W.R. Jeck, J.A. Sorrentino, K. Wang, M.K. Slevin, C.E. Burd, J. Liu, W.F. Marzluff, N.E. Sharpless, Circular RNAs are abundant, conserved, and associated with ALU repeats, Rna 19 (2) (2013) 141–157.
[52] X. Fan, X. Zhang, X. Wu, H. Guo, Y. Hu, F. Tang, Y. Huang, Single-cell RNA-seq transcriptome analysis of linear and circular RNAs in mouse preimplantation embryos, Genome Biol. 16 (2015) 148.
[53] J. Salzman, R.E. Chen, M.N. Olsen, P.L. Wang, P.O. Brown, Cell-type specific features of circular RNA expression, PLoS Genet. 9 (9) (2013) e1003777.
[54] J. Greene, A.M. Baird, L. Brady, M. Lim, S.G. Gray, R. McDermott, S.P. Finn, Circular RNAs: biogenesis, function and role in human diseases, Front Mol Biosci 4 (2017) 38.
[55] D. Siede, K. Rapti, A.A. Gorska, H.A. Katus, J. Altmuller, J.N. Boeckel, B. Meder, C. Maack, M. Volkers, O.J. Muller, J. Backs, C. Dieterich, Identification of circular RNAs with host gene-independent expression in human model systems for cardiac differentiation and disease, J. Mol. Cell. Cardiol. 109 (2017) 48–56.
[56] L. Szabo, R. Morey, N.J. Palpant, P.L. Wang, N. Afari, C. Jiang, M.M. Parast, C.E. Murry, L.C. Laurent, J. Salzman, Statistically based splicing detection reveals neural enrichment and tissue-specific induction of circular RNA during human fetal development, Genome Biol. 16 (2015) 126.
[57] S. Werfel, S. Nothjunge, T. Schwarzmayr, T.M. Strom, T. Meitinger, S. Engelhardt, Characterization of circular RNAs in human, mouse and rat hearts, J. Mol. Cell. Cardiol. 98 (2016) 103–107.
[58] W.L. Tan, B.T. Lim, C.G. Anene-Nzelu, M. Ackers-Johnson, A. Dashi, K. See, Z. Tiang, D.P. Lee, W.W. Chua, T.D. Luu, P.Y. Li, A.M. Richards, R.S. Foo, A landscape of circular RNA expression in the human heart, Cardiovasc. Res. 113 (3) (2017) 298–309.
[59] W. Guo, S. Schafer, M.L. Greaser, M.H. Radke, M. Liss, T. Govindarajan, H. Maatz, H. Schulz, S. Li, A.M. Parrish, V. Dauksaite, P. Vakeel, S. Klaassen, B. Gerull, L. Thierfelder, V. Regitz-Zagrosek, T.A. Hacker, K.W. Saupe, G.W. Dec, P.T. Ellinor, C.A. MacRae, B. Spallek, R. Fischer, A. Perrot, C. Ozcelik, K. Saar, N. Hubner, M. Gotthardt, RBM20, a gene for hereditary cardiomyopathy, regulates titin splicing, Nat. Med. 18 (5) (2012) 766–773.
[60] J. Haas, K.S. Frese, B. Peil, W. Kloos, A. Keller, R. Nietsch, Z. Feng, S. Muller, E. Kayvanpour, B. Vogel, F. Sedaghat-Hamedani, W.K. Lim, X. Zhao, D. Fradkin, D. Kohler, S. Fischer, J. Franke, S. Marquart, I. Barb, D.T. Li, A. Amr, P. Ehlermann, D. Mereles, T. Weis, S. Hassel, A. Kremer, V. King, E. Wirsz, R. Isnard, M. Komajda, A. Serio, M. Grasso, P. Syrris, E. Wicks, V. Plagnol, L. Lopes, T. Gadgaard, H. Eiskjaer, M. Jorgensen, D. Garcia-Giustiniani, M. Ortiz-Genga, M.G. Crespo-Leiro, R.H. Deprez, I. Christiaans, I.A. van Rijsingen, A.A. Wilde, A. Waldenstrom, M. Bolognesi, R. Bellazzi, S. Morner, J.L. Bermejo, L. Monserrat, E. Villard, J. Mogensen, Y.M. Pinto, P. Charron, P. Elliott, E. Arbustini, H.A. Katus, B. Meder, Atlas of the clinical genetics of human dilated cardiomyopathy, Eur. Heart J. 36 (18) (2015), 1123–35a.
[61] H. Maatz, M. Jens, M. Liss, S. Schafer, M. Heinig, M. Kirchner, E. Adami, C. Rintisch, V. Dauksaite, M.H. Radke, M. Selbach, P.J. Barton, S.A. Cook, N. Rajewsky, M. Gotthardt, M. Landthaler, N. Hubner, RNA-binding protein RBM20 represses splicing to orchestrate cardiac pre-mRNA processing, J. Clin. Invest. 124 (8) (2014) 3419–3430.
[62] S. Kelly, C. Greenman, P.R. Cook, A. Papantonis, Exon skipping is correlated with exon circularization, J. Mol. Biol. 427 (15) (2015) 2414–2417.
[63] H.J. Wu, C.Y. Zhang, S. Zhang, M. Chang, H.Y. Wang, Microarray expression profile of circular RNAs in heart tissue of mice with myocardial infarction-induced heart failure, Cell. Physiol. Biochem. Int. J. Exp. Cell. Physiol. Biochem. Pharmacol. 39 (1) (2016) 205–216.
[64] Y.-Y. Deng, W. Zhang, J. She, L. Zhang, T. Chen, J. Zhou, Z. Yuan, GW27-e1167 Circular RNA Related to PPARg Function as ceRNA of microRNA in human acute myocardial infarction, J. Am. Coll. Cardiol. 68 (16 Supplement) (2016) C51–C52.
[65] C.M. Tang, M. Zhang, L. Huang, Z.Q. Hu, J.N. Zhu, Z. Xiao, Z. Zhang, Q.X. Lin, X.L. Zheng, M. Yang, S.L. Wu, J.D. Cheng, Z.X. Shan, CircRNA_000203 enhances the expression of fibrosis-associated genes by derepressing targets of miR-26b-5p, Col1a2 and CTGF, in cardiac fibroblasts, Sci. Rep. 7 (2017) 40342.
[66] B. Zhou, J.W. Yu, A novel identified circular RNA, circRNA_010567, promotes myocardial fibrosis via suppressing miR-141 by targeting TGF-beta1, Biochem. Biophys. Res. Commun. 487 (4) (2017) 769–775.
[67] J. Cheng, F. Metge, C. Dieterich, Specific identification and quantification of circular RNAs from sequencing data, Bioinformatics 32 (7) (2016) 1094–1096.
[68] A.C. Panda, S. De, I. Grammatikakis, R. Munk, X. Yang, Y. Piao, D.B. Dudekula, K. Abdelmohsen, M. Gorospe, High-purity circular RNA isolation method (RPAD) reveals vast collection of intronic circRNAs, Nucleic Acids Res. 45 (12) (2017) e116.
[69] X. Zeng, W. Lin, M. Guo, Q. Zou, A comprehensive overview and evaluation of circular RNA detection tools, PLoS Comput. Biol. 13 (6) (2017) e1005420.
[70] F. Metge, L.F. Czaja-Hasse, R. Reinhardt, C. Dieterich, FUCHS-towards full circular RNA characterization using RNAseq, PeerJ 5 (2017) e2934.
[71] Y. Gao, J. Wang, Y. Zheng, J. Zhang, S. Chen, F. Zhao, Comprehensive identification of internal structure and alternative splicing events in circular RNAs, Nat. Commun. 7 (2016) 12060.
[72] X. Pan, K. Xiong, PredcircRNA: computational classification of circular RNA from other long non-coding RNA using hybrid features, Mol. Biosyst. 11 (8) (2015) 2219–2226.
[73] C.f.n.-c.R.i.T.a. Health, WebCircRNA: Assessing the Circular RNA Potential of Coding and Noncoding RNA.
[74] A. Jabeen, N. Ahmad, K. Raza, Machine learning-based state-of-the-art methods for the classification of RNA-seq data, in: N. Dey, A.S. Ashour, S. Borra (Eds.), Classification in BioApps, Springer International Publishing, 2018.
[75] D.B. Dudekula, A.C. Panda, I. Grammatikakis, S. De, K. Abdelmohsen, M. Gorospe, CircInteractome: a web tool for exploring circular RNAs and their interacting proteins and microRNAs, RNA Biol. 13 (1) (2016) 34–42.
[76] J. Si, J. Cui, J. Cheng, R. Wu, Computational prediction of RNA-binding proteins and binding sites, Int. J. Mol. Sci. 16 (11) (2015) 25952.
[77] S. Zhang, J. Zhou, H. Hu, H. Gong, L. Chen, C. Cheng, J. Zeng, A deep learning framework for modeling structural features of RNA-binding protein targets,

Nucleic Acids Res. 44 (4) (2016) e32.
[78] F.J. Azuaje, F.E. Dewey, D.L. Brutsaert, Y. Devaux, E.A. Ashley, D.R. Wagner, Systems-based approaches to cardiovascular biomarker discovery, Circ. Cardiovasc. Genet. 5 (3) (2012) 360–367.
[79] Y. Devaux, M. Vausort, G.P. McCann, J. Zangrando, D. Kelly, N. Razvi, L. Zhang, L.L. Ng, D.R. Wagner, I.B. Squire, MicroRNA-150: a novel marker of left ventricular remodeling after acute myocardial infarction, circulation, Cardiovasc. Genet. 6 (3) (2013) 290–298.
[80] Y. Devaux, E.E. Creemers, R.A. Boon, S. Werfel, T. Thum, S. Engelhardt, S. Dimmeler, I. Squire, n. Cardiolinc, Circular RNAs in heart failure, Eur. J. Heart Fail. 19 (6) (2017) 701–709.
[81] A. Salgado-Somoza, L. Zhang, M. Vausort, Y. Devaux, The circular RNA MICRA for risk stratification after myocardial infarction, Int. J. Cardiol. Heart Vasc. 17 (2017) 33–36.
[82] N. Abe, K. Matsumoto, M. Nishihara, Y. Nakano, A. Shibata, H. Maruyama, S. Shuto, A. Matsuda, M. Yoshida, Y. Ito, H. Abe, Rolling circle translation of circular RNA in living human cells, Sci. Rep. 5 (2015) 16435.
[83] Y. Yang, X. Fan, M. Mao, X. Song, P. Wu, Y. Zhang, Y. Jin, Y. Yang, L.L. Chen, Y. Wang, C.C. Wong, X. Xiao, Z. Wang, Extensive translation of circular RNAs driven by N6-methyladenosine, Cell Res. 27 (5) (2017) 626–641.
[84] X. You, T.O. Conrad, Acfs: accurate circRNA identification and quantification from RNA-Seq data, Sci. Rep. 6 (2016) 38820.
[85] J.O. Westholm, P. Miura, S. Olson, S. Shenker, B. Joseph, P. Sanfilippo, S.E. Celniker, B.R. Graveley, E.C. Lai, Genome-wide analysis of drosophila circular RNAs reveals their structural and sequence properties and age-dependent neural accumulation, Cell Rep. 9 (5) (2014) 1966–1980.
[86] Y. Gao, J. Zhang, F. Zhao, Circular RNA identification based on multiple seed matching, Briefings Bioinf. bbx014 (2017), https://doi.org/10.1093/bib/bbx014.
[87] K. Wang, D. Singh, Z. Zeng, S.J. Coleman, Y. Huang, G.L. Savich, X. He, P. Mieczkowski, S.A. Grimm, C.M. Perou, J.N. MacLeod, D.Y. Chiang, J.F. Prins, J. Liu, MapSplice: accurate mapping of RNA-seq reads for splice junction discovery, Nucleic Acids Res. 38 (18) (2010) e178.
[88] T.J. Chuang, C.S. Wu, C.Y. Chen, L.Y. Hung, T.W. Chiang, M.Y. Yang, NCLscan: accurate identification of non-co-linear transcripts (fusion, trans-splicing and circular RNA) with a good balance between sensitivity and precision, Nucleic Acids Res. 44 (3) (2016) e29.
[89] O.G. Izuogu, A.A. Alhasan, H.M. Alafghani, M. Santibanez-Koref, D.J. Elliott, M.S. Jackson, PTESFinder: a computational method to identify post-transcriptional exon shuffling (PTES) events, BMC Bioinf. 17 (2016) 31.
[90] M. Li, X. Xie, J. Zhou, M. Sheng, X. Yin, E.A. Ko, T. Zhou, W. Gu, Quantifying circular RNA expression from RNA-seq data using model-based framework, Bioinformatics 33 (14) (2017) 2131–2139.
[91] S. Hoffmann, C. Otto, G. Doose, A. Tanzer, D. Langenberger, S. Christ, M. Kunz, L.M. Holdt, D. Teupser, J. Hackermuller, P.F. Stadler, A multi-split mapping algorithm for circular RNA, splicing, trans-splicing and fusion detection, Genome Biol. 15 (2) (2014). R34.
[92] X. Song, N. Zhang, P. Han, B.S. Moon, R.K. Lai, K. Wang, W. Lu, Circular RNA profile in gliomas revealed by identification tool UROBORUS, Nucleic Acids Res. 44 (9) (2016) e87.
[93] P. Glazar, P. Papavasileiou, N. Rajewsky, circBase: a database for circular RNAs, Rna 20 (11) (2014) 1666–1670.
[94] S. Ghosal, S. Das, R. Sen, P. Basak, J. Chakrabarti, Circ2Traits: a comprehensive database for circular RNA potentially associated with disease and traits, Front. Genet. 4 (2013) 283.
[95] Y.C. Liu, J.R. Li, C.H. Sun, E. Andrews, R.F. Chao, F.M. Lin, S.L. Weng, S.D. Hsu, C.C. Huang, C. Cheng, C.C. Liu, H.D. Huang, CircNet: a database of circular RNAs derived from transcriptome sequencing data, Nucleic Acids Res. 44 (D1) (2016) D209–D215.
[96] J.H. Li, S. Liu, H. Zhou, L.H. Qu, J.H. Yang, starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data, Nucleic Acids Res. 42 (Database issue) (2014) D92–D97.
[97] X. Meng, Q. Chen, P. Zhang, M. Chen, CircPro: an integrated tool for the identification of circRNAs with protein-coding potential, Bioinformatics 33 (20) (2017) 3314–3316.
[98] L. Wang, H.J. Park, S. Dasari, S. Wang, J.P. Kocher, W. Li, CPAT: coding-Potential Assessment Tool using an alignment-free logistic regression model, Nucleic Acids Res. 41 (6) (2013) e74.
[99] M. Mokrejs, T. Masek, V. Vopalensky, P. Hlubucek, P. Delbos, M. Pospisek, IRESite–a tool for the examination of viral and cellular internal ribosome entry sites, Nucleic Acids Res. 38 (Database issue) (2010) D131–D136.
[100] R.D. Finn, P. Coggill, R.Y. Eberhardt, S.R. Eddy, J. Mistry, A.L. Mitchell, S.C. Potter, M. Punta, M. Qureshi, A. Sangrador-Vegas, G.A. Salazar, J. Tate, A. Bateman, The Pfam protein families database: towards a more sustainable future, Nucleic Acids Res. 44 (D1) (2016) D279–D285.
[101] M.F. Lin, I. Jungreis, M. Kellis, PhyloCSF: a comparative genomics method to distinguish protein coding and non-coding regions, Bioinformatics 27 (13) (2011) i275–i282.