Functions of Circular RNAs

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Circular RNAs (circRNAs) are a distinctive class of regulatory non-coding RNAs characterised by the presence of covalently closed ends. They are evolutionary conserved molecules, and although detected in different tissues, circRNAs resulted specifically enriched in the nervous system, where they might play an important role in neuronal specification and activity. Notably, deregulation of circRNAs expression has been linked with various neurological disorders. Little is known about circRNA mode of action, the few species characterized have been shown to act as molecular decoy for microRNAs (miRNAs) or RNA binding proteins (RBPs), to control transcription of their host genes and, although classify as ncRNAs, some of them hold the capacity to direct synthesis of short peptides/proteins.

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Although the functions of few circRNAs have been uncovered so far, a growing number of studies has revealed that circRNAs are involved in a wide range of cellular processes, as well as in human pathologies, strongly suggesting their potential role as major regulators of gene expression.

1. Circular RNAs as MicroRNA and RBP “Sponges”/Scaffold

Several studies have characterized a number of circRNAs that possess miRNA recognition elements (MREs) and through interaction with miRNA-Ago2 complexes act as effective “sponges”, thus, altering the expression of the natural miRNA targets (Figure 1a) [1][2][3][4]. A prime example is the cerebellar degeneration-related antigen 1-antisense circRNA, CDR1-AS. This circRNA, highly expressed in the mammalian brain and upregulated during neuronal development [5], has more than 70 sites for miR-7, most of them conserved across eutherian mammals [1][2]. The high number of MREs, together with the fact that CDR1-AS is much more expressed of any other housekeeping gene in mouse and human brain, suggests that the competing activity for miR-7 binding is stoichiometrically relevant in neuronal tissue [1]. Indeed, in zebrafish, which expresses mir-7, but not CDR1-AS, the ectopically expression of this circRNAs causes defects in midbrain development, phenocopying the miR-7 knock-down [2]. Intriguingly, mir-7 has been implicated, as a key regulator, in different cancers [6] and in neurological disorders, such as Alzheimer (AD) and Parkinson (PD) diseases . Indeed, the ubiquitin protein ligase A (UBE2A), the protein responsible for the clearance of AD-amyloid peptides and PD related α-synuclein are both targets of miR-7 [7][8], suggesting that the CDR1-AS - mir-7 regulatory network might have a role in these two pathological conditions.

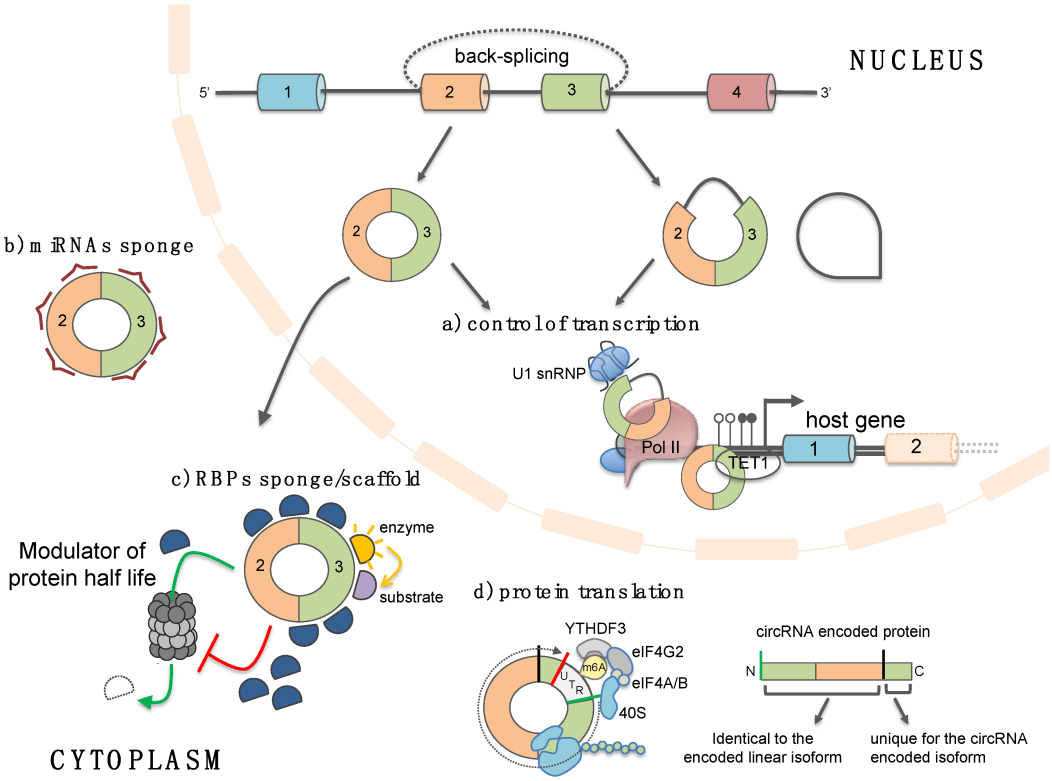

Figure 1. CircRNA functions. CircRNAs localized in the nucleus can function as a modulator of transcription of their host genes either by interacting with U1 small nuclear ribonucleoprotein (U1 snRNP) and enhancing the function of RNA polymerase II (Pol II) complex or by recruiting methylcytosine dioxygenase TET1 to the promoter region (**a**). When exported into the cytoplasm circRNAs can function as sponges or decoys for microRNAs and RBPs or alternatively can modulate the half-life of specific RBPs counteracting (red T line) or favoring their proteasome mediated degradation (green arrow) (**b**,**c**). CircRNAs have been shown to function also as protein scaffolds (**c**). By facilitating the colocalization of enzymes and their substrates are able to enhance the reaction kinetics (yellow arrow). Finally, circRNAs with internal ribosome entry site (IRES) elements and AUG sites (green line) may be translated through a CAP-independent mechanism (dashed arrow; red line depicts the STOP codon). This latter is promoted by the presence of methyl adenosine (m6A) and by the involvement of the reader protein YTHDF3 and the IRES-specialized translation initiation factor eIF4G2 (**d**). The protein isoform produced from circRNA translation will have part of the primary sequence in common with the linear encoded protein, while the rest of the polypeptide is unique for the circRNA encoded isoform.

Besides CDR1as, only few circRNAs are highly expressed and can function efficiently as miRNAs sponges; two examples are the circular Sry, which has 16 binding sites for miR-138 in mouse (but only one in human) [1], and circ-HIPK3, which, instead, has 18 putative binding sites for nine different miRNAs [3]. Therefore, it is not surprising that this field is very debated: the majority of the circRNAs described as “sponges” were indeed found to have only a single or very few binding sites for miRNAs raising the doubt regarding the effectiveness of their sponge activity [9][10].

In addition to miRNAs, circRNAs can bind to RBPs and sequester them from their natural targets or regulate their activity/stability (Figure 3b)[11][12][13][14]. Indeed, some circRNAs act as protein scaffolds favoring the colocalization of specific enzymes with their substrates[12][15]. CircMBL harbors numerous binding sites for the MBL protein that can, in turn, promotes the biogenesis of circMBL at the expenses of the production of the mature linear MBL mRNA. Therefore, it has been suggested that circMBL, by sequestering MBL, acts in a regulatory loop to finally fine tune the production and availability of the MBL protein [11]. Circ-FOXO3 and circ-ZNF609, are instead involved in controlling cell proliferation by inhibiting or promoting, respectively, the proteasome mediated degradation of specific cell cycle-related proteins [12][14]. In particular, circ-FOXO3 has been found to inhibit tumor genesis and progression and to be down-regulated in breast cancer, while an upregulation of circ-ZNF609 was described in Rhabdomyosarcoma [14][16][17]. The mechanism of action of circ-FOXO3 has been clarified: it has been described to act as a scaffold for mouse double-minute 2 (MDM2) and p53, thus, favoring the MDM2-dependent ubiquitylation of p53 [12]. Finally, a specific subgroup of circRNAs sharing 16–26 bp intra-double stranded RNA regions has been recently identified and shown to bind to PKR (dsRNA-activated protein kinase), thus, counteracting its activation in normal cultured cells. Liu and co-workers demonstrated that, upon viral infection, the activation of the endonuclease RNase L is responsible for circRNAs degradation and that this event is required for PKR release and activation in early cellular innate immune response [13]. Moreover, even though at its early stages, the work also revealed a correlation between a reduction of circRNAs expression and a stable activation of RNase L and PKR in patients with autoimmune disease systemic lupus erythematosus (SLE).

2. Circular RNAs as Templates for Protein Translation

Although generally considered “noncoding” molecules so far, circRNAs may hold the ability to serve as templates for protein translation (Figure 3c). This implies that new reading frames generated through circRNAs translation would expand the repertoire of protein isoforms in cells. Abe and colleagues were among the first to report the possibility for a circRNA molecule to be translated, revealing a rolling circle translation in rabbit reticulocyte lysate of an artificial circRNA with infinite open reading frame (ORF) [18]. Several other following studies demonstrated that endogenously produced circRNAs are indeed associated with polysomes and shifted to lighter fractions upon puromycin treatment [19][20][21]. Due to their circularity, circRNAs translation relies on a CAP-independent mechanism; moreover, it has been demonstrated that, *in vivo*, circRNAs must experience splicing to be competent for translation [20]. Further supports to a CAP-independent translation come from the work of Yang and colleagues which revealed that human circRNAs contain extensive m^6A modifications; this latter was shown to promote CAP-independent circRNAs translation through the involvement of the reader protein YTHDF3 and the IRES-specialized translation initiation factor eIF4G2 [21]. To date, only for a handful number of circRNAs the function of the translated protein isoform has been determined [22][23][24]. Nevertheless, the fact that the CAP-independent translation is enhanced in stress condition provides an interesting clue for the possibility that circRNAs-encoded proteins may play roles in a particular cellular condition, such as stress response.

3. Circular RNAs Regulate Gene Transcription

The regulation of gene expression through the miRNAs and RBPs sponge activity of circRNAs has been widely studied, since the majority of the identified circRNAs are localised in the cytoplasm; however, circRNAs have been reported to be also localised in the nucleus where they control gene expression at the transcriptional level (Figure 3d). For instance, two nuclear-localised circRNAs, that retain an intron (exon-intron circRNAs, EIcircRNA), circEIF3J and circPAIP2, through interactions with U1 small nuclear RNA (snRNA), the RNA polymerase II (RNAPII) and promoter regions are able to facilitate the expression of their parental genes[25] . The same function has been described for ci-ankrd52 and ci-sirt7, two intronic circRNAs; it has been demonstrated that they accumulate at the site of active transcription and through interaction with elongating RNAPII modulate the rate of transcription of their parental genes [26]. Fully spliced exonic circRNAs have also been detected in the nucleus [27] [28]. One example is FECR1 circRNA that regulates the *FLI1* gene by binding to the promoter region and by recruiting TET1 DNA demethylase to induce DNA demethylation [28].

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