



Circular RNA: non-coding RNA with emerging roles in stem cell differentiation

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Several different types of non-coding RNA have been shown to contribute to the regulation of pluripotency and renewal of stem cells and thereby participate in embryogenesis and development (1-4). Amongst these, the evidence for microRNA and long non-coding RNA as key players is particularly compelling. Several different individual or clusters of microRNAs have the potential to modulate embryonic stem cell pluripotency and reprogramming. These include microRNAs such as miR-372, miR-290 family, miR-302 family, miR-17-92, miR-106b-25 and miR-106a-363 clusters (5). Similarly, other types of non-coding RNA also have the potential to modulate the transcriptional networks that regulate pluripotency and lineage differentiation (6). The long non-coding RNA lncRNA-ROR, for example, can play a role in reprogramming of fibroblast into induced pluripotent stem cells (iPSCs) (7). LncRNA ROR can bind to pluripotency-associated transcription factors (PATFs) such as OCT4, SOX2 and NANOG that contribute to the maintenance of pluripotency in human ESC, as well as act as a sponge for miR-145, a microRNA that is implicated in the repression of genes involved in pluripotency (8).

Recent studies have implicated yet another type of non-coding RNA, the circular RNAs, in the control of pluripotency in humans. Circular RNAs are a class of non-coding RNA, so called because they differ from linear RNAs and assume a closed conformation. These RNAs form covalently closed loops generated from back splicing of exons or introns (or both) (Figure 1). The 3' and the 5' ends join together resulting in the formation of a continuous loop, and a conformation that renders them resistant to degradation by exonucleases. The potential contribution of circular RNA to regulation of gene expression is being recognized (9). A functional role of circular RNAs in

controlling pluripotency in hESC was described in a study by Yu *et al.* (10). This study identified circular RNAs that are enriched in undifferentiated human ESCs and iPSCs. Two of these circular RNAs, *circBIRC6* and *circCORO1C* were noted to be functionally related to maintenance of pluripotency. Modulation of circular RNAs using small hairpin RNA to specifically target circular junctions resulted in cellular differentiation and loss of pluripotency. This was associated with downregulated expression of PATFs such as *NANOG*, *OCT4*, *KLF4* and *MYC* and a corresponding increase in the expression of lineage-related transcription factors (LRTF) *Brachyury*, *SOX17* and *SOX1*.

Enforced expression of the three circular RNAs in hESCs using minigene constructs, in the absence of alterations in expression of the un-spliced circular RNA precursors, did not result in a loss of pluripotency. In differentiating hESCs, ectopic expression of *circBIRC6* or *circCORO1C* but not *circMAN1A2* increased the expression of PATFs and decreased LRTFs. Thus, *circBIRC6* or *circCORO1C* contribute to maintenance of pluripotency in differentiated hESCs. At the same time, expression of neither *circBIRC6* or *circCORO1C* was sufficient to reprogram somatic cells into iPSCs. However, the co-expression of circular RNAs and PATFs improved TF-mediated reprogramming in somatic fibroblasts.

The affinity of binding of miRNAs with circular RNAs is reported to be greater than that of binding to their endogenous target mRNA. Thus, circular RNA can act as a sponge to sequester miRNA and reduce their effects on endogenous targets. For example, ciRS-7 can sequester *miR7* thereby interfering with *miR7* effect on the suppression of epithelial to mesenchymal transition and cancer progression. Circular RNAs have also been reported to act as alternative splicing regulators or transcription factors (11). *CircBIRC6* but not *circCORO1C*

was enriched in AGO immunoprecipitates suggesting that *circBIRC6* could possess miRNA related functions. Indeed, *circBIRC6* interacted with *miR-34a* and *miR-145* in ESCs, acting as a sponge to maintain pluripotency (Figure 2). Both of these microRNAs are associated with pluripotency associated genes

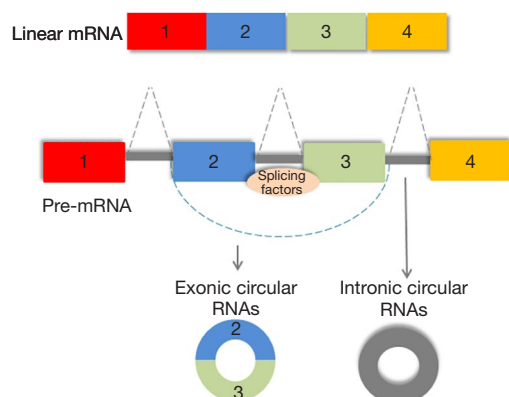


Figure 1 Biogenesis of circular RNAs. Linear splicing of pre-mRNA results in linear RNAs while a non-canonical splicing of pre-mRNA or backsplicing can result in formation of circular RNAs. Circular RNAs can consist of more than one exonic sequence, intronic sequences only, or both intronic and exonic sequences.

and can restrain somatic reprogramming by repressing the expression of these genes (3,4). Targeting sites for both of these miRNAs are also present on *circBIRC6*. A decreased expression of these miRNA was noted in undifferentiated hESCs but not in differentiated hESCs.

At first, eukaryotic circular RNAs were thought to represent artefacts in RNA sequencing. It is now recognized that these RNA molecules can be generated as a result of alternative splicing processes. Splicing results in the pairing of exons in a sequential order leading to linear RNA formation, but can also result in new transcripts such as circular RNA through back-splicing. Understanding the mechanisms of generation of circular RNAs, and the settings in which these processes occur will be necessary in elucidating their functional roles. Yu *et al.* also reported the involvement of splicing factors in generating circular RNA in human ESCs. The splicing factor ESRP1 was shown to be upregulated in ESCs, and involved in the formation of *circBIRC6*. Moreover, NANOG and Oct4 were shown to regulate ESRP1 expression. Further studies to extend this work may involve the generation and study of conditional knockouts of key proteins involved in splicing.

Improved techniques for the detection of circular RNA have resulted in characterization of their prevalence, expression in health and disease states, and their potential functional roles. The detection of circular RNA molecules

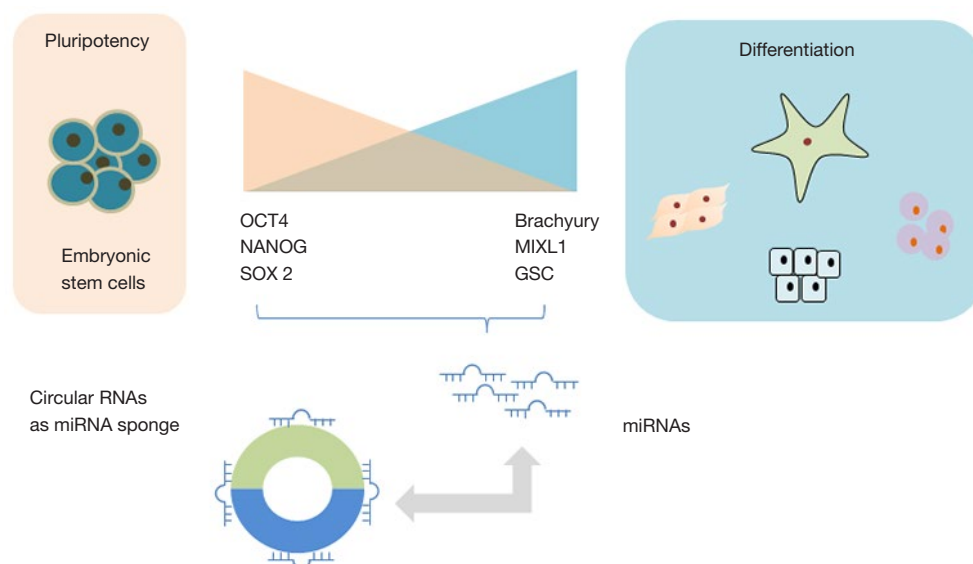


Figure 2 Potential mechanisms by which circular RNAs can regulate stem cell differentiation. Circular RNAs compete with endogenous mRNA targets of miRNA, acting as a sponge to miRNA. Some miRNAs participate in the differentiation of stem cells to more specialized cell types by inhibiting pluripotency associated transcription factors and activating lineage-related transcription factors. Circular RNAs can maintain pluripotency of stem cells by limiting these actions.

has been limited because of their low abundance and the lack of robust methods for their detection. However, the advent of gene sequencing technologies, and the use of specific analytical pipelines for the detection of circular RNA have facilitated the recognition and identification of these unique non-coding RNA. Widespread expression of circular RNAs have been observed, often in a cell type, tissue type or context-specific setting. Emerging data show that circular RNAs are stable, abundant and conserved among various biological systems. These studies are enhancing our knowledge of the role of these RNAs. Understanding the contributions of circular RNAs in the regulation of gene expression and control of pluripotency has relevance for other disease areas. For example, circular RNAs have been implicated in cancer where they may participate in tumorigenesis and disease progression (9). Pluripotent stem cells share many features with tumor cells, such as self-renewal and proliferation, while some of the transcriptional regulatory mechanisms and transcription factors involved in pluripotency may also participate in regulating tumorigenesis.

Understanding the processes that control the formation of circular RNA in different settings will be central to understanding their function. More studies are needed to elucidate the mechanisms by which a specific gene can result in various distinct transcripts that may have very different functional roles. We are only now beginning to recognize the incredible complexity of the human genome.

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Footnote

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