

## STEM CELLS

# Big roles for small RNAs

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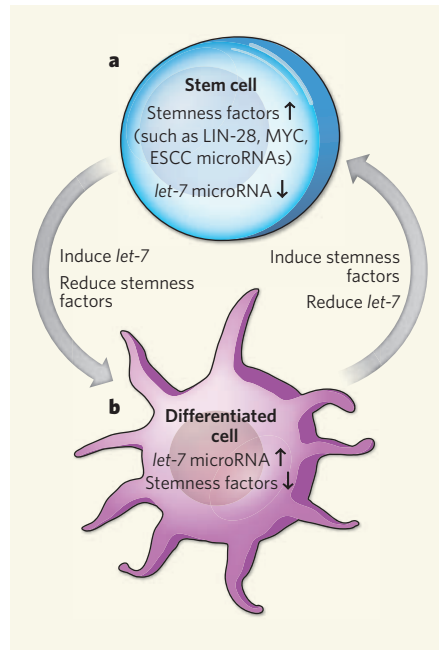
**Embryonic stem cells can create copies of themselves, but can also mature into almost any type of cell in the body. Tiny gene regulators called microRNAs are now shown to have a role in directing these properties.**

The single-celled, fertilized embryo is the source of all the trillions of specialized cells in our bodies, and the means by which this proliferation and specialization occur has fascinated developmental biologists for centuries. Moreover, the ability to artificially force mature, differentiated cells back into this naive state (called reprogramming) has huge potential in providing tools for regenerative medicine and for increasing our understanding of development. The work described on page 621 of this issue by Melton, Judson and Blelloch<sup>1</sup> indicates that small RNAs have a crucial role in stem-cell biology and therefore in future stem-cell-based therapies.

The cells that form soon after fertilization — embryonic stem cells — have a remarkable ability to divide rapidly, to make copies of themselves (self-renew) and to differentiate into any type of specialized cell — a property known as pluripotency. Once cells have differentiated, they express a set of genes encoding factors that inhibit self-renewal and that seal the differentiated state. Our understanding of the nature of this switch between pluripotent and differentiated states is still rudimentary, but several important regulatory genes are known to be required for the process. Moreover, when artificially expressed in differentiated cells (for instance, skin cells), a subset of these regulatory genes, which encode proteins known as ‘stemness’ factors, can reprogram the cells back to the pluripotent state<sup>2,3</sup>, generating what are known as induced pluripotent stem cells (iPSCs). In an interesting convergence of research, multiple lines of evidence point to a type of RNA, called microRNA, as an essential factor in this switch.

MicroRNAs, as their name suggests, are tiny RNA molecules that are encoded in our genome. MicroRNAs are not translated into protein; their function is to regulate gene expression<sup>4</sup> by binding to other RNAs, particularly messenger RNAs. Binding of microRNA to mRNA inhibits mRNA translation to protein. In humans, thousands of microRNAs regulate thousands of mRNAs in a complex network.

MicroRNAs were first discovered in the nematode worm *Caenorhabditis elegans*, when mutations in the microRNA genes *lin-4* and *let-7* were found to result in defective stem-cell maturation<sup>5,6</sup>. Specifically, in the absence of these genes, *C. elegans* epithelial stem cells known as seam cells failed to exit from the self-renewing state and to differentiate.



**Figure 1 | Proposed roles of microRNAs in embryonic stem cells.** **a**, Embryonic stem cells express high levels of ‘stemness’ factors, such as ESCC microRNAs and the proteins LIN-28 and MYC. Melton, Judson and Blelloch<sup>1</sup> now report that mouse embryonic stem cells also express *let-7* microRNAs at low levels. This microRNA must be induced to trigger cell differentiation, and seems to be essential for reducing the concentrations of the stemness factors. **b**, Differentiated cells, however, express low levels of the stemness factors and high levels of *let-7*. To dedifferentiate cells, stemness factors must be available and *let-7* must be inhibited.

Further work has revealed that microRNAs are involved in almost every biological process — including development, metabolism and ageing — in all multicellular organisms<sup>4</sup>. Moreover, microRNAs have been identified as being involved in many human diseases, most notably cancer<sup>7,8</sup>.

The *let-7* microRNA is expressed in seam cells and mammalian stem cells<sup>9</sup> just before the cells differentiate. Thus, it has been proposed that *let-7* is a conserved anti-stemness and pro-differentiation factor. Its mechanism of action, however, had remained elusive. Melton *et al.* now demonstrate<sup>1</sup> that *let-7* is a key factor in inducing the differentiation of mouse embryonic stem cells, and it seems to be essential for depleting the cell of several stemness factors whose encoding genes and mRNAs are

enriched in *let-7* binding sites. Decreasing the amounts of these stemness factors promotes differentiation (Fig. 1). In addition, the authors identify another family of microRNAs that prevents *let-7* from promoting differentiation. These findings form the basis of a model proposing that different types of microRNA have opposing effects on the fate of embryonic stem cells, one type promoting self-renewal and the other promoting differentiation.

In *C. elegans*, a mutation of the *lin-28* gene results in premature differentiation of stem cells<sup>10</sup>, indicating that the LIN-28 protein is also a stemness factor. Recent work<sup>11–15</sup> has shown that LIN-28 is an RNA-binding protein that inhibits the processing and maturation of *let-7* microRNA in both mammals and *C. elegans* (microRNAs are modified by various proteins before they become fully functional). Moreover, LIN-28 is needed for efficient iPSC production<sup>2</sup>. If one ‘joins the dots’, it’s not difficult to imagine that one of the roles of LIN-28 in iPSC production may be to suppress the maturation of *let-7* microRNA<sup>16</sup>. The current work<sup>1</sup> shows that inhibition of *let-7* promotes iPSC production from mature cells, supporting this model and highlighting the importance of this microRNA in maintaining the differentiated state (Fig. 1).

Melton and colleagues’ observations<sup>1</sup> raise interesting prospects for regenerative medicine. First, they suggest that transient manipulation of microRNA levels might be a preferred route for generating iPSCs. The authors’ findings will also interest workers in the cancer field, in which there is growing support for an emerging hypothesis that mutant stem cells are responsible for many cancers. The work<sup>1</sup> surely reinforces the strategy of manipulating the amounts of such microRNAs in tumours as anticancer therapy. These smallest of RNA molecules seem to have a big future ahead.

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