

# Stem Cells: From Epigenetics to microRNAs

## Minireview

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The complexity and cellular diversity of the adult brain arises from the proliferation and differentiation of a small number of stem cells. The intrinsic state of stem cells depends on their spatial and temporal history and affects their responsiveness to extrinsic signals from the microenvironment. Stem cell self-renewal and differentiation along neuronal and glial lineages are defined by the dynamic interplay between transcription, epigenetic control, and posttranscriptional regulators, including microRNAs, whose key role in stem cell biology is just emerging.

Stem cells are remarkable cells with two fundamental properties: self-renewal and multipotency. Stem cells persist in many tissues throughout life, including the brain. Neurons and glia, which comprise astrocytes and oligodendrocytes, arise from neural stem cells in a temporally defined sequence (Temple, 2001). As development proceeds, the early neuroepithelial stem cells transition into radial glia that, at the end of neurogenesis, transform into astrocytes. A subset of astrocytes persist as stem cells in specialized niches in the adult brain and continuously generate large numbers of neurons that functionally integrate into restricted regions (reviewed in Doetsch, 2003). Thus, neural stem cells are contained in the neuroepithelial → radial glia → astrocyte lineage (Figure 1).

Stem cells may generate differentiated progeny either directly or via rapidly dividing transit-amplifying cells. Progression along the lineage from stem cell to differentiated cell is characterized by striking morphological and functional changes at each stage in the lineage and the sequential expression of transcription factors and other signaling molecules, which elicit cascades of gene expression. Batteries of transcription factors have been proposed to control stem cell self-renewal and lineage progression and are also a powerful mechanism for generating cell diversity (Pearson and Doe, 2004).

The responsiveness of stem cells to extrinsic signals changes over time, and their developmental potential becomes more restricted, due to changes in their internal state (Temple, 2001). In the neuroepithelial → radial glia → astrocyte stem cell lineage, early neural stem cells are able to generate a greater diversity of cell types than adult neural stem cells upon transplantation into an earlier niche (Temple, 2001), suggesting

that the genetic program of stem cells is under tight control at the epigenetic level. In addition, different molecules may regulate embryonic and postnatal stem cell niches. Mice deficient in sonic hedgehog, Tlx, and Bmi, all exhibit significant deficits in postnatal stem cell niches, but not in embryos (Machold et al., 2003; Molofsky et al., 2003; Palma et al., 2005; Shi et al., 2004). As such, a combination of common and distinct mechanisms may regulate self-renewal in the embryo and in the adult.

How do cells with differentiated features of astrocytes retain stem cell potential? What underlies progenitor cell plasticity? How does a stem cell astrocyte rapidly transition into a morphologically and molecularly distinct cell type? Until recently, the analysis of stem cells and their lineages has largely focused on transcriptional regulation. Emerging evidence suggests that epigenetic control and posttranscriptional regulation, in particular by small noncoding RNAs, are essential components of stem cell biology.

### *The Role of Chromatin Structure in Regulating Self-Renewal and Differentiation*

The transition of stem cells from pluripotent to developmentally more restricted states is accompanied by global changes in gene expression. Genes active in earlier progenitors are gradually silenced at developmentally later stages, and subsets of cell type-specific genes are turned on. This progression is the result of selective expression of transcription factors in concert with chromatin remodeling and modification, which includes covalent histone modification, DNA methylation of CpG dinucleotides, and localization of chromatin to specialized nuclear domains (Li, 2002). This epigenetic memory allows cells to maintain their identity, even when exposed to extracellular environments that induce formation of other cell fates and is important for maintaining stem cells over time, and in preventing tumor formation (Valk-Lingbeek et al., 2004).

Covalent modifications to the amino terminal tails of histones, including acetylation, methylation, and phosphorylation, regulate the packing of chromatin into transcriptionally available or transcriptionally unavailable states (Jenuwein and Allis, 2001). Histone acetyltransferases (HATs) catalyze the addition of acetyl groups to conserved residues and are correlated with more transcriptionally accessible chromatin, whereas histone deacetylases (HDACs) catalyze the opposite reaction and are associated with transcriptional repression. Blocking global HDAC activity inhibits the differentiation of ES cells (Lee et al., 2004) and progression of oligodendrocyte progenitors into mature oligodendrocytes (Marin-Husstege et al., 2002) and promotes adult hippocampal progenitor differentiation into neurons (Hsieh et al., 2004). Recent work suggests that the intrinsic acetylation state of a cell is associated with transcription of biologically related genes (Kurdistani et al., 2004). Defining the genome-wide acetylation profiles and integrating them with the transcriptomes of different stem cells at distinct stages of development

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**Multipotency**

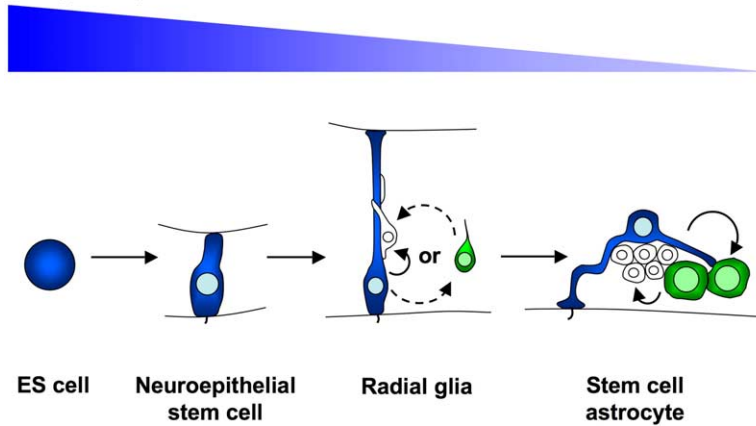


Figure 1. Stem Cells Become Progressively More Restricted over Time

Pluripotent ES cells derived from the inner cell mass of the blastocyst can differentiate into all cell types. During development, neural stem cells are contained in the neuroepithelial → radial glia → astrocyte lineage. However, stem cell potential is retained by the transit amplifying progeny (green cells) of adult stem cell astrocytes when exposed to appropriate growth factors (reviewed in Doetsch, 2003). The multipotency of stem cells is reduced over time due to progressive gene silencing. ES cell, embryonic stem cell.

will yield insight into global regulation of stem cell identity over time.

In addition to large-scale coregulation of gene expression, the particular combination of histone modifications and repertoire of transcription factors defines the heterogeneity and temporal identity of stem cells. During cortical development, neurogenesis and gliogenesis occur in sequential waves (Temple, 2001). Genes that promote one lineage must be inhibited when a stem cell generates a different lineage. Thus, neuronal genes must be inhibited until the onset of neurogenesis, and gliogenesis must be inhibited during neurogenesis. This is achieved through both transcriptional regulation and epigenetic changes that affect the ability of a cell to respond to extrinsic signals.

REST (RE1 silencing transcription factor, or NRSF), is a key regulator that binds to a conserved 23 bp motif, known as RE1, in the promoter region of many neuronal genes (Ballas et al., 2005). In nonneuronal cells, REST and its corepressors recruit HDACs, methyl binding proteins, and polycomb group repressors, which results in the spreading of silencing along adjacent chromatin and inhibition of expression of regionally proximal genes (Lunyak et al., 2002).

A novel role for REST and its corepressors is in the progression of neuronal differentiation from stem cells. REST is highly expressed in embryonic stem cells and is rapidly downregulated to very low levels upon differentiation into neural progenitors, such that neuronal genes are inactive but poised for transcriptional activation (Ballas et al., 2005). It is then released from the RE1 sites in differentiated neurons. Thus, REST and its corepressors exert differential regulatory control on its target genes depending on the cell's developmental stage.

The chromatin state can be developmentally regulated at the DNA level. Astrocyte differentiation occurs later in brain development and is revealed by the onset of GFAP expression. Activation of the GFAP promoter requires binding of the signal transducer and activator of transcription 3 (STAT3) to a consensus sequence (Ross et al., 2003). Early progenitors are refractory to astrocyte differentiation, even though STAT3 has been activated, which may be due to methylation of the

STAT3 binding site (Takizawa et al., 2001). At later developmental stages, this STAT3 binding element is not methylated and the GFAP promoter can be activated (Takizawa et al., 2001). A similar alteration in methylation pattern occurs at another STAT3 binding site in the S100β promoter, a calcium binding protein expressed in astrocytes (Namihira et al., 2004).

Extrinsic signals can also impact the chromatin state and alter the competence of progenitors to differentiate. Exposure of cortical progenitors to FGF2 increases astrocyte differentiation upon CNTF induction (Song and Ghosh, 2004). Dissection of the molecular mechanism reveals that FGF2 facilitates access of the STAT3/CBP/p300 complex to its binding site on the GFAP promoter via a switch in methylation of histone 3 lysine residue from K9 to K4 (Song and Ghosh, 2004). In this way, the GFAP promoter is transformed from a transcriptionally inactive state to a transcriptionally active state (demethylation of H3 K9 and hypermethylation of H3 K4). The endogenous HAT activity of CBP/p300 may initiate a cascade of histone acetylation to reinforce the transcriptional activation (Ross et al., 2003).

Multiple layers of epigenetic modification therefore regulate key transitions in the temporal development of stem cells and their differentiation, resulting in expression of unique repertoires of transcription factors at each stage of development and in different lineages.

**The Emerging World of Noncoding RNAs**

Transcription factors are essential players in stem cell self-renewal and differentiation (reviewed in Pevny and Placzek, 2005; Ross et al., 2003). However, posttranscriptional gene regulation is emerging as another essential and, until recently, unexpected regulator of development. Many different classes of small noncoding RNAs are present in the brain, with diverse roles including RNA modification and chromatin remodeling (Mattick and Makunin, 2005). Small double-stranded modulatory RNAs have been proposed to regulate the generation of neurons from adult neural stem cells by binding to REST (Kuwabara et al., 2004), although the mechanism by which this occurs remains unclear. Another recently identified large family of small noncoding RNAs are microRNAs (miRNAs), which are likely key posttranscriptional players in stem cells and their differentiated progeny.

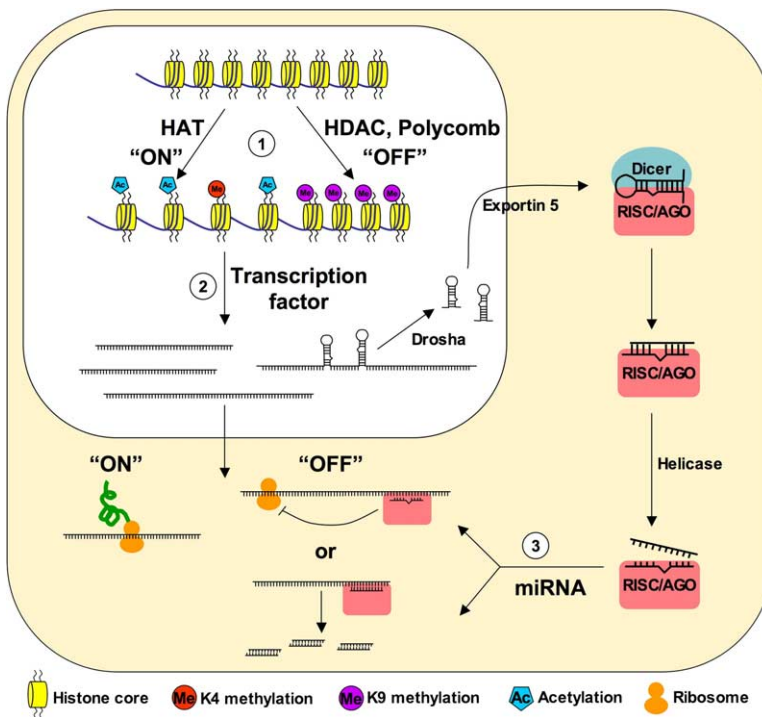


Figure 2. Global Regulatory Network

(1) DNA is wrapped around core histones as chromatin. HAT catalyzes acetylation on histone tails leading to a more transcriptionally active state ("ON"), whereas HDAC mediates the opposite reaction and causes transcriptional repression ("OFF"). Methylation of histone lysine residues is associated with different transcriptional accessibility. Methylated H3 K4 often promotes transcription and methylated H3 K9 leads to heterochromatin formation and long-term gene silencing by recruiting polycomb group members. (2) Selective expression of genes is mediated by a group of transcriptional activators and repressors. Note that the expression of miRNA genes is also under transcriptional control. (3) MiRNAs are transcribed as long primary transcripts, sometimes in clusters, which are subsequently cleaved by Drosha into hairpin precursors and are transported by exportin 5 into the cytoplasm. In the cytoplasm, Dicer generates the mature miRNAs in association with a RISC/Argonaute complex which then directs miRNAs to their targets resulting in posttranscriptional repression either by inhibiting translation or degradation of target mRNA.

MiRNAs are 21–25 nt, noncoding RNAs that are expressed in a tissue-specific and developmentally regulated manner and comprise ~1% of the total genes in the animal genome (Bartel, 2004). MiRNAs are derived from longer primary transcripts processed in the nucleus by the RNase III endonuclease Drosha into 60–75 nt hairpin-like precursors (pre-miRNAs), which are subsequently exported to the cytoplasm by Exportin 5 (Figure 2). The hairpin precursors are cleaved by Dicer, the same machinery that generates siRNAs, into mature miRNAs, which bind to effector complex RISCs that direct the miRNAs to their targets for posttranscriptional repression (Pasquinelli et al., 2005). Sequence complementarity with the target determines whether the miRNA inhibits protein translation by binding to the 3' UTR (less complementary) or degrades the mRNA (100% complementary) (Pasquinelli et al., 2005).

The first discovered miRNA genes in animals were *lin-4* and *let-7*, which were identified genetically and control the timing of *C. elegans* larval development and cell fate decisions (Ambros, 2004). Since then, miRNAs have been implicated in a wide variety of developmental and metabolic pathways in both invertebrates and vertebrates, including cell differentiation, proliferation, programmed cell death, and fat and insulin metabolism (He and Hannon, 2004), although the number of functional miRNA/target pairs identified to date is minimal.

Computational algorithms predict that each miRNA has many targets and that individual mRNAs can be targeted by many miRNAs (Bartel, 2004). Interestingly, transcription factors and other regulators of neural stem cell self-renewal, such as Ids, Notch, and Pten (Groszer et al., 2001; Ross et al., 2003), are among the predicted targets. However a comparison of the pre-

dicted miRNA targets generated with different computational algorithms reveals little overlap. In part, this is because the rules governing miRNA target recognition are still largely unknown, emphasizing the need for identifying the in vivo physiological miRNA/target pairs to assure relevance and further refine the algorithms.

miRNAs are especially attractive candidates for regulating stem cell self-renewal and cell fate decisions, as their ability to simultaneously regulate many targets provides a means for coordinated control of concerted gene action. Although direct evidence for a functional role for miRNAs in stem cell biology is just emerging, tantalizing hints regarding their involvement based on expression patterns, predicted targets, and overexpression studies suggest that they will be key regulators.

Components of the miRNA biogenesis machinery are expressed in brain germinal zones, such as DGCR8 (Shiohama et al., 2003), a dsRNA binding protein that forms a miRNA-processing complex with Drosha (Gregory et al., 2004). Intriguingly, several cloned miRNAs exhibit developmental temporal expression patterns that parallel the in vivo waves of neurogenesis and gliogenesis (Krichevsky et al., 2003; Miska et al., 2004; Sempere et al., 2004). A key role for miRNAs in brain formation has been shown by the rescue of brain morphogenesis in maternal-zygotic dicer zebrafish mutants by injection of miR-430 (Giraldez et al., 2005). This demonstrates that an individual miRNA can trigger large-scale changes in development, perhaps as a result of global changes in the transcriptome (Lim et al., 2005). miRNAs may also define regional patterning in the developing central nervous system. Sensor transgenic mice have been developed to visualize the spatial expression pattern of miRNAs and reveal that miRNAs

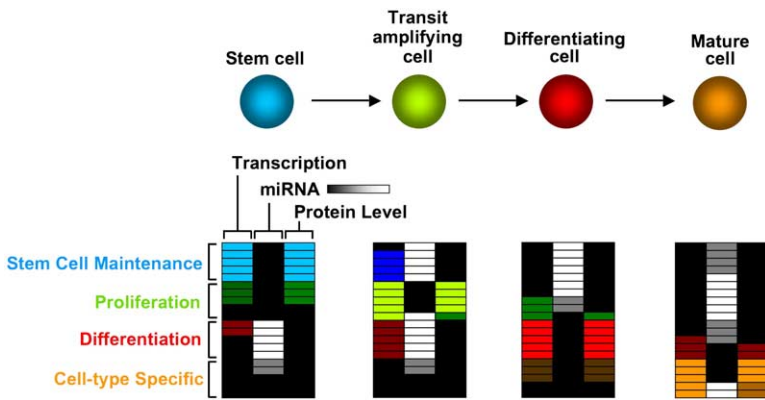


Figure 3. Possible Combinatorial Action of Transcription and miRNAs in Stem Cell Lineages

Each block represents a group of genes or miRNAs acting at distinct stages in the stem cell lineage: stem cell maintenance (blue), proliferation (green), differentiation (red), and terminal maturation of differentiated cells (orange). Gradient from bright colors to dark represents high to low expression (black is off). The first column depicts the level of transcription, and the second column expression of miRNAs targeting that class of genes. The combinatorial action of transcription and miRNA repression results in final protein level (third column). Note that miRNAs could act to refine gene activity by permitting only a subset of mRNAs to be translated. For example, only maintenance and proliferation genes are active in stem cells even though a few differentiation genes may also be transcribed at low levels (dark red in the first row).

mapping to homeobox clusters exhibit overlapping or inverse expression patterns with Hox genes during early embryogenesis (Mansfield et al., 2004). Thus the combinatorial expression of miRNAs and Hox genes may define domains of Hox gene action. The next crucial step will be to visualize miRNAs with single-cell resolution.

miRNAs are likely important regulators for stem cell self-renewal. Loss of Dicer1 causes embryonic lethality and loss of stem cell populations (Bernstein et al., 2003; Wienholds et al., 2003). Furthermore, Argonaute family members, key components of RISC complexes, are required for maintaining germline stem cells in different organisms (Carmell et al., 2002). Distinct sets of miRNAs are specifically expressed in pluripotent ES cells but not in differentiated embryoid bodies or in adult tissues, suggesting a role for miRNAs in stem cell self-renewal (Houbaviy et al., 2003; Suh et al., 2004). Interestingly, two of the novel ES miRNAs were common between mouse and human, whereas five were unique to mouse and seven to human, suggesting that some stem cell pathways are conserved and that others may be species specific. However, this may also indicate that the cloning has not yet reached saturation. Many of the ES-specific miRNAs are conserved miRNA gene families organized as gene clusters and cotranscribed as polycistronic transcripts (Houbaviy et al., 2003; Suh et al., 2004).

As stem cells differentiate, they downregulate stem cell maintenance genes and activate lineage-specific genes (Figure 3). These transitions require a rapid switch in gene expression profiles. Although the transcription factor pool is replaced, remaining transcripts that were highly expressed in the previous stage need to be silenced. miRNAs are uniquely poised to rapidly effect such changes through simultaneous repression of many targets of any remaining transcripts. This would predict that miRNAs are also transcriptionally regulated in different cell types such that there is extensive cross-talk between transcription and posttranscriptional regulation and that distinct miRNAs are active in particular

lineages. Indeed, loss of mature miRNAs in Dicer1 null mES results in their failure to differentiate into the three germ layers (Kanellopoulou et al., 2005). It will now be important to define which miRNAs are important for different lineages. In the hematopoietic system, ectopic expression of miR-181, which is highly expressed in thymus and not in most other tissues, increases the fraction of B-lineage cells both in vitro and in vivo (Chen et al., 2004), although it is not yet known whether this is due to the commitment of hematopoietic stem cells or survival of the B cell lineage. miRNAs also likely play important roles in maintaining mature cell function, as has been described in fat and insulin metabolism (He and Hannon, 2004). miRNAs are especially abundant in the adult brain, suggesting a key role for them in neuronal function and plasticity (He and Hannon, 2004).

#### Conclusion

A new integrated global regulatory network is currently emerging based on the dynamic interplay of chromatin remodeling components, transcription factors, and miRNAs. These three mechanisms synergize to choreograph stem cell self-renewal and the generation of cell diversity. Feedback loops between miRNAs and transcription factors reinforce cell fate decisions (Hobert, 2004), allowing for rapid transitions between the quiescent state and rapidly dividing cell. The coordinate regulation of clusters of genes that are stem cell, progenitor, or differentiated cell-specific likely relies on chromatin remodeling for initial expression and miRNAs for refinement. Defining the functional repertoires of miRNAs and their targets and integrating them into the transcription networks and global chromatin state are essential to understanding stem cell biology.

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