

Cell cycle micromanagement in embryonic stem cells

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Embryonic stem (ES) cells undergo rapid cell division without compromising their ability to differentiate into virtually all cell types. Using ES cells deficient for a microRNA biogenesis factor, *Dgcr8*, a new report uncovers the importance of specific microRNAs in the ES cell cycle transition from G1 to S phase.

Embryonic stem (ES) cells are derived from the inner cell mass of an early-stage embryo known as a blastocyst. ES cells can differentiate into all cell types of the adult body and, in the absence of stimuli, can infinitely divide without losing the capacity of self-renewal. Because of these capacities for differentiation and self-renewal, ES cells have been not only a potent tool for embryology but also a promising material for regenerative medicine. ES cells proliferate more rapidly than other cell types, as a result of the shortened G1 phase that lies between the cell division (M) and DNA synthesis (S) phases of the cell cycle. Because the transition from G1 to S phase is the major checkpoint during the cell cycle, ES cells need to be equipped with effective machinery in order to override the checkpoint. The involvement of microRNAs (miRNAs) in this process has been suggested, and a new study on page 1478 of this issue identifies microRNAs that have a role in the rapid transition from G1 to S phase in ES cells¹ (Fig. 1).

miRNAs under suspicion

miRNAs are regulatory RNAs of ~22 nucleotides in length that suppress their target genes through base-pairing to mRNAs. The miRNA sequence in the 2- to 7-nucleotide position relative to the 5' end of the miRNA (called the 'seed sequence') is critical for the specific base-pairing with the target mRNA. Biogenesis of miRNAs requires the RNase III-type enzymes Droscha and Dicer². Droscha, in a complex with its cofactor DGCR8, cleaves

out the hairpin embedded in the long primary miRNA transcript (pri-miRNA). The released small hairpin (pre-miRNA) is then cleaved by Dicer, generating mature miRNA. Mice deficient for *Dicer* or *Dgcr8* die during embryogenesis, indicating the critical roles of miRNAs in embryonic development. The loss of Dicer in mouse ES cells results in reduced proliferation and defective differentiation^{3,4}. Expression of Oct4, a hallmark of pluripotent stem cells, is partially reduced, and expression of endodermal and mesodermal markers is not detectable in differentiation conditions. The loss of *Dgcr8* causes similar defects in proliferation and differentiation of ES cells, albeit less severe compared to those of *Dicer* knockouts⁵. These miRNA-deficient ES cells show an accumulation of cells in the G1 phase, suggesting that miRNAs normally facilitate G1-S transition.

Uncovering a role in cell cycle control

Reporting in this issue, Blelloch and colleagues took advantage of the *Dgcr8* knockout ES cells to uncover the identity of the miRNAs responsible for the rapid proliferation of ES cells¹. To screen for miRNAs required for normal G1-S transition, a library of 266 known mouse miRNAs were chemically synthesized as forms of siRNA duplexes, and transfected individually into ES cells grown in 96-well plates. The screen revealed that most of the top-ranking miRNAs that rescued the G1 accumulation phenotype of *Dgcr8* null ES cells shared similar seed sequences. Notably, the list includes miRNAs that are known to be abundant in mouse ES cells and reduced in differentiated cells⁶: miR-291a-3p, miR-291b-3p, miR-294, miR-295, miR-302b, miR-302c and miR-302d. The homologs of these miRNAs are also specifically expressed in undifferentiated human ES cells⁷. The screen

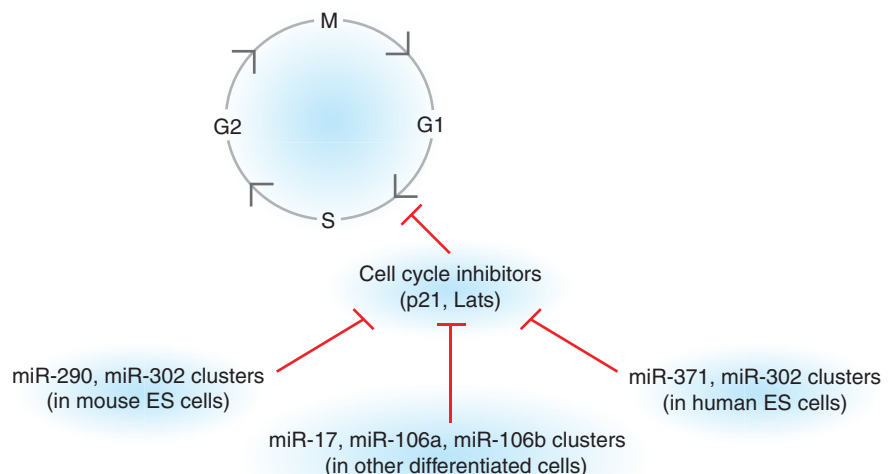


Figure 1 MicroRNAs regulate cell proliferation in ES cells. ES cells rapidly proliferate as a result of a shortened G1 phase in the cell cycle. ES cell-specific miRNAs such as miR-290 and miR-302 cluster miRNAs in mice (miR-371 and miR-302 clusters in humans) suppress multiple genes that inhibit the G1-S phase transition. Tumor cells also express the homologs or paralogs of miR-290 family miRNAs, resulting in enhanced cell proliferation.

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also identified several miRNAs that are not highly expressed in ES cells: miR-19a, miR-20a, miR-20b, miR-93 and miR-106a. Because these miRNAs share similar seed sequences with miR-290 and miR-302 cluster miRNAs, they would be expected to regulate an overlapping set of target genes and facilitate cell proliferation. However, as their expression patterns are different spatially and temporally, their biological functions are likely to be distinct.

ES cell-specific miRNAs containing an 'AAGUGC' motif regulate multiple genes that inhibit the G1-S transition¹. The gene encoding Cdkn1a (p21), the inhibitor of Cdk2, contains at least two binding sites for these miRNAs at the 3' UTR. The gene encoding tumor suppressor Lats2 also harbors several binding sites for ES cell-specific miRNAs. A shortened G1 phase promoted by miRNAs would be critical for normal embryogenesis, allowing the early embryo to proliferate before differentiation. Notably, it was previously shown in *Drosophila* that the miRNA pathway is important for division of germline stem cells, and that the homolog of p21 (called Dacapo) may be the critical miRNA target⁸.

Additional roles?

Recent studies have shown additional roles for miR-290 cluster miRNAs in DNA methylation. Dicer deficiency leads to reduced DNA

methylation, concomitant with increased telomere elongation and telomere recombination⁹. This is because miR-290 family miRNAs normally suppress retinoblastoma-like 2 (Rb12), which is a transcriptional repressor of *de novo* DNA methylation enzymes. DNA hypomethylation not only affects telomeres but also interferes with epigenetic silencing of protein-coding genes, such as the stem cell marker Oct4 (ref. 10). Failure of complete silencing of Oct4 in differentiation conditions explains (at least in part) why miRNA-deficient ES cells are defective in differentiation. Although Blelloch *et al.* did not find a rescuing effect of the ES cell-specific miRNAs on stem cell differentiation, this may be because differentiation requires the combination of multiple miRNAs. For instance, miR-1 and miR-133 are critical for muscle cell differentiation by suppressing non-muscle-lineage gene expression. Further studies will identify the individual miRNAs necessary for differentiation into specific cell lineages.

It is of note that the key ES cell transcription factors (Oct4, Sox2, Nanog and Tcf3) are associated with the promoters of ES cell-specific miRNAs¹¹. Therefore, miRNAs and transcription factors constitute a positive feedback loop, reinforcing the gene regulation network in undifferentiated stem cells.

The tumor connection

Expression of ES cell-specific miRNAs is highest in the blastocyst. Abnormal expression of these miRNAs would lead to inappropriate growth of differentiated cells. Indeed, miR-372 and miR-373, the human homologs of miR-290 cluster miRNAs, are highly expressed in testicular germ cell tumors, and induce cell transformation *in vitro*¹². Additionally, the miR-17 cluster is associated with B-cell lymphoma in humans, and ectopic expression of the miR-17 cluster along with c-Myc in mice causes lymphoma. Thus, understanding the role of miRNAs in the cell cycle control of ES cells has implications for both embryology and cancer biology.

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Phenotypic variations on the theme of CNVs

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Copy number variation has emerged as an important type of genetic risk factor for developmental disorders, including the neurodevelopmental disorders schizophrenia, autism and mental retardation. The highly pleiotropic effects observed for specific copy number variants (CNVs) challenge current classification of these disorders, but also provide opportunities to understand their origins and the relationships between them.

Insertions and deletions, collectively referred to as copy number variants (CNVs), have recently been recognized as an important source of human DNA polymorphism^{1–3}. It is not surprising then that the opportunities offered by new technologies to scan the genome for CNVs at increasing resolution have been seized with enthusiasm by researchers in many disciplines. The earliest studies were phenotype-led and applied genome-wide CNV analyses to cases

with a specified phenotype and a set of controls. Two recent papers, one on page 1466 of this issue by Nicola Brunetti-Pierri and colleagues⁴ and the other from Evan Eichler and colleagues in *The New England Journal of Medicine*⁵, have opted for a CNV-led approach with the aim of characterizing the associated phenotypes.

Developmental phenotypes at 1q21.1

The two studies both focus on 1q21.1, a region where CNVs had previously been shown to be associated with phenotypes including dysmorphic features, cardiac abnormalities, learning difficulties and mental retardation. The study of Brunetti-Pierri *et al.*⁴ was based upon 16,577 subjects referred for genetic testing on whom

array-based comparative genomic hybridization had been done. Although CNVs at this locus are rare, the exceptionally large sample allowed 27 subjects with 1q21.1 deletions (0.16%) and 17 with duplications (0.1%) to be identified. Deletions in particular were substantially more common than the rate of 0.02% observed in large population samples^{6,7}, albeit those had used a different CNV detection platform. The most striking finding with respect to phenotype was that deletion and duplication were associated with micro- and macrocephaly, respectively, although additional phenotypes including learning difficulties, mental retardation, autism, attention-deficit hyperactivity disorder and hallucinations were also observed. The other study⁵

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