STEM CELLS

Mobile elements control stem cell potency

A microRNA suppresses transposons and bars pluripotent stem cells from totipotency

By **Hidetoshi Hasuwa** *and* **Haruhiko Siomi**

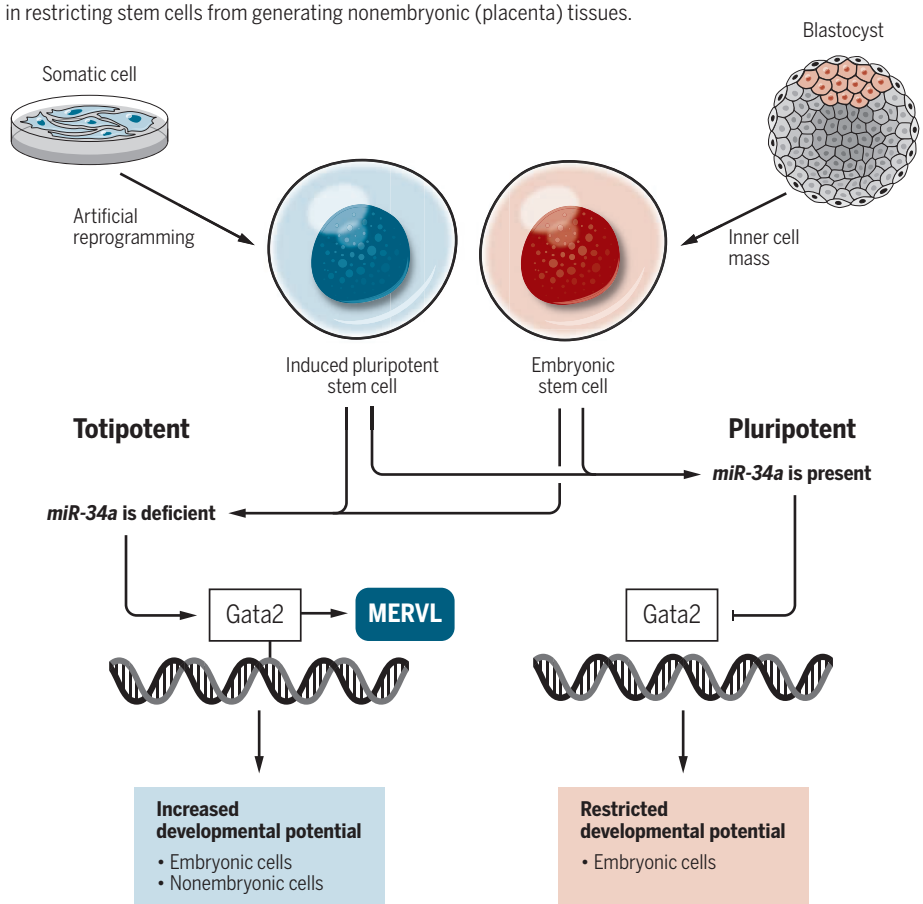
Embryonic stem cells (ESCs) are of great interest for tissue replacement therapies and for the study of mammalian development. Cultured ESCs as well as induced pluripotent stem cells (iPSCs), which are generated from somatic cells, can self-renew indefinitely and give rise to all cell types of the body including the germline. However, such stem cells are not totipotent because they are inefficient at generating extraembryonic tissues, such as the placenta (*1*). What underlies this developmental restriction? On page 596 of this issue, Choi *et al.* (*2*) report a mechanism that prevents totipotency. The barrier involves mobile genetic elements embedded throughout the genome and a small noncoding microRNA (miRNA).

Transposable elements are DNA insertions in the host genome that can rewire gene expression networks and drive genome evolution and speciation (*3, 4*). This is achieved because transposable element sequences carry potential regulatory information such as transcription factor–binding sites, and also because these mobile elements are targets for epigenetic modifications that can spread to flanking regions and influence the expression of nearby genes. Recently, a rare transient cell population (~2%) within mouse ESC and iPSC cultures was shown to express high amounts of a transposable element known as a murine endogenous retrovirus with leucine transfer RNA primer (MERVL) (*5*). These stem cells also expressed transcripts normally restricted to totipotent blastomeres of the two-cell (2C) embryo, and lacked three pluripotency factors: octamer-binding transcription factor 4 (Oct4), sex determining region Y-box 2 (Sox2), and Tir Na Nog (Nanog). In 2C-stage mouse embryos, more than 25% of the nearly 700 MERVL copies are transcribed, representing 3% of the total transcriptional output. Moreover, 307 protein-coding genes generate 626 chimeric transcripts containing MERVL sequences, suggesting that transcription of many genes in 2C-stage embryos is initiated by MERVL elements. Remarkably, this rare subpopulation of ESCs and iPSCs had the totipotent potential to produce both extraembryonic and embryonic cell lineages (*5*). This highlights a critical role for mobile elements in cell fate regulation by altering the profile of transcribed genes, and also indicates the existence of a molecular barrier that blocks the activity of MERVL and prevents pluripotent ESCs and iPSCs from entering a totipotent state.

The efficiency of iPSC generation from somatic cells is decreased if a miRNA called *miR-34a* is expressed, suggesting that *miR-34a* antagonizes reprogramming (*6*). miRNAs are small noncoding RNAs that associate with complementary sites on messenger RNAs (mRNAs) to repress their translation and promote their decay (*7*). These small noncoding RNAs contribute to cell fate decisions by controlling regulatory circuits that promote positive or negative feedback of gene expression (*8*). Choi *et al.* observed that *miR-34a* deficiency induces MERVL expression in cultured mouse ESCs (derived from the inner cell mass of the blastocyst embryo stage) and iPSCs. The authors found that teratomas generated from *miR-34a*–deficient ESCs and iPSCs not only contain derivatives of embryonic cell lineages but also display features of extraembryonic trophoblast-like cells of the placenta (see the figure). To corroborate these findings during early embryogenesis, Choi *et al.* microinjected *miR-34a*–deficient ESCs [labeled with green fluorescent protein (GFP)] into recipient morula-stage mouse embryos (just prior to the blastocyst stage) and generated chimeric blastocysts. By tracing GFP-positive

Developmental potential

A microRNA (*miR-34a*) and embedded mobile genetic elements (MERVL) play a role in restricting stem cells from generating nonembryonic (placenta) tissues.



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ESC progeny cells, the authors observed that more than 30% of injected *miR-34a*–deficient ESCs contributed to the inner cell mass, which gives rise to embryonic cell lineages, and to the trophectoderm, which develops into extraembryonic cell lineages. Thus, *miR-34a*–deficient ESCs had totipotent cell fate potential in vivo. Moreover, injected *miR-34a*–deficient ESCs contributed to multiple, fully differentiated cell lineages in the mouse embryo, yolk sac, and placenta at mid-gestation, demonstrating expanded cell fate potential that is functionally and molecularly distinct from wild-type pluripotent ESCs.

As is the case for 2C blastomeres (*5*), Choi *et al*. observed that induction of transposable element expression in *miR-34a*–deficient ESCs and iPSCs was largely specific to MERVL elements, and that among the most differentially expressed genes between *miR-34a*–deficient and wild-type iPSCs were those proximal to MERVL insertion sites, which generate chimeric transcripts containing MERVL sequences. Overexpression of *miR-34a* in *miR-34a*–deficient iPSCs decreased expression of these chimeric transcripts. This raises the question of whether this miRNA affects cell fate potential by directly or indirectly regulating the activity of MERVL.

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Choi *et al*. delineated a minimal fragment in the MERVL sequence required for its expression and found that it contained no substantial sequence complementary to *miR-34a*. This suggests a model in which transcription of MERVL is by transcription factors or chromatin modification factors that are repressed by *miR-34a*. Bioinformatic prediction identified binding sites for 70 discriminatory transcription factors within the MERVL minimal fragment. Among these factors, only GATA-binding protein 2 (Gata2) exhibits an expression pattern similar to that of MERVL during early preimplantation (2C-stage embryo) development. Mutations in the putative Gata2 binding site within the minimal fragment reduced its activity in *miR-34a*–deficient ESCs. Consistent with this, decreasing *Gata2* expression in *miR-34a*–deficient ESCs abolished the expression of MERVL and MERVL-proximal genes. Thus, Gata2 directly promotes expression of MERVL elements and their proximal genes in *miR-34a*–deficient ESCs. However, Choi *et al*. found no involvement of epigenetic modifications in this process, which is at odds with an earlier report of a histone demethylase as a negative regulator of MERVL in 2C blastomeres (*9*). The *Gata2* transcript harbors potential *miR-34a* binding sites, and Choi *et al*. observed that *Gata2* mRNA amounts increased in *miR-34a*–deficient iPSCs and decreased when *miR-34a* was overexpressed. Furthermore, decrease in *Gata2* in *miR-34a*–deficient ESCs abolished differentiation into both embryonic and extraembryonic cell lineages. Thus, *miR-34a* prevents pluripotent ESCs and iPSCs from entering a totipotent state by repressing Gata2, which otherwise activates the expression of MERVL.

It is not yet known how MERVL affects the process that determines pluripotent and totipotent cell states. It may be that proteins encoded by these elements, which include reverse transcriptase, and fusion proteins between MERVL and proximal genes are the key. Approximately 700 copies of MERVL exist in the mouse genome; therefore, it is one of the most abundant protein-coding genes in the genome. It was recently shown that a minor population of "naïve-like" cells exists among human ESCs that express human endogenous retrovirus type-H (HERV-H) transcripts (*10*). These naïve cells are capable only of embryonic cell fates. In contrast to mouse ESCs, human ESCs normally adopt a "primed" pluripotent state, meaning that they can develop embryonic cell fates but not germline cells. The expression of HERV-H appears to define naïve ESCs; however, it is not known how the HERV-H expression changes between naïve and primed cell states. Nonetheless, the findings of Choi *et al*. suggest that transposable elements have been co-opted to give rise to totipotent cells for early host development. ■

REFERENCES

1. K. Takahashi, S. Yamanaka, *Development* **142**, 3274 (2015).
2. Y. J. Choi, C.-P. Lin, D. Risso, S. Chen, T. A. Kim, M. H. Tan, J. B. Li, Y. Wu, C. Chen, Z. Xuan, T. Macfarlan, W. Peng, K. C. Kent Lloyd, S. Y. Kim, T. P. Speed, L. He, *Science* **355**, eaag1927 (2017).
3. N. V. Fedoroff, *Science* **338**, 758 (2012).
4. S. Schlesinger, S. P. Goff, *Mol. Cell Biol.* **35**, 770 (2015).
5. T. S. Macfarlan, W. D. Gifford, S. Driscoll, K. Lettieri, H. M. Rowe, D. Bonanomi, A. Firth, O. Singer, D. Trono, S. L. Pfaff, *Nature* **487**, 57 (2012).
6. Y. J. Choi *et al*., *Nature Cell Biol.* **13**, 1353 (2011).
7. H. Siomi, M. C. Siomi, *Mol. Cell* **38**, 323 (2010).
8. A. Mehta, D. Baltimore, *Nat. Rev. Immunol.* **16**, 279 (2016).
9. T. S. Macfarlan *et al*., *Genes Dev.* **25**, 594 (2011).
10. J. Wang, G. Xie, M. Singh, A. T. Ghanbarian, T. Raskó, A. Szvetnik, H. Cai, D. Besser, A. Prigione, N. V. Fuchs, G. G. Schumann, W. Chen, M. C. Lorincz, Z. Ivics, L. D. Hurst, Z. Izsvák, *Nature* **516**, 405 (2014).

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BIOPHYSICS

Flipping nanoscopy on its head

Fluorescent imaging with minimal photon flux can achieve single-nanometer resolution

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About the smallest object we can see with the naked eye is our own hair. With a magnifying glass, we can see about 10 times better, and light microscopy, until relatively recently, could resolve features about 300 times thinner than human hair (~250 nm). Recent developments in fluorescence "nanoscopy" made it possible to routinely image cellular structures at 20- to 30-nm resolution (*1*), but a gap remained at the molecular scale: Most proteins are smaller than 5 nm across. On page 606 of this issue, Balzarotti *et al*. (*2*) report a new concept in nanoscopy, termed MINFLUX, that achieves the true molecular resolution (2 to 3 nm) and dramatically reduces the number of photons required by "flipping" a common wisdom in nanoscopy on its head.

In traditional optical imaging, even a very tiny object such as a single fluorophore (<1 nm) becomes blurred because light diffraction makes it appear much larger—about half the wavelength of the light used. Nonetheless, the center of the imaged fluorescence spot can be determined with extremely high precision (down to 1.5 nm if 10,000 photons are used) (*3*). The Abbe diffraction limit can be overcome by determining the position of, or localizing, one molecule at a time, with single fluorescent molecules that can be switched on or off stochastically (*4*, *5*). This localization-based strategy, together with other nanoscopy approaches (*6*, *7*), ushered in the "resolution revolution" that enabled breakthrough biological discoveries in the past decade (*1*).

A common wisdom in nanoscopy is to localize a molecule near where the signal is the strongest, which requires high-emission photon flux that is often limited by the emission rate of the fluorophore. In MINFLUX, Balzarotti *et al*. devised the opposite strategy, in which they localize a molecule near