When Myc's asleep, embryonic stem cells are dormant

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Myc is one of the original reprogramming factors used to produce induced pluripotent stem cells. However, it is not necessary, instead its main role is to increase the efficiency of the reprogramming. The article by Scognamiglio *et al* (2016) helps clarify how. The authors show that Myc depletion leads to a reversible dormant state consistent with diapause. In this state, the cell sees its proliferation potential diminished but its pluripotency unchanged. The ability to coordinate the induction of this state should have important implications in cell differentiation.

See also: R Scognamiglio *et al* (February 2016)

Pluripotency can be divided into two states: naïve and primed. These two states represent different stages in development and are distinguishable in mice, with the naïve state reflecting cells from the inner cell mass, while those in the primed state reflecting cells post-implantation (Nichols & Smith, 2009). These different states describe different developmental potential, as naïve state pluripotent stem cells can be used to create chimeras, whereas primed state pluripotent stem cells cannot even though they can generate the three germ layers. Treatment with MEK and GSK3 inhibitors was shown to establish mouse embryonic stem cells (mESCs) and therefore the naïve ground state (Ying *et al*, 2008). Yet in humans, the naïve state has been much more challenging to acquire and even when it is, it is unstable (Takashima *et al*, 2014; Wang *et al*, 2016).

A number of factors are thought crucial for acquiring the naïve state, including Myc.

Myc is one of the four original reprogramming factors used to generate induced pluripotent stem cells (iPSCs) (Takahashi &

Yamanaka, 2006). Although iPSCs can be generated in its absence, the efficiency of the reprogramming is extremely low. The

Figure 1. A reduction in Myc expression levels shifts the embryo to the diapause state.

This state can be escaped, however, by increasing the Myc expression. 2i-treated naïve ground state mESCs have a similar signature as pluripotent embryonic cells and show the same behavior.

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new study by Scognamiglio *et al* (2016) provides important new insights that help explain why, as they show that deletion or inhibition of the Myc gene arrests proliferation without affecting pluripotency.

The authors found that naïve mESCs treated in 2i express low levels of two major Myc genes, c-Myc and N-Myc. To further investigate the Myc function, both Myc genes were deleted using the flox system. These double KO (dKO) mESCs ceased to proliferate and underwent apoptosis, yet maintained their pluripotency, suggesting that Myc expression levels regulate proliferation but not pluripotency maintenance. To confirm this conclusion, the authors next compared the transcriptome of the dKO mESCs, finding these cells had profiles that were consistent with those of diapause epiblasts. More specifically, the dKO mESCs showed a reduction in protein and nucleic acid synthesis while at the same time showing an increase in processes that promote their maintenance and survival; in other words, the cells maintained pluripotency, but had diminished differentiation propensity. Diapause describes a dormant state in which hormones signal the cell to arrest development in response to environmental factors (Renfree, 2015). It is especially beneficial in extreme environments or other conditions that indicate a strong competition for resources. Exogenously expressing Myc RNA, however, reversed the diapause state in the dKO cells, thus restoring protein synthesis and DNA replication. Similar findings of the transcriptome and the reversibility were found when using wild-type cells exposed to a MYC inhibitor that was later removed. Because diapause depends on hormone signaling, it will be interesting to investigate whether any specific hormones

can be used to regulate Myc expression and thus reprogramming.

Along with our scientific understanding of pluripotency, these findings should also have important technical implications. To maintain human ESCs/iPSCs, researchers have to passage the cells repeatedly. Each passage, however, puts the cells at risk of developing genetic mutations in response to cell divisions or other stresses. Incorporating into the passaging protocol, a way to maintain a dormant state via the suppression of Myc expression could eliminate this risk. Further, should the dormant state occur at the same time point of the cell cycle, it should be easier to standardize the efficiency of cell differentiation for later use.

While this study reports the function of c-Myc and N-Myc, it does not give consideration to the third major Myc gene, L-Myc. Like c-Myc, L-Myc is an important factor for iPSC generation especially for human iPSCs, as the efficiency of human iPSC generation is higher with L-Myc than with c-Myc and reduces the risk of tumorigenesis (Nakagawa *et al*, 2010, 2014). Considering that cell therapies based on iPSCs are shifting toward protocols that exclude c-Myc, future work that considers the expression of L-Myc on diapause could be of particular value for the study of human pluripotency.

Conflict of interest

S.Y. is a scientific advisor of iPS Academia Japan without salary.

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