Reprogramming by Cell Fusion: Boosted by Tets

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Pluripotent cells, when fused with somatic cells, have the dominant ability to reprogram the somatic genome. Work by Piccolo et al. (2013) shows that the Tet1 and Tet2 hydroxylases are important for DNA methylation reprogramming of pluripotency genes and parental imprints.

Experimental reprogramming has captured the imagination of biologists and medical practitioners alike because of the inherent fascination and scientific interest with turning one cell type into another and the implications this has for understanding disease processes and developing new ideas for therapy. Cloning by somatic cell nuclear transfer, induced pluripotency, and fusion of somatic cells with embryonic stem cells (ESCs) or embryonic germ cells (EGCs) can reprogram specialized cells (or their nuclei) to pluripotent ones that can potentially regenerate all of the differentiated cell types in an adult organism (Yamanaka and Blau, 2010). Although these techniques work (which sometimes still feels like a miracle), they are inefficient (typically one in a thousand to one in a hundred attempts succeed), and reprogramming is often incomplete. A number of bottlenecks to successful reprogramming have been identified, including some that are epigenetic, which appear to be critical. Hence, the epigenome of somatic cells needs to be reprogrammed into that of pluripotent cells (which is very different). For example, the promoters of pluripotency transcription factor genes such as *Oct4* or *Nanog* are DNA methylated in somatic cells and need to be demethylated during reprogramming. Insights into naturally occurring epigenetic reprogramming in primordial germ cells (PGCs), early embryos, and ESCs have indeed informed and resulted in improvements of experimental reprogramming. In a fascinating study by Piccolo et al. (2013), this thinking has now been applied to cell fusion reprogramming using ESCs and EGCs.

EGCs, derived from PGCs, are pluripotent and similar to ESCs in most respects; however, many EGC lines possess erased DNA methylation in imprinting control regions (ICRs) when they are derived from gonadal PGCs, which have undergone genome-wide demethylation (including in ICRs). Interestingly, these cells—when fused with somatic cells to form heterokaryons—can reprogram the somatic nuclei to a pluripotent state and erase methylation in the *Oct4* promoter and ICRs (Tada et al., 1997, Piccolo et al., 2013). ESCs, by contrast, dominantly reprogram somatic cell nuclei in fusions, but ICRs maintain their methylation, just as they do in preimplantation embryos and ESCs (Piccolo et al., 2013). The fused cells are tetraploid and divide as such (making them unsuitable for therapy approaches); however, they provide a convenient cell system for the study of epigenetic reprogramming, especially as the somatic genome can be genetically distinct from the stem cell genome.

Piccolo et al. (2013) observed demethylation of the *H19*, *Peg3*, and *Gtl2* ICRs in EGC, but not ESC, fusions; this is a protracted process that occurs over several rounds of cell division (Figure 1). This might therefore be compatible with passive (replication-linked) demethylation. *Oct4* demethylation occurred more quickly (48–72 hr after fusion), yet even here cells apparently undergo DNA replication. *Oct4* demethylation also took place in ESC fusions, which has previously been observed and attributed to the activation-induced cytidine deaminase (AID) (Bhutani et al., 2010). *Line1* retrotransposons were more demethylated in EGC than ESC fusions, but whether true genome-wide demethylation is induced in EGC fusions is not known. Since the hydroxylases Tet1 and Tet2 are highly expressed in ESCs and EGCs (and have a role in demethylation and pluripotency regulation in ESCs), the authors considered the possibility that hydroxylation was involved in demethylation. Indeed, they observed a rapid (48–72 hr) increase in 5-hydroxymethylcytosine (5hmC) in all target sequences, indicating that demethylation may be induced, at least in part, by conversion of 5mC to 5hmC.

In support of this conclusion, Piccolo et al. (2013) found that knockdown of Tet2 prior to EGC or ESC fusion reduced hydroxylation of the *Oct4* promoter and delayed its demethylation. This was associated with impaired induction of pluripotency, which may be linked with incomplete reprogramming of *Oct4* or of other pluripotency related transcription factors. Knockdown of Tet1 in EGC fusions, by contrast, diminished hydroxylation in ICRs but did not affect their demethylation (which, in any event, occurs more slowly than in *Oct4*). Hence, demethylation may occur in part by hydroxylation followed by passive dilution or further conversion to 5fC and 5caC. In parallel, it may also occur by passive dilution of 5mC. Differences in the kinetics of demethylation (faster in *Oct4* compared to ICRs, even though both are rapidly hydroxymethylated) could indicate differences in further metabolism of 5hmC. The discrepancy between the rapid accumulation of 5hmC at the ICRs and their delayed demethylation also potentially challenges the model of passive loss of hydroxymethylation at replication, suggesting that DNA methylation maintenance at ICRs

may be more dynamic than we think. A major unresolved question is how EGCs can reprogram ICRs while ESCs cannot, despite the same expression of Tets and all other relevant modifiers of DNA methylation. Perhaps the subtlety lies in factors that target Tets to their locations in the genome or, conversely, in factors that protect from demethylation such as Stella or Zfp57.

These new findings have interesting implications for both experimental and natural reprogramming. First, the Tet1 and Tet2 hydroxylases are also important for induced pluripotent stem cell (iPSC) reprogramming, in part because of how they may be targeted to the genome (Costa et al., 2013). Second, *Tet1* knockout mice do not appear to have problems with demethylating ICRs in their PGCs (while *Tet1*/*Tet2* double knockouts have a partially penetrant ICR erasure defect), and *Tet2* knockouts develop normally to adulthood (hence, without any apparent pluripotency defect). Nor does combined *Tet1*/*Tet2* deficiency abolish genome-wide erasure of methylation in PGCs, which seems to occur largely by a passive mechanism (Yamaguchi et al., 2012, Seisenberger et al., 2012, Hackett et al., 2013, Dawlaty et al., 2013, Kagiwada et al., 2013). Hence, demethylation, which is important in order to gain pluripotency and remove imprints, is regulated by a finely interleaved and at least partially redundant system of active and replication-coupled mechanisms. These new insights will allow researchers in this exciting field to apply demethylation strategies with increased subtlety and control and in different biological, and hopefully eventually medical, contexts.

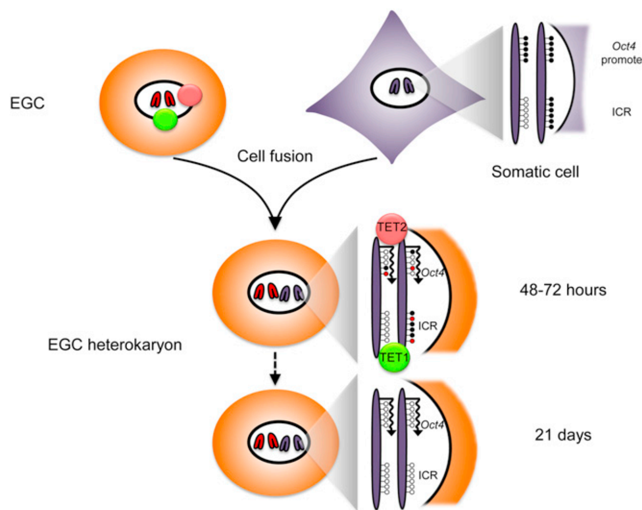

Figure 1. Involvement of Tet1 and Tet2 in Efficient Epigenetic Reprogramming in Heterokaryons
Fusion of EGCs with human B cells (somatic cells) leads to efficient reprogramming of the B cell genome to potential pluripotency with rapid hydroxylation and subsequent demethylation of the *Oct4* promoter by Tet2 and its transcription. Imprinting control regions (ICRs) also become hydroxylated, which requires Tet1, but their demethylation in the heterokaryons takes longer.

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