homeostasis, and therefore it seems appropriate that during states of chronic inflammation, cells may forgo their canonical functions to prioritize pathogen removal. What remains puzzling is the requirement for specialized stem cells to orchestrate immune recruitment, especially given the presence of supportive stromal populations.

Collectively, the work by Chen et al. identify a novel role for stem cell-mediated immune defense during chronic inflammatory states, at the expense of OSN replacement (Figure 1). Future work should seek to clarify the cross-talk between immune infiltrates and the adopted stem-cell phenotype, and whether this paradigm is conserved in additional neurogenic niches, as targeting of stem cell-derived chemokines could prove an attractive therapeutic target to limit immune-associated deficits in adult neurogenesis.

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Collisions on the Busy DNA Highway Set Up Barriers for Reprogramming

Xiao Hu[1,2] and Shangqin Guo[1,2,*]
[1]Department of Cell Biology, Yale University, New Haven, CT 06520, USA
[2]Yale Stem Cell Center, Yale University, New Haven, CT 06520, USA
*Correspondence: shangqin.guo@yale.edu
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Why most cells remain refractory to transcription factor (TF)-induced fate conversion remains largely mysterious, with the answers holding important instructions on how to effectively direct cell identities. In this issue of *Cell Stem Cell*, Babos et al. (2019) show that conflicts caused by simultaneous high transcription and high replication rates are to blame.

While rapid proliferation is well known to promote pluripotency induction by the Yamanaka factors (Guo et al., 2014), the involvement of active proliferation in other cell fate conversions is less clear, especially when the final cell types are not proliferative. In this issue of *Cell Stem Cell*, Babos et al. examined this question during mouse embryonic fibroblast (MEF) reprogramming into induced motor neurons (iMN) following the expression of six factors: Ascl1, Brn2, Myt1l, Ngn2, Isl1, and Lhx3 (6F). The bulk reprogramming culture displayed markedly reduced proliferation. The presence of the slow/non-dividing cells was accompanied by prevalent mitotic defects, such as micronuclei and chromatin bridges, indications of failed cellular attempts at proliferation. These results could easily be interpreted as proliferation being not beneficial for iMN conversion, as they had been previously (Son et al., 2011). However, live cell imaging and tracking revealed that the converting cells indeed underwent divisions, with some of the cell cycles appearing to last for only 14 h (refer to Figure S1A in Babos et al., 2019), a cell-cycle rate that is strikingly similar to the rare fibroblasts initiating pluripotency (Smith et al., 2010). The significance of the few proliferative cells became clear when a combination of RepSox (a TGFβ inhibitor), hRasG12V (a Ras mutant), and p53DD (a p53 mutant lacking a DNA-binding domain) (DDRR) promoted iMN reprogramming by 100-fold. Significantly, in the presence of DDRR, not only had many more cells undergone reprogramming, but reprogramming had also initiated mostly from the highly proliferative cells. DDRR in effect reduced the prevalence of genomic stress and allowed more cells to become proliferative. These new findings by Babos et al. flipped the view on how decreased proliferation at the bulk level is interpreted, and they suggest that caution should be exercised

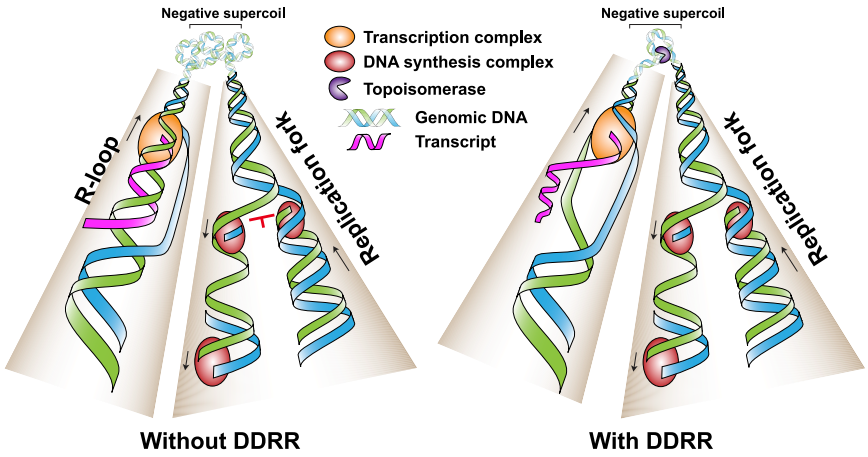

Figure 1. Schematic Model Illustrating the Genomic Conflicts Between the Transcription Machinery and the Replication Fork

Cells capable of hyper-transcription and hyper-proliferation convert to induced motor neurons highly efficiently. In most cells, transcription factor expression induces heightened transcription, which conflicts with the replication forks, yielding torsional stress on DNA. A chemical and genetic cocktail (DDRR) induces topoisomerases to help resolve the conflict and promotes highly efficient reprogramming.

when phenotypes of rare cells dominate the readout: cell number changes contributed by the meaningful few may not always be detectible without careful examination of the rare cells themselves.

How does 6F overexpression cause genomic stress early in iMN reprogramming, and how could DDRR help? 6F-expressing cells displayed heightened transcription, indicated by increased EU incorporation during pulse labeling. DDRR not only increased the proliferative cells, but among them, it increased the prevalence of EU-high cells to yield a population of hyper-transcribing and hyper-proliferating cells (HHCs). It is these HHCs that are responsible for most of the conversion into iMNs. As the transcription machinery could collide with the replication machinery when both systems are actively cruising along the same DNA region (García-Muse and Aguilera, 2016), it is plausible that the high demand for the DNA template could result in conflicts significant enough to have caused torsional stress on DNA. In support of this idea, the authors observed that two topoisomerases, *Top1* and *Top2a*, were among the genes upregulated by DDRR in the presence of 6F. Inhibiting *Top1* or *Top2a* reduced HHCs and iMN conversion even in the presence of DDRR. Conversely, *Top1* overexpression significantly promoted iMN conversion by 6F. As topoisomerases can release torsional stresses on DNA, these data support the notion that collisions on DNA are responsible for the failed iMN conversion from most MEFs. It is important to note that the high transcription state and high proliferation state were mostly defined in relative terms, i.e., cells with the highest transcription rate and highest DNA replication rate among a heterogeneous bulk population. More quantitative measures will be helpful to appreciate the critical rates of transcription and replication required to set off the genomic conflicts.

The conflicts between hyper-transcription and hyper-proliferation came in at least three flavors: negative DNA supercoiling, R-loops, and stalled replication forks. Expression of 6F alone renders many cells able to bear these conflicts, and DDRR reduced their prevalence. It is likely that the types of genomic stresses may not be limited to these three (Hamperl et al., 2017). More in-depth detection of these genomic stresses would await techniques with sufficient sensitivity and resolution for genomic conflicts in the same cells, around the same genomic regions or possibly even on the same DNA fibers. Nonetheless, the results strongly support a model where collisions between transcription and replication are responsible for many failed iMN conversion attempts. As a further support, DDRR addition also significantly promoted the cell fate conversion across multiple initiating cell types (mouse and human, adult and fetal) to multiple final cell types (induced neurons, induced dopaminergic neurons, and induced hair cells) by multiple TF cocktails. DDRR also promoted the functionality of the iMNs. The widespread increase in reprogramming supports the model's general validity.

The model proposed by Babos and colleagues provides a vivid depiction for the nature of a reprogramming barrier: collisions between two major systems traveling on the DNA highway (Figure 1). The importance of genomic conflict resolution illustrates why many of the highly proliferative cells still fail to reprogram. It remains to be determined whether a similar mechanism operates in cell fate reprogramming of lineages beyond those of the neuroectoderm, and whether and/or which additional enzymes could help clear the collisions (Schwab et al., 2015). The model will become more fully fleshed out when we understand where in the genome the collisions occur and whether certain constrains on the chromatin (e.g., binding to the nuclear lamina; van Steensel and Belmont, 2017) or heterochromatin condensates formation (Larson et al., 2017; Strom et al., 2017) make collisions more or less likely. Due to the oncogenic potential of hRasG12V and p53DD, the DDRR cocktail is unlikely to be directly applicable for cells intended for clinical use, but the insightful mechanistic revelation that has come with DDRR will undoubtedly spur further investigations that will eventually lead to better cell fate engineering.

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