STEM CELLS

Roadblocks to reprogramming, cleared

The removal or knockdown of an epigenetic repressor, *Mbd3*, results in near-complete reprogramming of mouse and human somatic cells to pluripotency.

In the years that have elapsed since Shinya Yamanaka first reported that somatic cells can be converted to a pluripotent state by the introduction of a few reprogramming factors, much has been learned about ways to enhance and modulate this process. Yet reprogramming to pluripotency remains, by most accounts, stochastic and inefficient. New work by Jacob Hanna and colleagues at the Weizmann Institute now changes all that.

Hanna's previous postdoctoral research on the kinetics of reprogramming to induced pluripotency had suggested that there could be a single rate-limiting factor responsible for the stochasticity that is typically observed. In his own lab, Hanna and his colleagues set out to identify what this factor might be.

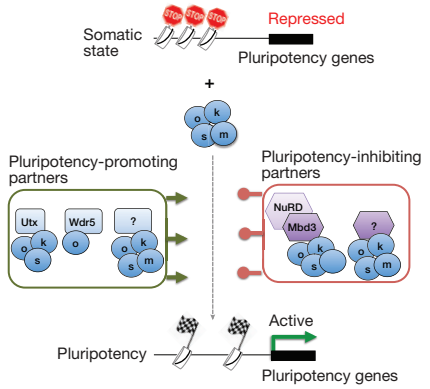
Prompted by the growing understanding in the field that pluripotent stem cells have relatively derepressed chromatin, the researchers designed a loss-of-function screen in which they knocked down candidate epigenetic repressors with short interfering RNA (siRNA) and asked whether one or more of these repressors is the roadblock to efficient reprogramming. Knockdown of *Mbd3*, a component of the NuRD repressor complex, had dramatic effects.

The researchers conducted their initial screen on mouse epiblast stem cells (EpiSCs), searching for factors that, upon siRNA knockdown, would result in more efficient conversion of the EpiSCs into embryonic stem cells in culture conditions that promote full pluripotency. (Under these conditions, EpiSCs spontaneously convert at low frequency to embryonic stem cells, which are capable of chimera formation.) Having identified Mbd3 as the only hit in this screen, and having then confirmed its role using genetic depletion, Hanna and colleagues examined the effects of Mbd3 on reprogramming somatic cells to pluripotency.

In the mouse, they observed that genetic depletion of Mbd3 combined with induction of the standard Yamanaka factors (Oct4, Sox2, Klf4 and Myc) and culture in 2i/LIF conditions (where 2i is ERK1/2 and GSK3β inhibitors, and LIF is leukemia inhibitory factor) yielded almost complete (nearly 100%) conversion of mouse embryonic fibroblasts to pluripotency within about 1 week. Furthermore, they used a 'secondary', doxycycline-inducible system to express the reprogramming factors in cells also expressing an Oct4-GFP reporter, which permitted the reprogramming process to be monitored at the single-cell level. Using either microscopy or flow cytometry, the researchers observed synchronous and near-complete activation of the Oct4 reporter upon reprogramming factor expression in Mbd3-depleted cells.

Notably, reintroduction of Mbd3 within the first 5 days of reprogramming, but not after that, inhibited reprogramming in the Mbd3-depleted cells. It therefore appears that although Mbd3 depletion removes a block to reprogramming, the protein is not required to maintain the pluripotent state. This is consistent, Hanna points out, with the expression pattern of *Mbd3 in vivo*: the gene is downregulated during very early mouse development and then comes back up at the late morula stage and is later ubiquitously expressed in somatic tissues.

The effect of Mbd3 depletion applies to reprogramming of human cells as well. The researchers generated biallelic *Mbd3* mutant human induced pluripotent stem cells (iPSCs) using a transcription activator–like effector nuclease, again in the context of a secondary, doxycycline-inducible system. As for the mouse, secondary fibroblasts derived from the *Mbd3* mutant cells could be reprogrammed to pluripotency with near-100% efficiency upon reprogramming factor induction, under optimized culture conditions.



The 'gas-and-brakes' model of reprogramming to induced pluripotency. Adapted from *Nature*.

It should be noted that in both mouse and human, Mbd3 depletion alone is insufficient for reprogramming: the reprogramming factors, several other positive regulators and optimized culture conditions are necessary.

Being able to rapidly, completely and synchronously convert somatic cells to iPSCs has several implications. First, it will be useful to be able to generate iPSCs more efficiently. Furthermore, Hanna maintains, conversion by this method will enable deeper insight into what is going on mechanistically during reprogramming. "Reprogramming cells with Mbd3 around is like driving a car with the handbrake on," he says. "I think you should get rid of it if you want to study the process." The researchers propose a 'gas-and-brakes' model, whereby reprogramming factors interact with both positive and negative epigenetic regulators that have opposing effects on the fascinating process of converting a somatic cell to a pluripotent one.

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RESEARCH PAPERS
Rais, Y. *et al.* Deterministic direct reprogramming of somatic cells to pluripotency. *Nature* **502**, 65–70 (2013).