ESSAY

REGENERATIVE MEDICINE

Back to basics

Refined nuclear reprogramming techniques yield higher-quality stem cells

by **Yosef Buganim**

The "hyperdynamic" chromatin state, which characterizes embryonic stem cell (ESC) epigenetic state, facilitates a rapid and efficient reaction to external and internal cues. These cues lead to the activation of key master regulators that drive the cells into their developmental fate. Thus, ESCs hold great promise as a source of diverse differentiated cell types for cell therapy and regenerative medicine.

Two major bottlenecks to realizing such potential are allogenic immune rejection of ESC-derived cells and ethical concerns related to the use of fetal tissues. In 2006, two Japanese scientists, Kazutoshi Takahashi and Shinya Yamanaka, made progress toward addressing both of these hurdles when they showed that introducing four transcription factors—Oct4, Sox2, Klf4, and Myc (OSKM)—can reprogram fibroblasts derived from adult mice into functional embryonic stem-like cells, known as induced pluripotent stem cells (iPSCs). (Resetting the epigenome of a somatic cell to a pluripotent state has already been achieved with somatic cell nuclear transfer, but this type of transformation is mediated by the actions of a large number of genes expressed within the host oocyte.)

The notion that as few as four factors are sufficient to reset the epigenome of a cell has inspired scientists to attempt to convert different adult cells into other somatic cell types by avoiding the pluripotent state, using a specific combination of key master regulators. Several subsets of cell types—including hematopoietic cells, various neuronal cells, cardiomyocytes, hepatocytes, embryonic Sertoli cells, endothelial cells, and retinal pigment epithelial cells—have been generated from somatic cells by using the direct conversion approach pioneered by Takahashi and Yamanaka.

Unfortunately, the majority of directly converted cells are not stable and represent mostly incomplete reprogramming states, and the vast majority of iPSCs exhibit poor potential for incorporation into all developing tissues in mice (*1*). This suggests that the current prevailing reprogramming methods are not ideal and must be improved before we consider using these cells in the clinic.

During my postdoctoral training in the laboratory of Rudolf Jaenisch at the Massachusetts Institute of Technology (MIT), I explored the reprogramming process at the single-cell level. I analyzed the expression of 48 genes in single mouse embryonic fibroblasts (MEFs) at various stages during the reprogramming process (*2*). Analysis of early stages revealed considerable variation in gene expression between cells, in contrast to late stages, which exhibited more homogeneous expression between reprogrammable cells. We found that cells that expressed *Esrrb*, *Utf1*, *Lin28*, and *Dppa2* were more likely to become iPSCs than were those that expressed previously suggested reprogramming markers such as *Fbxo15*, *Fgf4*, and *Oct4*. We also noticed that stochastic gene expression early in reprogramming is followed by a late deterministic phase, in which *Sox2* is the upstream factor in the gene expression network. Exploiting this network allowed us to reprogram MEFs to iPSCs in the absence of "Yamanaka" key pluripotency genes and Nanog, a transcription factor that had previously been considered crucial for reprogramming, demonstrating for the first time that the core pluripotency circuitry can be activated via different entries.

In a follow-up study, we reasoned that a combination of key factors derived from the later phase of reprogramming would convert cells in a more controlled way and therefore might uniformly yield iPSCs of high quality. We chose *Sall4*, *Esrrb*, and *Lin28*—key downstream players during the late reprogramming phase—and *Nanog* for our initial experiment. We observed that ectopic expression of *Sall4*, *Nanog*, *Esrrb*, and *Lin28* (SNEL) in MEFs generated iPSCs of superior quality to iPSCs derived from OSKM (*3*). SNEL-iPSCs contributed to high-grade chimeras and produced "all-iPSC" mice by means of 4n complementation at a significantly higher frequency than did OSKM-derived iPSCs. These results demonstrate that bioinformatic models derived from in vitro single-cell data can aid in improving the quality of iPSCs.

In January 2014, I became an independent faculty member at the Hebrew University of Jerusalem. My first goal was to determine whether a high degree of nuclear reprogramming can be achieved even in cells undergoing conversion to nonpluripotent cells. Indeed, in a paper that we published in *Cell Stem Cell* (*4*), we showed for the first time that a high nuclear resetting state can be attained in cells undergoing conversion to induced trophoblast stem cells (iTSCs) and that this complete nuclear reprogramming process is independent from the pluripotent state. The iTSCs resemble blastocyst-derived trophoblast stem cells in all examined parameters, including transcription, methylation, epigenome, and function. As such, we believe that these cells hold great promise for modeling and treating placental dysfunction diseases and for mitigating recurrent miscarriage.

Overall, the main goal of my laboratory is to identify and investigate the components that regulate cell plasticity and epigenome resetting. To that end, we study several con-

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Yosef Buganim received undergraduate degrees from Bar-Ilan University and a Ph.D. from the Weizmann Institute of Science. As a postdoctoral fellow at the Whitehead Institute for Biomedical Research at MIT, he used single-cell technologies and bioinformatic approaches to shed light on the molecular mechanisms that underlie the reprogramming of somatic cells to iPSCs. Currently the leader of his own laboratory at The Hebrew University of Jerusalem, Buganim uses somatic cell conversion models to identify and investigate the elements that facilitate safe and complete nuclear reprogramming.

version models in order to identify common and more global elements that facilitate nuclear reprogramming and improve the quality of converted cells. We investigate both nearly complete conversion models, such as conversion of fibroblasts into iPSCs and iTSCs, together with less stable, partial-reprogramming models, such as the conversion of somatic cells into induced embryonic Sertoli-like cells, induced neurons, and induced hepatocytes. To capture rare reprogrammable cells, we have established triple/quadruple fluorescent knock-in reporter systems, using the CRISPR/Cas9 technique, to mark the cells that are destined to become converted. We have applied cutting-edge single-cell technologies such as RNA-sequencing, Fluidigm-BioMark, and single-molecule-mRNA–fluorescence in situ hybridization to probe the transcriptome of multiple individual reprogrammable cells from the various conversion models. To understand how different key master regulators define a new epigenome and reshape the chromatin, we have performed chromatin immunoprecipitation–sequencing on various transcription factors and histone marks from different models. These approaches allow us to dissect the most global and fundamental events that facilitate complete nuclear reprogramming and will ultimately improve the quality of iPSCs and converted cells available for clinical use.

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10.1126/science.aag1215

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Yosef Buganim (June 16, 2016)

Science **352** (6292), 1401. [doi: 10.1126/science.aag1215]

Editor's Summary

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.