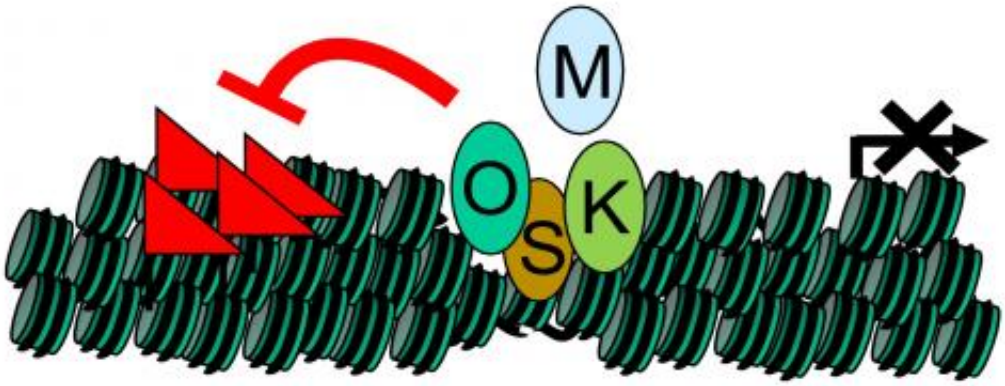
New study decodes molecular mechanisms underlying stem cell reprogramming

November 15 2012



At the beginning of cellular reprogramming, gene regulatory proteins Oct 4 (O), Sox2 (S) and Klf4 (K) enter the chromosomes at silent genes and allow c-Myc (M) binding. However, there are large chromosomal domains that contain particular epigenetic marks (red triangles) that prevent O, S, K, M from binding. Erasing the marks allows the regulatory proteins to bind and promotes the process of cellular reprogramming. Credit: Kenneth Zaret, PhD, at the Perelman School of Medicine, University of Pennsylvania

Fifty years ago, British researcher John Gurdon demonstrated that genetic material from non-reproductive, or somatic, cells could be reprogrammed into an embryonic state when transferred into an egg. In 2006, Kyoto University researcher Shinya Yamanaka expanded on those findings by expressing four proteins in mouse somatic cells to rewind their genetic clocks, converting them into embryonic-like stem cells

called induced pluripotent stem cells, or iPS cells.

In early October, Gurdon and Yamanaka were awarded the 2012 Nobel Prize in Physiology or Medicine for their discoveries. Now, thanks to some careful detective work by a team of scientists led by Kenneth Zaret, PhD, at the Perelman School of Medicine, University of Pennsylvania, researchers can better understand just how iPS cells form – and why the Yamanaka process is so inefficient, an important step to work out for regenerative medicine. Zaret is associate director of the Penn Institute for Regenerative Medicine and professor of Cell and Developmental Biology.

The findings, which appear in the Nov. 21 issue of the journal *Cell*, uncover cellular impediments to iPS cell development that, if overcome, could dramatically improve the efficiency and speed of iPS cell generation.

"These studies provide detailed insights into how reprogramming factors interact with the chromatin of differentiated cells and start them down the path toward becoming stem cells," said Susan Haynes, PhD, National Institute of General Medical Sciences, which partially funded the work. "Dr. Zaret's work also identified a major structural roadblock in the chromatin that the factors must overcome in order to bind DNA. This knowledge will help improve the efficiency of reprogramming, which is important for any future therapeutic applications."

Human iPS cells are generated by expressing four DNA-binding proteins – Oct4, Sox2, Klf4, and c-Myc (O, S, K, and M) – in human non-reproductive, or somatic cells, such as skin cells. These factors have generated intense interest in the stem cell and medical communities, not least because they offer the promise of embryonic stem cells with none of the messy ethical and moral dilemmas. Just as significantly, patient-specific iPS cells from individuals with genetic disorders can be used to

study disease origin and to develop drugs for a range of conditions such as Huntington's and Parkinson's diseases.

Yet, the process of generating iPS cells is highly inefficient. It can take a month to fully reprogram somatic cells into iPS cells, and as few as one in 1,000 cells that take up the four factors will successfully convert. What's more, some studies indicate that, for all their plasticity, iPS cells are not precisely equivalent to embryonic stem cells. Zaret, with Penn postdoctoral fellow Abdenour Soufi, PhD, and bioinformatician Greg Donahue, PhD, decided to find out why.

Destination Determination

The team analyzed the destination in the human genome of the four reprogramming factors 48 hours after the initiation of iPS cell reprogramming and compared those locations to four cell types: the starting cell population; the fully reprogrammed iPS cells; cells nearing the end of the reprogramming process (pre-iPS); and embryonic stem cells.

They found that at 48 hours the factors tended to bind gene regulatory elements called enhancers, far removed from the genes they regulate, rather than the target genes themselves. That suggests that O, S, and K serve as "pioneer factors" that open closed chromatin structures on the DNA itself, facilitating the reprogramming process by making target sections of the genome available to be read by messenger RNA.

The team also found large regions of the genome that were "refractory" to the binding of reprogramming factors at 48 hours, but which were eventually activated in, and are in fact required, for the formation of iPS cells.

"Basically, large chunks of the human genome were physically resisting

these factors from entering," Zaret explained. "That provided some understanding that you've got to overcome the binding impediment to get these factors to their final destination."

These refractory sequences tended to be chemically marked with a histone modification called H3K9me3. When the team blocked the enzymes that create that modification, they "significantly accelerated" the reprogramming process.

According to Zaret, these findings reveal genetic roadblocks that slow and impede the iPS cell reprogramming process, as well as factors that may underlie the subtle differences between iPS and embryonic stem cells. They also suggest a potential workaround to these issues, by adding inhibitors of H3K9me3.

But the findings also reveal a normal cellular mechanism that cells may be using to repress blocks of genes that are contrary to the cell's biology, Zaret said. "We went into this thinking we were going to learn something about the mechanism of conversion to pluripotency, but at the end of the day we ended up discovering new ways that cells control gene expression by shutting down parts of their genome."

Provided by University of Pennsylvania School of Medicine