

NOVEMBER 08, 2006

## Researchers Map Protein Network that Regulates "Stemness"

Howard Hughes Medical Institute (HHMI) researchers have created a map that charts the largely unexplored protein landscape that regulates a stem cell's ability to differentiate into multiple types of mature cells.

Understanding this protein network in greater detail could give stem cell biologists a new set of tools to coax mature cells to revert to an embryonic state, said the researchers. Reprogramming adult cells in this way could provide an alternative source of stem cells to use in regenerating tissues damaged by disease or trauma, rather than employing embryonic cells, they said.

HHMI investigator Stuart Orkin and his colleagues at Children's Hospital Boston and Harvard Medical School published their findings November 8, 2006, in an advanced online publication in the journal *Nature*.

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- **Stuart H. Orkin**

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Orkin said that thus far experiments aiming at reprogramming mature cells into a stem cell-like state have yielded cells that imperfectly resemble embryonic stem cells. "However, with this new understanding of the network of regulatory factors, it might be possible to refine this approach to reprogramming," he said.

The regulatory network that maintains a stem cell's ability to become many different cell types - a characteristic called pluripotency - also prevents the cell from inappropriately differentiating into a mature cell, while keeping it poised to undergo maturation when required. This precise control relies on intricate circuits of interacting proteins that both regulate one another and govern the activity of genes.

To create a detailed map of this network, the researchers used mouse embryonic stem cells. Orkin noted that although the researchers used mouse embryonic stem cells as their model, the circuitry regulating pluripotency is likely similar in all stem cells.

As the jumping-off point of their mapping effort, Orkin and his colleagues used a protein called Nanog, which other researchers' experiments had indicated was central to regulation of stem cell pluripotency. The researchers first tagged Nanog so that when they removed it from cells, they would simultaneously remove any proteins that were attached to it.

These experiments enabled them to identify numerous proteins that interact with Nanog, including some already known to regulate pluripotency. To confirm that the proteins they had found functioned to maintain stem cell pluripotency, they depleted the levels of several proteins in embryonic cells and observed that the cells then expressed markers of differentiation.

Next, the researchers created a protein interaction map that showed the relationships among the various proteins. The map will provide stem cell biologists with an important guide for future studies, said Orkin. "Even though some of these factors were known to be important in pluripotency, exactly how they work and who they talk to and interact with was completely unknown," he said.

Orkin said the fact that so many of the proteins seemed to be concentrated in a single self-contained regulatory circuit came as a surprise. The proteins in the network seem to be regulated as a group in embryonic cell differentiation. Also, many proteins work closely together in complexes that repress genes that trigger stem cell differentiation.

"It could well have been that some of these proteins were distributed elsewhere in the cell proteome, and that they interacted with other factors that only subsequently converged in the genes that they regulated. But instead, our findings suggest that not only do these proteins interact with the target genes together in various ways, but they are also continuously talking to one another as proteins. Thus, this seems to be a sort of holistic network that is almost grafted onto the rest of the cellular machinery.

"The whole network is very convoluted. The proteins are not only regulating one another, but many of their genes are regulatory targets of the same proteins," said Orkin. "So, in one sense the system is stable because the proteins are interdependent, but on the other hand it is unstable, because if you take out a critical element, the whole circuit falls apart. But having such a tightly controlled, interdependent circuit makes sense, because the stem cell doesn't want to be completely frozen as a stem cell -- it has to be poised to differentiate in response to the right signals."

The intimate contact between the proteins in the network should make it easier to characterize their function and identify additional members, he said. Orkin added that thorough understanding of the characteristics of pluripotent embryonic stem cells is the field's "gold standard." "We would like to understand as much as we can about the network of components required to establish and maintain pluripotency, because it might be feasible to establish that whole network within a somatic cell and reprogram it to closely resemble an embryonic stem cell," he said.

"The major controversy over using human embryonic stem cells is that it requires obtaining oocytes and perhaps destroying embryos," said Orkin. "However, if one could reprogram, say, a skin cell to revert to become identical to an embryonic-type cell, it would obviate these ethical issues."