STEM CELLS

RNA-based reprogramming

RNA molecules can both induce pluripotency and direct differentiation.

The ability to make person-specific pluripotent stem cells opens up many possibilities for research tools and perhaps even therapies. But most reprogramming techniques alter cells' genomes, making cells less predictable and more prone to cancer-like growth. Methods to reprogram cells without changing their genomes exist but are inefficient and labor-intensive; most also rely on DNA and so still carry the risk of unintended genetic alterations.

A reprogramming technique recently reported by Derrick Rossi of Harvard University uses synthetic RNA molecules and eliminates the chance of unwanted genetic modification. Moreover, it is about twice as fast and a hundred times more efficient than standard techniques that use viruses to insert genes into cells. Researchers at the Harvard Stem Cell Institute plan to start using the RNA-based technique instead of viral techniques for much of its induced pluripotent stem cell production.

Getting the technique to work required a lot of trial and error, says Rossi. His first attempts to reprogram cells with RNA failed because cells responded as if they had been infected by a virus, committing apoptosis or launching other antiviral defenses. Swapping out two nucleosides with similar analogs mitigated the response and allowed the researchers to reprogram cultured human cells by applying daily doses of RNAs coding for four to five pluripotency genes. The extent of protein expression depended on the amount of RNA delivered, offering the researchers more precise control over the process.

Modified RNAs can direct differentiation as well as induce pluripotency. Induced pluripotent stem cells dosed with RNA encoding MYOD, an important factor for muscle development, began producing myotubes. In fact, Rossi expects that techniques using RNA for differentiation will probably be adopted more quickly than using RNA for reprogramming. "I think people will have an easier time in differentiation because it's often one factor rather than several," he says.

Making the modified RNA itself is easy, says Rossi, but researchers will need to spend some time tinkering to find the best conditions for their systems. Every aspect of culture conditions affects how cells are transfected with RNA, he says. "Whenever a new cell type is approached, everything needs to be optimized."

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RESEARCH PAPERS

Warren, L. *et al.* Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell* **7,** 618–630 (2010).