Synthetic mRNAs: Powerful Tools for Reprogramming and Differentiation of Human Cells

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In this issue of *Cell Stem Cell*, Warren et al. (2010) describe a new methodology, using synthetic mRNA, for efficiently generating iPSCs without compromising genomic integrity. This powerful approach can also be used for directed differentiation of iPSCs, or even for trans-differentiation to generate clinically relevant differentiated cell types.

The character of a fully differentiated somatic cell is no longer considered an irreversible terminus. We now appreciate how the many routes toward differentiation can be experimentally manipulated to bring the cells back to an embryonic-like pluripotent state, followed by respecification into cells of another tissue type. In this issue of *Cell Stem Cell*, Warren et al. (2010) demonstrate that both tasks can be accomplished by synthetic mRNAs, as long as appropriate modifications are introduced in both the molecule and culture conditions.

Almost 5 years ago, the Yamanaka laboratory first demonstrated that adult skin cells could generate embryonic-like stem cells, known as induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka, 2006; Takahashi et al., 2007) simply by enforcing ectopic expression of a few transcription factors (reprogramming factors [RFs]). Because iPSCs can be also generated from somatic cells derived from patients harboring genetic diseases (patient-specific iPSCs), they provide an opportunity to perform pharmacological screens and autologous cell-based therapy approaches.

However, before reprogrammed cells can be considered realistic clinical tools, several major challenges must be overcome, not the least of which is the method of RF delivery into cells. The first generation of iPSCs used integrating viral vectors to deliver RFs, which compromised the integrity of the target cell genome, and thus generated mutant iPSCs. To overcome this hurdle, a number of strategies were pursued, including (1) reprogramming by protein delivery, (2) delivering RFs via the nonintegrating Sendai virus, (3) use of adenoviruses as vectors, and (4) using transient plasmid deliveries. Although all of these advances improved the technology, they did not completely bypass the preclinical roadblock, as each approach is still hampered by relatively minor but specific caveats, in particular with respect to the efficiency of reprogramming. Furthermore, even when the inserted transgenes were removed using the Cre-LoxP system, as attempted in the mouse, permanent modifications were left in the host genome (Kaji et al., 2009), again creating mutant iPSCs.

The new and innovative approach presented here addresses all of the above concerns and provides what seems to be the best strategy so far to reprogram mutation-free pluripotent cells at a high frequency (Warren et al., 2010). The approach relies on the delivery of a cocktail of in vitro-generated, modified synthetic mRNA that encodes the RFs (Klf4, c-Myc, Oct4, Sox2, and Lin28). Given that these mRNAs are translated in the cytoplasm, their transfection into human cells does not cause permanent genetic changes. Interestingly, as also briefly referenced by the authors, the introduction of synthetic mRNA as a means to manipulate protein expression in target cells was first used in the context of gain-of-function experiments to dissect early embryogenesis of the *Xenopus laevis* (Yisraeli et al., 1989; Vize et al., 1991). However, although this approach worked robustly in frogs, it proved to be rather inefficient in human cells, given that it led to cytotoxicity due to the interferon-mediated innate immune response and poor protein yield. Undeterred, Rossi and colleagues developed three main modifications of the original protocol (Warren et al., 2010), which collectively led to the resolution of these problems. The first protocol adaptation was at the molecular level, whereby 5-methylcytidine was substituted for cytidine, and pseudouridine for uridine, followed by phosphatase treatment. These alterations solved the protein yield issue, probably by stabilizing the delivered mRNAs, and also enabled the researchers to produce the appropriate RF stoichiometry. The second modification was made at the cellular level: the interferon response was blocked on target cells with a Vaccinia virus decoy protein, B18R, which inhibits type I interferon and eliminated cytotoxicity. These approaches simplify a previous protocol based on the cotransfection of RF mRNAs with a siRNA cocktail designed to knock down immune-related factors (Angel and Yanik, 2010). The final adaptation involved changing a variety of cell culture and RF delivery conditions, which included switching to low oxygen and daily transfections of the modified synthetic mRNAs encoding the RF factors. Interestingly, these alterations to the protocol drastically improved both the efficiency and the kinetics of reprogramming in multiple cell types. The combination of these thoughtful changes ultimately led to successful RNA-mediated reprogramming of target cells.

Another study, published earlier this year, had shown that transfection of unmodified mRNAs encoding the RFs could induce expression of pluripotency markers in human fibroblasts, without suppression of the interferon response (Yakubov et al., 2010). However, whether these cells—termed RiPSCs—were functionally pluripotent remained an open question. Importantly, Warren et al. (2010) show here that RiPSC lines generated

with their modified protocol satisfied all molecular and functional tests of pluripotency, thus validating their identity. Moreover, in addition to their capacity to generate progeny from all three germ layers, RiPSC exhibited advantages relative to iPSCs obtained by viral delivery of RFs, given that they displayed: (1) a fast 2 week output, (2) greater homogeneity, and (3) a global molecular signature that clustered closer to those of human embryonic stem cells (hESCs). Although the long-term implications of the findings that RiPSCs arise more frequently and homogeneously—and with faster kinetics—remain to be seen, the final point emphasizes the crucial importance of using hESCs as the gold standard by which to measure the results of comparative studies among sources of pluripotent cells.

Also, the findings of this study demonstrate the feasibility of the RNA-mediated approach to support directed differentiation, given that introduction of modified, synthetic mRNA for MyoD was able to convert RiPSCs or mesenchymal stem cells into myotubes (Warren et al., 2010). Further application of this technology will lead to the simplification of RNA-mediated differentiation of target cells toward specific, desired cell fates.

Another route to change fate of one cell into another, involves transdifferentiation, of somatic cells without passing through an embryonic state. This strategy was originally utilized when ectopic expression of a single transcription factor, MyoD, led to the transdifferentiation of fibroblasts into terminally differentiated myofibers (Davis et al., 1987). The parallel approach has since been applied to a variety of cells, such as the in vivo conversion of mouse pancreatic exocrine cells to pancreatic β cells after the delivery of three factors (Ngn3, Pdx1, and Mafa; Zhou et al., 2008). Thus, transdifferentiation may provide yet another platform for cell-based therapy strategies in clinical settings. It will be interesting to assess whether the synthetic mRNA technology could be applied to transdifferentiate adult somatic cells into useful cell types.

The use of synthetic mRNAs to change cell fate has come a long way since its debut in the amphibian system. The ingenious adaptation of this technology to the human system in order to mediate reprogramming and directed differentiation, including the additional problem solving conducted by the authors in order to derive integration-free RiPSCs, makes the modified RNA approach a powerful new tool that brings cell-based therapy ever closer to reality.

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Extinction of Xist Improves Cloning

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Mammalian cloning by somatic cell nuclear transfer is a notoriously inefficient process with a low birth rate. Recently in *Science*, Inoue et al. (2010) report that somatic cell nuclear transfer fails to regulate *Xist* expression from the X chromosome.

Three broad categories encompass the varied experimental approaches used to reprogram somatic nuclei to a pluripotent state: cell fusion between somatic and embryonic stem cells (ESCs), transcription-factor transduction, and somatic cell nuclear transfer (SCNT). Cell fusion has been useful for exploring the regulatory mechanisms responsible for reprogramming; however, the tetraploid nature of fused cells prevents a stringent in vivo test of the cell's developmental potential. On the other hand, induced pluripotent stem cells (iPSCs) generated by defined transcription-factor transduction have been successfully assayed for their ability to generate completely stem cell-derived embryo proper by tetraploid embryo complementation. SCNT reprograms somatic cell nuclei through nuclei introduction into enucleated oocytes,