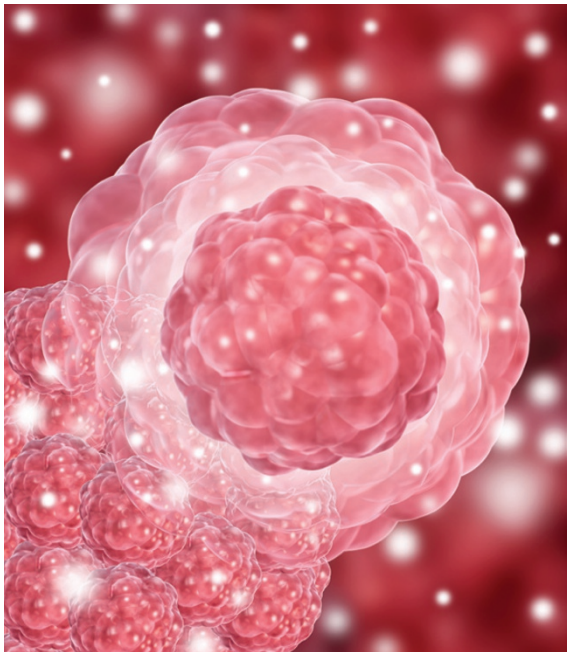
A new route to human embryonic stem cells

There is much excitement surrounding pluripotent stem cells for their potential in regenerative medicine and the possibility of their providing improved cell-based systems to study the mechanisms of disease. One approach for reprogramming to embryonic stem cells (ESCs) is the transfer of a nucleus from a somatic cell to an oocyte (somatic cell nuclear transfer; SCNT). However previous attempts to produce human ESCs by this method have failed after arrest of SCNT-derived embryos. In a new study, Tachibana *et al.*[1] designed an optimized strategy that allowed them to efficiently generate such reprogrammed cell lines from human oocytes. We asked three experts for their viewpoint on these findings and their implications for stem cell–based therapies and the study of human disease.



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Fertilization is a remarkable reprogramming event involving the sperm and oocyte. It is also interesting that lineage-committed adult somatic cell types can be efficiently and rapidly reprogrammed in amphibians and mammals by SCNT. Despite a lack of optimism and considerable opposition to human 'therapeutic cloning' (via SCNT) by various groups, Tachibana *et al.*[1] have recently shown that human SCNT efficiently results in the production of euploid embryonic stem cells (SCNT-ESCs). Is SCNT made redundant by the availability of transcription factor–transduced induced pluripotent stem cells (iPSCs)[2], or will it challenge iPSCs as an optional method for reprogramming adult cells? First, further generation of SCNT-ESCs is needed to evaluate their relative differences from iPSCs using carefully constructed experiments and to analyze their robustness for producing differentiated and histocompatible transplant products for cell therapy, their relative genetic stability and freedom from epigenetic memory, and their accuracy as human disease models for the discovery of new drugs. Second, SCNT uniquely enables the production of cells for therapy for patients with inheritable mitochondrial diseases because the mutated somatic mitochondrial DNA is replaced by the mitochondria from the reprogramming oocyte.

The reprogramming of cell lineage commitment is very different in SCNT-ESCs and iPSCs. SCNT recapitulates totipotency so that early embryonic development proceeds to reset the complete capability of cells to form an

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Had the derivation of human embryonic stem cells (ESCs) by somatic cell nuclear transfer been reported by Tachibana *et al.*[1] a decade ago, my commentary would have been markedly different. Then, several groups including mine were seeking to reprogram cells to pluripotency by SCNT to advance our ability to model human disease *in vitro* and to produce rejection-proof tissues for autologous repair. But in 2006, Takahashi and Yamanaka taught us to derive customized stem cells by the simple transfer of the genes encoding Oct4, Sox2, KLF4 and c-Myc (OSKM), transcriptional regulators active in ESCs[5]. With the advent of iPSCs, and under pressure from dwindling funding, we abandoned our SCNT work, as did all but a small band of intrepid researchers, because deriving iPSCs is far less cumbersome.

> "Crucial questions about the fidelity of reprogrammed cells relative to the gold standard—embryo-derived ESCs—remain to be answered."

However, there remain a few lingering concerns that iPSCs may not be entirely equivalent to embryo-derived ESCs. When we compared mouse iPSCs and SCNT-ESCs to ESCs derived from control mouse embryos, we found that SCNT-ESCs were subtly closer to ESCs, as defined by the methylation marks on their

organism. By contrast, iPSCs recapitulate pluripotency, which is the ability to form all the basic embryonic tissue lineages but not the whole organism. The factors responsible for efficiently reprogramming totipotency are very efficient and rapid when compared to the factors that reprogram cells to iPSCs[3]. Data from ESCs were used to discover factors for reprogramming

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to iPSCs, and developments in SCNT will probably allow further refinement and improvement of iPSCs. The debates about SCNT will pivot around reimbursement for women donating oocytes and the ethics of accessing human oocytes for SCNT research. These are likely to be issues until oocytes can be produced from ESCs *in vitro*[4].

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DNA and their behavior in differentiation assays[6]. And several recent papers have suggested that human iPSCs may indeed harbor aberrant epigenetic marks that are related to the challenges of reprogramming the regions around telomeres and centromeres[7,8]. Although I doubt that these subtle molecular differences will ever prove problematic for any of the research or clinical applications we envision for iPSCs, crucial questions about the fidelity of reprogrammed cells relative to the gold standard—embryo-derived ESCs—remain to be answered. And finally, there are fundamental biological questions and certain medical applications that can only be addressed through SCNT. Oocytes reprogram by a different mechanism than OSKM, and studying this process may yield new factors that will improve iPSCs. Although no fertility specialist should embark on reproductive cloning because of the inherent dangers, the enucleation and spindle transfer methods pioneered by Mitalipov and his colleagues[9] are a legitimate strategy for avoiding mitochondrial disease. Sadly, these essential medical research questions cannot be studied in the United States using federal funds because of ongoing restrictions on embryo research. Perhaps it's time to revisit these restrictions.

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The outstanding study by Tachibana *et al.*[1] demonstrates that human somatic cells can be reprogrammed by SCNT (also called cloning), enabling the derivation of cloned human embryonic stem cells (SCNT-ESCs)[1]. These cells can be generated from healthy and diseased donors and, like conventional ESCs, are able to differentiate into many different cell types.

The reprogramming of somatic cells by SCNT followed by ESC derivation was previously achieved in animal models such as mice and rhesus macaques. However, attempts in the human system were not successful until now[1]. The recent accomplishment of Tachibana *et al.*[1] arose from their identification of multiple technical issues with SCNT and their careful optimization of the method, which took advantage of past and new findings, especially those in the monkey SCNT system[1,10]. In addition, donor oocyte quality is also crucial for the success of the procedure. Perfecting SCNT means that human SCNT-ESCs can now be created at very high efficiency[1].

"This will have important implications for disease modeling, drug discovery and regenerative medicine."

Importantly, the new approach establishes a second method for reprogramming human somatic cells to a pluripotent state. This can also be achieved by the overexpression of a few key transcription factors, yielding iPSCs[2]. The extent to which iPSCs are molecularly equivalent to ESCs derived from fertilized embryos is still being debated. Studies in mice suggest that iPSCs, at low passage, can show tissue-of-origin differences from ESCs that are not apparent when using mouse SCNT-ESCs[6]. Similar studies have not been possible in the human system until now. We expect that the derivation of human iPSCs and SCNT-ESCs from the same person will allow an in-depth comparison of the two reprogramming technologies on the same genetic background. In addition, given the high reported efficiency of human SCNT-ESC derivation (35%) compared with that of iPSC derivation (0.1–1%), the recent report[1] will certainly spur new studies directed at understanding how the oocyte reprograms the somatic nucleus, which should lead to the improvement of the quality and kinetics of reprogramming to generate patient-specific pluripotent cells. This will have important implications for disease modeling, drug discovery and regenerative medicine.

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