Production of Pluripotent Stem Cells by Oocyte-Assisted Reprogramming

Joint Statement with Signatories

As described in the President’s Council on Bioethics’ May 2005 White Paper, altered nuclear transfer (ANT) is a broad conceptual proposal for producing pluripotent stem cells without creating and destroying embryos. In the description set forth below, we outline a research program for a form of ANT that should allow us to produce pluripotent stem cells without creating or destroying human embryos, and without producing an entity that undergoes or mimics embryonic development. The method of alteration here proposed (“oocyte-assisted reprogramming,” or OAR) would immediately produce a cell with positive characteristics and a type of organization that from the beginning would be clearly and unambiguously distinct from, and incompatible with, those of an embryo. Incapable of being or becoming an embryo, the cell produced would itself be a pluripotent cell that could be cultured to establish a pluripotent stem cell line. Significantly, this cell would not be totipotent, as a zygote is.

Our proposal is for initial research using only nonhuman animal cells. If, but only if, such research establishes beyond a reasonable doubt that oocyte-assisted reprogramming can reliably be used to produce pluripotent stem cells without creating embryos, would we support research on human cells.

With few exceptions, all human cells contain a complete human genome; i.e., the complete DNA sequence characteristic of the human species. Specifically, one-celled human embryos, pluripotent human embryonic stem (or ES) cells, multipotent

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NOTE: In this presentation of the statement, we have denoted the names of genes with italicization and the names of proteins and transcription factors without italicization.—Ed.

human adult stem cells, and differentiated (specialized) adult human cells such as
neurons all contain a complete human genome. Thus, possession of a human ge-
nome is a necessary but not sufficient condition for defining a human embryo with
its inherent dignity. Rather, the nature of each cell depends on its epigenetic state,
i.e., which subset of the approximately thirty thousand human genes is switched on
or off and, if on, at what level. For example, the gene for albumin, a liver-specific
protein, is found both in human embryos and in adult human liver cells called hepato-
cyes. However, neither the messenger RNA (mRNA) for albumin nor the protein
itself is found in single-celled embryos, because in them the gene is silenced.

This fundamental observation has given rise to the concepts of cell fate plastic-
ity and epigenetic “reprogramming.” If successful, reprogramming converts a cell
from one kind to another by changing its epigenetic state. The ability to clone ani-
mals, such as Dolly the sheep, by transfer of a specialized adult nucleus to an enucle-
ated oocyte, demonstrates the power of epigenetic reprogramming: the oocyte cyto-
plasm is sufficient to reprogram the somatic nucleus to a totipotent state. Human
cloning has been proposed as a means of generating human embryos whose pluripo-
tent stem cells would be used in scientific and medical research. Here, through a
form of altered nuclear transfer, we propose to utilize the power of epigenetic repro-
gramming in combination with controlled alterations in gene expression to directly
produce pluripotent cells using adult somatic nuclei, without generating and subse-
quently destroying embryos.

How do pluripotent stem cells differ from totipotent single-celled embryos?
Several key transcription factors essential for establishing and maintaining the pluri-
potent behavior of ES cells have been identified. Importantly, some of these are
specifically expressed only in pluripotent cells, such as embryonic stem cells or the
cells found in the inner cell mass (ICM) of the week-old embryo or blastocyst. They
are not expressed in oocytes or single-celled embryos. Expression of these factors
therefore positively defines and distinguishes mere pluripotent cells from embryos.
These factors instruct a cell to have the identity of a pluripotent cell. Currently, the
best studied example is the homeodomain transcription factor called Nanog.2  Nanog

2Kaoru Mitsui et al., “The Homeoprotein Nanog Is Required for Maintenance of
[Abstract: Embryonic stem (ES) cells derived from the inner cell mass (ICM) of blasto-
cysts grow infinitely while maintaining pluripotency. Leukemia inhibitory factor (LIF)
can maintain self-renewal of mouse ES cells through activation of Stat3. However, LIF/
Stat3 is dispensable for maintenance of ICM and human ES cells, suggesting that the path-
way is not fundamental for pluripotency. In search of a critical factor(s) that underlies
pluripotency in both ICM and ES cells, we performed in silico differential display and
identified several genes specifically expressed in mouse ES cells and preimplantation
embryos. We found that one of them, encoding the homeoprotein Nanog, was capable of
to generate epiblast and only produced parietal endoderm-like cells. Nanog-deficient ES
cells lost pluripotency and differentiated into extraembryonic endoderm lineage. These
data demonstrate that Nanog is a critical factor underlying pluripotency in both ICM and
ES cells.]
is not present in oocytes or single-celled embryos, but first becomes expressed weakly in the morula and then highly in the ICM. Deletion of the Nanog gene does not prevent early cleavage stages of embryogenesis, including formation of the ICM, but does prevent the formation of an epiblast. ES cells in which Nanog is blocked lose their pluripotency—which clearly shows that Nanog is a positive factor instructing cells to be pluripotent, i.e., to behave like ES cells. Furthermore, ES cells which constitutively express Nanog can no longer be differentiated, i.e., they are forced to remain in their undifferentiated state.

We propose a procedure that combines epigenetic reprogramming of a somatic nucleus with forced expression of transcription factors characteristic of embryonic stem cells, to produce a pluripotent stem cell. As a result of this procedure, Nanog and/or other, similar factors would be expressed at high levels in somatic cells prior to nuclear transfer, to bias the somatic nucleus towards a pluripotent stem cell state. Such altered nuclei would then be epigenetically reprogrammed by transplantation into enucleated oocytes. Alternatively or concomitantly, the mRNA for these same factors could be introduced into the oocyte prior to nuclear transfer. This procedure could ensure that the epigenetic state of the resulting single cell would immediately be different from that of an embryo and like that of a pluripotent stem cell: the somatic-cell nucleus would be formed into a pluripotent stem-cell nucleus and never pass through an embryonic stage. Therefore, unlike some other proposed methods of

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3Ibid. See also Shin-ya Hatano et al., “Pluripotential Competence of Cells Associated with Nanog Activity,” *Mechanisms of Development* 122.1 (January 2005): 67–79. [Abstract: Nanog is a novel pluripotential cell-specific gene that plays a crucial role in maintaining the undifferentiated state of early postimplantation embryos and embryonic stem (ES) cells. We have explored the expression pattern and function of Nanog and a Nanog-homologue, Nanog-ps1. Nanog-ps1 was mapped on Chromosome 7 and shown to be a pseudogene. Immunocytochemical analysis in vivo showed that the Nanog protein was absent in unfertilized oocytes, and was detected in cells of morula-stage embryos, the inner cell mass of blastocysts and the epiblast of E6.5 and E7.5 embryos, but not in primordial germ cells of early postimplantation embryos. In monkey and human ES cells, Nanog expression was restricted to undifferentiated cells. Furthermore, reactivation of the somatic cell-derived Nanog was tightly linked with nuclear reprogramming induced by cell hybridization with ES cells and by nuclear transplantation into enucleated oocytes. Notably, mouse Nanog (+/-) ES cells, which produced approximately half the amount of Nanog produced by wild-type ES cells, readily differentiated to multi-lineage cells in culture medium including LIF [leukemia inhibitory factor]. The labile undifferentiated state was fully rescued by constitutive expression of exogenous Nanog. Thus, the activity of Nanog is tightly correlated with an undifferentiated state of cells even in nuclear reprogrammed somatic cells. Nanog may function as a key regulator for sustaining pluripotency in a dose-dependent manner.]

4Mitsui et al., “The Homeoprotein Nanog.”

5Ibid.

6Nanog is only one example of a growing list of candidate factors, numbering probably at least ten. Oct3/4 is another well-studied example, and is noteworthy because it is also expressed at high levels in pluripotent adult stem cells.
ANT, this method would achieve its objective, not by a gene deletion that precludes embryonic organization in the cell produced, but rather by a positive transformation that generates, ab initio, a cell with the distinctive molecular characteristics and developmental behavior of a pluripotent cell, not a totipotent embryo. This should allow us to produce a pluripotent stem cell line with controlled genetic characteristics.

**Endorsers**

Institutional affiliations are provided for purposes of identification only and do not necessarily represent the views of organizations with which endorsers are affiliated. Endorsers who are not themselves specialists in biomedical science do not put themselves forward as experts in that field. Their endorsement of the proposal pertains to the ethics of ANT-OAR, assuming its technical feasibility.

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