

Therapeutic Somatic Cell Reprogramming by Nuclear Transfer

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Abstract In the course of normal development, cells rarely are able to revert from a differentiated state back to an embryonic state. However, techniques exist that allow this reversal to take place. In an experiment performed over 50 years ago, single cell nuclear transfer from somatic cells to enucleated eggs was able to yield successful development of cloned *Xenopus laevis* (Gurdon et al., Nature 182:64–65, 1958). Through somatic cell nuclear transfer (NT), several cell divisions occur before the onset of new gene transcription; moreover, new cell types and even organisms can be derived (Campbell et al., Nature 380:64–66, 1996). More recently, terminally differentiated cells could be induced to reprogram to a pluripotent, embryonic stem (ES) cell-like state via overexpression of a particular subset of transcription factors (TF) (Takahashi and Yamanaka, Cell 126:663–676, 2006). These induced pluripotent stem (iPS) cells can then be re-differentiated into various tissue types, including both somatic and germ cells. A possible advantage that somatic cell NT harbors over iPS is that factors present in the egg have been shown to directly remove silencing of genes via chromatin decondensation, removal of histone modifications, and activation of gene transcription prior to cell division. Therefore, an improved understanding of how the egg facilitates nuclear reprogramming by natural means may identify components that can be used for more efficient reprogramming by this and other means.

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Therapeutic Limitations of Reprogramming Techniques

Although iPS cells hold great promise for the generation of patient-specific pluripotent stem cells, several challenges currently exist that limit their direct application in human therapy. Though increasing with recent techniques, the efficiency of generating iPS cells remains low (Wang et al. 2011; Chen et al. 2011). In addition, iPS cells can harbor increased tumorigenic potential due to the use of genome-incorporating viruses in the original reprogramming process, along with having an increased presence of oncogenes (Zhang et al. 2012). This issue has since been remedied by implementation of integration-free methods, such as episomal plasmid vectors (Okita et al. 2011). Furthermore, iPS cells have been demonstrated to have issues regarding increased copy number variation, somatic mutations, and aberrant epigenetics (Hussein et al. 2011; Gore et al. 2011; Lister et al. 2011). However, some recent data indicate that these could be the result of abnormalities already present in the original cell lines (Young et al. 2012).

Nuclear reprogramming via NT provides a few distinct advantages. These include the use of natural egg components, which avoids the use of viral vectors, small molecules, or chemical factors altogether. Moreover, ES cells derived via somatic cell NT, when compared to iPS cells, are able to be reprogrammed at higher efficiency and are of higher equality, as shown through having less epigenetic memory via each respective reprogramming step (Kim et al. 2010; Polo et al. 2010).

Recent headway made in human somatic cell NT – where non-enucleated human eggs were able to successfully reprogram diploid human somatic cells – suggests the utility of NT as a technique for generating pluripotent stem cells (Noggle et al. 2011). However, the aforementioned technique generated triploid cells, which would not be compatible with therapeutic application. Coupled with the ethical challenges associated with ES cell research and the scarcity of human embryos available for research purposes, these roadblocks provide a great challenge in the application of NT to human therapy (Egli et al. 2011). How can we transition from the above to develop useful somatic cell NTs?

NT to Enucleated Eggs

Originally established in amphibia, the initial NT experiments had a nucleus from a ruptured cell injected into an enucleated and unfertilized egg (Briggs and King 1952). A proportion of these were able to develop normally through embryogenesis, reaching adulthood (Gurdon et al. 1958). When the donor nuclei were taken from more embryonic cells, such as from a blastula, a higher proportion was able to reach blastula stages, along with adulthood (Gurdon 1960). Thus, in general, less terminally committed cells are less resistant to nuclear reprogramming by eggs. Furthermore, it has been demonstrated that transplantation of a mammalian somatic cell nucleus into an enucleated egg in second meiotic metaphase (MII) can lead to

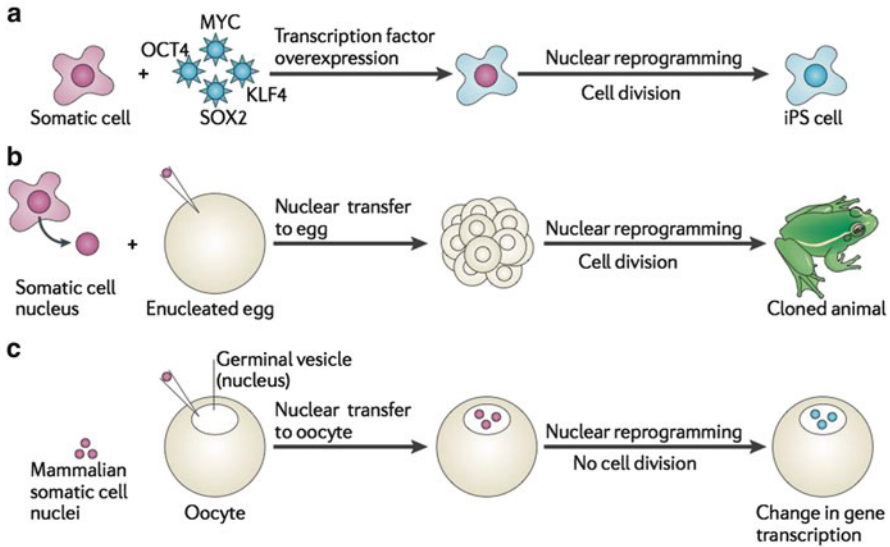


Fig. 1 Nuclear reprogramming methods. (a) Transcription factor overexpression. Oct4, Klf4, Sox2, and c-Myc expression reprograms somatic cells to an ES cell-like state, generating iPS cells. (b) Somatic cell NT. A somatic cell nucleus is transplanted into an enucleated egg in second meiotic metaphase, which allows nuclear reprogramming of the transplanted genome towards a pluripotent state. (c) When mammalian somatic cell nuclei are transplanted into the germinal vesicle (GV) of *Xenopus laevis* oocytes at first meiotic prophase, no cell division occurs. However, factors present in the oocyte directly reprogram gene expression (Jullien et al. 2011)

successful development of NT embryos (Campbell et al. 1996; Dominko et al. 1999). Various efficiencies of mammalian NT have been extensively reviewed (Beyhan et al. 2007).

Nuclear reprogramming via NT to MII oocytes can involve a high number of cell divisions with DNA synthesis. As such, inefficient reprogramming can result from the inability of transplanted nuclei to synchronize with these rapid cell cycle changes, leading to abnormal chromosomes and failure of further development (Mizutani et al. 2012). Still, *Oct4* reactivation during mouse somatic cell NT is able to occur 1–2 cell divisions post-NT, whereas derivation of germ cells requires an average 25 cell divisions post-NT (Boiani et al. 2005; Egli and Eggan 2006). Therefore, a system wherein cell division is not a compounding factor would be better for studying mechanisms of reprogramming in NT (Fig. 1).

NT to Oocytes

A different design for NT exists, wherein multiple nuclei are transplanted into the germinal vesicle (GV) of amphibian first meiotic prophase oocytes (Byrne et al. 2003). The oocyte GV contains a high concentration of components, eventually distributed to the rest of the egg post-meiotic maturation, that is necessary for embryonic development (Gao 2002). Of particular note, and as opposed to the previous NT system, this one does not generate new cell types. Somatic cell nuclei injected into an oocyte GV do not undergo DNA synthesis or cell division; however, they become intensely active in RNA synthesis along with the host oocyte. Thus, it is possible to transplant multiple nuclei from mammalian cells into the amphibian oocyte and see activation of genes that are active during normal early development, such as pluripotency genes. Furthermore, it is possible to observe direct activation of silenced genes in adult somatic nuclei without the complication of DNA replication. A direct switch in gene transcription from somatic to oocyte-type occurs without the intervention of or need for DNA replication. Thus, the exchange of factors involved directly reflects the process of transcriptional reprogramming. Conversely, as previously described, the NT experiments to unfertilized eggs are complicated by a period of intense DNA replication and cell division, along with the absence of transcription immediately following NT. As such, the timing of transcriptional reprogramming in egg NT experiments is difficult to analyze.

Using the NT to oocyte system, the oocyte-type linker histone B4 was identified as a necessary factor for efficient gene reactivation (Jullien et al. 2010). Following NT, B4 is incorporated into transplanted nuclei, a process that is associated with the loss of somatic linker histone H1. Furthermore, it was found that polymerization of nuclear actin, which is especially abundant in the oocyte GV, is necessary for transcriptional reactivation of *Oct4* in the oocyte system during reprogramming (Miyamoto et al. 2011).

It can be concluded that *Xenopus* oocytes are able to efficiently induce gene reactivation, without cell division and within a short window of time, utilizing natural oocyte components. Thus, the transplantation of multiple mammalian nuclei to *Xenopus* oocytes can be seen as a model system for investigating the mechanisms of transcriptional reprogramming. Additionally, their size (1.2 mm in diameter) allows for easier manipulation and an abundant source of material for identification of novel factors in reprogramming.

Potential Therapeutic Benefits of NT Reprogramming

The three main routes by which nuclear reprogramming can be achieved are induced pluripotency by transcription factor overexpression, cell fusion, and NT to eggs or oocytes. Cell fusion involves retention of one of the nuclear donors and

does not, therefore, yield a cell whose genetic material is all derived from the intended donors. Transcription factor overexpression for induced pluripotency is an excellent procedure but the yield of reprogrammed cells is initially very small, which could present problems if large numbers of iPS cells are required.

Reprogramming by NT has some disadvantages and some advantages. The disadvantages are that NT to unfertilized eggs (in MII) is generally followed by extensive abnormalities of development when donor nuclei from differentiated cells are used. The yield of normal cells is, therefore, very small. NT to oocytes (in first meiotic prophase) does not yield new growing cells, although it does achieve pluripotency gene expression in transplanted nuclei. A further disadvantage of NT is that it is unlikely to be practical for humans because of the great difficulty in obtaining sufficient numbers of human eggs (Egli et al. 2011). There is also the problem that, so far, NT in humans has succeeded only when the egg nucleus is retained to make a fusion with an introduced somatic nucleus; until a procedure is developed by which the egg nucleus can be eliminated and NT achieved with only donor nuclear material, this also presents an obstacle to practical use.

The advantages of a NT route for reprogramming are that it makes use of natural components contained in eggs. It is important to remember that eggs are able to reprogram (or activate) pluripotency genes in the highly specialized and condensed sperm nuclei with 100 % efficiency. A sperm nucleus is more specialized than any somatic nucleus. The egg, therefore, possesses a remarkable supply of components that are able to achieve this activation of a sperm nucleus. It might well be that the natural gene-activating components of eggs could yield more perfectly reprogrammed somatic nuclei than enforced reactivation by overexpressed transcription factors. An advantage of using oocytes (as opposed to eggs) for reprogramming somatic nuclei is that it might well be possible, in future, to identify the actual components of oocytes or eggs that provide the reprogramming effect. This point is particularly true of amphibian oocytes, because 1 frog contains some 25,000 oocytes, each of which is over a millimeter in diameter. Therefore, the amount of material available for analysis is enormous compared to mammals, most of which have eggs of <100 μm and a limited number of these are available.

In conclusion, the NT route towards reprogramming is likely to be of eventual therapeutic value if oocyte or egg components can be identified, purified, and used alone or in conjunction with transcription factor overexpression to achieve large numbers of well-reprogrammed somatic cells.

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