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## Nuclear Reprogramming by Human Embryonic Stem Cells

**Embryonic stem cells have two unique properties. They are capable of indefinite self-renewal and, being pluripotent, they can differentiate into all possible cell types, including germ cells. A new study by Cowan et al. (2005) published in *Science* shows that human embryonic stem cells are able to reprogram the nuclei of fully differentiated human somatic cells, apparently conferring on them a pluripotent state.**

The possibility of regenerating the entire repertoire of diverse cell types from an adult somatic cell, such as a skin cell, is an alluring thought. However, development from a totipotent fertilized egg involves progressive loss of pluripotency as cells commit to become defined cell types, such as nerves or muscle. Essentially, there is no way back for these differentiated cells without the help of experimental manipulation. In contrast, pluripotent embryonic stem cells (ESCs) derived from early mouse or human embryos consisting of 60 to 100 cells at the blastocyst stage can proliferate indefinitely in culture and retain the capacity to differentiate into all cell types present in adults (Smith, 2001). Is it possible somehow to confer this unique property from ESCs directly to adult somatic cells? Building on the work of Tada et al. (2001), Cowan and colleagues now report in *Science* that the answer to this question is a qualified yes. The process involved is called nuclear reprogramming as it entails a journey back to the beginning of development.

The process of development and cellular differentiation is accompanied by heritable modifications to the DNA sequence, principally methylation of CpG dinucleotides. The histone proteins that are bound to DNA are also subject to modifications, including methylation and acetylation. All of these modifications ensure that appropriate genes are selected for expression or repression as part of the generation of diverse cell types. These are epigenetic modifications, as they do not alter the genetic blueprint, which remains essentially unchanged in all cells. They are robust and heritable modifications, and as such ensure that differentiated cells in adults retain their appropriate properties and individual

identity throughout adulthood. These modifications have to be erased and re-set during nuclear reprogramming.

The most dramatic demonstration of nuclear reprogramming is seen following the transfer of a somatic cell nucleus from an adult to an enucleated oocyte (unfertilized egg) (Rideout et al., 2001), a technique that has been used to generate a number of different cloned mammals since the breakthrough with Dolly the sheep (Wilmut et al., 1997). The efficiency of the process, however, remains disappointingly low, and the underlying mechanisms involved are virtually unknown. Perhaps oocytes are deficient in factors necessary to erase some types of epigenetic modifications efficiently. Oocytes are also very complex cells as they undergo a stereotypic developmental program to which a donor somatic cell nucleus must adapt rapidly. Furthermore, mammalian oocytes are both relatively small and not very numerous, which precludes some studies, such as biochemical approaches to identify the key reprogramming factors present in the oocyte. The use of ESCs by comparison represents a relatively uncomplicated alternative for studying nuclear reprogramming and for identifying key nuclear reprogramming factors.

One approach to examine whether ESCs can reprogram somatic cell nuclei is to generate heterokaryons by fusing ESCs with somatic cells (Blau and Blakely, 1999). In one such study where mouse ESCs were fused with T cells, analysis of such hybrid cells showed that the somatic nucleus did indeed acquire characteristics of the ESCs with which the somatic cells were fused (Tada et al., 2001). In the new work, Cowan and colleagues investigated whether human ES cells (hESCs) fused to human somatic fibroblasts can reprogram the fibroblast nuclei and render these cells pluripotent (Cowan et al., 2005). Their study reveals that the human fibroblast nucleus is reprogrammed in these hybrid cells. First, the original somatic fibroblast nucleus showed expression of genes associated with pluripotency, while the expression of fibroblast-specific genes was repressed. Second, the ESC-fibroblast hybrid cell exhibited properties akin to those of hESCs, including the capacity for self-renewal over at least 50 passages and the ability to differentiate into a variety of cell types. Finally, *OCT4*, a key pluripotency-specific gene that is repressed in mature fibroblasts with a methylated promoter region, became unmethylated, a necessary step for reinitiation of its expression. This is a heritable epigenetic modification, which suggests that the effects may not be just transient but could endure subsequently. Overall, the experiments suggest that hESCs probably contain key reprogramming factors that can modify a somatic cell nucleus, returning it to a pluripotent state.

This approach while encouraging requires careful considerations. First, the efficiency of the fusion process between hESCs and somatic cells is very low. This necessitates the use of drugs to select for productive hybrids from among the vast pool of unfused cells. The selection process takes about 10 days, which is a disadvantage as it precludes systematic investigation of the early events leading up to nuclear reprogramming. On the other hand, the prolonged exposure of the somatic nucleus to “reprogramming” factors from the ESC nucleus in hybrid cells may be an advantage. Sec-

ond, we cannot say unequivocally that the somatic cell nucleus is reprogrammed as it coexists with the original chromosomes from the ESC in the hybrid cell. To be sure, the ESC chromosomes need to be eliminated from the hybrid cell to see if the somatic cell nucleus continues to display its new identity. Of course, being able to remove the ESC chromosomes while retaining the reprogrammed somatic nucleus would make this procedure extremely valuable even if we do not understand the mechanism. However, this is not possible at present. Nevertheless, the likelihood that hESCs have the potential to reprogram somatic cell nuclei is a big step forward because it means that there is a large volume of biological material available for biochemical characterization of the reprogramming factors present in these cells. There is also the potential to develop cell-based assays to hunt for these key reprogramming factors.

But we also need to know the nature of the reprogramming factors themselves. A key requirement during reprogramming is the efficient erasure of existing epigenetic modifications associated with DNA and histones. Are oocytes and ESCs equivalent in this respect? Some studies suggest that certain forms of histone modifications may be erased more efficiently in the pluripotent epiblast compared to oocytes (Bao et al., 2005; Santos et al., 2003). If ESCs inherit this property from epiblast cells from which they are derived, then this could make ESCs in some respects more efficient at reprogramming somatic cells. The recent identification of a histone demethylase called LSD1 (Lee et al., 2005; Metzger et al., 2005) could be an important step forward if this or similar molecules have a role in nuclear reprogramming. Indeed, we need to understand the mechanism of nuclear reprogramming in much greater detail, and the Cowan et al. study is certainly a step in this direction.

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#### Selected Reading

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