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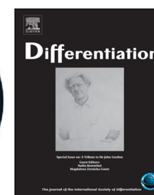

Epigenetic memory in somatic cell nuclear transfer and induced pluripotency: Evidence and implications

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ABSTRACT

Six decades ago, seminal work conducted by John Gurdon on genome conservation resulted in major advancements towards nuclear reprogramming technologies such as somatic cell nuclear transfer (SCNT), cell fusion and transcription factor mediated reprogramming. This revolutionized our views regarding cell fate conversion and development. These technologies also shed light on the role of the epigenome in cellular identity, and how the memory of the cell of origin affects the reprogrammed cell. This review will discuss recent work on epigenetic memory retained in pluripotent cells derived by SCNT and transcription factor mediated reprogramming, and the challenges attached to it.

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1. Introduction to nuclear reprogramming

Nuclear reprogramming refers to the phenomenon whereupon the nuclear content of a somatic cell can be reprogrammed into another cellular state. Reprogramming can be achieved by three different methods: somatic cell nuclear transfer (SCNT) (Gurdon et al., 1958), cell fusion (Blau et al., 1985) and transcription factor (TF) mediated reprogramming (Takahashi and Yamanaka, 2006). Herein we will focus on SCNT and TF mediated reprogramming. The first experiments on nuclear transfer, performed by Briggs and King (1952), successfully produced phenotypically normal swimming tadpoles, by transferring nuclei from embryonic cells into enucleated eggs. Most notably, Briggs and King (1952) used the nuclei from embryonic cells rather from the mature somatic cells in this study. These experiments were then followed by Gurdon et al. (1958), who used cells from later developmental stages of the African clawed frog *Xenupus laevis*. This led to the remarkable discovery that the nucleus of a fully differentiated cell from the intestinal epithelium could be injected into an enucleated egg and give rise to viable cloned swimming tadpoles (Gurdon, 1962; Gurdon and Uehlinger, 1966). In this way, Gurdon and colleagues demonstrated that genetic information was not lost during development. Instead, the mature somatic cell was able to revert back into a more primitive state upon exposure to factors inside the cytoplasm of the enucleated egg.

This discovery revolutionized our understanding of cellular plasticity in development. Since then, further refinements in cloning methods via SCNT and subsequent adaptation to the mammalian system resulted in the cloning of Dolly the sheep in 1997. This was achieved by transplanting the nuclei of adult mammary gland cells to enucleated sheep eggs (Wilmut et al., 1997). Another important achievement in the field was the derivation of mouse embryonic stem cells (ESCs) from cloned embryos. The ESCs obtained by SCNT were able to be cultured *in vitro* and differentiated into various adult cell types (Jaenisch, 2004; Munsie et al., 2000). SCNT technology also led to the successful derivation of SCNT-ESCs in primate rhesus macaques (Byrne et al., 2007; Sparman et al., 2009) and most recently human SCNT-ESCs were successfully generated. In this particular case, the nuclei of fetal fibroblasts were transplanted into human oocytes at metaphase II (Tachibana et al., 2013). The derivation of human SCNT-ESCs was also confirmed by Chung et al. (2014) and Ma et al. (2014) using adult donor fibroblasts and by Yamada et al. (2014) using fetal and adult somatic cells from Type 1 diabetes patients (Ma et al., 2014; Chung et al., 2014; Yamada et al., 2014).

Takahashi and Yamanaka (2006) revolutionised the field with the discovery that the overexpression of four transcription factors Oct4, Sox2, c-Myc and Klf4 could induce differentiated somatic cells to become ESC-like pluripotent cells, subsequently termed induced pluripotent stem cells (iPSCs). This method of TF mediated reprogramming facilitated the widespread use of the reprogramming technology in and outside of the field. iPSCs hold great potential in producing patient-specific cell types for use in disease modelling, drug screening and cellular replacement therapies (Robinton and Daley, 2012). Since its discovery, a large body of

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research has focused on understanding the mechanisms underlying the reprogramming process, in particular, the epigenetic changes underpinning this process.

2. Epigenetic memory in SCNT and TF mediated reprogramming

Differentiated cells are remarkably stable in nature and it is believed that proper cell differentiation is regulated by epigenetic mechanisms such as DNA methylation, histone variants and posttranslational histone modifications to ensure the correct genes are expressed at the precise time during development (Halley-Stott and Gurdon, 2013; Ng and Gurdon, 2008a). Epigenetic regulation patterns for a particular cell type are inherited through successive cell cycles from one generation to the next and through a specific lineage. This 'epigenetic memory' plays an important role in modulating gene function, rather than altering the DNA sequence (Halley-Stott and Gurdon, 2013).

During reprogramming, the epigenome of mature somatic cells undergo massive rearrangements to re-establish the pluripotent stem cell network (Maherali et al., 2007). Nevertheless, it has been shown in both reprogramming methods that these epigenetic rearrangements are not adequate to fully erase the epigenetic memory. This could bias the potential uses of these cells for downstream applications, as such memory affects the fidelity and quality of reprogrammed cells (Blelloch et al., 2006; Ng and Gurdon, 2005, 2008a).

For natural or *in vitro* fertilisation (IVF) reproductive technologies, approximately 95% of born offspring are phenotypically normal, but this is not the case for SCNT-derived clones where only 1% were found to be normal in mice (Wakayama and Perry, 2002) and approximately 3% for cows (Gurdon, 2006; Tsunoda and Kato, 2002). For successful SCNT reprogramming, the transplanted donor nucleus must supress the genes that have been transcribed in the donor cell, and simultaneously activate totipotency-related genes in an ordered and timely manner through a number of mechanisms. Such mechanisms include DNA replication and epigenetic modifications (e.g. chromatin remodelling and DNA methylation and demethylation) (Reik et al., 2001; Rideout et al., 2001; Shi et al., 2003). Gene-analyses have shown that 4% to 5% of the whole genome and 30–50% of imprinted genes are incorrectly transcribed in cloned mice (Hochedlinger and Jaenisch, 2003; Humpherys et al., 2002). Therefore, a comprehensive understanding of the mechanisms underpinning nuclear reprogramming, especially in aspects of epigenetic remodelling, is crucial for deriving good quality cells for clinical uses. It was observed by Gurdon and colleagues that, upon transplantation, a dramatic swelling of the donor nucleus in *Xenopus* oocytes occurred, similar to changes observed in sperm chromatin during natural fertilisation. This suggests that the relaxation of chromatin is a crucial step in both reprogramming and normal development (Byrne et al., 2003; Pfeiffer et al., 2011). Studies in *Xenopus* also showed that oocytes have a strong demethylating activity and this activity is limited to specific loci in the genome such as the promoter region of *Oct4* (Simonsson and Gurdon, 2004). In addition, high levels of enzymatic activity for histone modifications have been detected, and these enzymes provide extensive epigenetic modifications such as histone phosphorylation, methylation and acetylation upon transplantation (Bian et al., 2009; Murata et al., 2010; Pfeiffer et al., 2011). In summary, despite SCNT being able to generate functional totipotent cells which are able to give rise to entire organisms, such epigenetic modifications do not seem to be able to fully erase the donor cell specific epigenetic patterns resulting in retention of 'epigenetic memory'.

Moreover, it has been demonstrated that the epigenetic memory may persist in a cell type-specific manner. For instance, if a gene is activated during differentiation, this activated state is partially remembered and can be passed on through several cell divisions. However, there is significant variation observed for this persistence (Ng and Gurdon, 2005). Ng and Gurdon transplanted a nucleus of an endodermal cell expressing the gene *edd* (an endoderm-specific marker) to an enucleated egg. Not only did they observe expression of *edd* in a non-endodermal lineage, but they showed epigenetic memory was very stable, with no loss of such memory even after 24 cell divisions and an additional two rounds of nuclear transfer experiments. Further investigation revealed that *edd* gene expression was retained, even after the treatment of donor cells with the potent demethylating agent 5-aza-deoxycytidine. These findings indicate that DNA methylation was not the only mechanism resulting in the retention of *edd* memory, as neither the regulatory nor promoter regions of the *edd* gene required DNA demethylation for inactivation. Complementary experiments with the well-defined regulatory regions of the *MyoD* gene also suggested that memory is not regulated by DNA methylation but maintenance of *MyoD* memory required histone modifications; in particular, the incorporation of H3.3 variant in the *MyoD* gene promoter region (Ng and Gurdon, 2008b). This study suggests that gene memory may be maintained through a specific mechanism that requires either one or more epigenetic changes.

Alternatively, iPSCs generated though the forced expression of the four Yamanaka transcription factors (Takahashi and Yamanaka, 2006) can also be used to dissect the molecular mechanisms of reprogramming epigenetics. Early passage iPSCs were found to harbour residual DNA methylation, histone modifications, and consequently preserving transcriptional and metabolic signatures from the donor cell, favouring their *in vitro* differentiation along lineages related to the donor cell type (Bar-Nur et al., 2011; Kim et al., 2010a; Panopoulos et al., 2012; Polo et al., 2010; Rizzi et al., 2012). However, continuous passaging is able to attenuate epigenetic memory. (Polo et al., 2010).

In addition to the re-differentiation bias, cellular memory can affect the functional properties of iPSCs. Work by Rizzi *and collaborators* conducted a functional comparison of cardiomyocytes (CM) differentiated from cardiomyocytes-derived iPSCs (CM-iPSCs) to CM from cardiac-fibroblasts-derived iPSCs (CF-iPSCs). They compared stimulus-induced Ca^{2+} release in response to caffeine, which acts on muscle-specific ryanodine receptor channels, and they found the responsiveness levels were significantly higher in CM differentiated from CM-iPSCs than from CF-iPSCs (Rizzi et al., 2012).

The importance of cellular memory is not restricted to mice as human iPSCs (hiPSCs) also showed somatic memory persistence (Lister et al., 2011; Ohi et al., 2011). Moreover, hiPSCs derived from umbilical cord blood cells and neonatal keratinocytes were compared in terms of DNA methylation pattern and differentiation propensity. Similar to the results observed in the murine system, these hiPSCs exhibited donor cell-specific DNA methylation patterns and a propensity to differentiate into the original cell type. However, unlike early passage of mouse iPSCs, a subset of hiPSCs retained epigenetic memory even after extended passaging. In late passages, keratinocyte-derived iPSCs showed a decrease in HOXD8 gene expression, a highly methylated gene in keratinocytes. The decrease in HOXD8 restored blood-forming potential (Kim et al., 2011). Another study evaluated the levels of histone H3 acetylation, in beta cell-derived iPSCs (BiPSCs) and non-beta derived iPSCs (nbiPSCs). They found that BiPSCs had H3 acetylated *INSULIN* and *PDX1* genes, despite both genes not being expressed. This was not the case for nbiPSCs which, like ESCs, did not show H3 acetylation of these genes. Also, somatic genes like *ZNF44, CD40, NUEB1, WRD52* and *MTH1* remained

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