The use of *Xenopus* oocytes and embryos as a route towards cell replacement

J B GURDON

Wellcome/Cancer Research UK Gurdon Institute, Tennis Court Road, University of Cambridge, Cambridge CB2 1QR, UK

(Fax, 44-1223-334185*; Email, j.gurdon@gurdon.cam.ac.uk)*

When nuclei of somatic cells are transplanted to enucleated eggs of *Xenopus*, a complete reprogramming of nuclear function can take place. To identify mechanisms of nuclear reprogramming, somatic nuclei can be transplanted to growing meiotic oocytes of *Xenopus*, and stem cell genes activated without DNA replication. The combination of somatic cell nuclear transfer with morphogen signalling and the community effect may lead towards the possibility of cell replacement therapy. When mechanisms of nuclear reprogramming are understood, it may eventually be possible to directly reprogramme human somatic cell nuclei without the use of eggs.

[Gurdon J B 2005 The use of *Xenopus* oocytes and embryos as a route towards cell replacement; *J. Biosci*. **30** 11–14]

1. Introduction

Genetic determinism is a term that concerns many aspects of gene function, but it must surely include the remarkable stability of somatic cell differentiation. Soon after the beginning of development, embryonic cells enter a 'determined' state, well before they undergo differentiation into terminal cell-types. Their determined state is evident from experiments in which such cells are explanted and grown in culture, or are transplanted to parts of an embryo where they are surrounded by entirely unrelated cell-types. Once a cell has undergone determination, it will never reverse or substantially change its pathway of differentiation under explant or transplant conditions. However, if the nuclei of such determined cells are transplanted to egg cytoplasm, a dramatic reversal of their determined state takes place, and rejuvenated cells of this kind have all or nearly all pathways of differentiation open to them – thus they become totipotent or multipotent.

For this reason, nuclear transplantation offers an approach, by reversal of the determined or committed cell state, to the possibility of cell replacement, so that an individual might be supplied with cells of their own genetic constitution, but able to be made to differentiate in a wide variety of ways. This article reviews work of this kind.

2. The conservation of the genome during cell differentiation

Over 50 years ago, the first success was achieved in transplanting a living nucleus from one cell to another. In 1952, Briggs and King reported the development of normal tadpoles by the transfer of the nuclei of *Rana pipiens* blastula cells into enucleated eggs of the same species. It is important to understand the state of knowledge at that time. The structure of DNA had just been proposed that year. It was not known whether or not the different cell-types that compose a multicellular organism all have the same genetic composition. It had indeed been proposed that, as cells diversify during development, genes could be selectively lost or permanently inactivated, when there was no longer any need for their function. For example, intestine cells would permanently lose the activity of

Keywords. Community effect; morphogen signalling; nuclear transfer; *Xenopus*

genes needed for nerve and muscle differentiation, and muscle cells would lose irreversibly the potential activity of genes for lens, etc. A definitive test of this idea would be to transfer the nucleus of a cell committed to one pathway of differentiation into an egg lacking both egg and sperm pronuclei, and to ask if the egg could develop into a normal animal containing all different cell types. If it could, then clearly the somatic cell nucleus could replace the function of a multipotent zygote nucleus, and this would show that not only is the genome conserved during cell differentiation, but also that dormant genes, never to be used in the rest of the life of a somatic cell, could be reactivated by exposure to egg cytoplasm.

Briggs and King's 1952 experiment showed that it was possible to transplant a living cell nucleus. It demonstrated the multipotency of a blastula cell nucleus, but this did not test whether, as cells begin to differentiate after the blastula stage, they might lose their multipotential state. This was a very real question because, when Briggs and King transplanted nuclei from post blastula stages, such nuclei became progressively unable to support normal development, and by the neurula stage, no normal development was obtained from the nuclei of endoderm cells (Briggs and King 1957). In the meantime, success had been achieved in transplanting nuclei in the South African frog, *Xenopus laevis* (Fischberg *et al* 1958). Surprisingly the results of nuclear transfer in *Xenopus* differed from those of Briggs and King in *R. pipiens*. Although the success of nuclear transfer declined as cells became more differentiated in both *Rana* and *Xenopus*, normal adult, sexually mature *Xenopus* frogs were obtained by transplanting nuclei from endoderm and even intestine cells (Gurdon 1962). It should be realized that endoderm cells beyond the gastrula stage, and also of course intestine cells, cannot be made to change into unrelated cell-types such as muscle or nerve. Indeed a characteristic of the process of cell differentiation is its remarkable stability. Cells never normally go back from a specialized to an embryonic state, nor do they switch from one differentiated state to another. However, in contrast, the nucleus of a committed or even differentiated cell is dramatically reversed in its epigenetic state when it is made experimentally to occupy a new type of cytoplasm. This process is now called nuclear reprogramming.

The success of nuclear reprogramming is not complete. Indeed the frequency with which completely normal sexually mature animals are obtained from the nuclei of differentiated cells is very low, being about 1%. However, the success of nuclear reprogramming is much greater than this if we consider, for example, the frequency with which muscle and nerve cells can be derived from intestine cell nuclei. By combining the results of serial nuclear transfer with those of grafting cells from imperfect first-transfer embryos, it can be shown that up to 30% of intestine-derived nuclear transfers can generate functional muscle and nerve cells, something that could never happen by transplanting whole intestine cells to any part of the body or by explanting such cells and growing them in culture.

The extent of nuclear reprogramming by transplanting nuclei to enucleated eggs is very clear when gene expression assays are carried out. Long after the early nuclear transfer experiments in *Rana* and *Xenopus*, that were judged by the morphology of developing embryos, molecular assays were developed. At first, it was possible to test only the expression of multiple copy genes such as those encoding 28 s, 18 s, 5 s, and 4 s RNAs. Later, single copy 'differentiation' genes, such as those encoding muscle proteins, could be tested in nuclear transplant embryos. When this was done in *Xenopus* it was found that nuclei had become fully rejuvenated by the blastula and early gastrula stages of nuclear transplant embryo development (Gurdon 1986). This process is not perfect, and transplanted nuclei often show quantitatively incorrect reprogramming (Byrne *et al* 2003). In mammals, in which nuclear transplantation has become very successful in the last decade, it has been found that 96% of genes tested by microarray are adequately reprogrammed and 4% are not (Humpherys *et al* 2002). Interestingly, the incorrectly reprogrammed genes include oct4, a key gene needed for the maintenance of stem cell expression in mammals (Niwa *et al* 2000). It may indeed be that the lower than normal expression of oct4 in mammalian nuclear transfer embryos may help to account for the failures of nuclear transplantation from differentiated cell donors (Boiani *et al* 2002; Bortvin *et al* 2003).

3. Mechanisms of nuclear reprogramming

A matter of great current interest in this field concerns the mechanisms by which a somatic cell nucleus is reprogrammed or rejuvenated in its pattern of gene expression. An understanding of these mechanisms would throw light on the basis of the stability of cell differentiation, and would also open the way to cell replacement therapy. At present only very general statements can be made about the process of nuclear reprogramming. It has been established in mammals, that even such differentiation events as X-chromosome inactivation in female mammals, gene imprinting, and telomere replacement are reversed when somatic cell nuclei are reprogrammed (Rideout *et al* 2001; Gurdon and Byrne 2003). A number of changes associated with nuclear reprogramming take place soon after transplanted nuclei are surrounded by egg cytoplasm. These include a remarkable swelling of the nucleus and dispersion of nuclear chromatin. Also proteins are lost from transplanted nuclei, and egg cytoplasmic proteins migrate into transplanted nuclei.

A difficulty that affects attempts to identify the molecular events that accompany nuclear transfer is that the primary response of transplanted nuclei to egg cytoplasm is DNA replication and chromatin assembly. In mammals and amphibia, no new gene transcription takes place, in either nuclear transplant embryos or in embryos grown from fertilized eggs, until the mid-blastula stage (4000 cells) in amphibia, or until the 2-cell or later stage (24 h or more) in mammals. Therefore it is not at all clear when transcriptional reprogramming takes place, and the most immediate post-nuclear-transfer events may well be associated with DNA replication, since this precedes transcription.

Sorting out the role of DNA replication from other events in nuclear reprogramming has been facilitated by transplanting nuclei to oocytes of amphibia. Oocytes are the growing egg cells in an ovary, and are in the prophase of first meiosis. Oocytes cannot be fertilized, and do not divide when nuclei are transplanted into them. Eggs are deposited in the metaphase of second meiosis, and their nuclei undergo immediate DNA replication, whether eggs are fertilized or are given a transplanted nucleus. Multiple somatic cell nuclei can be injected into an oocyte, and when this is done, substantial changes in gene expression take place in the complete absence of DNA replication. Such changes were first observed by 2D protein analysis when nuclei from amphibian or mammalian species were transplanted to *Xenopus* oocytes (De Robertis and Gurdon 1977). More recently, this type of analysis has been pursued, and it has been found that nuclei of even the most differentiated mammalian cells such as those of the thymus are induced to express the stem cell marker gene oct4 (Byrne *et al* 2003). This happens in the complete absence of DNA replication, and indeed the same chromosomal DNA that was present in thymus cells is directly reprogrammed to new gene expression by exposure to an oocyte. This therefore provides an opportunity to recognize molecular events truly associated with transcriptional reprogramming, and not with DNA replication. One event of this kind appears to be the demethylation of DNA. Genes that become inactive in the course of cell differentiation, as does oct4, often become methylated in somatic cells. It seems that oocytes have an activity that can demethylate repressed genes and that this may be an essential part of the nuclear reprogramming process (Simonsson and Gurdon 2004).

Looking ahead, it may become possible to identify the mechanisms and genes responsible for DNA demethylation and other reprogramming events, so that such genes can be over-expressed in somatic cells, opening the way to initiating the rejuvenation of somatic cell nuclei, and hence to the derivation of embryonic stem cells from adult cells. This might lead to a means of providing people with rejuvenated cells of their own genetic constitution, and hence of avoiding the need for immunosuppression after cell or tissue replacement.

4. Directed differentiation of cells obtained by nuclear reprogramming

For the purposes of cell replacement, it will be necessary not only to derive rejuvenated cells of the same genetic constitution as adult donor cells, but also to be able to proliferate these and to obtain a homogeneous population of cells of one type. Since the pioneering work of Evans *et al* in 1981, it is known that mammalian embryo cells can be made to enter an indefinite state of proliferation, while retaining an ability to differentiate into a variety of different cell-types, when for example transplanted back to host embryos. These 'embryonic stem cells' (ES cells) can now be derived from a variety of mammalian species including humans. Extensive efforts are currently in hand to find ways of directing embryonic stem cells into homogeneous groups of cells of one kind. Almost all of this work is being undertaken with mammalian ES cell lines in culture.

Work with amphibia, which I now summarize, has given two particular insights into this problem. Most of the early events that lead to the differentiation of embryonic cells in normal development depend on the emission of secreted signalling molecules from certain regions of an embryo. Such 'signalling centres' include the Nieuwkoop Centre and the Spemann Centre. Molecules that are released from these regions act as 'morphogens', in that they form a concentration gradient spreading out from their sources. Most importantly, responding cells in the pathway of such a concentration gradient are able, with extraordinary sensitivity, to detect a concentration of the extracellular morphogen, and to select an appropriate differentiation pathway. Very often these morphogens are members of the TGF*b* class of signal factors. Responding cells will choose one of as many as 5 fundamentally different fates according to the concentration of morphogen that they detect. Following a detailed analysis of how this might be done, we have come to the view that the key mechanism is the steady state concentration of nuclear transduction molecules (Bourillot *et al* 2002; Gurdon and Bourillot 2001). For example, in signalling by the TGF*b* factor activin, the intracellular transduction molecule is activated Smad2, and this is continuously transmitted to the nucleus at a rate corresponding to the absolute number of occupied activin receptors. This may be a basic mechanism by which embryonic cells, whether obtained by nuclear reprogramming or from fertilized eggs, embark on a selected differentiation pathway.

But it turns out that this is not the whole story. When cells have read an extracellular concentration of morphogen,

they undergo only the first step of their differentiation pathway. This entails expressing the first of a cascade of transcription factors, but this is not sufficient to force a population of such cells to complete a differentiation process. It has turned out that these cells must also communicate with each other by secreting another different extracellular signal factor. This secondary factor also works in a concentration-dependent way, such that cells must experience an above-threshold concentration of it to progress further down the chosen differentiation pathway. All the cells that have experienced a particular concentration of the primary morphogen (e.g. activin) must secrete the secondary factor (in this case, a particular member of the FGF family), so that all cells in close proximity to each other receive the sufficient concentration to express the next gene in the pathway (in this case, MyoD, in the muscle pathway). Because cells must be in close proximity to each other to experience a sufficient concentration, this process is called a 'community effect' (Standley *et al* 2001). If cells releasing the community factor are too far apart from each other, they are not part of a community, and the required concentration is not reached. By the combined effect of the interpretation of a morphogen gradient, and a subsequent community effect, a group of naïve blastula cells can be directed to make a homogeneous group of cells of one differentiated type.

5. Conclusion

The nuclear transfer experiments outlined above constitute only the beginning of the long-term aim of understanding the molecular mechanisms of nuclear reprogramming, and of signal factor interpretation by embryonic cells. It seems inevitable, since somatic cells possess the same genome, that ways will eventually be found of rejuvenating readily accessible adult cells to an embryonic state, and of directing such cells into new desired directions of differentiation.

It seems unlikely that we will be able to rejuvenate the memory of neural cells, but the physical properties of cells should be reversible, and 'young' cells, capable of differentiation into various adult cell-types should be obtainable. It is obvious that this reversal of cell differentiation might have profound benefits for mankind.

References

Boiani M, Eckardt S, Scholer H R and McLaughlin K J 2002 Oct4 distribution and level in mouse clones: consequences for pluripotency; *Genes Dev.* **16** 1209–1219

Bortvin A *et al* 2003 Incomplete reactivation of Oct4-related genes in mouse embryos cloned from somatic nuclei; *Development* **130** 1673–1680

Bourillot P Y, Garrett N and Gurdon J B 2002 A changing morphogen gradient is interpreted by continuous transduction flow; *Development* **129** 2167–2180

Briggs R and King T J 1952 Transplantation of living nuclei from blastula cells into enucleated frogs' eggs; *Proc. Natl. Acad. Sci. USA* **38** 455–463

Briggs R and King T J 1957 Changes in the nuclei of differentiating endoderm cells as revealed by nuclear transplantation; *J. Morph.* **100** 269–312

Byrne J A, Simonsson S, Western P S and Gurdon J B 2003 Nuclei of adult mammalian somatic cells are directly reprogrammed to oct-4 stem cell gene expression by amphibian oocytes; *Curr. Biol.* **13** 1206–1213

De Robertis E M and Gurdon J B 1977 Gene activation in somatic nuclei after injection into amphibian oocytes; *Proc. Natl. Acad. Sci. USA* **74** 2470–2474

Evans M J and Kaufman M H 1981 Establishment in culture of pluripotential cells from mouse embryos; *Nature* (*London*) **292** 154–156

Fischberg M, Gurdon J B and Elsdale T R 1958 Nuclear transplantation in *Xenopus laevis*; *Nature* (*London*) **181** 424

Gurdon J B 1962 The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles; *J. Embryol. Exp. Morphol.* **10** 622–640

Gurdon J B 1986 Nuclear transplantation in eggs and oocytes; *J. Cell Sci. Suppl.* **4** 287–318

Gurdon J B and Bourillot P Y 2001 Morphogen gradient interpretation; *Nature* (*London*) **413** 797–803

Gurdon J B and Byrne J A 2003 The first half-century of nuclear transplantation; *Proc. Natl. Acad. Sci. USA* **100** 8048–8052

Humpherys D, Eggan K, Akutsu H, Friedman A, Hochedlinger K, Yanagimachi R, Lander E S, Golub T R and Jaenisch R 2002 Abnormal gene expression in cloned mice derived from embryonic stem cell and cumulus cell nuclei; *Proc. Natl. Acad. Sci. USA* **99** 12889–12894

Niwa H, Miyazaki J and Smith A G 2000 Quantitative expression of Oct-3/4 defines differentiation dedifferentiation or self-renewal of ES cells; *Nat. Genet.* **24** 372–376

Rideout W M 3rd, Eggan K and Jaenisch R 2001 Nuclear cloning and epigenetic reprogramming of the genome; *Science* **293** 1093–1098

Simonsson S and Gurdon J 2004 DNA demethylation is necessary for the epigenetic reprogramming of somatic cell nuclei; *Nat. Cell Biol.* **6** 984–990

Standley H J, Zorn A M and Gurdon J B 2001 eFGF and its mode of action in the community effect during Xenopus myogenesis; *Development* **128** 1347–1357

*e*Publication: 7 January 2005