Nuclear Transfer to Eggs and Oocytes

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We review experiments in which somatic cell nuclei are transplanted singly to enucleated eggs (metaphase II) in amphibia and mammals and as multiple nuclei to the germinal vesicle of amphibian oocytes (prophase I). These experiments have shown the totipotency of some somatic cell nuclei, as well as switches in cell type and changes in gene expression. Abnormalities of nuclear transplant embryo development increase greatly as nuclei are taken from progressively more differentiated donor cells. The molecular changes that accompany the reprogramming of transplanted nuclei help to indicate the mechanisms used by eggs and oocytes to reprogram gene expression. We discuss the importance of chromosomal protein exchange, of transcription factor supply, and of chromatin access in reprogramming.

Cells of the female germline are unique because they form eggs that are the direct ancestors of all cells of the body including the future eggs and sperm. After normal fertilization, 100% of eggs can form complete fertile individuals. Even when not fertilized, activated eggs of most species, especially when made diploid, can develop into remarkably complete organisms with nearly all somatic cells though usually not the germline. In accord with this totipotency, eggs of many species can also develop entirely normally when provided with the nucleus of a somatic cell in place of egg chromosomes or a sperm nucleus. Somatic cells do not have the ability to generate a complete organism and the nucleus of a somatic cell must be reprogrammed if it is to participate in normal development with an enucleated egg. We review the extent to which a transplanted somatic nucleus can, in combination with an enucleated egg, generate a normal individual. We first describe the extent to which normal development results from somatic cell nuclear transfer. We then discuss the extent to which this does not happen, especially when nuclei from differentiated somatic cells are used. Finally, we discuss possible mechanisms by which the reprogramming of the somatic nucleus is induced after transfer to eggs or oocytes.

The original reason for wishing to carry out nuclear transfer to eggs was to determine whether the genome of somatic cells is complete in the sense of containing copies of all genes in the genome. Up until the 1950s, it was thought possible that genes could become lost or permanently inactivated in those cells that follow different lineages in which certain genes would never normally be required. Over the last half
century, nuclear transfer and some other proce-
du res have established the general principle that
the genome is conserved during development,
so that almost all somatic cells contain a com-
plete copy of the original zygote genome (Gur-
don and Byrne 2003). In more recent time this
situation has been used as a basis of procedures
for cell replacement. It has become possible to
derive all kinds of cells of the body from a
somatic cell already committed to a particular
lineage (Takahashi and Yamanaka 2006). This
ability has opened up the possibility of provid-
ing replacement cells of many different kinds
starting from a specialized somatic cell. In this
way it is, in principle, possible to provide an
individual with replacement cells of their own
 genetic constitution, thereby avoiding the need
for immunosuppression in any cell replacement
therapy.

We have not attempted to give a detailed
review of the nuclear transfer literature, but
refer to several other reviews for different as-
pects of this problem (Kikyo and Wolfe 2000;
Cibelli et al. 2002; Morgan et al. 2005; Collas
and Taranger 2006; Meissner and Jaenisch 2006;
Yang et al. 2007).

EXPERIMENTAL SYSTEMS
The basic procedure, by which a living cell
nucleus is transplanted to an egg or oocyte,
was established by Briggs and King (1952).
They used Rana padiens and sucked a blastula
cell into a micropipette so that the cell wall
was broken but the nucleus remained intact
and covered by cytoplasm. The whole cell was
injected into an unfertilized egg in second mei-
otic metaphase (M2). The egg was enucleated
manually by removing the metaphase spindle
with its chromosomes from the surface of the
egg. The same procedure is used for eggs of
Xenopus, except that the egg chromosomes are
destroyed by ultraviolet irradiation; the large
egg size of amphibia and the low penetrance
of ultraviolet irradiation ensures that no sig-
nificant damage is done to the rest of the egg.
A key characteristic of early Xenopus egg nuclear
transfer experiments was the ability to make
use of the one-nucleolated mutant as a genetic
marker to prove that development resulted
from the implanted nucleus and not from a
failure of enucleation (Elsdale et al. 1960).
Amphibian eggs do not need activation when
they have been penetrated with a nuclear trans-
fer pipette. In nearly all mammalian nuclear
transfer experiments, unfertilized eggs in sec-
ond meiotic metaphase are used as recipi-
ents. Nuclear injection in mammals is usually
achieved manually or by cell fusion with a
micropipette as in amphibia, but in some spe-
cies piezo-electric needle penetration is used.
Enucleation is achieved by removal of the fe-
nale pronucleus in second meiotic metaphase
with a microinjection pipette. Activation is by
procedures that differ between the species, and
in the mouse is usually performed by the addi-
tion of SrCl2 to the medium. Activation is nor-
 mally performed within a few hours of nuclear
injection and enucleation.

An altogether different nuclear transfer pro-
cedure is to use growing eggs in first meiotic
prophase (M1); in amphibia, these cells (usually
called oocytes) are present in huge abundance
in the ovary. Somatic nuclei can be injected
into the specialized and enlarged nucleus (ger-
minal vesicle, GV) of the ovary (Byrne et al.
2003) the contents of the GV are essential for
normal development (Gao et al. 2002). Injected
nuclei remain in the germinal vesicle for many
days; there is no cell division and no DNA
replication but intensely active transcription.

An advantage of this experimental system is
that it is useful for analyzing the changes under-
gone by transplanted nuclei as they become
transcriptionally reprogrammed. Twenty-five
 thousand oocytes per frog, and up to five
hundred injected nuclei per oocyte, make
molecular analysis of transcriptional reprog-
ramming practicable. In contrast to oocytes,
eggs are primarily active in DNA replication,
and transcription does not start until the four
 thousand-cell stage of the amphibian blastula
(5 h) or until the two-cell stage in mouse
embryos (20 h). The immediate responses of
somatic nuclei to injected eggs (M2) are there-
fore more likely to be associated with the induc-
tion of DNA synthesis than with transcriptional
reprogramming.
BACKGROUND

The background to the field of somatic cell nuclear transfer has been outlined by Gurdon (2006). Following the initial success of Briggs and King (1957), subsequent work with *Rana pipiens* found that nuclei from postgastrula cells could no longer support normal development (Briggs and King 1957). *Xenopus* experiments on the other hand continued to give entirely normal development in some nuclear transplant embryos even with nuclei from differentiated intestinal epithelial cells of tadpoles. The sequence of events in mammalian nuclear transfer has been summarized by Meissner and Jaenisch (2006) and others. The finding of McGrath and Solter (1984) that even nuclei from eight cell mouse embryos did not support normal development was later attributed to the use of fertilized eggs as recipients rather than of unfertilized eggs as used in earlier amphibian experiments and in most subsequent mammalian work. Initial success was obtained with embryo nuclei transplanted to eggs of sheep (Willasden et al. 1986) and to those of cows and pigs (Prather et al. 1989). A major advance took place when adult sheep were obtained from nuclei of a cultured line of cells grown from sheep embryo cells (Campbell et al. 1996) and then from nuclei of an adult sheep cell line (Wilmut et al. 1997). Since then a wide range of mammalian species has been cloned (production of fertile adults). Nuclear transfer, and hence evidence for nuclear programming, has been successful in a wide range of mammalian species (Lanza et al. 2001b; Wakayama et al. 1998). In some cases, technical maneuvers such as the timing and type of activating stimulus are needed to give the best results. In a few mammalian species tested, a small proportion of nuclei from differentiated or adult tissues have given normal adult nuclear transfer development (terminal lymphocytes [Hochedlinger and Jaenisch 2002]; postmitotic neurons [Eggan et al. 2004; Li et al. 2004]). In these cases tetraploid rescue was used, but Inoue et al. (2004) overexpression has the great practical benefit of deriving proliferating ES cells, and hence all other cells types, from accessible adult cells without the need to use eggs which would be hard to obtain in humans. Nuclear transplantation into eggs or oocytes is of interest because it uses the natural reprogramming activity of eggs on the sperm after normal fertilization without the need for induced gene overexpression, and because it gives a relatively high efficiency of transcriptional activation. Eventually, it may be possible to identify the molecules and mechanisms of nuclear reprogramming used by eggs, and hence to use these molecules to enhance the efficiency of reprogramming by other procedures.

NORMAL DEVELOPMENT

Totipotency

The ultimate test of reprogramming of a transplanted somatic cell nucleus is to ask if it can yield a normal fertile sexually mature adult when transplanted to an enucleated egg. This was performed in *Xenopus* when a considerable number of fertile males and females were grown from enucleated eggs injected with nuclei from intestinal epithelial cells of feeding tadpoles (Gurdon and Uehlinger 1966). The demonstration that the nucleus of a cell in an adult animal can yield, after nuclear transfer, a complete sexually mature adult was achieved with sheep nuclear transfers (Wilmut et al. 1997). Dolly, the sheep, lived for 6 years, a life span within the normal range of sheep reared indoors. Dolly was as fertile as a normal sheep raised indoors. Since that time, a somatic cell nuclear transfer has been successful in a wide range of mammalian species (Lanza et al. 2001b; Wakayama et al. 1998). In some cases, technical maneuvers such as the timing and type of activating stimulus are needed to give the best results. In a few mammalian species tested, a small proportion of nuclei from differentiated or adult tissues have given normal adult nuclear transfer development (terminal lymphocytes [Hochedlinger and Jaenisch 2002]; postmitotic neurons [Eggan et al. 2004; Li et al. 2004]). In these cases tetraploid rescue was used, but Inoue et al. (2004)
obtained adults from postmitotic neurons without tetraploid rescue. In conclusion, at least 1%–3% of both differentiated and adult cells contain totipotent nuclei. Embryonic stem cell lines have been derived in the mouse, cow, and monkey (Munsie et al. 2000; Wakayama et al. 2001; Byrne et al. 2007). A claim of human ES cells derived from nuclear transfers was retracted (Kennedy 2006).

There is one exception to the results summarized above, and this is the particular case of antibody-forming cells, in which gene rearrangement accounts for the huge diversity of antibodies that cells can make. Nuclei transplanted from T-cells form normal mice except that they make only one kind of antibody (Hochedlinger and Jaenisch 2002).

We can conclude that, with the special exception of cells in which genes are known to undergo rearrangement, the genome is conserved during cell differentiation.

Switches in Cell Type

From most points of view including the prospect of cell replacement therapy, we are much more interested in the extent to which one particular type of cell can be derived from cells of another unrelated cell-type, rather than in totipotency. For example, can brain or heart cells be derived from skin or blood? The efficiency of reprogramming at this more limited level is much higher than when assessed by the production of fertile adults. In *Xenopus* nuclear transfer experiments from intestinal epithelium, we can ask with what frequency functional muscle and nerve cells are obtained, as judged by the formation of embryos that make muscular movements when stimulated with a needle. We can take into account those embryos that result from serial nuclear transfer after taking donor nuclei from the normal-appearing cells of an otherwise defective nuclear transplant embryo, as well as muscle and nerve cells resulting from the grafting of such abnormal embryo-derived cells to fertilized egg-derived recipients. GFP-marked donor nuclei in the combination of these experiments give an overall efficiency of about 30%; that is to say, nearly one third of all intestinal epithelial cell nuclear transfers produce functional muscle and nerve cells. In other *Xenopus* work, nuclei from a range of adult tissues including foot-web skin, lung, heart, etc., yielded normal feeding tadpoles (Laskey and Gurdon 1970; Gurdon et al. 1975). Although the proportion of total nuclear transfers that developed to feeding tadpoles was very small (2%–3%), these results established the principle that the nuclei of differentiated and adult cells can be reprogrammed to generate a wide range of unrelated cell-types. The same conclusion is probably true of mammals, because ES cells, and hence a range of somatic cell types, can be derived from many adult mammalian tissues.

Early Gene Expression

It was found long ago that embryos derived from transplanted muscle nuclei discontinue muscle gene transcription soon after nuclear transfer, but recommence muscle gene expression in muscle but not in other cell-types (Gurdon et al. 1984). Microarray analyses give an approximate measure of transcription of many genes; these conclude that over 95% of several thousand genes assayed are correctly transcribed in early mouse nuclear transplant embryos (Humpherys et al. 2002; Beyhan et al. 2007; Vassena et al. 2007). We can conclude that nuclear transfer to eggs causes a major reprogramming of the great majority of genes. The genes that are not reprogrammed correctly may account for some of the abnormal embryo development always seen in nuclear transfer experiments.

Cancer Nuclei

Nuclei transplanted from the Herpes virus related frog Lucké adenocarcinoma cells yielded tadpoles with a wide range of normal cell types (McKinnell et al. 1969). Blelloch et al (2004) transplanted nuclei from mouse embryonal carcinoma cells to mouse eggs; the resulting blastocysts gave rise to embryonic stem cells that, when transferred to hosts, displayed the same range of abnormalities as the donor embryonal carcinoma cells. There is therefore no evidence that nuclear transfer changes the genetic state
of donor cells. We can conclude that a cancerous state is compatible with normal early development and with some kinds of somatic cell differentiation.

Cross-Species Nuclear Transfer

An appealing idea is to generate human ES cells from a human cell nucleus transplanted to enucleated eggs of cows, pigs, rabbits, etc. The earliest nuclear transfer experiments across related species within the genera *Rana* or *Xenopus* always resulted in embryo death (Moore 1960; Gurdon 1962b). In mammals, most such experiments have not been published, but it is generally agreed that nuclear transfers between mammalian species (defined as ones in which cross-fertilization gives no survival) die at close to the stage when zygotic transcription starts; this is at the two-cell stage in mice, at the 8–16 cell stage in cows, pigs, and humans. In spite of this, very early postnuclear transfer events may succeed in cross-species combination, such as sperm demethylation (Beaujean et al. 2004b). A report of proliferating ES cells from a human-rabbit nuclear transplant combination (Chen et al. 2003) has yet to be confirmed.

ABNORMAL DEVELOPMENT

Increase with Progressive Cell Differentiation

It has been known since the earliest nuclear transfer experiments that the normality of nuclear transplant embryo development decreases dramatically as nuclei are taken from increasingly differentiated cells (Briggs and King 1957; Gurdon 1962a). The same is true of mammalian experiments (Hochedlinger and Jaenisch 2002).

A question of great interest is whether the type of nuclear transplant abnormalities is related to the type of donor cells. In amphibia, nuclear transplant embryos die progressively throughout development and there is no morphological indication of any particular type of developmental abnormality in relation to donor cell-type. In mammals, the most obvious defects all relate to the placenta and it appears that reprogramming is much less successful in the trophectoderm than in the inner cell mass. Commonly observed abnormalities include shorter life span, enlarged placenta, obesity, respiratory problems, and large offspring syndrome (Yang et al. 2007). It was suggested that developmental abnormalities observed in amphibian nuclear transplant embryos might be inversely related to the cell-type from which nuclei were taken (Briggs and King 1960). However, the numbers in this experiment were too small to be significant, and subsequent experiments with *Xenopus* neural cell nuclei failed to show any morphological correlation (Simnett 1964). When assessing the developmental abnormalities of nuclear transplant embryos it is important to appreciate that such embryos have had their zona layers removed and their development should therefore be compared to that of ICSI embryos (intracytoplasmic sperm injection), because the culture medium of zona-free mouse embryos can adversely affect the normality of their development.

Epigenetic Memory

An entirely unexpected result was encountered when a transcriptional analysis was made of *Xenopus* nuclear transplant embryo development (Ng and Gurdon 2008). In this case, nuclei were transplanted from a defined cell type such as somite muscle, neural cells, or from the endoderm (future intestine). When the resulting embryos reached the blastula stage, they were divided into future neurectoderm, mesoderm (including) muscle, and endoderm regions and the embryo parts were cultured for another day until they reached a stage of lineage-specific gene expression, when they were analyzed for marker gene expression. The surprise was that, in about half of all embryos analyzed, an excessive abundance of transcripts characteristic of the donor cell-type was observed in an inappropriate lineage. For example, the neurectoderm and endoderm parts of embryos resulting from the transfer of muscle nuclei had expressed muscle genes to an excessive extent, even though the same regions of control embryos grown from fertilized eggs showed no such aberrant transcription. This
memory of a former state of gene expression was especially remarkable because Xenopus embryos, whether from fertilized eggs or from transplanted nuclei, are inactive in transcription for their first 12-cell cycles. This example of epigenetic memory is therefore able to persist through multiple cell divisions in the absence of transcription. This effect is seen in genes that mark lineage determination, such as MyoD, and not in those that represent terminal differentiation. A somewhat related phenomenon has been described in mouse muscle nuclear transfers when the culture of these in myoblast culture medium promotes a continued expression of muscle cell markers (Gao et al. 2002). We can conclude that, judged by gene expression, reprogramming by eggs is about 50% efficient, at least for nuclei of cells that are undergoing, but have not yet completed, differentiation. Incomplete reprogramming may account for some of the nuclear transplantation abnormalities observed.

Early Gene Expression

Most attempts to estimate the normality or defects in early gene expression of nuclear transplant embryos have used a microarray technology. The general finding is that the vast majority of genes, which are active in early development but not in adult cells, show clear evidence of reprogramming. Humpherys et al. (2002) saw abnormalities in 4% of the genes tested. Smith et al. (2005) report that 99% of genes tested appeared to be normally expressed and therefore potentially reprogrammed. The methods used are not very quantitative and an incomplete reprogramming of a gene would not always be seen. For genes in which the amount of product may be critical for early development (e.g., Oct4) incomplete reprogramming may be damaging (Boiani 2002; Bortvin et al. 2003).

MOLECULAR CHANGES

Somatic Mutation

Assuming a gene mutation rate of $10^{-6}$ per gene per cell, a mouse embryo containing $10^9$ cells might have accumulated $10^3$ mutations in its cells. Most of these are unlikely to be in genes needed for early development, and most mutations are also unlikely to inactivate a gene. Furthermore, even nuclei transplanted from embryo cells, with a theoretically much lower load of accumulated mutations, result in a substantial number of early developmental abnormalities. We conclude that naturally accumulated mutations in donor cells are very unlikely to account for the majority of nuclear transplant embryo abnormalities.

DNA Methylation

It is well established that the control regions of genes that are wholly inactive in most somatic cells undergo methylation, and that this contributes to the silencing of those genes. A good example is the promoter of Oct4, a gene that must be expressed soon after nuclear transfer to eggs if development is to proceed (Niwa et al. 2000). DNA demethylation hardly ever takes place in differentiated somatic cells, but takes place to some extent, though not efficiently, in nuclear transplant embryos (Beaupre et al. 2004a; review by Morgan et al. 2005). DNA demethylation is known to be imperfect in some embryos as judged by antibody staining of transplanted nuclei (Bortvin 2003; Boiani 2002). Because the level of Oct4 expression is critical for early mouse development, it is likely that incomplete Oct4 DNA demethylation may account for abnormalities of development in nuclear transplant embryos. Because demethylation of the Oct4 promoter is seen in nuclei transplanted to nondividing Xenopus oocytes (Simonsson and Gurdon 2004), the process of demethylation cannot depend on loss by nonreplacement in dividing cells as it is supposed to do for the mammalian female pronucleus. A candidate facilitator for DNA demethylation that has been proposed is Gadd45a (Barreto et al. 2007). Other candidates include components of the DNA repair process (Niehrs 2009).

DNA methylation is believed to be involved in mammalian female X chromosome inactivation, and in mammalian imprinting. Although some degree of X chromosome reactivation has been reported in transplanted nuclei (Nolen
et al. 2005) the efficiency of this process is not clear and an incomplete reactivation of Xi probably contributes to the abnormal development of nuclear transplant embryos.

Telomere Replacement

The low expression of telomerase in most somatic cells compared to the high level in embryo and embryo stem cells leads us to expect that normal nuclear transplant embryos would contain an increased content of telomeres on their chromosomes compared to the somatic cells from which they are derived. The terminal restriction fragment of DNA (that contains telomeres) was smaller in all three nuclear transfer sheep analyzed than in age-related controls (Wilmut et al. 1997). On the other hand, six calves had a higher level of an age-related gene transcript than controls of similar age (Lanza et al. 2000). The fact that the serial transfer of nuclei in mice is successful for seven generations argues against an irreversible shortening of telomeres (Wakayama 1998). In general, telomere length seems to be restored in cloned fetuses. However, it is not possible to determine the telomere content of a single cell that is used to donate a nucleus for successful nuclear transfer; the telomere characteristic of that donor nucleus must be assumed to be like the average of the donor cell population. We can therefore conclude that some telomere replacement probably occurs after nuclear transfer.

Epigenetic Modification

Modifications to histones in transplanted nuclei have been studied in both mammals and frogs (review by Morgan et al. 2005). The most commonly used procedure is to stain transplanted nuclei with antibodies specific for particular histone modifications. This type of assay has revealed variable changes in nuclei transplanted to eggs (Wang et al. 2007). For example, H3K9 is deacetylated but later reacetylated but other acetylations show no change. H3K9 is demethylated in some cases, but not in others.

A general observation is that these changes are seen in nuclei transplanted from differentiated cells. These then come to resemble, in this respect, transplanted ES nuclei, which do not themselves undergo these changes. It has to be assumed that these changes are made on histones that are components of chromatin rather than in the nucleoplasm of transplanted nuclei. These changes are seen in nuclei transplanted to both eggs and oocytes, and are not therefore correlated with transcription rather than DNA replication. In future it will be important to know whether these are global changes to the whole chromatin or are localized to those particular genes that undergo reprogramming. There is some evidence that tri-chostatin A treatment of nuclear transplant embryos can improve their survival (Kishigami et al. 2006).

It is interesting that some of the chromatin features of embryo nuclei are already seen in sperm (Hammoud et al. 2009). Because transplanted somatic nuclei are not exposed to conditions that prevail in sperm, it may be hard for these chromatin characteristics to be correctly imposed by eggs.

Chromatin Decondensation and Protein Exchange

The most obvious first response of transplanted nuclei to eggs and oocytes is a huge increase in volume. In frogs, this can be as much as 30-fold in 1 h for eggs and similarly for oocytes on a slower time scale. The same effect is seen in mammalian eggs, but more slowly. In Xenopus oocytes this enlargement includes the dispersion of chromatin and is correlated with transcriptional activation (Gurdon et al. 1976). A massive nuclear volume increase is also seen in sperm soon after fertilization in mammals and frogs. Nucleoplasmin is present at an enormous concentration of 7 mg/mL in Xenopus eggs (Leno et al. 1996), and is in part, but not wholly, responsible for chromatin dispersal (Tamada et al. 2006). The major function of nucleoplasmin is to act as a chaperone for histone H2a and b. N1 and N2 serve a similar function for histone H3 and H4. The oocyte-specific linker histones are also present in a very high concentration in eggs and oocytes. The loosening or decompaction of chromatin is a primary event in nuclear
reprogramming, and seems to set the stage for ensuring transcription or DNA replication.

It has long been known that somatic nuclei transplanted to eggs and oocytes of *Xenopus* soon exchange proteins between nucleus and cytoplasm; about 80% of the proteins brought in with a transplanted nucleus are lost within a few hours, and there is an obvious immigration of cytoplasmic proteins into transplanted nuclei (Gurdon et al. 1976). This may reflect the normal rapid exchange of proteins between nucleus and cytoplasm in most kinds of cells; the very large volume of eggs and oocytes will result in loss being much more evident than uptake. The abundant proteins of oocytes are among those taken up by transplanted nuclei; labeled histones present in oocyte cytoplasm, whether by protein or mRNA injection, become intensely concentrated in the germinal vesicle and in transplanted nuclei. The rapid replacement of the mammalian linker histone H1foo may be related to chromatin decondensation (Teranishi et al. 2004). There is, at present, no clear evidence that the loss or gain of a particular protein is causally connected with induced transcription or replication of transplanted nuclei.

**Cell Extracts**

A preferred route by which to identify functional components of cells is to use extracts of cells that have a desired activity. Depletion of an extract by antibodies or by other means can identify components of the extract that make a necessary contribution to the activity being analyzed. This experimental design has been particularly successful in identifying components of *Xenopus* eggs that are required for DNA replication (Gonzalez et al. 2005). However, a substantial problem is that, so far, no one has succeeded in making an extract of cells that can carry out transcriptional reprogramming in vitro. Three types of experiment go some way toward this objective.

One is to use a cell-free extract derived from eggs or oocytes to describe changes that take place in nuclei or permeabilized cells, even though these changes do not include new transcription. Important work within this category is that of Kikyo and Wolffe (2000) who described the ISWI-induced loss of TBP (TATA box binding protein) from *Xenopus* cultured cell nuclei incubated in *Xenopus* egg extracts. Subsequent work found that FRGY2 in egg extracts causes a reversible disassembly of nucleoli in somatic nuclei (Gonda et al. 2003), and that nucleoplasmin in eggs is required for chromatin dispersal (Tamada et al. 2006). These and other changes are listed in Table 1.

A second route based on cell-free extracts involves the addition of extracts to lightly permeabilized cells, which are then resealed by Ca\(^{++}\) and cultured until transcription starts (Teranger et al. 2005; Collas and Taranger 2006). With this experimental design, Hansis et al. (2004) found that Brg-1 is needed to permit reactivation of Oct4. All these experiments analyze extracts of eggs, and may therefore identify components needed for DNA replication as opposed to transcription (see above). Extracts of porcine oocytes show the entry of linker histone B4 and lamin III into

<table>
<thead>
<tr>
<th>Donor nuclei</th>
<th>Extract of eggs or oocytes</th>
<th>Duration</th>
<th>Responses</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Xenopus</em> XTC cells</td>
<td><em>Xenopus</em> S phase</td>
<td>2 h</td>
<td>Tata protein release by ISWI</td>
<td>Kikyo and Wolffe 2000</td>
</tr>
<tr>
<td>Bovine fetal fibroblast</td>
<td><em>Xenopus</em> egg or oocyte</td>
<td>3 h</td>
<td>Lamin A/C removed; PolII transcription maintained</td>
<td>Alberio et al. 2005</td>
</tr>
<tr>
<td><em>Xenopus</em> embryo fibroblasts</td>
<td><em>Xenopus</em> S phase egg</td>
<td>2 h</td>
<td>FRGY2a+b; nucleolus disassembly</td>
<td>Gonda et al. 2003</td>
</tr>
<tr>
<td><em>Xenopus</em> embryo fibroblasts</td>
<td><em>Xenopus</em> eggs</td>
<td>2 h</td>
<td>Chromatin decondensation; H3 histone phosphorylation + acetylation; nucleoplasmin</td>
<td>Tamada et al. 2006</td>
</tr>
</tbody>
</table>
permeabilized fibroblasts, which when resealed and cultured, can activate pluripotency genes (Miyamoto et al. 2007, 2008, 2009). An extract of axolotl oocytes is able to demethylate DNA to reduce H3K9Me3 and to increase H3K9Ac so that nuclei of differentiated cells become like those of ES cells (Bian et al. 2009). These latter changes are seen in extracts of oocytes, and are therefore likely to be relevant to transcriptional reprogramming. See Table 2 for a list of these results.

**MECHANISMS**

The aim of this section is to discuss possible mechanisms by which eggs or oocytes can successfully reprogram somatic cell nuclei to behave like embryo nuclei. In view of the slow or nil division rate and specialized gene expression of most somatic cells, it is remarkable that normal development ever results from nuclear transfer. However, there are two respects in which reprogramming does not succeed well. Compared to fertilized eggs, almost all of which develop normally, only about 30% of even blastula or blastocyst nuclei elicit normal development, and with the nuclei of differentiated cells only about 2% of total transfers become adults. The incomplete functioning of any of the mechanisms we discuss could help to explain developmental defects in nuclear transplant embryos.

### The Initiation of DNA Replication

In amphibia, the first cell division takes place 1.5 h after fertilization or nuclear transfer, and DNA replication of transplanted nuclei in GI needs to precede this. Eggs have a strong DNA synthesis inducing activity (Graham et al. 1966). This is brought about by a great decrease in the spacing of origins of replication (Lemaitre et al. 2005). Even nondividing adult brain nuclei respond to egg cytoplasm by initiating DNA synthesis, but chromosome replication is sometimes incomplete by the time of the first cell division, and partially replicated chromosomes are pulled apart leading to damaged cleavage nuclei and defective cleavage (Di Berardino and King 1965). In mammals, the long (20 h) first cell cycle gives no reason to attribute the 50% of nuclear transplants, even from cumulus nuclei that fail to reach the two-cell stage, to failures of DNA replication.

### Chromosomal Protein Exchange at Mitosis

An interesting idea that has surfaced over many years is that the exchange of chromosomal proteins that takes place in chromosomes during mitosis may facilitate changes in cell differentiation, and these could include nuclear reprogramming. There is indeed a general release of chromosomal proteins at mitosis. In the course of normal development, it is supposed that cells remaining in the same lineage

#### Table 2. In vitro culture followed by resealing of cells

<table>
<thead>
<tr>
<th>Extract source</th>
<th>Nuclei</th>
<th>Duration</th>
<th>Responses</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xenopus eggs or oocytes</td>
<td>Human 293 T and leucocytes</td>
<td>30 min, then 7 d</td>
<td>Oct4 and GCAP; Brg required</td>
<td>Hansis et al. 2004</td>
</tr>
<tr>
<td>Xenopus egg</td>
<td>Porcine fibroblast</td>
<td>2 h, then 10 d</td>
<td>B4 histone and nuclear lamin uptake; Oct4 &amp; Sox2 activation</td>
<td>Miyamoto et al. 2007</td>
</tr>
<tr>
<td>Porcine oocytes (GV)</td>
<td>Porcine fibroblast</td>
<td>2 h, then 10 d</td>
<td>Nanog activation &amp; demethylation; histone acetylation</td>
<td>Miyamoto et al. 2009</td>
</tr>
<tr>
<td>Axolotl oocytes (GV)</td>
<td>Mouse embryo fibroblast</td>
<td>5 h, then 3 d</td>
<td>H3K9Me3, HP1α &amp; DNA methylation reduced; Oct4 and Nanog activation</td>
<td>Bian et al. 2009</td>
</tr>
<tr>
<td>Mouse oocytes (GV)</td>
<td>Cumulus cells</td>
<td>45 min, then 4 wk</td>
<td>H3K9Me reduced; Oct4 activation</td>
<td>Bui et al. 2008</td>
</tr>
</tbody>
</table>

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pathway will lose regulatory chromosomal proteins as they enter mitosis, but will regain the same kinds of proteins as they progress to interphase with no change of gene control. In the case of nuclear transfer to eggs, the proteins lost at the first mitosis will be massively diluted in the huge volume of an egg, and will be replaced by proteins that represent the maternal content of the egg, and a change in gene control would result. In amphibia, the egg is 100,000 times larger than a somatic cell, and a mouse egg is 350 times larger. Because there is no transcription for the first cell cycle in the mouse, nor during the first 12-cell cycles in the frog, chromosomal proteins of a transplanted somatic nucleus should be diluted out. This concept has been most recently presented by Eggan (2004). We point out that mitotic exchange of this kind cannot be the only mechanism of nuclear reprogramming, because the latter takes place efficiently in nuclear transfer to oocytes (no DNA synthesis of mitosis) and in heterokaryon cell fusion experiments (no cell division), as explained above. If chromosomal protein exchange in eggs worked perfectly, nuclear transplant embryos should all develop normally and the phenomenon of epigenetic memory (above) would not exist. It is likely, nevertheless, that mitotic chromosomal protein exchange is a contributory factor in reprogramming when this takes place over multiple cell cycles and in iPS and other transcription factor overexpression experiments.

Transcription Factor Supply

An obvious explanation for a dramatic switch in gene expression is the provision of new transcription factors. As noted above, the overexpression of transcription factors has long been known to be able to activate genes in inappropriate cell types. The remarkable results of Yamanaka (Takahashi et al. 2006) and Melton (Zhou et al. 2008) illustrate this effect very clearly. This would provide a simple explanation for the transcriptional activation of pluripotency genes in nuclear transfers to eggs or oocytes; for example Xenopus oocytes that can directly activate transcription of Sox2, Oct4, and Nanog might contain a maternal content of transcription factors required for these genes. Because these factors are not yet identified for Xenopus, this proposition cannot be tested directly. It has, however, been possible to test this idea in respect of the very well studied myogenic gene MyoD. This gene is, surprisingly, strongly expressed in Xenopus oocytes, and furthermore it is strongly induced in nonmuscle nuclei transplanted to oocytes (Biddle et al. 2009). MyoD and other myogenic genes are activated in normal development by MyoD protein itself, of which there is a maternal content in Xenopus oocytes. It is possible to eliminate the function of MyoD in Xenopus oocytes by overexpressing the inhibitory protein Id. When this is performed, it has no effect at all on the transcriptional activation of MyoD or other myogenic genes in nuclei transplanted to oocytes. This suggests that gene activation in nuclei transplanted to oocytes may not be achieved by transcription factor overexpression.

In contrast to this result, the overexpression of TPT-1, an oncogenic factor, does enhance Oct4 activation in somatic nuclei in oocytes (Koziol et al. 2007). However, the genes immediately downstream from TPT-1 are not known, and the TPT-1 effect may be indirect.

We conclude that the maternal provision of gene-specific transcription factors may not be a general reprogramming mechanism in oocytes and eggs. At present, it seems more likely that oocytes (and perhaps eggs) cause a global derepression of genes, so that most genes become available for transcription by a nonspecific transcriptional apparatus. This would be consistent with the intensely active transcription of most of the genome during the lampbrush phase of amphibian oogenesis (Davidson 1986; Alberts et al. 2008).

Chromatin Access

It is generally believed that, as cells differentiate, an increasing proportion of genes become transcriptionally inactive, compared to embryonic cells. In concert with this, chromatin becomes increasingly condensed and compacted. An especially obvious effect of transplanting...
somatic nuclei to eggs or oocytes is that the nuclei undergo a huge volume increase that is accompanied by an increased dispersion of chromatin in the transplanted nuclei (see above). This could be interpreted as a general loosening of chromatin structure that might give the transcriptional apparatus of the cell more ready access to repressed genes.

FRAP provides a valuable technique for determining the dynamics of nuclear protein mobility (Hager et al. 2009). It has not yet been possible to apply this method to single copy proteins bound to individual genes; multiple fluorescent proteins need to be present at one point on a chromosome for fluorescence to be strong enough to detect loss and replacement of a protein. This technique has therefore been used when multiple copies of a protein, such as histones, HP1, etc., are associated with a gene in chromatin. In all such cases, the residence time of proteins has turned out to be remarkably short, in the order of seconds or minutes. If this is also true of repressor complexes such as components of the polycomb series, we would expect such complexes to be rapidly replaced in transplanted nuclei, which would then be rapidly derepressed. As explained above, nuclei from differentiated cells are remarkably resistant to reprogramming compared to nuclei of embryonic cells.

One explanation for this, in terms of chromosomal protein mobility is the following (Gurdon and Melton 2008). We suggest that the numerous proteins that comprise a repressor complex like PC1 are only loosely connected to chromatin in embryo nuclei. Each component of the complex would have a short dwell time, and so, in a limited time, all such components would have dissociated from chromatin; being only loosely associated with each other, the gene region that they occupy would soon become vacated, allowing access of the cell’s transcriptional apparatus. In contrast, the components of a repressor complex in a differentiated cell are supposed to be extensively bound to each other in a compact structure. Each component of the complex would partially dissociate, but would not be fully released because of its association, at the same time to another member of the complex. As a result, a complete vacation of the site would require a far longer time before the transcriptional apparatus would have access to this site. It would therefore be understandable that a gene in a very specialized cell with highly condensed chromatin would need a very long time, compared to the same gene in an embryonic cell, before it could be transcribed. This concept seems to be in agreement with the results of somatic cell nuclear transfer and with iPS experiments.

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REFERENCES


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transcriptome of cloned embryos during the first two cell cycles. **Dev Biol** 304: 75–89.


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