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Nuclei of Adult Mammalian Somatic Cells Are Directly Reprogrammed to *oct-4* Stem Cell Gene Expression by Amphibian Oocytes

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Summary

Nuclear reprogramming by the transplantation of somatic cell nuclei to eggs (in second meiotic metaphase) is always followed by a phase of chromosome replication and cell division before new gene expression is seen. To help understand the mechanism of nuclear reprogramming, we have asked whether the nuclei of normal, nontransformed, nondividing, and terminally differentiated mammalian cells can be directly reprogrammed, without DNA replication, by *Xenopus* oocytes. We find that nuclei of adult mouse thymocytes and of adult human blood lymphocytes, injected into *Xenopus* oocytes, are induced to extinguish a differentiation marker and to strongly express *oct-4*, the most diagnostic mammalian stem cell/pluripotency marker. In the course of 2 days at 18°C, the mammalian *oct-4* transcripts are spliced to mature mRNA. We conclude that normal mammalian nuclei can be directly reprogrammed by the nucleus (germinal vesicle) of amphibian oocytes to express *oct-4* at a rate comparable to that of *oct-4* in mouse ES cells. To our knowledge, this is the first demonstration of a stem cell marker being induced in a differentiated adult human cell nucleus. This is an early step toward the long-term aim of developing a procedure for reprogramming readily accessible human adult cells for cell replacement therapy.

Results and Discussion

When amphibian somatic cell nuclei are transplanted to enucleated unfertilized eggs of *Xenopus*, the early embryos obtained come to express genes characteristic of blastulae and gastrulae grown from fertilized eggs [1]. The extent of this remarkable reprogramming activity is particularly clear when nuclei transplanted from differentiated amphibian [2] or mammalian [3, 4] cells result in the production of normal adult animals. The nuclear reprogramming mechanism is currently unidentified, and a biochemical analysis of nuclear reprogramming molecules by egg fractionation is complicated by the fact that a major activity of eggs is to induce DNA replication [5, 6]. Thus, many components and activities of eggs are likely to have functions connected with replication [7] and chromatin assembly [8], not necessarily associated with transcriptional reprogramming. It has indeed been suggested that DNA replication may be central to the transcriptional reprogramming process [9].

To try to identify an abundant and DNA replication-free source of biological material with transcriptional reprogramming activity and to investigate whether DNA replication is in fact required for transcriptional reprogramming, we have analyzed the reprogramming activity of *Xenopus* oocytes. These cells are in meiotic prophase, and their nuclei are inactive in replication but are intensely active in transcription of their lampbrush chromosomes [10, 11]. We found, some years ago, that somatic cell nuclei injected into growing oocytes become enhanced in transcriptional activity [12] and, in the case of nucleocytoplasmic combinations within amphibia, that the pattern of protein synthesis is changed [13]. Mammalian HeLa nuclei injected into *Xenopus* oocytes also undergo enlargement and a change in the pattern of protein synthesis [14, 15]. However, in none of these experiments have the newly synthesized proteins been identified, and the significance of the changes in protein synthesis is not known. In the light of this background, we have investigated whether mammalian *oct-4* is induced in normal adult mammalian cells injected into *Xenopus* oocytes. *oct-4* (Figure 1A) is a gene whose expression is required for, and restricted to, pluripotent stem cells in mice [16–18].

Two sets of PCR primers were designed for mouse *oct-4* (Figure 1A). The first set of primers encapsulated the second set, permitting nested PCRs. Both sets of primers were designed to span the 183 base pair (bp) intron 2 and to test whether the PCR products detected were from spliced or unspliced transcripts. The first mouse *oct-4* primer set produced a 238 bp fully spliced PCR product and a 421 bp partially unspliced PCR product (intron 3 spliced, intron 2 remaining). The second mouse *oct-4* primer set produced a 178 bp fully spliced PCR product and a 361 bp unspliced PCR product (Figure 1A). As a primer control, the second mouse *oct-4* primer set was used in an RT-PCR analysis of mouse embryonic stem cells (should be *oct-4* positive) and adult mouse thymocytes (should be *oct-4* negative). A single 178 bp PCR product was obtained from the mouse embryonic stem cell cDNA, and no PCR product was observed in the adult mouse thymocyte cDNA (Figure 1B).

Our first experiments used nuclei of mouse fetal fibroblasts passaged three times. *Xenopus* oocytes were injected with 10–20 streptolysin permeabilized mouse fibroblasts and were cultured for 7 days at 18°C or were immediately frozen for analysis. RT-PCR analysis showed no *oct-4* expression in the fibroblast-injected oocytes that were immediately frozen, demonstrating that *oct-4* is not expressed to a detectable level in mouse fetal fibroblasts. Of the incubated oocytes, RT-PCR analysis of three groups of four injected oocytes gave evidence of spliced *oct-4* transcripts in only one group (Figure 1C). The *Xenopus* oocyte-specific marker *vegT*

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was used as a loading control. This sporadic reprogramming of *oct-4* suggested to us that the position in an oocyte where nuclei are deposited might be important for *oct-4* activation. However, due to the small number of nuclei injected (10–20 per oocyte), the accumulation of spliced *oct-4* transcripts was very low, and unspliced *oct-4* was not even detectable. Although injecting more nuclei decreases oocyte survival, we found that 100–200 nuclei could be injected with over a one third survival rate (Figure 1D). This enabled us to ask whether the position in an oocyte where nuclei are deposited is important for *oct-4* activation. With practice, it is possible to deposit at least some injected nuclei in the nucleus (germinal vesicle) of an oocyte, in the majority of attempts. Alternatively, nuclei can be injected into the cytoplasm, away from the germinal vesicle (GV). When 100–200 mouse fetal fibroblast nuclei were injected toward the GV, oocytes cultured for 4 days at 18°C yielded both spliced and unspliced *oct-4* transcripts. These transcripts were on average 5–10 times more abundant, relative to the oocyte marker *vegT*, in GV-injected oocytes than in cytoplasmic injections (see Table S1 in the Supplemental Data available with this article online). We conclude that the induction of *oct-4* expression in somatic cell nuclei depends primarily on a component of the oocyte nucleus or on material released by it after injection.

Using GV-injected samples, we observed no murine *oct-4* in any immediately frozen oocytes, but we observed a high level of murine *oct-4* transcription (both spliced and unspliced) in every 4 day incubated sample (Figure 1E). We note that by day 4, there is much more spliced than unspliced *oct-4* (Figure 1E); this explains why no unspliced *oct-4* was detected in our original "low nuclear number" experiment (Figure 1C). This cross-species reprogramming result has proven to be consistent. In 15 groups of GV-injected oocytes from 10 different females, we have seen mature spliced *oct-4* transcripts in every group in which the oocytes have been cultured for more than 4 days at 18°C.

To identify more fully the transcripts recognized by our *oct-4* primers, we extracted the spliced 238 bp PCR product from gels (Figure 1E) and sequenced this DNA. Figure 1F shows that in each case, identical sequences were obtained that exactly matched the published mouse *oct-4* sequence [19], and that the splicing junctions are exactly correct. Thus, we can conclude that *Xenopus* oocytes possess the cross-species reprogramming ability to induce the expression of murine *oct-4* in fetal mouse fibroblasts.

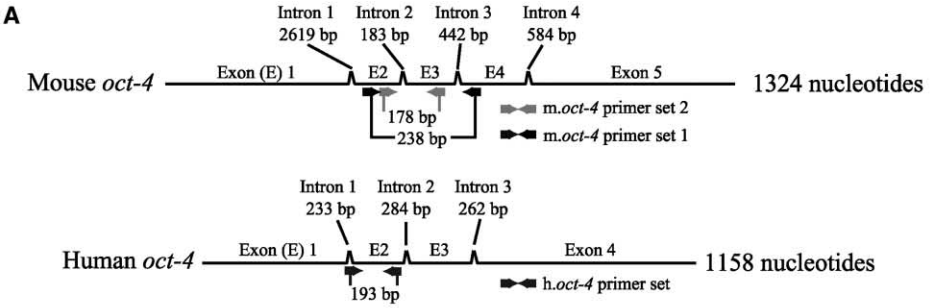
We next considered the specificity of the gene-activating effect of the oocyte germinal vesicle. Are all mouse genes nonspecifically activated to an equivalent extent, or does the oocyte preferentially activate embryo- or oocyte-expressed genes and downregulate other somatically expressed genes? We assayed all the samples so far discussed for the expression of the housekeeping gene cytoskeletal β-*actin* by using PCR primers on the same cDNA material that was tested for *oct-4* expression. We found that, just as *oct-4* expression increases with the duration of incubation, the expression of β-*actin* goes down (Figure 1C). β-*actin* expression is not eliminated in the oocyte, and the persisting level of β-*actin* mRNA may represent a low level of continuing expression or the persistence of stable mRNA introduced with the permeabilized donor cells. We conclude that the *Xenopus* oocyte downregulates murine β-*actin* at the same time as activating murine *oct-4*.

Having achieved the preceding results with fetal cell nuclei, we investigated whether the reprogramming activity of the *Xenopus* oocyte germinal vesicle is also effective on the nuclei of differentiated cells from adult mice, since, to be useful in the context of cell replacement, it is important that the effect we have described can be applied to cells obtained from adults. About 100 permeabilized cells isolated directly from the thymus of 6-week-old mice were injected into the germinal vesicle region of oocytes. Mouse thymocytes are *oct-4* negative (Figure 1B) and are terminally differentiated. In Figure 2A, we see that fully spliced mouse *oct-4* is indeed expressed in injected oocytes incubated for 5.5, 7, or 9 days. The fact that the injected thymocytes that were immediately frozen (day 0) showed no *oct-4* expression (Figure 2A) and that those incubated in *Xenopus* oocytes for 5.5 (or more) days did so (Figure 2A) demonstrates that transcriptional reprogramming had occurred. A time course of gene activation shows that, in some experiments, fully spliced transcripts can be seen at less than 2 days at 18°C (Figure 2B).

The use of differentiated thymocytes enables us to investigate further the specificity of the reprogramming activity, because these cells express the thymus-specific differentiation marker *thy-1* [20]. Using RT-PCR, we analyzed the same samples that were found positive for *oct-4* by using appropriate primers for *thy-1*. It can be seen that injected oocytes frozen immediately after nuclear injection contain enough *thy-1* mRNA to be readily detected (Figure 2A). However, this mRNA is absent in all samples by day 5.5 (Figure 2A). The *Xenopus* oocyte-specific marker, *vegT*, was used as a loading control. The inverse relationship between the activation of *oct-4* expression and the extinction of *thy-1* expression demonstrates the gene-specific nature of the *Xenopus* oocyte's cross-species reprogramming ability. We conclude that this differentiation-specific marker is extinguished at the same time as the activation of the stem cell marker *oct-4*.

Using known amounts of murine *oct-4* transcripts and quantitative RT-PCR analysis, we were able to investigate the extent of the mouse thymocyte transcriptional reprogramming and compare the *oct-4* transcription level in the reprogrammed thymocytes to that observed in mouse embryonic stem cells (Figure 2C). Three different groups of injected oocytes were analyzed, one incubated for 4 days, one incubated for 5.5 days, and the other incubated for 9 days. Each group contained 5 oocytes, and each oocyte contained approximately 100 injected thymocyte nuclei. The highest *oct-4* expression was detected in the day 5.5 incubated samples; this finding suggests that by day 9, the oocytes have begun to senesce and thus their transcriptional ability was reduced. We have calculated (see the Experimental Procedures) that the amount of murine *oct-4* in the 5.5 day reprogrammed mouse thymocytes is about 1000 transcripts per cell (ranging from 500–1500 transcripts),

A



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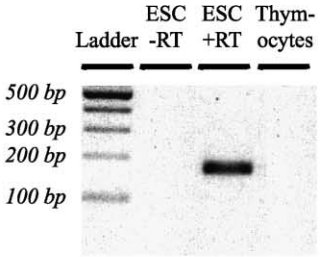

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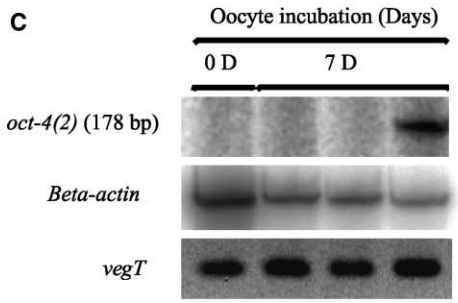
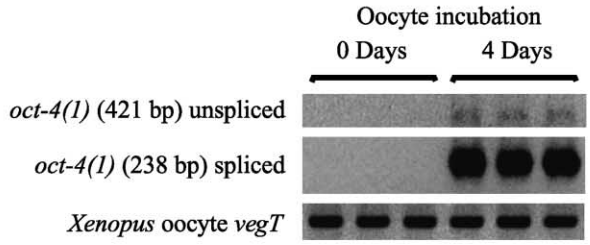


D

Survival of Xenopus oocytes injected with different numbers of mouse nuclei into their GVs			
No. of nuclei inj. into oocytes	**No. of oocytes injected**	**Oocyte survival 1 day after inj.**	**Oocyte survival 5.5 days after inj.**
0	**16**	**100%**	**75%**
10-20	**13**	**92%**	**67%**
100-200	**9**	**67%**	**34%**
1000-2000	**15**	**47%**	**7%**

E



F

PCR product sequence spanning intron 2 splice junction

PCR sample 1	TGGGCGTTCTCTTTG < Intron 2 spliced out > GAAAGGTGTTCAGCC
PCR sample 2	TGGGCGTTCTCTTTG < Intron 2 spliced out > GAAAGGTGTTCAGCC
PCR sample 3	TGGGCGTTCTCTTTG < Intron 2 spliced out > GAAAGGTGTTCAGCC
Mouse *oct-4*	TGGGCGTTCTCTTTG < Intron 2 spliced out > GAAAGGTGTTCAGCC

PCR product sequence spanning intron 3 splice junction

PCR sample 1	CCGACAACAATGAGAACCTTCAGGAG < Intron 3 spliced out > ATAT
PCR sample 2	CCGACAACAATGAGAACCTTCAGGAG < Intron 3 spliced out > ATAT
PCR sample 3	CCGACAACAATGAGAACCTTCAGGAG < Intron 3 spliced out > ATAT
Mouse *oct-4*	CCGACAACAATGAGAACCTTCAGGAG < Intron 3 spliced out > ATAT

a value comparable to the average value of 1000 *oct-4* transcripts that each mouse ES cell is calculated to contain (Figure 2C).

We next investigated whether the reprogramming activity of a *Xenopus* oocyte germinal vesicle could be effectively applied to cells obtained noninvasively from adult humans. We injected about 100 permeabilized human lymphocytes (purified from blood) into each oocyte and analyzed the results after 4 or more days of incubation by using RT-PCR primers specific for the human gene *oct-4*. One of the pair of human *oct-4* primers was designed to physically span the intron 1 splice site, so that only the fully spliced human *oct-4* would be recognized (Figure 1A). RT-PCR analysis demonstrates that the human *oct-4* gene [21] is indeed activated just as in mouse fetal fibroblasts and adult mouse thymocytes (Figure 2D). Sequence analysis of the human *oct-4* PCR product confirmed that fully spliced human *oct-4* had been reprogrammed (Figure 2E). The cross-species reprogramming activity of an amphibian oocyte is therefore effective on cells readily obtainable from adult humans.

Xenopus embryos express three members of the *oct* gene family, namely, *oct-25*, *oct-60*, and *oct-91* [22]; *oct-60* has the widest region of significant sequence homology with mouse *oct-4* (72% homology over 229 nucleotides). Like mouse *oct-4*, *Xenopus oct-60* has a very high expression level in oocytes, eggs, and early embryos and is not expressed in any adult tissue (with the exception of the kidneys) [22]. *oct-60* is the closest candidate to a *Xenopus* "*oct-4*" pluripotency marker. Blast analysis shows that all three *Xenopus oct* genes have less than 80% sequence homology to either mouse or human *oct-4*. In our analyses, the mouse and human *oct-4* sequences obtained following RT-PCR were over 97% the same as the published mouse [19] and human [21] *oct-4* sequences. This rules out the possibility that the introduced mammalian nuclei induced the expression of one of the three *Xenopus oct* genes and that it was these transcripts that were detected via RT-PCR analysis and subsequently sequenced.

To begin to understand how nuclear reprogramming takes place, we need to answer several questions at the cellular level. Which parts of an oocyte do nuclei aimed at the germinal vesicle actually come to occupy? Can injected nuclei be seen to undergo any cytological changes during reprogramming? How many nuclei undergo such changes, and hence how efficiently is *oct-4* transcribed? To answer these questions, we fixed and sectioned injected oocytes from the same experimental series as were analyzed by RT-PCR. Staining sections with Hoechst readily identifies injected nuclei above the very weak background staining of the germinal vesicle, whose DNA is spread through such a large volume. Our first observation is that the injection of somatic nuclei into the germinal vesicle causes no immediate visible disturbance to the structure of an oocyte (Figures 3A and 3B). However, after 2 days or more, most germinal vesicle-injected oocytes have a ruptured germinal vesicle, such that its contents are in contact, and probably mixed, with the adjacent cytoplasm. We see that the majority of injected nuclei lie in this region of ruptured germinal vesicle contents and perinuclear cytoplasm (Figure 3D). The location of the nuclei raises the possibility that reprogramming activity results from an interaction between components of the germinal vesicle and cytoplasm. Second, we find that injected nuclei in the perinuclear cytoplasm undergo a significant enlargement in the course of 3 days of incubation (Figures 3C and 3D). However, nuclei in the germinal vesicle undergo a greater enlargement (Figures 3E and 3F), and, from 5 days onward, they often become dispersed as chromatin (Figures 3G and 3H), bearing some resemblance to the oocytes' own dispersed chromatin structure. Third, we have calculated the number of *oct-4* transcripts in oocytes injected with about 100 thymocyte nuclei (following 5.5 days incubation), and we find it to be comparable to that of the same number of cultured mouse ES cells (Figure 2C). The oocyte's reprogramming activity works at a remarkably high efficiency, and we therefore exclude the possibility that we are detecting, by RT-PCR analysis, a very low level of traumatic response to the manipulations involved.

The large size of an oocyte's germinal vesicle enables it to be manually removed from the oocytes (Figure 4A), and the injected nuclei within it can be recovered intact, stained, and examined (Figure 4B). The mammalian nuclei can be dissected from the GV in small groups and gently shaken free from the nuclear matrix. By this means, we have compared adult mouse thymocyte nuclei immediately after injection with those that have been in a germinal vesicle for 2 days. The 2 day time point was chosen because we find cell nuclei become increasingly difficult to recover from the germinal vesicle matrix after day 2 and spliced transcripts can be detected within 2 days (Figure 2B). A considerable increase in nuclear volume is already observed at this time (Figures 4B and 4C). The ability to recover somatic cell nuclei from an

Figure 1. Mammalian *oct-4* Transcripts in *Xenopus* Oocytes

(A) Intron/exon structure of mouse [19] and human [21] *oct-4* transcripts and primers used for PCR analysis.

(B) PCR recognition of mouse *oct-4*. The mouse *oct-4* primer set 2 (see Figure 1A) recognizes transcripts in ES cells, but not in thymocytes.

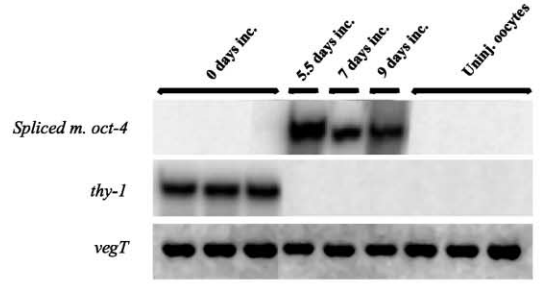
(C) Mouse *oct-4* is expressed in one group of four injected oocytes that had been incubated for 7 days, but not in the two other groups of 7 day oocytes, nor in the group of 0 day oocytes that had been frozen immediately after injection. Oocytes were injected in an approximately central position.

(D) Survival of *Xenopus* oocytes injected with different numbers of mouse nuclei into their GVs.

(E) Six groups of four oocytes, GV injected with mouse fetal fibroblast nuclei, contain both unspliced and spliced *oct-4* transcripts on day 4, but not on day 0. This analysis was done without label, and the gel was stained with ethidium bromide. Oocyte *vegT* was used as a loading control.

(F) The sequence of mouse *oct-4* transcripts in *Xenopus* oocytes. The 238 bp bands from three different samples of oocytes were cut out from the gel shown in Figure 1E and were sequenced. The transcripts and splice junctions of each series are identical to the published mouse *oct-4* sequence [19].

A



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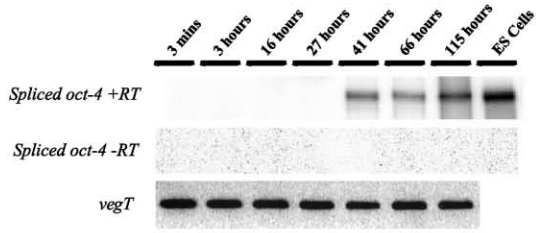

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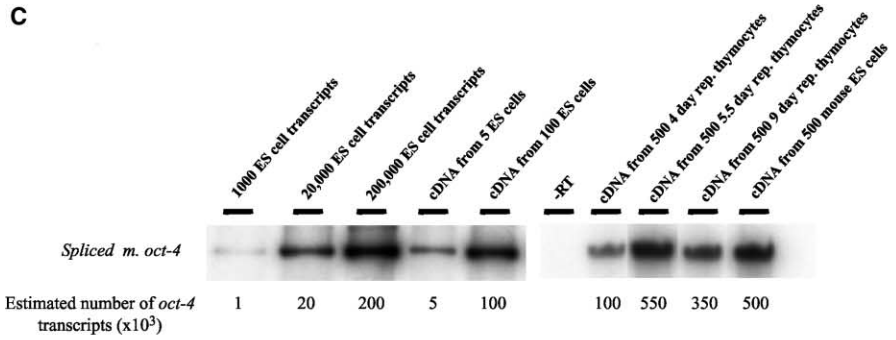

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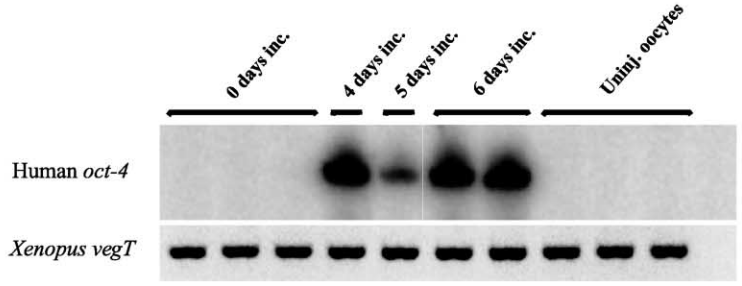


E

PCR sample 1	...AAATCTTCAGGAGATATGCAAAGCAGAAACCCTCGTGCAG...
PCR sample 2	...ACATCTTCAGGAGATATGCAAAGCAGAAACCCTCATGCAG...
Human *oct-4*	...ACATCTTCAGGAGATATGCAAAGCAGAAACCCTCGTGCAG...

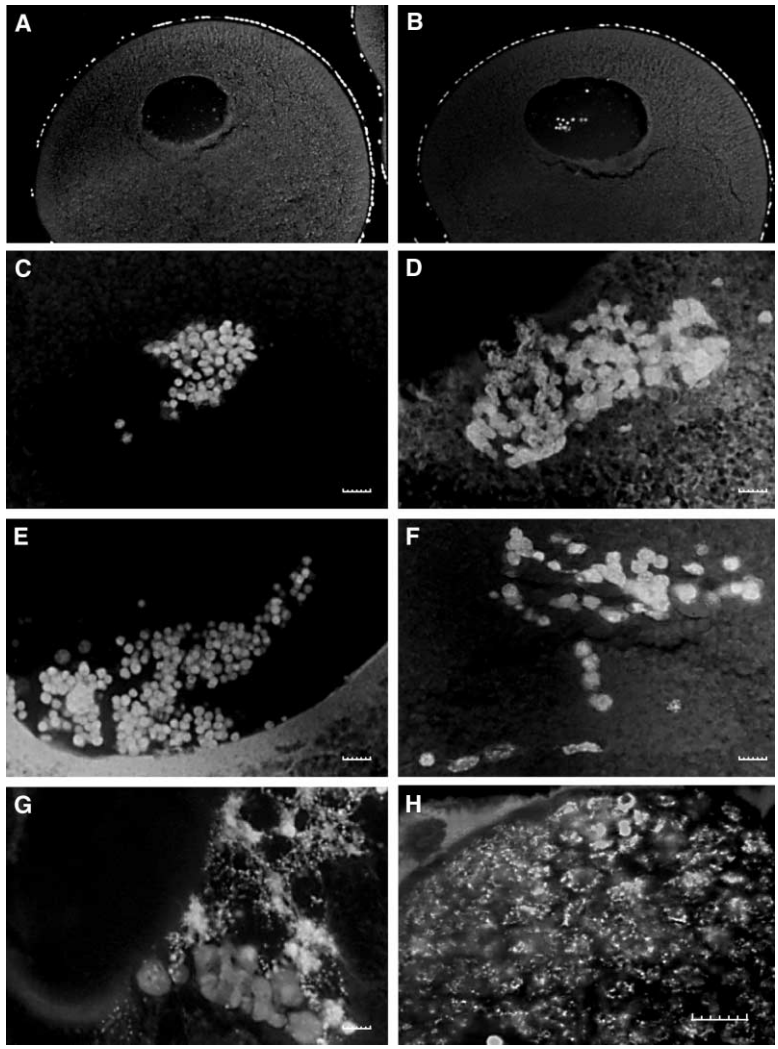


Figure 3. Mouse Thymus Cell Nuclei in *Xenopus* Oocytes

(A) A transverse section of an uninjected oocyte surrounded by follicle cells (white nuclei) and containing a large central germinal vesicle (dark circle). The small gray spots in the germinal vesicle are the oocytes' own extranucleoli.

(B) An injected oocyte containing mouse thymocyte nuclei in the germinal vesicle, fixed 10 min after injection.

(C) Mouse thymocyte nuclei 10 min after injection into the perinuclear cytoplasm of an oocyte.

(D) Mouse thymocyte nuclei 3 days after injection into the perinuclear cytoplasm of an oocyte.

(E) Mouse thymocyte nuclei 10 min after injection into the germinal vesicle of an oocyte.

(F) Three days after injection into the germinal vesicle of an oocyte, mouse thymocyte nuclei are located in a mixture of germinal vesicle contents and cytoplasm.

(G) Mouse thymocyte nuclei 5 days after injection into the germinal vesicle of an oocyte are seen as highly enlarged nuclei or as dispersed chromatin.

(H) Mouse thymocyte nuclei 6 days after injection into the germinal vesicle of an oocyte have been converted into a mass of dispersed chromatin. ([A] and [B], oocyte diameter = 1100 μm; Figures [C]–[H], scale bar = 20 μm).

oocyte germinal vesicle (after reprogramming to *oct-4* expression) opens up the possibility of transplanting such nuclei to enucleated mammalian eggs. The resulting cloned embryos might be more suited for the derivation of nuclear transfer ES cells than is currently the case for most mammalian species. It has indeed been suggested that *oct-4* expression by transplanted nuclei is critical for successful development and for embryonic stem cell derivation following somatic cell nuclear transfer [23].

As far as we know, the introduction of a human somatic cell nucleus into the germinal vesicle of an oocyte is the only means known, at present, of directly inducing stem cell gene expression in a differentiated adult human cell nucleus. No incompatibility affecting *oct-4* induction is seen between mammalian nuclei and amphib-

Figure 2. Reprogramming of Adult Mouse Thymocyte Nuclei by *Xenopus* Oocytes

(A) Six groups of GV-injected oocytes (five oocytes per group) show a strong activation of mouse *oct-4* in samples incubated at 18°C for 5.5, 7, or 9 days, but not in samples frozen directly after injection (day 0). The *thy-1* marker characteristic of differentiated mouse thymocytes is extinguished before day 5.5.

(B) Time course of appearance of spliced *oct-4* transcripts. Oocytes were injected with about 100 mouse thymocyte nuclei, cultured at 18°C, and groups of five oocytes frozen for analysis at the times shown.

(C) Quantification of *oct-4* transcripts. Tracks 1–3 show the intensity of gel bands that correspond to known numbers of transcripts. The values for the other seven samples were determined by comparison with tracks 1–3. Details of the procedures are provided in the Experimental Procedures under Transcript Quantification. rep., reprogrammed after injection into oocytes.

(D) Reprogramming of adult human lymphocytes. Seven groups of GV-injected oocytes (five oocytes per group) show a strong activation of human *oct-4* 4–6 days after injection, but not on day 0. *Xenopus vegT* is a loading control.

(E) Sequence of human *oct-4* transcripts from *Xenopus* oocytes. Human *oct-4* PCR products from two of the 6 day incubated samples shown in Figure 2D show an almost complete (98%) similarity with the known human *oct-4* sequence [21]. The human *oct-4* sequence is quite different from the *oct-4* sequence of the mouse (Figure 1F), and from that of the nearest *Xenopus* homolog (not shown).

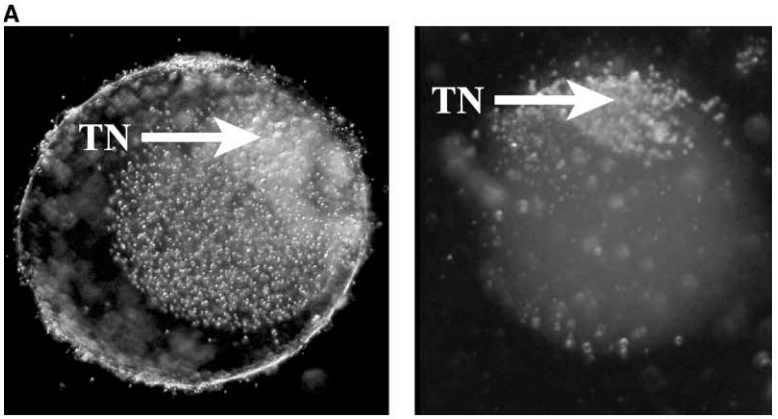
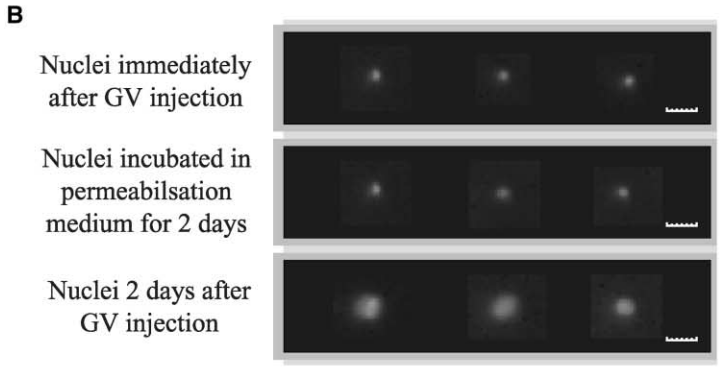


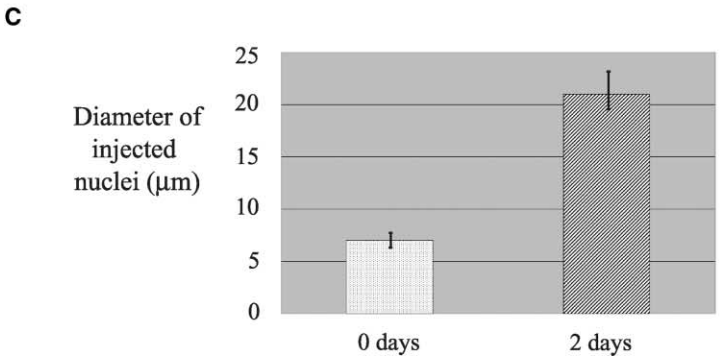
Figure 4. Recovery of Adult Mouse Somatic Cell Nuclei from Injected *Xenopus* Oocytes

(A) A view of living oocyte germinal vesicles, with (left) and without (right) nuclear envelope, isolated 2 days after injection of Hoechst-stained adult mouse thymocyte nuclei (TN).

(B) Retrieved adult mouse thymocyte nuclei, stained with Hoechst. Adult mouse thymocyte nuclei recovered 2 days after injection into an oocyte germinal vesicle have decondensed and increased their volume about 25-fold; no such increase takes place in nuclei maintained in the nuclear permeabilization medium (see the Experimental Procedures). (The scale bar represents 40 μm).

(C) An early response of mammalian nuclei to an oocyte GV is a 25-fold volume increase in 2 days at 18°C. Volume calculated by using: $4/3\pi$ (diameter/2)^3.





ian oocytes. The mechanism of reprogramming is direct, in the sense that new gene expression is induced in the same nucleus as was part of a somatic cell; DNA synthesis and replication, as happens in egg nuclear transfers, is not required for stem cell gene expression. The reprogramming that we have described is relatively rapid considering the temperatures used. Our injected oocytes were cultured at 18°C. Spliced *oct-4* transcripts are seen following 2 days of incubation at 18°C, equivalent in metabolic terms to 12 hr of incubation at 37°C. The only directly comparable work known to us is that of Gall and Murphy [24], who saw lampbrush chromosome-like structures in sperm nuclei injected into oocytes, and that of Blau [25], who was able to directly reprogram human amnion cells to muscle gene expression by making heterokaryons between mouse myotubes and human cells. Other examples of reprogramming include work by Tada et al. [26–28], who were able to reactivate several genes in fusion cell hybrids that had proliferated in culture for a few days, and by Hakelien et al. [29], who observed the expression of *Il-2* in cultured fibroblast cell nuclei incubated in vitro with T cell extracts. In these, as in our own earlier experiments with oocytes, there is no evidence that a mammalian stem cell marker gene

was activated directly, in the absence of DNA replication and cell division [12–15]. In future work, it will be interesting to test the expression of genes other than *oct4* in the experimental system described here. We might expect to see some other oocyte-specific genes induced, but not, for example, those expressed in the proliferating stem cell of embryos.

Our work opens up a number of future possibilities. The main objective must be to identify the molecules and mechanisms of nuclear reprogramming. In this respect, amphibian oocytes should be particularly favorable. Most mammalian eggs are $\sim$70 μm in diameter compared to 1200 μm for a full-size *Xenopus* oocyte, a volume advantage of 5000. A *Xenopus* ovary contains about twenty thousand large oocytes. Compared to the volume of oocytes in a mouse, this gives a *Xenopus* ovary a volume (and protein content) advantage of about 10^7 for the identification of molecules that can activate *oct-4* expression in mouse nuclei. We believe that the ability of amphibian oocyte components to induce stem cell gene expression in normal mouse and human adult somatic cells, and the abundant availability of amphibian oocytes, encourages the long-term hope that it may eventually be possible to directly reprogram cells, easily obtained from adult human patients, to a stem cell condition.

Supplemental Data

Supplemental Data including Experimental Procedures and data suggesting that the *Xenopus* oocyte germinal vesicle possesses greater murine *oct-4* reprogramming ability than the oocye cytoplasm are available at http://www.current-biology.com/cgi/content/full/13/14/1206/DC1/.

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References

1. Gurdon, J.B. (1986). Nuclear transplantation in eggs and oocytes. J. Cell Sci. Suppl. *4*, 287–318.
2. Gurdon, J.B. (1962). The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. J. Embryol. Exp. Morph. *10*, 622–640.
3. Campbell, K.H., McWhir, J., Ritchie, W.A., and Wilmut, I. (1996). Sheep cloned by nuclear transfer from a cultured cell line. Nature *380*, 64–66.
4. Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J., and Campbell, K.H. (1997). Viable offspring derived from fetal and adult mammalian cells. Nature *385*, 810–813.
5. Graham, C.F., Arms, K., and Gurdon, J.B. (1966). The induction of DNA synthesis by frog egg cytoplasm. Dev. Biol. *14*, 349–381.
6. Blow, J.J., Dilworth, S.M., Dingwall, C., Mills, A.D., and Laskey, R.A. (1987). Chromosome replication in cell-free systems from *Xenopus* eggs. Philos. Trans. R. Soc. Lond. B Biol. Sci. *317*, 483–494.
7. Almouzni, G., and Mechali, M. (1988). *Xenopus* egg extracts: a model system for chromatin replication. Biochim. Biophys. Acta *951*, 443–450.
8. Almouzni, G., Clark, D.J., Mechali, M., and Wolffe, A.P. (1990). Chromatin assembly on replicating DNA in vitro. Nucleic Acids Res. *18*, 5767–5774.
9. Tada, T., and Tada, M. (2001). Toti-/pluripotential stem cells and epigenetic modifications. Cell Struct. Funct. *26*, 149–160.
10. Callan, H.G., and Lloyd, L. (1960). Lampbrush chromosomes of crested newts *Triturus Cristatus*. Philos. Trans. R. Soc. Lond. B Biol. Sci. *243*, 135–219.
11. Gall, J.G., Murphy, C., Callan, H.G., and Wu, Z.A. (1991). Lampbrush chromosomes. Methods Cell Biol. *36*, 149–166.
12. Gurdon, J.B., Partington, G.A., and De Robertis, E.M. (1976). Injected nuclei in frog oocytes: RNA synthesis and protein exchange. J. Embryol. Exp. Morphol. *36*, 541–553.
13. 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.
14. Gurdon, J.B. (1976). Injected nuclei in frog oocytes: fate, enlargement, and chromatin dispersal. J. Embryol. Exp. Morphol. *36*, 523–540.
15. De Robertis, E.M., Partington, G.A., Longthorne, R.F., and Gurdon, J.B. (1977). Somatic nuclei in amphibian oocytes: evidence for selective gene expression. J. Embryol. Exp. Morphol. *40*, 199–214.
16. Nichols, J., Zevnik, B., Anastassiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., Scholer, H., and Smith, A. (1998). Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell *95*, 379–391.
17. Pesce, M., and Scholer, H.R. (2001). Oct-4: gatekeeper in the beginnings of mammalian development. Stem Cells *19*, 271–278.
18. Pan, G.J., Chang, Z.Y., Scholer, H.R., and Pei, D. (2002). Stem cell pluripotency and transcription factor Oct4. Cell Res. *12*, 321–329.
19. Gidekel, S., and Bergman, Y. (2002). A unique developmental pattern of oct-3/4 DNA methylation is controlled by a cis-demodification element. J. Biol. Chem. *277*, 34521–34530.
20. Nishimura, H., Nozawa, S., Awaji, M., Saitoh, J., Abe, M., Hattori, S., Hirose, S., and Shirai, T. (1992). Exogenous Thy-1.1 expression as a marker for thymocyte maturation in transgenic mice. Thymus *19*, 35–44.
21. Nichols, J., Zevnik, B., Anastassiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., Scholer, H., and Smith, A. (1998). Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell *95*, 379–391.
22. Hinkley, C.S., Martin, J.F., Leibham, D., and Perry, M. (1992). Sequential expression of multiple POU proteins during amphibian early development. Mol. Cell. Biol. *12*, 638–649.
23. 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.
24. Gall, J.G., and Murphy, C. (1998). Assembly of lampbrush chromosomes from sperm chromatin. Mol. Biol. Cell *9*, 733–747.
25. Blau, H.M., Chiu, C.P., and Webster, C. (1983). Cytoplasmic activation of human nuclear genes in stable heterocaryons. Cell *32*, 1171–1180.
26. Tada, M., Tada, T., Lefebvre, L., Barton, S.C., and Surani, M.A. (1997). Embryonic germ cells induce epigenetic reprogramming of somatic nucleus in hybrid cells. EMBO J. *16*, 6510–6520.
27. Tada, T., Tada, M., Hilton, K., Barton, S.C., Sado, T., Takagi, N., and Surani, M.A. (1998). Epigenotype switching of imprintable loci in embryonic germ cells. Dev. Genes Evol. *207*, 551–561.
28. Tada, M., Takahama, Y., Abe, K., Nakatsuji, N., and Tada, T. (2001). Nuclear reprogramming of somatic cells by in vitro hybridization with ES cells. Curr. Biol. *11*, 1553–1558.
29. Hakelien, A.M., Landsverk, H.B., Robl, J.M., Skalhegg, B.S., and Collas, P. (2002). Reprogramming fibroblasts to express T-cell functions using cell extracts. Nat. Biotechnol. *20*, 460–466.