Mitosis Gives a Brief Window of Opportunity for a Change in Gene Transcription

Richard P. Halley-Stott1,2, Jerome Jullien1,2, Vincent Pasque3, John Gurdon1,2

1 Wellcome Trust/Cancer Research UK Gurdon Institute, The Henry Wellcome Building of Cancer and Developmental Biology, Cambridge, United Kingdom, 2 Department of Zoology, University of Cambridge, Cambridge, United Kingdom, 3 Department of Biological Chemistry, David Geffen School of Medicine, The Eli and Edith Broad Center of Regenerative Medicine and Stem Cell Research, University of California, Los Angeles, California, United States of America

Abstract

Cell differentiation is remarkably stable but can be reversed by somatic cell nuclear transfer, cell fusion, and iPS. Nuclear transfer to amphibian oocytes provides a special opportunity to test transcriptional reprogramming without cell division. We show here that, after nuclear transfer to amphibian oocytes, mitotic chromatin is reprogrammed up to 100 times faster than interphase nuclei. We find that, as cells traverse mitosis, their genes pass through a temporary phase of unusually high responsiveness to oocyte reprogramming factors (mitotic advantage). Mitotic advantage is not explained by nuclear penetration, DNA modifications, histone acetylation, phosphorylation, methylation, nor by salt soluble chromosomal proteins. Our results suggest that histone H2A deubiquitination may account, at least in part, for the acquisition of mitotic advantage. They support the general principle that a temporary access of cytoplasmic factors to genes during mitosis may facilitate somatic cell nuclear reprogramming and the acquisition of new cell fates in normal development.

Introduction

Normal development, as well as nearly all cases of experimentally induced changes in gene transcription, is accompanied by cell division. It is therefore hard to distinguish those molecular events which prepare cells for, or engage them in, mitosis from those that are required specifically for transcriptional reprogramming. The relationship between the cell cycle and cell fate decisions has for a long time attracted interest [1]. Transition through mitosis is a time when many transcription factors are displaced from chromatin, potentially permitting new transcription factors to occupy chromatin sites on mitotic exit and so direct a postmitotic cell fate change [2–5]. Mitotic remodelling has been shown to be of great importance for the efficient replication of erythrocyte nuclei by Xenopus egg extracts [6,7]. For new transcription, cell division seems to be needed in some cases [8,9] but not in others [10,11]. Here we have used nuclear transfer to amphibian oocytes to compare directly the ability of mitotic chromatin or interphase nuclei to reprogramme in the absence of cell division.

Germinal vesicle (GV) stage oocytes do not replicate or divide. They therefore provide an opportunity to test whether the cell cycle phase of donor nuclei affects the efficiency of nuclear reprogramming as judged by active transcription of previously silenced genes [12]. To our surprise, we found that a mitotic state of donor nuclei dramatically increases the efficiency of activating certain quiescent pluripotency genes in these nuclei. Our results support an idea that a brief period during mitosis facilitates an exchange of gene regulatory factors on chromatin and that this could be an important mechanism to help cells embarking on new cell lineages during normal development.

Results

Mitotic Chromatin Is Reprogrammed Much More Rapidly Than Interphase Nuclei

Permeabilized mouse C2C12 cells, a cultured myoblast cell line which we have used extensively in our oocyte nuclear transfer experiments, were arrested at specific stages of the cell cycle (Figure S1a) and were injected into the GV of oocytes (Figure 1a). The DNA content of these donor cell populations (Figure 1b) confirmed cell cycle arrest in each of the cell cycle stages. The transcriptional reactivation of three silent genes quiescent in C2C12 cells (Nanog, Oct4, and Sox2) was assessed by RT-qPCR 38 h after nuclear transplantation (Figure 1c). Nuclei at a late stage of the cell cycle (M) show greatly enhanced transcription of each of the genes when compared to unsynchronized nuclei (predominantly G1 and S), whereas an already active gene (c-jun) shows little increase in transcript level. Particularly impressive is...
 Sox2 is a gene that is more widely expressed than most others, much affected by the exact duration of nocodazole treatment. 25–48 h after injection to oocytes than unsynchronized donor always generated more Sox2 transcripts from reactivated genes at nuclei when compared to interphase donor nuclei (Figure 1c). In the 100-fold enhancement in Sox2 expression from mitotic donor type (C2C12 myoblasts) or is a non specific effect of nocodazole, (Figure S1b) or prepared mitotic C2C12 donor cells without any inhibitors by a shake-off procedure (Figure S1c). In both cases, enhanced transcription from mitotic donor was observed, although the magnitude of mitotic advantage was lower (particularly in the case of the shake-off samples, many cells of which appeared to be apoptotic by visual inspection). Mitotic donor nuclei were also prepared using another cell synchronization agent (Taxol), and the mitotic advantage was again seen (Figure S1d). When G1/G0 cells were exposed to nocodazole for the same period of time as used to prepare mitotic cells, no enhancement of transcription of the genes was observed (Figure S1c). These results indicate that the observed mitotic advantage is not due to a nonspecific activity of nocodazole nor to a peculiarity of one line of cells (C2C12).

To ask if this mitotic advantage applies more widely in the genome than to the pluripotency genes so far tested, we compared by RNAseq the genes transcribed in injected oocytes by interphase nuclei or mitotic chromatin. We focussed our analysis on genes that were found to be consistently expressed by interphase nuclei after nuclear transfer. One experiment indicated that 617 genes were transcribed in oocytes at least 2-fold more in mitotic nuclei compared to interphase nuclei. Of these mitotically up-regulated genes, Sox2 was 4-fold more transcribed than in interphase nuclear transfers, and over half of the 617 genes were more strongly transcribed than Sox2. The list of these genes is in Table S1.

Mitotic Advantage Is Independent of Nuclear Membrane Permeability

The most obvious explanation for this mitotic advantage is the absence of a nuclear envelope in the mitotic karyoplasts. We have quantified this difference in membrane permeability by time course imaging a mixture of injected interphase nuclei and mitotic karyoplasts. We carried out a “double permeabilization,” in which both the cell and nuclear membranes, of interphase or mitotic donor cells, were permeabilized as illustrated in the scheme in Figure 3a. We then compared the rate of oocyte factor uptake with the rate of reprogramming by RT-qPCR. A difference in the amount of B4 and H2B uptake is indeed seen after plasma permeabilization with digitonin (Figure 3b) but is no longer seen after double permeabilization of the nuclear envelope with Triton (Figure 3c). Nevertheless, the mitotic difference between interphase and mitotic chromatin does persist in respect of the transcriptional reprogramming of silenced genes (Figure 3d).

We have confirmed this conclusion using permeabilization by different reagents. Streptolysin 0 (SLO) permeabilizes the plasma membrane but not the nuclear membrane; SLO and Lysolecithin (LL) together permeabilize the plasma membrane and nuclear membrane [15]. Permeabilization was tested using different sizes of dextran (Figure S3a). We then compared transcription from transplanted nuclei, comparing those treated with SLO alone and
Figure 1. Mitotic nuclei are reprogrammed much more efficiently than interphase nuclei. (a) Nuclear transplantation procedure used in this and the following experiments. (b) DNA content analysis of donor cells used for nuclear transplantation to oocytes confirms enrichment of specific cell cycle stages. (c) Donor nuclei in the later stages of the cell cycle reprogram better than those from earlier stages. Nuclei from C2C12 cells arrested at each stage of the cell cycle or growing in the absence of inhibitor were used as donor material for NT to oocyte GVs. The figure shows the relative expression for each of the indicated genes at 38 h after transplantation compared to unarrested donor cells (n = 3). Supporting data can be found in Data S1.

doi:10.1371/journal.pbio.1001914.g001
those treated with SLO and LL. The transcription ratio following these two procedures shows no advantage when the nuclear envelope is permeabilized (Figure S3b).

We conclude that the presence of an intact interphase nuclear envelope does not explain the mitotic advantage.

The Mitotic Advantage Is Due to Chromatin Composition

Because the difference in reprogramming rate between interphase and mitotic donor cells is maintained after extensive permeabilization of the interphase nuclear membrane, we asked if the source of the difference lies in the chromatin of the two donor cell preparations. To answer this, we mildly sonicated both interphase and mitotic donor cell preparations to give fragments of chromatin of similar sizes (Figure 4a and b), injected these preparations in parallel with a permeabilized cell preparation into oocyte GVs, and assessed gene reactivation by RT-qPCR (Figure 4c). It is clear that the difference in the rate of gene reactivation from interphase and mitotic nuclei is maintained when the injected material is sonicated chromatin as opposed to whole nuclei. This suggests that the “mitotic advantage” is present in the chromatin of mitotic cells. This result also confirms that the difference between interphase and mitotic donor cells is not due to the interphase nuclear membrane, nor to any other aspect of nuclear organization that is eliminated by sonication.

The difference between interphase and mitotic reprogramming is, however, abolished when genomic DNA prepared from donor nuclei is injected into oocyte GVs (Figure 4d); this excludes differences at the DNA level (sequence and DNA methylation for example) as possible sources of the difference in reprogramming between interphase and mitotic samples. The possibility of DNA methylation accounting for the mitotic effect was further excluded by bisulphite analysis of specific loci on mitotic and interphase DNAs, as this revealed no mitosis-specific differences (Figure 4e).

These two results indicate that whatever accounts for the difference between mitotic and interphase donor cells is not present at the level of genomic DNA itself but is in non-DNA components of chromatin.

Figure 2. Mitotic advantage is due to an increased rate of reprogramming. (a) Live imaging of mixed mitotic and interphase nuclei injected into the GVs of oocytes expressing fluorescently labelled histone B4 or histone H2B. Mitotic chromatin becomes decorated with oocyte-derived factors to a far greater degree than interphase nuclei 2 h after nuclear transplantation. The arrows indicate interphase (I) or mitotic (M) nuclei. (b) Mitotic donor nuclei (M) display actively transcribing RNA Pol II far more rapidly than interphase nuclei (I) when these nuclei are co-injected into oocyte GVs. Immunofluorescent staining against a mixture of interphase and mitotic donor nuclei injected into the oocyte GV and fixed at 3 min or between 1 and 2 h after transplantation using antibodies against active poI II (magenta). (c) A time course of expression by mitotic and interphase donor nuclei shows that the difference between these nuclei decreases with time, suggesting that the eventual amount of reprogramming is similar in the two nuclear types but that initiation of transcription is much more rapid in mitotic nuclei. Supporting data can be found in Data S1.

doi:10.1371/journal.pbio.1001914.g002
Figure 3. Mitotic advantage is independent of nuclear membrane permeability. (a) Design of permeability assay. (b) Under normal conditions of plasma membrane permeabilization by digitonin with no nuclear permeabilization, mitotic chromatin (M arrows) takes up histones B4 and H2B faster than interphase nuclei (I arrows). (c) When double permeabilized by Digitonin and Triton X, interphase nuclei and mitotic chromatin take up these histones at a similar rate. (d) After double permeabilization, the mitotic advantage of mitotic nuclei is still very large, as judged by RT-qPCR. Supporting data can be found in Data S1.

doi:10.1371/journal.pbio.1001914.g003
Mitotic Advantage Is Independent of Most Salt-Released Chromatin Factors

As the mitotic advantage is likely to be due either to the loss or gain of chromatin binding factors, we removed most of these from our donor suspension of interphase nuclei by incubating such nuclei in a high-salt Triton buffer. We thereby tested whether a loss of chromatin binding factors at mitotic entry could remove the mitotic advantage. We also largely depleted chromatin binding factors from permeabilized mitotic cells and thus removed many chromatin factors that may be gained by cells entering mitosis. The depletion of chromatin binding factors was achieved with 300 mM salt and Triton, which removed most nonhistone DNA binding factors. A scheme of the cell preparation and examples of the proteins removed are shown in Figure 5a and 5c. It can be seen that the great majority of the nonhistone chromosomal proteins that have been tested and that normally exist in interphase nuclei have been removed from mitotic chromatin by 300 mM salt and Triton. Nevertheless interphase nuclei depleted of salt soluble nuclear protein (300 mM sample) do not acquire the same reprogramming responsiveness as mitotic donor material (Figure 5b). Likewise, extensive protein removal from mitotic donor material (Figure 5c) before nuclear transplantation does not abolish the mitotic advantage (mitotic 300 mM; Figure 5b), indicating that the acquisition of chromosomal proteins by mitotic nuclei does not account for this advantage.

Independently of salt release experiments, we tested topoisomerase II whose activity increases from S phase to the end of G2. The inhibition of topoisomerase II and of its adaptor molecules 14-3-3z and H3S10ph by inhibitors, inhibitory peptides, and antibody injection in transplanted mitotic nuclei did not reduce the mitotic advantage. We also found that salt release removes topoisomerase from mitotic chromatin, as in experiments shown in Figure 5c, but does not change the mitotic advantage.

We conclude that a loss of salt-soluble chromatin binding factors does not account for the mitotic advantage. It is likely therefore that the source(s) of the difference is either a non-salt-soluble factor (gained or lost at mitotic entry), a covalent modification of chromatin, or the spatial arrangement of nucleosomes.

Histone Acetylation, Phosphorylation, and Methylation

We next considered covalent histone modifications that may be lost or gained on mitotic chromatin compared to interphase chromatin. A large number of histone modifications are associated with mitotic entry [16,17], as well as changes in nucleosome positioning and in chromatin compaction. We first tested the most striking changes involving global histone deacetylation, phosphorylation, and some small increases in histone H3 lysine 4 and 9 methylation that have been seen on mitotic chromatin [16,18,19]. Histone deacetylation in mitotic cells is successfully inhibited during mitotic synchronisation by the histone deacetylase inhibitor TSA (Figure 6a). Histone phosphorylation in mitotic cells is inhibited by the Aurora B/JAK inhibitor AT9823 (Figure 6b). Nevertheless, the mitotic advantage persists after both of these treatments (Figure 6c and 6d). Similarly, the removal of mitotic histone phosphorylation from the Sox2 gene by protein phosphatase treatment of mitotic and interphase donor cells before nuclear transplantation also failed to abrogate the mitotic effect (Figure 6a and b). A small (2-fold) local increase in Sox2 locus histone methylation at mitosis (Figure 6c and d), seen by ChIP in mitotic chromatin, is eliminated by the methylation of MTA (not shown), but the mitotic advantage is retained (Figure 6c).

Histone Ubiquitination

In normal cells, histone ubiquitination [primarily H2AK119Ub and H2BK120Ub] is dramatically reduced at mitotic entry [16]. H2AK119Ub is associated with transcriptional repression [20]. Thus, a reduction in H2A ubiquitination at mitosis is an attractive candidate to explain the enhanced reprogramming of mitotic chromatin. We first tested the effect of increasing ubiquitination of mitotic chromatin, by preparing nuclei for injection in the presence of iodoacetamide (IAA), which inhibits deubiquitinasises (Figure 7a) [21]. Under normal conditions, interphase chromatin is at least five times more ubiquitinated than mitotic chromatin (Figure 7a,b). The inhibition of mitotic deubiquitination by IAA increases the ubiquitin level in mitotic chromatin, so that it is nearly equal to that of interphase nuclei (Figure 7c). When tested for transcription in oocytes, hyperubiquitinated mitotic chromatin by IAA does not show an advantage over interphase chromatin (Figure 7d), in accord with the idea that the deubiquitinated state of normal mitotic chromatin could account for its special transcriptional advantage.

As further support for this idea, we tried to remove ubiquitin from interphase nuclei with a recombinant deubiquitinase (Ubp-M), and then tested the effect of this by oocyte injection followed by RT-qPCR. Treatment of interphase nuclei with Ubp-M removes histone ubiquitination (Figure 7g). However, unexpectedly, we see that the removal of histone ubiquitination by Ubp-M does not significantly enhance the reprogramming of interphase nuclei, so that they behave the same, in this respect, as mitotic chromatin (Figure 7h). This suggested that deubiquitination itself is not sufficient to confer mitotic advantage. We hypothesized that H2A deubiquitination is a required step in a series of chromatin remodelling events that eventually lead to mitotic advantage. We therefore chose to reduce the ubiquitinated state of interphase nuclei in living cells in order to allow events downstream of ubiquitin-depleted chromatin to take place. The inhibitor MG-132 is thought to lower histone ubiquitination by reducing the pool of free ubiquitin through inhibition of the proteasome. MG-132 treatment of interphase nuclei before injection to oocytes (Figure 7a) gave a partial but significant reduction in H2A ubiquitination on Sox2 (Figure 7c compared to 7b); it also resulted in a substantial enhancement of oocyte-induced transcription from interphase, but not mitotic chromatin (Figure 7f).

The increase in ubiquitination of transplanted mitotic nuclei, coupled with the removal of the mitotic advantage, shows that chromatin ubiquitination contributes to the mitotic advantage. It may, however, not be sufficient to explain the whole phenomenon, because we do not achieve a complete mitotic advantage in interphase nuclei by deubiquitination.

Discussion

Our results show a substantial effect of the cell cycle stage of donor nuclei in nuclear transfer experiments. The reason why this has not been seen before in some of the older nuclear transfer experiments with eggs is probably for several reasons. One is that the somatic nuclei used as donors were not able to be well synchronized [22–24]. Another is that tests have involved the normality of development, rather than gene activity. Third, and most importantly, tests have been carried out on cell dividing eggs, whereas our work has tested gene transcription in the complete absence of DNA replication or cell division. The more recent results of [6] are in agreement with the work described here. The success of the first mammal cloning work was attributed in part to the use of donor cells in G0 [25,26]; this result was not, however, found by others [27–30]. Lemaître et al. [6] have described a
dramatic effect of mitosis on the efficiency of DNA replication by egg cytoplasm, but transcription could not be tested in their extract experiments. Egli et al. [3] have proposed an important role for mitosis in permitting chromosomal protein exchange in mouse nuclear transfer experiments but not necessarily on gene transcription. Our results are therefore in accord with previous work, but reveal a specific effect of mitosis on gene transcription. An important component responsible for the acquisition of mitotic advantage appears to be the removal of ubiquitin from histone H2A or H2B in mitotic chromatin. Ubiquitination of...
Figure 5. Removal of salt-soluble factors from interphase C2C12 nuclei. (a) Design of salt depletion procedure. (b) 300 mM salt does not remove the mitotic advantage of mitotic nuclei, nor does it make interphase nuclei behave like mitotic chromatin by loss of DNA-binding factors. Salt-treated samples were injected to oocytes and cultured for 40 h and then analyzed by RT-qPCR. Supporting data can be found in Data S1. (c) Two independent experiments show that the majority of chromatin binding factors can be depleted from nuclei by 300 mM salt and Triton when compared to 75 mM salt, which should not remove chromatin-bound factors. The blemish for topoisomerase II (75 mM,M) is not in the position of this protein.

doi:10.1371/journal.pbio.1001914.g005
histones is primarily monoubiquitination, and we assume that this is the modification involved in mitotic advantage. H2A ubiquitination on lysine 119 is associated with transcriptional repression, particularly of lineage-specifying genes in ES cells [31], possibly through its association with members of the polycomb repressive proteins [32]. It could be envisaged that the deubiquitination of chromatin seen at mitotic entry permits this mitotic advantage by removing this inhibitory mark or the associated binding proteins.

Figure 6. Most mitotic histone modifications do not distinguish mitotic chromatin from interphase nuclei. (a) Western blot showing TSA inhibition of the normal mitotic deacetylation of histone H3K9ac. (b) Western blot showing that AT2983 prevents the normal mitotic phosphorylation of histone H3. (c) Mitotic nuclei prepared in the presence of TSA maintain the mitotic advantage. Supporting data can be found in Data S1. (d) Inhibiting normal mitotic phosphorylation by AT2983 has no adverse effect on mitotic advantage. Supporting data can be found in Data S1. (e) MTA inhibits mitotic methylation of H3K4me3 and H3K9me3, both of which are normally increased at the Sox2 locus in C2C12 cells. This does not change the mitotic advantage. Supporting data can be found in Data S1.

doi:10.1371/journal.pbio.1001914.g006
Figure 7. Histone ubiquitination can explain the mitotic advantage. (a) Western blot for histone H2AK119Ub and H2B120Ub from interphase and mitotic donor cells treated with IAA or MG132. (b) ChIP analysis for ubiquitinated histone H2A shows a large difference between mitotic chromatin and interphase nuclei for several genomic regions. (c) IAA largely removes the deubiquitinated state of mitotic chromatin in several gene regions. None of these values are significantly different from one another. Supporting data can be found in Data S1. (d) Mitotic advantage is eliminated by IAA treatment. Supporting data can be found in Data S1. (e) H2AK119 ubiquitination in interphase nuclei is reduced by MG132. Supporting data can be found in Data S1. (f) RT-qPCR of interphase and mitotic nuclei treated with MG132 after nuclear transfer to oocytes; interphase transcription is much enhanced. Supporting data can be found in Data S1. (g) Western blot to show in vitro deubiquitination of interphase and mitotic donor nuclei. (h) RT-qPCR transcription analysis of Ubp-M-treated donor nuclei 36 h after nuclear transfer. Supporting data can be found in Data S1.

doi:10.1371/journal.pbio.1001914.g007
iodoacetamide (IAA) made freshly and used at 10 mM. Aphidicolin (A0781, Sigma) at 1 mM. Taxol (T7402, Sigma) at 100 nM. ICRF-90 (I4659, Sigma) used at 4 mM. Spermine; SuNaSP-BSA, SuNaSP with 3% (w/v) BSA; and 0.25 M Sucrose, 75 mM NaCl, 0.5 mM Spermidine, 0.15 mM Spermine; SuNaSP-BSA, SuNaSP with 3% (w/v) BSA; and MG-132 ([12]) in buffer (50 mM NaCl, 50 mM Tri-Cl pH 8.0, 1 mM DTT, 1 mM EDTA-free Protease inhibitor [Roche]).

Materials and Methods

Cell Culture

Cells were cultured in DMEM (D5796, Sigma; E15-810, PAA; 41965-062, Invitrogen) with 10% FCS (10270106, Invitrogen), 100 units/ml Penicillin-Streptomycin (15140-122, Invitrogen), and 0.25 µg/ml Fungizone (15240-096, Invitrogen). Inhibitors used for various experiments included the following: 5'-Deoxy-5'-(methylthio)adenosine (MTA)/D5011, Sigma) used at 1 µg/ml, APhidicolin (A0781, Sigma) at 1 µg/ml, AT9823 (gift from M. Dawson) used at 100 nM; IRF-90 (I4659, Sigma) at 1 µg/ml, iodoacetamide (IAA) made freshly and used at 10 µM, MG-132 (Sigma) used at 4 µM, Nocodazole (M1404, Sigma) at 75–100 mM; Taxol (T7402, Sigma) at 1 µM, Thymidine (T1895, Sigma), and Trichostatin A (T8552, Sigma) at 1 µg/ml.

Synchronization

Cell synchronization was achieved according to the scheme in Figure S1A. In general, medium containing the desired inhibitor was applied to unsynchronized cells a day after seeding for 16–20 h. For mitotic cells, seeded cells were initially arrested in 2 mM thymidine for 16–24 h, washed 3× in PBS, released into fresh media for 6–12 h, and then media replaced with Nocodazole or Taxol containing media for 10–16 h, after which rounded cells were detached by “shake-off” and the culture media harvested for the mitotic cell fraction. G1 arrest was achieved by Serum starvation for 72 h.

Nuclear and Karyoplast Preparation and Transplantation to Oocyte GVs

Cells in suspension (either from mitotic shake-off or trypsinization of adherent cells) were washed twice in PBS, transferred to SuNaSP, and permeabilized with Digitonin (40–100 µg/ml) for 3 min on ice. The reaction was stopped by addition of and excess of SuNaSP-BSA and the nuclei concentrated to an appropriate volume for GV transfer [12]. Nuclear transplantation to oocyte GVs was performed as described in [12].

Supporting Information

Data S1 Supporting histogram data. (XLSX)
Figure S1  (a) Design of cell cycle synchronization procedure. (b) 10T1/2 cells show a mitotic advantage by transcription assay after injection into oocytes. Supporting data can be found in Data S1. (c) Shake-off procedure to enrich for mitotic cells without inhibitors also displays some mitotic advantage when compared to interphase cells. The proportion of cells in mitosis for these samples is indicated in the adjacent table. Supporting data can be found in Data S1. (d) Taxol-synchronized cells give the same transcriptional enrichment as nocodazole-arrested cells after transplantation to oocyte GVs. Supporting data can be found in Data S1. (e) G1/G0-arrested cells, treated with Nocodazole, show no enhancement of pluripotency gene transcription after nuclear transplantation to oocyte GVs. Supporting data can be found in Data S1. (TIF)

Figure S2  Design of experiments for nuclear incorporation assays. (a) A mixture of mitotic and interphase donor nuclei are injected into oocyte GVs, 24 h after the oocytes have been injected with RNAs encoding fluorescently labelled (GFP or Cherry) proteins. The resulting GVs are then isolated from the oocyte and the transplanted nuclei examined by confocal microscopy. I, interphase; M, mitotic. (b) The oocyte-specific transcription factor TBP2 (red) is taken up by mitotic nuclei to a far greater extent than interphase nuclei by 48 h. GFP labelled histone H4 is used to mark the position of transplanted mitotic and interphase nuclei. The arrows indicate examples of one interphase (yellow) and one mitotic nucleus (white). (TIF)

Figure S3  Permeabilization of the nuclear membrane does not reduce mitotic advantage. (a) 3 KDa dextran enters SLO-permeabilized nuclei, but 70 KDa dextran does not (black spheres). SLO and LL together permit entry of 70 KDa dextran nucleoplasm. The table illustrates the proportion of permeabilized plasma and nuclear membranes by these treatments. (b) Transcriptional reprogramming 24 h after transplantation of SLO or SLO+LL permeabilized nuclei to oocyte GVs is similar. Supporting data can be found in Data S1. (TIF)

References

1. Holtzer H, Rubinsteinn N, Fellini S, Yeoh G, Chi J, et al. (1975) Lineages, quantal cell cycles, and the generation of cell diversity. Q Rev Biophys 8: 523–537.
2. Martínez-Balbas MA, Dey A, Rabindran SK, Ozato K, Wu C. (1995) Displacement of sequence-specific transcription factors from mitotic chromatin. Cell 83: 29–38.
3. Egli D, Birkhoff G, Eggan K (2008) Mediators of reprogramming: transcription factors and transitions through mitosis. Nat Rev Mol Cell Biol 9: 505–516.
4. Egli D, Rosains J, Birkhoff G, Eggan K (2007) Developmental reprogramming after chromosome transfer into mitotic mouse zygotes. Nature 447: 679–685.
5. Egli D, Eggan K (2010) Recipient cell nuclear factors are required for reprogramming by nuclear transfer. Development. 137(12): 1953–1963.
6. Lemaire J-M, Danis E, Pasero P, Vassetzky Y, Mechali M. (2005) Mitotic remodeling of the replication and chromosome structure. Cell 123: 787–801.
7. Gaveri Q, Bocquot S, Poller I, Brochard V, Arnaud P (2011) Synergic reprogramming of mammalian cells by combined exposure to mitotic Xenopus egg extracts and transcription factors. Proc Nat Acad Sci USA 108: 17221–17336.
8. Tsoukouchi T, Soza-Kied J, Brown K, Piccolio FM, Cantone I, et al. (2013) DNA synthesis is required for reprogramming mediated by stem cell fusion. Cell 152: 873–883.
9. Foshay KM, Looney TJ, Chai S, Mao FF, Lee JH, et al. (2012) Embryonic stem cells induce pluripotency in somatic cell fusion through biphasic reprogramming. Mol Cell 46: 159–170.
10. Chinn CP, Blau HM (1984) Reprogramming cell differentiation in the absence of DNA synthesis. Cell 37: 879–887.
11. Vierbuchen T, Wernig M (2011) Direct lineage conversions: unnatural but useful? Nat Biotechnol 29: 892–897.
12. Halley-Stott RP, Pasque V, Astrand G, Miyamoto K, Simeoni I, et al. (2010) Mammalian nuclear transplantation to germinal vesicle stage Xenopus oocytes—a method for quantitative transcriptional reprogramming. Methods 51: 56–65.
13. Driesens G, Blanpain C (2011) Long live Sox2: Sox2 lasts a lifetime. Cell Stem Cell 9: 283–284.
14. Jullien J, Astrand C, Halley-Stott RP, Garrett N, Gordon JB (2010) Characterization of somatic cell nuclear reprogramming by oocytes in which a linker histone is required for pluripotency gene reactivation. Proc Natl Acad Sci USA 107: 5403–5408.
15. Coverley D, Downes CS, Romanowski P, Laskey RA (1993) Reversible effects of nuclear membrane permeabilization on DNA replication: evidence for a positive licensing factor. J Cell Biol 122: 985–992.
16. Wang F, Higgins JMG. (2013) Histone modifications and mitosis: countermarks, landmarks, and bookmarks. Trends Cell Biol 23: 175–184.
17. Xu D, Bai J, Duan Q, Costa M, Dai W (2009) Covalent modifications of histones during mitosis and meiosis. Cell Cycle 8: 3688–3694.
18. Valls E, Sánchez-Molina S, Martínez-Balbas MA (2005) Role of histone remodeling factor. J Cell Biol 122: 985–992.
19. Terrenoire E, Sanchez-Molina S, Martinez-Balbas MA (2005) Role of histone modifications in marking and activating genes through mitosis. J Biol Chem 280: 42592–42600.
20. Terrenoire E, McDonald F, Halsall JA, Page P, Illingworth RS, et al. (2010) Immunosubstitution of modified histones defines high-level features of the human metaphase epigenome. Genome Biol 11: R110.
21. Bannister AJ, Kouzarides T (2011) Regulation of chromatin by histone modifications. Cell 141: 11–20.
22. Ke Q, Ellen TP, Costa M (2008) Nickel compounds induce histone ubiquitination by inhibiting histone deubiquitinating enzyme activity. Toxicol Appl Pharmacol 228: 190–199.
22. McAvoy JW, Dixon KE, Marshall JA (1975) Effects of differences in mitotic activity, stage of cell cycle, and degree of specialization of donor cells on nuclear transplantation in *Xenopus laevis*. Dev Biol 45: 330–339.

23. Ellinger MS (1978) The cell cycle and transplantation of blastula nuclei in *Bombina orientalis*. Dev Biol 65: 81–99.

24. Berrelding von CH (1981) The developmental potential of synchronized amphibian cell nuclei. Dev Biol 81: 115–126.

25. Campbell KH, Lui P, Otaegui PJ, Wilmut I (1996) Cell cycle co-ordination in embryo cloning by nuclear transfer. Rev Reprod 1: 40–46.

26. Campbell KH, McWhir J, Ritchie WA, Wilmut I (1996) Sheep cloned by nuclear transfer from a cultured cell line. Nature 380: 64–66.

27. Wakayama T, Rodriguez I, Perry AC, Yanagimachi R, Mombaerts P (1999) Mice cloned from embryonic stem cells. Proc Nat Acad Sci USA 96: 14984–14989.

28. Cibelli JB, Stice SL, Golueke PJ, Kane JJ, Jerry J, et al. (1998) Cloned transgenic calves produced from nonquiescent fetal fibroblasts. Science 280: 1256–1258.

29. Yabuuchi A, Yasuda YU, Kato Y, Tsunoda Y (2004) Effects of nuclear transfer procedures on ES cell cloning efficiency in the mouse. J Reprod Dev 50: 263–266.

30. Oback B, Wells DN (2007) Donor cell differentiation, reprogramming, and cloning efficiency: elusive or illusive correlation? Mol Reprod Dev 74: 646–654.

31. Endoh M, Endo TA, Isono K, Sharif J, et al. (2012) Histone H2A mono-ubiquitination is a crucial step to mediate PRC1-dependent repression of developmental genes to maintain ES cell identity. PLoS Genet 8(7): e1002774.

32. Wang H, Wang L, Erdjument-Bromage H, Vidal M, Tempst P, et al. (2004) Role of histone H2A ubiquitination in Polycomb silencing. Nature 431: 873–878.

33. Joo H-Y, Zhai L, Yang C, Nie S, Erdjument-Bromage H, et al. (2007) Regulation of cell cycle progression and gene expression by H2A deubiquitination. Nature 449: 1068–1072.

34. Zhao R, Nakamura T, Fu Y, Lazar Z, Spector DL (2011) Gene bookmarking accelerates the kinetics of post-mitotic transcriptional reactivation. Nat Cell Biol 13: 1296–1304.