Supporting Information

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Relative gene expression 24h after NT

Fig. S1. Quantification of *Sox2, Oct4,* and *Nanog* reactivation *from* nuclei of retinoic acid-differentiated ES (RA-ES)cells transplanted into a *Xenopus* oocyte. The graphs summarize the results of 15 experiments analyzed as in Fig. 1*B* and show the extent of *Sox2, Oct4,* and *Nanog* gene transcription in RA-ES nuclei 24 h after nuclear transfer to oocytes. The results are shown as the per cent of experiments in which, after nuclear transfer, transplanted nuclei regained 0%, 10%, 30%, or 100% of the transcription level of ES nuclei.



Fig. S2. Real-time monitoring of histone H2B and oocyte linker histone following nuclear transfer into oocyte germinal vesicle. (*A*) Core histone H2B remains associated with transplanted nuclei. Real-time monitoring of H2B-GFP (present in HeLa nuclei before transplantation) and B4-RFP (expressed in the oocyte by mRNA injection) was carried out during the first 6 h of reprogramming (a complete series of images is shown in Movie S1). (*B*) Average change in fluorescence intensity with time in the experiment shown in (*A*). Also shown is the decondensation of nuclei as measured by changes in nuclear area. Error bars indicate the mean \pm SEM (n = 15 nuclei).



Fig. S3. H3 and H3.3 incorporation into the chromatin of transplanted nuclei. C2C12 nuclei were transplanted into oocytes preinjected with mRNA for GFP-H3.3, GFP-H3, or H1o-GFP. Six hours after nuclear transfer (*Left*), chromatin is labeled with H1° (*Bottom Row*) but not with H3.3 or H3 (*Top and Middle Rows*). At 24 h after nuclear transfer (*Right*), chromatin labeling by H3.3 or H3 is observed.

DN A C

ssDNA injection



Myc tagged competitor





Fig. 54. Characterization of chimeric B4/H1 linker histone. (*A*) Experimental design for tagged linker histone ChIP analysis from chromatinized plasmid following injection of ssDNA into the oocyte. (*B*) Quantification of tagged linker histone overexpression. The level of B4-GFP and Myc-B4 proteins obtained by mRNA injection to the oocyte is compared with the level of endogenous B4 in the oocyte nucleus by Western blot with anti-B4 antibody. (*C*) ChIP analysis shows that B4 binds to chromatinized plasmid DNA. ChIP analysis was performed with anti-GFP or anti-MYC antibody on samples prepared from oocytes containing various amounts of B4-GFP and Myc-B4. (In the table below the graph, the tagged B4 protein level is shown as the per cent of endogenous B4.) (*D*) Structure of chimeric linker histones. Amino-terminal, globular, and carboxy-terminal domains of oocyte and somatic linker histones were swapped to generate chimeric linker histones (*Upper*). mRNA encoding these constructs were injected into the oocyte to obtain more than 5-fold overexpression over endogenous nuclear B4 (*Lower*).

Q-RT-PCR

- Nanog-F: TCTCTCAGGCCCAGCTGTGT
- Nanog-R: GCTGGAGGCTGAGGTACTTCTG
- Nanog probe: FAM- CACTCAAGGACAGGTTT -NFQ-MGB
- Sox2-F: TCAGGCTGCCGAGAATCC
- Sox2-R: TCAAACTGTGCATAATGGAGTAAAAAC
- Sox2 probe: FAM- TGAACTAATACCATCCTTATAAC -NFQ-MGB
 - Oct4 F: GAAGGGCAAAAGATCAAGTATTGAG
- Oct4-R: GCCCCCCTGGGAAAG
- Oct4 probe:FAM- CCCAACGAGAAGAGTATGAGGCTACAGGGAC -BHQ
- G3PDH-F: CATGGCCTTCCGTGTTCCT
- G3PDH-R: GCGGCACGTCAGATCCA
- C-Jun F: CCTGTCCCCTATCGACATGG
- C-Jun R: CTTTTCCGGCACTTGGAGG
- C-Jun probe: FAM- TCCTCATGCGCTTCCTCTGCCT -BHQ1
- (FAM= dye for probe
- BHQ: Black hole Quencher 1 from Sigma-Aldrich
- NFQ: Non-Fluorescent quencher from ABI
 MGB: Minor Groove Binder)
- Q-PCR (CHIP)
- Major sat-F: GACGACTTGAAAAATGACGAAATC
 Major sat-R: CATATTCCAGGTCCTTCAGTGTGC
- Major sat-R: CATATICCAGGICCTICAGIGIC
- Octp-F: TCTAGACGGGTGGGTAAGCAA
- Octp-R: CCTAAAACATCCATTGAATGTTCGT
- Sox SSR2-F:CAGGTTCCCCTCTAATTAATGC
- Sox SSR2-R:CTGTGCTCATTACCACGTGAA
- Nanogp-F:GTAAAGCCTCTTTTTGGGGG
- Nanog-R:TCACACTGACATGAGTGTGG
- M13-F:CGCCTCTGCGCGATTTT
- M13-R:CAGATCCTTTTACATCGGGAGA

RT-PCR

- Oct4-F: GTGAGCCGTCTTTCCACCAG
- Oct4-R: TTCTCCAACTTCACGGCATT
- Nanog-F: CCAGTCCCAAACAAAGCTC
- Nanog-R: GCTTGCACTTCATCCTTTGG
- Sox2-F: GGAGTGGAAACTTTTGTCCGAGAC
- Sox2-R: TGGAGTGGGAGGAAGAGGTAACC
- C-Jun-E: TGAAAGCTGTGTCCCCCTGTC
- C-Jun-R: ATCACAGCACATGCCACTTC
- G3PDH-F: TCAACGACCCCTTCATTGAC
- G3PDH-R: ATGCAGGGATGATGTTCTGG
- VgT-F: AGAAACTGCTGTCGGGAA
- VgT-R: CGGATCTTACACTGAGGA

Fig. S5. List of primers used in PCR and real-time PCR analysis.

Other Supporting Information Files



Movie S1. Real-time monitoring of gene activation following nuclear transplantation. Reporter nuclei (1) were transplanted in oocyte expressing CFP-LacR (green), MS2-YFP (yellow), and oocyte linker histone B4-RFP (red). Images were collected every 10 minutes, starting 10 minutes after transplantation.

1. Janicki SM, et al. (2004) From silencing to gene expression: Real-time analysis in single cells. Cell 116:683-698.

Movie S1 (MP4)



Movie S2. Real-time monitoring of linker histone exchange in transplanted nuclei. NIH3T3 nuclei expressing H1o-GFP were transplanted into *Xenopus* oocyte expressing oocyte linker histone B4-RFP. Images were collected every 20 minutes, starting 10 minutes after transplantation.

Movie S2 (MP4)



Movie S3. Real-time monitoring of histones exchange in transplanted nuclei. Hela nuclei expressing H2B-GFP were transplanted into *Xenopus* oocyte expressing oocyte linker histone B4-RFP. Images were collected every 20 minutes, starting 10 minutes after transplantation.

Movie S3 (MP4)