Supplementary Materials and Methods

Embryo collection and microinjection

Zygotes and 2-cell stage embryos were collected at 17-18 h post-hCG and 46 h post-hCG injection, respectively, from F1 (C57BL/6 × CBA/H) crosses on superovulation. Human H3.1(Santenard et al. 2010; aa 1 to 135), H3.2 (aa 1 to 135), H3.3 (Santenard et al. 2010), and H2A (HIST1H2AK) cDNAs were subcloned into pRN3P plasmid and corresponding mRNAs were transcribed in vitro, as described previously (Santenard et al. 2010). The H3.2 plasmid was generated by mutating the H3.1 construct. 1-2 pl of corresponding mRNAs at 230 ng/µl were microinjected. Embryos were then cultured in KSOM medium at 37ºC and 5% CO₂ until appropriate stage and were subject to FRAP analysis. Embryos were subsequently placed back in the incubator, allowed to develop to the blastocyst stage and developmental progression was scored on the 3rd day. All fusions were cloned with EGFP in C-terminal and all plasmids have identical 3’ and 5’UTRs. For the FRAP analysis in blastocysts, the levels of GFP in the blastocyst were too noisy when microinjection was performed in the zygote. Thus, to overcome this limitation, both blastomeres of 2-cell stage embryos, collected at 45 hphCG, were injected with identical concentrations of mRNA. We previously determined that recovery rates are independent of protein levels (see Supplementary Figure S3). For the CARM1 double microinjection experiments, zygotes were injected with appropriate mRNAs 18 h post-hCG and cultured until the late 2-cell stage (46 h post-hCG), at which point one of the blastomeres was injected with HA.CARM1 mRNA (0.8 µg/µL) with mRFP mRNA as tracer (Torres-Padilla et al. 2007). Embryos were cultured until the 8-cell stage and double-positive embryos were subject to FRAP. In each embryo, one H3.1-GFP+/RFP- and one H3.1-GFP+/RFP+ blastomere was analysed. We have checked that the presence of RFP does not influence the GFP recovery curves (not shown). To verify the incorporation of histones into chromatin, zygotes were injected with H3.1, H3.2 and H2A-GFP mRNAs, as described and embryos were fixed in mitosis (48-50 h post-hCG). Fixed embryos were mounted in Vectashield (Vector Laboratories) containing 4′-6- Diamidino-2-phenylindole (DAPI) for visualizing DNA. Furthermore, H2A, H3.1 and H3.2-positive 2-cell stage embryos were subject to Triton pre-extraction, as described (Hajkova et al. 2010), fixed, mounted, and GFP signal was analysed by confocal microscopy. Note that it is known that the inside of the NLBs tends to accumulate non-incorporated histones or
overexpressed proteins. Therefore, we systematically avoided the NLBs during our FRAP experiments.

**Immunostaining**

ES cells were fixed with 4% paraformaldehyde in PBS for 20 min at room temperature. After washing with PBS, cells were permeabilized with 0.5% TritonX-100 in PBS for 10 min and then incubated in blocking solution (0.2% BSA in PBS) for 30 min. Primary antibody used was Oct4 (611202, BD Phamingen). After incubation in blocking solution containing primary antibody for 1 hour, cells were washed three times with 0.01% TritonX-100 in PBS for 5 min each and then incubated in blocking solution containing secondary antibody labeled with Cy3 (Jackson ImmunoResearch Laboratory). After washing with PBS, mounting was done in Vectashield (Vector Labs).

**Microscopy**

All experiments were performed using a Leica SP2 confocal microscope and at 37°C using a 63.0 x 1.4 oil objective. For FRAP, embryos were placed in drops of M2 medium on a glass bottom dish. A rectangular region of interest of 2.28 µm² was chosen randomly within a nucleus avoiding the nucleolar-like bodies and the surrounding heterochromatin regions and was subject to FRAP. Ten prebleach frames were acquired followed by 2 bleach pulses without acquisition. Recovery of fluorescence was followed during 60 seconds, with 1 frame/second. The raw data was processed with Fiji software (ImageJ). All analysis was done on background-subtracted values, using EasyFRAP software (Rapsomaniki et al. 2012). Briefly, the signal of the bleached region was normalised to its prebleach value, which was set to 1. The fluorescence intensity of the whole nucleus at each timepoint was used to correct the decrease in signal intensity in the ROI during the imaging process. The obtained curves were normalized using the Full-scale normalization method so that the first postbleach frame was set to zero. Normalized curves were then subject to curve fitting. For fixed samples, confocal microscopy was performed using a 63x oil objective on Leica SP2 AOBS MP inverted microscope. Z-sections were taken every 1 µm.
Curve fitting and statistical analysis

Experimentally obtained and normalized recovery curves were fit using Prism6 software (GraphPad Software). Two-phase exponential association equation \( Y = Y_{\text{max}1} * (1 - e^{-K1*X}) + Y_{\text{max}2} * (1 - e^{-K2*X}) \) was used to obtain mobile fractions and reaction rates, as this has been previously described to be appropriate for nuclear proteins (Phair and Misteli 2000). Accordingly, throughout the manuscript, \( Y_{\text{max}1} \) values are used for mobile fraction estimation, as they reflect the steady-state protein pool, unless otherwise stated. However, all the \( Y_{\text{max}1} \) and \( Y_{\text{max}2} \) and their statistical comparisons are shown in the Supplementary Information. All the fit data are presented as mean ± S.E.M. Statistical analysis on mobile fractions between different stages was performed using and QuickCalcs software (GraphPad Software). Unpaired t-test was used for comparing two groups.

Inhibitor treatment

To address if histone mobility is regulated through the action of the G9a histone methyltransferase or through histone acetylation, zygotes were collected as detailed above, microinjected with H2A-GFP or H3.1-GFP mRNA and allowed to develop till the 8-cell stage. Embryos were then treated with TSA (100 nM) for 2 hours to inhibit HDAC activity (Ma et al. 2001; Maalouf et al. 2009) or BIX-01294 (4.1 mM) for 4 hours to inhibit G9a (Kubicek et al. 2007), after which they were subject to FRAP as above. Control embryos were treated with vehicle (DMSO) and subject to FRAP under identical conditions in parallel. After FRAP analysis, embryos were fixed as described (Torres-Padilla et al. 2006) and the effect of inhibitors was verified by immunostaining. Antibodies used were: anti-H4K8,12ac (kindly provided by M. Oulad-Abdelghani, IGBMC) and anti-H3K9me2 (Upstate 07-441), at 1:200 dilution.

Electron microscopy

Embryos at the 2-cell (n=3) and 8-cell (n=3) stages were collected after natural matings of B6CBAF1/J mice. Embryos were fixed in 2% formaldehyde + 2.5% glutaraldehyde in 0.1M cacodylate buffer for 2h at 37°C, postfixed 1h at 4°C in 1% osmium tetroxide and en bloc stained with 1% uranyl acetate for 1h at 4°C. Samples were then dehydrated in graded ethanol solutions (50%, 70%, 90%, 100%) to be then infiltrated with epoxy resin by a graded series of dilutions (30%, 70%, 100%). Due to the size of the embryos, they were flat embedded in a
sandwich of Aclar (200µm) in order to be observed using binoculars. Ultrathin sections (70 nm) were performed using an ultracut UCT ultramicrotome (Leica Microsystems, Vienna, Austria) and mounted on pioloform coated slot grids to avoid crossing mesh in the nucleus. They were then stained for 20 minutes with uranyl acetate and 5 minutes with lead citrate and observed with a transmission electron microscope (CM12, Philips; FEI Electron Optics, Eindhoven, the Netherlands) operated at 80kV. Images were acquired using an Orius 1000 ccd camera (Gatan, Pleasanton, CA). MEFs (DMEM 1g/ml + 10% FCS + gentamycin) were fixed and treated as described above (n>40).

Quantification of electron dense regions from TEM micrographs.
The evolution of heterochromatin compaction between 2-cell and 8-cell embryos was quantified by comparing the relative areas of electron-dense (ED) regions in transmission electron microscopy (TEM) images inside the nucleus. Assuming the separability of ED and non-ED regions into two classes, these regions can be classified by thresholding methods. To ensure a proper classification, the illumination bias of TEM images was corrected using an automatic method based on intensity gradients and a bivariate polynomial modeling (Tasdizen et al. 2008) prior to the classification procedure. To define a proper region of interest (ROI) corresponding to the nucleus without the nucleolus or the nucleolar-like bodies (Supplementary Figure S8a) the classification task was performed using three steps. First, the boundaries of the nucleus were estimated. Because the homogeneity of the intensities inside and outside the nucleus and the poor boundary definition precluded the use of an automatic method, this step was done manually. Secondly, the area occupied by the nucleolus was removed based on two observations i) the nucleoli are large ED regions in TEM images with relatively smooth edges (in contrast with heterochromatin) and ii) the nucleoli are separable from the background of the nucleus (the histogram is bimodal). These two parameters allowed the automatic segmentation of the nucleoli based on a minimum thresholding (Prewitt and Mendelsohn 1966) followed by a morphological opening in order to remove remaining small ED regions (Supplementary Figure S8b). Finally, the third step was to classify ED/non-ED regions from the ROI defined by the nucleus minus the nucleoli using the isodata thresholding method (Ridler and Calvard 1978), which resulted in the segmentation of ED and non-ED regions (Mask, Figure 4b). Subsequently, the proportion of ED area over the whole ROI area was computed as a percentage. The whole procedure was
implemented as a macro of ImageJ. The thresholding methods and the morphological opening used in the experiments are the ImageJ implementation. The minimum and isodata thresholding procedures correspond to Minimum and Default methods of Auto global thresholding menu. The illumination bias correction has been implemented as a plugin of ImageJ.

ES cell culture and generation of 2C::tdTomato MuERVL reporter cell line
Mouse E14 ES cell line was cultured without feeders on gelatin-coated glass-bottom dishes (MatTek) in DMEM with GlutaMax (Invitrogen) containing 15% FCS, LIF, non essential aminoacids, penicillin/streptomycin, and 0.1 mM 2-mercaptoethanol supplemented with 3 μM CHIR9901 and 1 μM PD0325901. The 2C::tdTomato plasmid (addgene) (Macfarlan et al. 2012) was transfected into E14 ES cells by Lipofectamine 2000 and cells were selected with 200μg/mL hygromycin. After selection, several colonies were picked and the clones in which tdTomato was expressed in a small proportion of cells (Macfarlan et al. 2012) were chosen for further experiments. For FRAP experiments, cells were transiently transfected with H3.1-GFP or H2A-GFP plamids using Lipofectamine 2000 (Invitrogen) according to manufacturer’s instructions. The H3.1-EGFP plasmid was constructed by inserting the human H3.1 cDNA (Santenard et al. 2010) into the pEGFP-N2 plasmid (Clonetech). FRAP was performed 24 h after transfection on tdTomato+/GFP+ as well as control (tdTomato-/GFP+) cells.

References for Supplementary Methods and Figures


