

Supplementary Materials and Methods

Mouse strains and embryo culture

For EdU labelling of paternal chromatin male from C57BL/6J x C3H/He mice were supplied continuously with drinking water containing 0.5 mg/ml EdU. The EdU-containing water was changed twice per week. EdU is incorporated in place of thymidine into the replicating DNA of mitotically dividing somatic and pre-meiotic cells. The cycle time to produce mature spermatozoa from EdU-labelled pre-meiotic cells in mice is ~35 days. After continuous EdU feeding for at least 6 weeks, EdU-treated males were mated with superovulated B6C3F1 females. Embryos were isolated 20 hours after HCG injection and cultured in G1 medium (Vitrolife) up to the 8-cell stage.

Embryos used for live imaging of microtubule tips were isolated from C57BL/6J x C3H/He F1 females mated with C57BL/6J x C3H/He F1 males.

Immunofluorescence

To visualise EdU-substituted DNA embryos were fixed with 1%PFA for 30 minutes and then washed three times in PBS. For staining of EdU labelled DNA embryos were treated with Click-iT® Labelling Technologies (Thermo Fisher Scientific C10340) according to the manufacturer's instructions.

Paternal chromatin was labelled with Alexa Fluor 647 and the whole nucleus counter stained with 100nM Sytox Green (Molecular probes S7020).

For immunofluorescence of H3K9me3 and Ring1B embryos were fixed as described ¹. Embryos were incubated overnight at 4 °C at the following primary antibody dilutions: 1:2000 rabbit anti Ring1B (Abcam 101273) or 1:200 rabbit anti-H3K9me3 (Abcam 8898) in 5% normal goat serum, 3% BSA in PBST. Embryos were washed with 0.3%BSA in PBST for 3x 5 minutes then incubated with 1:1000 anti-rabbitAlexa 647 and 5ug/ml Hoechst (Sigma) for 1 hour at room temperature. Embryos were washed with 0.3%BSA in PBST for 3x 5 minutes before imaging.

Embryo Imaging

Fixed EdU treated embryos were imaged using the in-house-built inverted light-sheet microscope. 101 images with 520nm between planes were acquired consecutively for Sytox Green and Alexa 647 signals. Fixed embryos stained for epigenetic marks were imaged on a SP8 Leica confocal microscope equipped with a 63x C-Apochromat 1.2 NA water immersion objective lens. Images were acquired at 90 nm XY and 360 nm Z resolution. For imaging of growing microtubule tips 5 images with 500 nm between planes were acquired simultaneously for mCherry and EGFP at 800 ms intervals using the in-house-built inverted light-sheet microscope.

Image processing and analysis

Segmentation and analysis of EDU signal

The nucleus was segmented from the DNA signal and used as a mask to segment the EdU signal. The centre of nuclear mass was detected and a plane was fitted through the centroid to achieve maximum separation of the EdU signal in the two resulting hemispheres. Then, the volume of the smallest EdU signal was divided by the volume of the largest EdU signal.

Segmentation and analysis of maternal and paternal genomes

The chromosome mass was segmented from the DNA channel and used as mask to crop maternal and paternal chromosome signals. These signals were then segmented using intensity threshold without considering the regions masked out. Segmented maternal and paternal chromosomes were bounded by two separate 3D convex hulls. The mixing between the two was computed by the volume of overlapping voxels between two convex hulls divided by the volume of the union of the two convex hulls.

Simulation of random distribution of paternal chromatin signal at interphase

Three spheres were generated representing chromosome masses of 2-cell, 4-cell and 8-cell embryos. The average volume of cells in each stage was calculated from the original data in order to select the radius of each sphere to achieve comparable relative volumes of chromosome masses in different stages. 40, 20 and 10 small spheres representing paternal chromatin signal were generated with the chromosome masses of 2-cell, 4-cell and 8-cell embryos, respectively.

Simulation of random distribution of maternal and paternal centromere signals at metaphase

A model for chromosome mass was generated by combining the shape information of several individual metaphase chromosome masses. 40 spheres with the same radius were generated in random location within the model chromosome mass of which 50% represent maternal and 50% paternal chromosomes. A minimum distance between the centroids of any two spheres was enforced in order to avoid generating multiple spheres in the same location.

Correlation of maternal and paternal chromosome congression (15-frame window)

To segment chromosomes, original images were filtered first using a 2D Gaussian filter of size 3 and SD=2. Filtered images were interpolated along Z to obtain isotropic pixel size. Pixels at a distance from the centre of mass $>13 \mu\text{m}$ were discarded to reduce the effect of noisy blobs to be detected as chromosomes. Each slice of the interpolated stack was binarised using an adaptive threshold value

determined by combining both 2D and 3D threshold values as described in ². Chromosome masses were detected after removing very small and scattered pixels by connected component analysis. Maternal and paternal chromosome masses were represented by their three orthogonal Eigen vectors and associated eigenvalues where the eigenvector with the smallest eigenvalue represented the shortest elongated axis of the chromosomal volume. This smallest Eigen value was used as a measure for chromosome congression over time. Since duration of individual phases in different embryos may vary, they were normalised to average phase time. This resulted in uniform phase wise progression among all the embryos and allowed comparison in time. Correlation (Pearson) of the smallest Eigen values between maternal and paternal chromosomes was computed over time considering a sliding window of size 15.

Correlation between 5mC and 5hmC signals

The Yoyo channel was interpolated first to obtain isotropic voxels. A 3D Gaussian filter of size 3 and SD=2 was applied to reduce the effect of noise. Interpolated stacks were binarised by combining 2D and 3D threshold values and chromosome masses were detected by connected component analysis. Interpolated slices were removed to keep only original slices in order to detect 5mC and 5hmC signals inside the chromosome mass. Spatial correlation between these two signals was performed considering only their bright pixels (top 25%) detected from their histograms. Then their correlation were computed as below

$$\frac{N_{5mC \cap 5hmC}}{\sqrt{N_{5mC} \times N_{5hmC}}}$$

Where N_{5mC} and N_{5hmC} represent the number of 5mC and 5hmC top 25% positive pixels, respectively.

Volume occupied by Top 50% of pixels for 5mC, 5hmC, Ring 1B and H3K9me3

Segmentation of 5mC, 5hmC, Ring1B and H3K9me3 regions was performed as described above, however a number of top pixels that added up 50% of the total intensity of the signal were selected. The volume occupied by these pixels was divided by the total volume of the signal.

Average intensity for maternal and paternal pro-nuclei 5mC and 5hmC labelling

Chromosome regions were segmented from the DNA channel. The average background intensity was calculated from the intensity in chromosome free regions and was subtracted from the original signal. The average intensity of pro-nuclei was calculated from the background subtracted signal.

Analysis of directionality of growing microtubule tips

To analyse the directionality of growing microtubule tips the Laplacian of Gaussian, also known as the Mexican hat filter, was computed using the ImageJ plugin LoG3D³. Kymographs were computed using the kymograph analyze feature of ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <https://imagej.nih.gov/ij/>, 1997-2016.).

EB3 comets were tracked using the Matlab tool u-track2.0 from the Danuser Lab (www.utsouthwestern.edu/labs/danuser/software/). Due to the 4D nature of the problem the track length was typically short with approximately 80% of the tracks shorter than 5 frames. For the analysis we used tracks longer than 3 frames that were sufficient to determine the overall direction of microtubule growth, resulting in > 7000 tracks per embryo. As the tracks followed straight lines, we used the vector determined by the initial and end point of the track for further processing. To remove tracks from astral microtubules we performed a density based clustering using the average track position and chose the largest cluster (Michael Hahsler and Matthew Piekenbrock (2017). *dbscan: Density Based Clustering of Applications with Noise (DBSCAN) and Related Algorithms*. R package version 1.1-1. <https://CRAN.R-project.org/package=dbscan>). To cluster similar trajectories (Supplementary Fig. 3B) we performed spectral clustering using the absolute cosine distance between trajectories to construct the similarity matrix (David Meyer and Christian Buchta (2017). *proxy: Distance and Similarity Measures*. R package version 0.4-17. <https://CRAN.R-project.org/package=proxy>). Then we computed eigenvalues and eigenvectors of the random-walk normalised graph-Laplacians (Chung, F. (1997). *Spectral graph theory* (Vol. 92 of the CBMS Regional Conference Series in Mathematics). Conference Board of the Mathematical Sciences, Washington.). The Eigen gap heuristic indicated that three clusters were sufficient to describe our data (not shown).

- 1 Kitajima, T. S., Ohsugi, M. & Ellenberg, J. Complete kinetochore tracking reveals error-prone homologous chromosome biorientation in mammalian oocytes. *Cell* **146**, 568-581, doi:10.1016/j.cell.2011.07.031 (2011).
- 2 Heriche, J. K. *et al.* Integration of biological data by kernels on graph nodes allows prediction of new genes involved in mitotic chromosome condensation. *Mol Biol Cell* **25**, 2522-2536, doi:10.1091/mbc.E13-04-0221 (2014).
- 3 Sage, D., Neumann, F. R., Hediger, F., Gasser, S. M. & Unser, M. Automatic tracking of individual fluorescence particles: application to the study of chromosome dynamics. *IEEE Trans Image Process* **14**, 1372-1383 (2005).