Histone Acetylation Controls the Inactive X Chromosome Replication Dynamics

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Supplementary Figure S1. Karyotype of C2C12 cell line stably expressing GFP-PCNA

M-FISH on transgenic C2C12 lines exhibited an almost tetraploid karyotype\textsuperscript{61}. The line probably originated from tetraploid cells, which eventually lost some chromosomes, and underwent an additional chromosome 11 and a t(4;15) translocation. The original tetraploidy of the cell line explains, why generally two inactive X chromosomes can be expected. The non-transgenic original C2C12 cell line showed a similar hypotetraploid karyotype.
Supplementary Figure S2. Effects of loss of Xist RNA, H3K27m3 accumulation and histone hypoacetylation on cell cycle progression

BrdU incorporation was used to identify the S-phase cells. The percentage of replicating cells in the population was quantified in control and recombined Xist conditional KO and Ezh2 conditional KO, as well as in control and TSA treated C2C12. While knocking out Xist and treating cells with TSA did not significantly disturb the relative S-phase duration, Ezh2 KO cells show a strong impairment in cell cycle progression.
Supplementary Figure S3. *Xist* conditional KO cells showing the Xi replication pattern before and after recombination

*Xist* conditional KO (control and recombined) cells after 15 minutes incorporation of 10 µm EdU were co-stained for replication and H3K27m3. *Xist* KO cells still show a clear synchronous replication pattern during mid S, demonstrating that Xist RNA coating the Xi is dispensable to maintain the Xi replication dynamics. Scale bar: 10 µm.
Supplementary Figure S4. Global and Xi specific hyperacetylation after TSA treatment

(a) C2C12 cells were treated with 20 nM TSA for four days, fixed and double stained with anti-H4K8ac and anti-H3K27m3 specific antibodies followed by FISH with a chromosome X specific probe. DNA was counterstained with DAPI. Confocal microscopy images showing the X chromosome territories and H3K27m3 marking the two Xi chromosomes (left panels, maximum intensity projections) and corresponding acetylation signal (single mid section). Arrows point at Xi chromosomes. Scale bar: 5 μm. (b) The integrated histone acetylation signal intensity over the whole nucleus or exclusively at the Xi territory was measured in confocal mid sections of TSA treated and untreated cells (n = 34) by measuring the integrated acetylation signal intensity at the whole nucleus/inactive territory as defined by the X chromosome FISH signal and H3K27m3 accumulation. The results were normalized to the value in control cells. Statistical analysis was performed using Microsoft Excel. Results are plotted relative to control untreated cells. Error bars represent 95% confidence interval.
Supplementary Figure S5. Effect of TSA treatment on Xist RNA accumulation

(a) Maximum intensity projections of confocal images of Xist RNA FISH (green) and DAPI (red) on C2C12, mouse and human female primary fibroblasts, with and without TSA. Scale bar: 10 µm. (b) Quantification of the percentage of cells showing accumulation of Xist RNA on the Xi territories with and without TSA (20 nM TSA for four days); n > 78.
Supplemental References

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