Symmetry-breaking mechanisms at the *Xist* locus at the onset of X-chromosome inactivation

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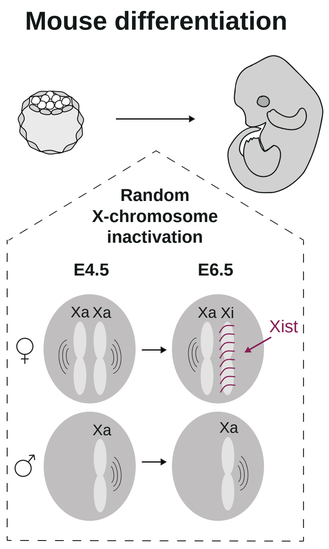
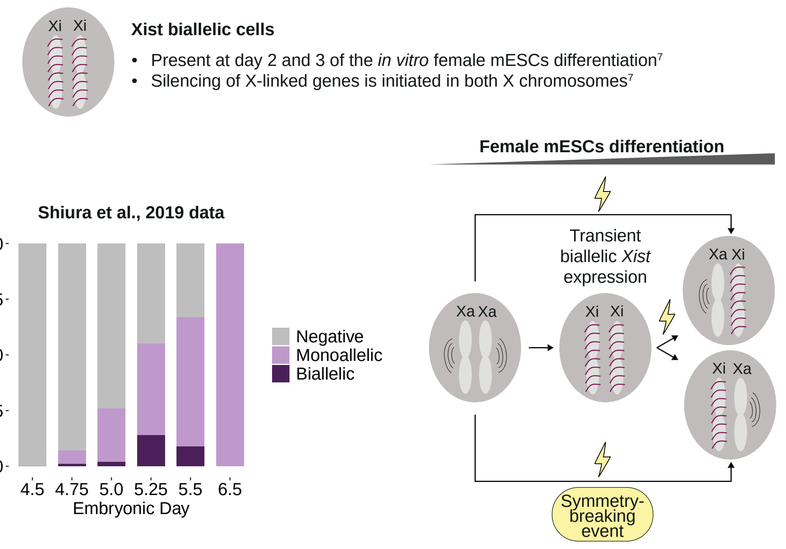
Background

- X-chromosome inactivation (XCI) is the process by which female mammals compensate for the dosage imbalance of X-linked genes between the sexes[1]. *Xist* acts as the master regulator of XCI by silencing one X chromosome *in cis*[2].
- During mouse embryonic development, before establishing the *Xist*-monoallelic expression state, the epiblast undergoes a transitional stage during which *Xist*-negative, monoallelic (MA) and biallelic (BA) *Xist* expressing cells coexist[3-5]. **A study of *Xist* intrinsic dynamics with high temporal resolution is needed to understand how *Xist* is regulated and how the different *Xist*-expressing cells fluctuate before becoming all MA.**
- *Xist* is positively regulated by X-linked factors in a X-dosage-dependent manner and repressed by antisense transcription and heterochromatization of the promoter[6]. A symmetry-breaking event must occur between the two *Xist* alleles to the extent that *Xist* is only expressed in one of the two X chromosomes, which will subsequently be inactivated. Even though the process has been studied intensively, **the specific event responsible for symmetry-breaking is unknown.**





How is the symmetry between the two X chromosomes broken to adopt two opposite states within the same cell?

Research aims

- Quantify the dynamics of mono- and biallelic *Xist* upregulation using live-cell imaging techniques
- Characterize the molecular state of *Xist*-expressing and *Xist*-silent alleles at the onset of XCI through the development of a cell sorting system with allelic specificity
- Investigate candidate mechanisms for symmetry-breaking through quantification and perturbation

1. *Xist* upregulation dynamics through live-cell imaging

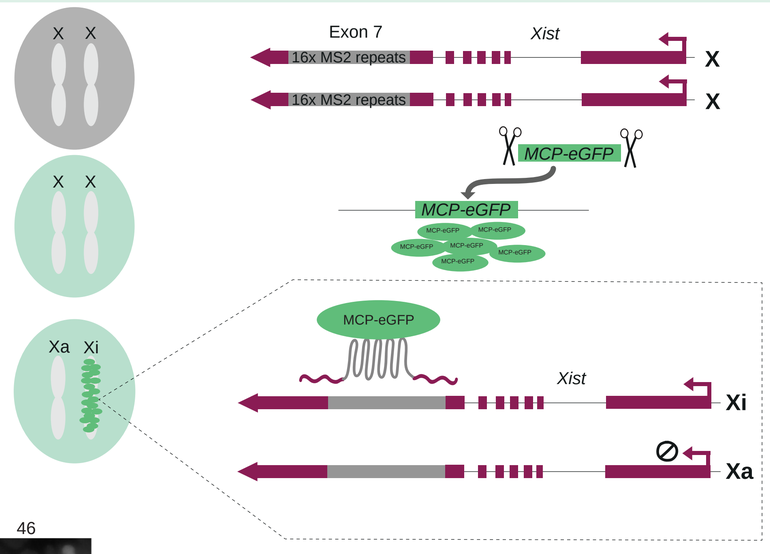
When is *Xist* upregulated? Do all cells undergo a BA state? How long does the BA state last? Is the BA state resolved to MA?

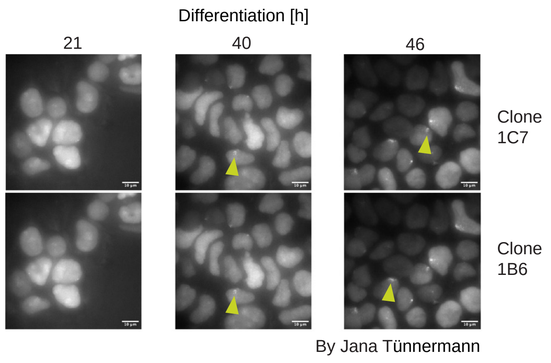
1. Generating the 1.8 XX *Xist*-MS2 MCP-eGFP cell line

1.8 XX cell line with a MS2 tag in both *Xist* alleles (Schulz et al., 2014)[8]

MS2-coat protein (MCP) random integration in the genome using PiggyBac system (by Jana Tünnermann)

Visualization of the Xist cloud in the inactive X chromosome through the accumulation of MCP-eGFP

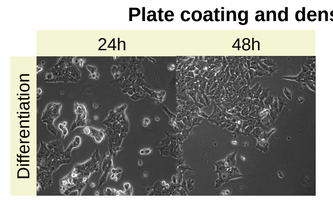




By Jana Tünnermann

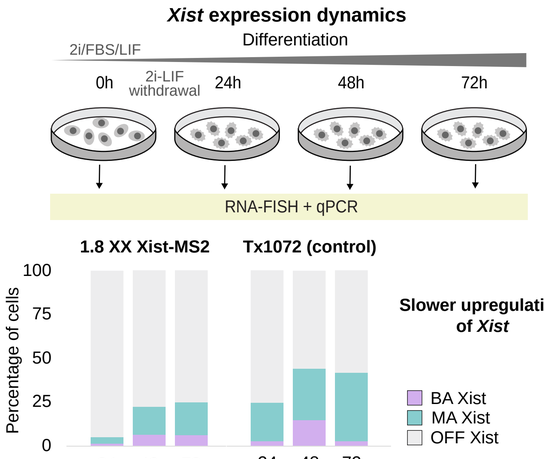
2. Characterizing 1.8 XX *Xist*-MS2

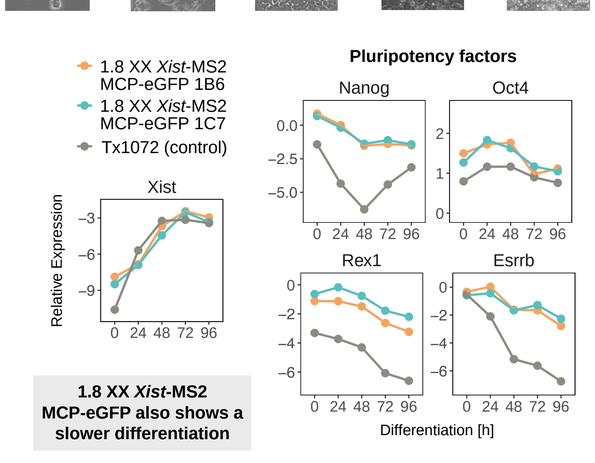
Plate coating and density tests



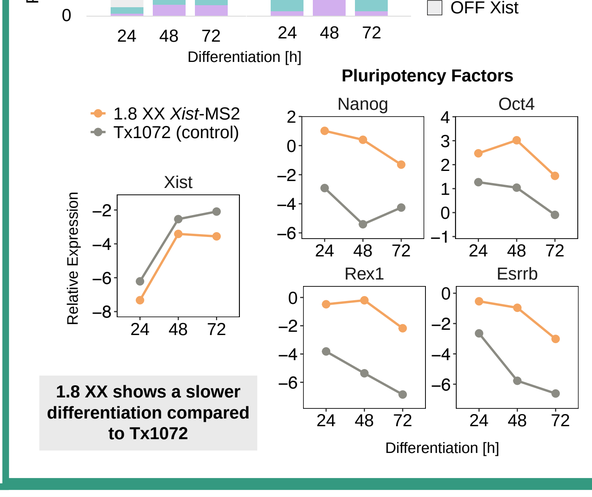
Optimal conditions for cell attachment to the glass-bottom dishes used for imaging

***Xist* expression dynamics**





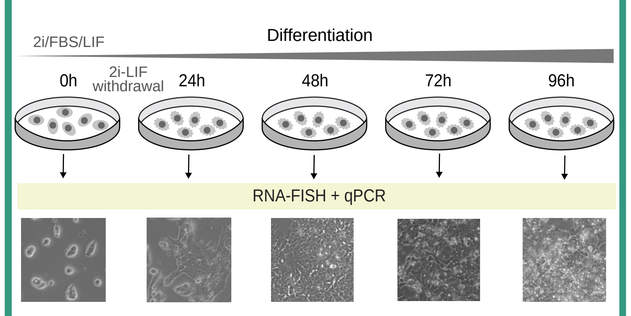
1.8 XX *Xist*-MS2 MCP-eGFP also shows a slower differentiation



1.8 XX shows a slower differentiation compared to Tx1072

3. Characterizing 1.8 XX *Xist*-MS2 MCP-eGFP

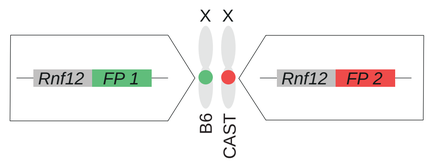
Xist expression dynamics



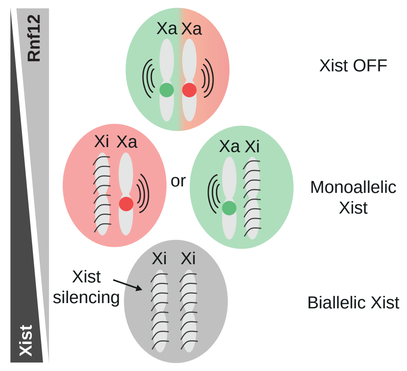
2. Allele-specific characterization of the *Xist* locus during XCI

What are the molecular differences between the Xi and Xa during early *Xist* upregulation time-points?

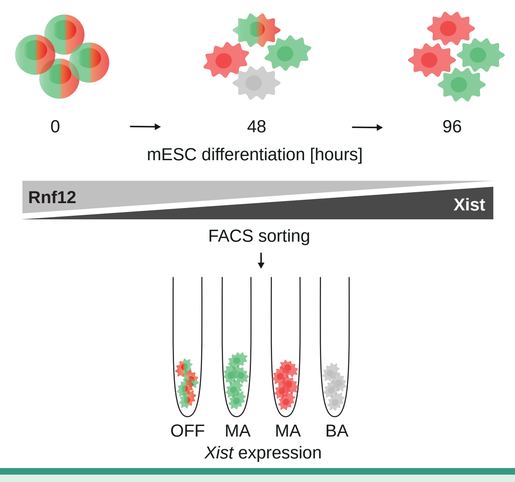
1. System design



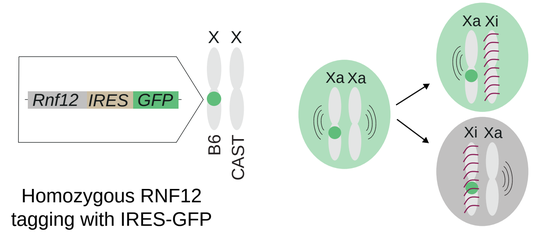
Proxy for *Xist* expression tagging an X-linked gene that is highly-expressed and fast-silenced (e.g., *Rnf12*).



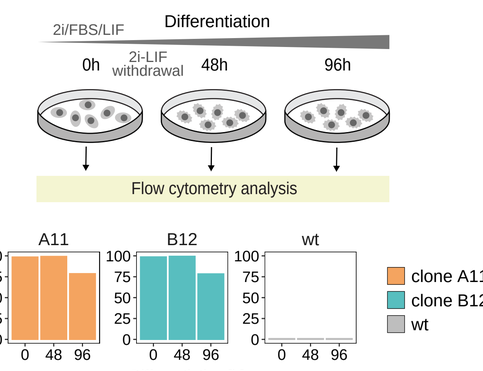
Sorting of the cells depending on *Rnf12* expression, which negatively correlates with *Xist* expression



2. First test



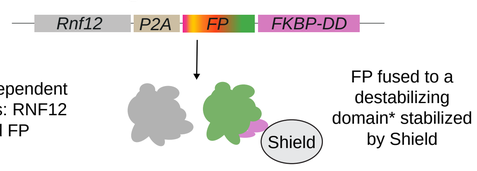
Homozygous RNF12 tagging with IRES-GFP



The system works → We can detect a decrease in the GFP signal during differentiation → But we need a faster system

3. System optimization

Design of the construct



Two independent products: RNF12 and FP

FP fused to a destabilizing domain* stabilized by Shield

Co-transfection with Cas9 and *Rnf12* specific guides

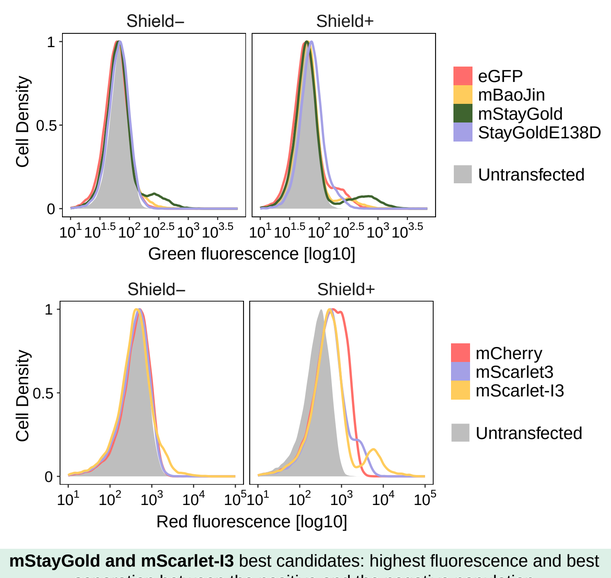
*To mimic RNF12 half-life

Select the brightest fluorescent proteins and test in cells

Transfect mESCs with different constructs → Screening by flow cytometry

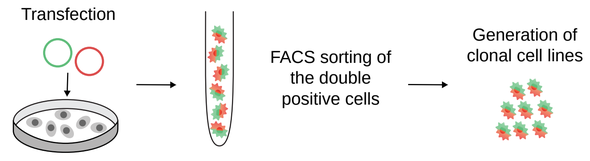
4. Fluorescent proteins screening

Flow cytometry analysis of the transfected cells with ~ 9% of positive cells



mStayGold and mScarlet-I3 best candidates: highest fluorescence and best separation between the positive and the negative population

Next step: double tagging



Outlook

Aim 1

- Disentangle the dynamics of *Xist* upregulation at the onset of XCI
- Establish a system to track Xist that can be potentially applied to study Xist regulators interactions and dynamics.

Aim 2

- Establish a system to sort the cells depending on the X chromosome that is inactivated without altering the stochasticity of the process.
- Analysis of the sorted populations to assess the accessibility of the *Xist* locus (ATAC-seq), the binding of regulatory molecules or histone modifications (CUT&Tag) and the 3D contacts (Tiled-MCC).

References

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