# The behavior of fourth chromosome in *Drosophila melanogaster* Spermatogenesis

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# Introduction

Meiotic Sex Chromosome Inactivation (MSCI) is a process of transcriptional silencing of the sex chromosomes in the early stages of meiosis in spermatogenesis. Recently, the IV chromosome of *D. melanogaster* (dot) was described as an ancient X chromosome. This finding suggests that possibly the IV chromosome has characteristic sequences used in regulations related to the sex chromosome, including regulations during meiosis. In order to verify the behavior and the activity of the IV chromosome during meiosis I, cytogenetic experiments (double immunofluorescence-FISH) were performed in meiotic cells of adult testis using different types of RNA polymerase II.

# Methods

We used fluorescent *in situ* hybridization to identify both X and IV chromosomes (figure 1). Immunofluorescence was used to identify different activities of RNA polymerase II (patterns of phosphorylation) and different stages of spermatogenesis (Asterless).

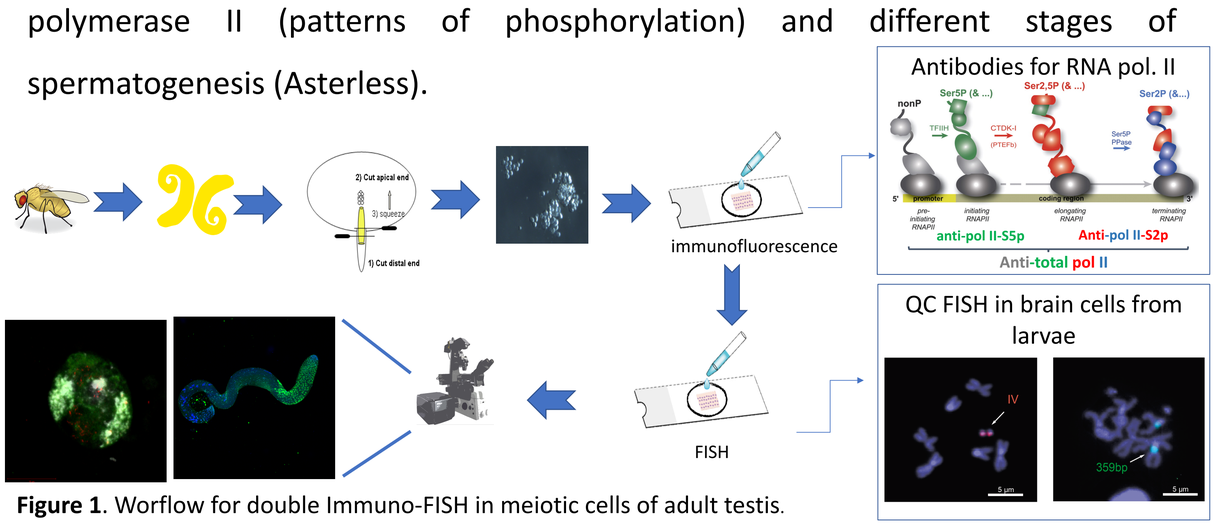



**Figure 1**. Worflow for double Immuno-FISH in meiotic cells of adult testis.

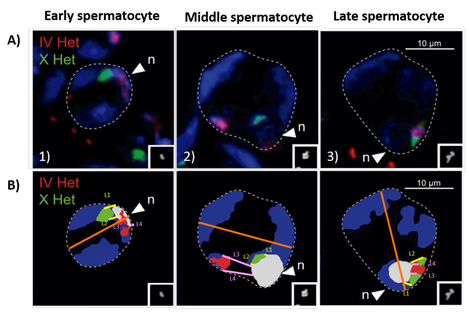



**Figure 2**. X and IV chromosomes localizations in early, middle and late spermatocyte, respectively. A) FISH detecting X(green) and IV (red) heterochromatin regions with respective probe. B) Scheme for distance measurements.

In order to assert whether there is an association between the IV/X chromosomes and the nucleolus, 135 cells were analyzed and three measurements were made: (1) the distance between X and nucleolus, (2) the distance between IV and nucleolus, and (3) the longest diameter of the cell.

To ensure that 2D images are not generating artificial distances between chromosome probes and the nucleolus, 50 cells from confocal microscope photos were analyzed to generate a three-dimensional figure by layer integration.

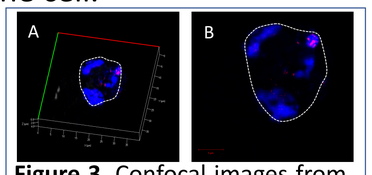



**Figure 3**. Confocal images from late spermatocytes showing the DAPI (blue) and IV chromosome (red). A) 3D image generated by ZEN black software. B) 2D image generated with the maximum projetion of the layers.

# Results

## 2D measurements confidently reflect 3D distances in spermatocytes

It was found a significant 1 to 1 correlation between 2D and 3D measurements.

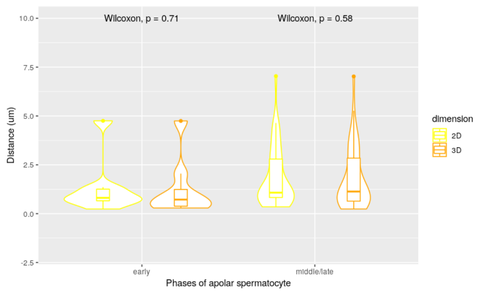
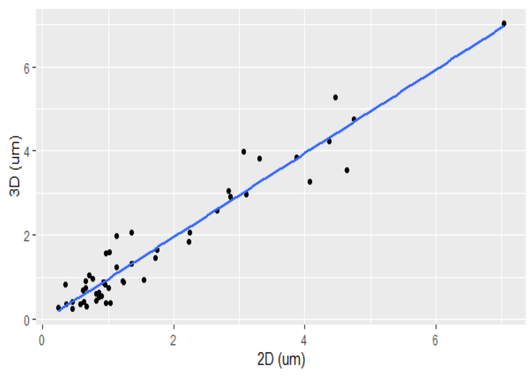



**Figure 5**. Linear Regression of the IV-nucleolus distance between measurements in 2D and 3D cells. Regression made by the ggplot2 package from R. R-squared: 0.9251 , Intercept : -0.03105 (p-value: 0.738 ), slope: 0.99388 (p-value: <2e-16 ***).

**Figure 4**. Violin graph showing the distributions of distances between the IV chromosome to the nucleolus in 2D and 3D dimensions in different stages




## The distances between the X and IV chromosomes to the nucleolus

The distances distributions revealed that IV chromosome is observed more frequently far from the nucleolus than the X chromosome (p-values =0.0037 and 9.4e-09 for early and middle/late stage stages, respectively – figure 6).

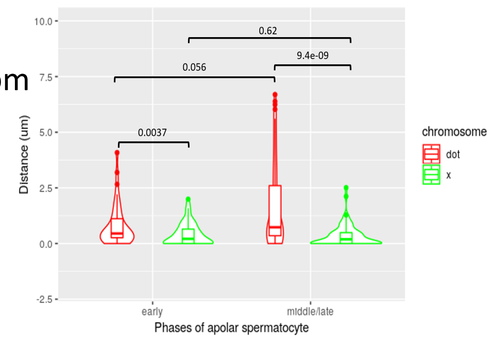
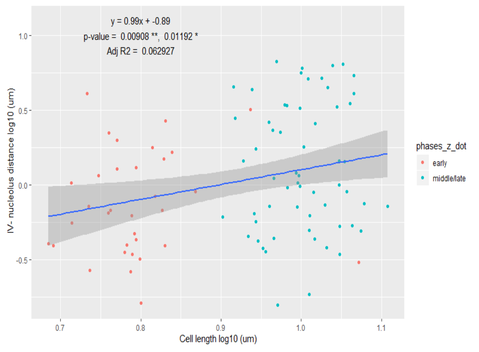



**Figure 6**. Violin graph showing the distributions of distances between the X (green) and IV (red) to nucleolus.

Although the X chromosome distance from the nucleolus does not change along spermatocyte development (p-value=0.62), there is a marginal significant increase of the distance between IV chromosome and the nucleolus between early and middle/late phases (p-value =0.056, respectively).

## The increase of distance between the IV and the nucleolus is associated with cell growth occurring in late stages




It was observed a positive association between cell size and the distance between the IV chromosome and the nucleolus (p-value=0.009). Interestingly, the X chromosome-nucleolus distance does not present a significant association with cell size (figure 7).

**Figure 7**. Association between cell length and IV chromosome probe -nucleolus distances.

## RNA polymerase II depletion caused by a failure of mRNA elongation

We found that the evidence of a depletion of active RNA polymerase II (Pol-IISer2p) on IV chromosomes in the late stages of meiotic prophase I, compared to the autosomes (figure 8). Surprisingly, the antibody for active and non-active RNA polymerase II (total RNApol) is was not observed depletion of staining in IV chromosome.

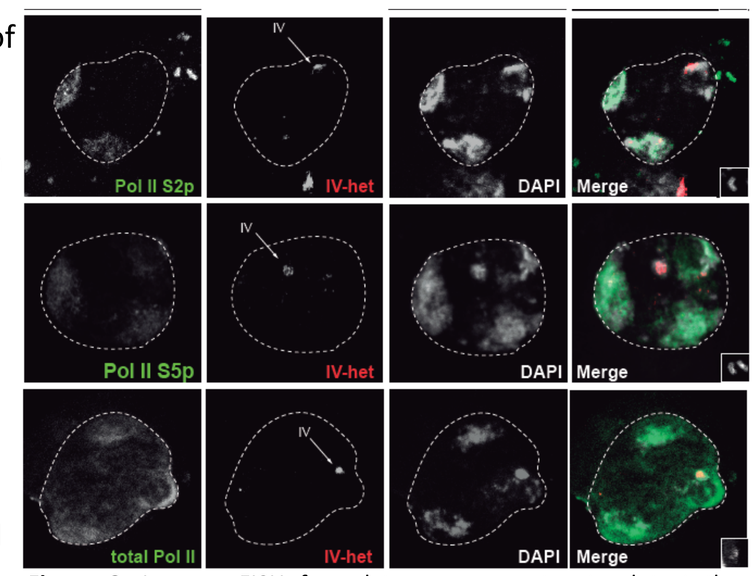



**Figure 8**. Immuno-FISH from late spermatocytes to detect the activity of the chromosomes with different status of RNA polymerase. DAPI is in grey,the IV chromosome is in red, and RNA polymerase in green.

# Conclusions

Our results for the measurements suggest that the increase of distance between the IV and the nucleolus is associated with cell growth occurring in middle and late stages, which does not happen to the X chromosome probably due to its tight association to the nucleolus. The depletion of RNA polymerase II-S2p in IV chromosome suggests that occurs a binding of RNA polymerase II, but generally fails to initiate or elongate the mRNA in late spermatocytes. Therefore, those observations show evidence which suggests that the dot chromosome participates in the MSCI mechanism as an ancestral sex chromosome.

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