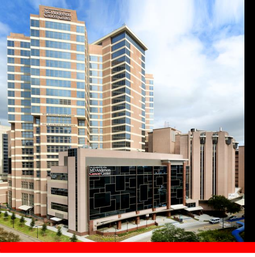

Role of ATM in Regulating Meiotic Crossovers in Sex Chromosomes and Autosomes

Lindsey Ran, Parijat Chakraborty, Lakshmi Paniker, Maria Sandoval, and Francesca Cole

The Department of Epigenetics & Molecular Carcinogenesis, M.D. Anderson Cancer Center, Houston, TX

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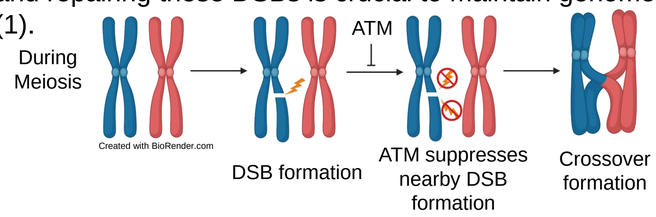
Introduction

ATM is a kinase that plays crucial roles in the DNA repair pathway of homologous recombination (HR) (1,2). Mutations in ATM gene can lead to the disease of ataxia telangiectasia, which is implicated in multiple cancers (1). Thus, investigating the roles of ATM in HR is crucial for understanding human disease mechanisms.

While multiple pathways for DNA repair are functioning in somatic cells, meiotic spermatocytes primarily use HR, making spermatocytes an excellent system to study roles of ATM in HR. ATM is essential in limiting the programmed self-inflicted double stranded breaks (DSBs) during meiosis (1) that are required for proper chromosome segregation. Spermatocytes rely on crossovers, products of HR, to connect and segregate homologous chromosomes to generate haploids. Therefore, failure to form a crossover results in aneuploidy (3) and regulating and repairing these DSBs is crucial to maintain genome integrity (1).



Background and Approach

Aged mouse spermatocytes have defective crossover formation and thus have higher levels of aneuploidy as seen in Figure 1. Considering that ATM deficiency results in a tenfold increase in DSBs in spermatocytes, I hypothesize that *Atm*$^{+/-}$ spermatocytes will have increased DSB levels, which will rescue the age-associated loss of crossovers and increase in aneuploidy in spermatocytes.

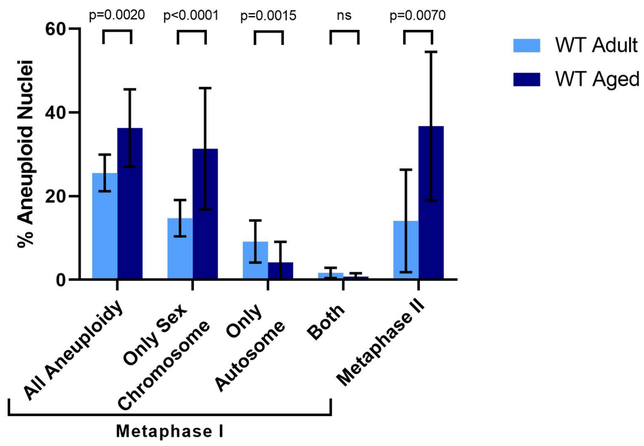
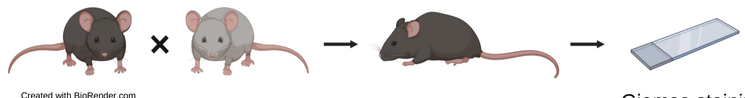


Figure 1. Aged spermatocytes of WT mice have significantly more overall and sex chromosome aneuploidy than adult WT mice.

Materials and Methods



We used B6xDBA F1 hybrid *Atm*$^{+/-}$ mice and wildtype littermate controls. The testes of adult (2-6 m) and aged (18-24m) mice were dissected to generate chromosome spreads that were stained with Giemsa to assess aneuploidy level or immunofluorescence staining for RPA foci, which are an upstream marker of crossovers.

Results

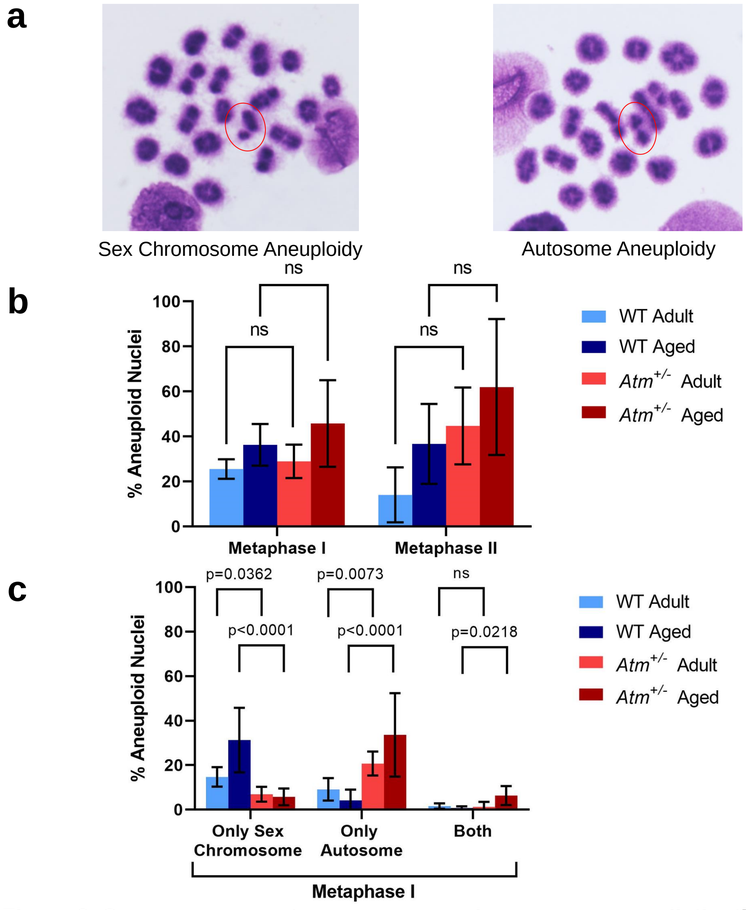


Figure 2. Crossovers on autosomes vs sex chromosomes are distinctly regulated by ATM

[a] Representative metaphase images showing sex chromosome (left) and autosomal (right) aneuploidy.

[b] Adult and aged *Atm*$^{+/-}$ spermatocytes show similar levels of aneuploidy to WT spermatocytes.

[c] Decrease in aneuploidy was observed on sex chromosomes of both adult and aged *Atm*$^{+/-}$ spermatocytes as compared to WT, indicating that decrease in ATM allelic content may rescue crossover defects.

Together, this implies that crossovers on sex chromosomes are likely independently regulated from autosomes by ATM.

Results continued

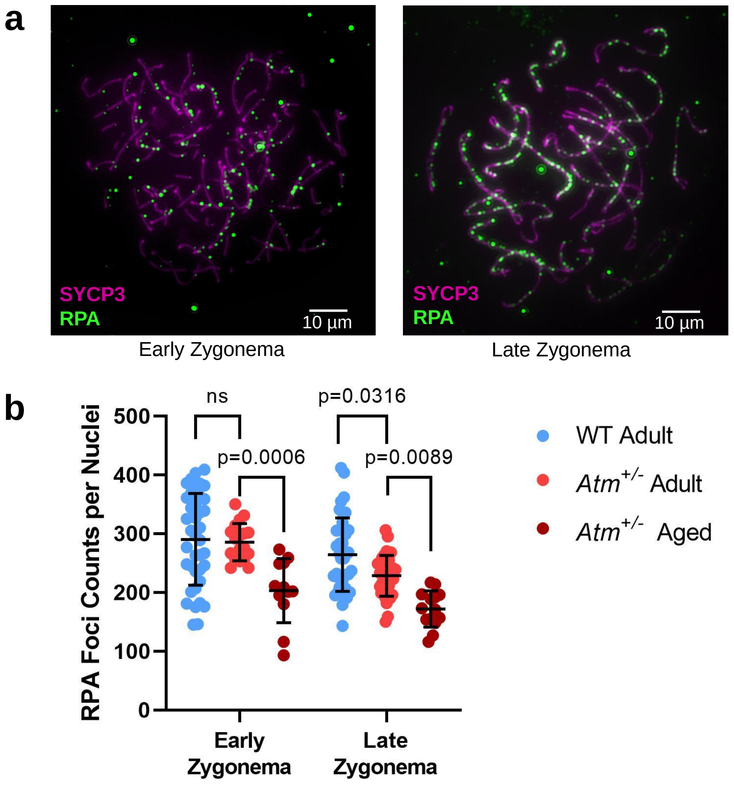


Figure 3. ATM-dependent crossover regulation begins upstream

[a] Representative RPA foci images showing early zygonema (left) and late zygonema (right).

[b] Both early and late zygonema show a significant decrease in the number of RPA foci per nucleus between *Atm*$^{+/-}$ aged spermatocytes and *Atm*$^{+/-}$ adult spermatocytes. RPA binds to earlier recombination intermediates as they are processed to form crossovers. This suggests that ATM may act earlier in crossover regulation.

Conclusions

The crossover pathway in sex chromosomes is differentially regulated from autosomes. Prior reports have shown that the dynamics of DNA break formation are independently regulated in autosomes as compared to sex chromosomes (4). My observation suggests that the downstream DNA repair and crossover formation may also be independently regulated on sex chromosomes in a manner dependent upon ATM.

Future studies could investigate further upstream intermediates to determine the functional time period of ATM.

References

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