

Undergrad Research Symposium

The RNAi – Centromere – Recombination Interactome

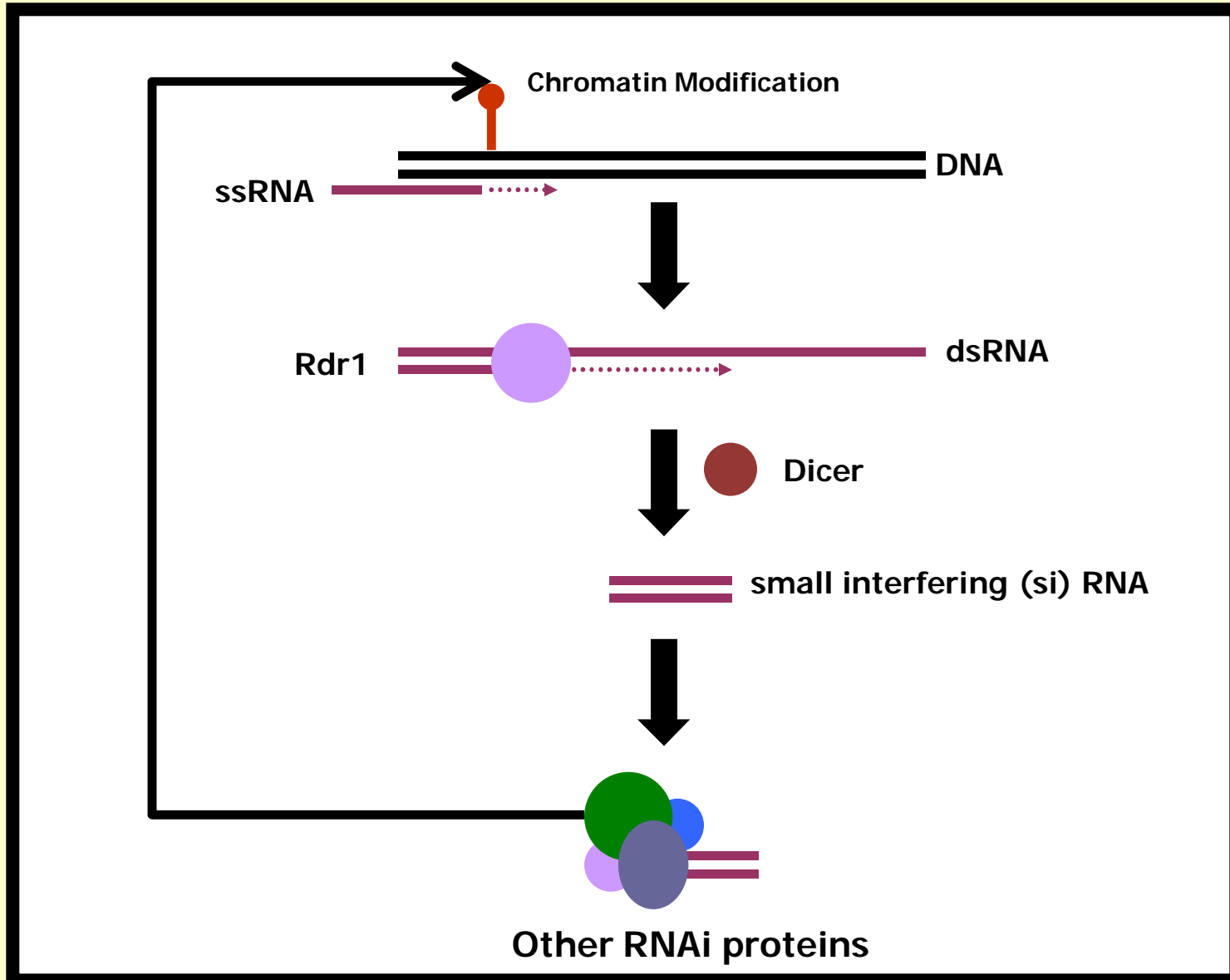
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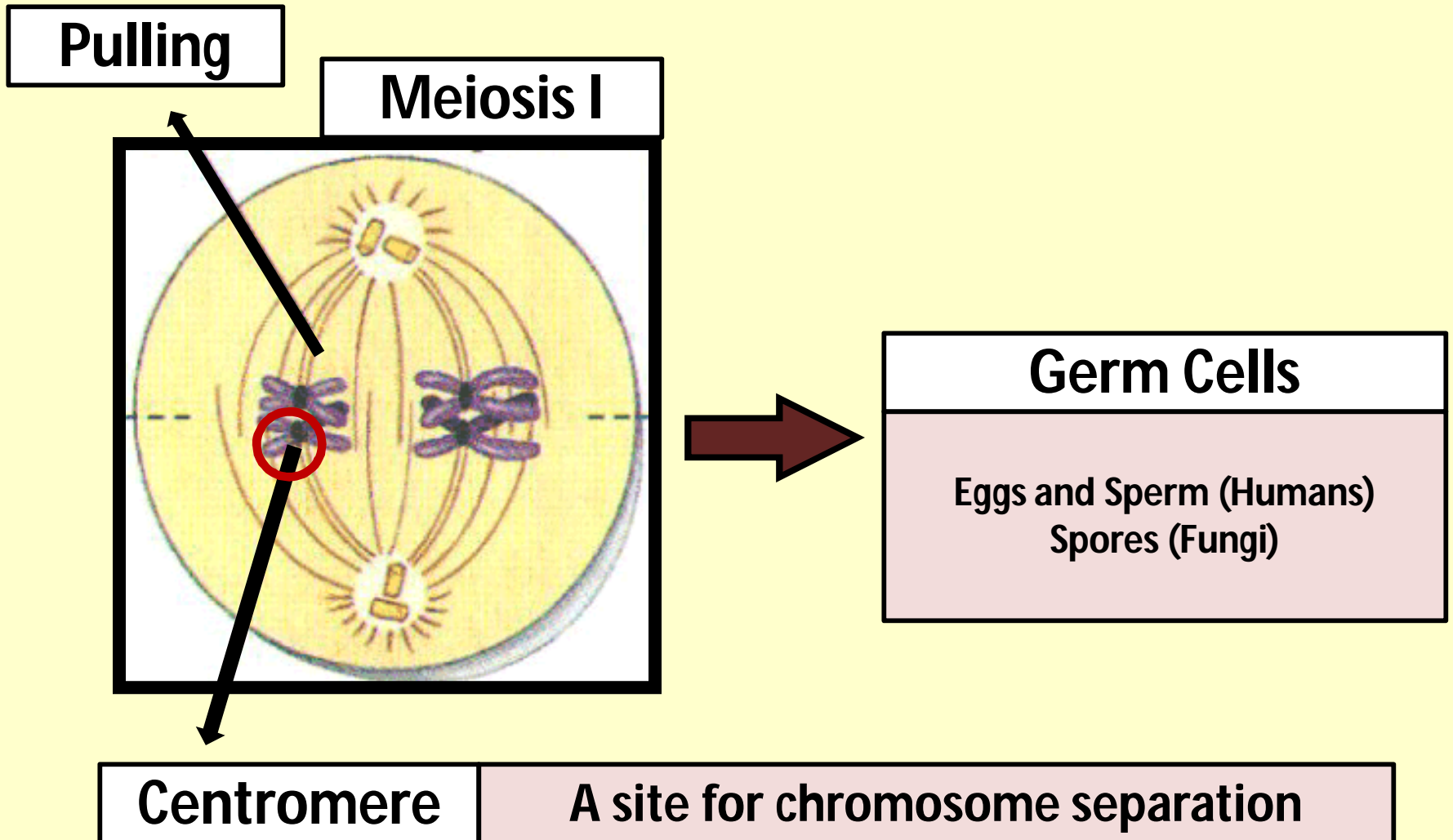
What is RNAi?

The RNAi – Centromere – Recombination Interactome



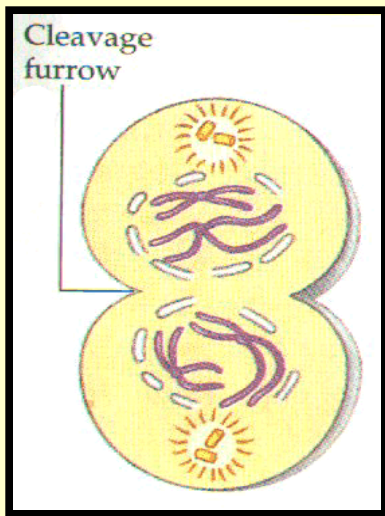
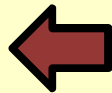
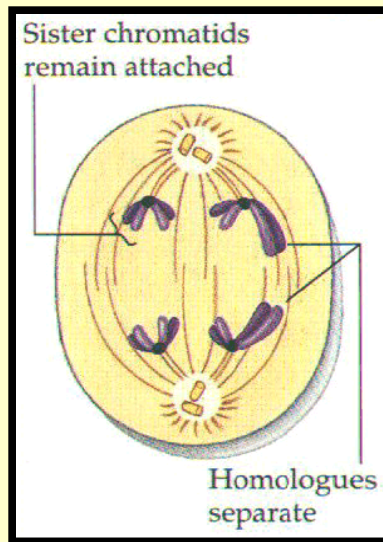
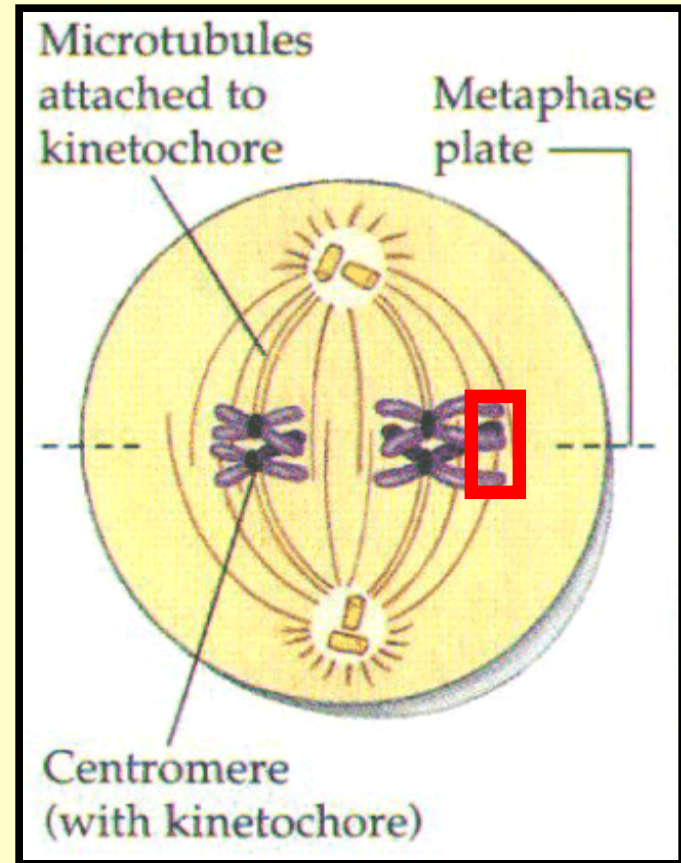
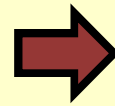
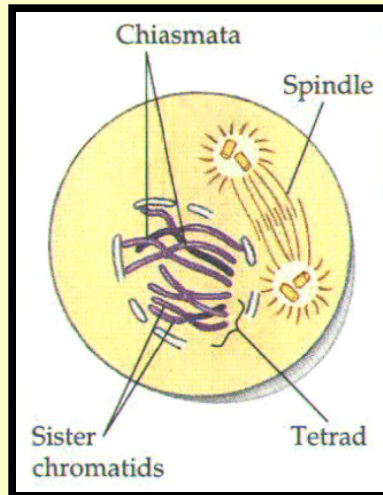
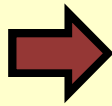
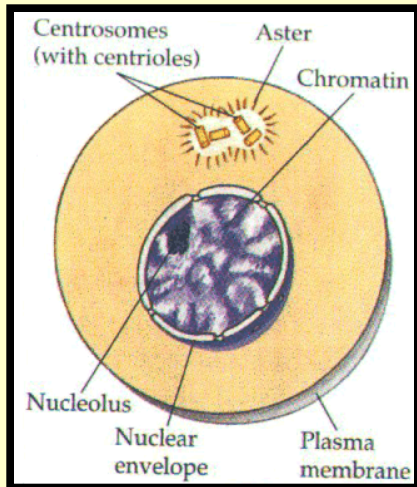
What is a centromere?

The RNAi – **Centromere** – Recombination Interactome



Homologous chromosomes must recognize each other before separating.

Chromosomes in Meiosis

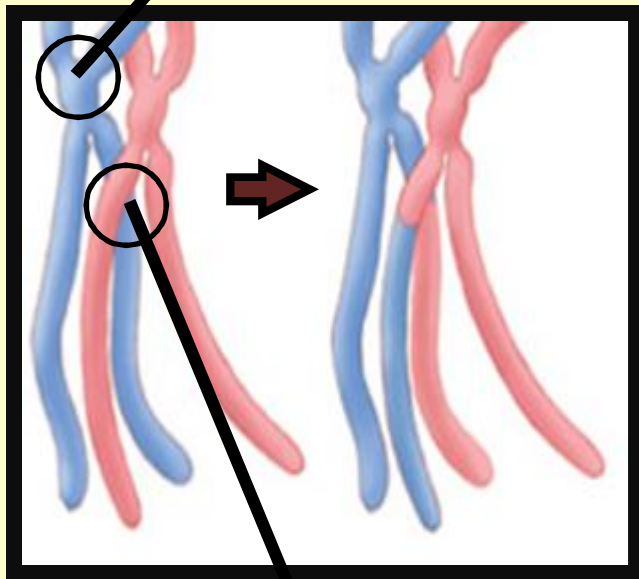


Recombination uses a homologous template for recognition between tetrads

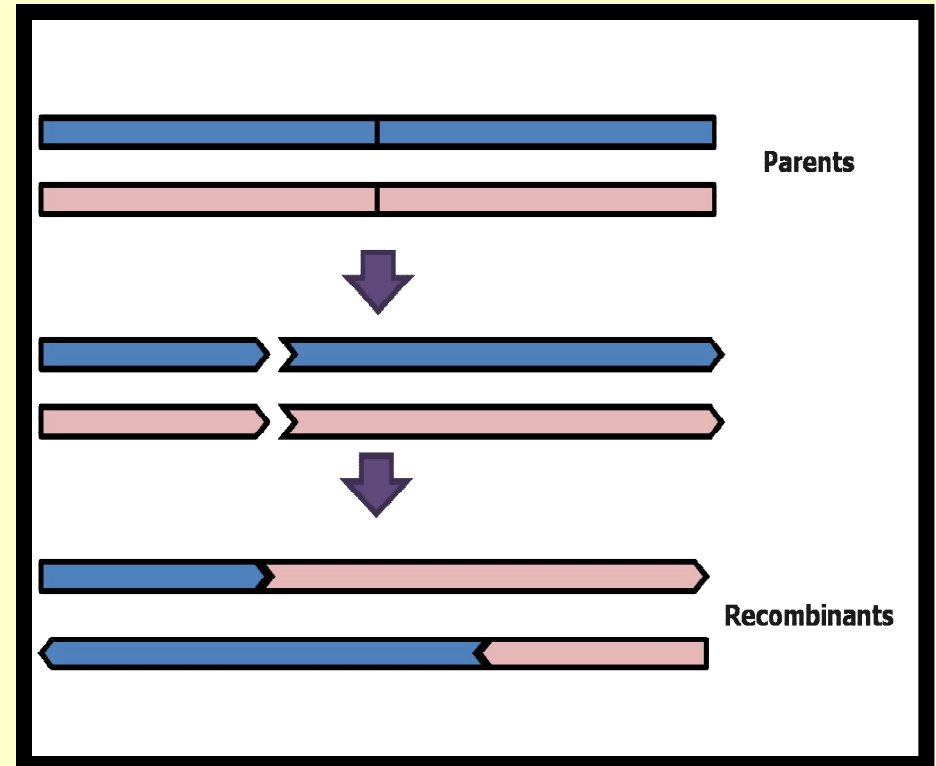
What is Recombination?

The RNAi – Centromere – **Recombination** Interactome

Centromere



Connection generates tension



Recombination at the centromere can interfere with chromosome segregation!

Relevant Pathways

The RNAi – Centromere – Recombination Interactome

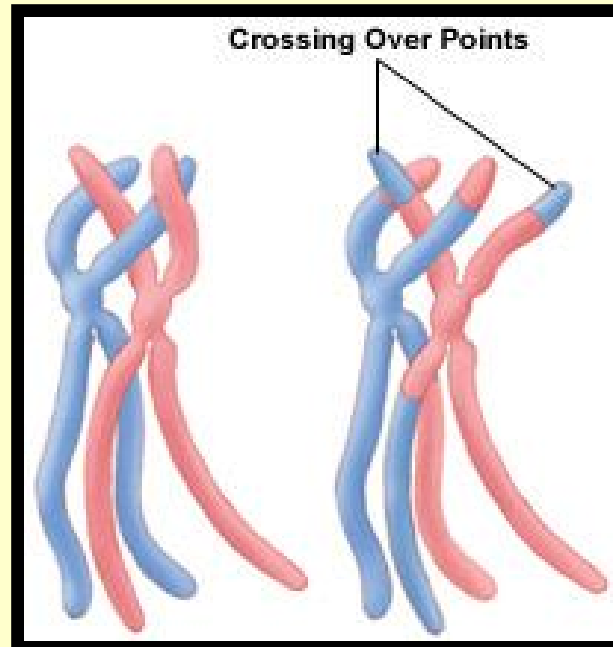
Dicer-1

Clr-4

Argonaut

Rd-RNA Pol

Rik-1



Recombination here is toxic.

Hypothesis:
Inhibited by RNAi – induced chromatin modification.

RNAi

- Gene silencing by condensation of chromatin structure

Recombination

- Breaks induced during Meiosis
- Breaks repaired

Centromere

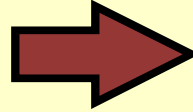
- Repetitive DNA
- Transcriptionally Silent

Recombination at the Centromere?

The RNAi – Centromere – Recombination Interactome

Atypical segregation of chromosomes can lead to...

- Miscarriage
- Birth Defects
- Cancer
- Sterility
- Irregular Development

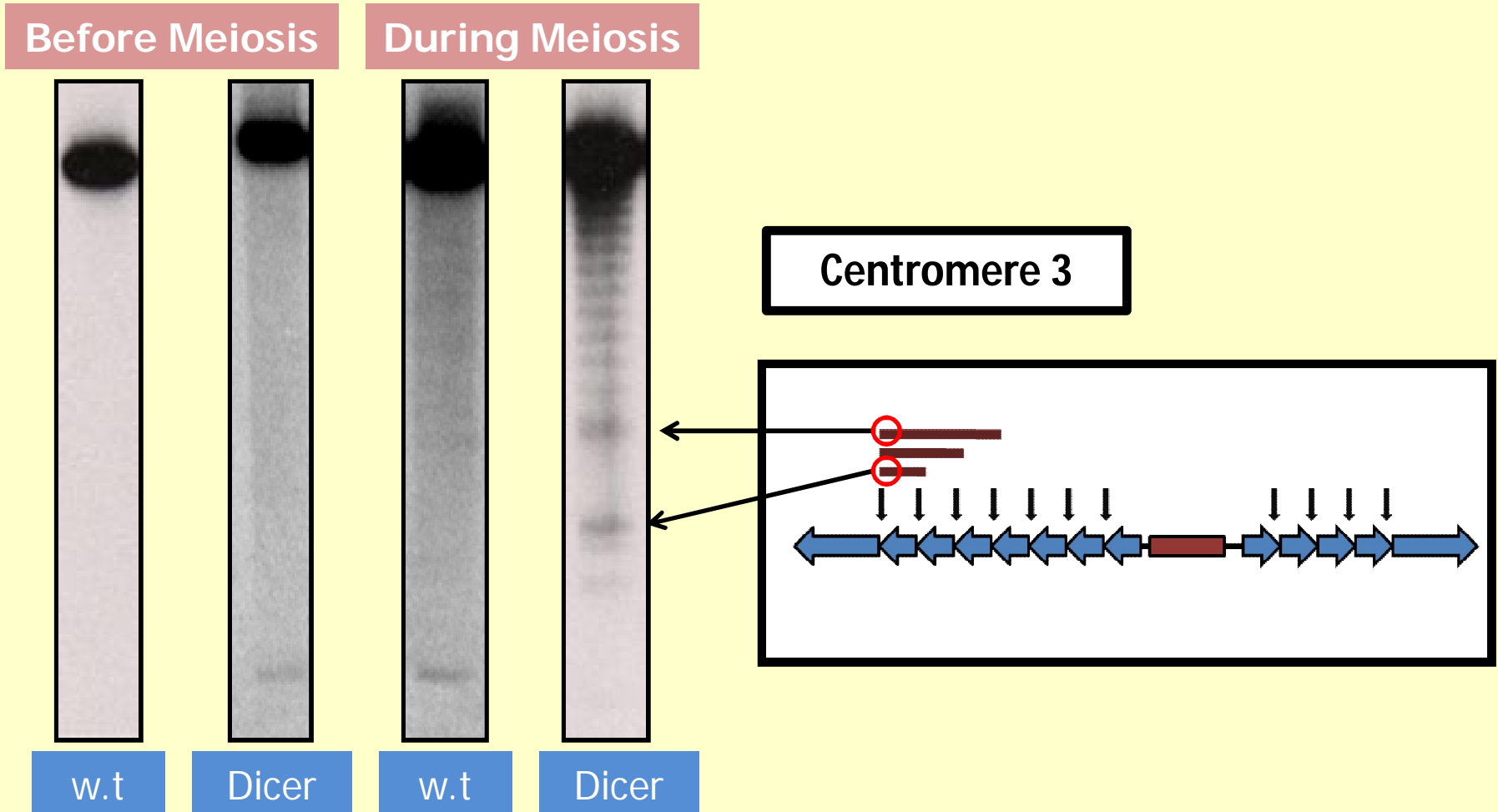


Which means...

The cell prevents recombination at the centromere.

How?

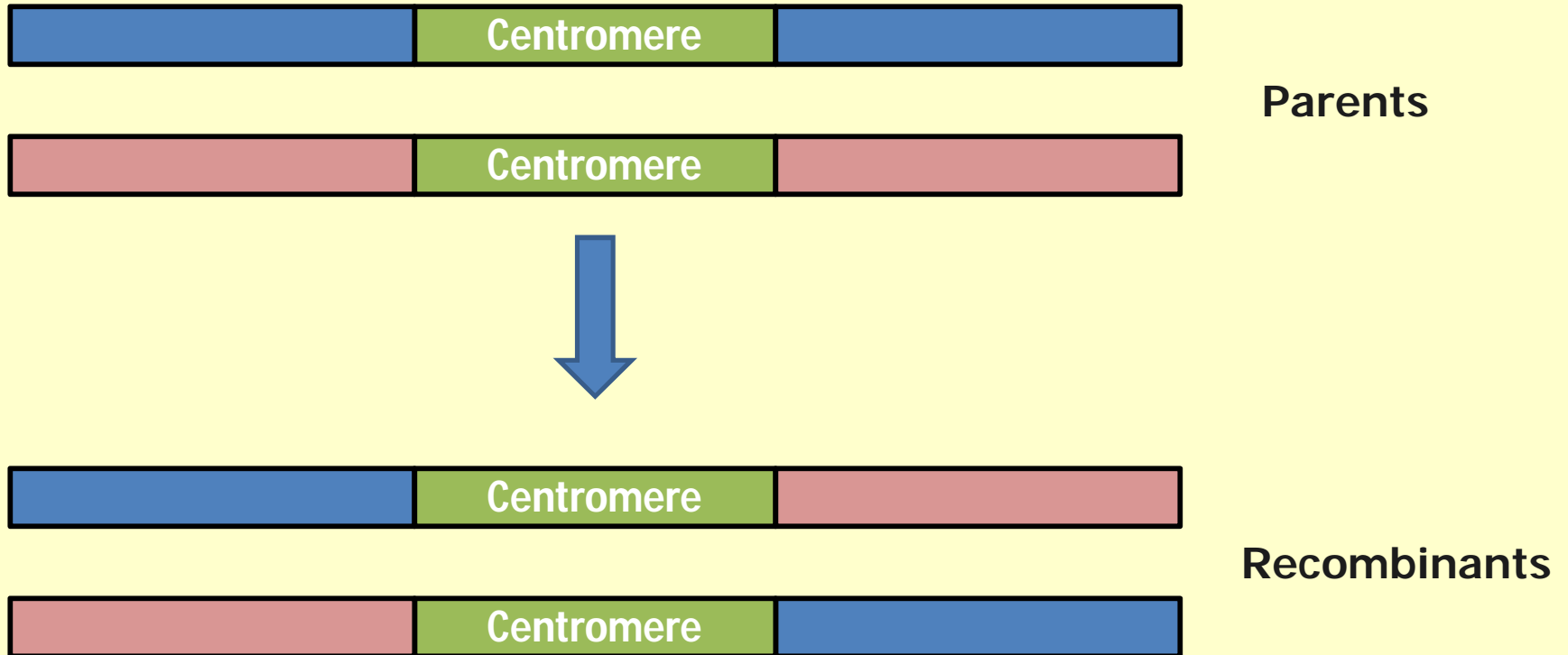
Centromere breaks in RNAi mutant (Dicer Δ)



Project Question 1

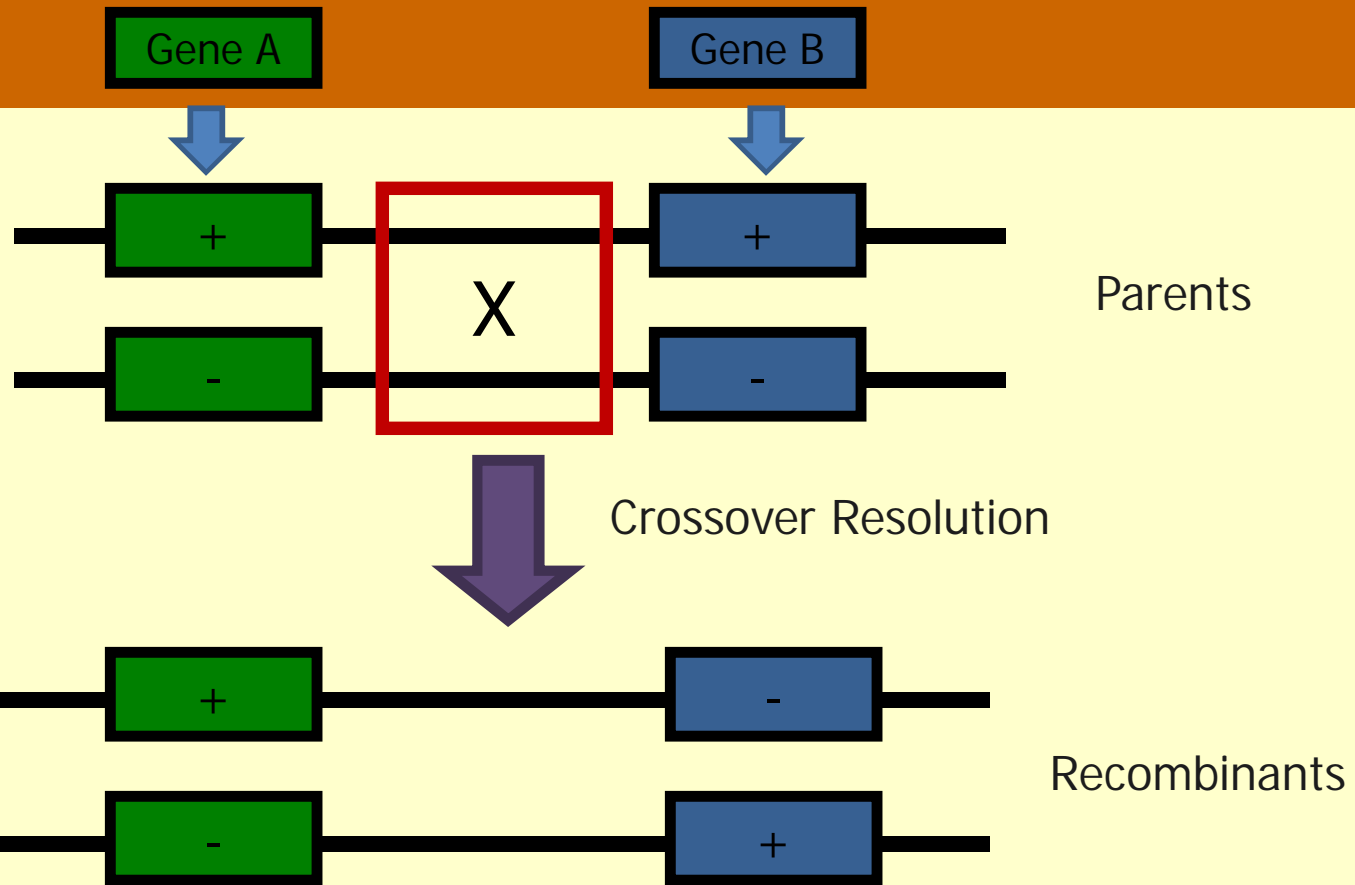
Are these breaks recombinogenic, and if so, is the effect specific to the centromere?

Experiments in RNAi deletion mutants



$$\text{Frequency} = \frac{\text{Recombinants}}{\text{Recombinants} + \text{Parental}}$$

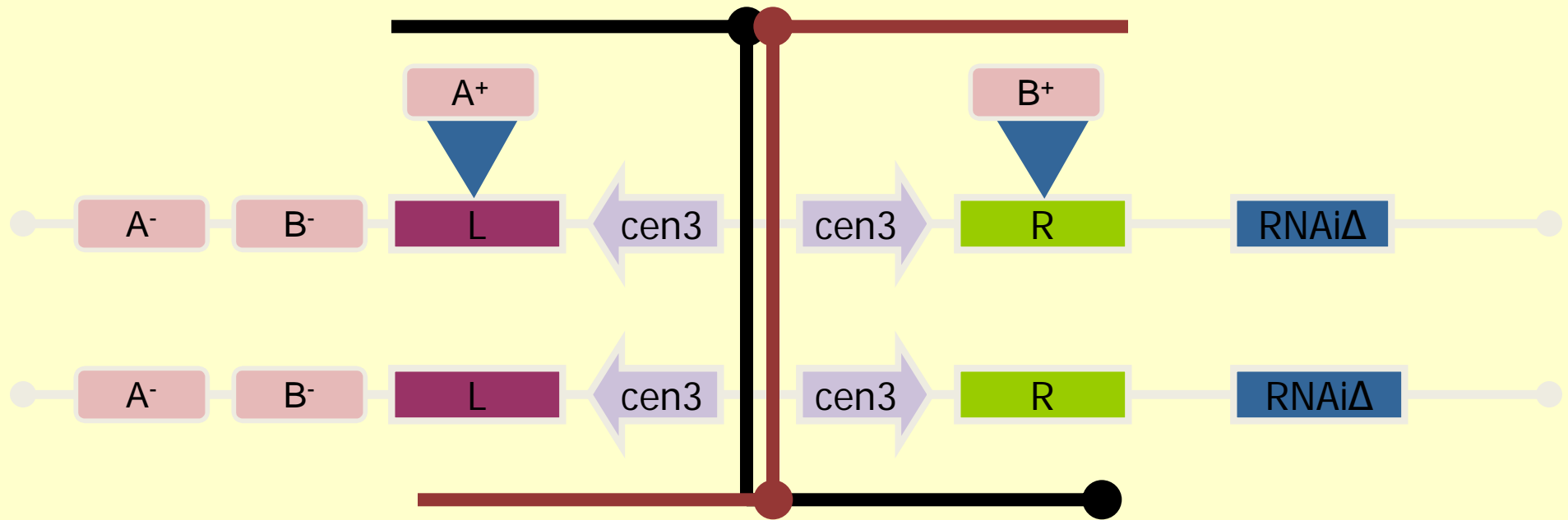
Measuring Recombination



Frequency =

$$\frac{\begin{matrix} \boxed{+} \boxed{-} + \boxed{-} \boxed{+} \\ \boxed{+} \boxed{+} + \boxed{-} \boxed{-} + \boxed{+} \boxed{-} + \boxed{-} \boxed{+} \end{matrix}}{4}$$

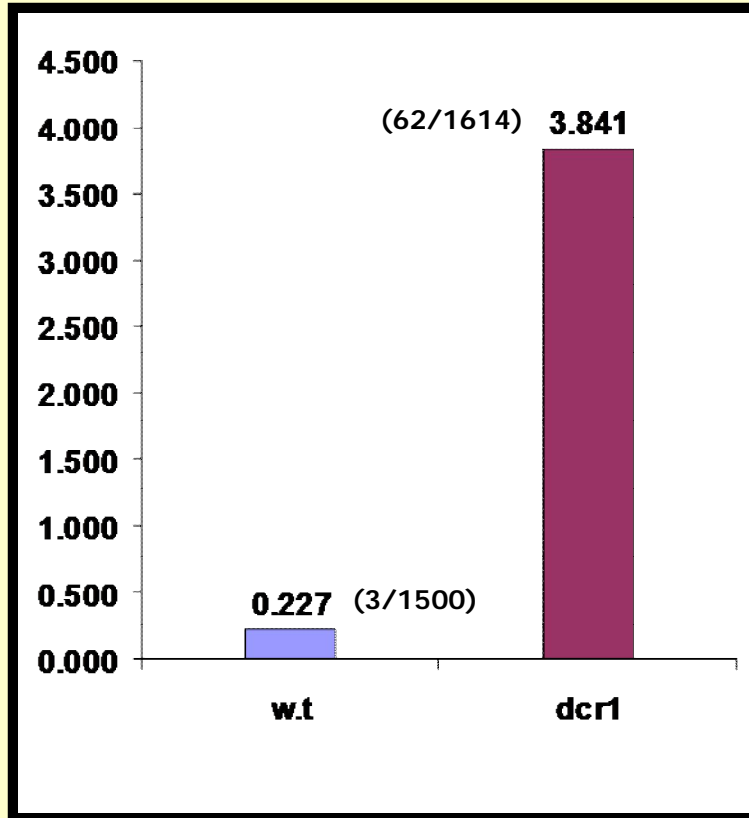
Experiments with Full Deletion Mutants (RNAi Δ)



Decoupling of *A* and *B* insertions from *cen3* is a good assay for recombination

Results

Frequency of recombination across *cen3* in *dcr1*-deletion mutants



20-fold increase

- Other RNAi mutants are being tested

Experiments with Dicer Nuclease Mutant

D → A

E. Coli RNAase III
H. Sapiens RNAase III
S. Pombe Dicer-1

980 990 1000 1010 1020

FLGDAVVEFLTSVHLYYLFPSLEEGGLATYRTAIVQNH LAMLAKKLELDPFML
FLGDSILSYVIANALYHRFPRVDEGDMSRMRATLVRGNTLAELAREFELGECLR
FYGDCFLKLGASITVFLKFPDTQEYQLHFNRRKII SN CNLYKVAIDCELPKYAL

Catalytic sites inferred from structural homology

Project Question 2

Does the nucleolytically inactive Dicer mutant show the same phenotype as the full deletion?

Acknowledgements

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