

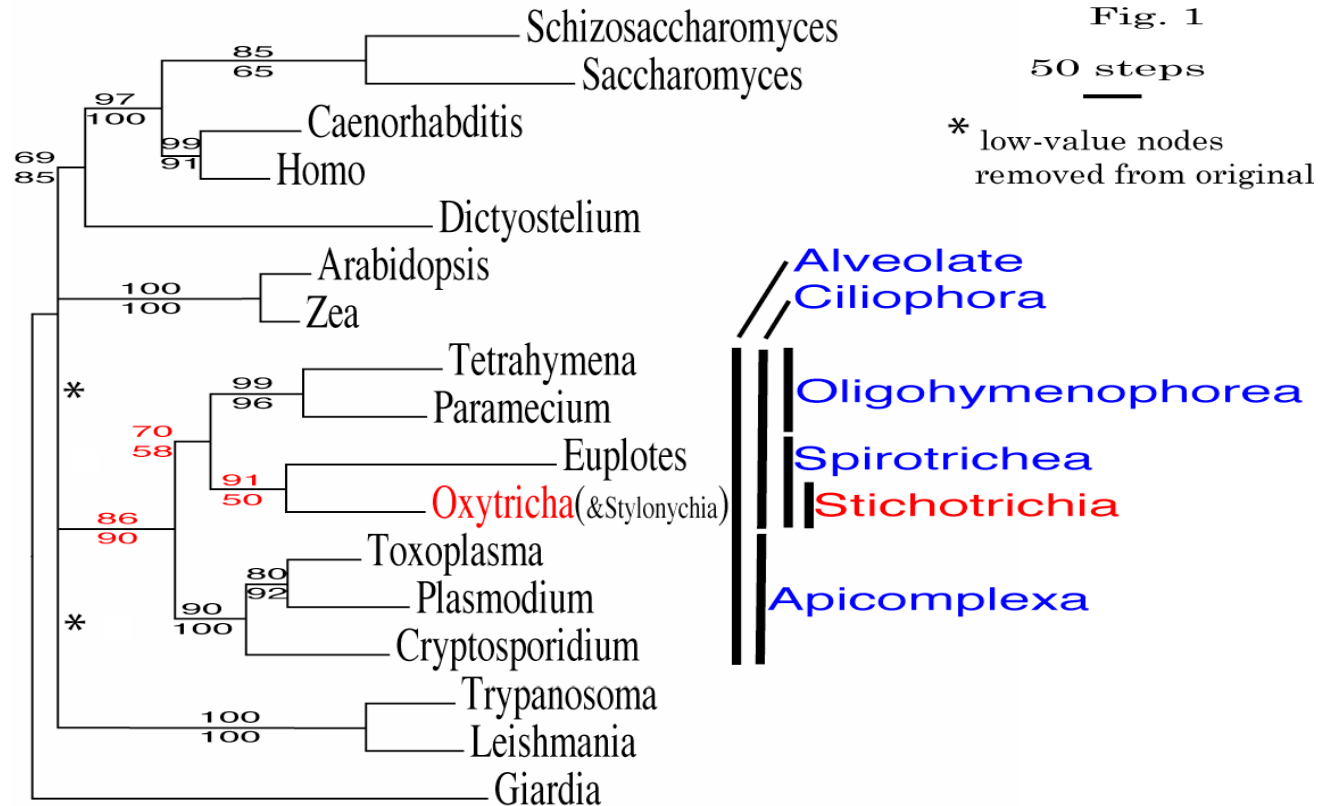
RNA-mediated Transgenerational Epigenetic Inheritance of DNA Rearrangements and Copy Number

John Bracht, Ph.D
Landweber Lab
Princeton University

Plant and Animal
Genome XX
San Diego, CA
Jan 14 2012

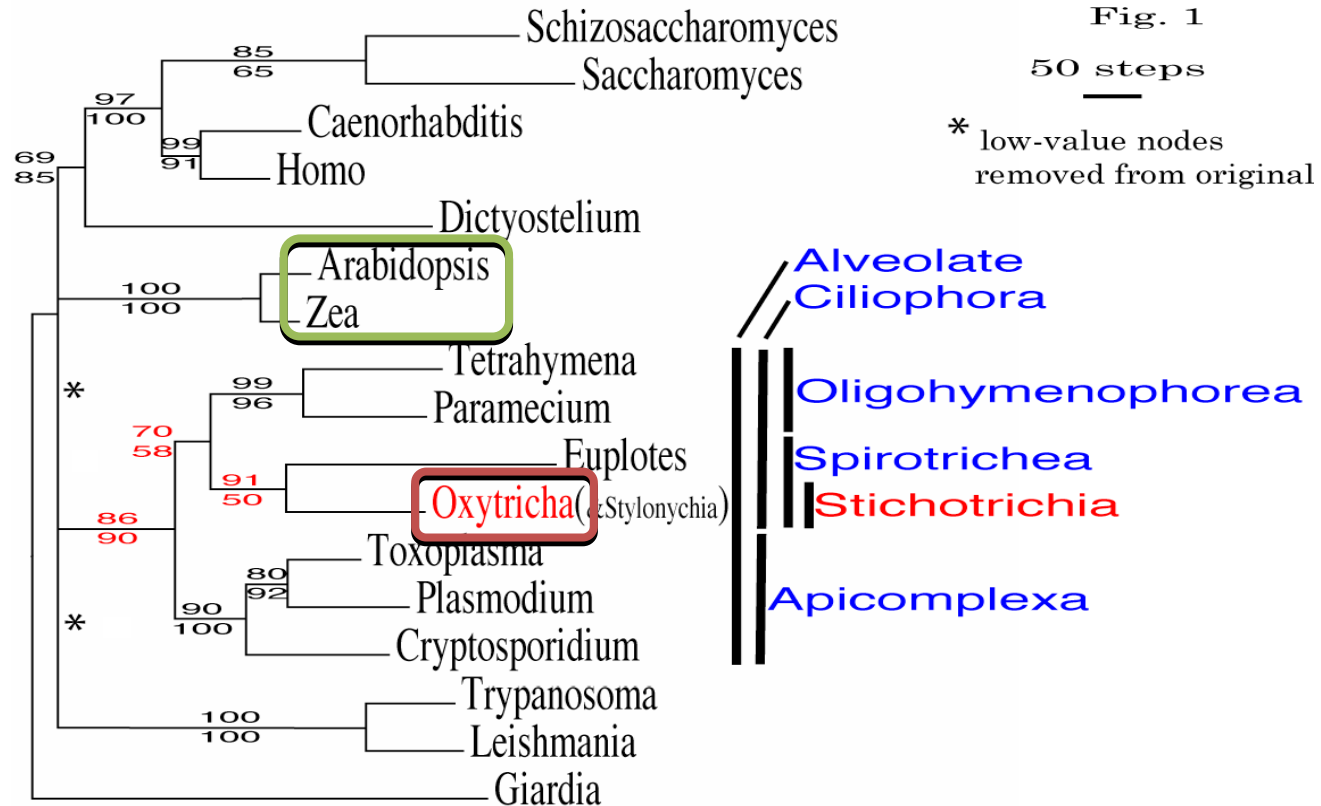


Oxytricha trifallax



Modified from Baldauf et al. (2000)

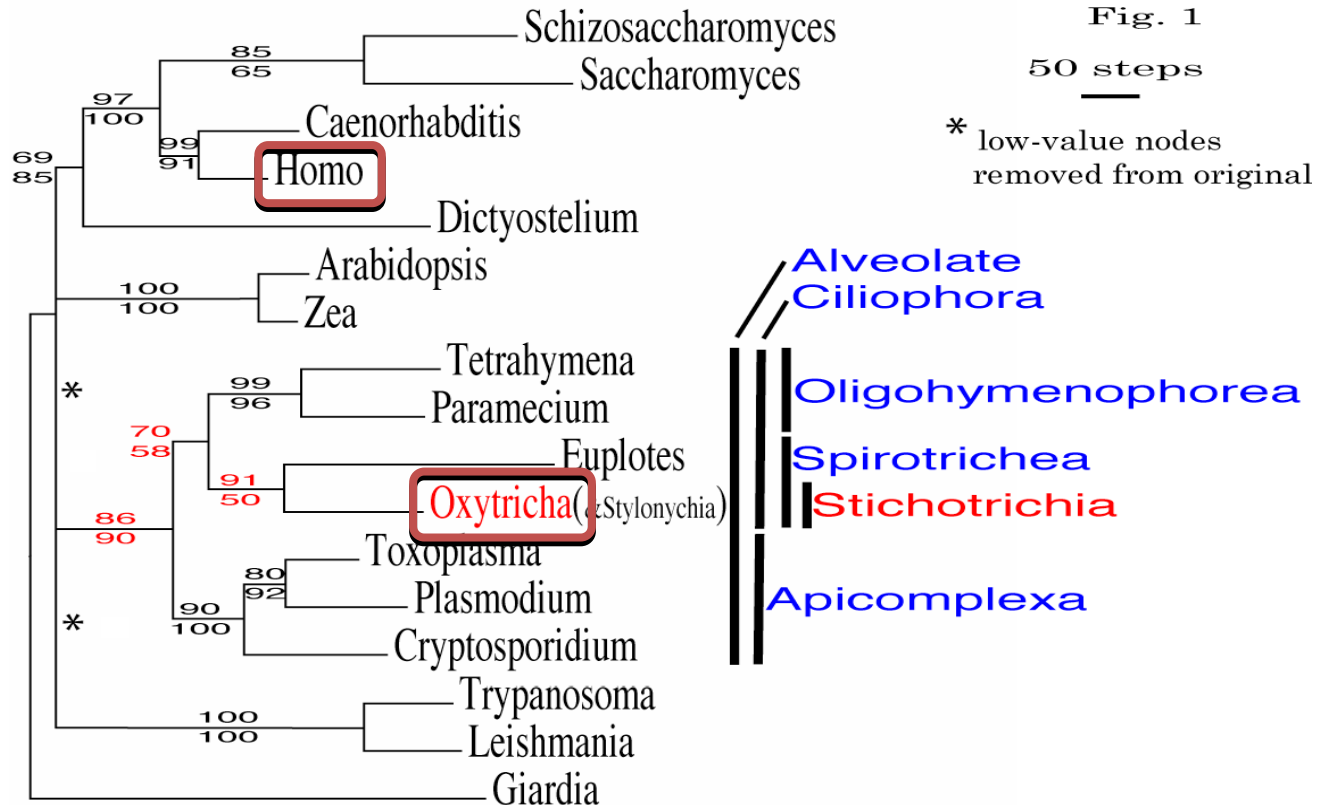
Oxytricha trifallax



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Oxytricha trifallax

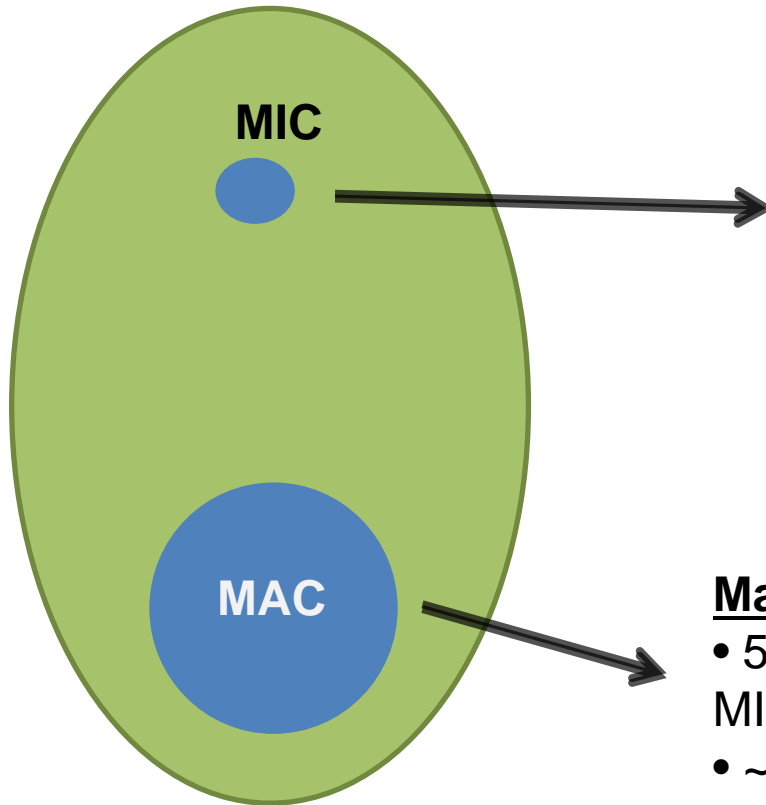
Oxytricha to *Homo* represents approximately 1.8 billion years of independent evolutionary history



Nei M, Xu P, Glazko G.
PNAS. 2001 Feb 27;98(5):2497-502.

Modified from Baldauf et al. (2000)

Oxytricha Nuclear Dimorphism



Oxytricha cell

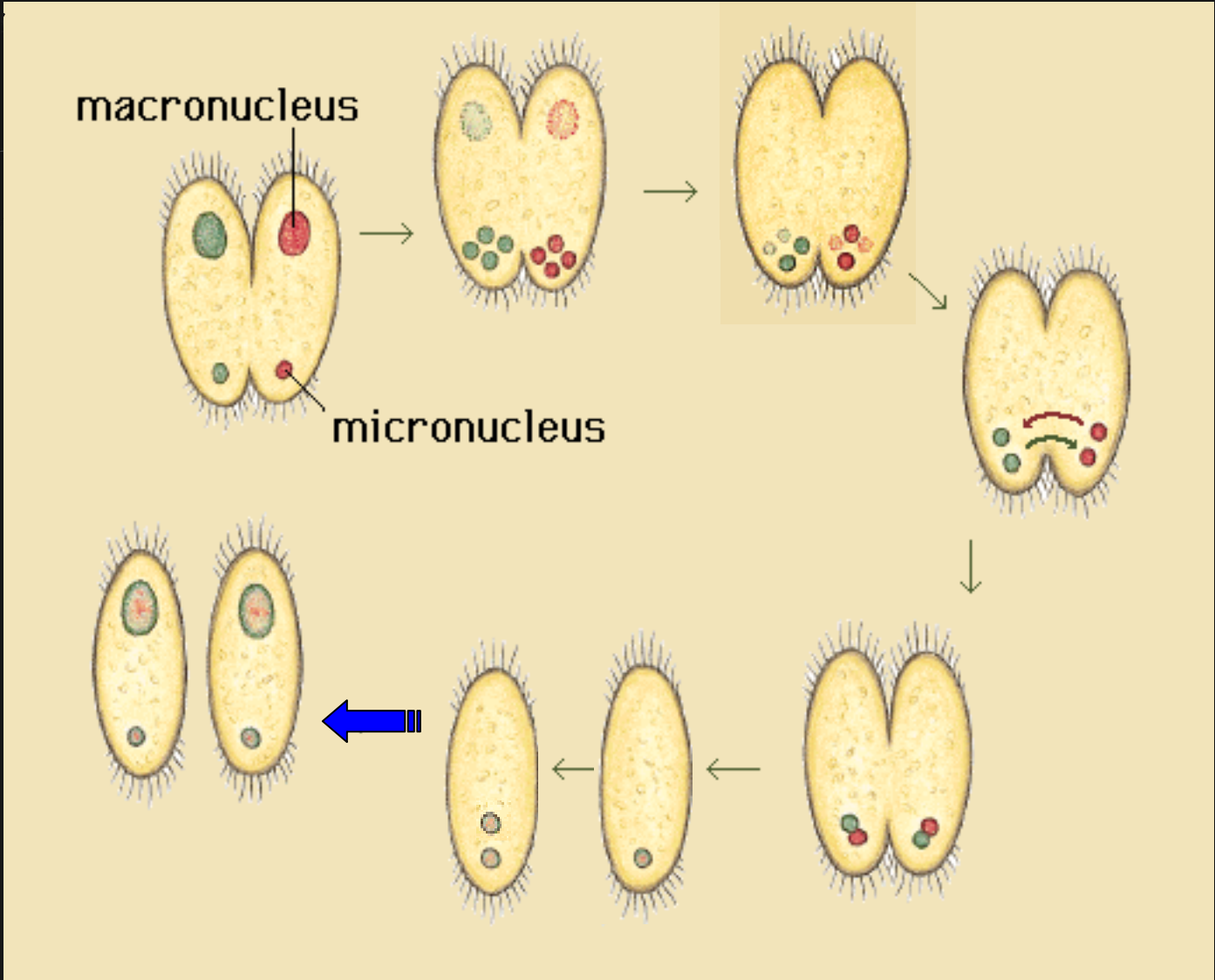
Micronucleus:

- 1 gigabase genome (1×10^9 bp)
- 120 euchromosomes
- diploid
- scrambled genes
- direct repeats, transposons
- transcriptionally silent
- “dark matter”

Macronucleus:

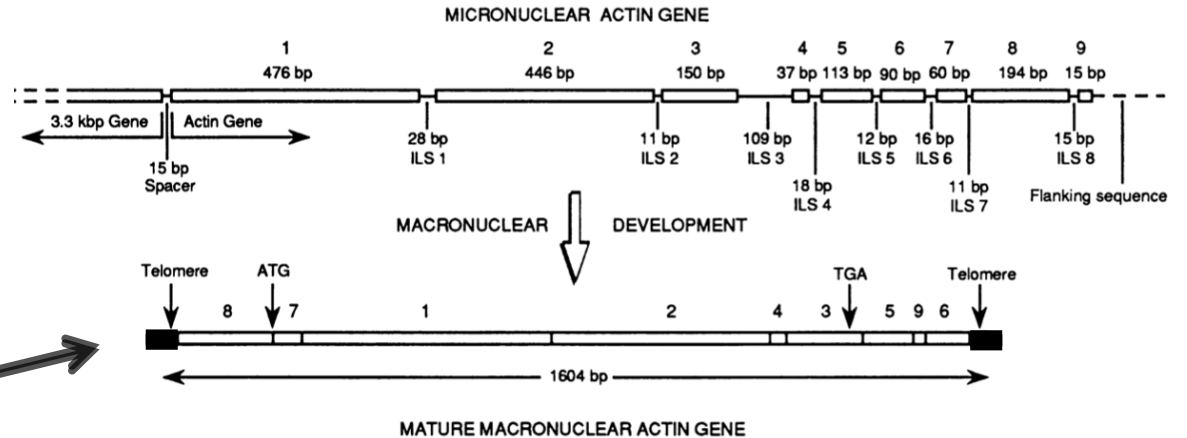
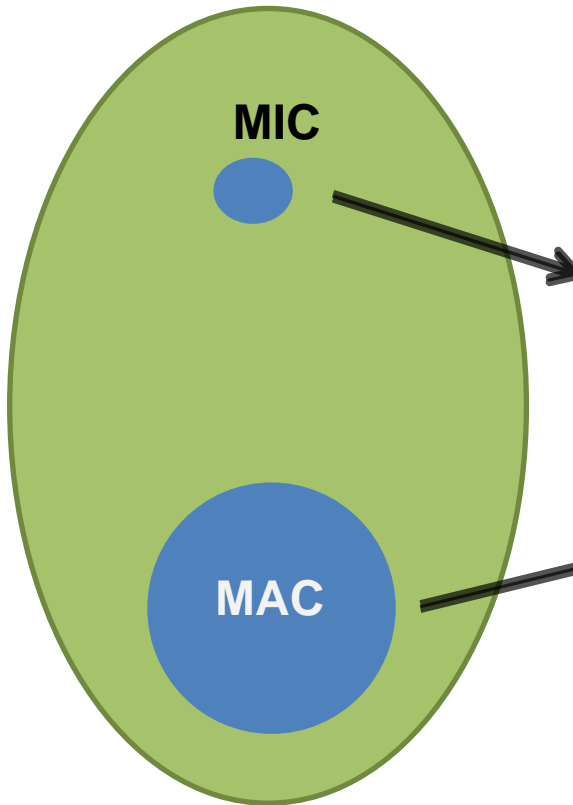
- 50 megabase genome (50×10^6 bp) (20x smaller than MIC)
- ~20,000 gene-sized nanochromosomes
- nanochromosomes average 3.2kb long
- Usually 1 gene per nanochromosome
- 1900n
- Short telomeres
- No centromeres
- Protein expression

The Oxytricha Sexual Cycle



SEM courtesy Bob Hammersmith

Oxytricha Nuclear Dimorphism



Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 6264-6268, August 1989
Genetics

Reordering of nine exons is necessary to form a functional actin gene in *Oxytricha nova*

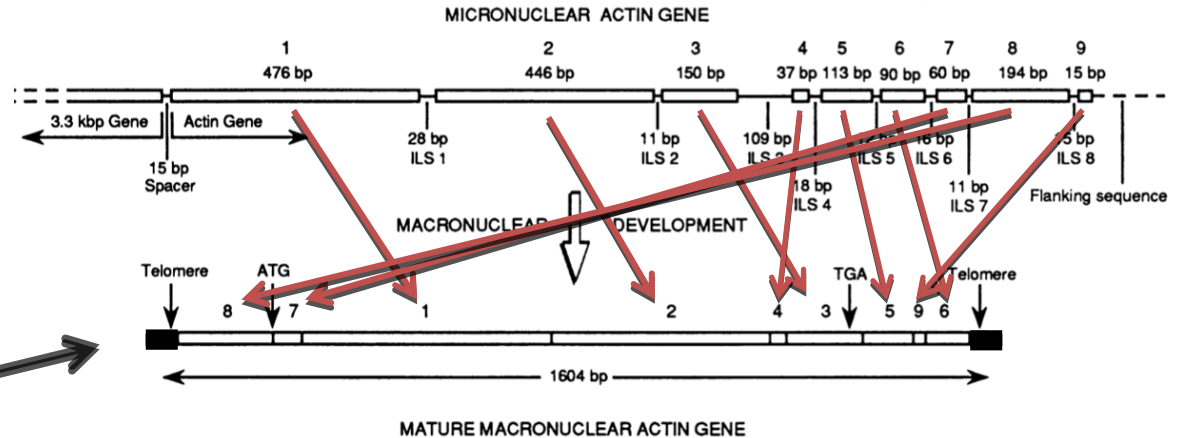
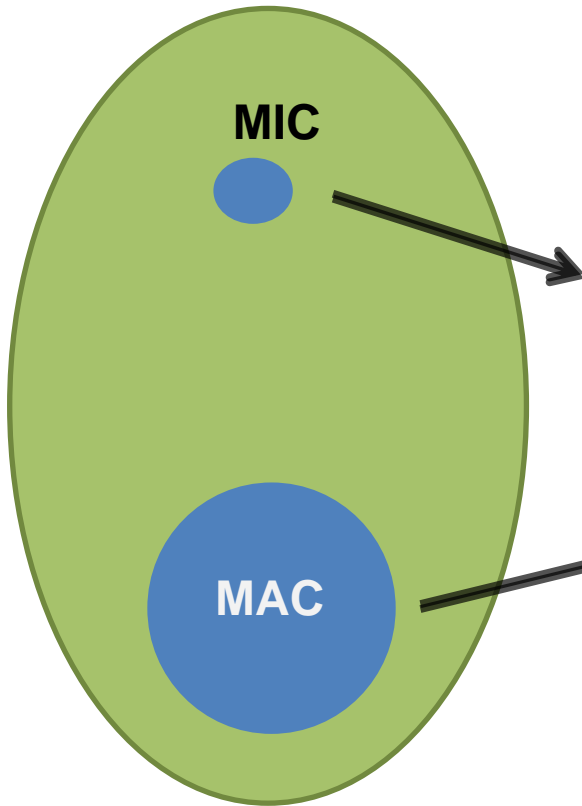
(macronuclear development/micronucleus/hypotrichs)

ARTHUR F. GRESLIN, DAVID M. PRESCOTT, YOSHIO OKA*, STEPHEN H. LOUKIN†, AND JAMES C. CHAPPELL
Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309

Contributed by David M. Prescott, May 30, 1989

Oxytricha cell

Oxytricha Nuclear Dimorphism



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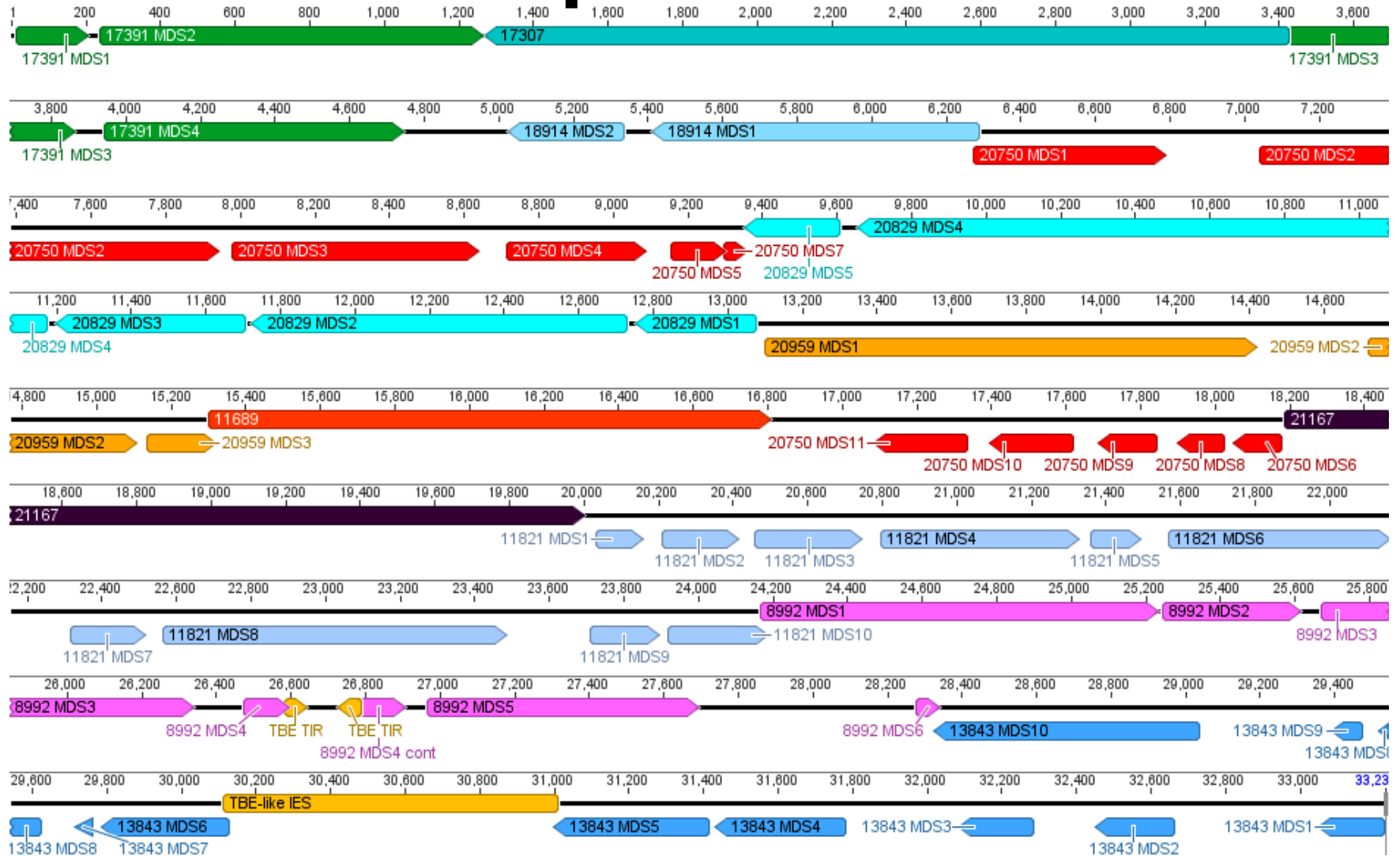
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Oxytricha cell

A MIC mashup of 13 genes in 55 pieces



Questions

- How does the cell decide to delete or retain a given DNA segment (g

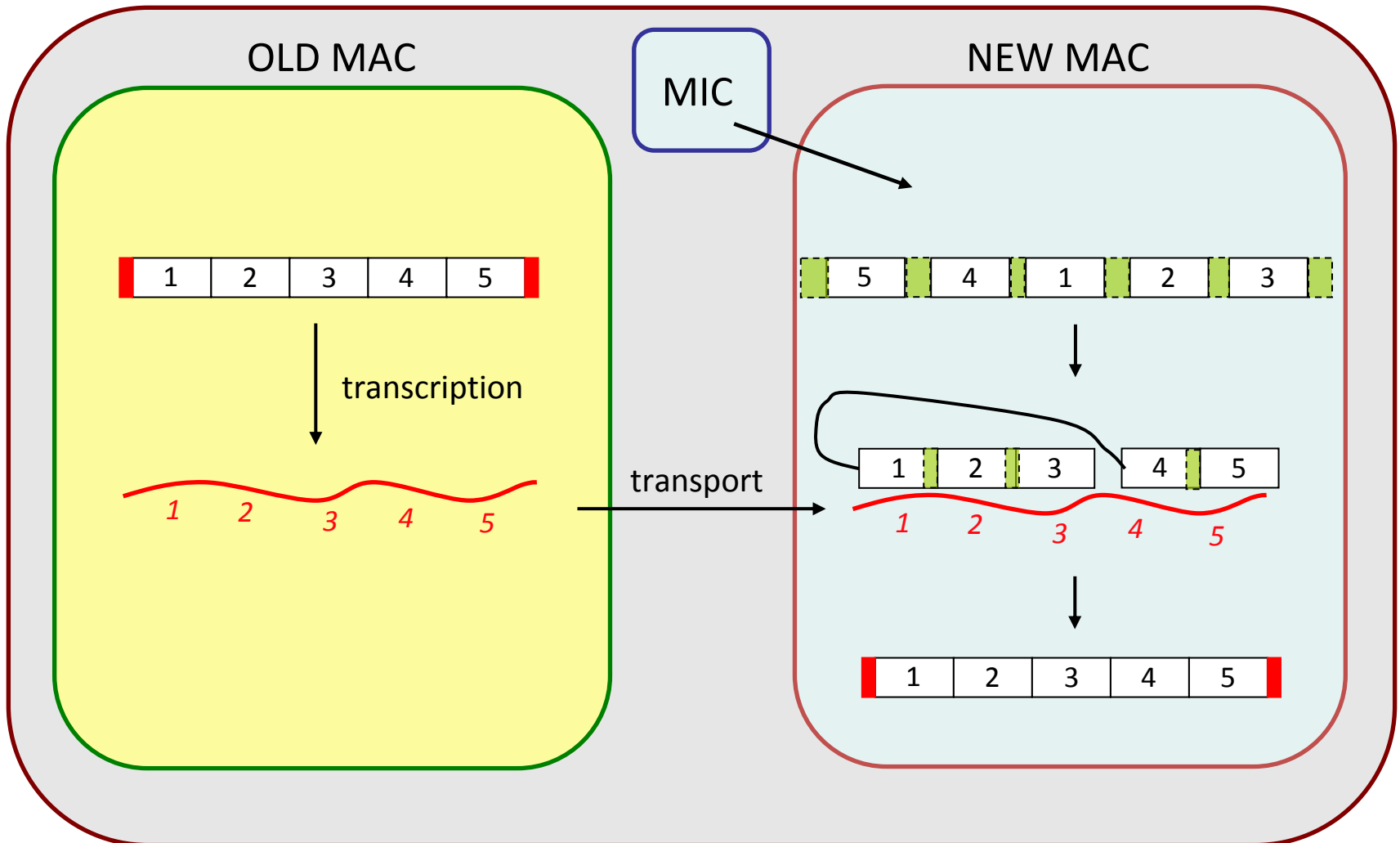
95%
deleted!



- How does the cell know the correct order
for pieces of genomic DNA?
?

- What machinery performs genome rearrangements?

Template Model



Mariusz Nowacki



University of Bern

Telomere RT-PCR can detect putative Template RNA

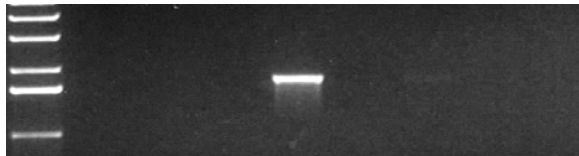
TEBP α (scrambled)

	V		T5		T30		T55	
RT	-	+	-	+	-	+	-	+

sense



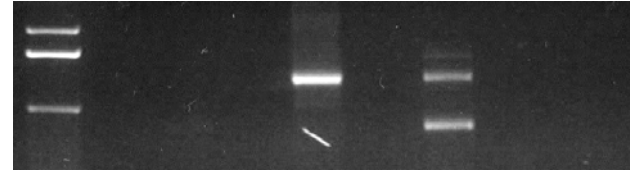
antisense



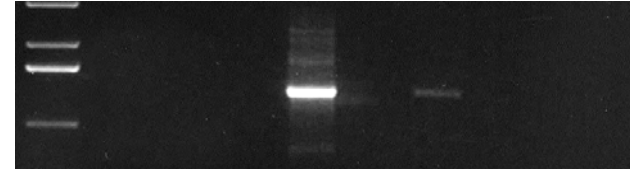
TEBP β (non-scrambled)

	V		T5		T30		T55	
RT	-	+	-	+	-	+	-	+

sense



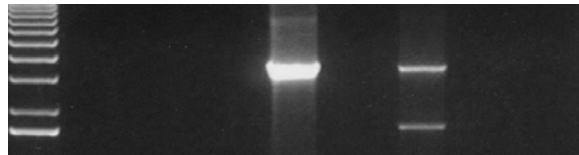
antisense



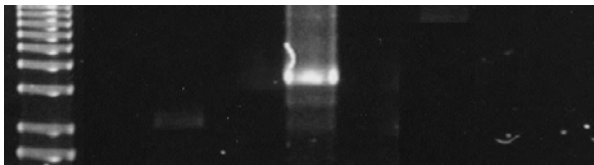
pol- α (scrambled)

	V		T5		T30		T55	
RT	-	+	-	+	-	+	-	+

sense



antisense



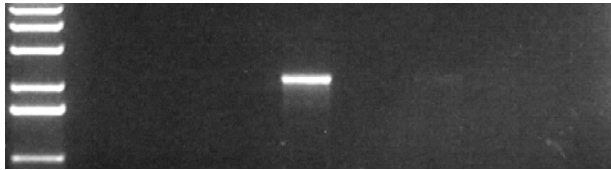
An RNA cache of the entire genome?

Telomere RT-PCR can detect putative Template RNA

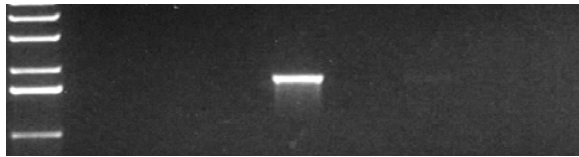
TEBP α (scrambled)

V T5 T30 T55
RT - + - + - + - +

sense



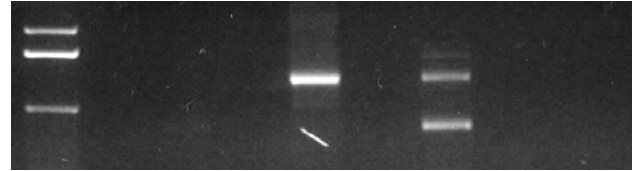
antisense



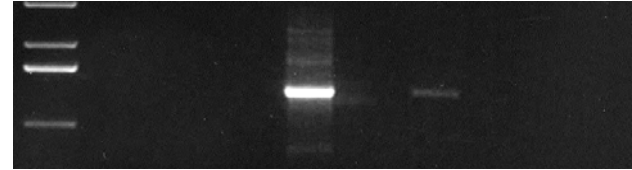
TEBP β (non-scrambled)

V T5 T30 T55
RT - + - + - + - +

sense



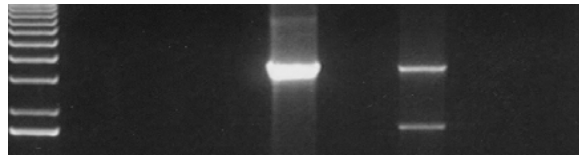
antisense



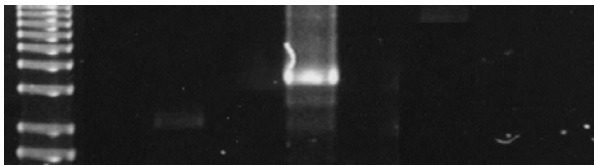
pol- α (scrambled)

V T5 T30 T55
RT - + - + - + - +

sense

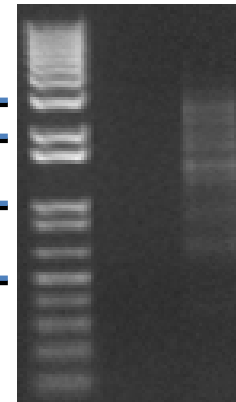


antisense



An RNA cache of the entire genome?

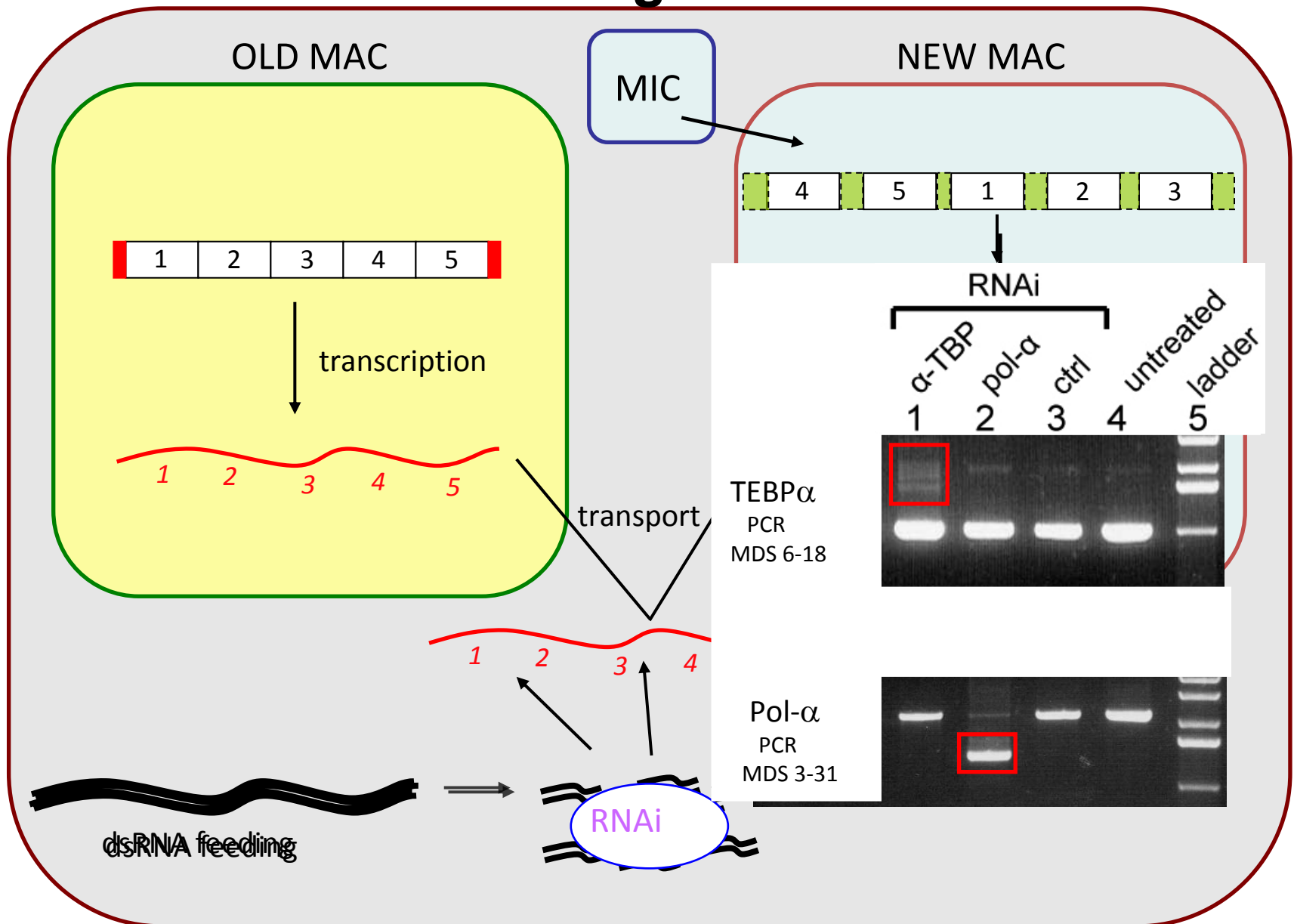
3000bp
2000bp
1000bp
500bp



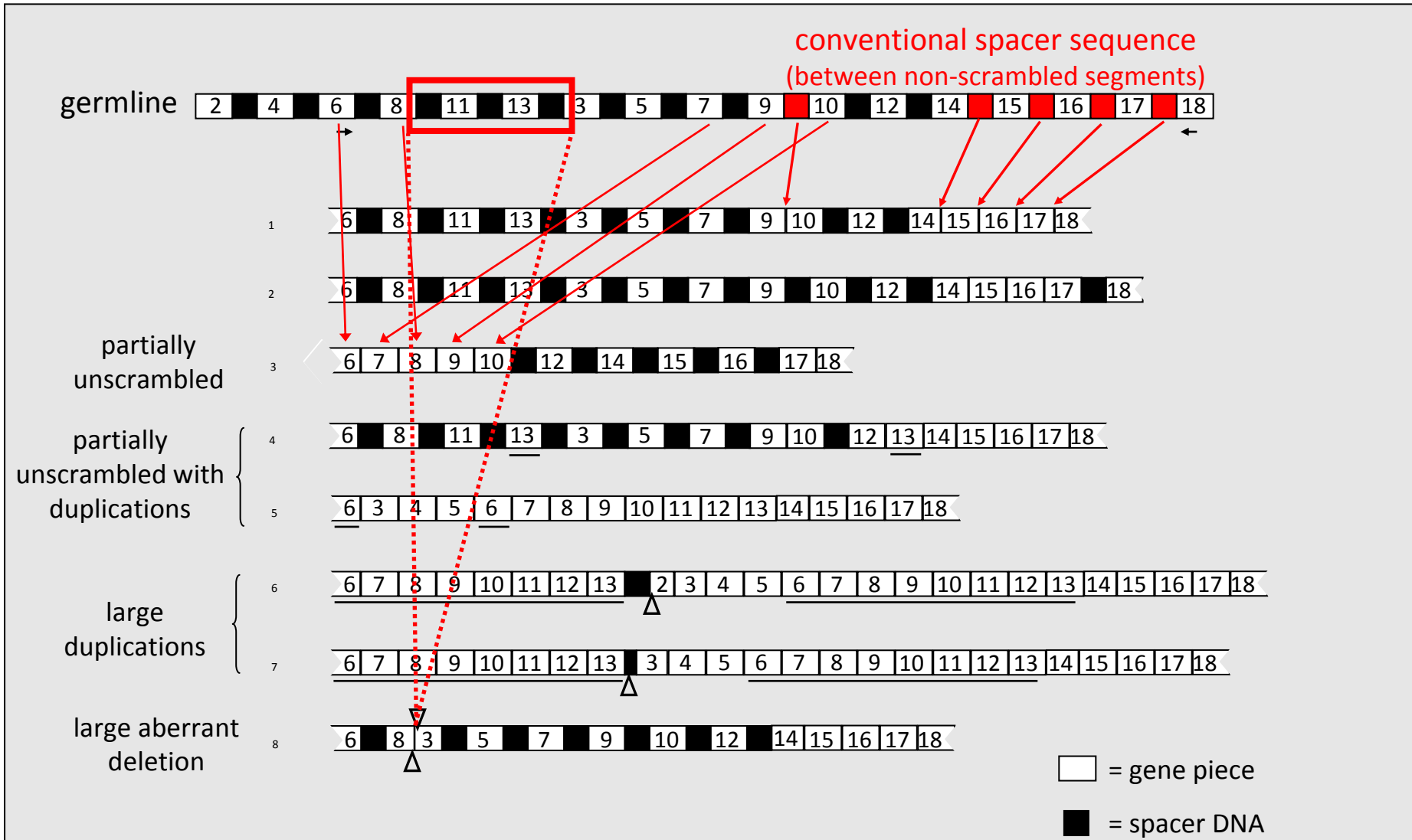
0hr

10hr

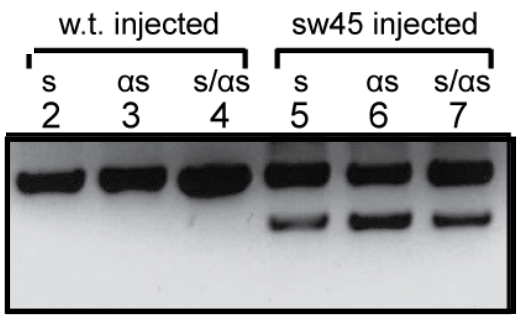
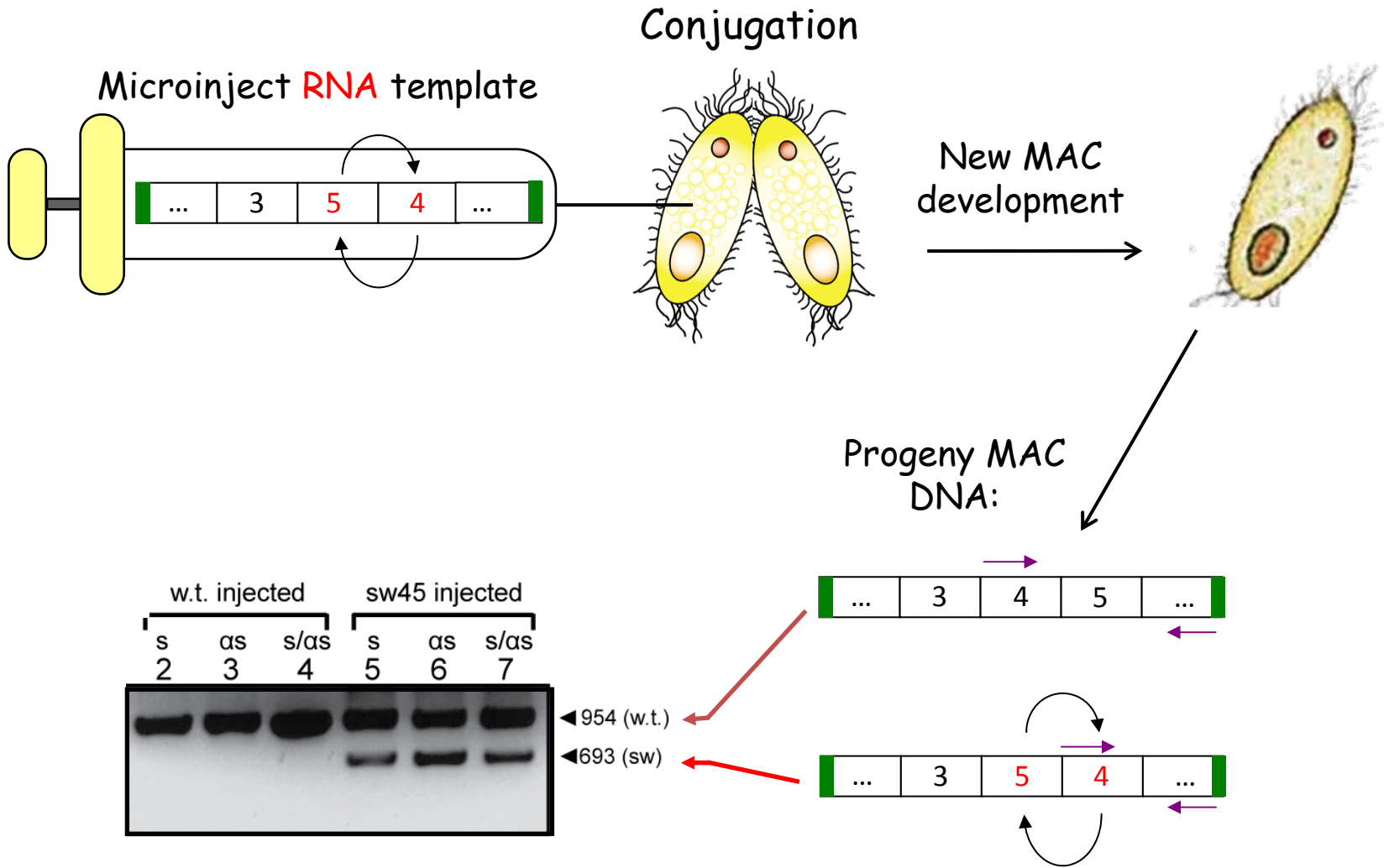
RNAi against putative templates stalls rearrangements



Examples of aberrantly rearranged molecules

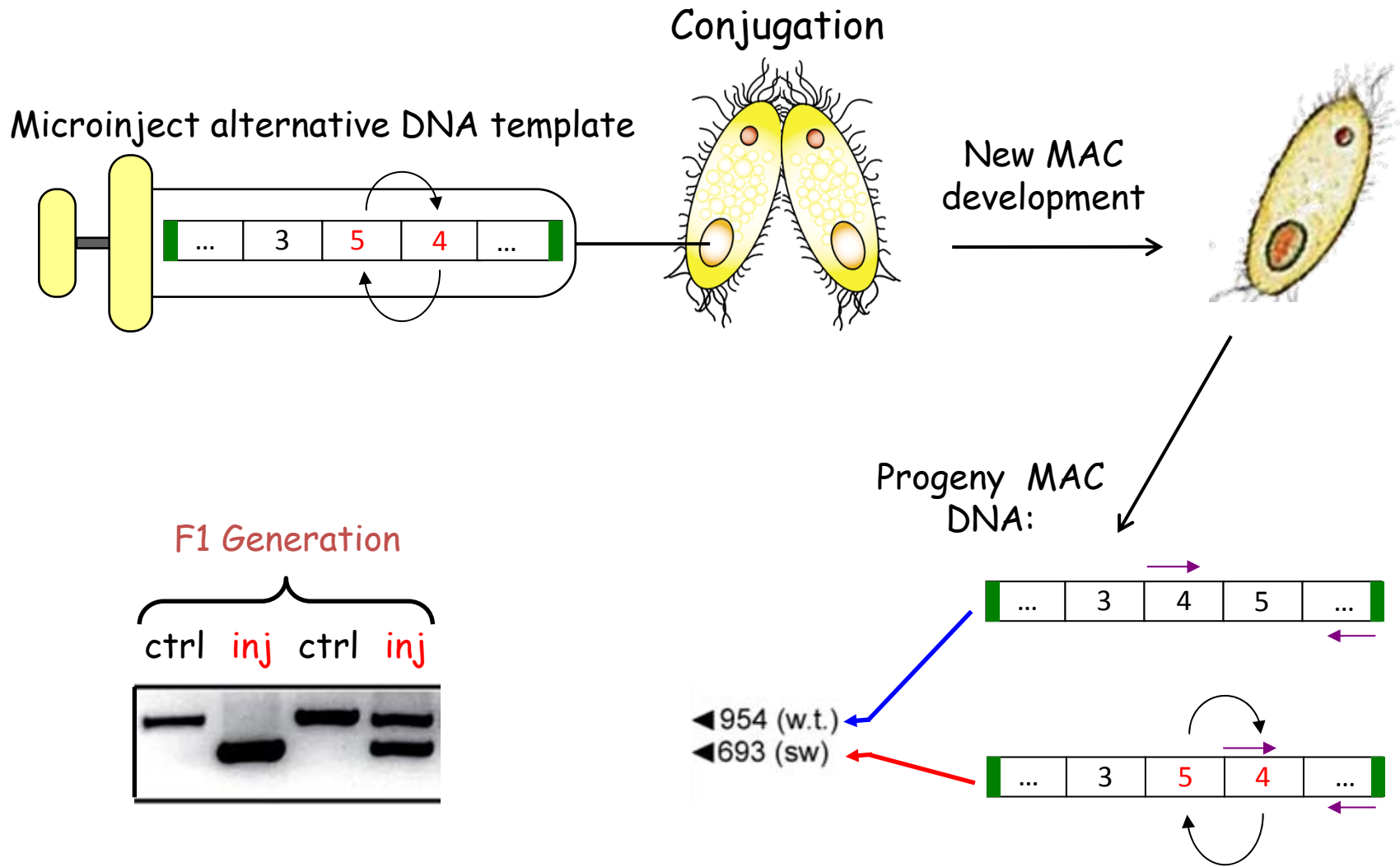


Artificial RNA template microinjection re-programs the genome rearrangement pathway



← 954 (w.t.)
 ← 693 (sw)

Switched chromosomes are transmitted to next generation



Point substitutions are inherited by F2 and F3 generations

MDS 4 (MAC = Soma)

310 allele_1 ...ATTCAAG...CATCCAACppp...
 310 allele_2 ...ATTCAAG...CATCCAACppp...
 510 allele_1 ...ATTCAAG...CATCCAACppp...
 510 allele_2 ...ATTCAAG...CATCCAACppp...
 sw45 temp ...ATTCTAG...CATCTAACppp...

F1 | ...ATTCAAG...CATCTAACppp...
 | ...ATTCAAG...CATCTAACppp...
 | ...ATTCAAG...CATCTAACppp...
 | ...ATTCAAG...CATCTAACppp...
 | ...ATTCAAG...CATCTAACppp...

F2 | ...ATTCAAG...CATCTAACppp...
 | ...ATTCAAG...CATCTAACppp...
 | ...ATTCAAG...CATCTAACppp...
 | ...ATTCAAG...CATCTAACppp...
 | ...ATTCAAG...CATCTAACppp...

F3 | ...ATTCAAG...CATCTAACppp...
 | ...ATTCAAG...CATCTAACppp...
 | ...ATTCAAG...CATCTAACppp...
 | ...ATTCTAG...CATCTAACppp...
 | ...ATTCAAG...CATCTAACppp...

MDS 4 (MIC = Germline)

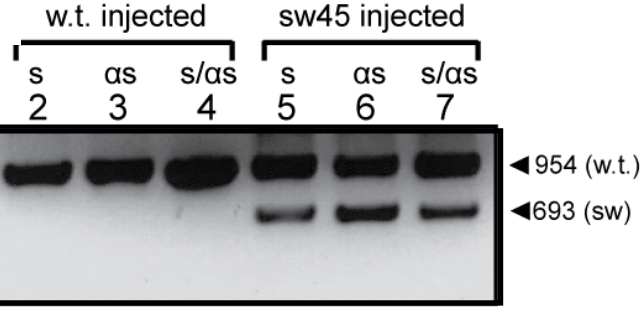
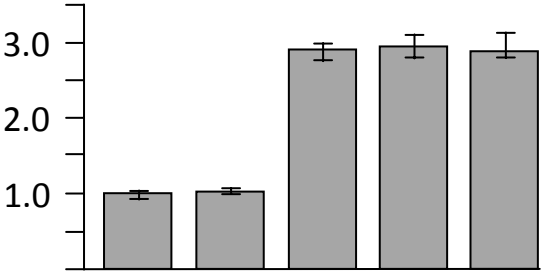
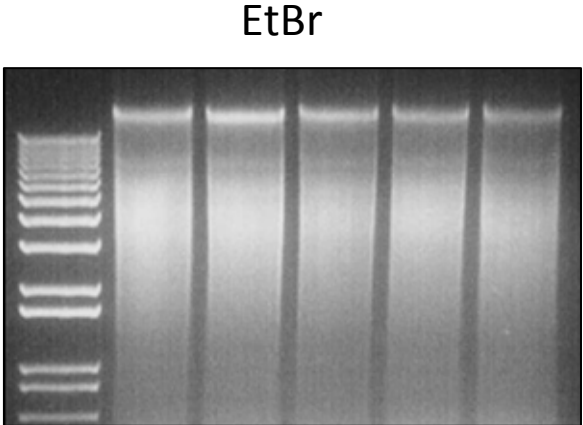
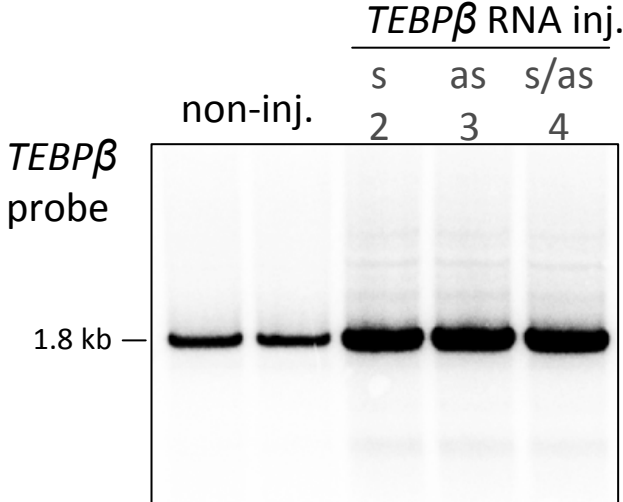
310 allele_1 ...ATTCAAG...CATCCAAC...
 310 allele_2 ...ATTCAAG...CATCCAAC...
 510 allele_1 ...ATTCAAG...CATCCAAC...
 510 allele_2 ...ATTCAAG...CATCCAAC...
 sw45 temp ...ATTCTAG...CATCTAAC...

F1 | ...ATTCAAG...CATCCAAC...
 | ...ATTCAAG...CATCCAAC...
 | ...ATTCAAG...CATCCAAC...
 | ...ATTCAAG...CATCCAAC...
 | ...ATTCAAG...CATCCAAC...

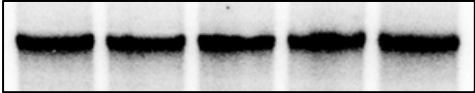
F2 | ...ATTCAAG...CATCCAAC...
 | ...ATTCAAG...CATCCAAC...
 | ...ATTCAAG...CATCCAAC...
 | ...ATTCAAG...CATCCAAC...
 | ...ATTCAAG...CATCCAAC...

F3 | ...ATTCAAG...CATCCAAC...
 | ...ATTCAAG...CATCCAAC...
 | ...ATTCAAG...CATCCAAC...
 | ...ATTCAAG...CATCCAAC...
 | ...ATTCAAG...CATCCAAC...

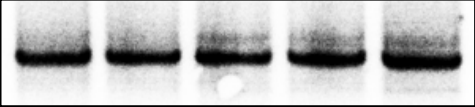
Epigenetic changes in DNA copy number



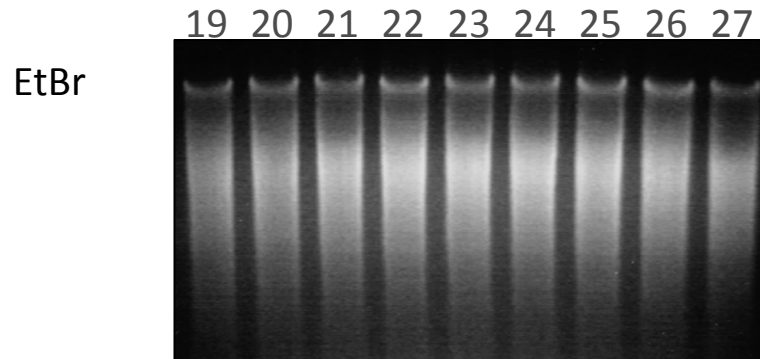
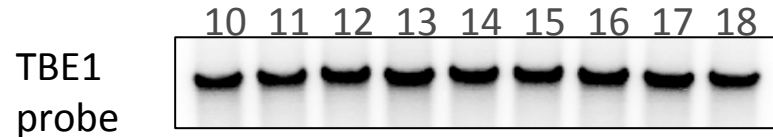
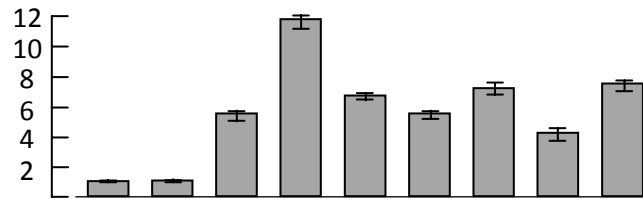
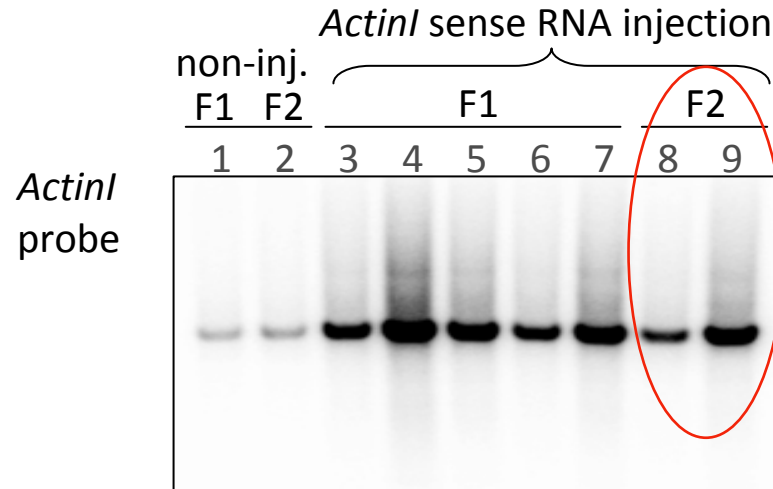
TBE1 probe (load control)



Actin1 probe (load control)

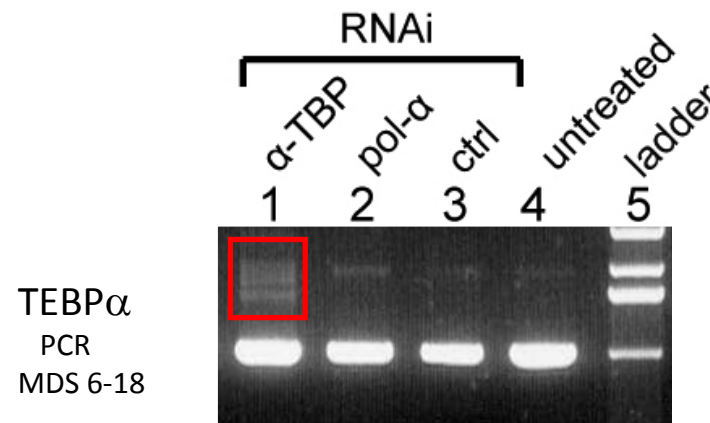
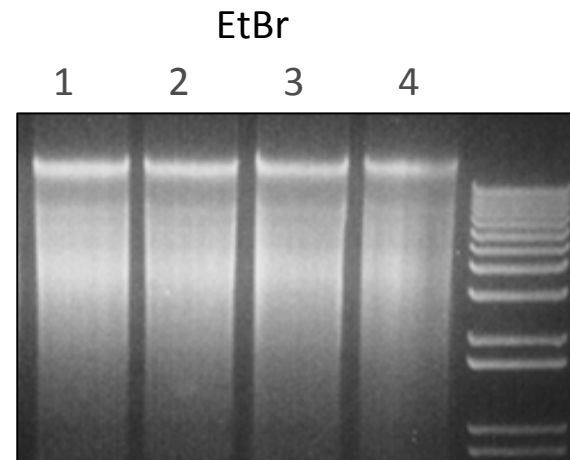
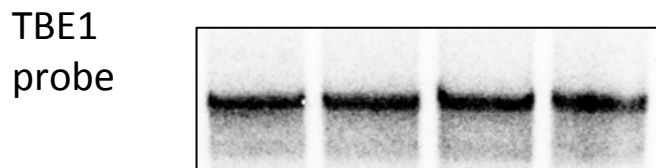
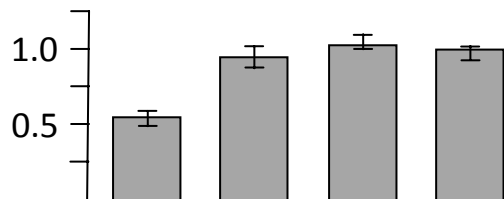
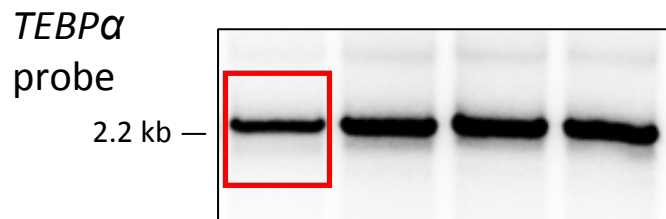
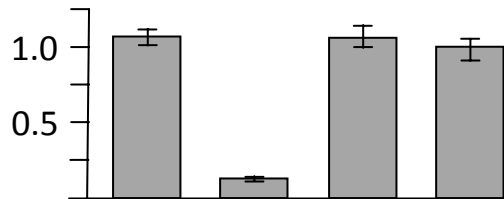
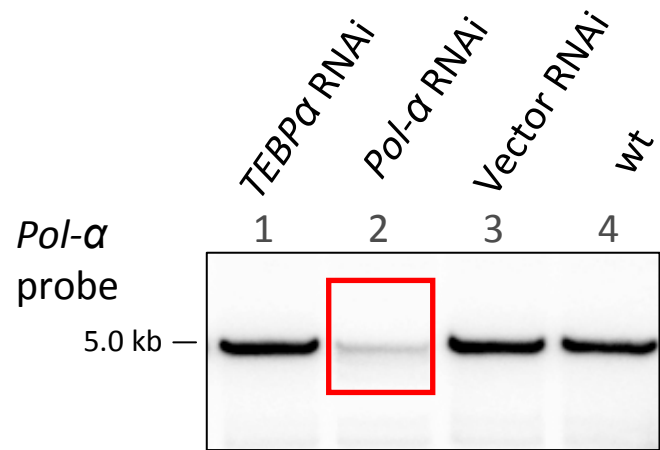


Epigenetic inheritance of DNA copy number changes

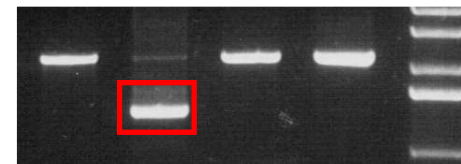


Nowacki, Haye *et al.*,
PNAS 2010

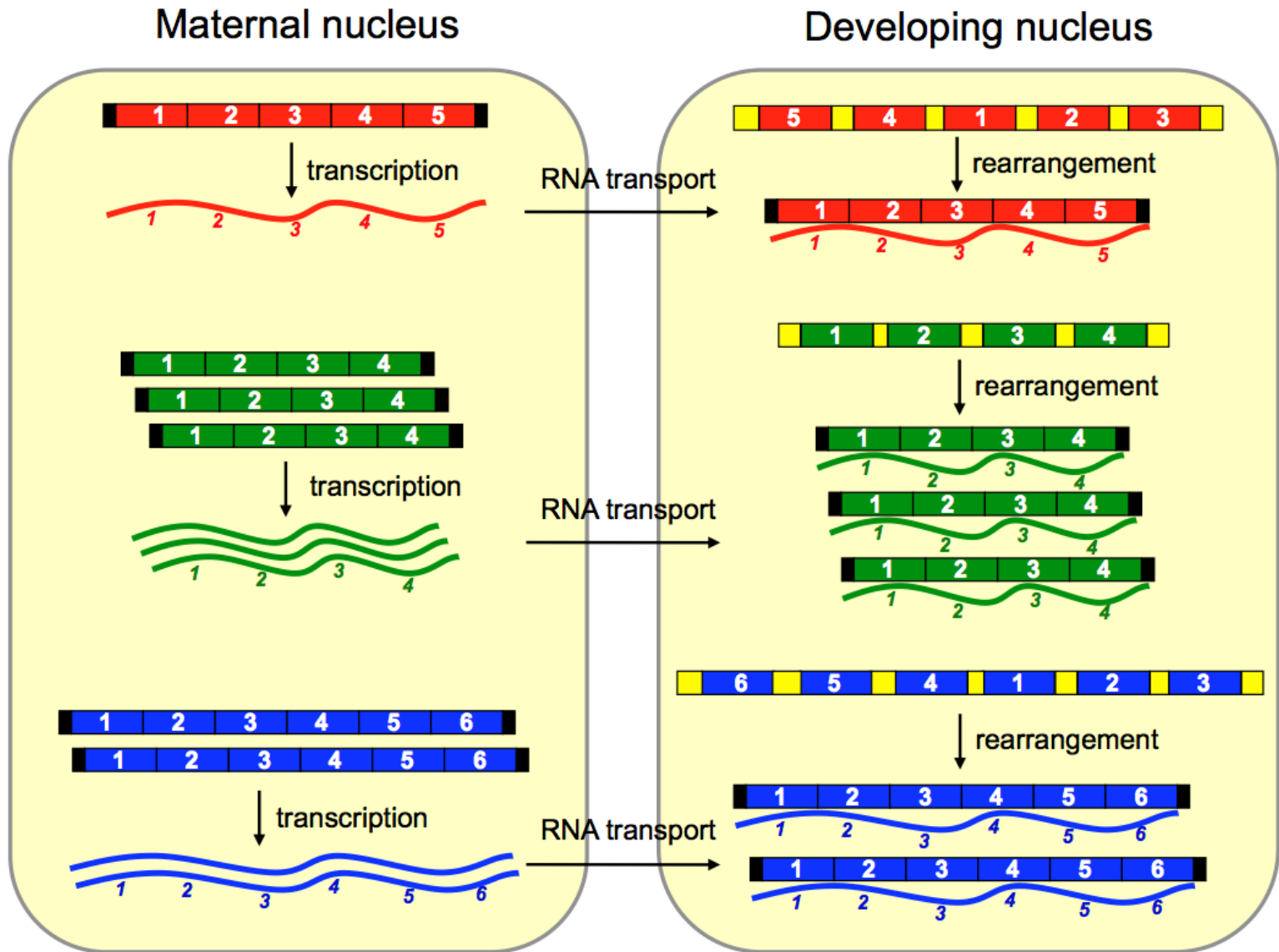
Reduced RNA template levels reduce DNA copy number



Pol- α PCR MDS 3-31



Templates as epigenetic regulators of genome architecture



Open Questions

- What polymerase makes templates?
 - How do RNA template-introduced mutations affect *Oxytricha* evolution?
- What machinery mediates the rearrangement process?
 - A role for transposases only found in the MIC genome (Nowacki *et al.*, 2009)
- How are deleted and retained regions specified?
 - A role for small RNAs (Fang *et al.*, in revision)

Acknowledgements

Laura Landweber

Mariusz Nowacki – University of
Bern



Joanna Haye

Wenwen Fang

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Yi Zhou

Klaas Schotanus

Thomas Doak

Xing Wang

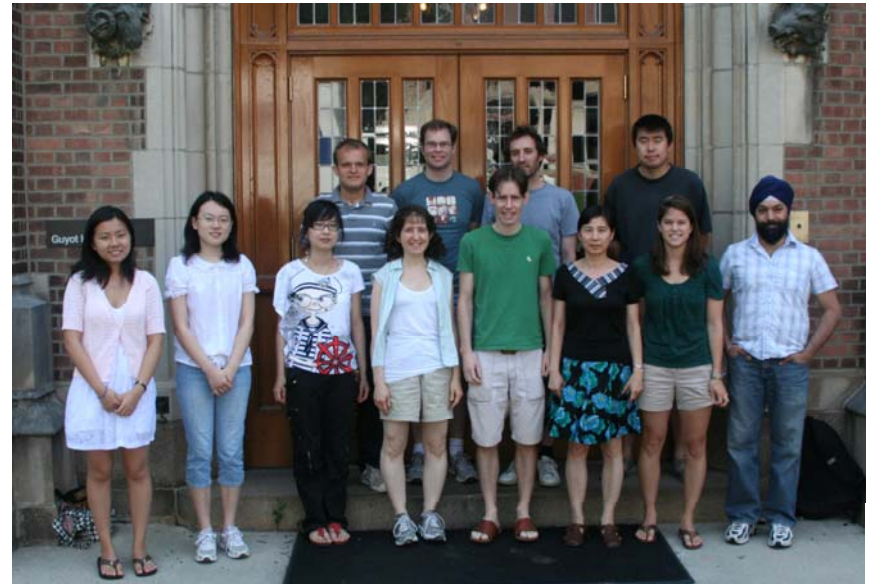
Estienne Swart

Xiao Chen

Aaron Goldman

Jaspreet Khurana

Jingmei Wang



**Princeton High Throughput Sequencing
Center**

Jessica Buckles

Benaryoya Research Institute

Chris Amemiya

Andrew Stuart