

Possibilities for Ancient Genome Evolution: Lessons from *Oxytricha*

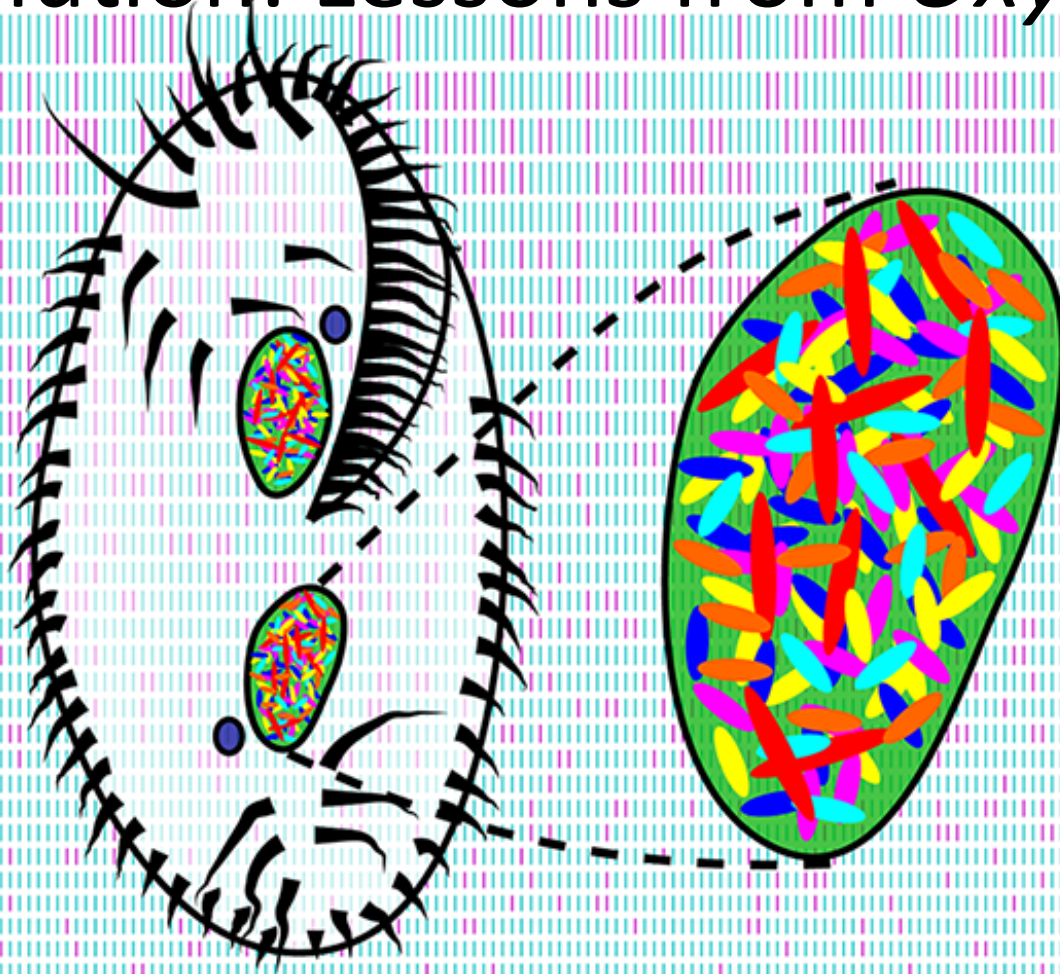
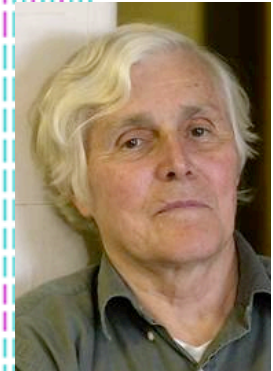
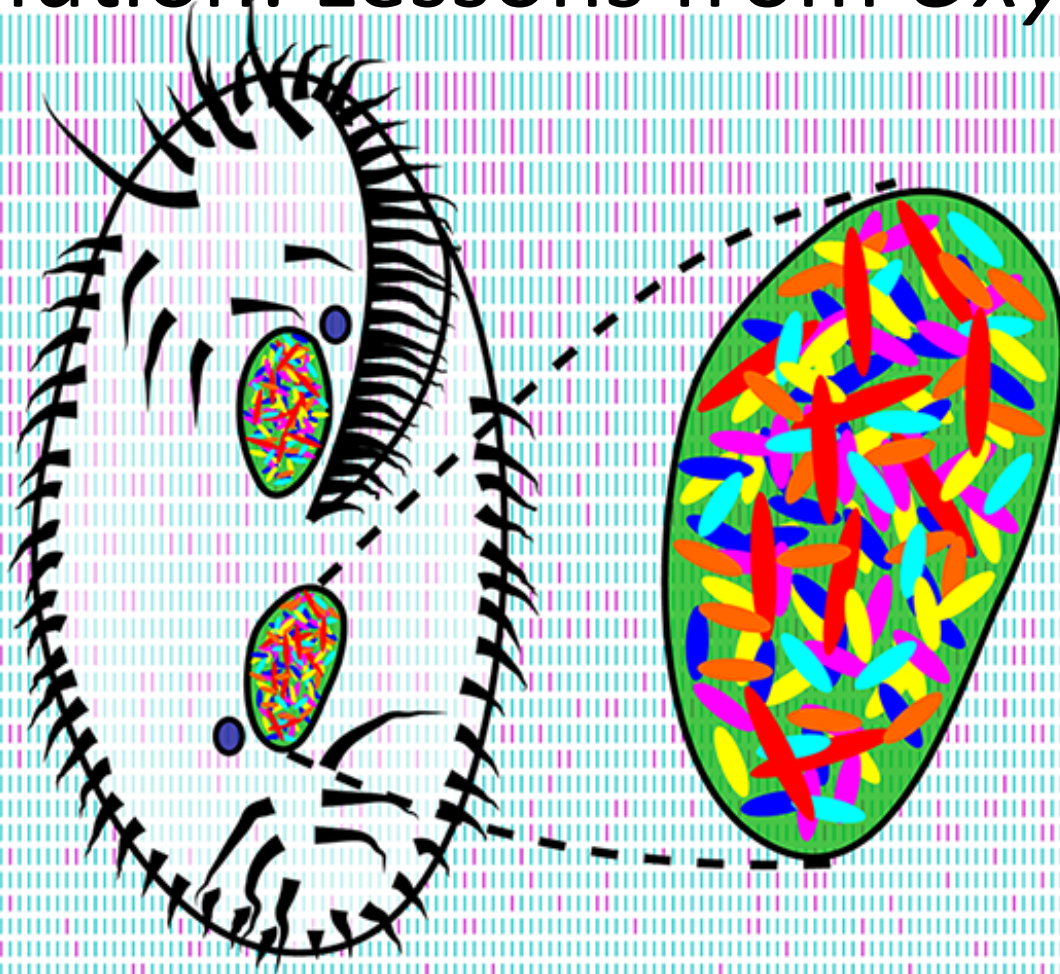


image: Estienne Swart, *PLoS Biology*, Jan. 29, 2013

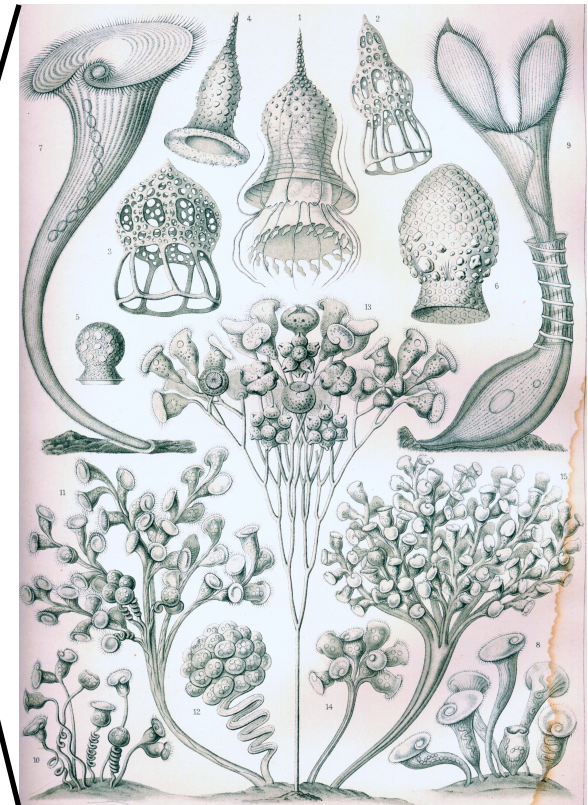
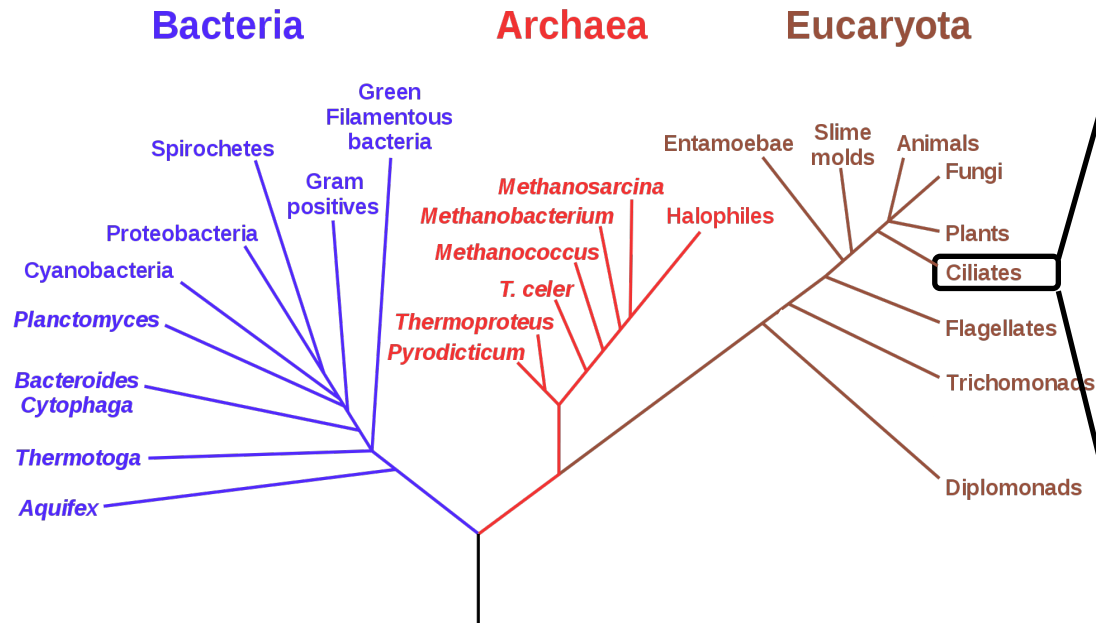
Possibilities for Ancient Genome Evolution: Lessons from *Oxytricha*



古代のゲノム進化の可能性: *Oxytricha* からの教訓

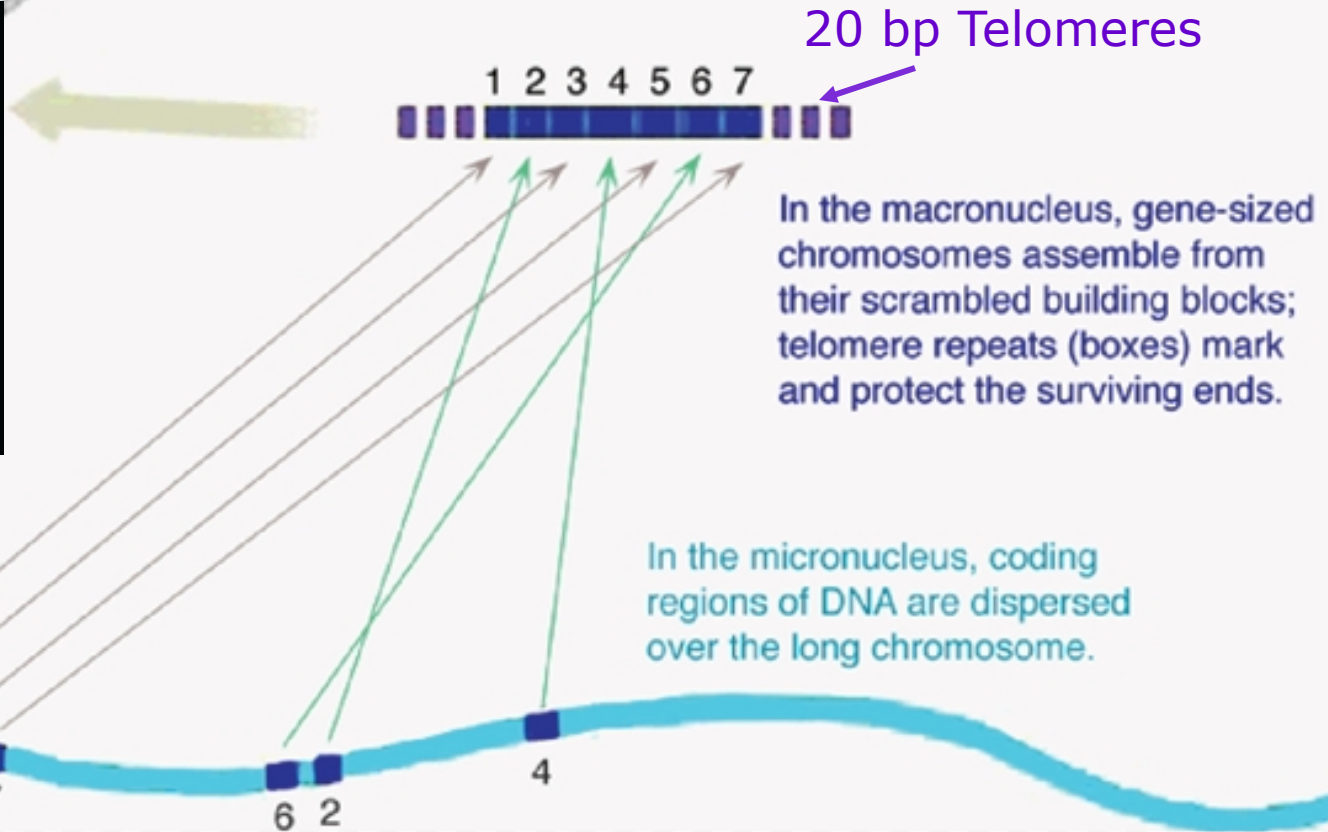
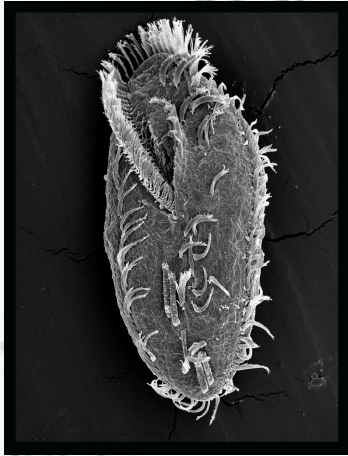
Swart et al., *PLoS Biology*, 2013

Ciliates are microbial eukaryotes

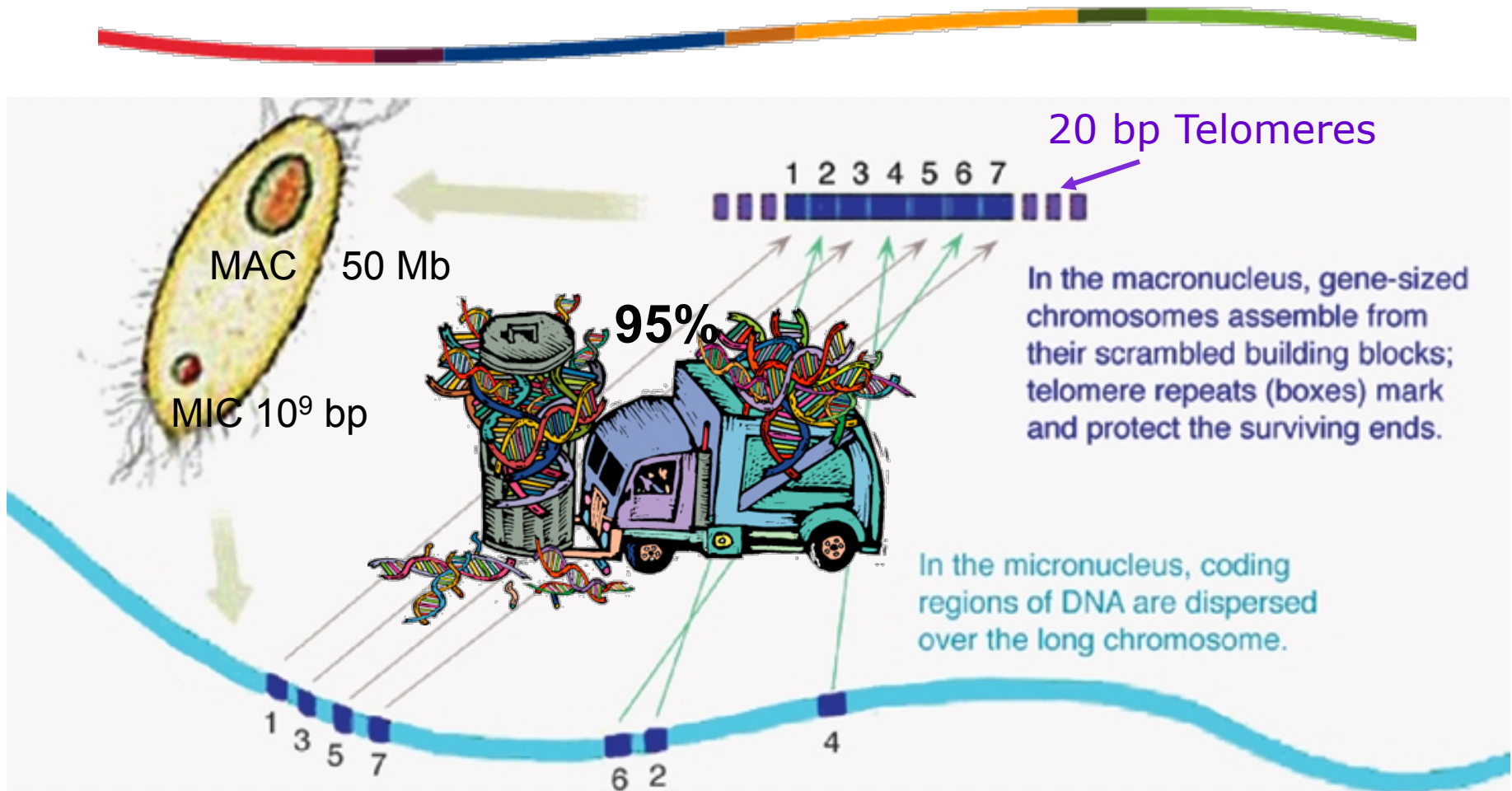


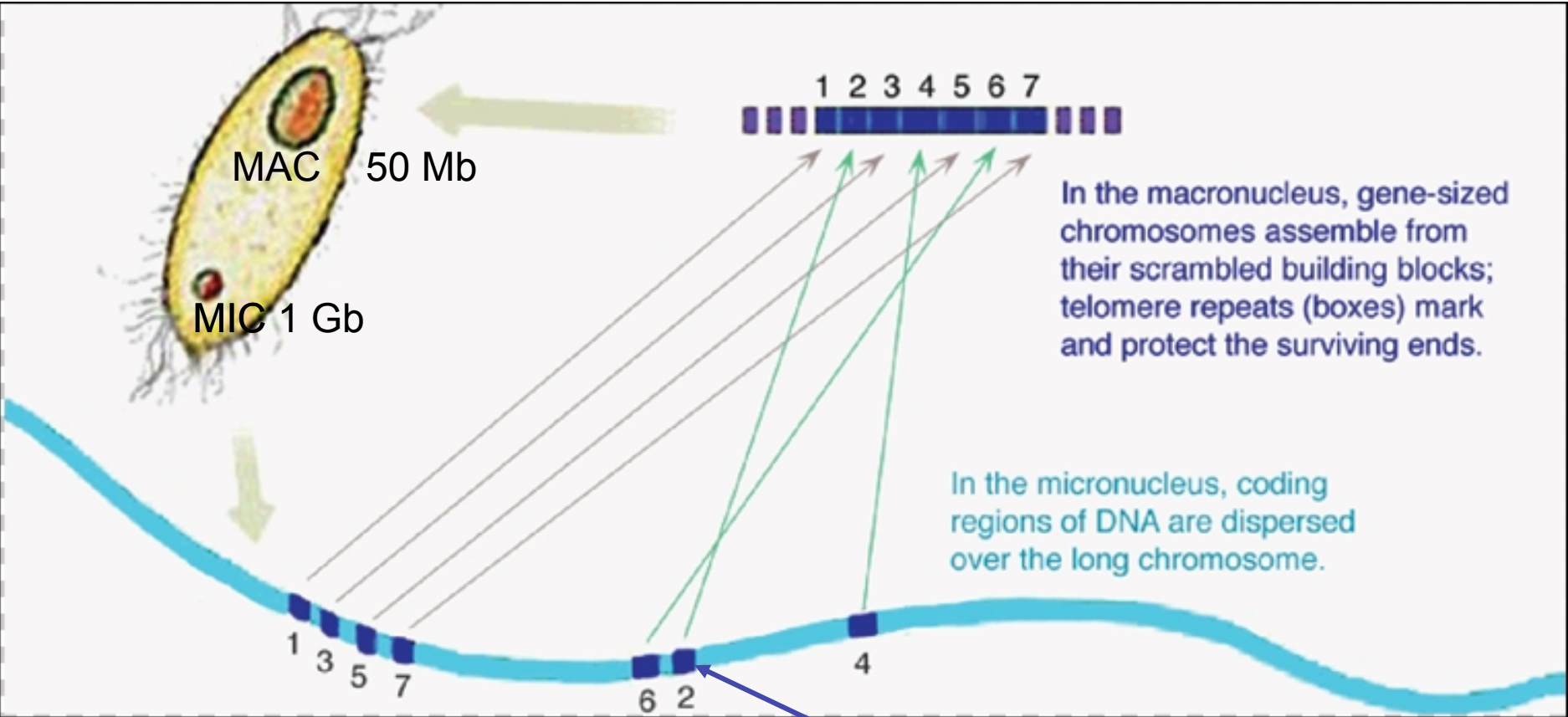
Ernst Haeckel, Kunstformen der Natur, 1904

Diverse Genome Architectures: Dual genomes in *Oxytricha*



A Minimal Eukaryotic Genome Architecture: Sequence compression in the MAC

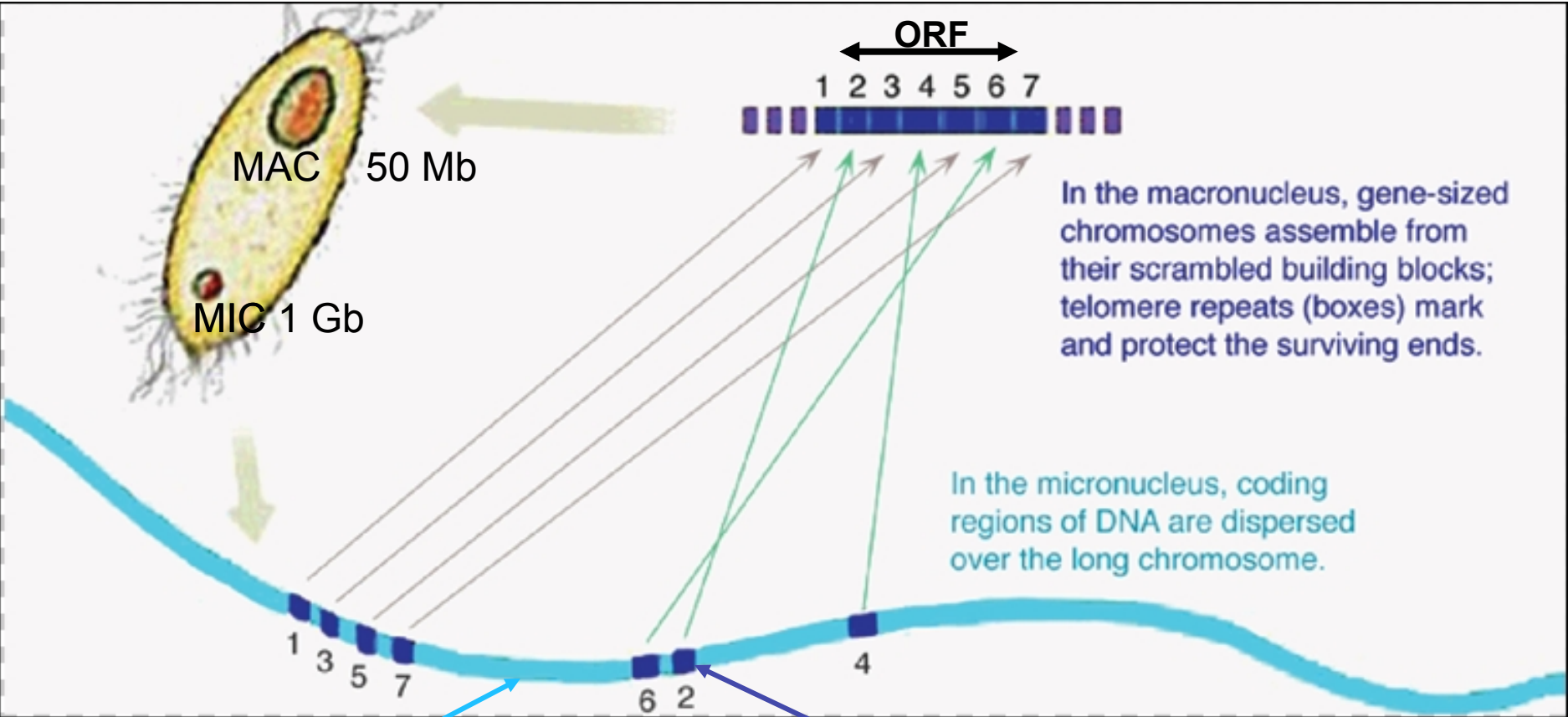




In the macronucleus, gene-sized chromosomes assemble from their scrambled building blocks; telomere repeats (boxes) mark and protect the surviving ends.

In the micronucleus, coding regions of DNA are dispersed over the long chromosome.

MDS: macronuclear destined sequences

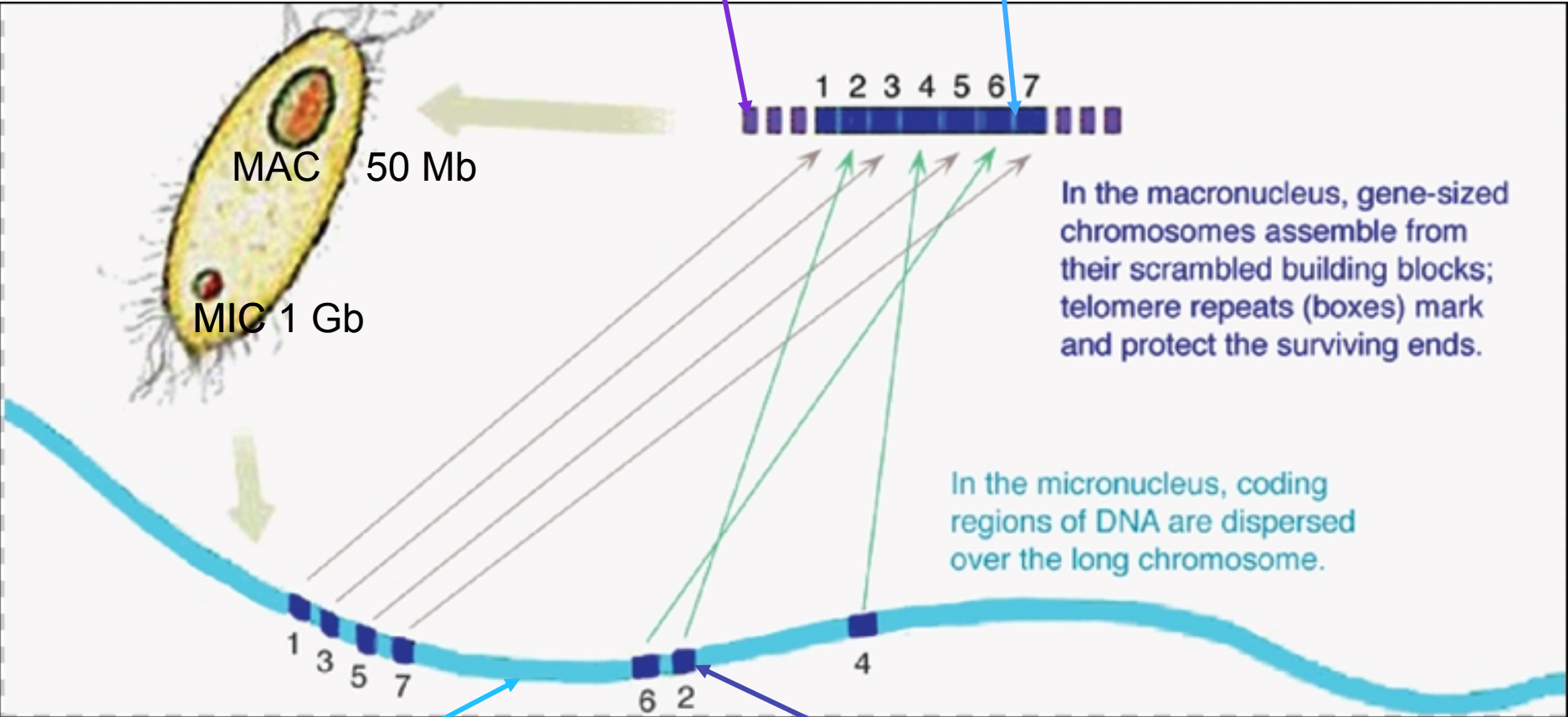


MDS: macronuclear destined sequences

IES: internal eliminated segments

Telomere

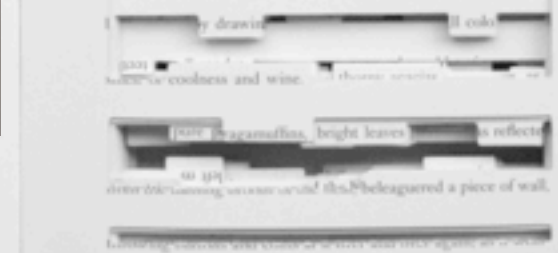
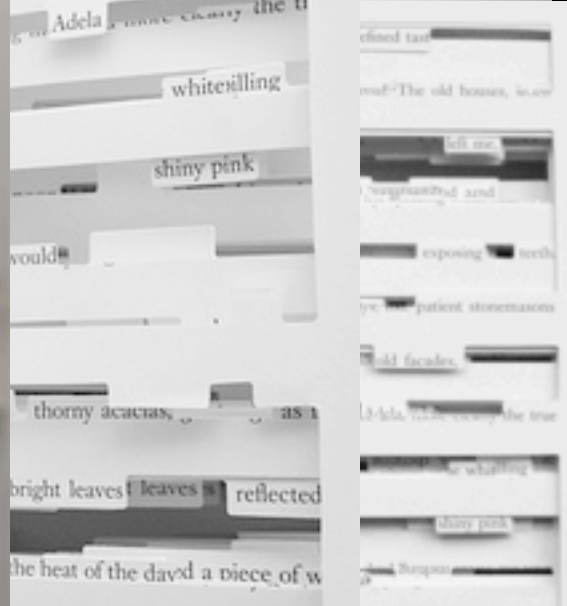
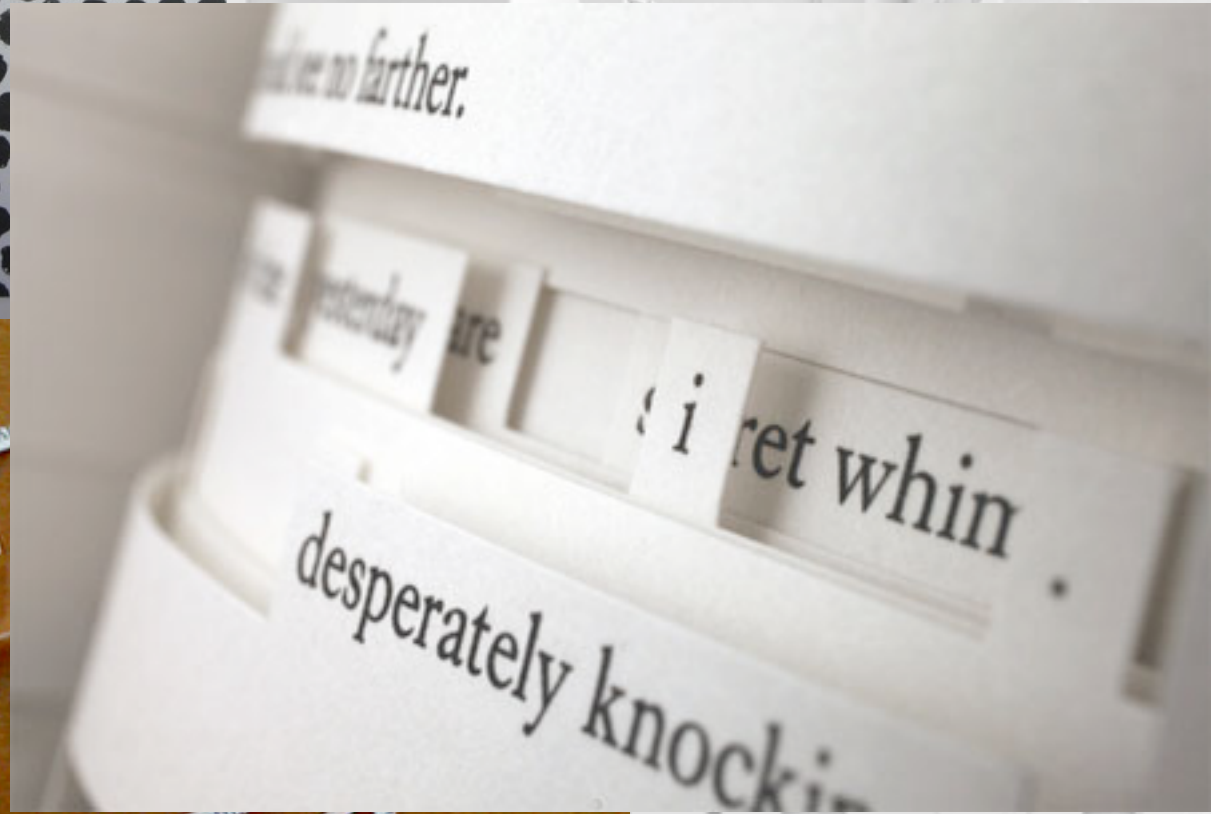
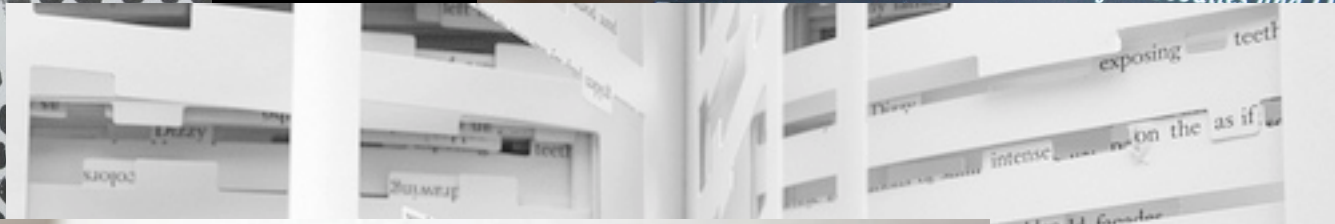
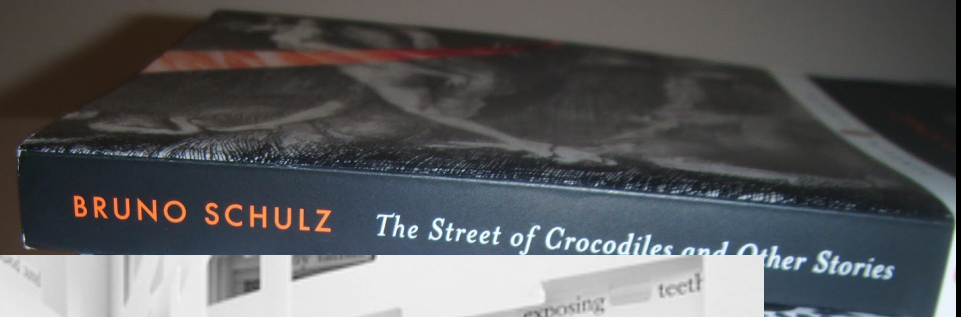
Pointers



In the macronucleus, gene-sized chromosomes assemble from their scrambled building blocks; telomere repeats (boxes) mark and protect the surviving ends.

In the micronucleus, coding regions of DNA are dispersed over the long chromosome.

MDS: macronuclear destined sequences
IES: internal eliminated segments

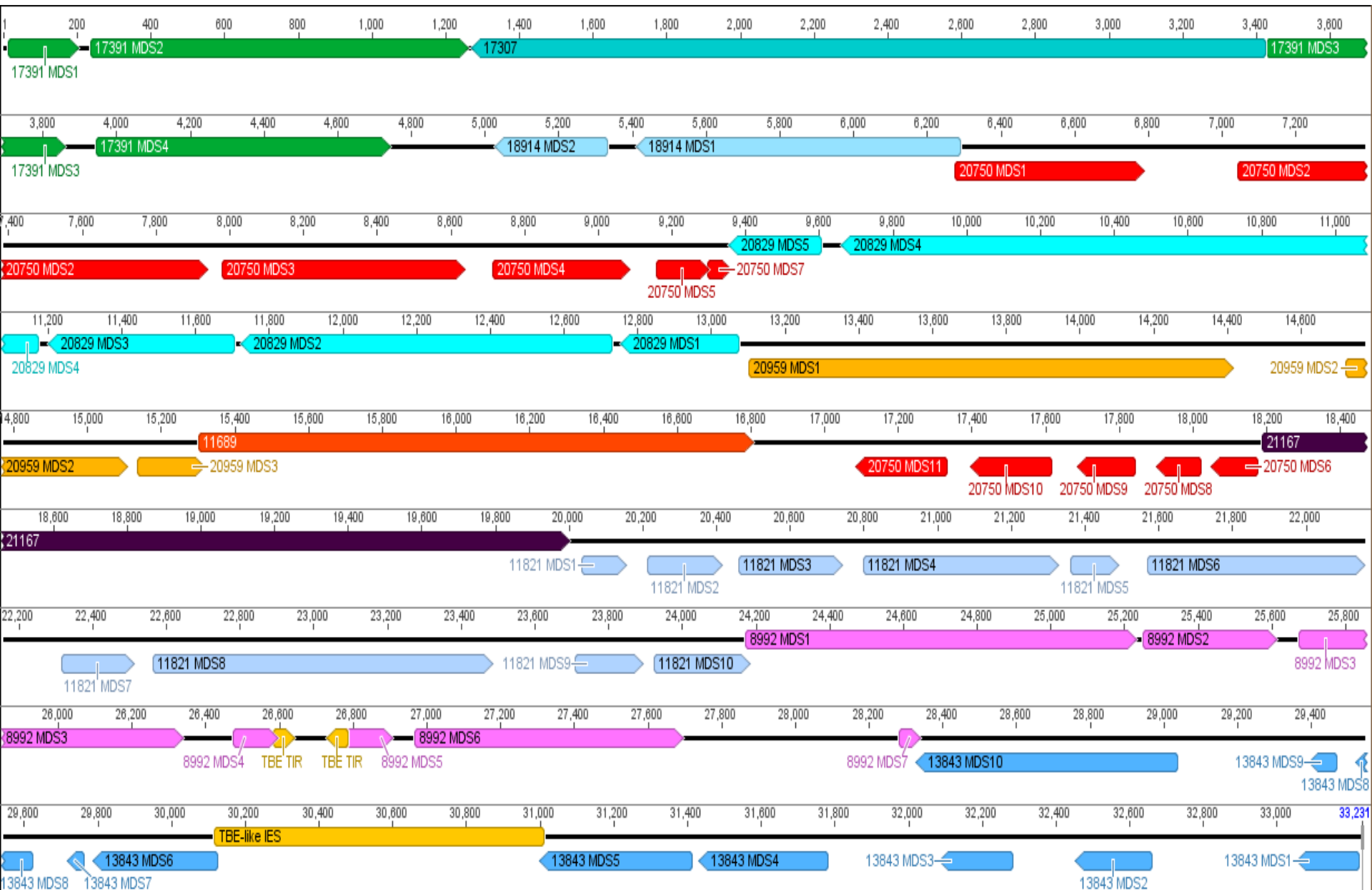




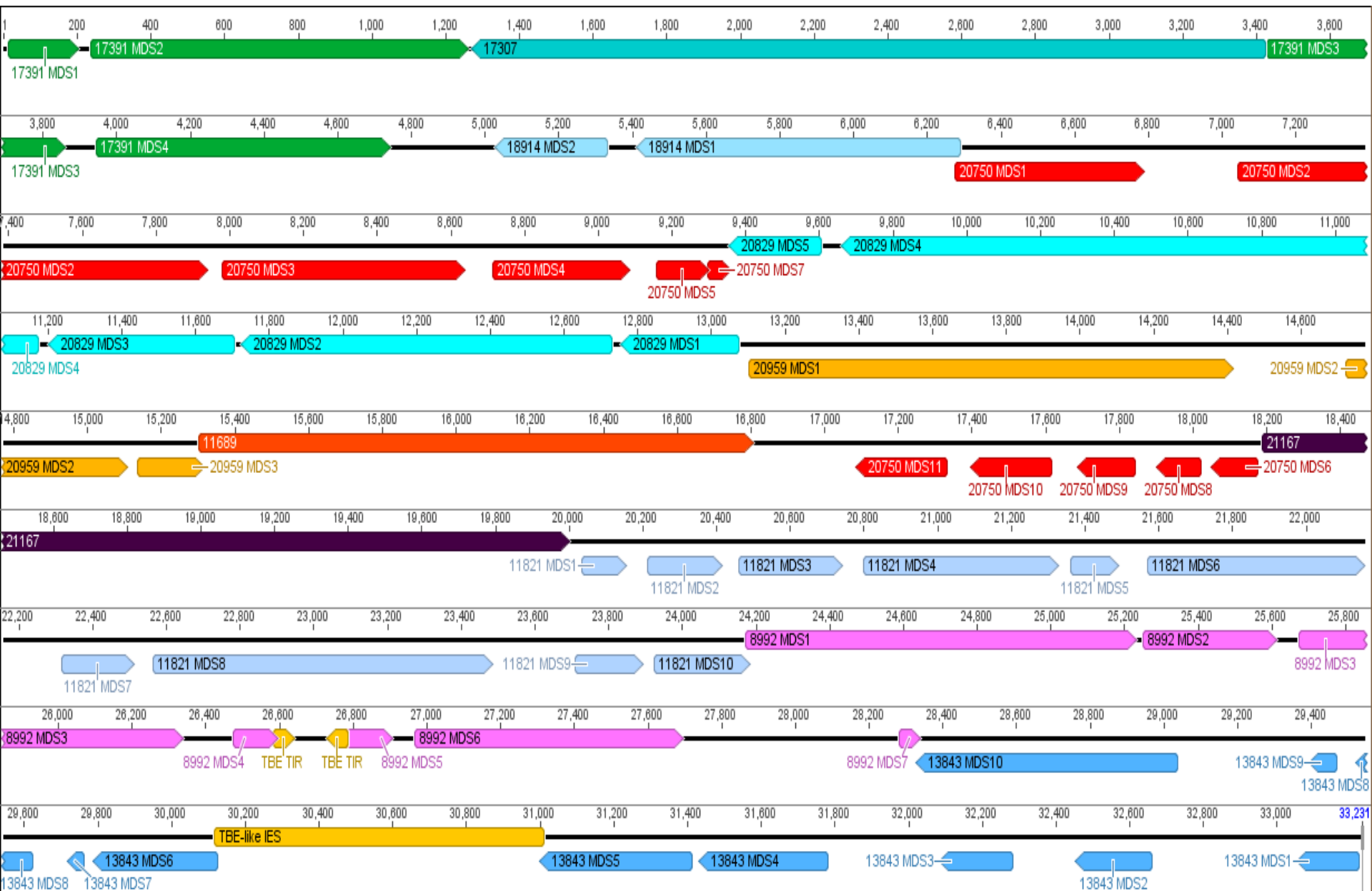
Oxytricha trifallax: 2 nuclear genomes

- **MACronucleus** complete [Swart *et al.*, PLoS Biol, 2013]
 - 67 Mb (half *Tetrahymena*)
 - average MAC “nano-chromosome” 3.2 kb (469 bp - 66 kb)
 - 90% contain 1 gene, 10% contain 2-8 genes
 - >**16,000** completely sequenced nanochromosomes, ~18,400 genes
 - amplified: ~1000 copies each
 - 65% A+T
- **MICronucleus** 100x draft [Chen, Bracht, Goldman, Amemiya *et al.*]
 - 10⁹ bp (20-50x more complexity than MAC)
 - ~100 small equal-sized chromosomes
 - 75% is highly repetitive
 - ~8% are known transposon families, 67% satellite
 - ~20% genes **scrambled (>3000 scrambled genes)**
(MDSs in permuted order, inverted, or unlinked)

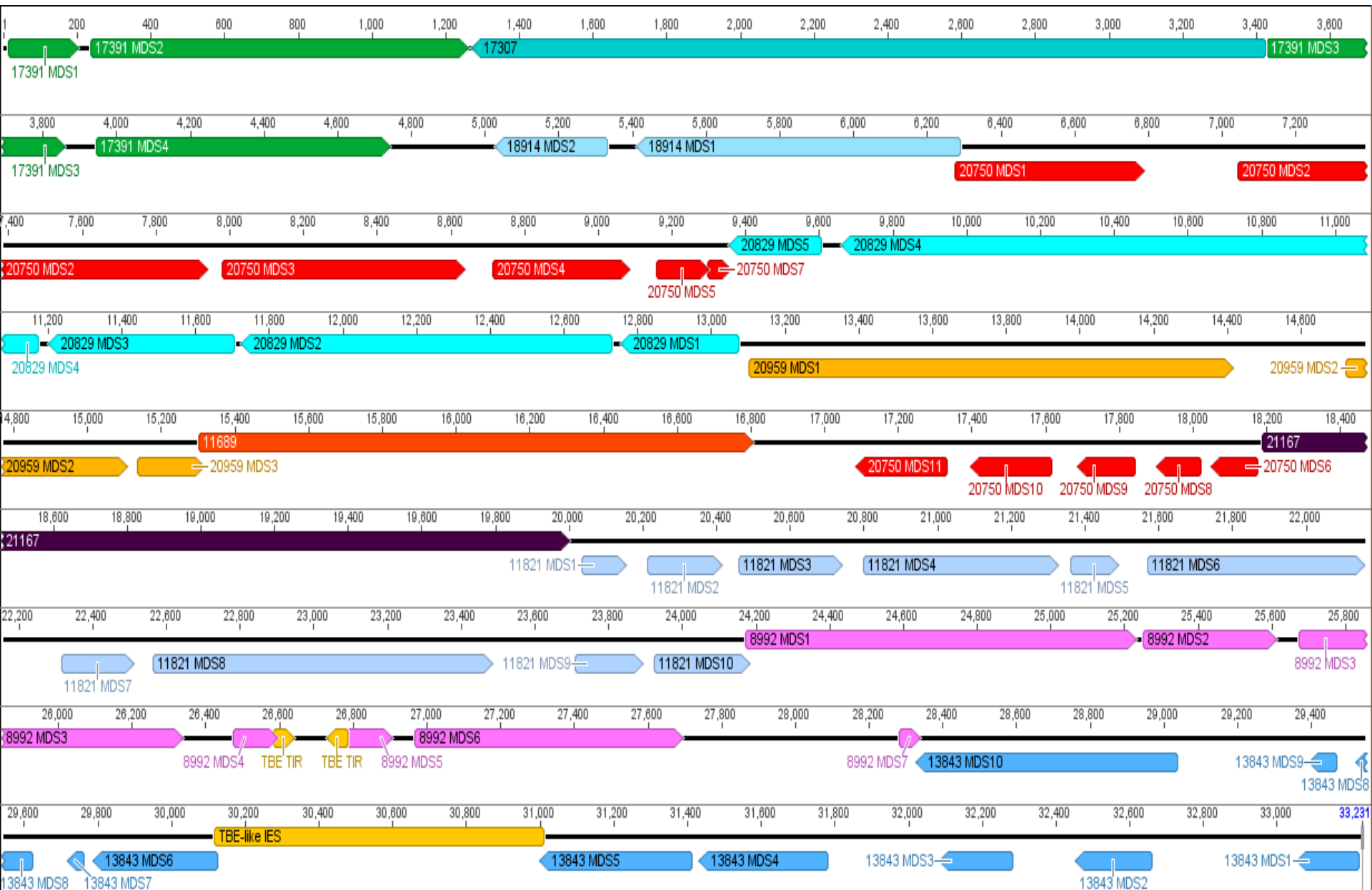
A Gene-Dense Micronuclear Region



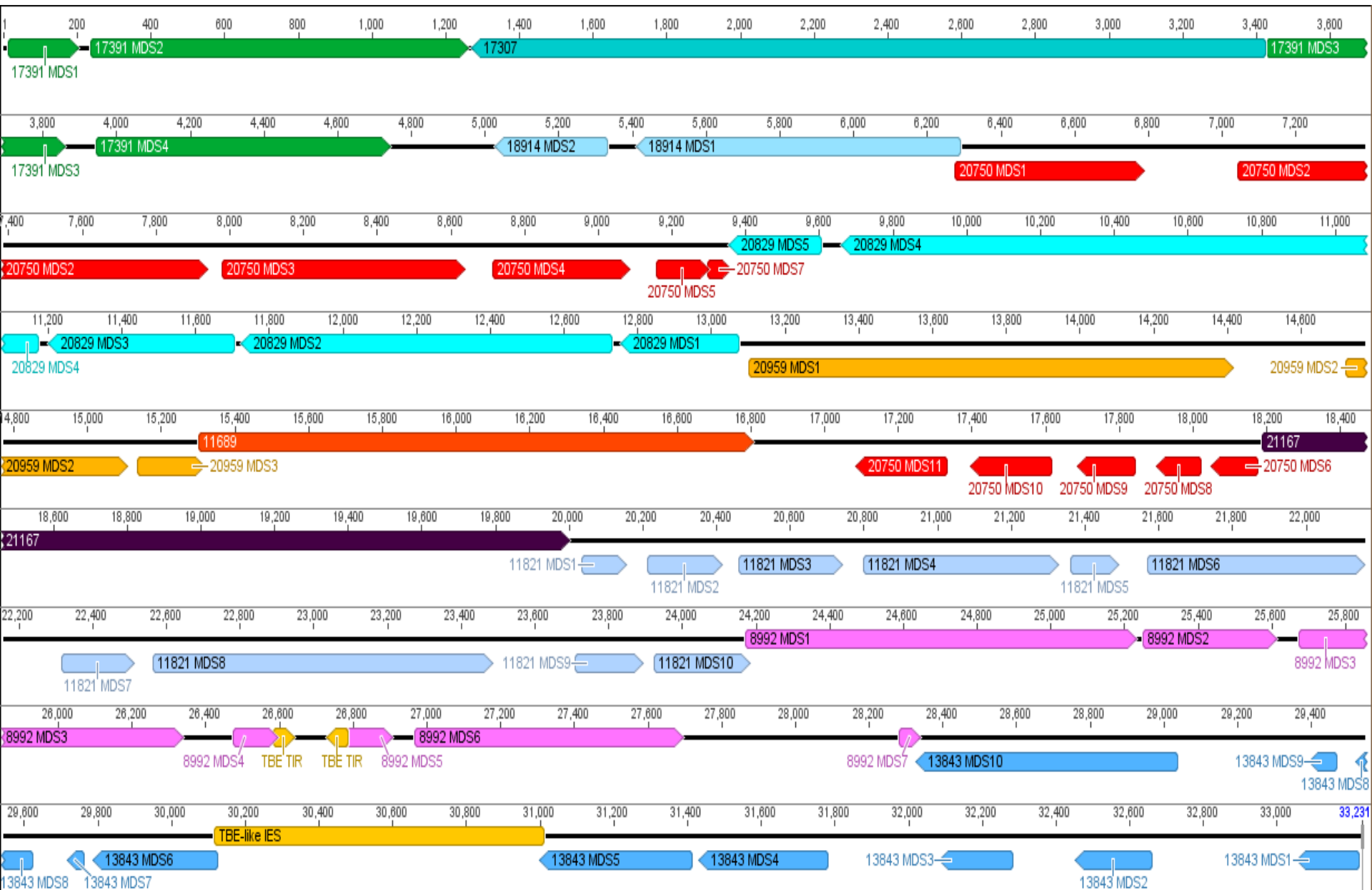
11 complete genes, 3 IES-less, 1 scrambled (red)



Gene pieces (MDSs) interrupt other genes



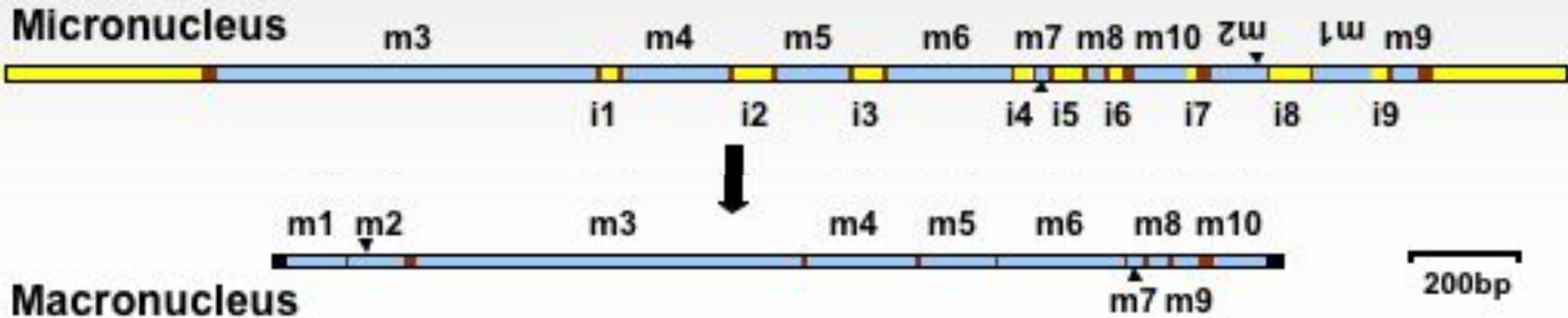
(851 cases so far of overlapping genes)

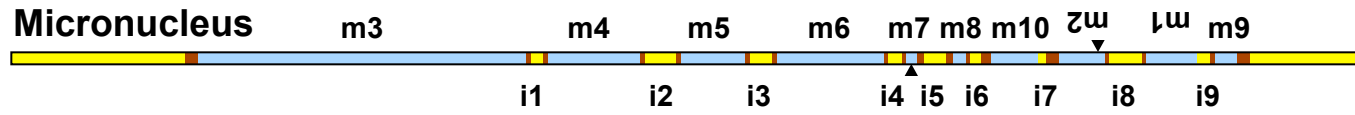


MDS shuffling: origin of a new gene



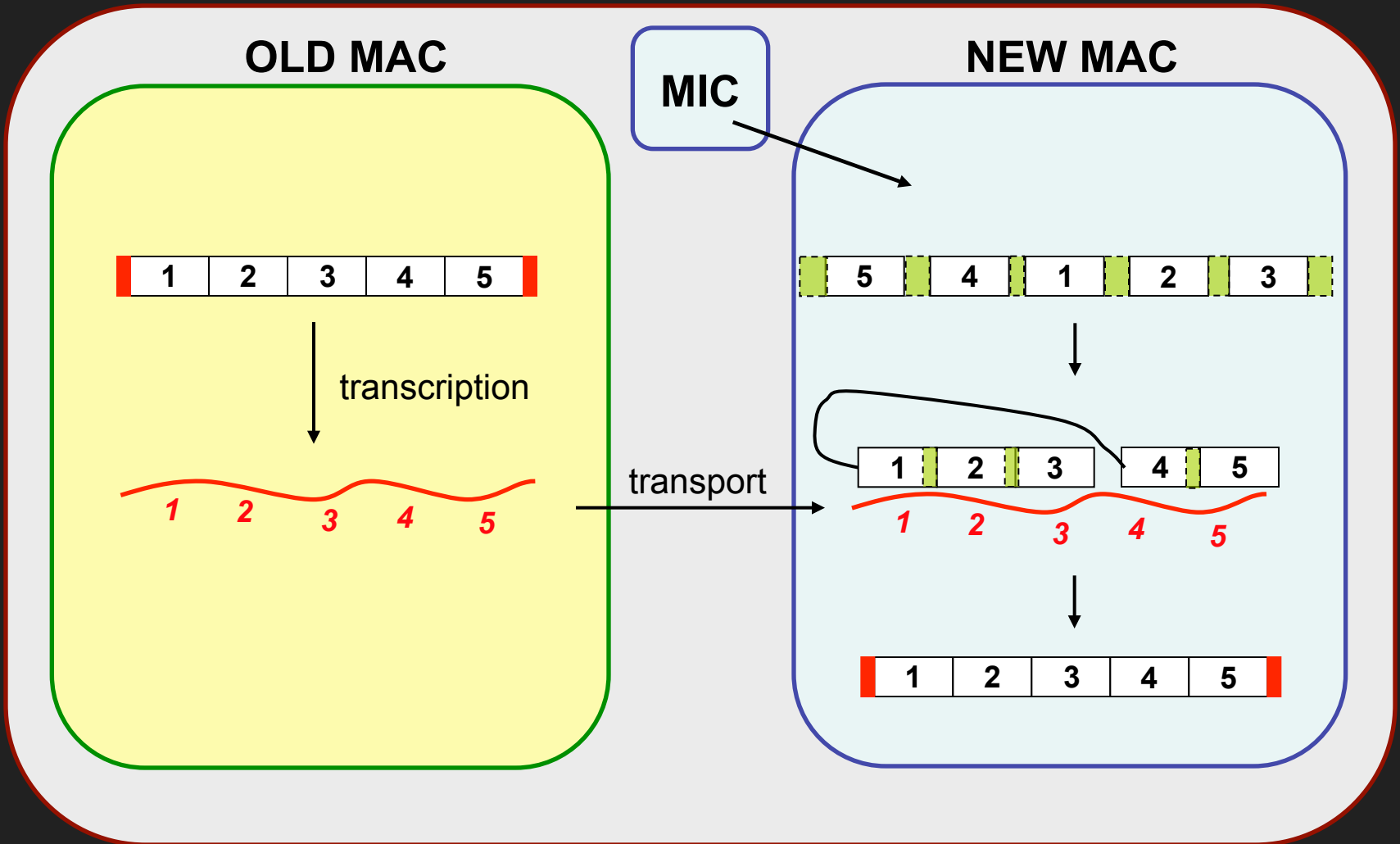
The scrambled Actin I gene in Stylonychia lemnae:
a simplest case to examine the timecourse of rearrangement





[Especially error prone...!]

Template Model





www.rasiel.com

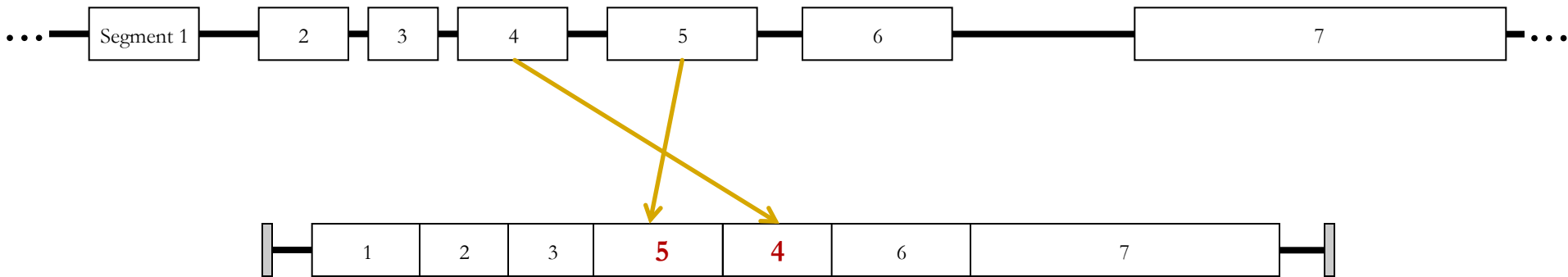
Sunday Afternoon on the Island of Grand Jatte, 1884-6 - Seurat

8

3

Switching adjacent DNA segments: scrambling a conventional gene

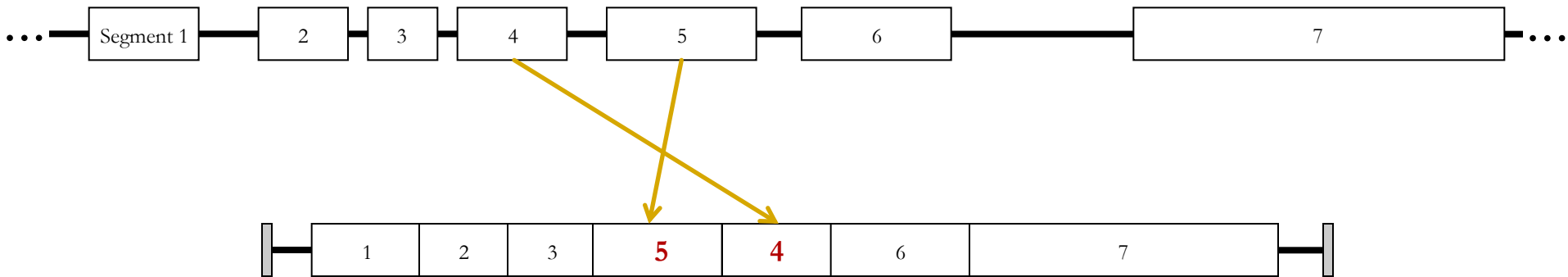
β -TBP precursor DNA



Rearranged β -TBP product DNA

Epigenetic re-programming of a DNA rearrangement pathway

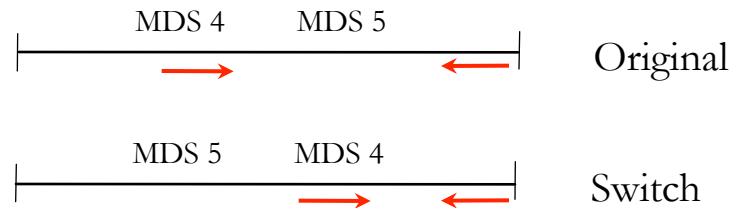
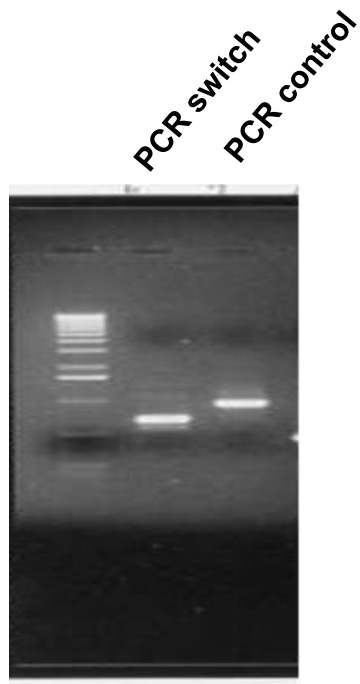
β -TBP precursor DNA



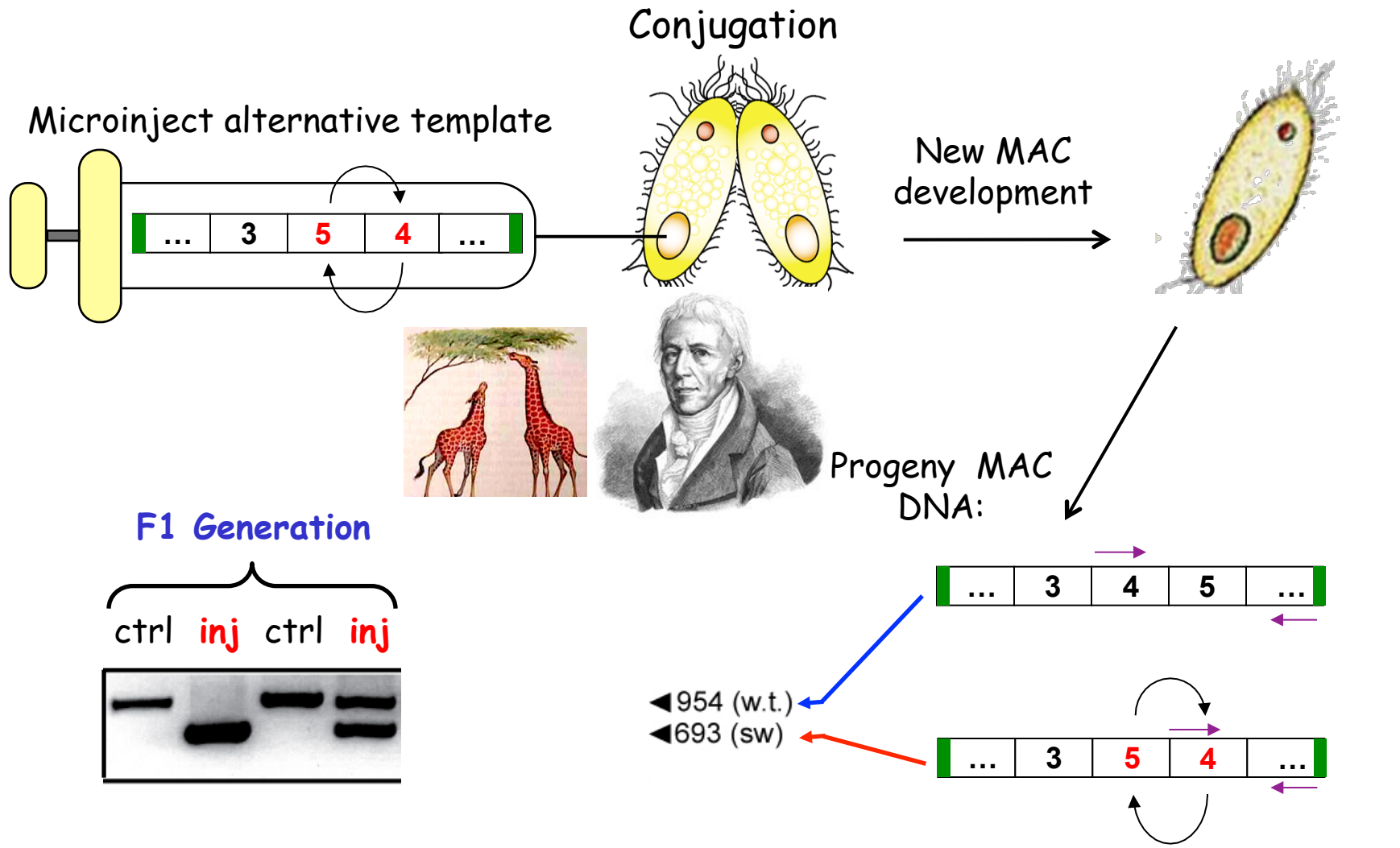
Rearranged β -TBP product DNA



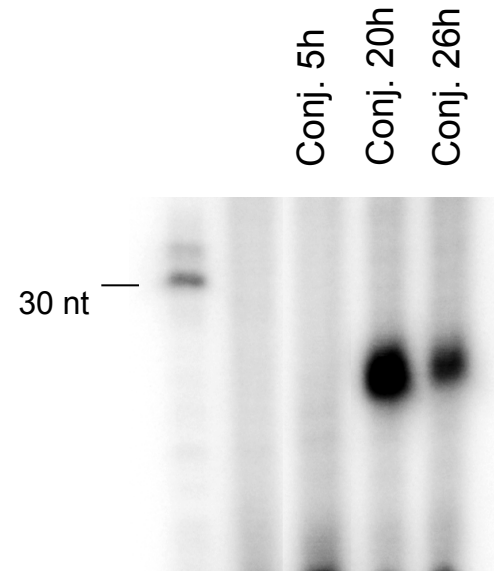
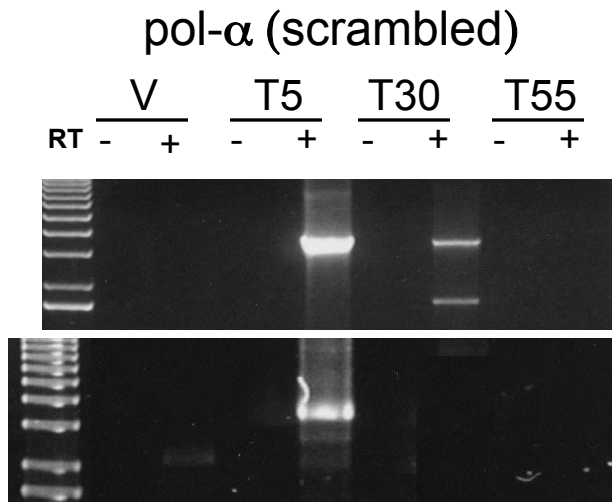
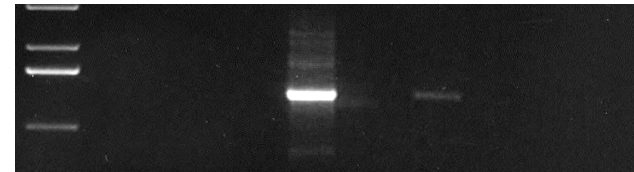
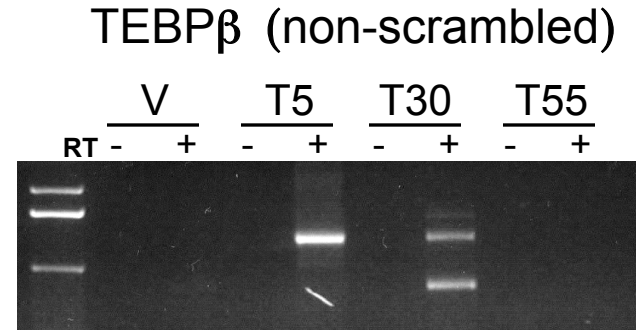
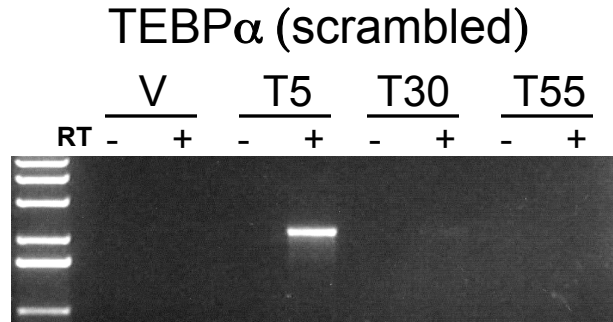
Rearrangement or original?



Microinjection of alternative DNA or RNA templates: *Inheritance of acquired somatic characters*



Telomere RT-PCR can detect putative Template RNAs

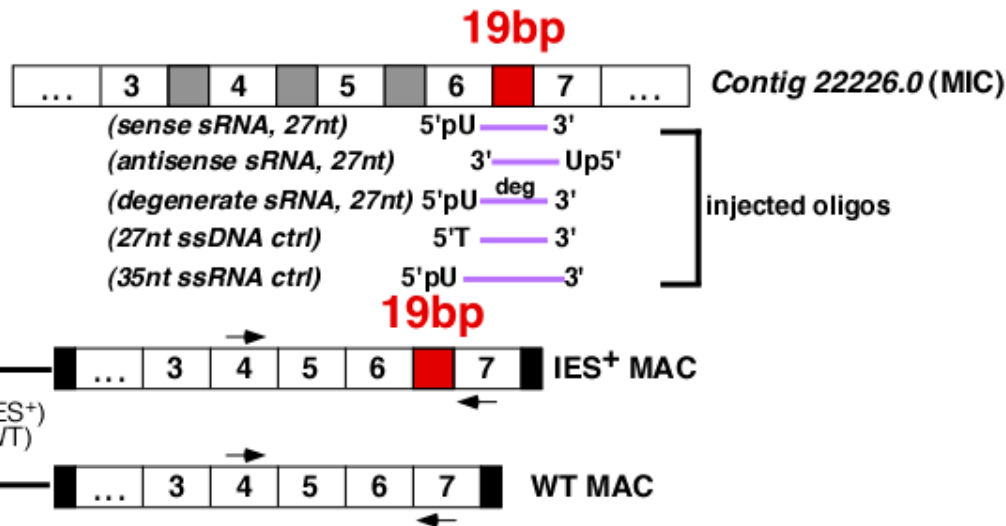
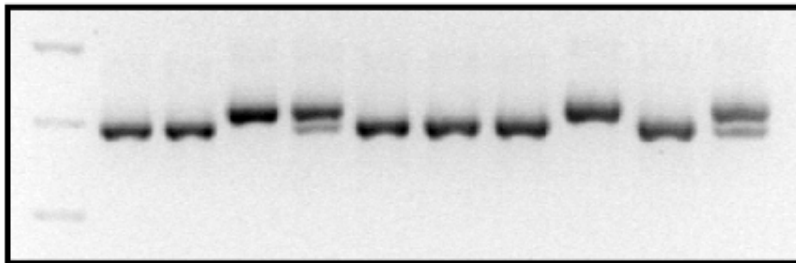


Oxytricha piRNAs
Fang *et al.*, *Cell*, 2012

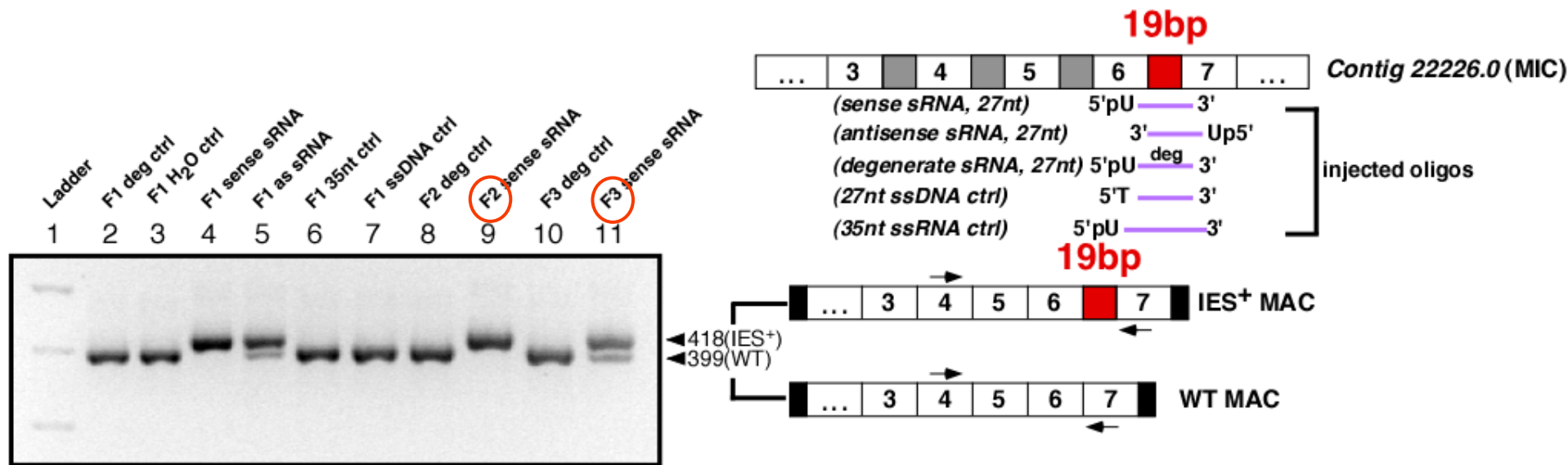
Injection of small RNAs leads to heritable DNA fusions lasting 3 generations

Ladder F1 deg ctrl F1 H₂O ctrl F1 sense sRNA F1 as sRNA F1 35nt ctrl F1 ssDNA ctrl F2 deg ctrl F2 sense sRNA F3 deg ctrl F3 sense sRNA

1 2 3 4 5 6 7 8 9 10 11

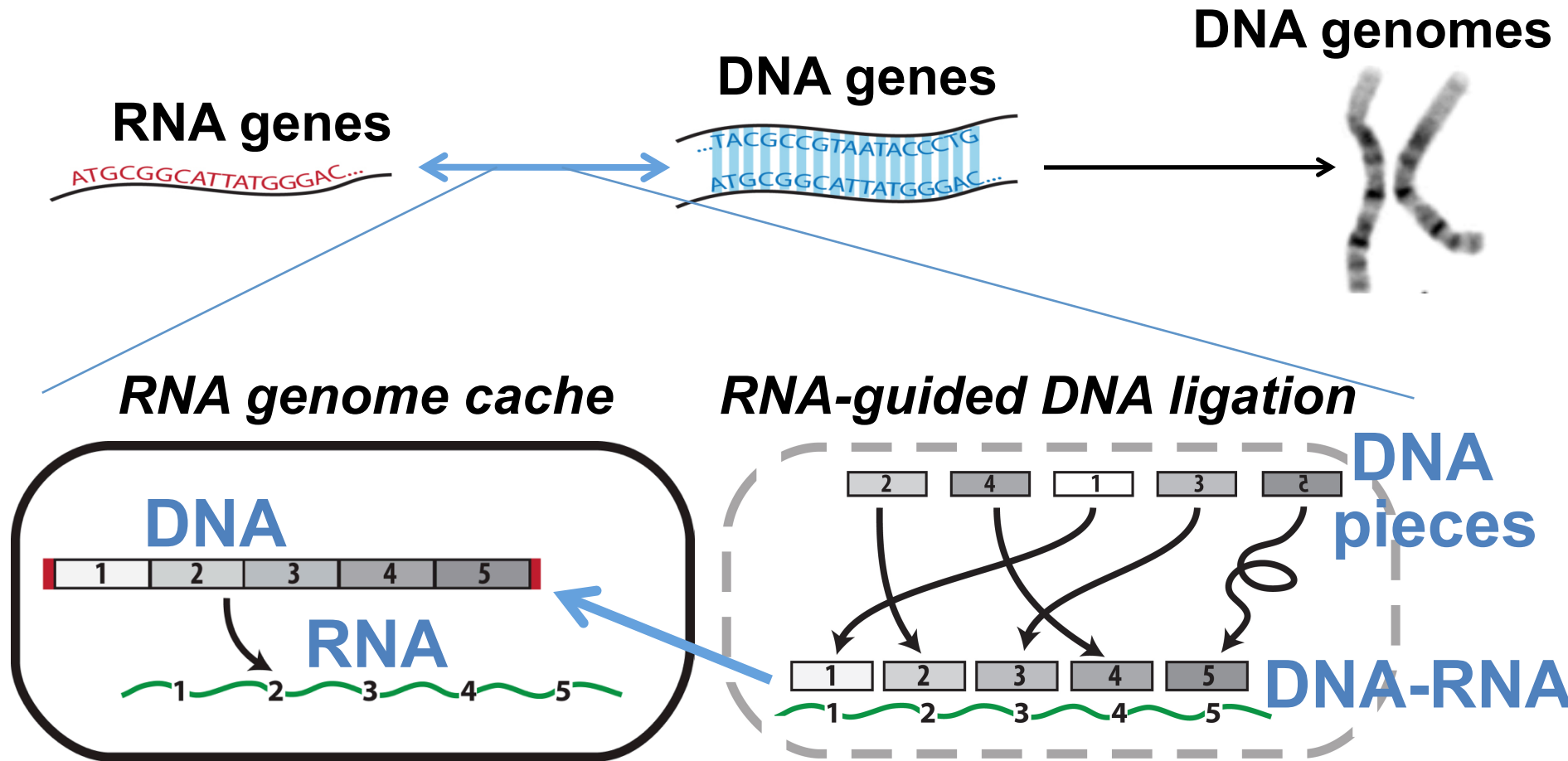


Injection of small RNAs leads to heritable DNA fusions lasting 3 generations



Small RNAs are powerful transgenerational carriers of epigenetic information!

Oxytricha versus early genomes



Conclusions for early genomes

- ❖ **RNA genes are subject to epigenetic regulation:**
 - **Epigenetics preceded genetics**
- ❖ **Complex life is possible with tiny chromosomes:**
short enough to be copied by inefficient polymerases
- ❖ **Ligases, much easier to arise than polymerases,**
could have copied genomes before polymerases



www.youtube.com/watch?v=5YHVP2xDbS0 (DNA bypass)

ありがとう



Mariusz Nowacki Wash U GC Chris Amemiya funding: NSF/NIH

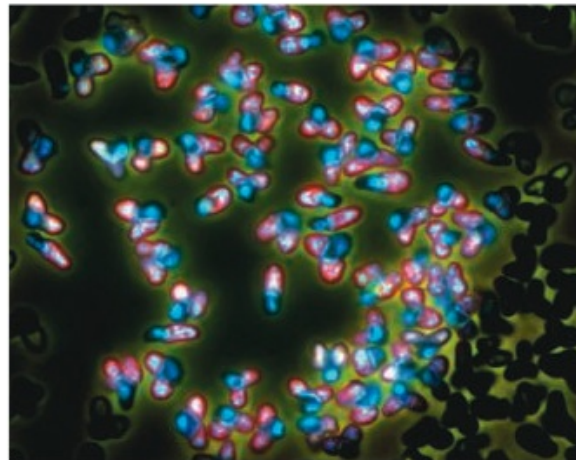
Why Genomes in Pieces?

Laura F. Landweber

Microbial eukaryotes take ample detours along the route from DNA to messenger RNA (mRNA) and protein. Some of their tricks continue to erode the notion of a gene beyond its natural subdivision into functional exons and noncoding introns (1). Two discontinuous genetic systems described in this issue further challenge this dogma. On page 415 of this issue, Marande and Burger report a fully scrambled mitochondrial genome in *Diplonema papillatum*, a free-living relative of disease-causing trypanosomes (2), and on page 450, Soma *et al.* describe a set of scrambled transfer RNA (tRNA) genes in the nuclear genome of the red alga *Cyanidioschyzon merolae* (3). The findings are reminders that a genome sequence can be a far cry from knowledge of gene products.

Marande and Burger explode the notion of a gene with mRNA building blocks present as “modules” of ~165 base pairs, each on a sepa-

rate chromosome in the mitochondria of the protist *D. papillatum*. Construction of a complete mRNA requires joining up to nine modules through a mechanism that appears distinct from known forms of RNA splicing, the processes that join exons in eukaryotic mRNA. Although split genes occur in other systems (including *Chlamydomonas*, *Euglena*, Alveolata, plants, and Diptera), rarely are the



Backwards. tRNAs in *C. merolae* assemble from scrambled genes. Red plastids autofluoresce; blue color is DAPI stain (14).

Some microorganisms are evolutionary puzzles in that their genomes contain encrypted genes that are descrambled into gene products.

scrambled pieces “sewn” back together to create a contiguous gene or RNA. Some exceptions are gene unscrambling in ciliates (4) and the *bursicon* gene in mosquitoes (5). Trans-splicing of RNA (6) and even proteins (7) can also merge functional regions located on dispersed elements of prokaryotic (7) or eukaryotic genomes.

The pathway for gene assembly in diplone-mid mitochondria may provide a clue to the origin of U-insertional RNA editing, which makes a modest appearance in the report by Marande and Burger as six non-DNA-encoded uracil (U) residues that join two RNA modules. Perhaps the ancestral role of guide RNAs that direct U insertion and deletion in the related kinetoplastid protozoa was to provide a template scaffold to link modules. Such small antisense RNAs may later have gained a role in RNA editing, possibly under selective pressure to repair a region or restore a reading frame after loss or erosion of a module.

Soma *et al.* describe a new layer of tRNA processing in the red alga *C. merolae*: circularly permuted tRNAs, with the coding region

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Why not? – (almost) neutral

Novelty – combinatorial products

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Molecular fossils – primitive

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Atavism – reverting to primitive form

Why not? – (almost) neutral

Novelty – combinatorial products

Molecular fossils – primitive

Atavism – reverting to primitive form

Reductive evolution – consequence of

Gene scrambling solutions to ecological challenges are neither neat nor elegant, but nevertheless seem to be favored by selection processes.

DAVE SPEIJER

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References

1. W. Marande, G. Burger, *Science* **318**, 415 (2007).
2. A. Soma *et al.*, *Science* **318**, 450 (2007).
3. D. Speijer, *IUBMB Life* **58**, 91 (2006).
4. D. Speijer, in *RNA Editing*, U. Goring, Ed. (Springer Verlag, Berlin, 2007), pp. 199–227.
5. T. Cavalier-Smith, *Trends Genet.* **13**, 6 (1997).
6. L. F. Landweber, W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 918 (1994).

Making Sense of Scrambled Genomes

IN HER INTERESTING PERSPECTIVE, APTLY called “Why genomes in pieces?” (19 October 2007, p. 405), L. F. Landweber discusses possible explanations for the existence and maintenance of evolutionary puzzling modes of gene expression, as exemplified by the fully

Response

SPEIJER OFFERS, TO MY KNOWLEDGE, THE FIRST plausible explanation for why possession of a chaotically organized genome, a hallmark of some microbial eukaryotes, may actually confer increased evolutionary fitness. He proposes a model in the context of extensive RNA

Why not? – (almost) neutral

Novelty – combinatorial products

Molecular fossils – primitive

Atavism – reverting to primitive form

Reductive evolution – consequence of

Protection against DNA loss

- MIC genome generally unexpressed
- MAC genes unlinked and prone to loss
- The only way to regenerate the MAC genome is to recoup all the pieces from the germline.
- Consistent with maximal fragmentation of essential genes, such as rDNA

Why not? – (almost) neutral

Novelty – combinatorial products

Molecular fossils – primitive

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Reductive evolution – consequence of

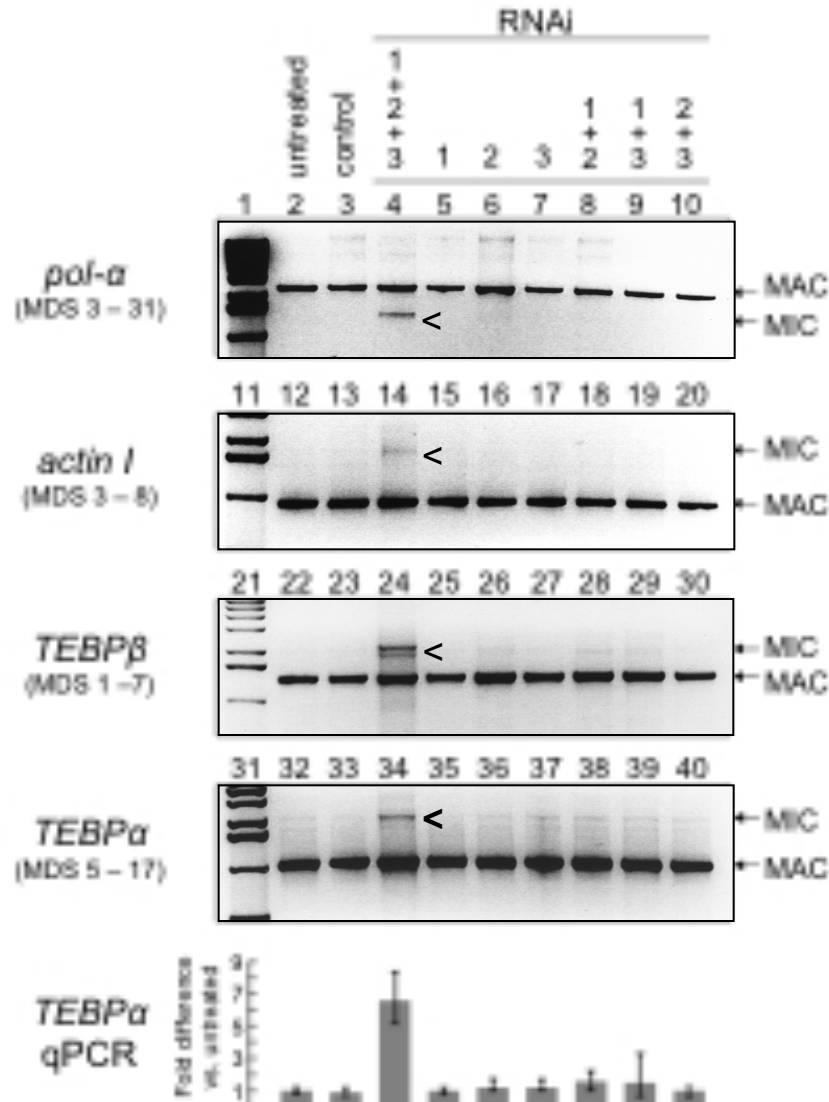
Protection against DNA loss

Rapid adaptation – somatic fixation?

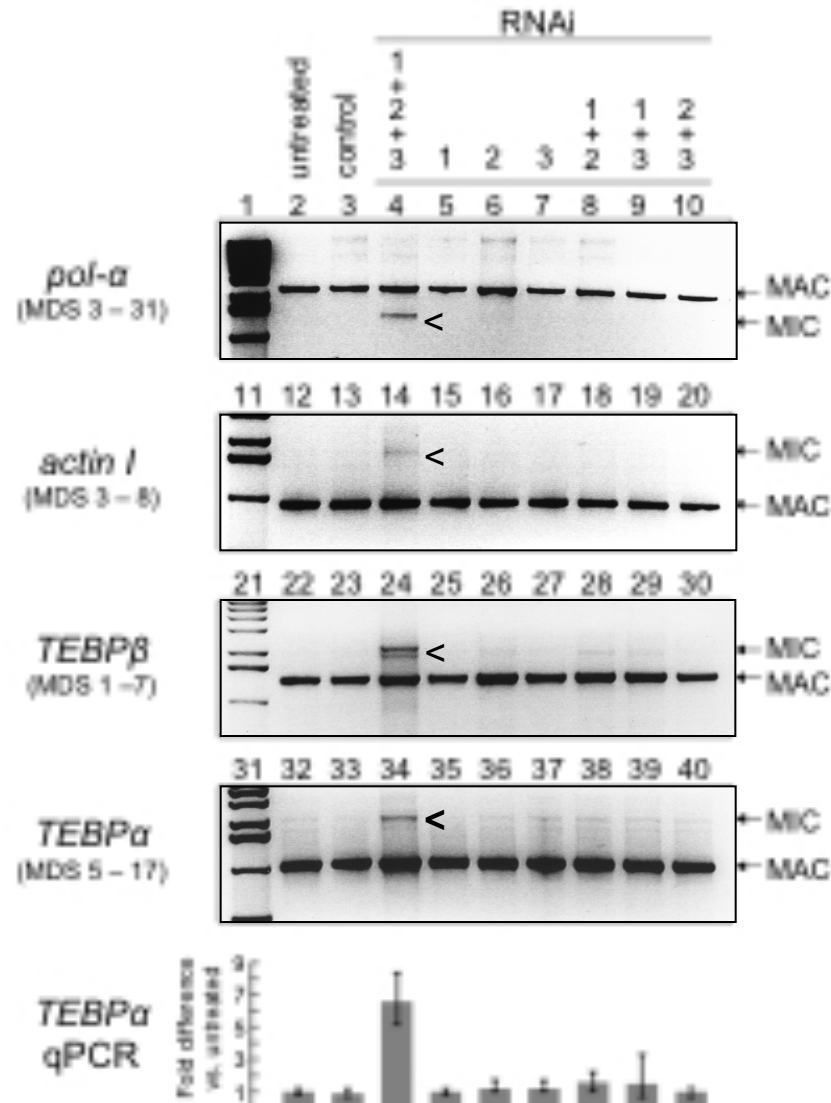
What protein machinery is involved in mediating rearrangements ?



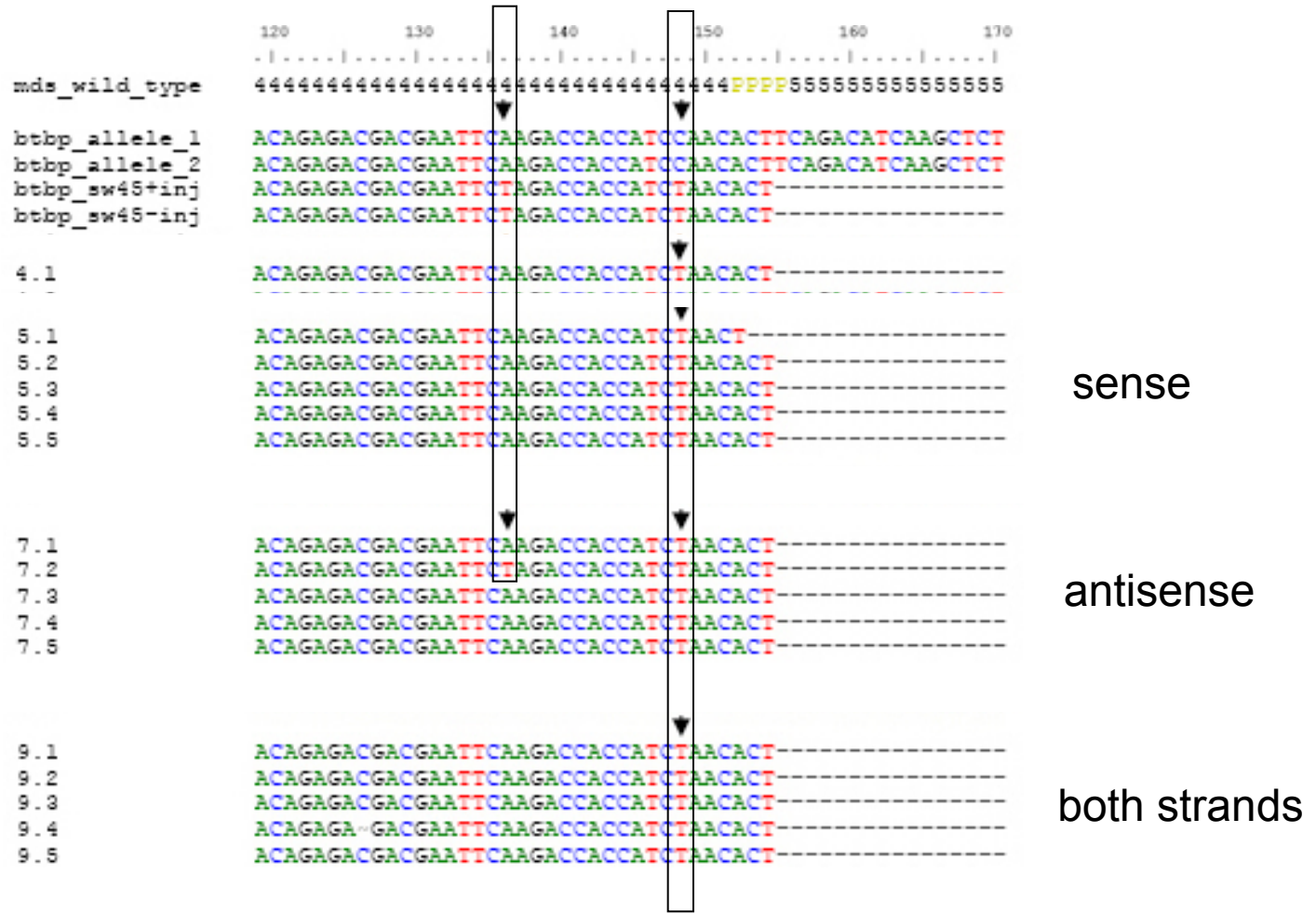
RNAi knockdown of TBE 1, 2 & 3 transposase reduces genome-wide rearrangements



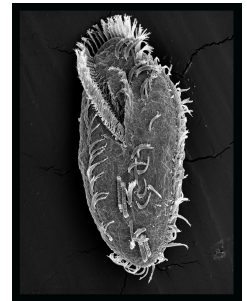
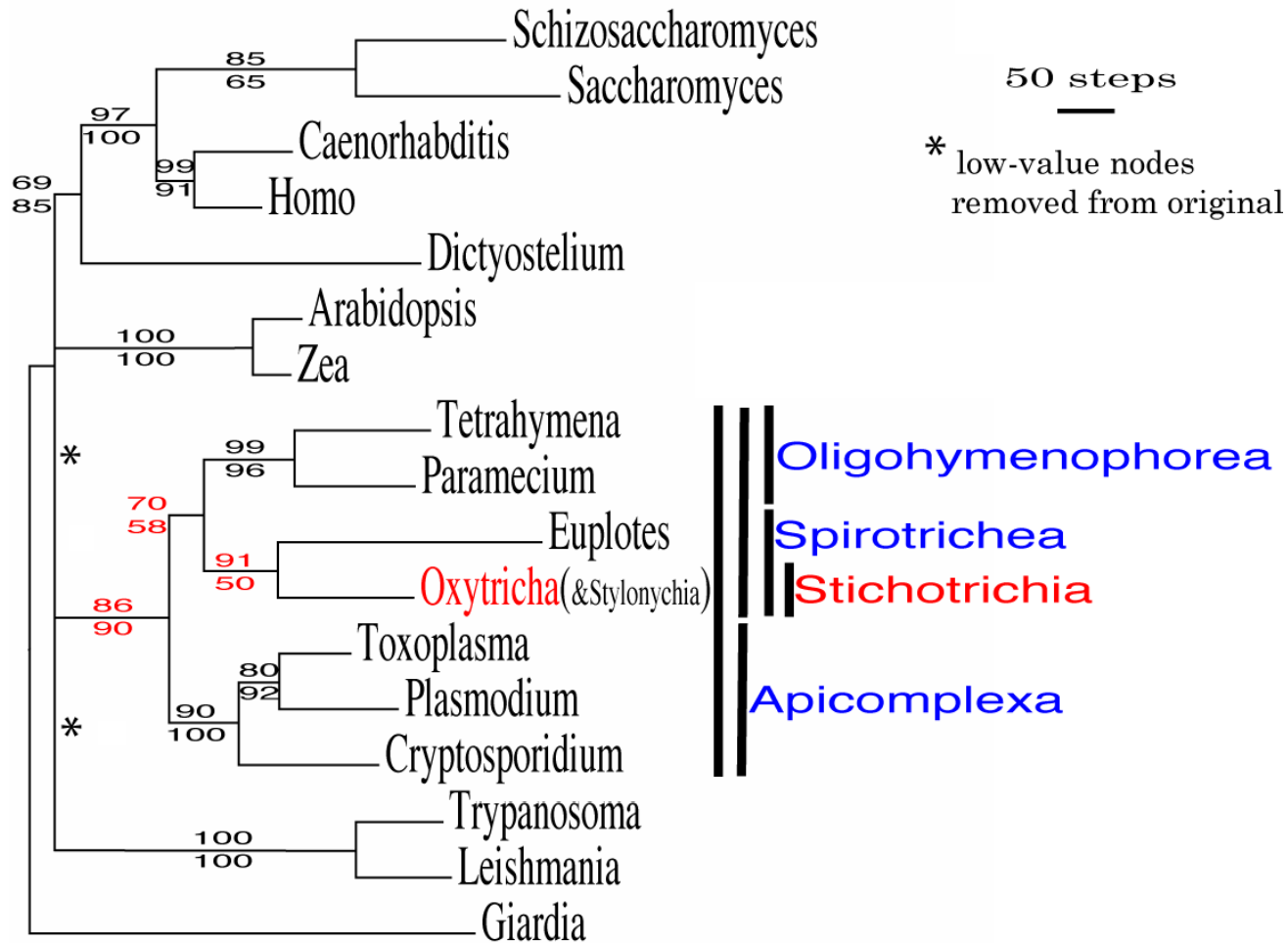
“Genome-wide” RNAi knockdown of TBEs implicates a role for the “dispensable genome”



Transfer of artificial nucleotide substitutions from RNA templates to F1 progeny DNA



RNA-directed DNA proofreading



Genome Rearrangements in Cancer

