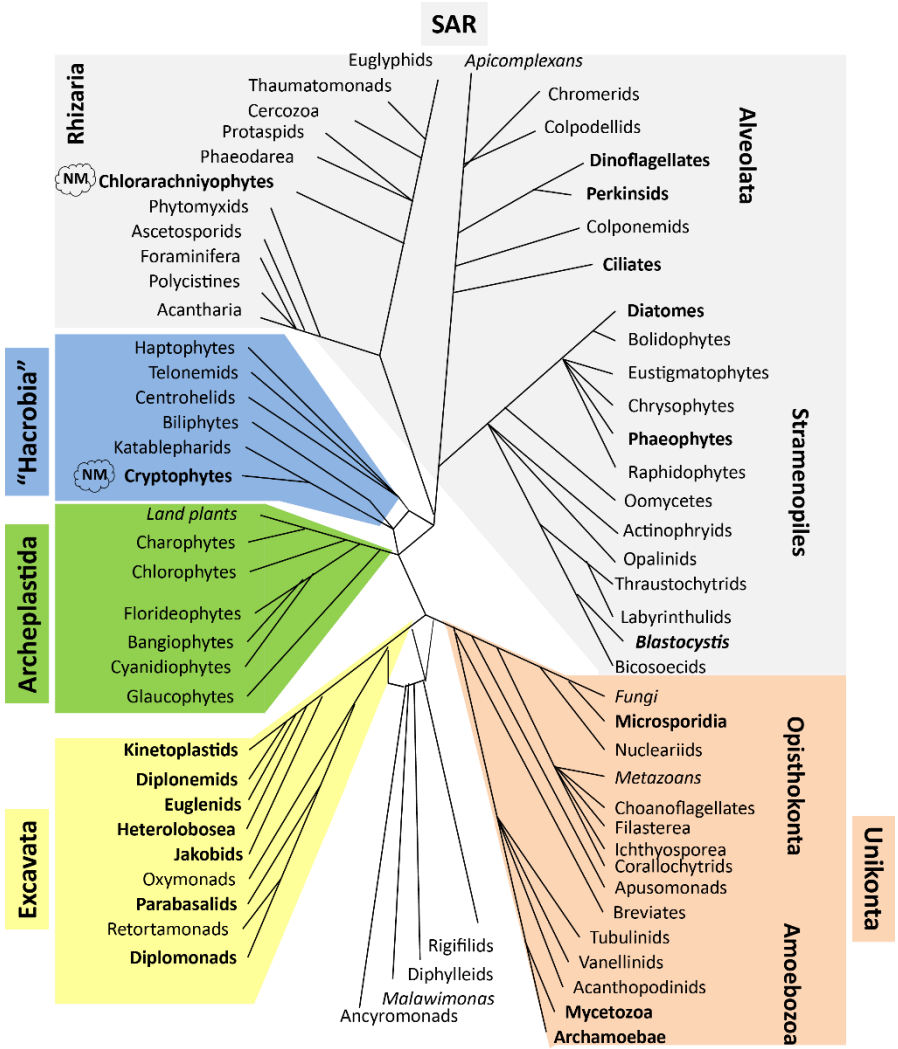
# THE PHYSICAL GENOME ACRROSS EVOLUTION

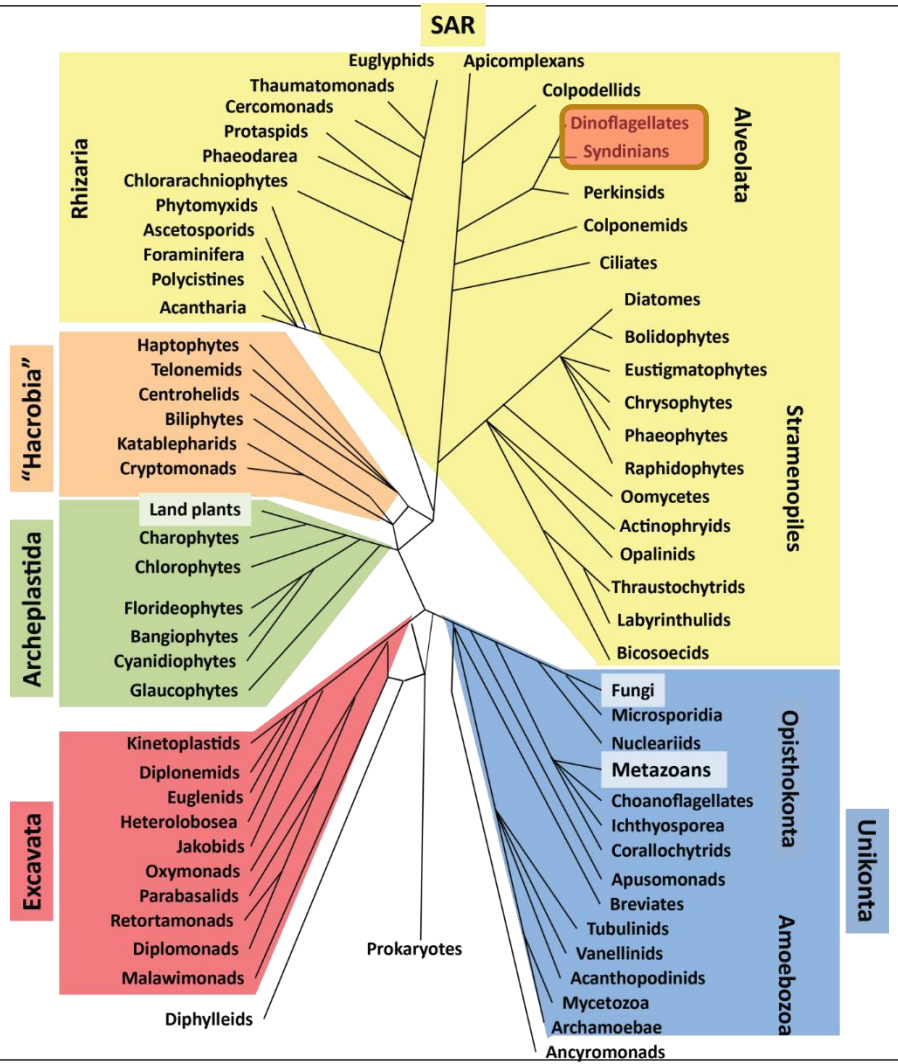
# EUKARYOTE TREE



# GENERAL QUESTIONS:

- What are the deepest principles of chromatin organization and gene expression? Studying its extremes can make apparent previously obscured features.
- Such deviations from the norm are known, but have generally not been studied at all with modern tools
- How did the regulatory apparatus and mechanisms evolve across the eukaryotic tree of life
- How many times did distal enhancers originate? Why and how?
- What are the conserved and derived chromatin states across different lineages?
- What is the relationship between genome organization and organismal complexity?
- What does all of that tells us about mammalian genomes?
- Finally, some things are just too cool on their own to not be studied.

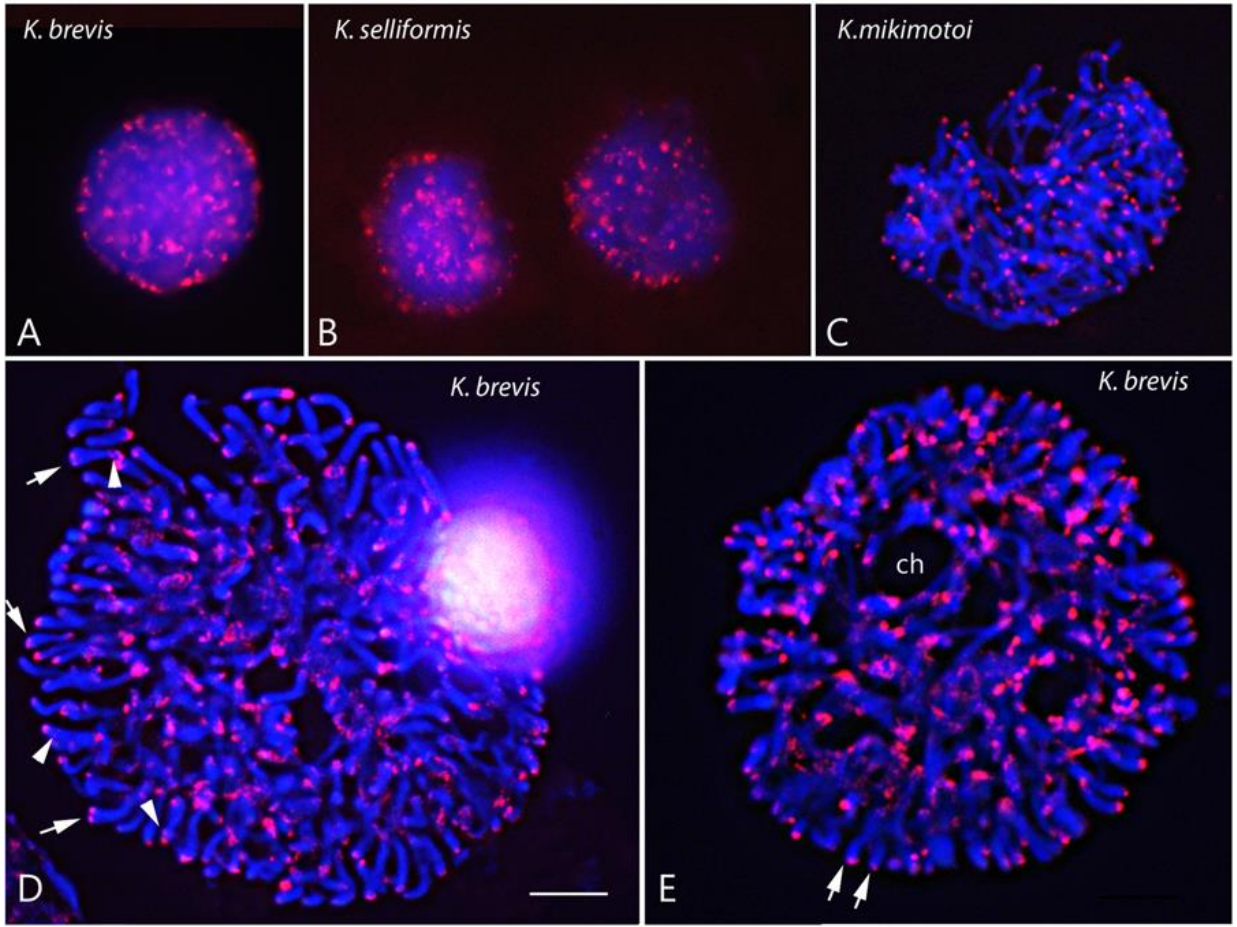
# DINOFLAGELLATES



# DINOFLAGELLATES AND THEIR SPECIAL FEATURES

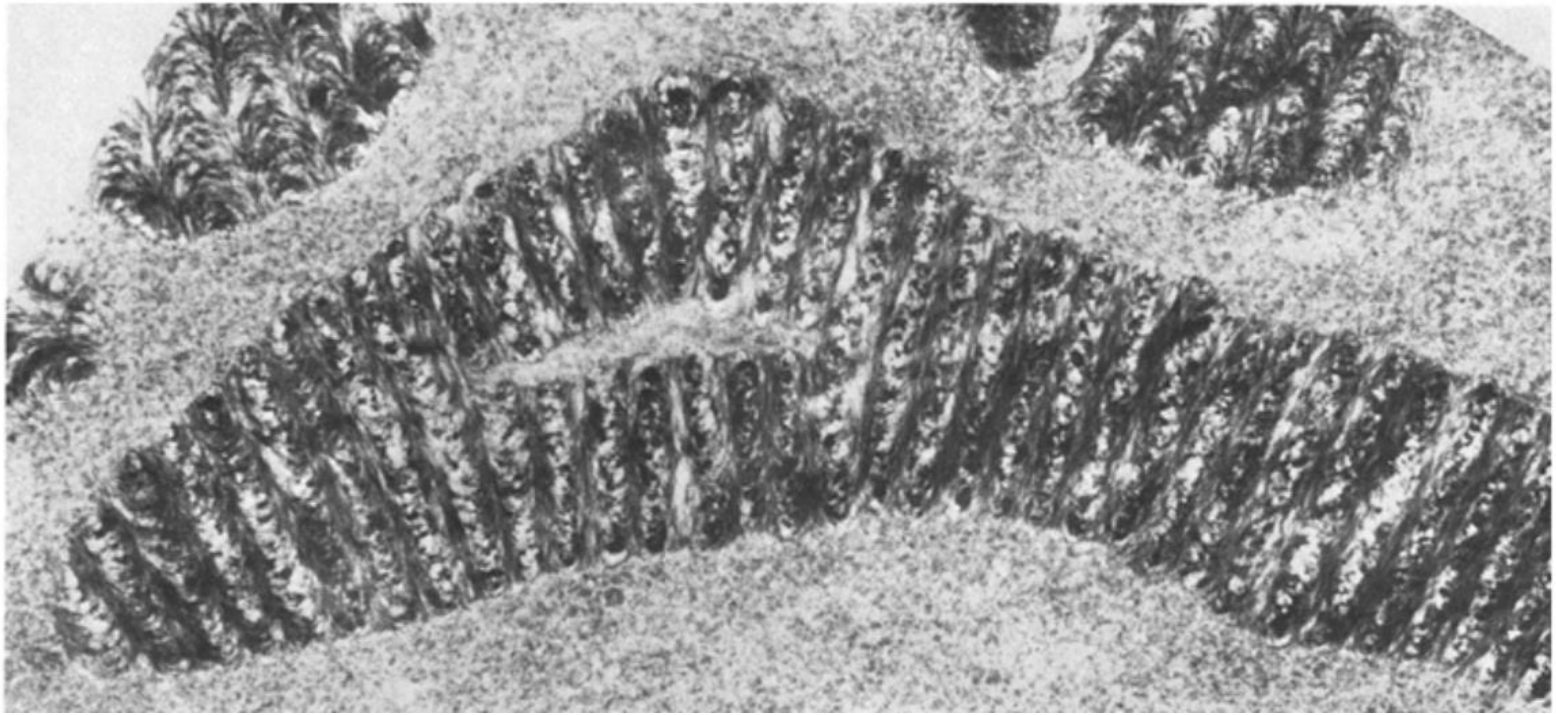
- Permanently condensed fibrilar chromosomes
- Very low protein-to-DNA ratio (~1/10$^{th}$ of the usual)
- Histones are in low abundance, long thought to be completely absent
- High percentage of 5-hydroxymethyluracile (up to 40%)
- Huge genomes, often with tandem arrays of the same gene
- Extremely intron rich (19 introns per gene on average); unique splice sites
- Few transcription factors
- Gene regulation is hypothesized to happen either at the posttrascriptional level or at the level of the control of chromatin looping

# DINOFLAGELLATE CHROMOSOMES




- Note: these are interphase chromosomes!

Cuadrado et al. 2019



Livoland & Bouligand, 1978

# GENERAL DINOFLAGELLATE QUESTIONS:

- How is chromatin organized in 3D space in permanently condensed chromosomes mostly without histones?
- How is transcriptional regulation (if it exists) accomplished in such an environment?
- How has the transcriptional machinery adapted to transcribing through DVNPs
- What is the role of the very divergent histones?
- What is the role of dhmU?
- and many others

# SYMBIODINIUM HI-C

# CORAL SYMBIOSIS

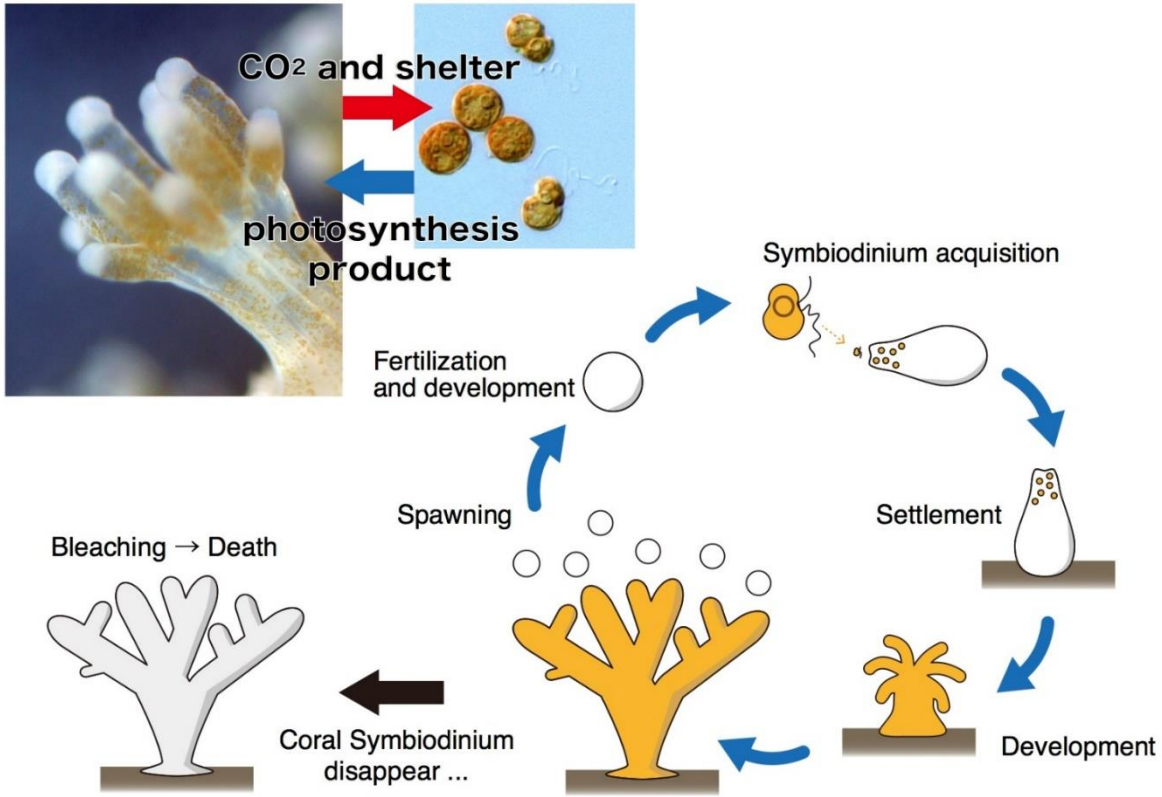
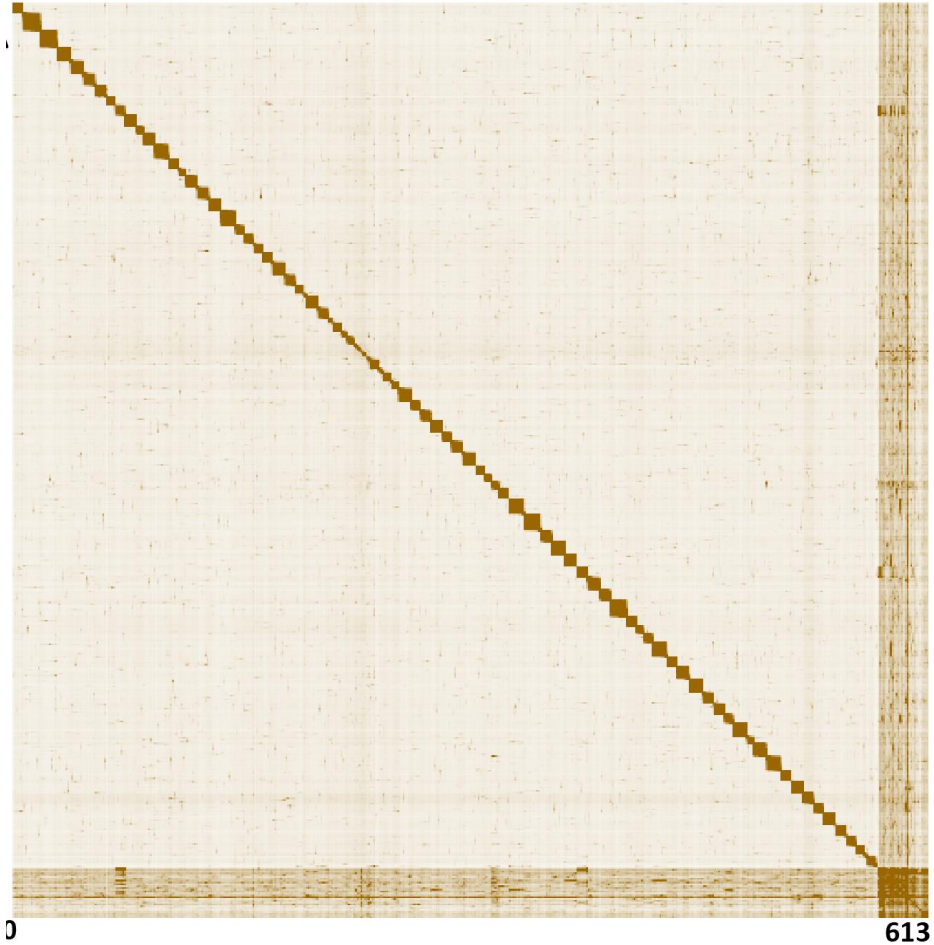


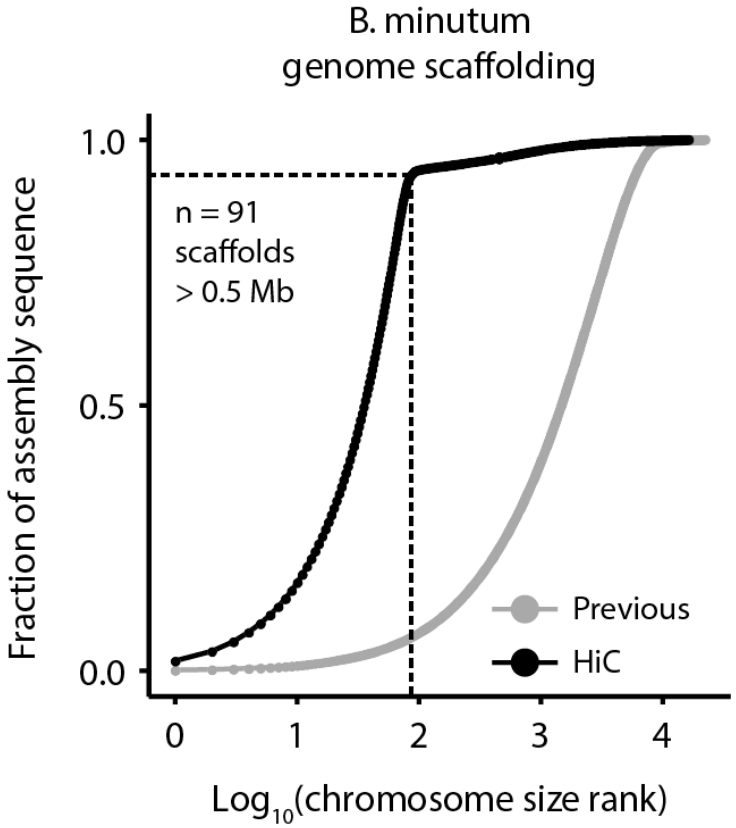

Figure 2. A symbiotic relationship between corals and Symbiodinium

# CORAL BLEACHING

# HI-C SCAFFOLDING

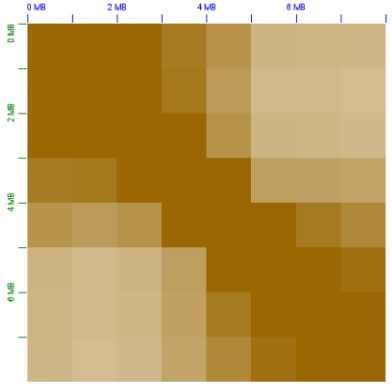


# HI-C SCAFFOLDING

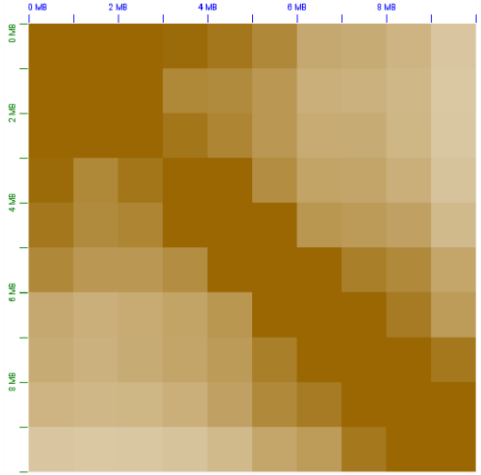


# BROAD STRUCTURE:

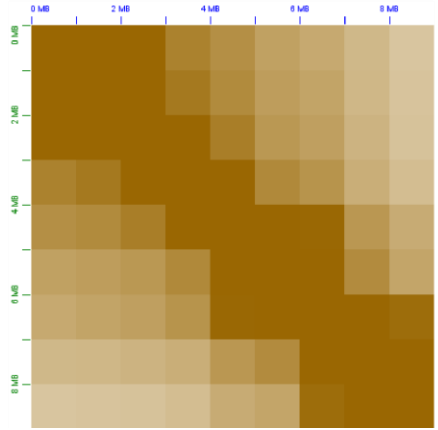
pseudochromosome 10



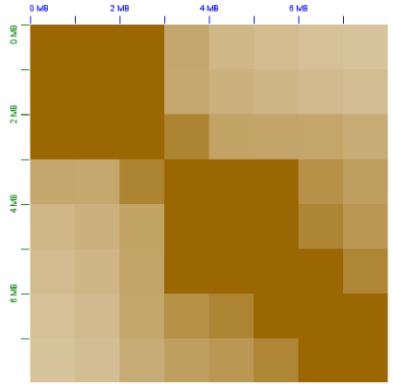

pseudochromosome 4



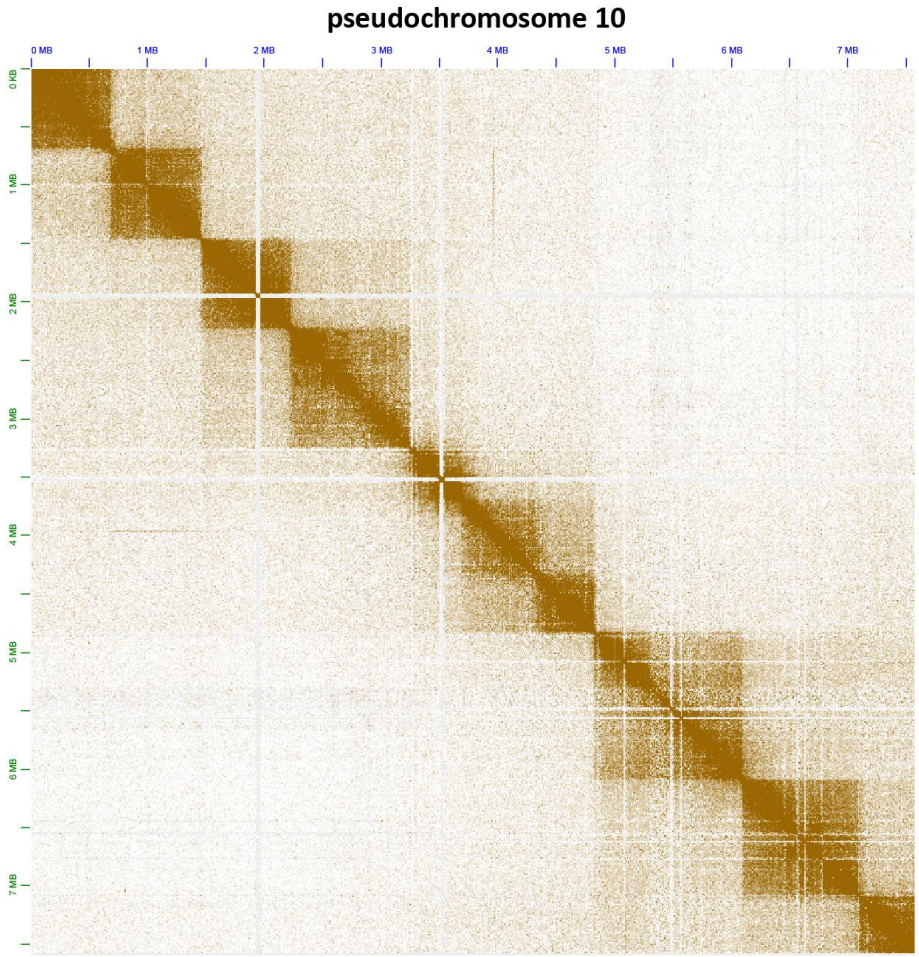

pseudochromosome 5

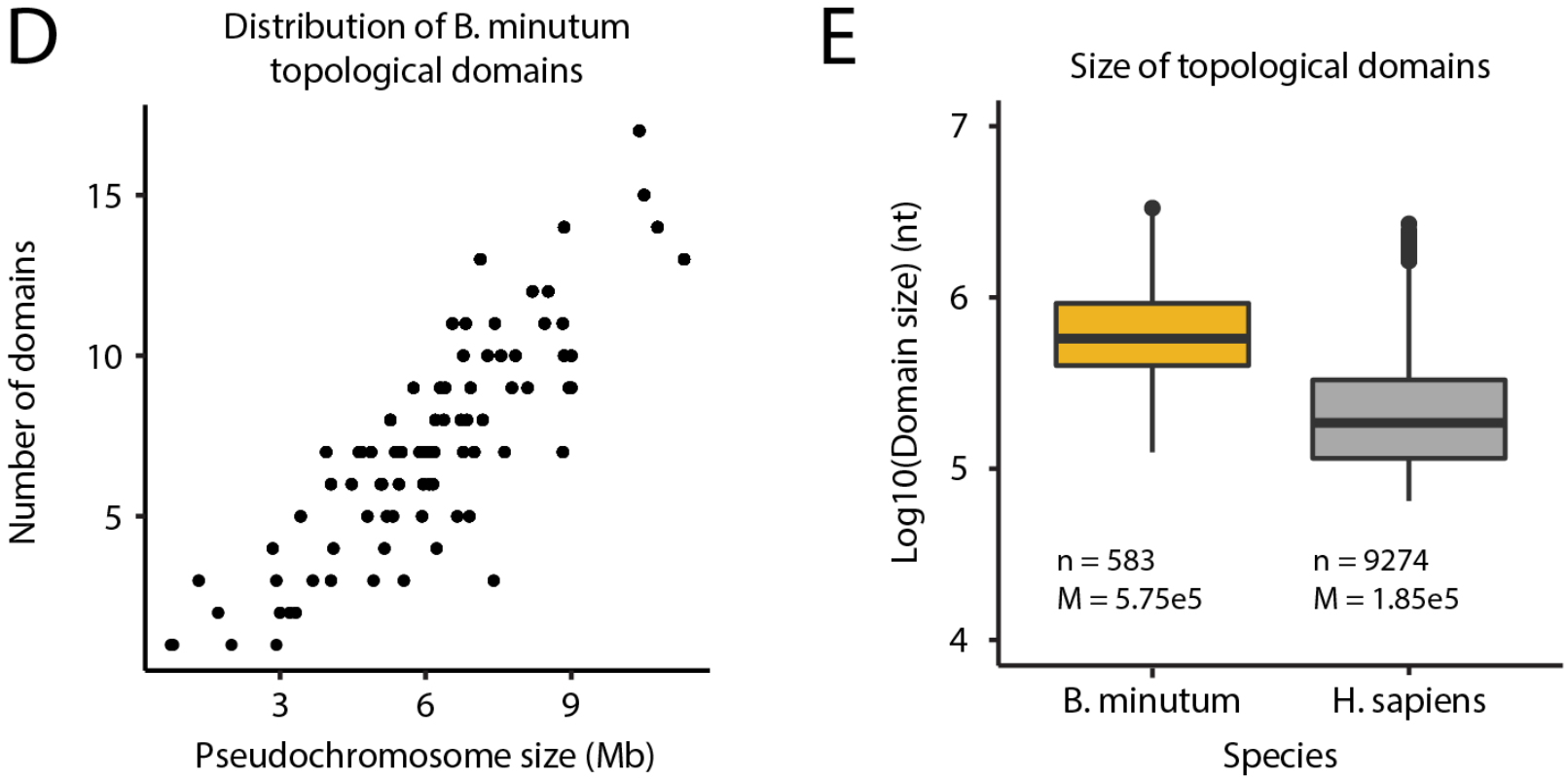



pseudochromosome 6



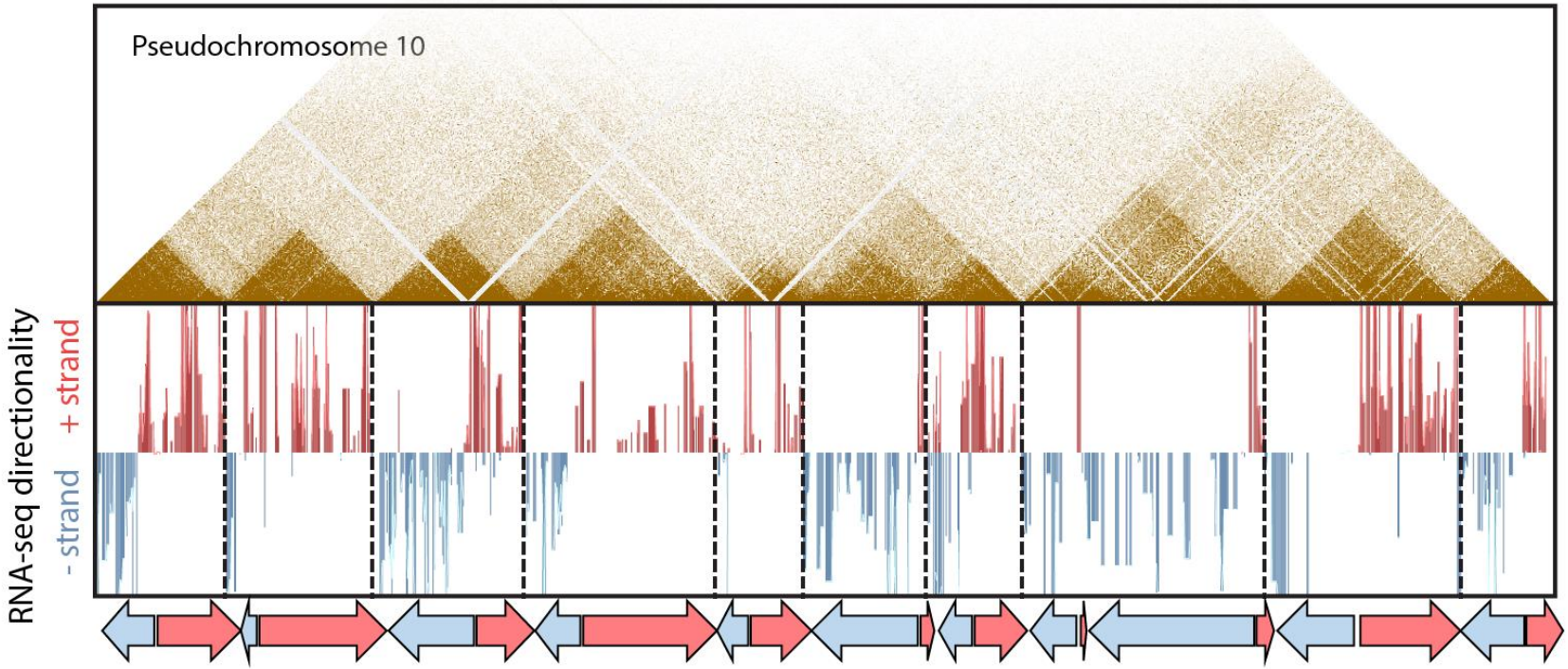
# DOMAIN STRUCTURE:




D
Distribution of B. minutum topological domains
Number of domains
15
10
5
3
6
9
Pseudochromosome size (Mb)
E
Size of topological domains
Log10(Domain size) (nt)
7
6
5
4
n = 583
M = 5.75e5
n = 9274
M = 1.85e5
B. minutum
H. sapiens
Species

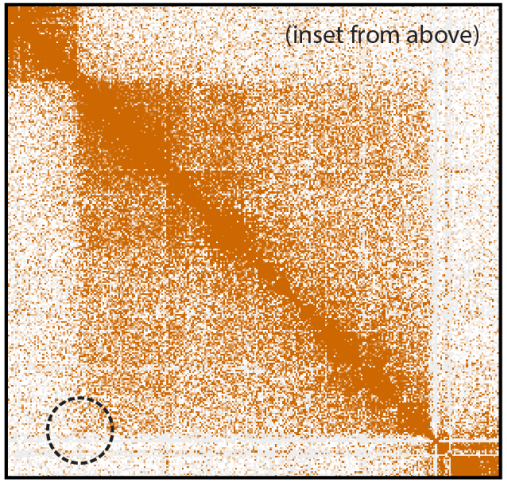
F

Gene directionality and topological domains

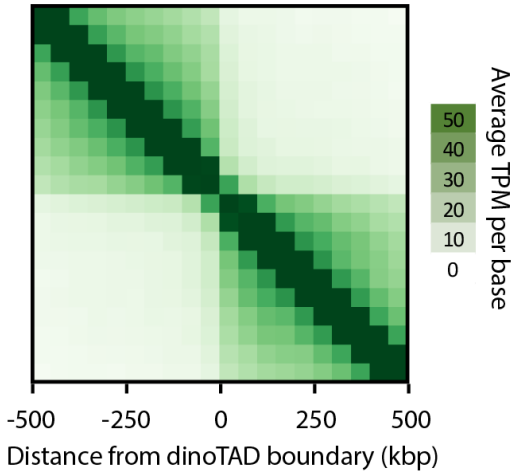


# DOMAIN BOUNDARIES AND ABSENCE OF LOOPS

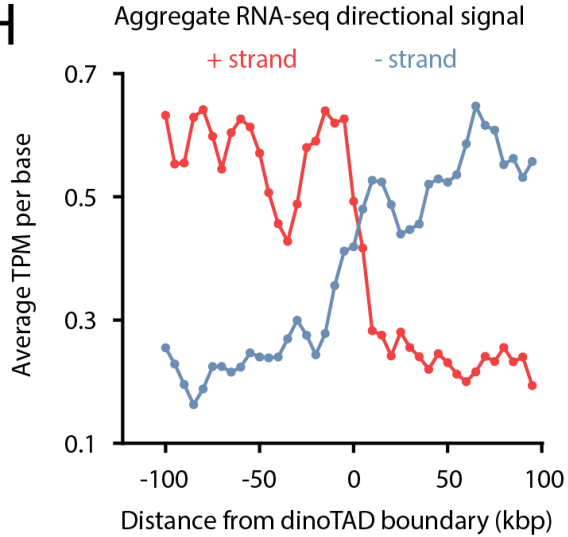
C




G

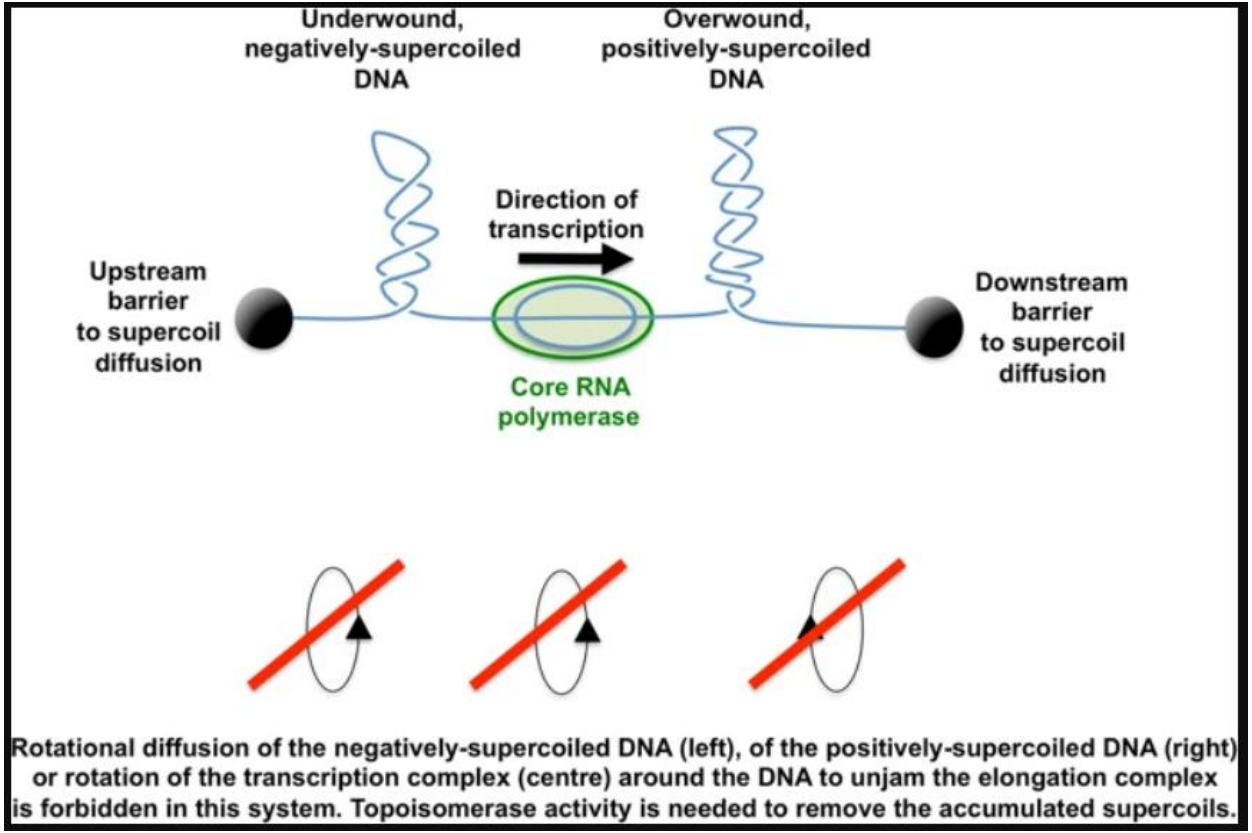



H



# WHAT IS THE MECHANISM?

- Transcription-induced supercoiling is one possibility

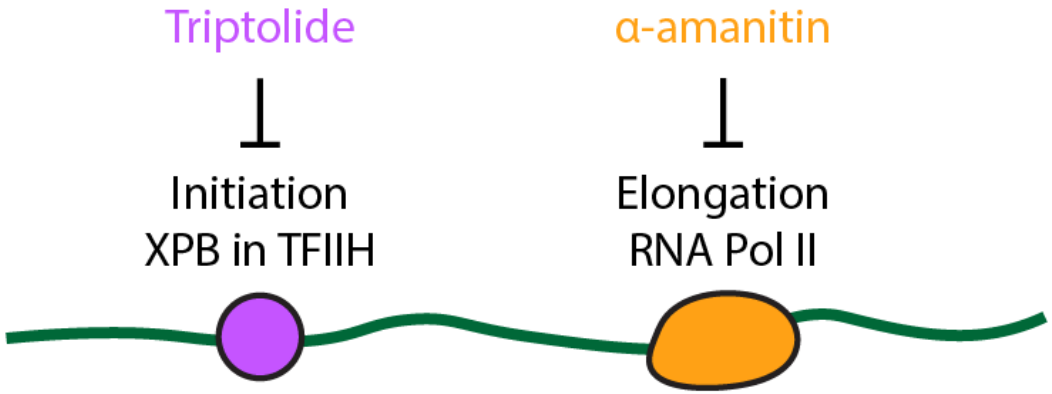



Rotational diffusion of the negatively-supercoiled DNA (left), of the positively-supercoiled DNA (right) or rotation of the transcription complex (centre) around the DNA to unjam the elongation complex is forbidden in this system. Topoisomerase activity is needed to remove the accumulated supercoils.

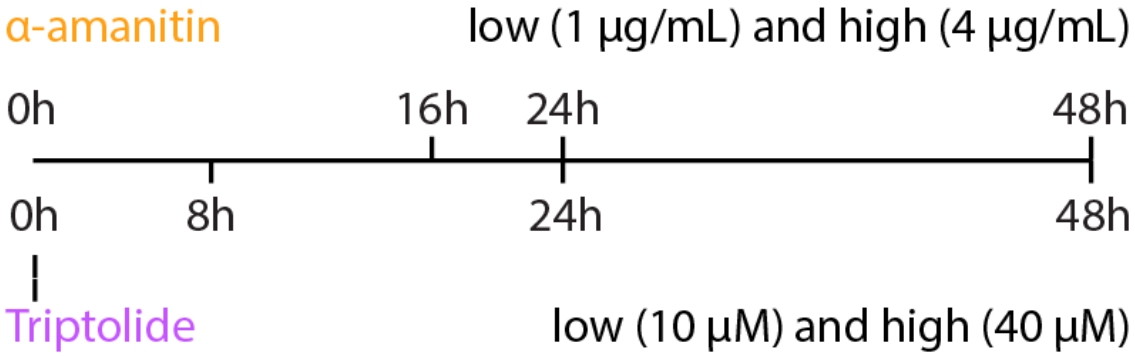
# PREDICTION:

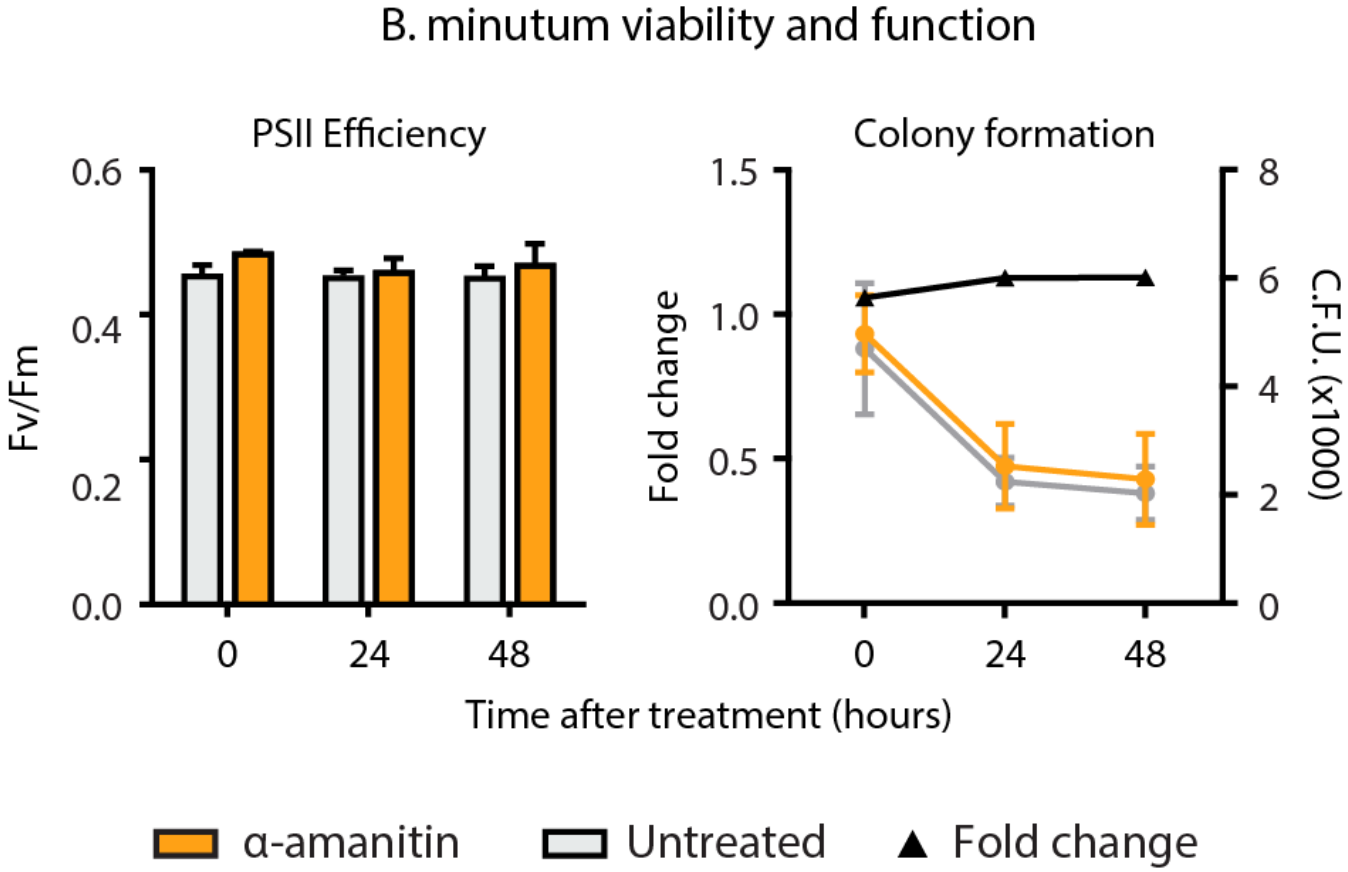
- If transcription is blocked, domains should disappear

## Transcriptional inhibitors

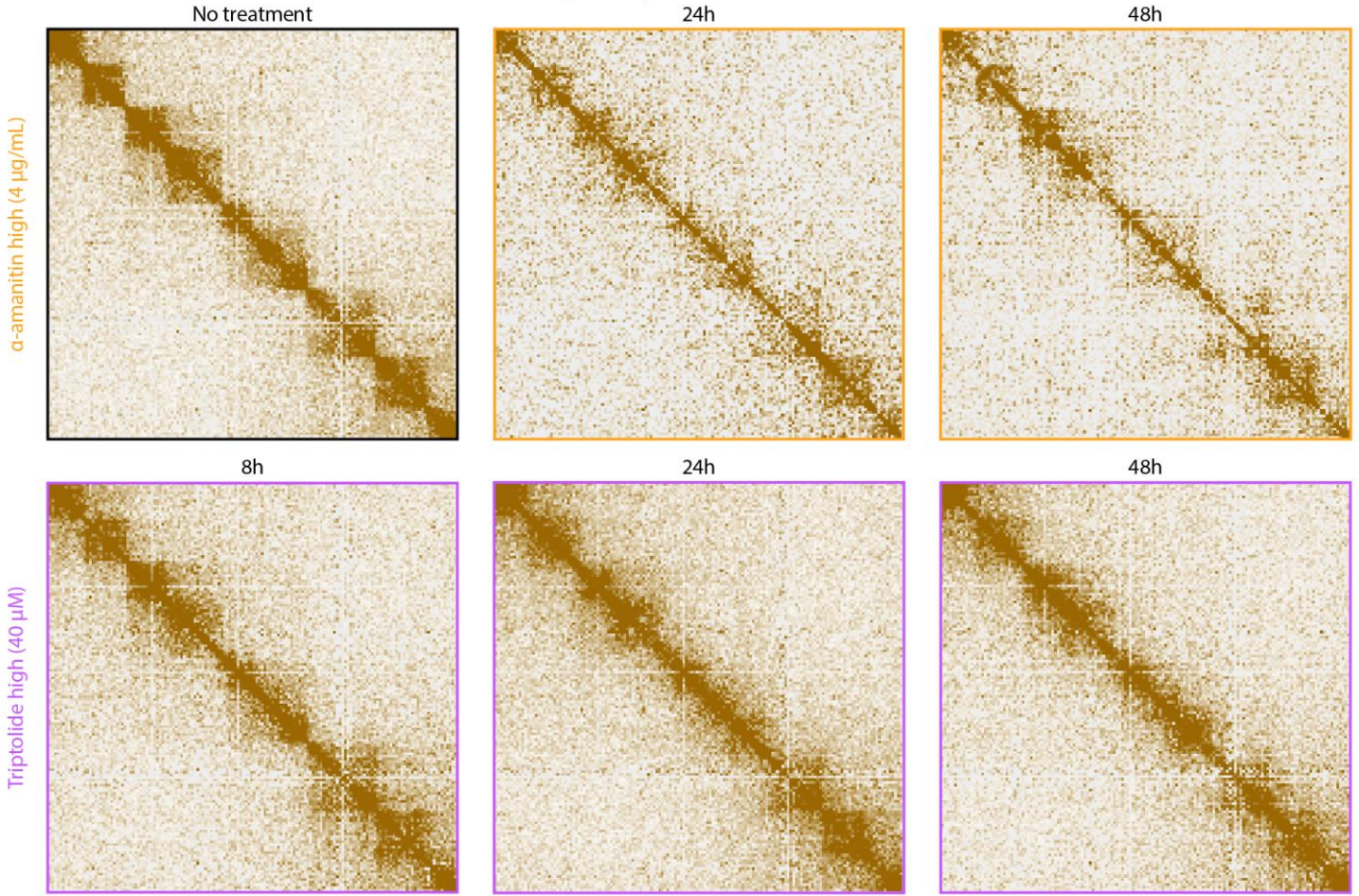


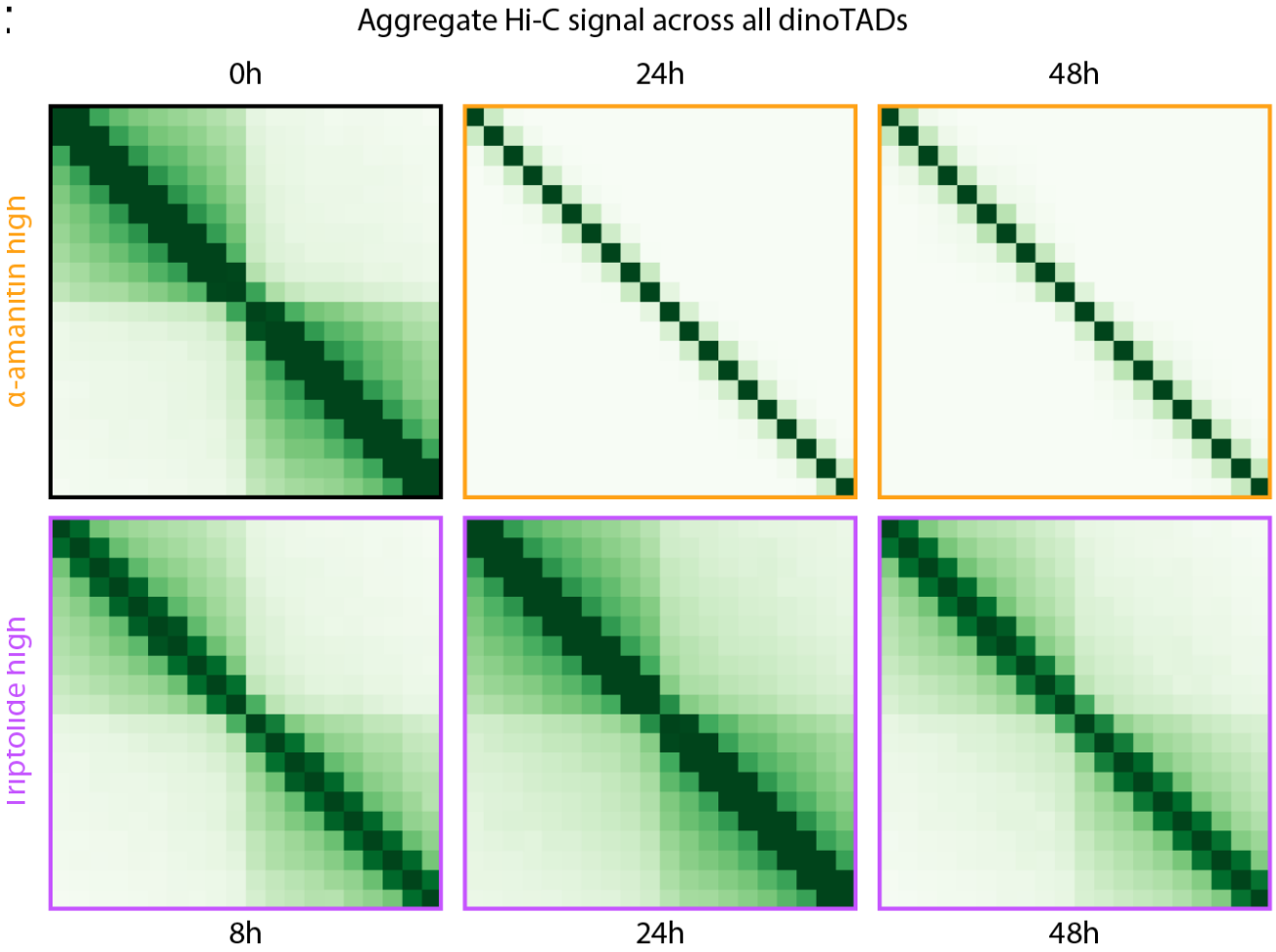

## Experimental overview




B. minutum viability and function
PSII Efficiency
Fv/Fm
0.6
0.4
0.2
0.0
0
24
48
Colony formation
Fold change
1.5
1.0
0.5
0.0
C.F.U. (x1000)
8
6
4
2
0
Time after treatment (hours)
α-amanitin
Untreated
Fold change

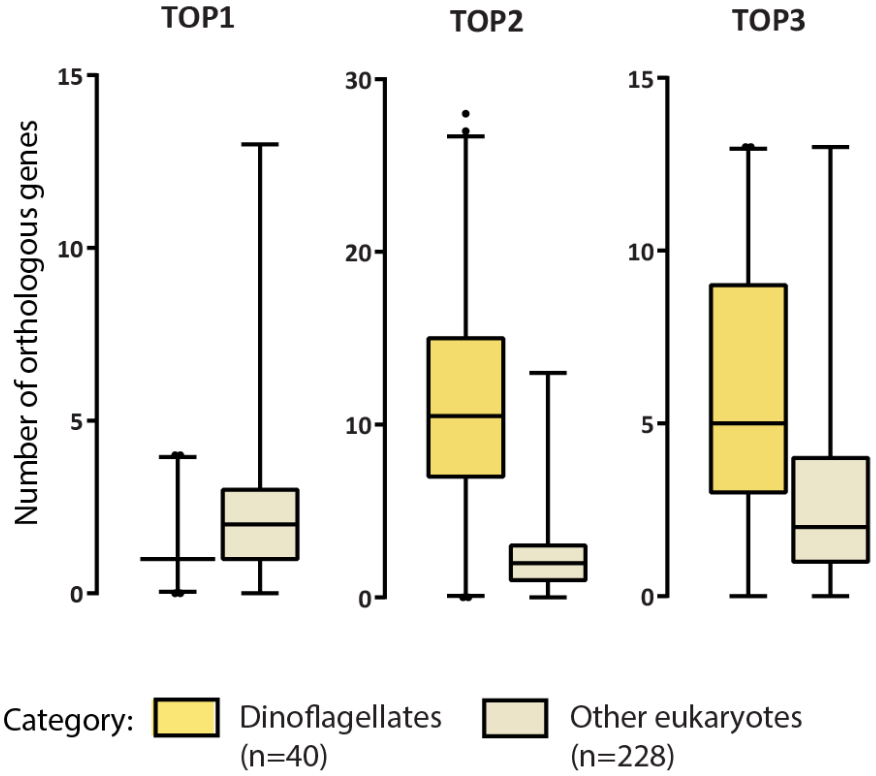
HiC Contacts following transcriptional inhibition (Pseudochromosome 10)



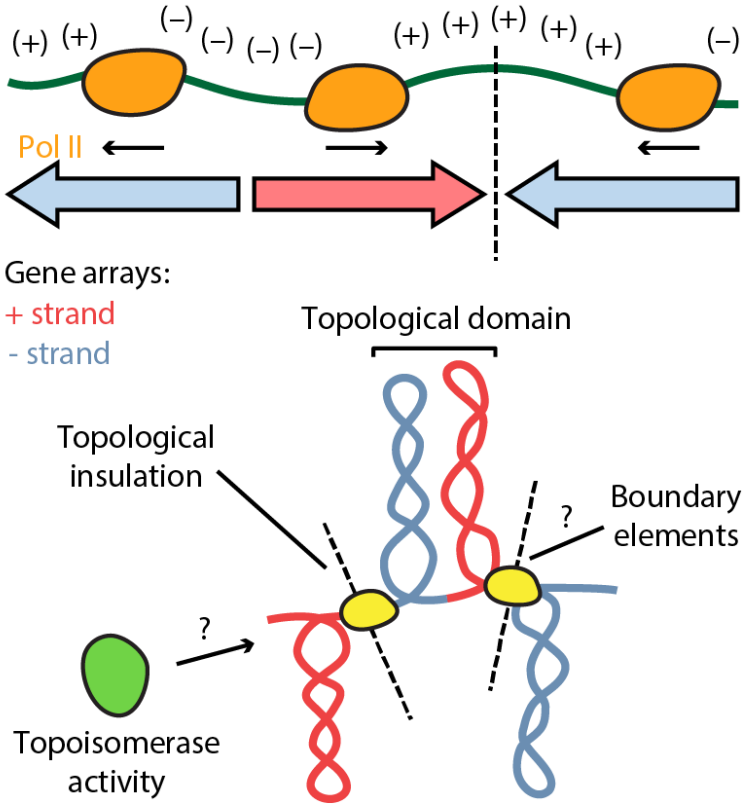

Aggregate Hi-C signal across all dinoTADs
0h
24h
48h
α-amanitin high
Triptolide high
8h
24h
48h

G

Topoisomerase gene amplification
in dinoflagellates

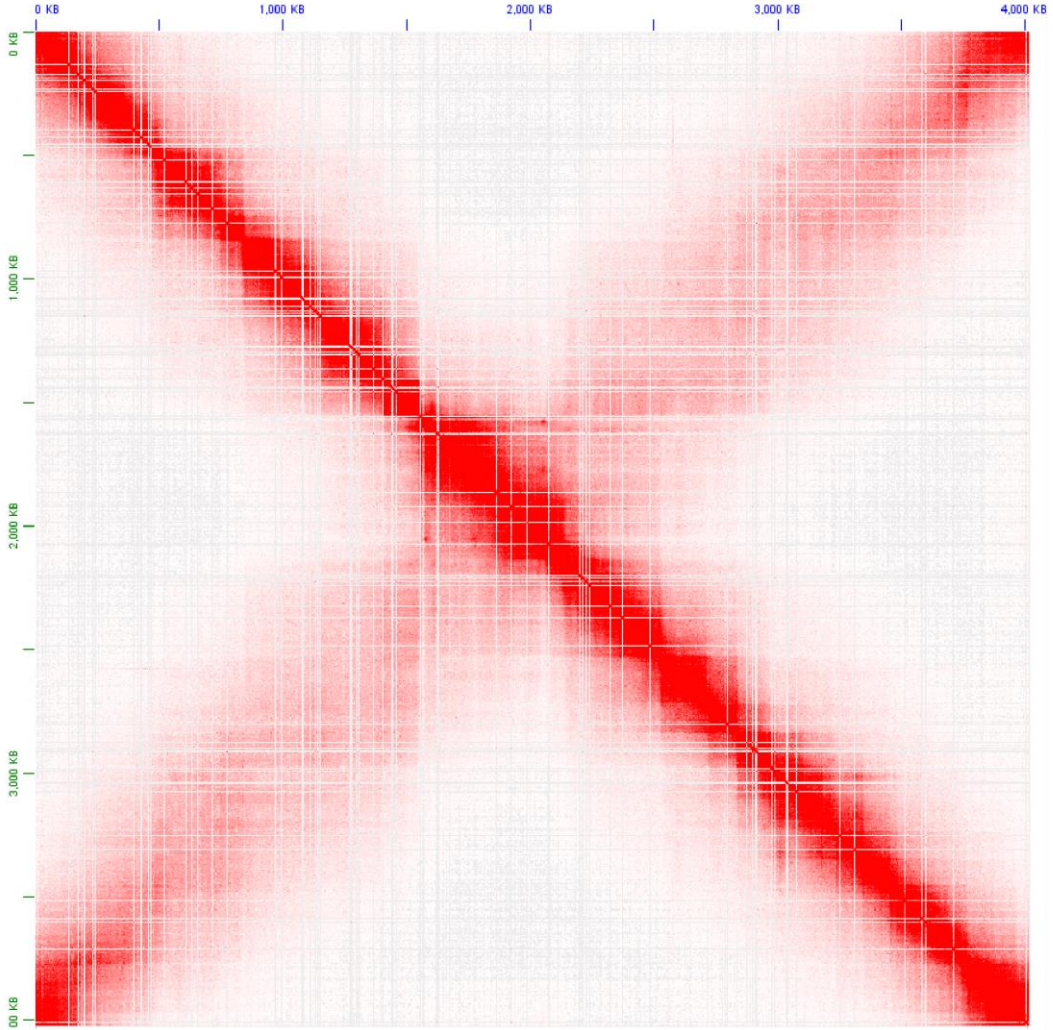


Model of transcription-induced
plectonemes and domain formation



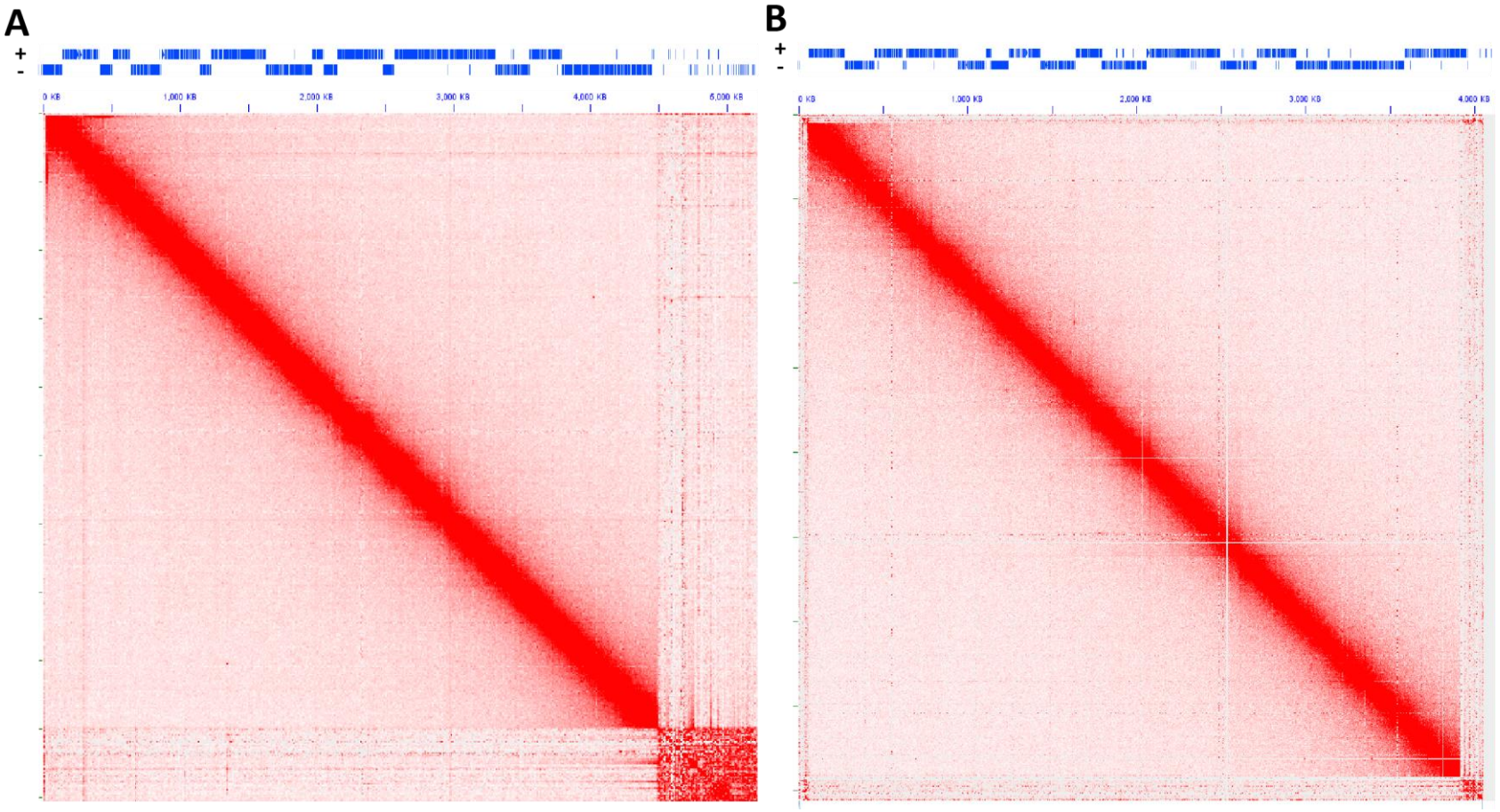
# Caulobacter

- prokaryote



# Trypanosoma

- eukaryote with gene arrays but also with histones



# CONCLUSIONS

- Strong insulation domains are observed in the *Symbiodinium* genome
- These appear to correspond to pairs of divergent multicistronic gene arrays
- Clear loop contacts are not immediately obvious
- Transcription-induced supercoiling appears to be the mechanism driving their formation
- Supercoiling emerges as another fundamental topological force shaping genome folding
- Its effects are masked in most eukaryotes by the presence of nucleosomes – interactions between histones block the formation of plectonemes

# OPEN QUESTIONS

- How exactly is supercoiling creating self-interacting domains?
- How could supercoiling create sharp boundaries?
- Can these patterns be reproduced computationally?
- Alternative hypotheses: it is possible that there are specific boundary elements with a distinct chromatin state, e.g. perhaps that is where the histones are
- As it is at present not possible to answer that question without extensive additional experiments involving raising antibodies, ChIP-seq, mass-spectrometry, etc., we hope to use computational modelling to gain insights into the possible mechanisms