Using statistical profiling to decipher hidden chromatin contacts resulting from repeated sequences

26/11/2024

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Spatial Regulation of Genomes - Genomes & Genetics - UMR3525

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INCEPTION

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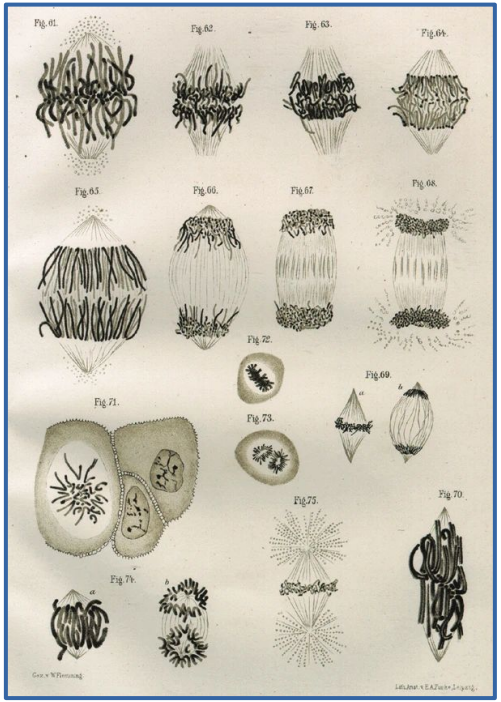
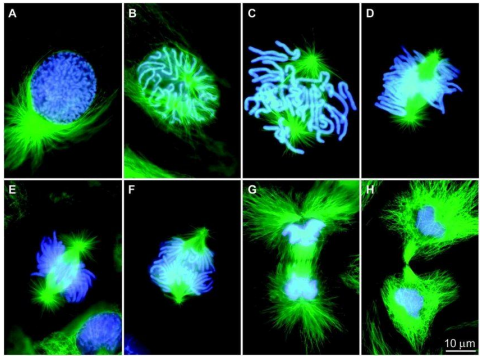
Early observation of genomes organisation

Lilium corceum drawings





Human cell

3.10^9 bp = 2m

yeast

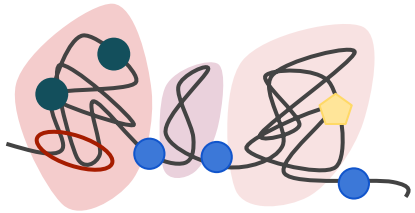
12.10^6 bp = 8 mm

DNA folding is a highly organized process necessary to fit DNA in nucleus

Zellsubstanz, Kern und Zelltheilung, Flemming, 1882
Risler, 2011

Chromosome folding influences or regulates dynamic processes?

Genome folding



Biological functions

- Gene transcription
- DNA Repair ; Replication
- Chromosome segregation

ADN

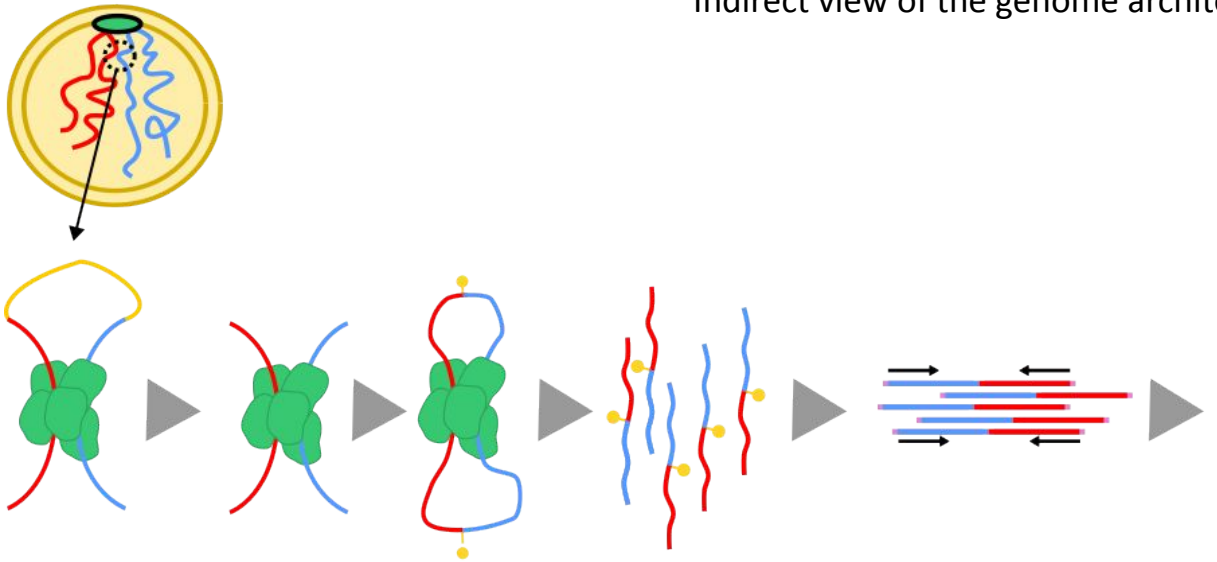
DNA binding proteins

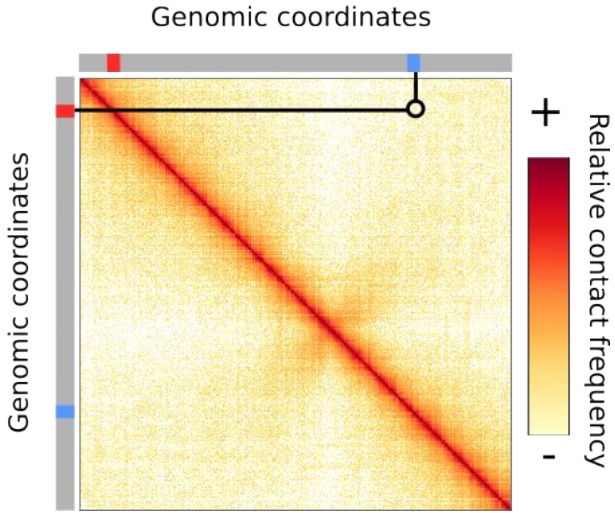
Physical constraints (supercoiling, etc.)

Cause or consequence ?

Hi-C to capture genomes 3D structures

Indirect view of the genome architecture

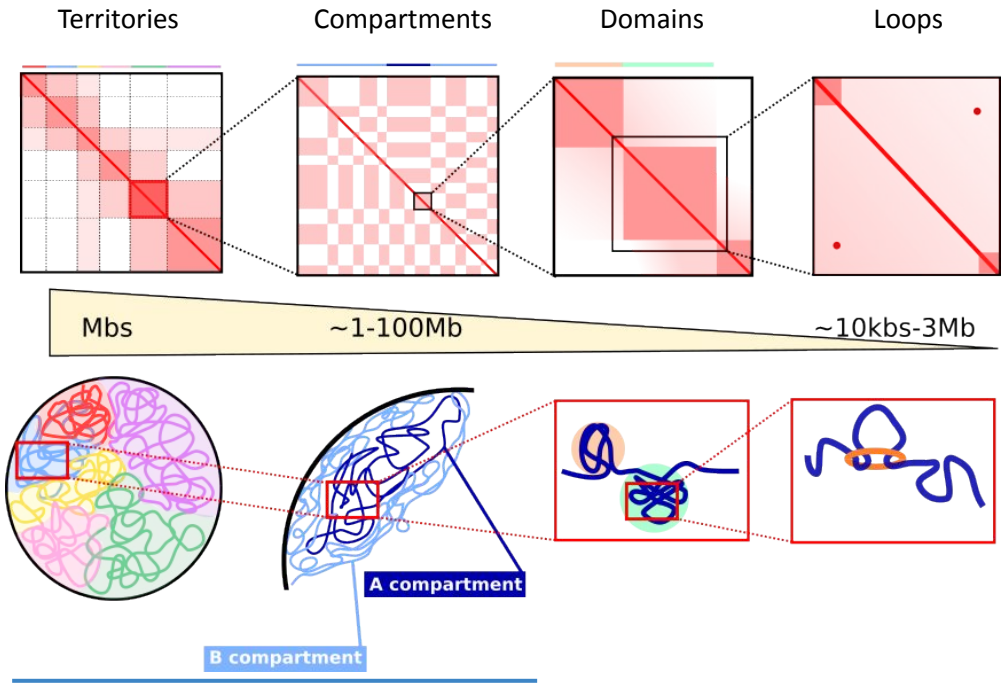




Adapted from Lieberman-Aiden, et al. 2009

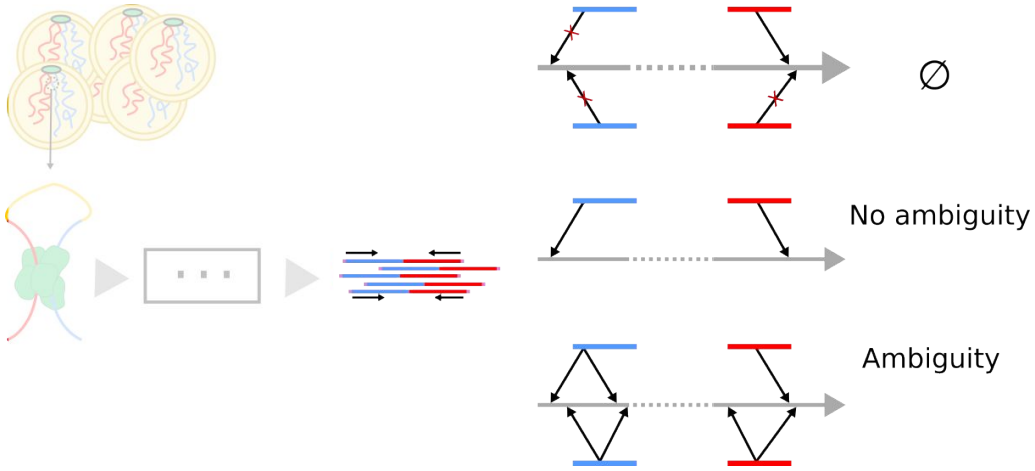
Chromosome Configurations: Influencing Genome Organization

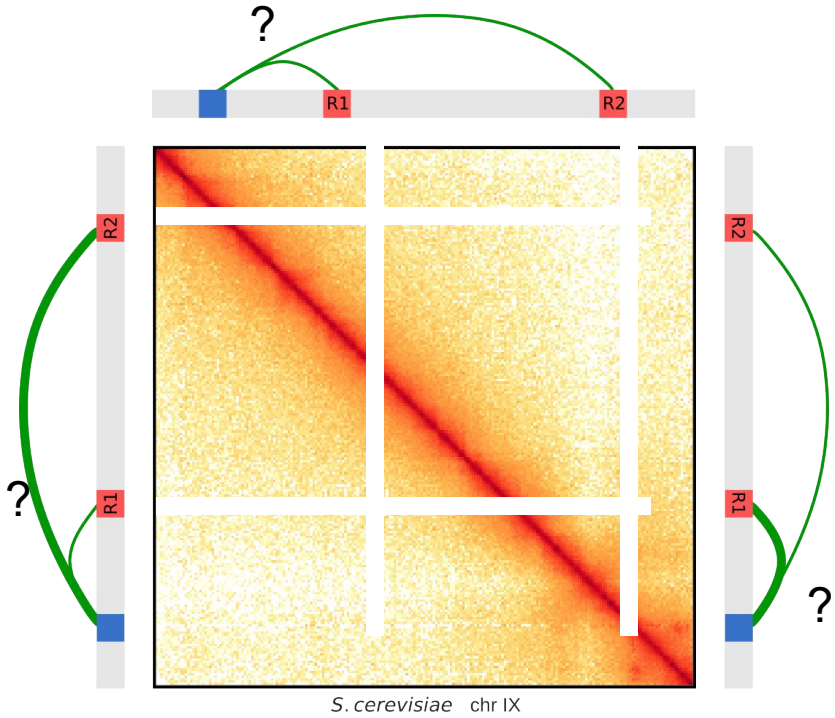
How these different spatial structures correlates with biological processes?



- Cell cycle phase dependence
- Response to stresses
- Quiescence / replicating?
- Viral infection?

Limitations of Traditional Hi-C



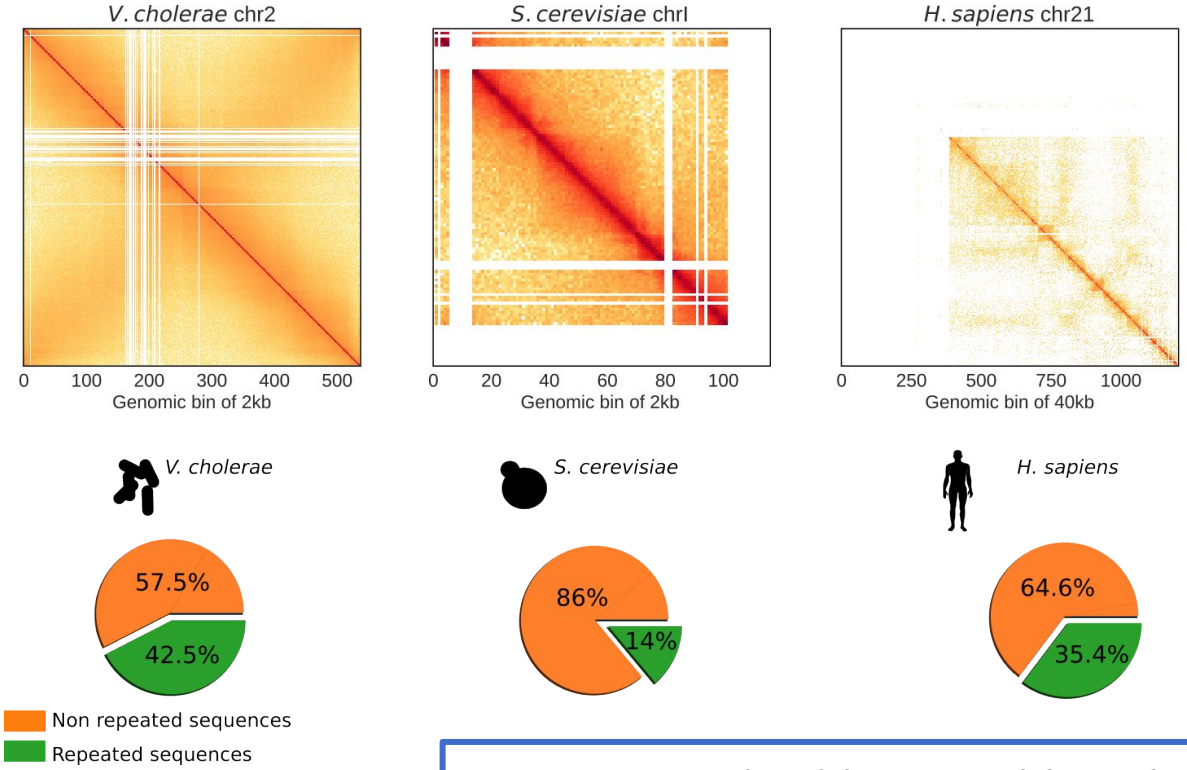


- Pair-end sequencing produces short reads ~ 50 bp
- Genomes carry repeated regions

  → Some reads can have multiple genomic positions

Statistical approach for ambiguous contacts prediction?

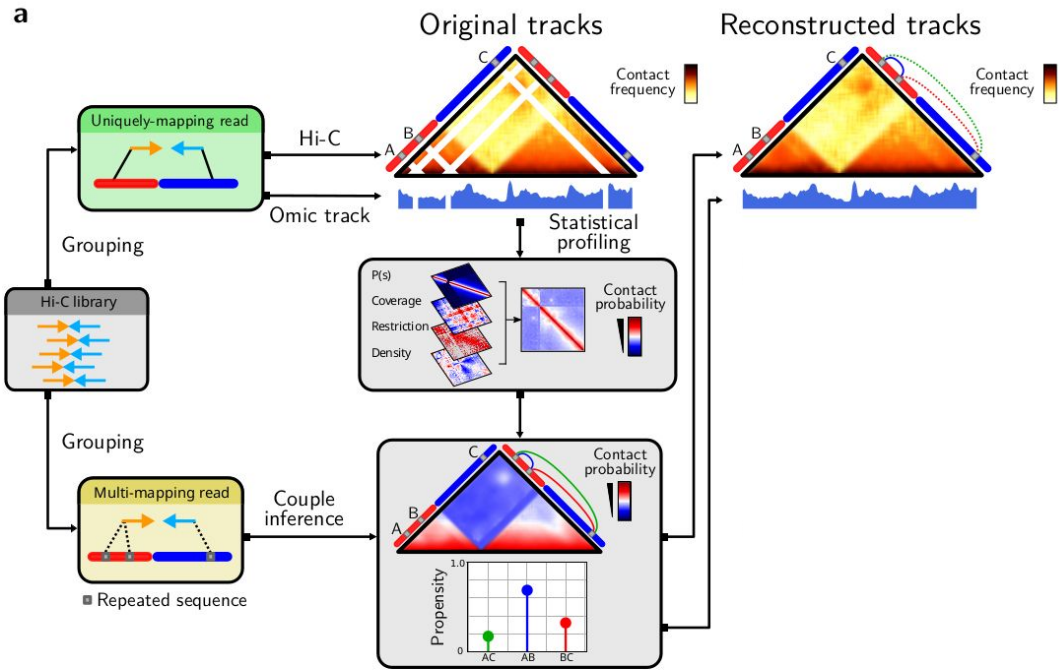
Thesis Objectives



Can a statistical modeling approach be used to infer missing information in Hi-C data and accurately reconstruct the 3D interactions of repeated sequences?

Hicberg: Reconstruction of genomic signals from repeated elements

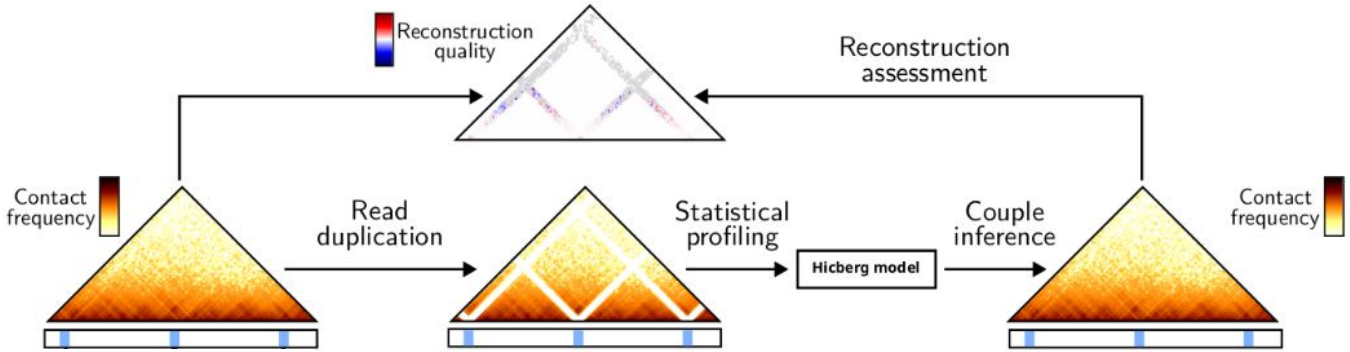
Hicberg: a novel tool for (3D) genomics



- **Hicberg:** Reconstruction of Hi-C data, and more!
- **On-the-fly statistical profiling:** extraction of statistical tendencies from unambiguous data.
- **Probability Mass Function estimation:** Guides accurate placement of ambiguous reads.

Gradit et al., in prep.

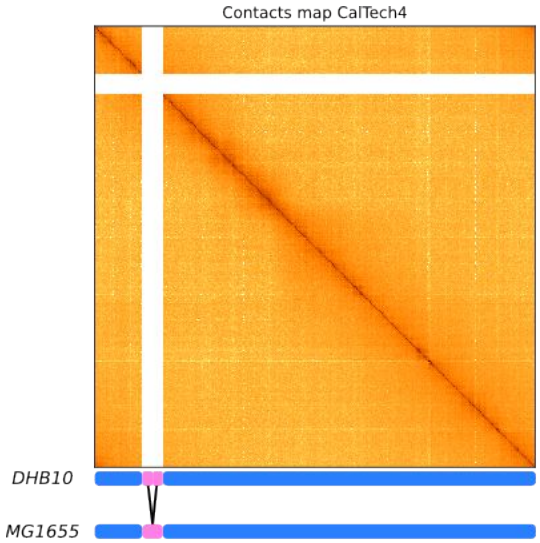
Benchmarking & In-silico Evaluation

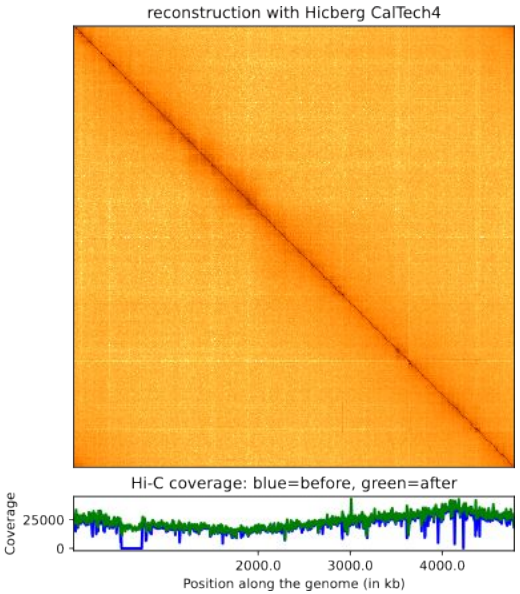


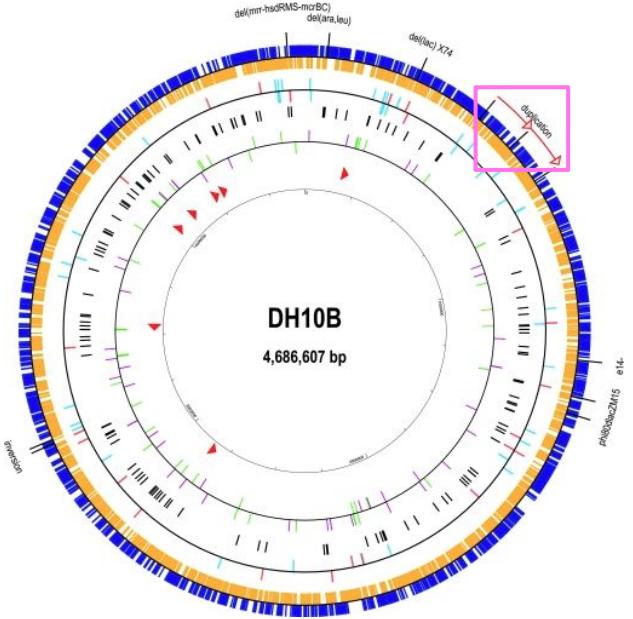
- **Simulated** repeated sequences by duplicating unambiguous reads.
- **Robust** across various repeat sizes, numbers, and spacing.
- **Polymeric** behavior component is key for accurate inference.

Does Hicberg perform as well in real-context?

Biological validation



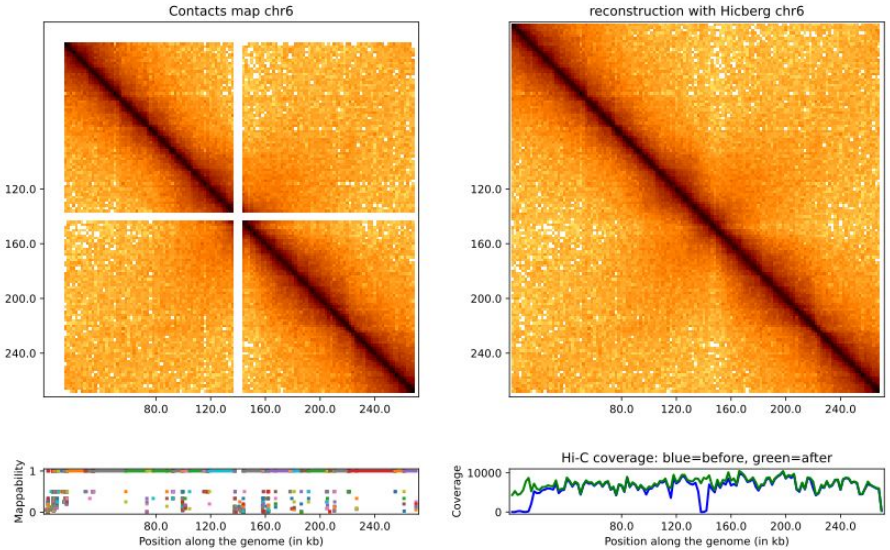




- Accurate reconstruction of the complex 3D structure of *E. coli* carrying tandem duplication.
- Hicberg captured expected DNA polymer behavior, demonstrating versatility and power.

Adapted from Durfee, et al. 2008

Biological validation



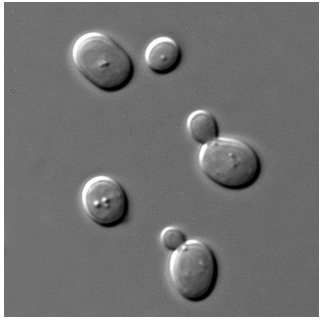
- Accurate reconstruction of the complex 3D structure of *S. cerevisiae* chr VI carrying repeats.
- Hicberg captured expected DNA polymer behavior, demonstrating versatility and power.
- Hicberg recovered expected mean coverage despite low theoretical mappability

Unveiling Hidden Connections: Hicberg in action

How do repetitive elements shape the 3D genome and influence its function?

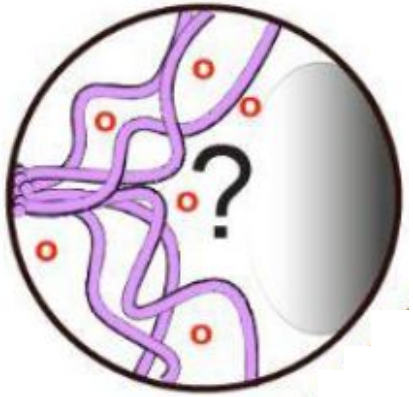
1) *Study of Ty1 Retrotransposons: 3D Organization and Cohesin Loading*

2) *Study of rDNA Contact Dynamics in Response to Stress*

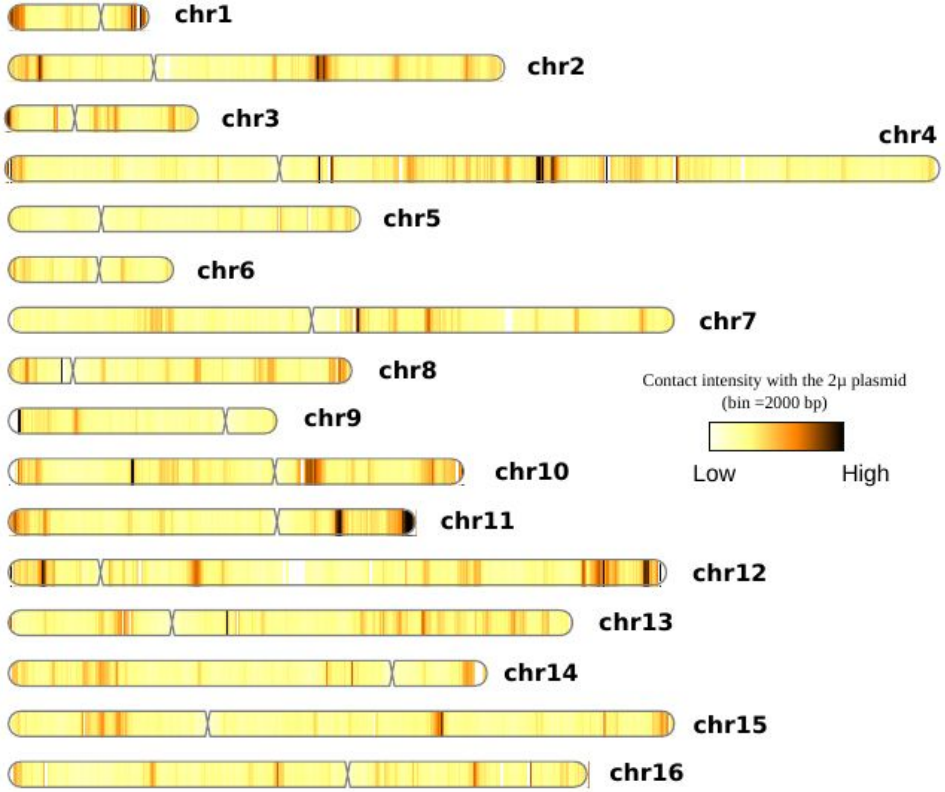


Beyond Parasitic Elements: Ty1 Retrotransposons as Organizers of Nuclear Architecture

Application : *S. cerevisiae* TYs as new 2µ preferencial contact spots



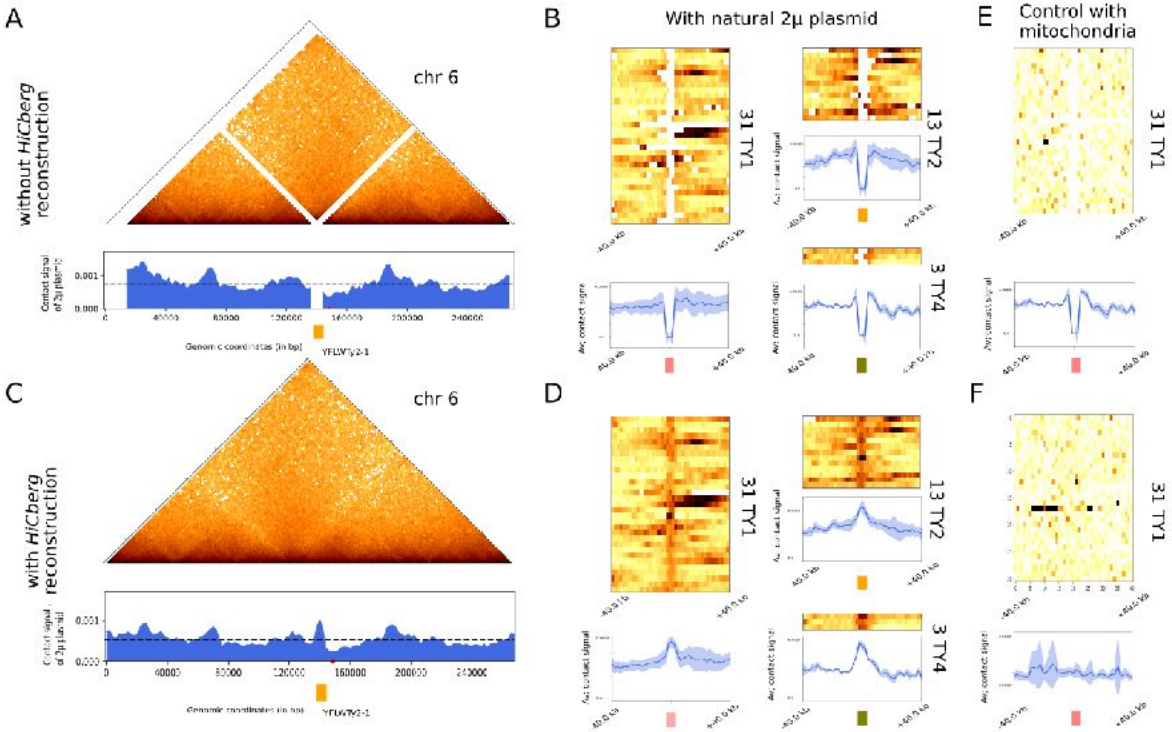
Presence of natural 2µ plasmid in *S. cerevisiae*



Detection of ~ 75 hotspots of contact

Adapted from Girard, et al. 2023

Application : *S. cerevisiae* TYs as new 2µ preferencial contact spots

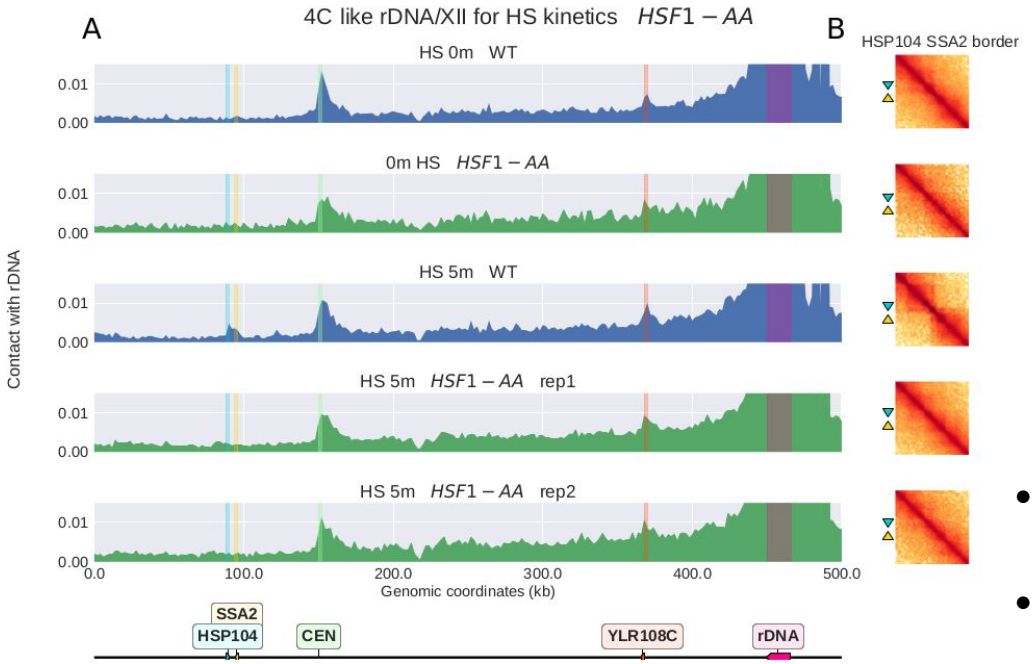


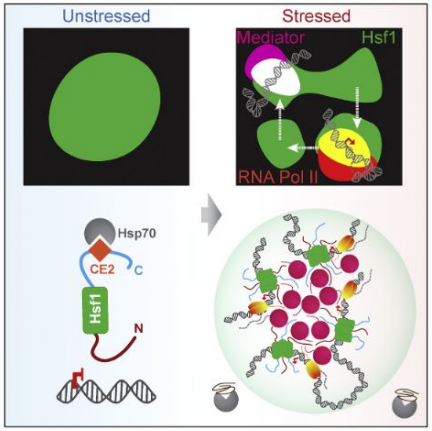
Retrotransposons (TYs) appear to be **new hotspots** of *2µ plasmid*

Gradit et al., in prep.

Unveiling the rDNA Network: Key Players and Choreographers of its Spatial Organization

Application : *S. cerevisiae* TYs as new 2µ preferencial contact spots





- **Hsf1 Mutant:** Transient rDNA-HSP104/SSA2 interaction and border pattern are lost.
- **Dual Requirement:** Both transcription and Hsf1 are essential for this stress-induced structure.
- **Mechanism:** Supports Chowdary's hypothesis of micro-aggregates, not loop extrusion, for contact formation.

Adapted from Chowdary, et al. 2022

HiC-BERG - Conclusion & Perspectives

- Hicberg showed **good performances** both in **simulated** and in **biological** data
- Several genomic features have been unveiled in *S. cerevisiae:*
    - 2µ plasmid **hotspots of contacts** of yeast retrotransposons
    - **New rDNA** interaction during **heat shock**, involving *Hsf1* protein complex

→ First link about spatial organization of rDNA and **stress**

Régulation Spatiale des Génomes

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Hélène Bordelet
Jacques Serizay
Pauline Larrous
Devon Conti
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Collaborators

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INCEPTION

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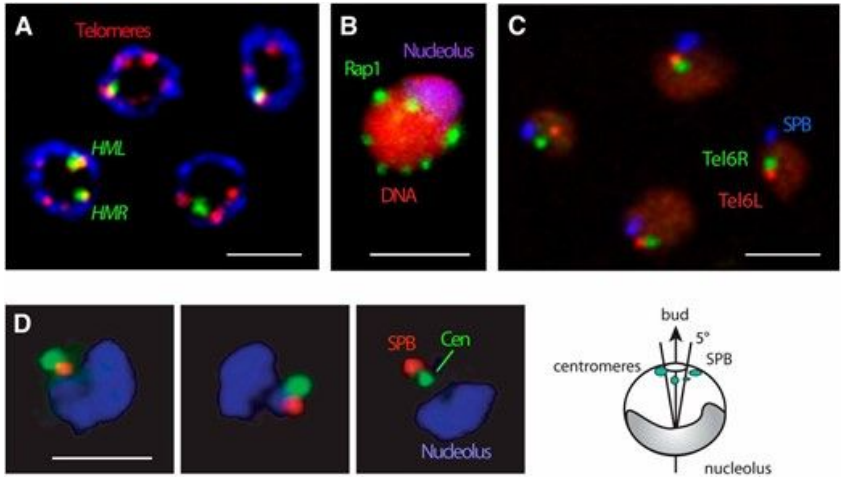
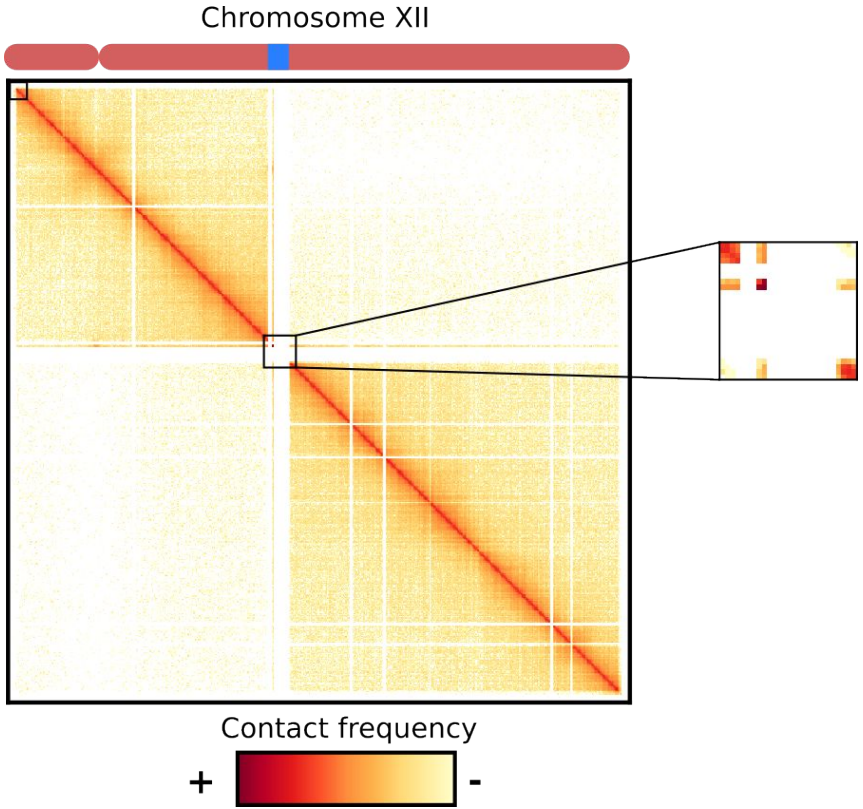
rDNA: A Landmark in the Nuclear Landscape

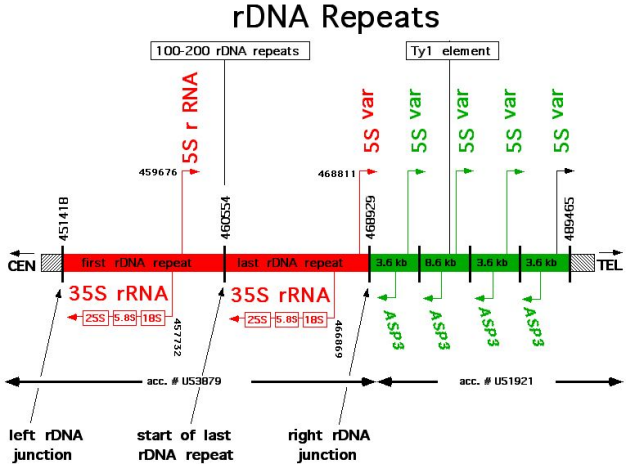




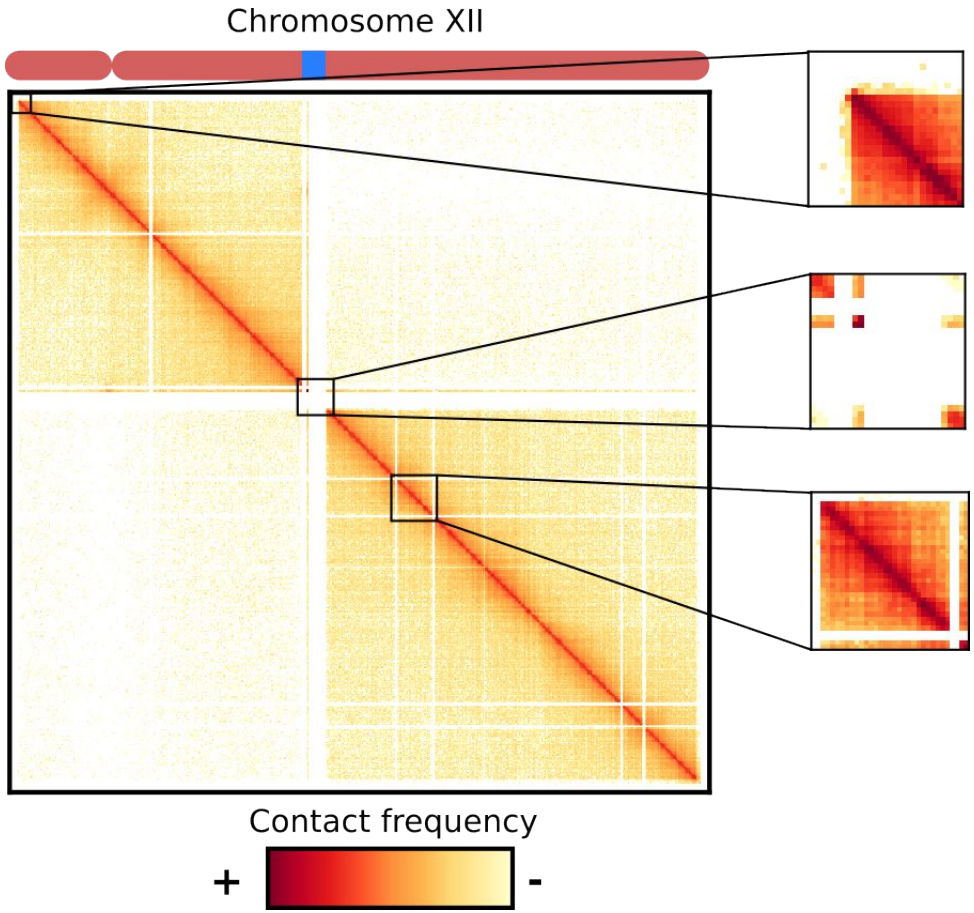
- rDNA as an organizational feature within the nucleus
- Ribosomes biogenesis
- Often exhibits interactions with nuclear landmarks (telomere, centromeres)

Adapted from Taddei, et al. 2012

The Challenge of Repeated Sequences

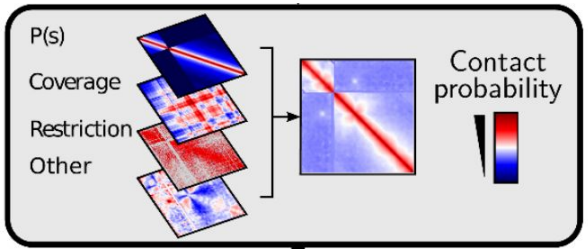


Why are repetitive sequences, particularly challenging to study using traditional Hi-C?

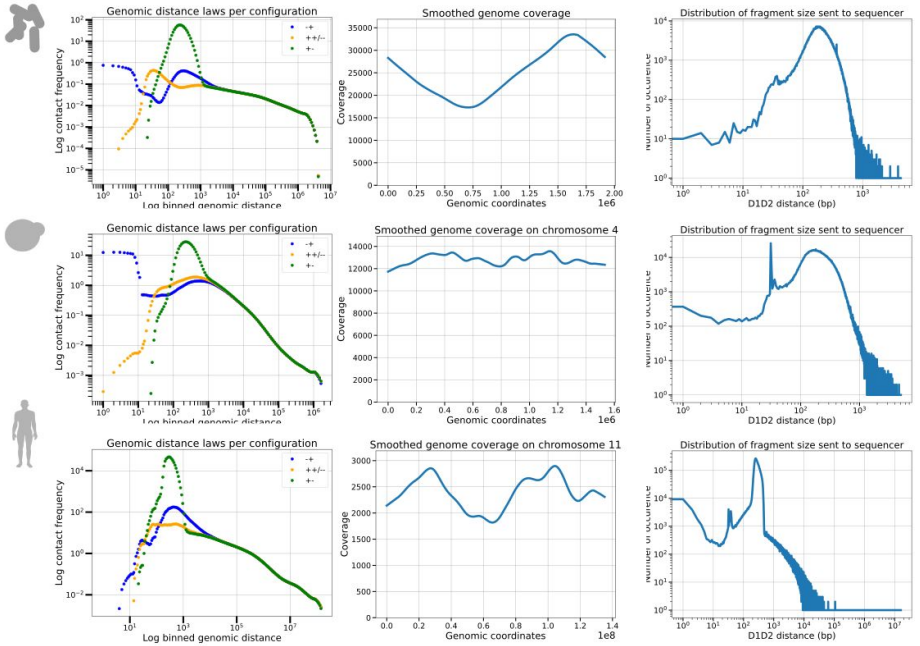


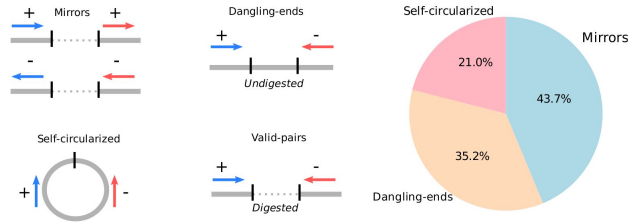
Adapted from Johnston, et al. 1997

Hicberg: a novel tool for (3D) genomics

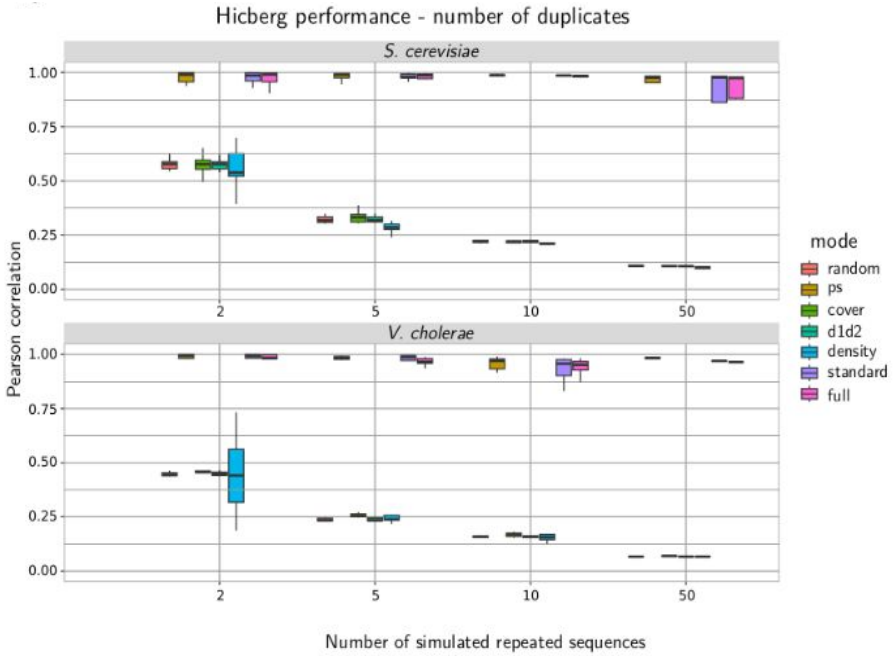


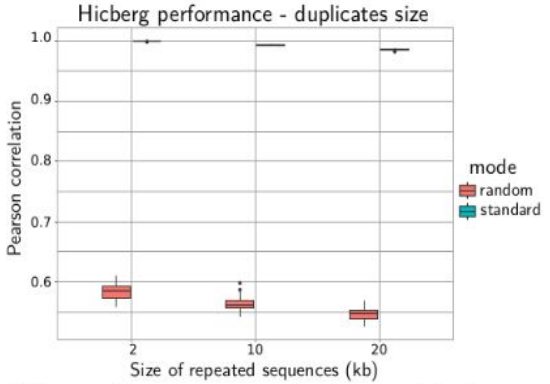
- **Polymer Behavior:** Captures DNA flexibility with 3 species/condition-specific sub-laws.
- **Coverage Bias:** Accounts for uneven read distribution; higher coverage increases interaction probability.
- **Restriction Site Proximity:** Accounts for fragment length biases.

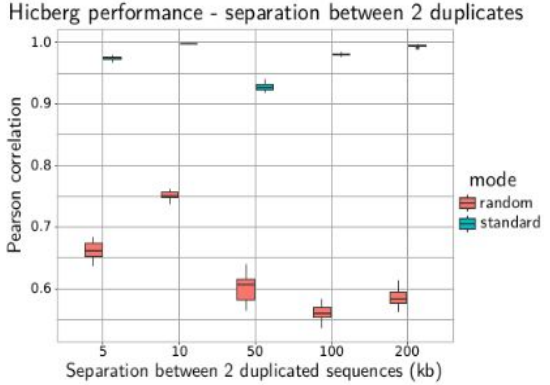




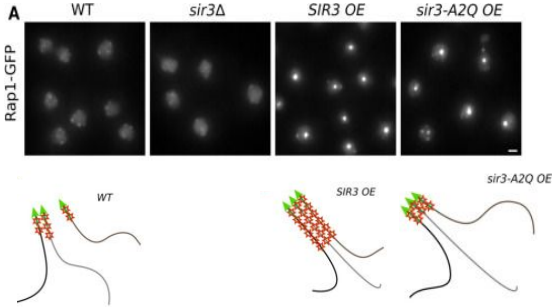
In-silico Evaluation

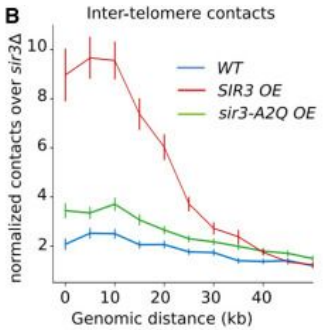




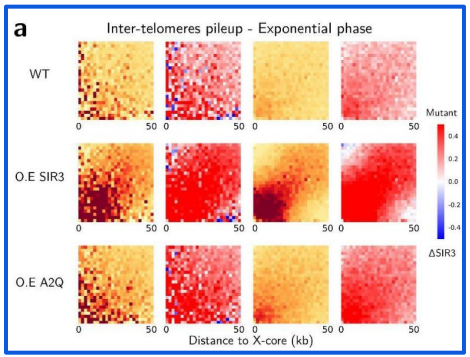


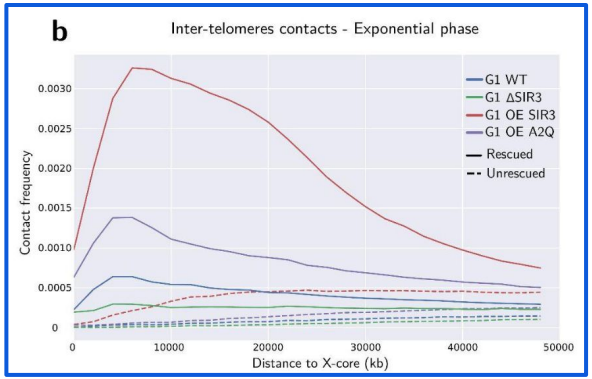
Biological validation





Hicberg to re-analyze yeast Hi-C data on telomere clustering under SIR3 over-expression studied with a diffusion-based method (Serpentine)



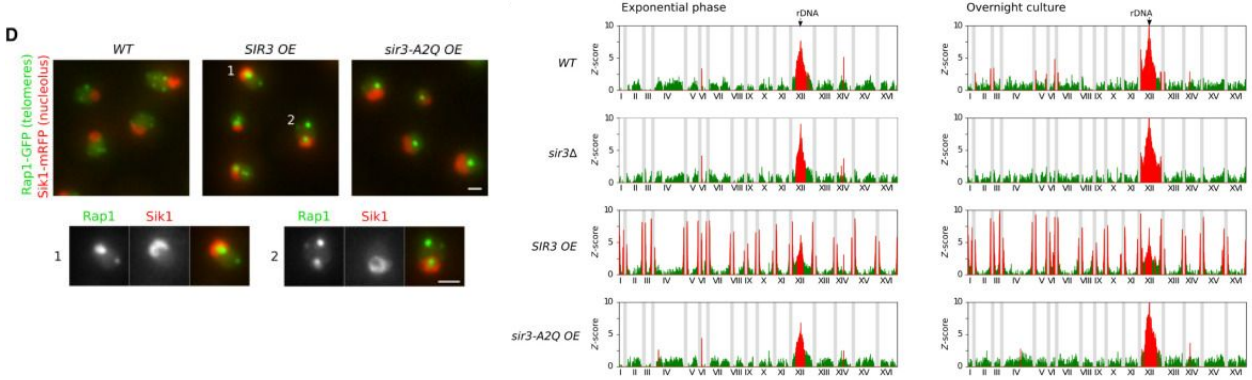


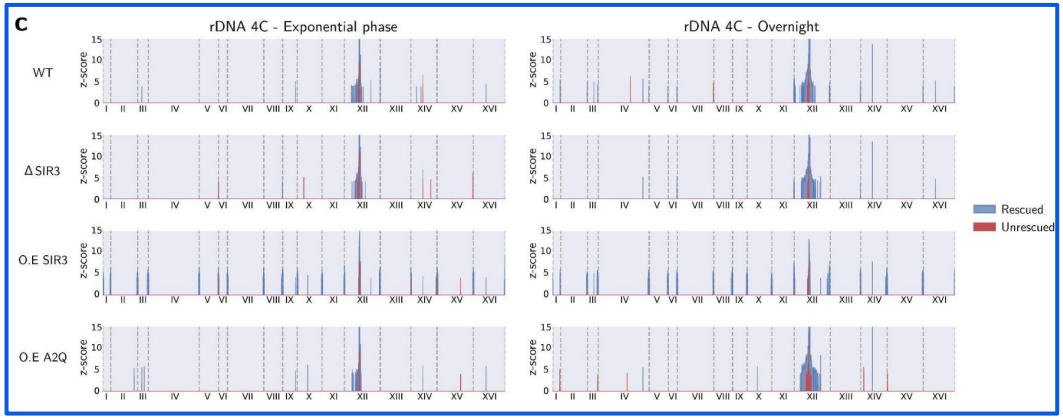
Hicberg reconstruction: Accurately reproduced Ruault's findings:

- SIR3 overexpression → telomere hyper-clustering.
- Altered SIR3 (A2Q) → reduced clustering.

Adapted from Ruault, et al. 2021

Biological validation





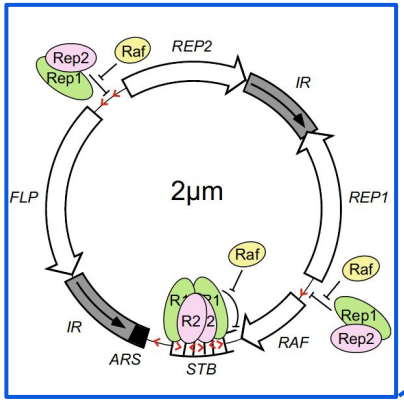
Hicberg reconstruction: Reproduced Ruault's findings:

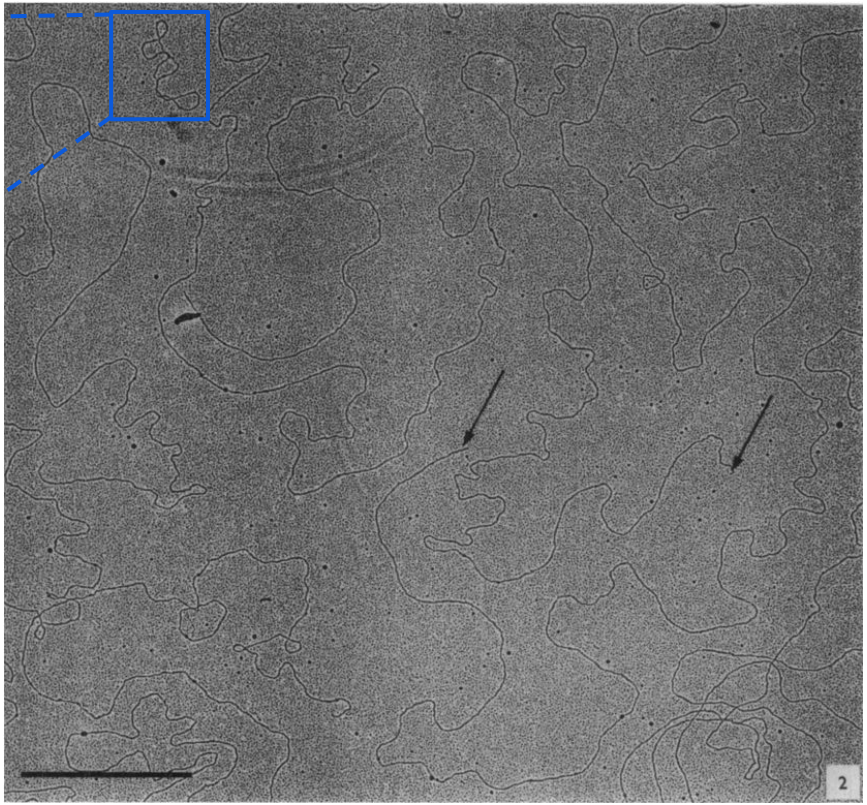
- SIR3 overexpression → increased rDNA-telomere clustering (exponential and overnight cultures).
- Altered SIR3 (A2Q) → partially reduced clustering.

Hicberg accurately captures complex, condition-specific 3D interactions in repeat rich regions, further validating its biological relevance.

Adapted from Ruault, et al. 2021

Discovery of 2µ plasmid in Saccharomyces cerevisiae





Observation in the 1970's of **enriched fraction of small circular DNA** found as different molecules species

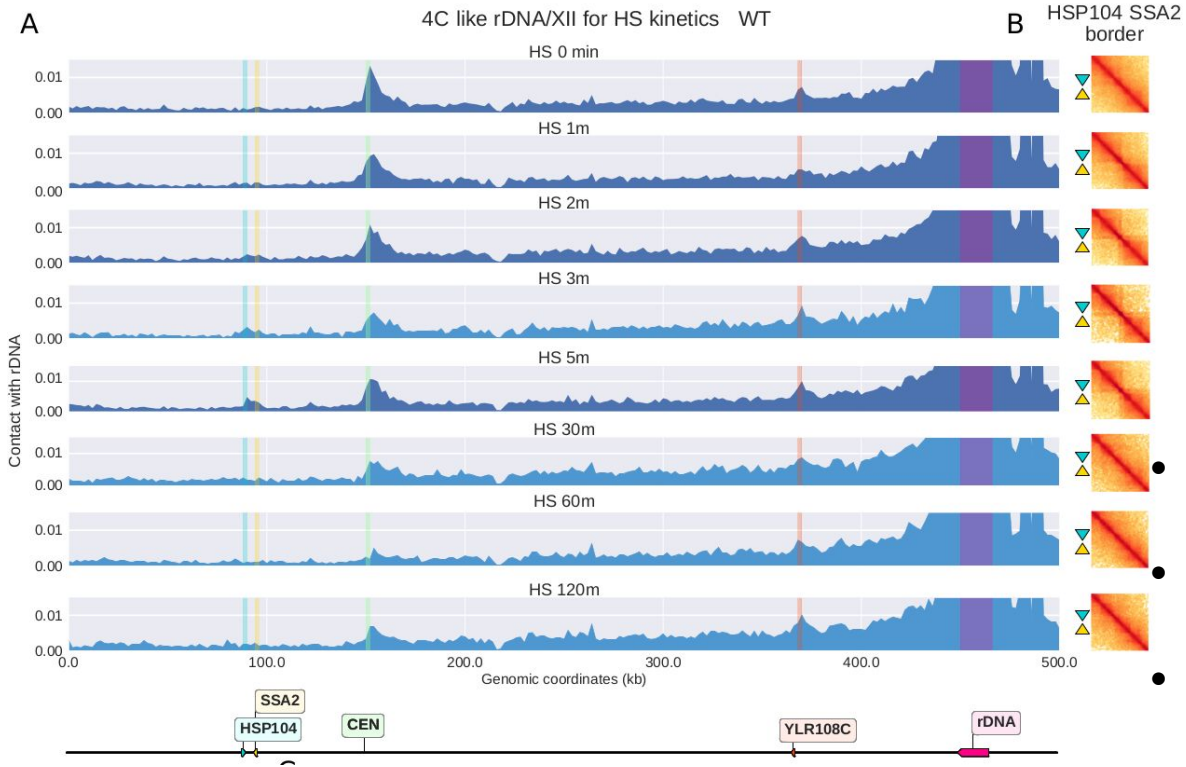
Predominant class of with **mean contour length of 1.88 ± 0.11 µm**

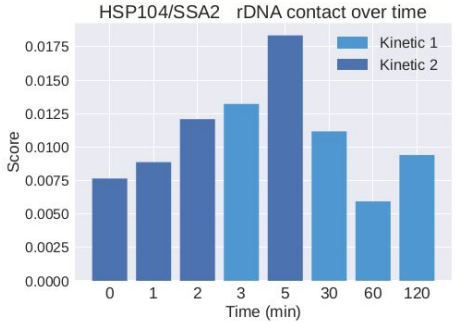
Conserved through generations

Specific locations in *S.cerevisiae* genome (long genes, low transcription spots)

Adapted from Girard, et al. 2023

rDNA-HSP104/SSA2: A Transient Tango During Heat Shock





- **rDNA in Heat Shock:** Hicberg reveals transient interaction with HSP104/SSA2 genes.
- **Dynamic contact:** Forms and dissolves during heat shock, suggesting a role in stress response.
- **Border pattern:** Emerges around HSP104/SSA2, indicating potential transcriptional activation.

HiC-BERG - Conclusion & Perspectives

- Hicberg showed good performances both in simulated and in context data
- Hicberg outperformed mHiC on the definition of reconstructed structures, and number of retrieved Hi-C reads
- Several genomic features have been unveiled (2µ hotspots on TYs, new rDNA interaction during heat shock, transcriptionally induced aggregates)
- First link about spatial organizationof rDNA and stress

Expand Applications:

- Explore diverse stress conditions in yeast.
- Apply to other organisms and datasets.
- Investigate various "OMICs" data.

Enhance Performance:

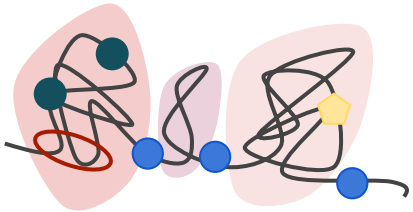
- Optimize for speed and efficiency.
- Facilitate routine use across species.

Chromosome folding influences or regulates dynamic processes?

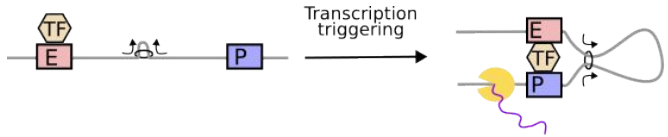
DNA binding proteins

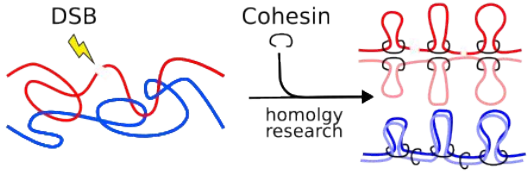
Physical constraints (supercoiling, etc.)

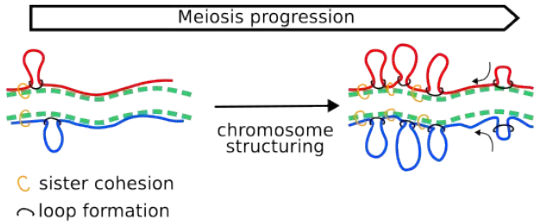
Non-coding RNA



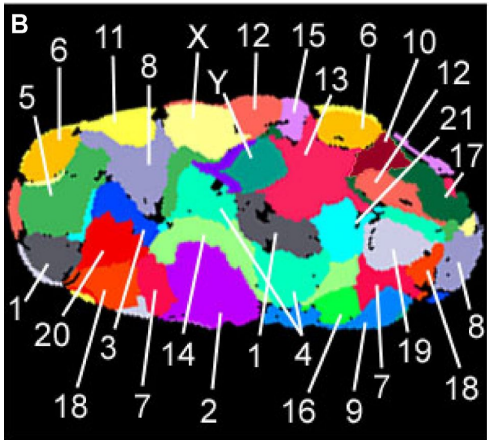
Cause or consequence ?

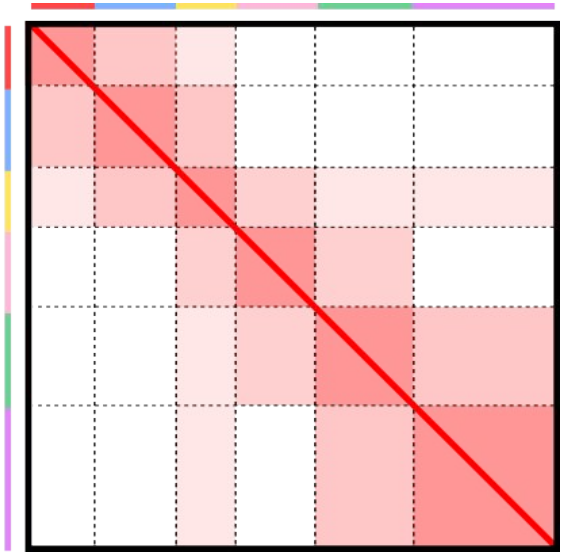






Chromosome Location in Genomes





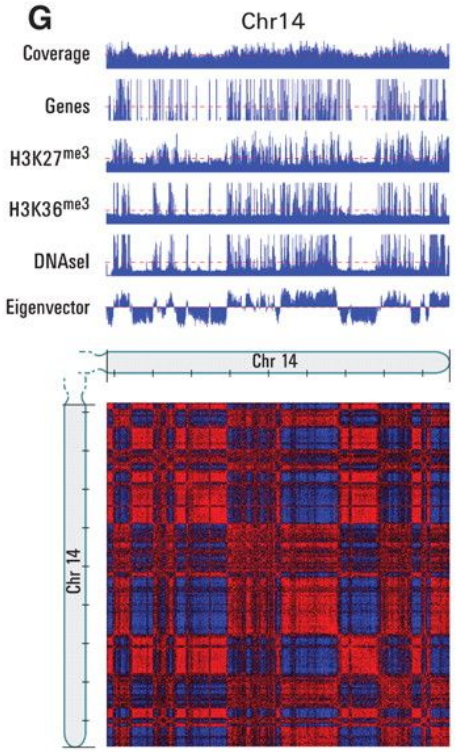
- Size/shape vary → chromosome, cell type
- Dynamic state (cellular processes, cycle, …)
- Gene rich toward interior

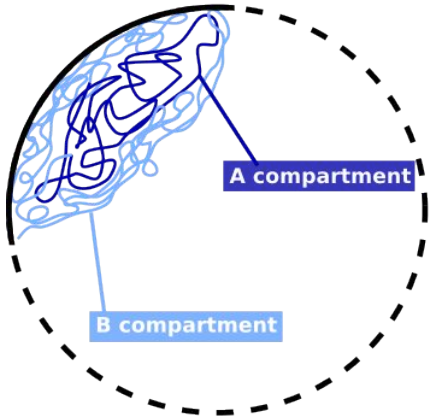
Response to biological processes:

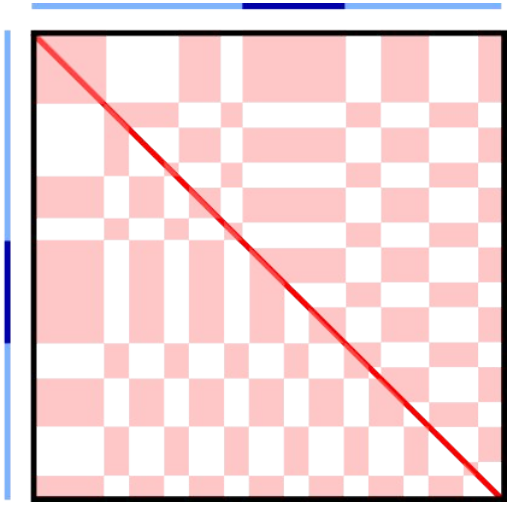
- ➢ Efficiency of DNA replication and repair
- ➢ Prevent inter-chromosomes translocations

Adapted from Bolzer, et al. 2005

From chromosomes to compartments







A compartments:

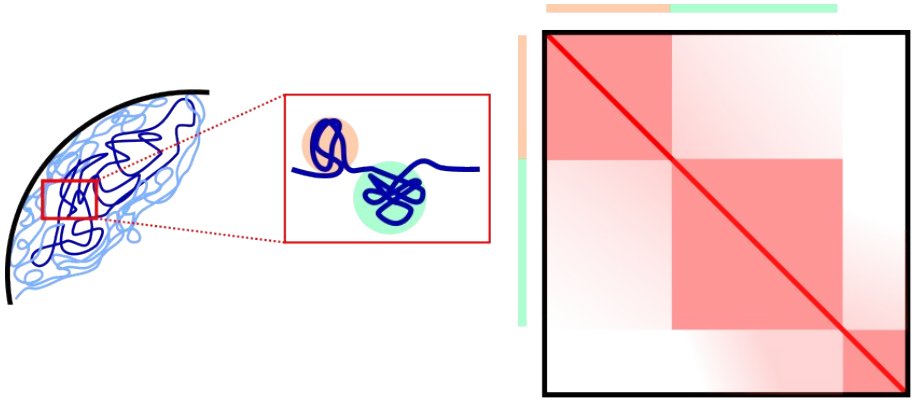
- Open chromatine
- Gene rich
- Acetylation marks
- Interior of nucleus

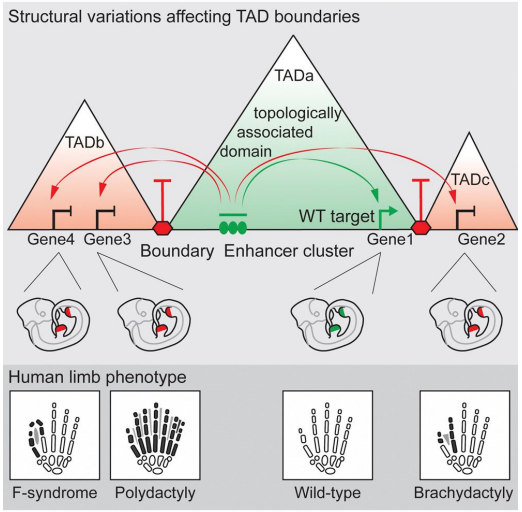
B compartments:

- Dense chromatine
- Gene devoid
- Methylation marks
- Toward lamina

Adapted from Lieberman-Aiden, et al. 2010

Topological Associated Domains





High level of self-interaction:

- Boundary regions insulator elements : separators
- Enriched for CTCF or architectural proteins

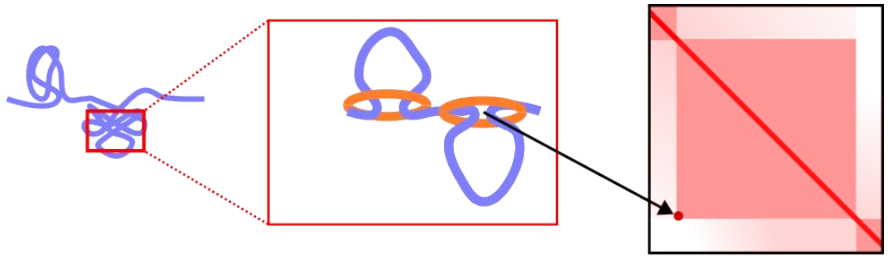
- Regularity units: appropriate enhancer/promoter
- Boundary disruption = inappropriate gene activation

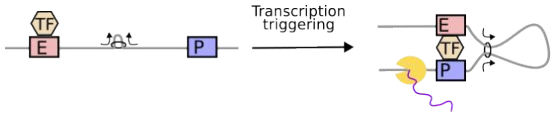
  → Disorders (development, cancer, …)

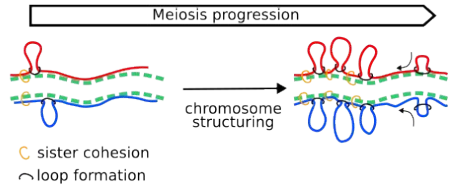
How do distant regulatory elements communicate with their target genes to control gene expression?

Adapted from Lupianez et al., 2015
Dixon et al., 2012

Chromatin loops: Bringing Elements Together







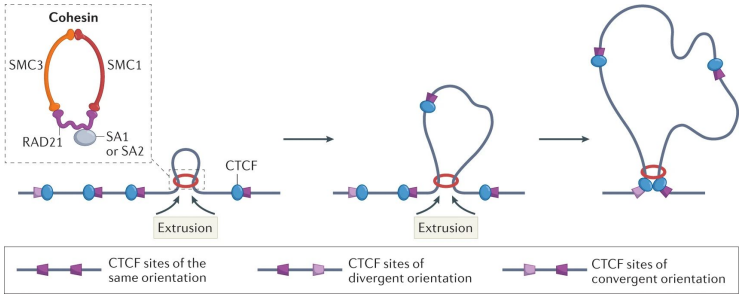
Regulatory loops

- Modulate gene expression
- Small range, gene expression precise control
- Dynamic: TF, chromatine remodelers, signaling

Structural loops

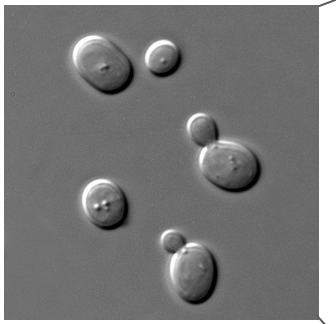
- Establish, maintain territories
- Long range, overall genome structuration
- More stable ⟶ slower timescale (cellular cycle)

Dual role, different scales but shared molecular mechanisms



Adapted from Zhang, et al. 2022

S. cerevisiae as a model for 3D genomics studies



- ~ 12 millions bp
- ~ 6000 genes
- Haploid and diploid states
- Easy to handle