Summer School: *Introduction to Biological Physics*, WI 2018

Physics of the cell’s nucleus:

Large-scale organization of living information

→ For small scale organization: lecture by Bar-Ziv

Large-scale organization of DNA in the nucleus

- The packing problem:

What are the basic scales and geometry of cellular DNA?

- What is the underlying physics?

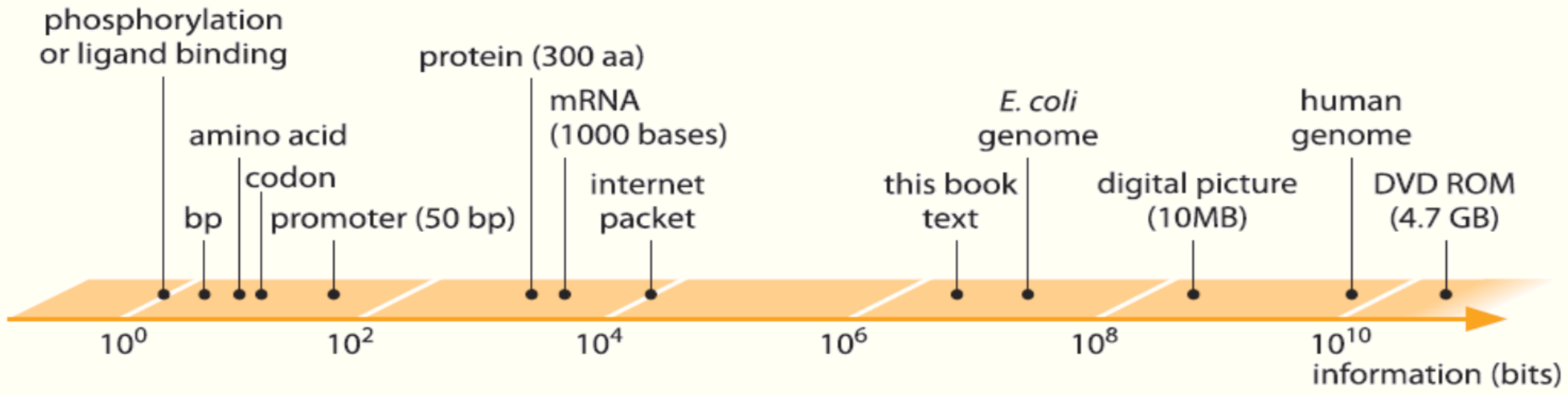
- What are the building blocks of chromosome organization?

- How can physical forces affect biological function?

Living matter carries blueprint for self-construction

- Living matter is active and heterogeneous, with numerous diverse species of:
  - Protein machines: enzyme, motors, information processors.
  - RNAs: ribosomes, mRNA, tRNA, small RNAs
  - Signaling and control molecules…

- The information required to construct all this is huge



information, $I = \log_2$ (# possible configurations)

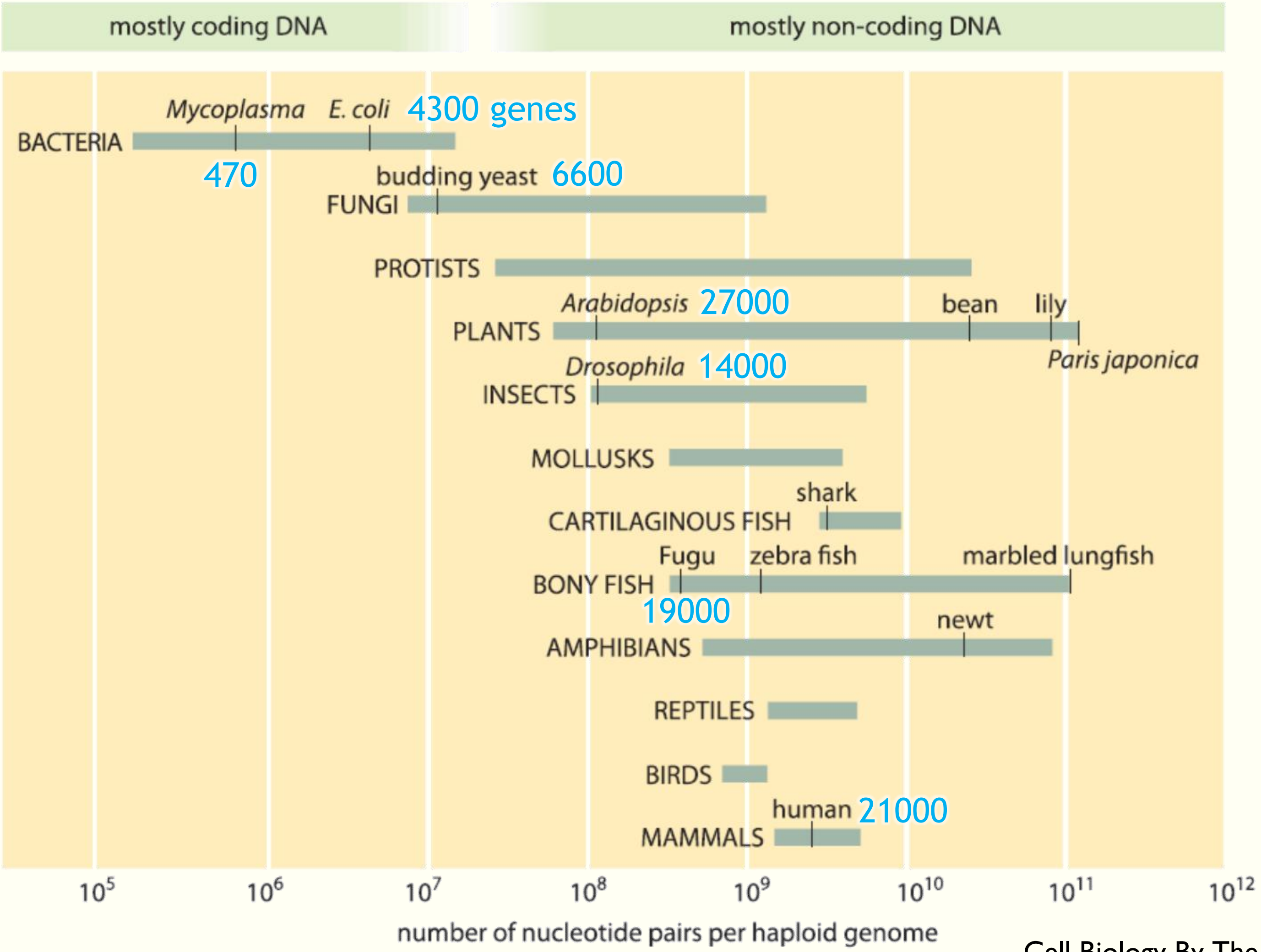
e.g. base pair (bp) has four possibilities $\Rightarrow I = \log_2(4) = 2$

- How this information is stored and processed in the cell?

Fig. from Cell Biology By The Numbers - Milo & Philips

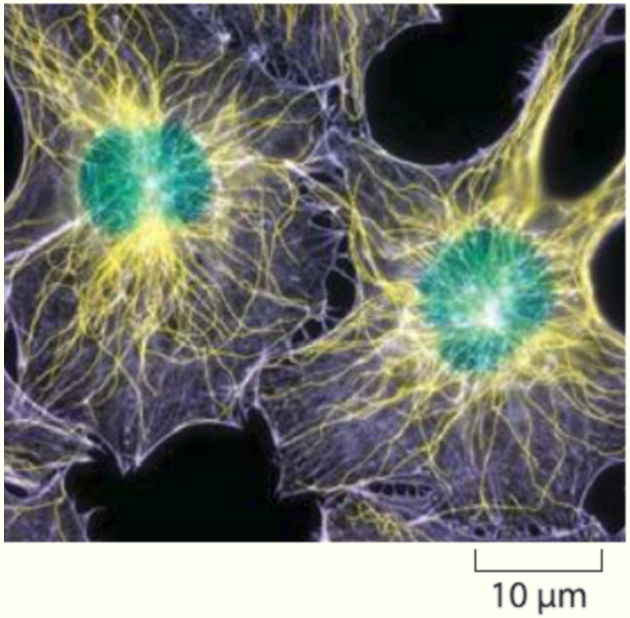
Genome lengths spread over 5-6 orders of magnitude

- But the number of genes varies much less.



Cell Biology By The Numbers (Milo & Philips)

Long genome are packed into micron-scale nuclei



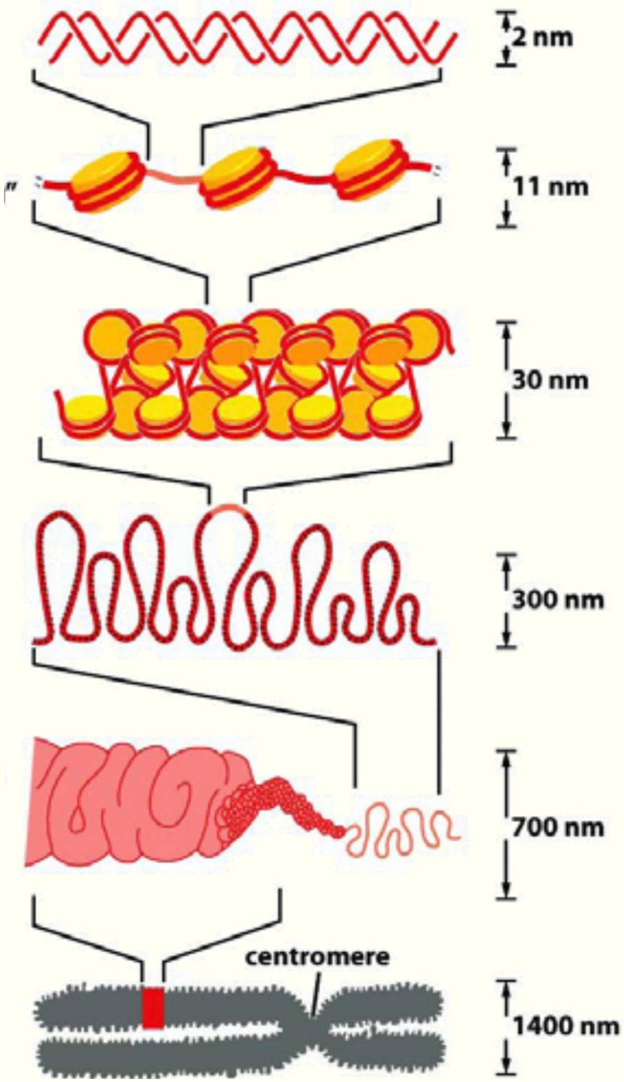
Human fibroblast cells (nuclei in green)
(CBBTN – Milo & Philips)

Species	Length (μm)	Nucleus (μm)	Folding ratio
Human	$2 \cdot 10^6 = 2$ m	10	$2 \cdot 10^5$
Mouse	$1.8 \cdot 10^6$	8	$2 \cdot 10^5$
Fruit Fly	$5 \cdot 10^4 = 5$ cm	5	10^4
Yeast	$4 \cdot 10^3$	2	$2 \cdot 10^3$
E. coli	$1.5 \cdot 10^3$	1	$1.5 \cdot 10^3$
T4 (virus)	50	0.05	10^3

- Base-pair length ≈ 3Å
- Base-pair volume ≈ 1nm^3.
- 10^9 bp ≈ 1 μm^3.
- Volume fraction DNA/nucleus ≈ 1%
- In virus, volume fraction o(1).

- The nucleus is hallmark of the eukaryotes.
- The nucleus contains most of the cell's genetic material + with many other proteins.

DNA is packed in a hierarchical structure



- **Nucleosome** = octamer of histone proteins.
- ~150 bp are rolled around each nucleosome.
- 10-nm beads-on-a string fiber

- "**30-nm fiber**": disks of ~6 nucleosomes.
- ~1000 bp per disk.

- **Chromatin** = DNA+histone+… complex.
- Functions:

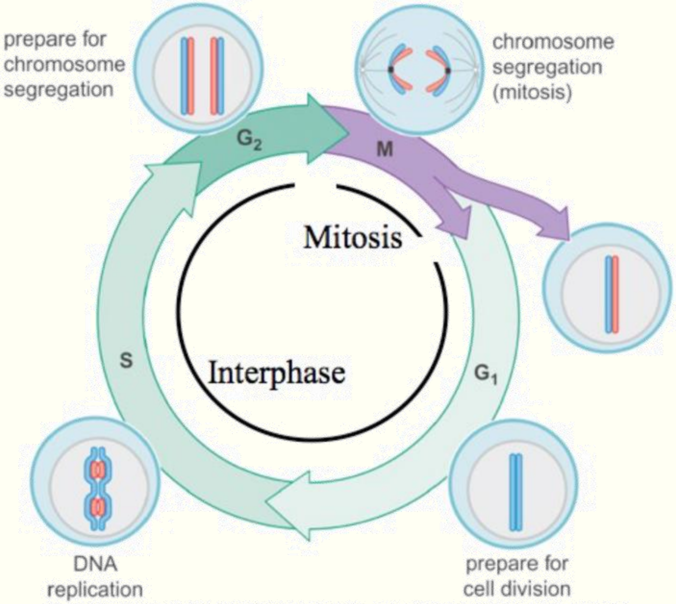
1. packaging DNA.
2. Strengthening DNA for mitosis.
3. Preventing DNA damage.
4. Controlling gene expression and replication.

- **Chromosome** = packaged DNA molecule.

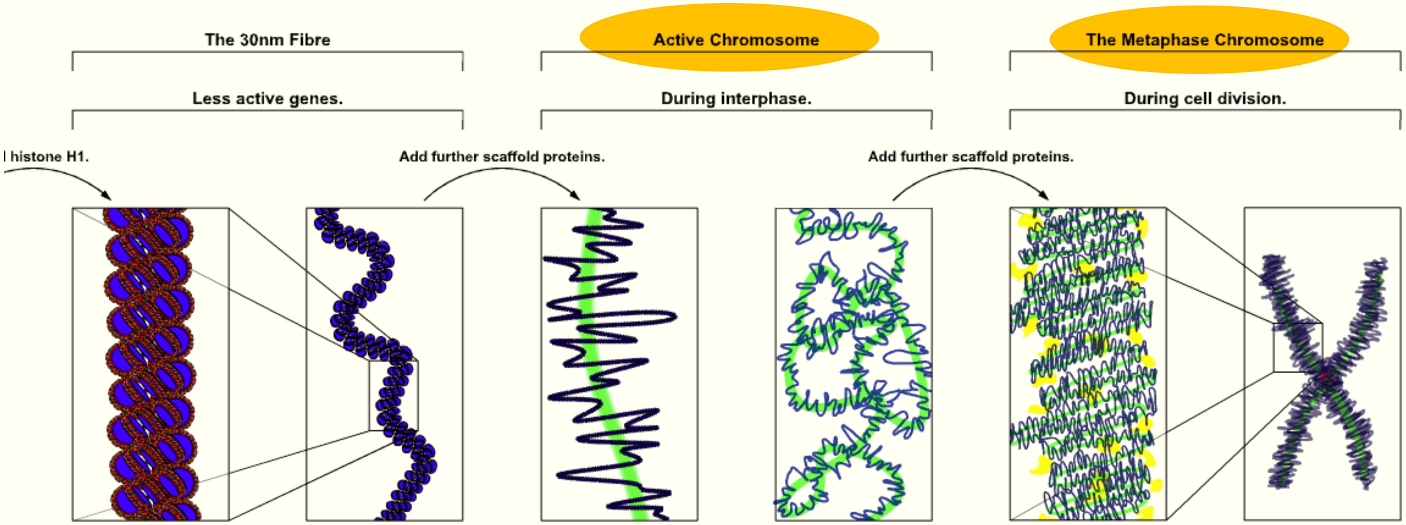
Alberts et al. – Molecular Biology of the cell 2008

The geometry of chromatin depends on cell cycle and gene expression

- **Global structure** depends on cell cycle:
  - During interphase: chromatin is loose to allow access to RNA and DNA polymerases.
- **Local structure** of chromatin during interphase depends on genes expression:
  - **Euchromatin =** Regions of actively transcribed genes ("turned on") are loosely packaged.
  - **Heterochromatin** = regions of inactive genes ("turned off") are more condensed.
- chemical modification of histones also affects packaging.



Copyright © 2004 Pearson Education, Inc., publishing as Benjamin Cummings

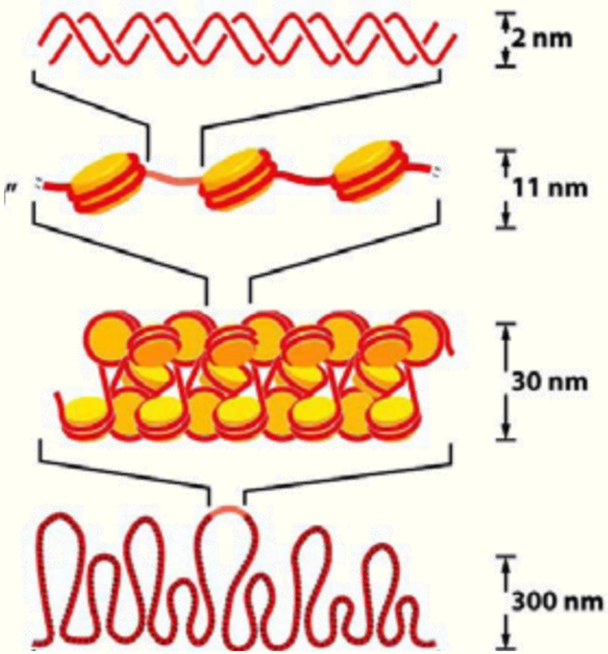


Chromosomes are long DNA polymers

- **Polymers** = long molecules dominated by coiling and flexibility.

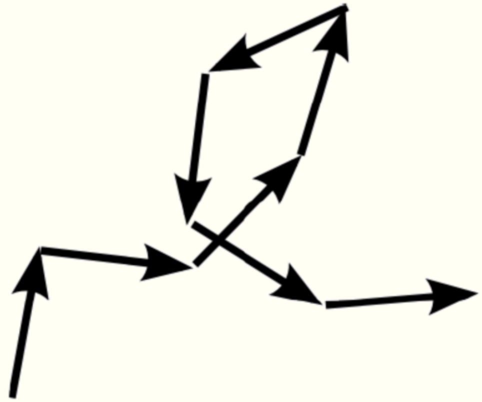
- Persistence length **a** = the scale of the random walk.
- For dsDNA: **a** = 50 nm = 150 bp
- For chromatin fiber: **a** = 150 nm (rough estimate).

- Density in 30-nm fiber ~ 100 bp/nm

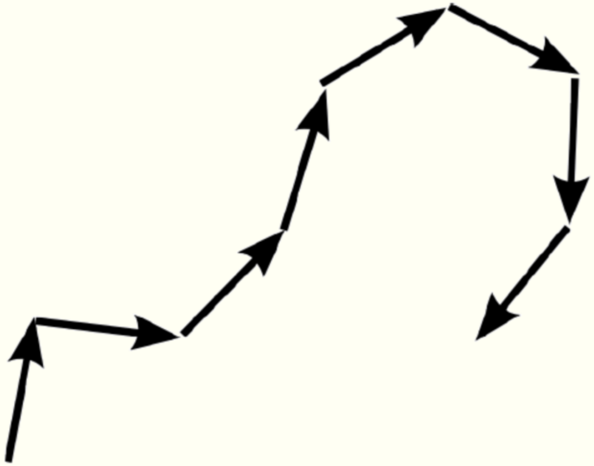


Basics of polymer physics: a crash course

- Random walk (“ideal”): $R \sim a\, s^{1/2}$



- Self-avoiding walk: $R \sim a\, s^{3/5}$



M . Kardar MIT : http://www.mit.edu/~kardar/teaching/IITS/lectures/lec6/AG-compacts.pdf

In equilibrium polymer “melts” the chains are ideal

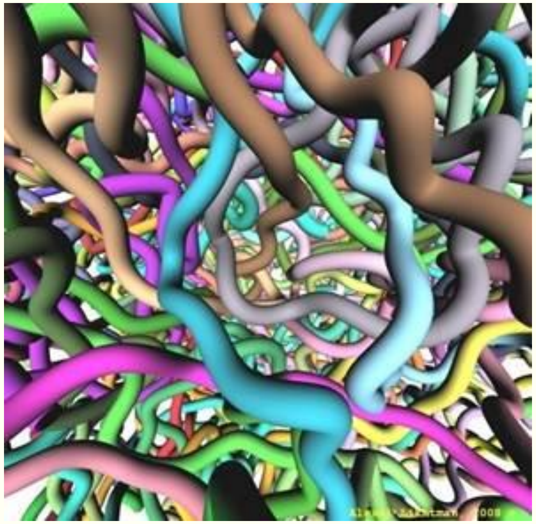
- Inside dense globule:
- Self-repulsion is screened.

→ Density of monomers ~ const.

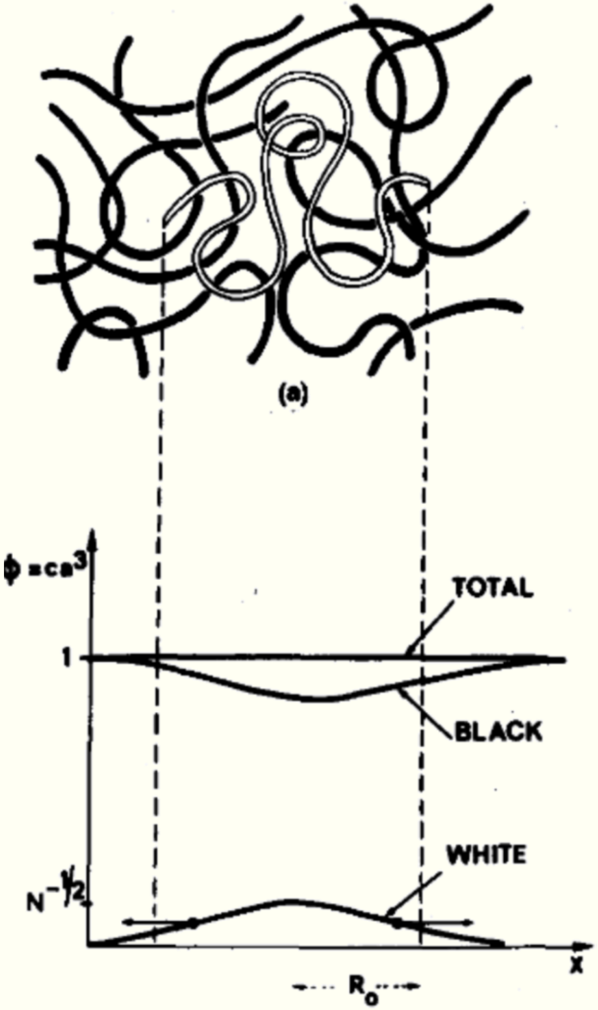
- Scales as ideal random walk:

  $R \sim a\, s^{1/2}$

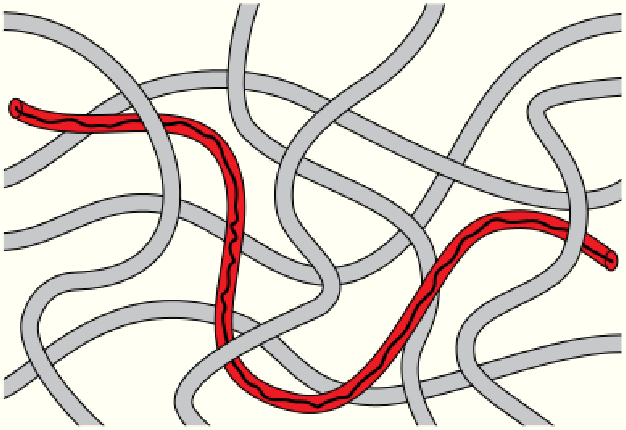
- Reach equilibrium by reptation



Alexi Likhtman homepage



de Gennes, Scaling concepts in polymer physics, 1979



In equilibrium globule chains are intertwined and knotted

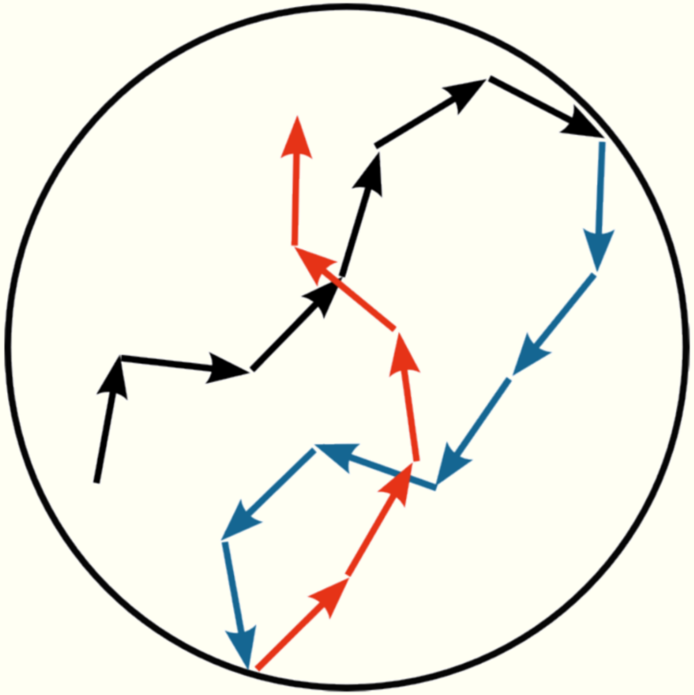
- Globule size: $R_{\max} \sim a\, N^{1/3}$.

- Inside globule ($R < R_{\max}$ or $s < N^{2/3}$):

$$R \sim a\, s^{1/2}.$$

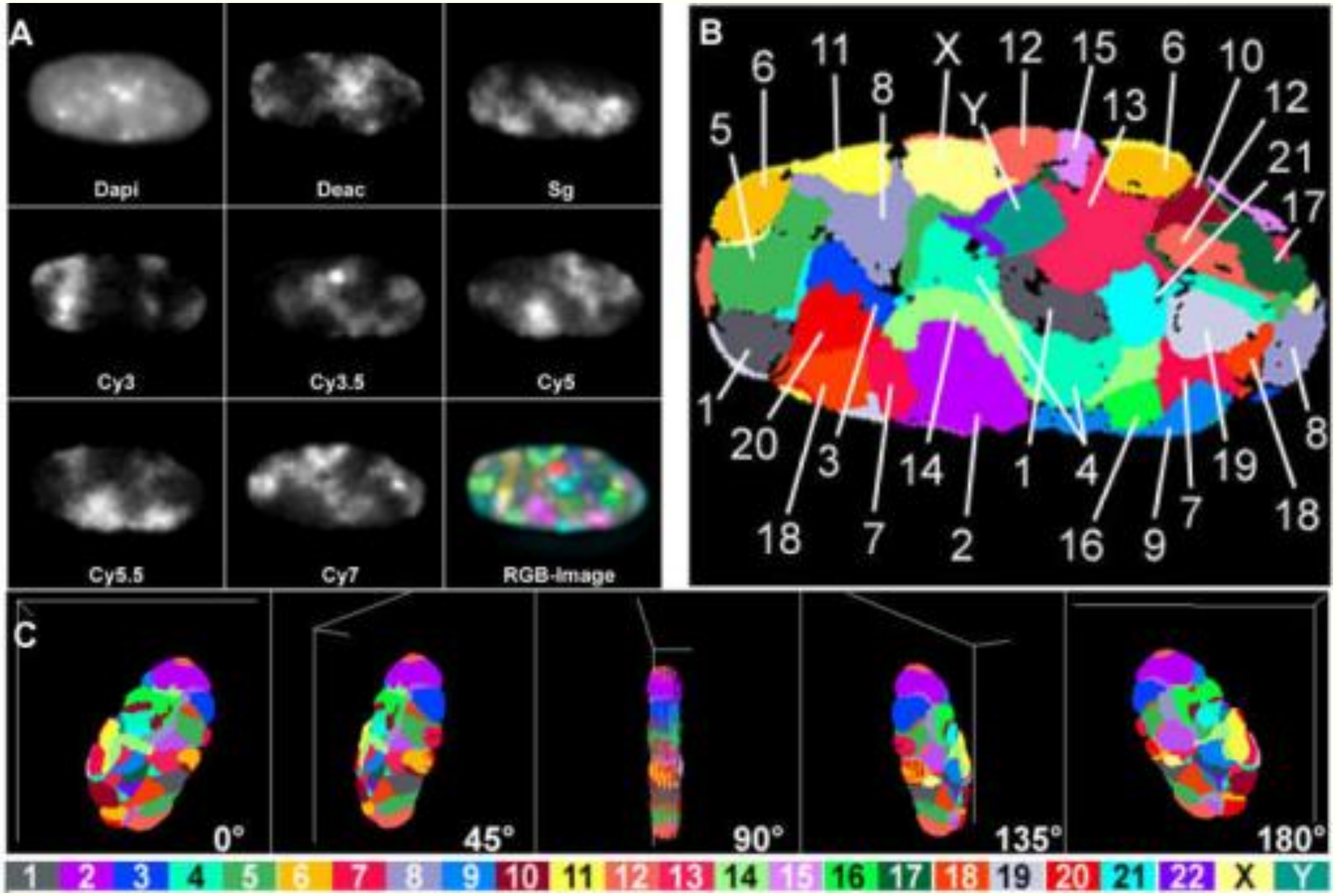
- Longer scales ($R > R_{\max}$ or $s > N^{2/3}$):

$$R \sim R_{\max}$$



From Sam Safran, Physics soft condensed matter: Stat Mech II

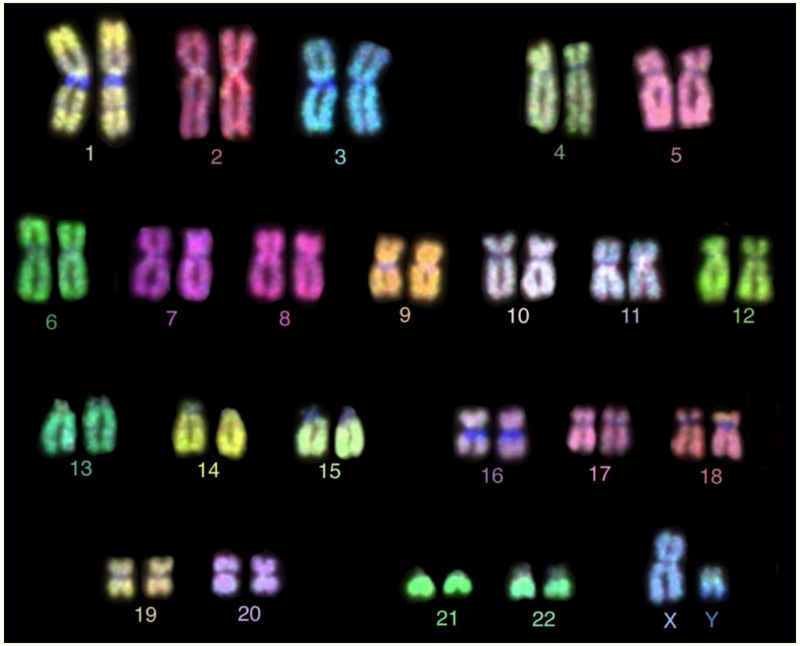
But chromosomes are territorial: each chromosome occupies a distinct region in the nucleus



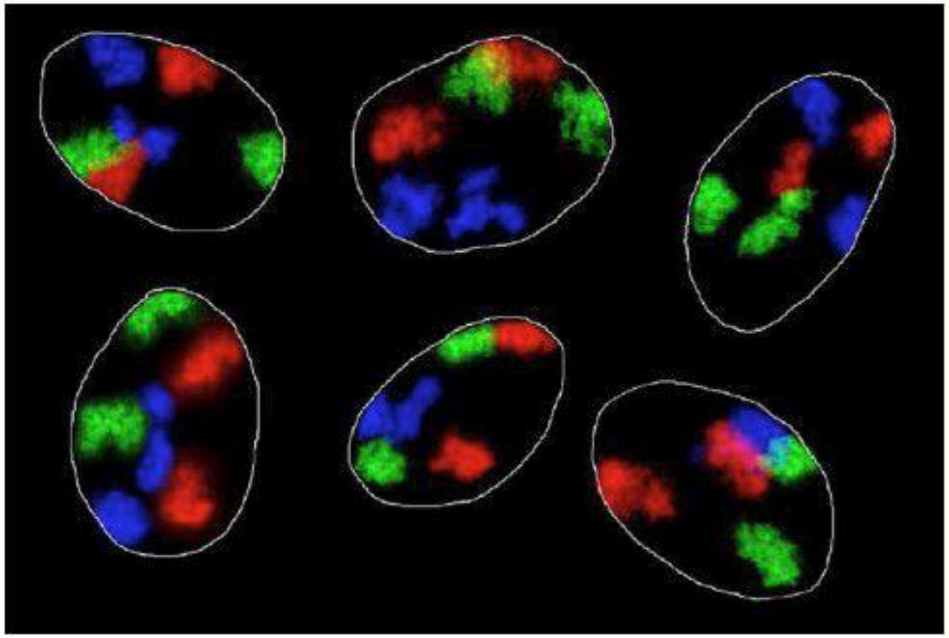
TA.Bolzer, G.Kreth, I.Solovei, D.Koehler, K.Saracoglu, C.Fauth, S.Muller, R.Eils, C.Cremer, MR.Specher, PLoSBiol. 2005

Interphase chromosomes spread into territories

Mitotic Chromosomes



Interphase Chromosomes

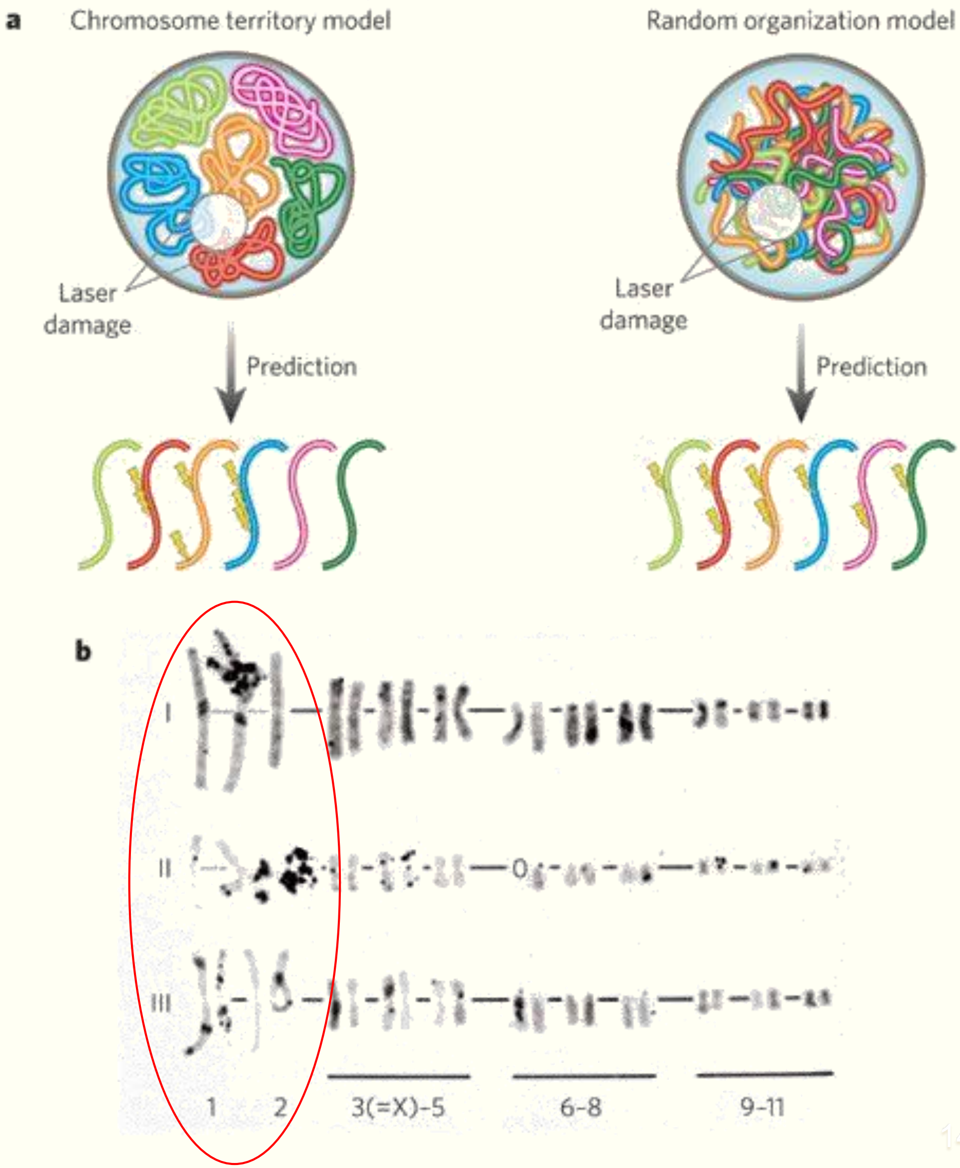


Chromosome territories in HeLa cells (3, 5, 11)

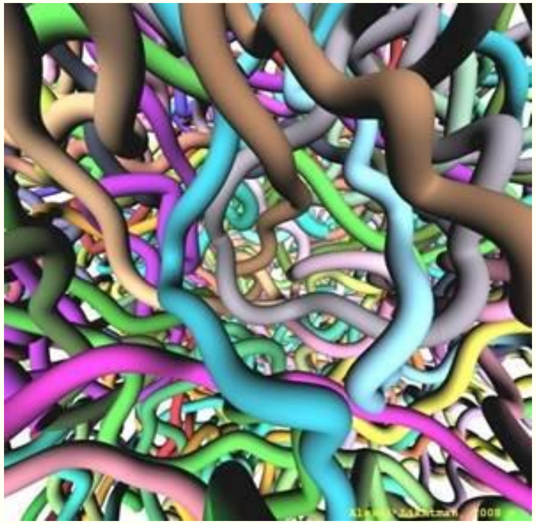
Foster&Bridger 2005

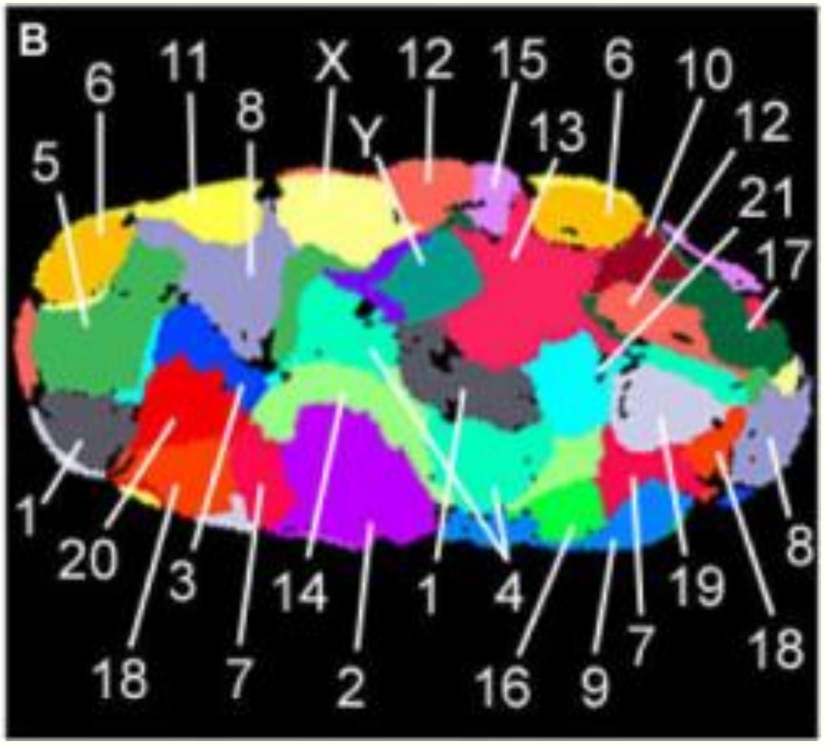
Chromosome territories were discovered by measuring the spread of laser damage

- Old idea: Rabl and Boveri suggested 'chromosome territories' ~100 years ago.
- CTs were demonstrated in the early 1980s in micro-laser experiments by the brothers Thomas and Christoph Cremer.



Chromosome territories contradict the equlibirium globule picture (mixed pasta)

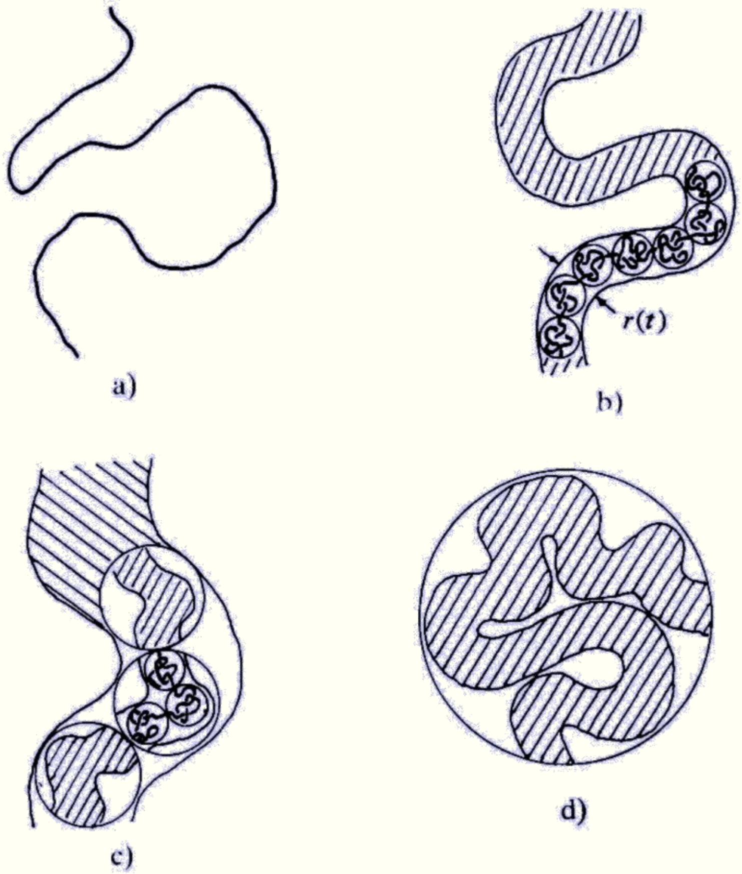


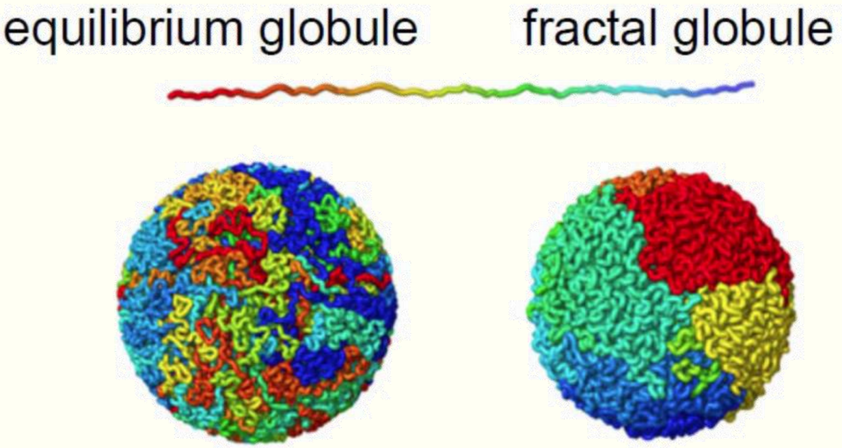


» **What happens if the reptation is very slow?**

Crumpled (fractal) globule is a long-lived non-equilibrium state of a collapsed polymer

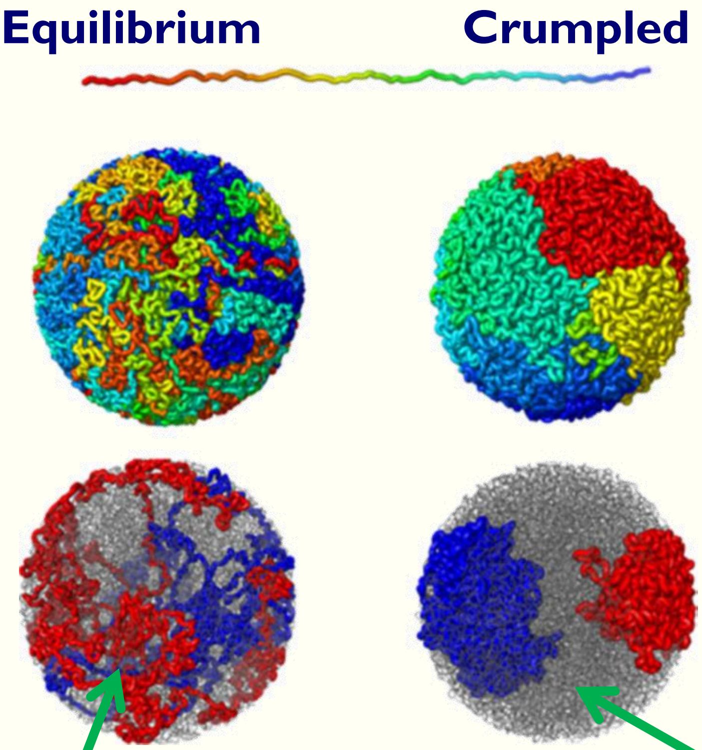
Fast collapse: no time to reptate…

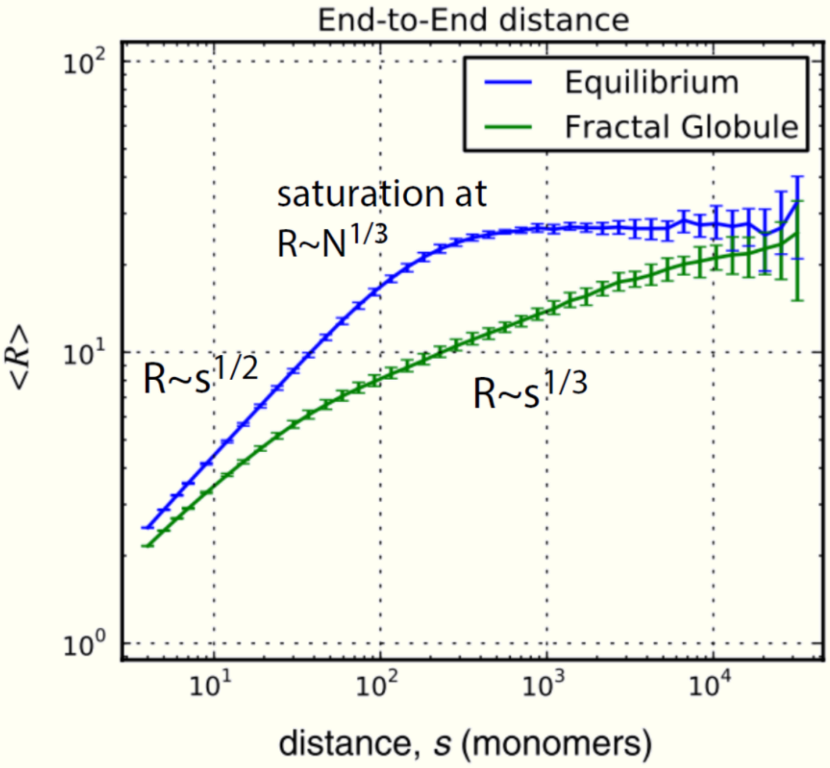




Grosberg, Nechaev, Shakhnovich, *J de Physique*, 1988.

Chromatin is territorial like the crumpled globule



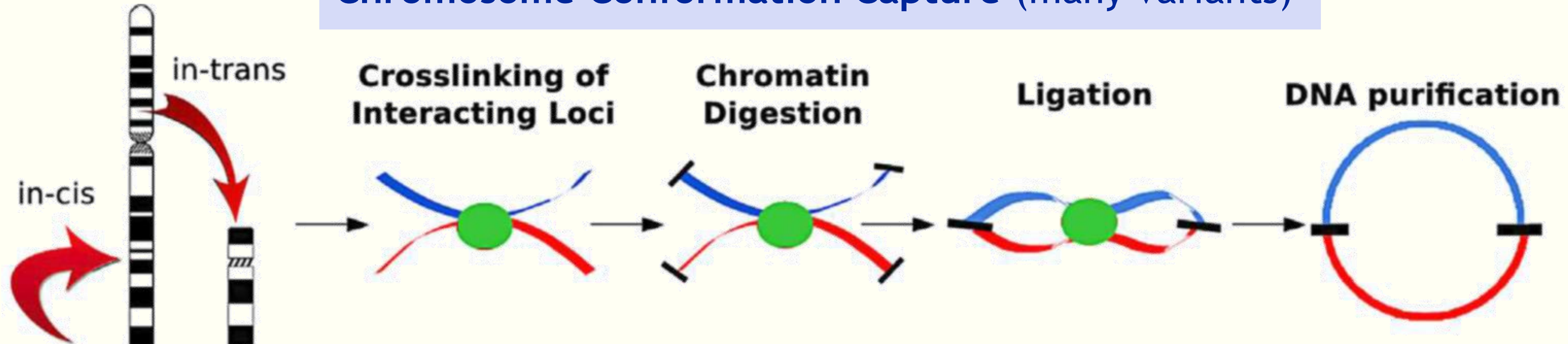


- Scale R_{max}
- Knots
- Screening
- Intertwining, $P(s) \sim s^{-3/2}$

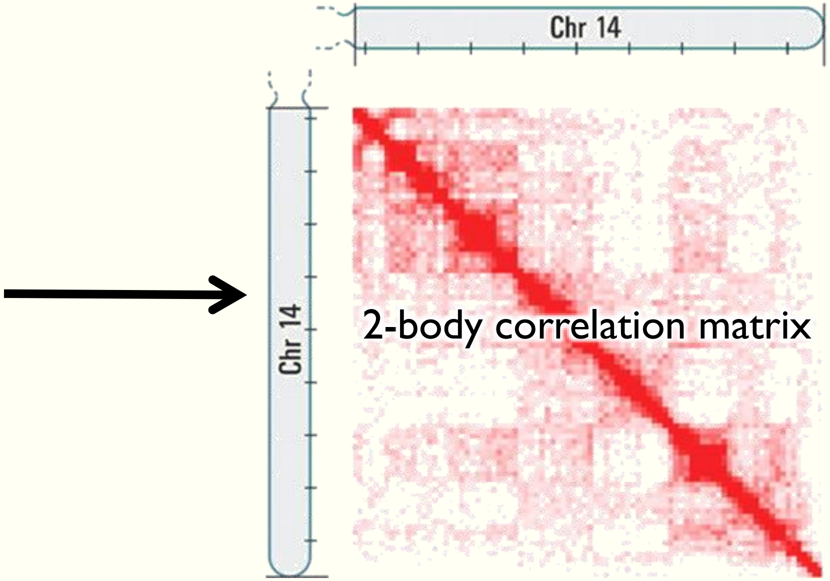
- Self-similar, fractal $R(s) \sim s^{1/3}$
- No knots.
- No screening
- Local, **territorial,** $P(s) \sim 1/R(s)^3 \sim 1/s$

DNA sequence provides natural coordinate for measuring two-body spatial correlations in the chromosome

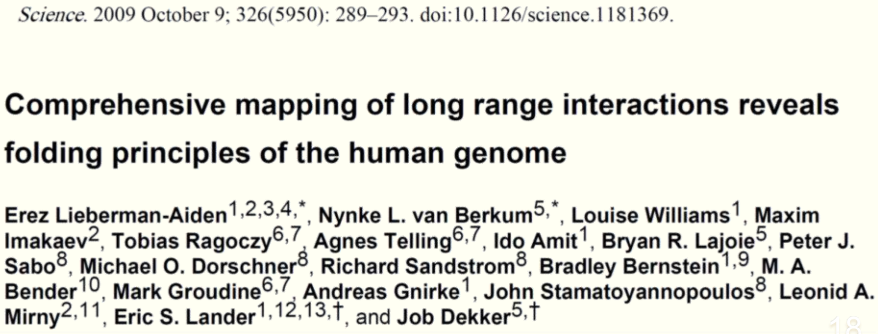
Chromosome Conformation Capture (many variants)



Barutco et al. , J Cell Phys 2016



2-body correlation matrix

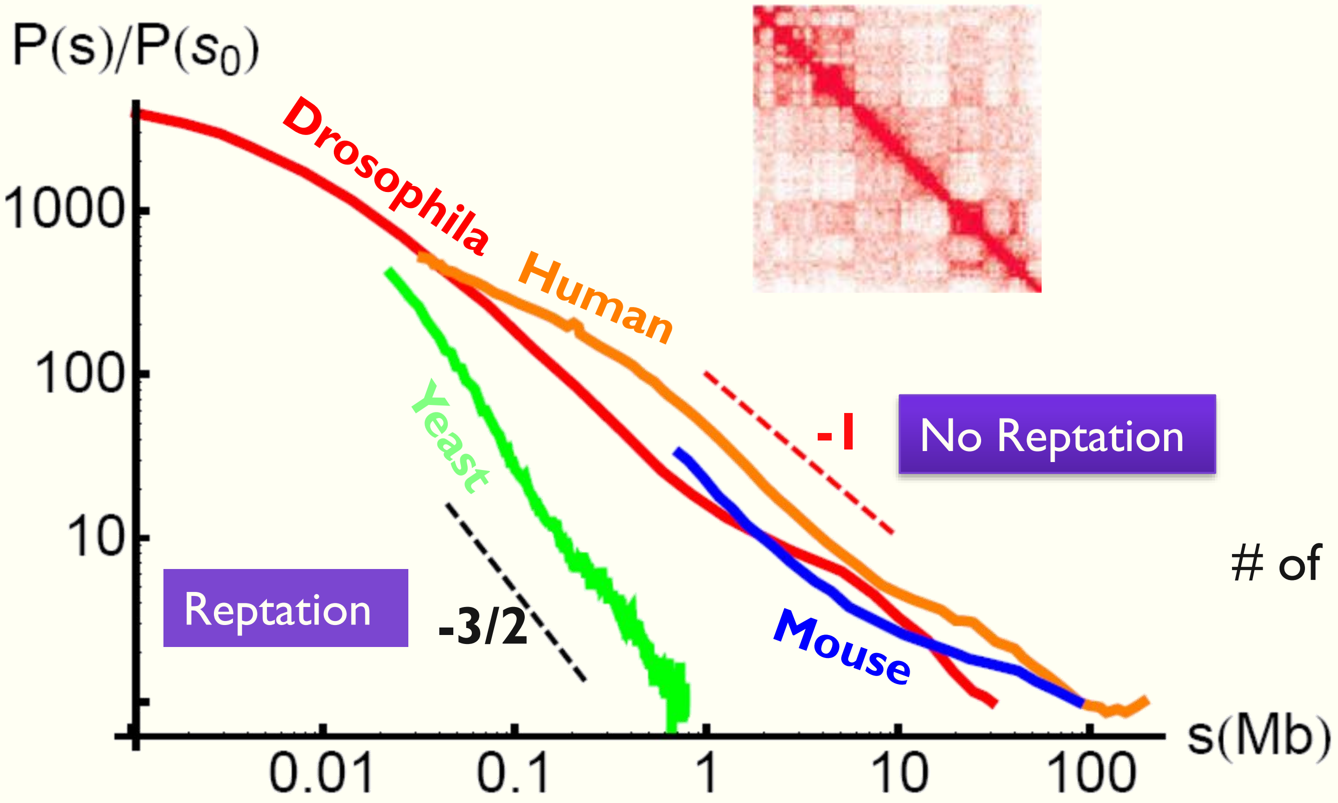


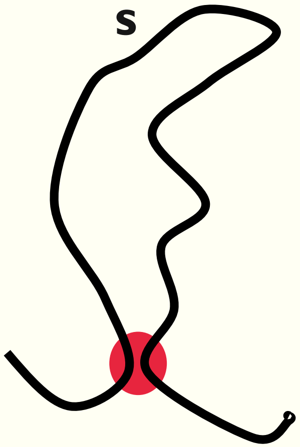
Science. 2009 October 9; 326(5950): 289–293. doi:10.1126/science.1181369.

Comprehensive mapping of long range interactions reveals folding principles of the human genome

Erez Lieberman-Aiden[1,2,3,4,*], Nynke L. van Berkum[5,*], Louise Williams[1], Maxim Imakaev[2], Tobias Ragoczy[6,7], Agnes Telling[6,7], Ido Amit[1], Bryan R. Lajoie[5], Peter J. Sabo[8], Michael O. Dorschner[8], Richard Sandstrom[8], Bradley Bernstein[1,9], M. A. Bender[10], Mark Groudine[6,7], Andreas Gnirke[1], John Stamatoyannopoulos[8], Leonid A. Mirny[2,11], Eric S. Lander[1,12,13,†], and Job Dekker[5,†]

Chromosome Conformation Capture reveals the scaling of the 2-body correlation (meeting probability of loci)



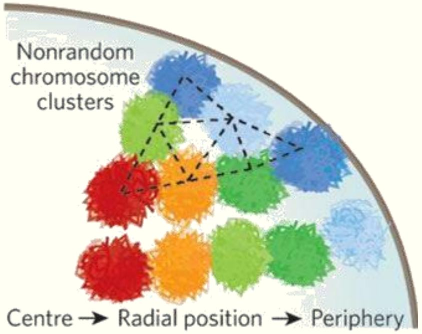


# of neighbors = Σ_s P(s)

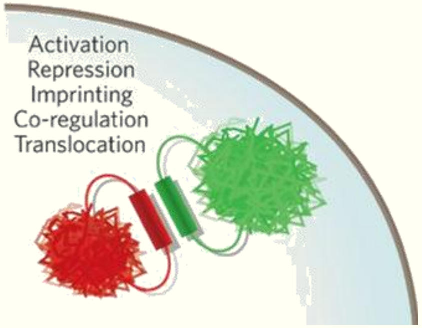
- Alexander Grosberg, *Topology in physics of polymers and biopolymers*, data from J. Dekker.

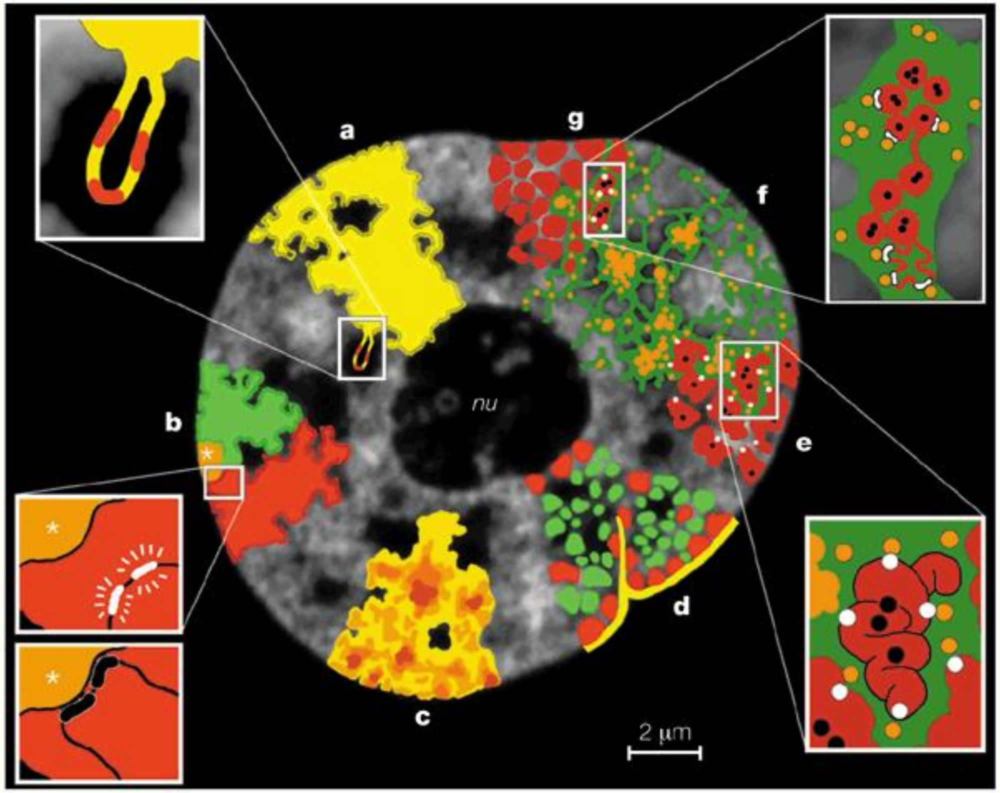
Chromosome Territories are units of nuclear organization with preferred position in the nucleus

- Non-random neighbors: to facilitate proper gene expression.
- Variability between cell types.



- Complex folded surface: active genes extend into the inter-chromatin space.



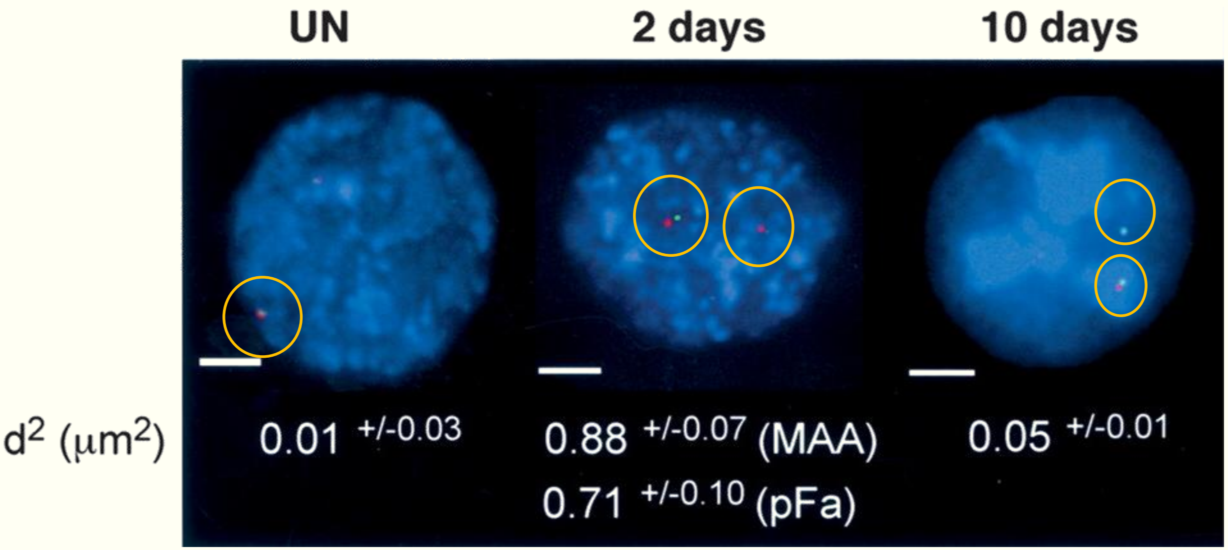


Nature Reviews | Genetics

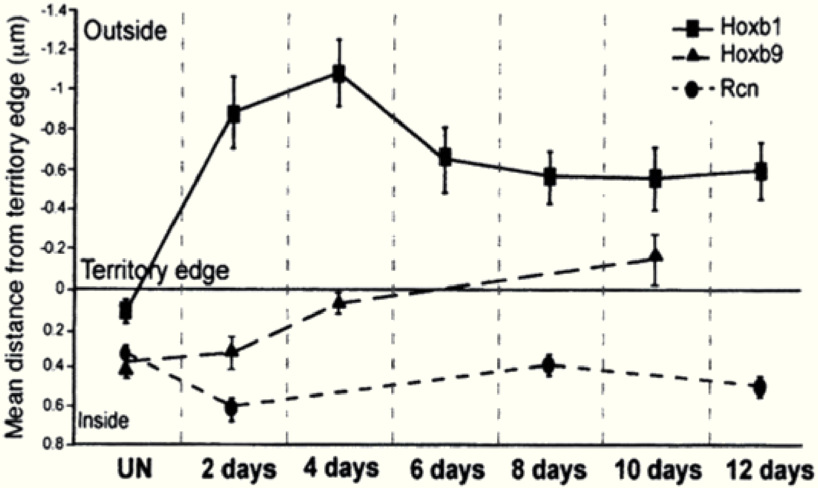
Cremer & Cremer 2001

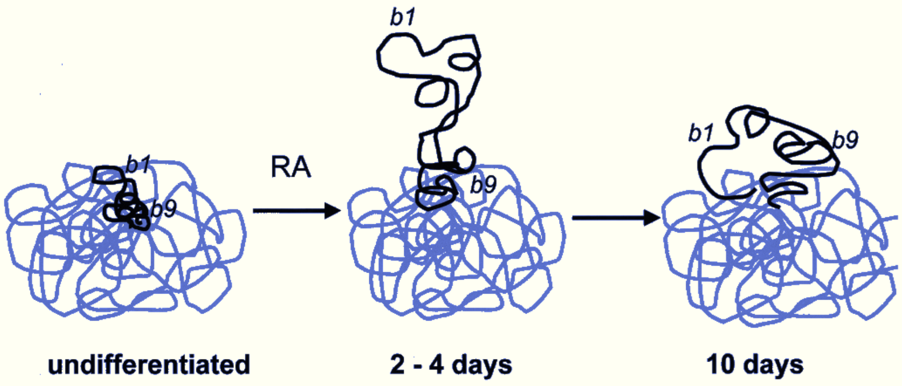
Chromosome territories are dynamic structures that change following gene activity

- Example: the movement of Hoxb1 and Hoxb9 as development progresses.

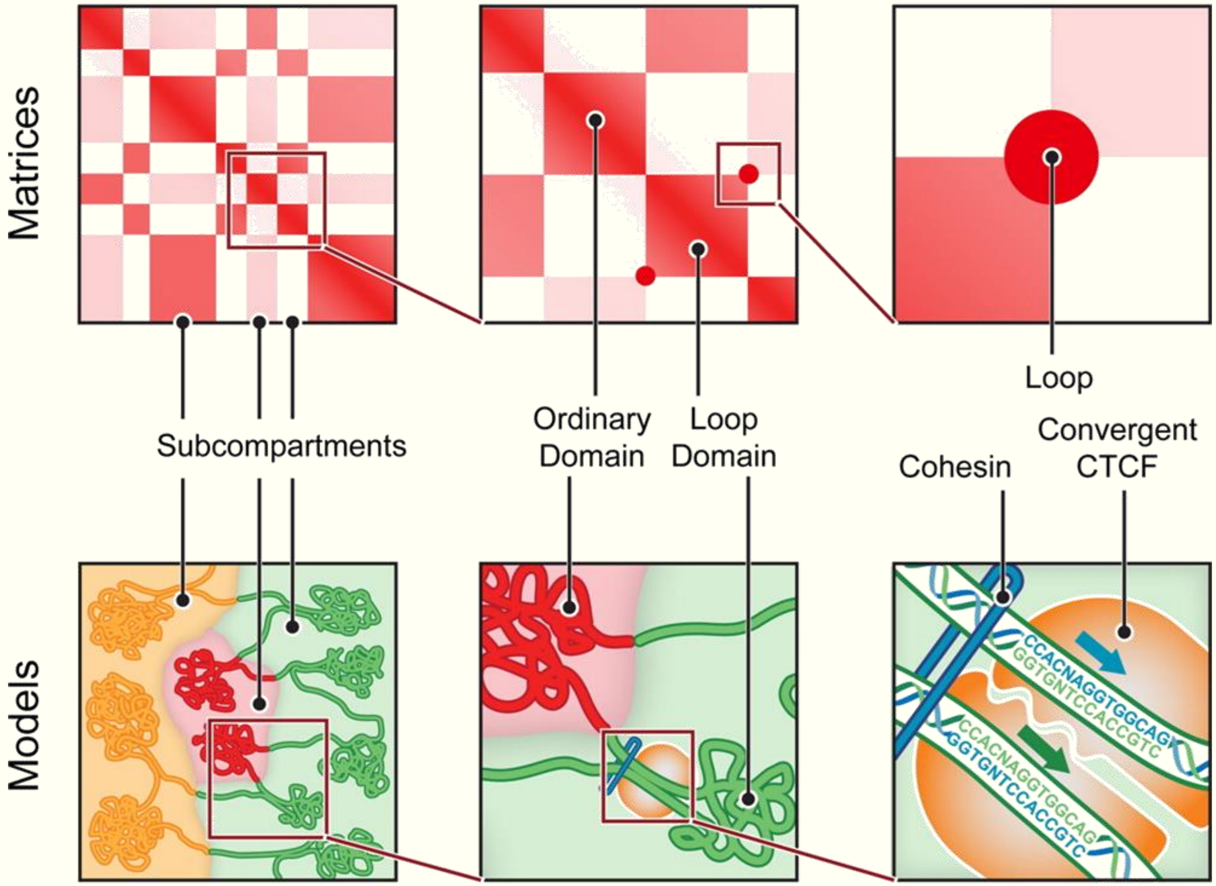


Chamberyon & Bickmore, Gen Dev 2004





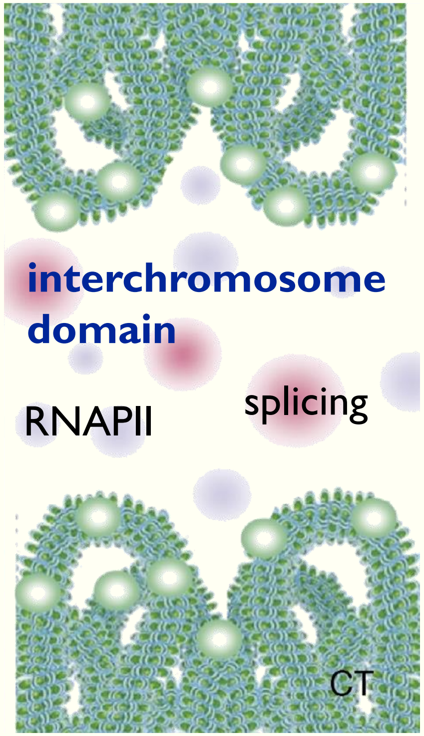
Hi-C measurements at kilo-bp resolution reveals multiscale organization, in particular chromatin looping



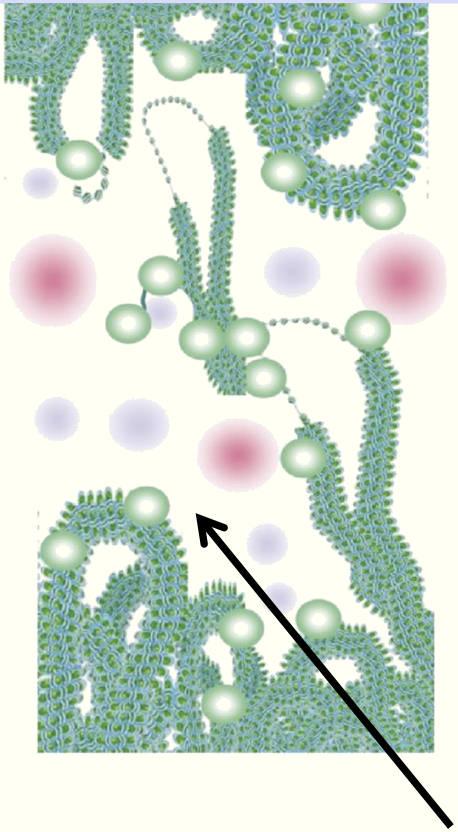
Suhas et al. Cell 2014

The inter-chromatin domain is full of activity: three models

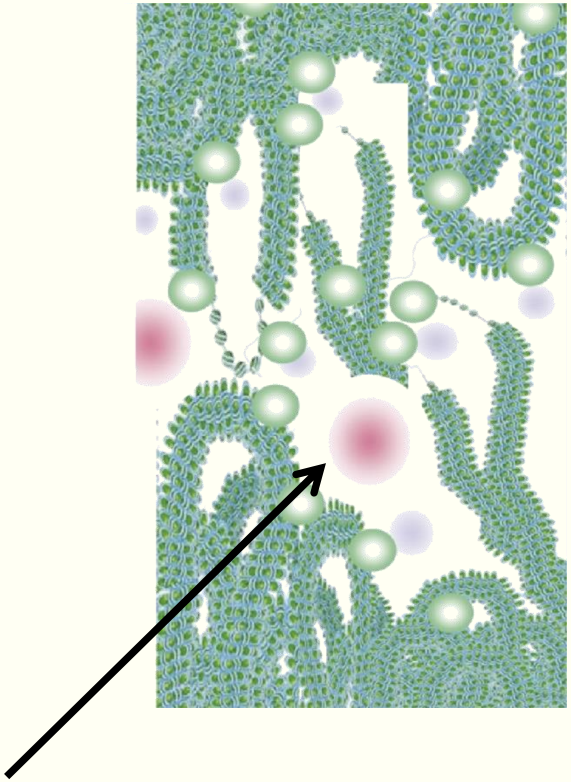
Interchromosome domain: active genes at CT surface.



Inter-chromatin compartment: CT surface loops into ICD for better access of transcription machinery



Lattice model: extensive intermingling of chromatin fibers of adjacent CTs.

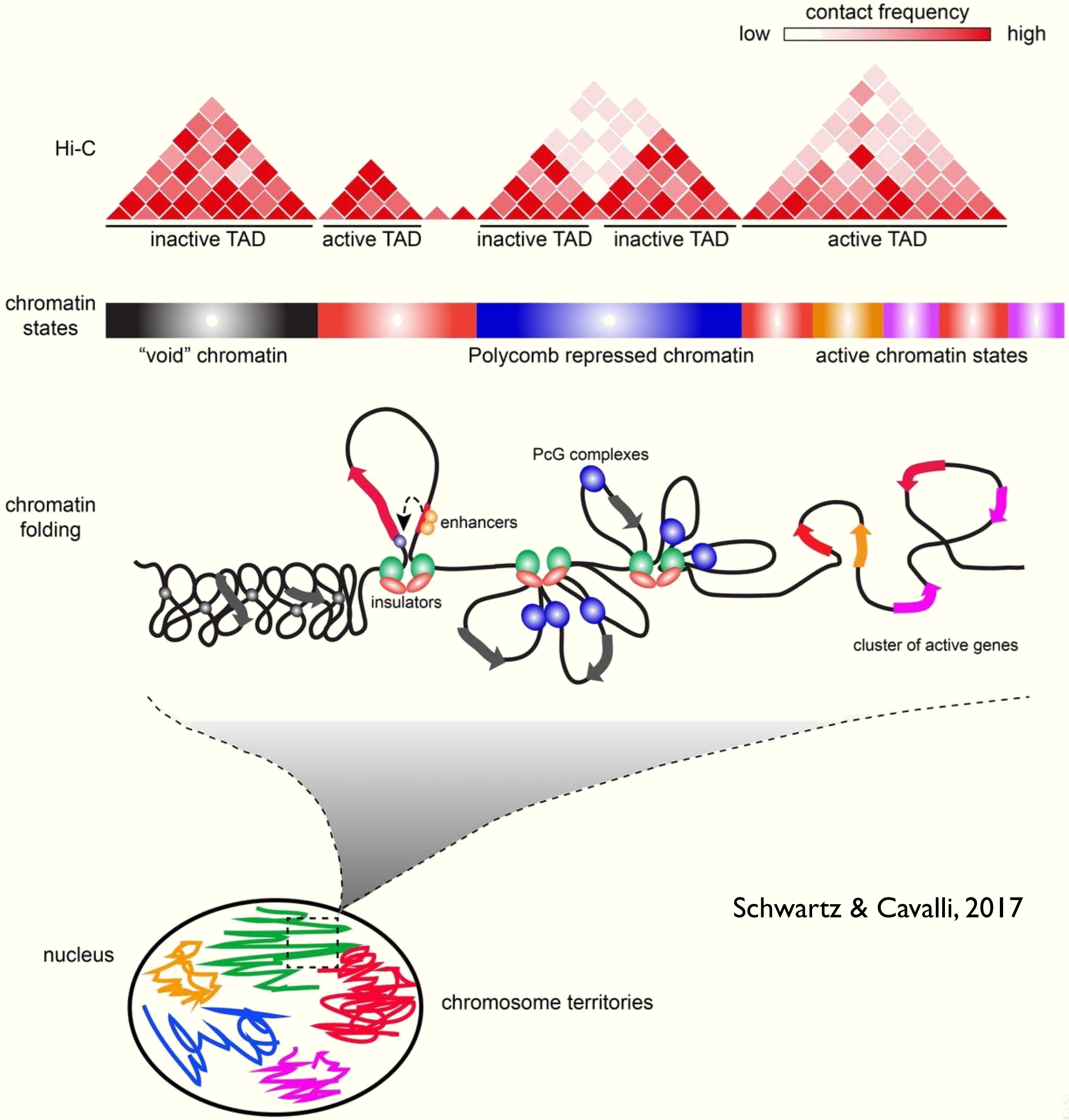


genes from different CTs **co-localize** with transcription factories

Heard & Bickmore 2007

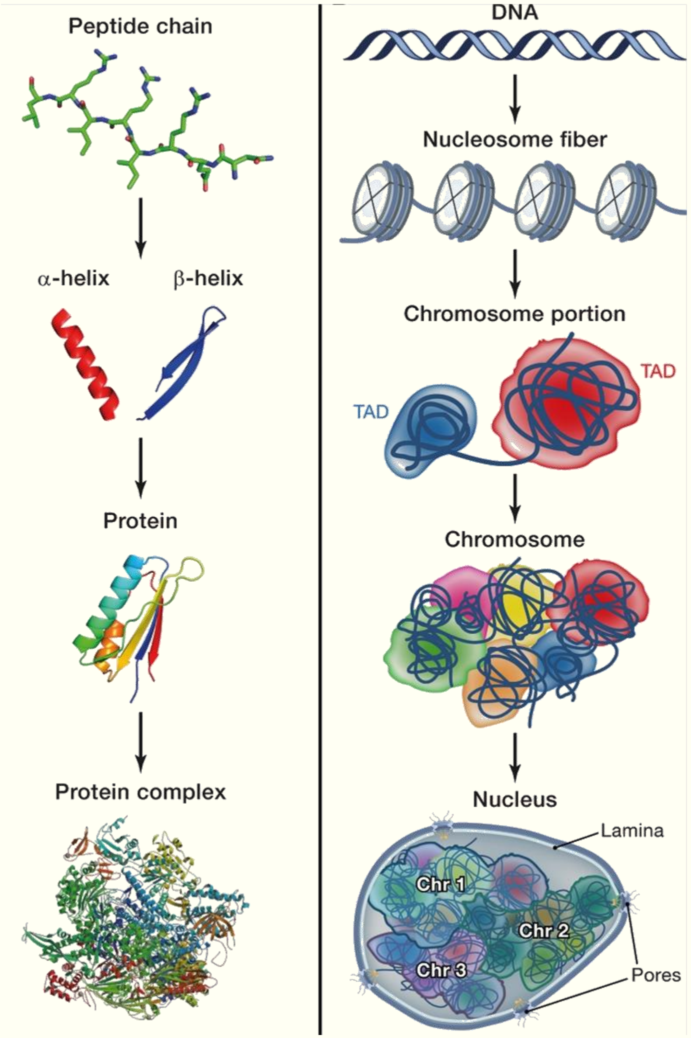
Topologically Associating Domains (TAD) are subunits of chromosome organization

- **TADs** = domains of chromatin fibers with different degrees of folding and contacts.
- TADs are determined by DNA composition and transcriptional activity.
- Void and repressed chromatin has more contacts than chromatin of active genes.
- TAD partition correlates with histone modifications.

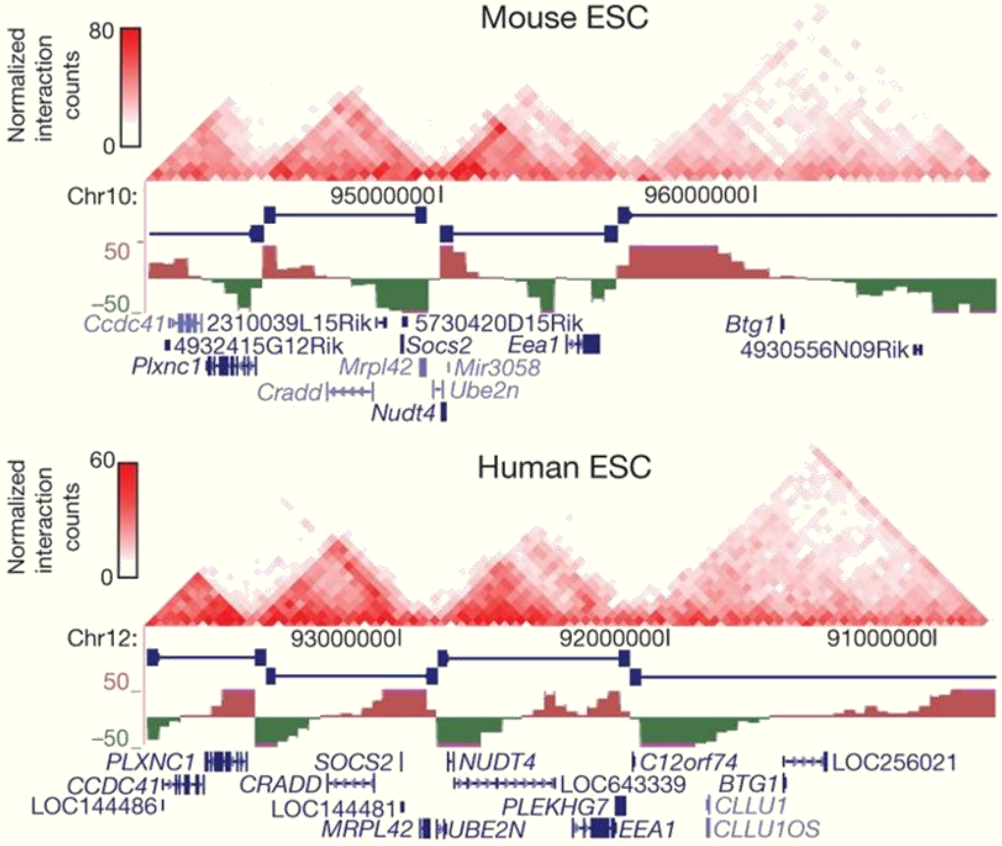


Schwartz & Cavalli, 2017

TADs define hierarchical organization of genome that is conserved across species



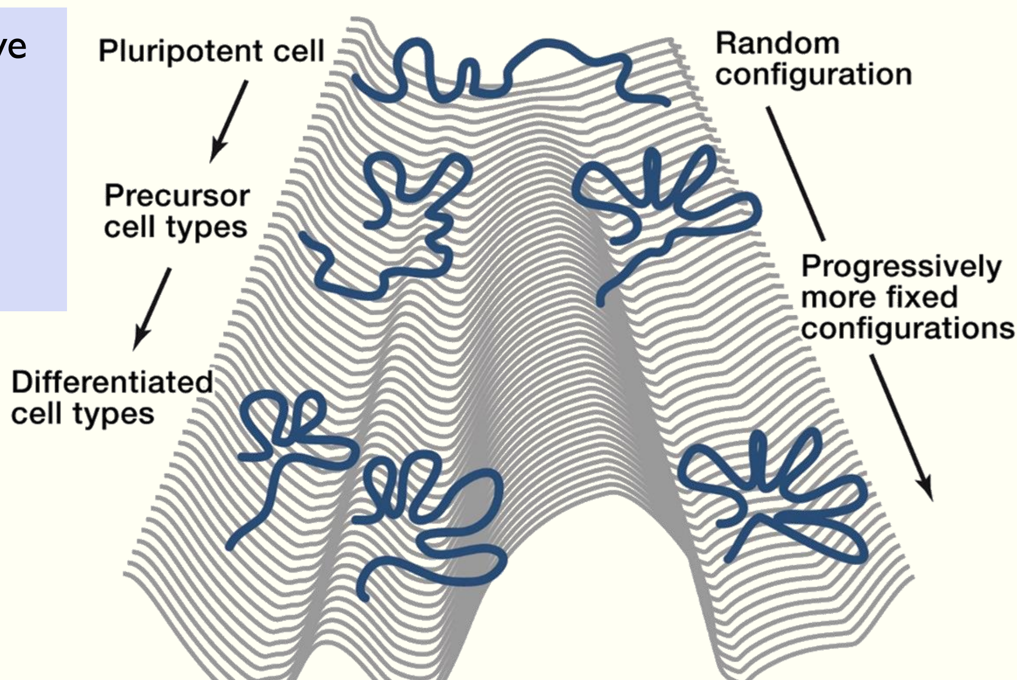
Sexton and Cavalli, Cell 2015



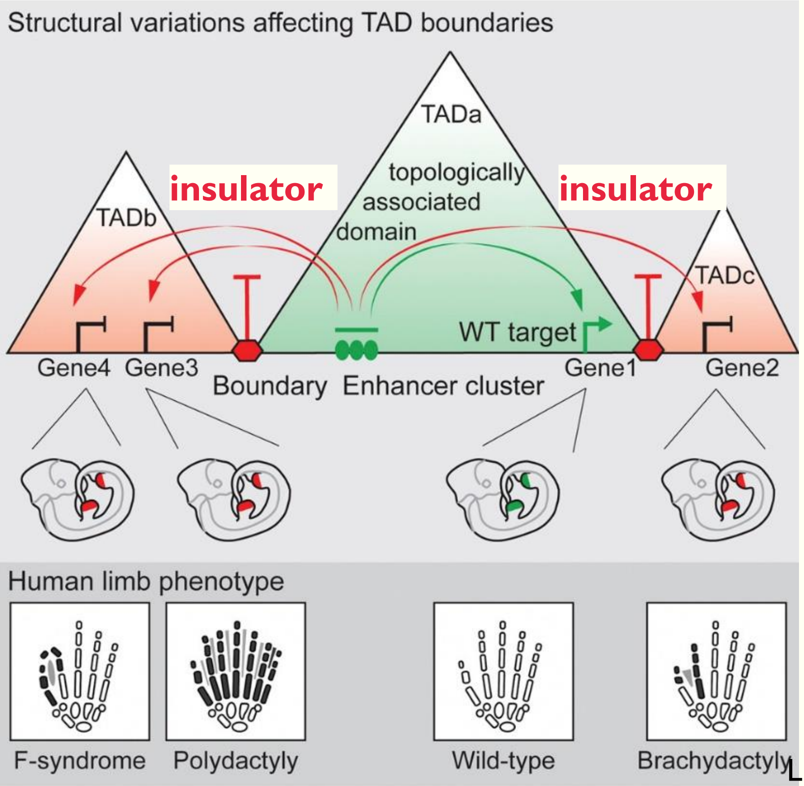
Dixon et al. , Nature 2012

Chromatin loop configurations change through development

- Pluripotent cells able to form any lineage have unstructured local chromatin topologies.
- Progressive lineage restriction throughout development accompanied by progressive constraint of chromatin loop topologies.



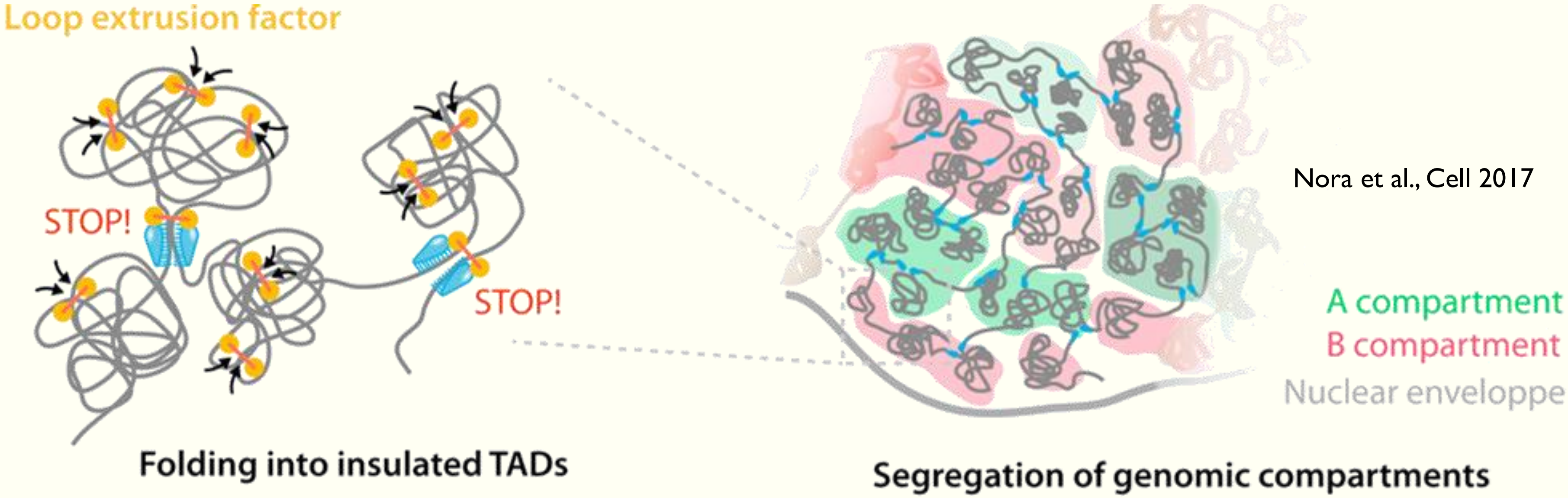
Sexton and Cavalli, Cell 2015



Lupianez et al. Cell 2015

- Disruptions of TADs lead to de novo enhancer-promoter interactions, misexpression and malformation

Loop extrusion actively self-organizes the TADs



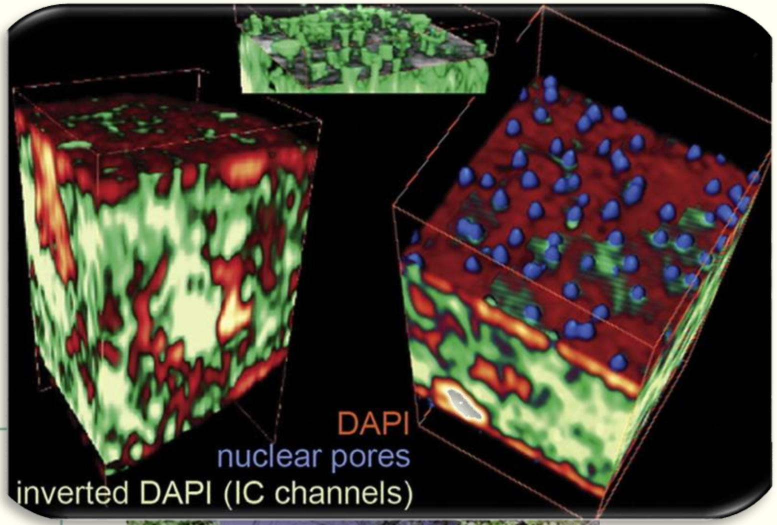
Nora et al., Cell 2017

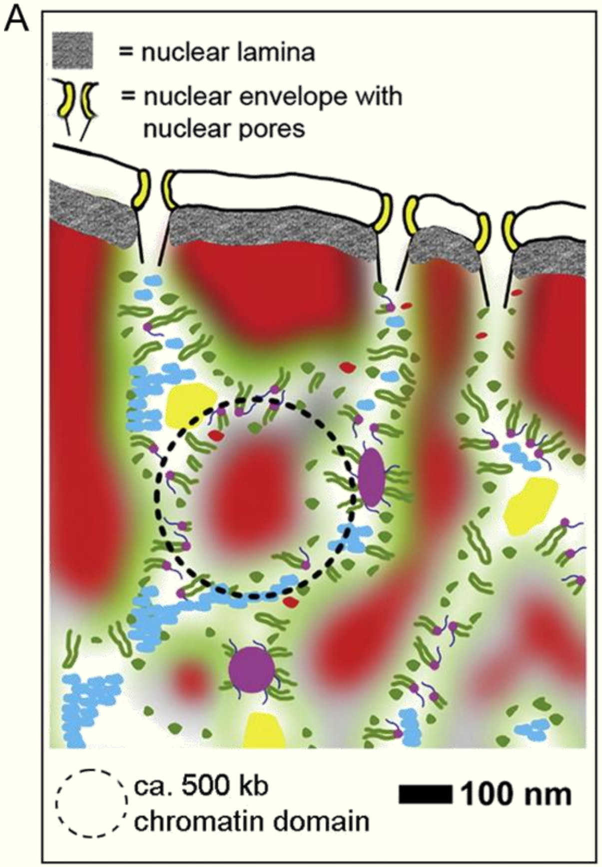
May answer several questions:

- How “insulators” prevent cross-talk among TADs.
- Checkerboard patterns of TADS.
- $R(s) \sim s^x$ $(x<1)$: another scale s_*

Super-resolution microscopy reveals a “marshland” of TADs

- Cremer, Cremer et al., FEBS Lett 2015





active nuclear compartment

ANC

🟩 Transcriptionally competent decondensed chromatin marked by „active“ histone marks

transcriptionally competent chromatin loops,

transcriptionally active chromatin loops

☐ Interchromatin compartment, harboring

● Transcription factories,

🟨 splicing speckles,

🟦 architectural proteins, e.g. CTCF, SAF-A, Matrin

inactive nuclear compartment

INC

🟥 Compacted part of chromatin domain clusters (CDCs) marked by repressive histone marks


1

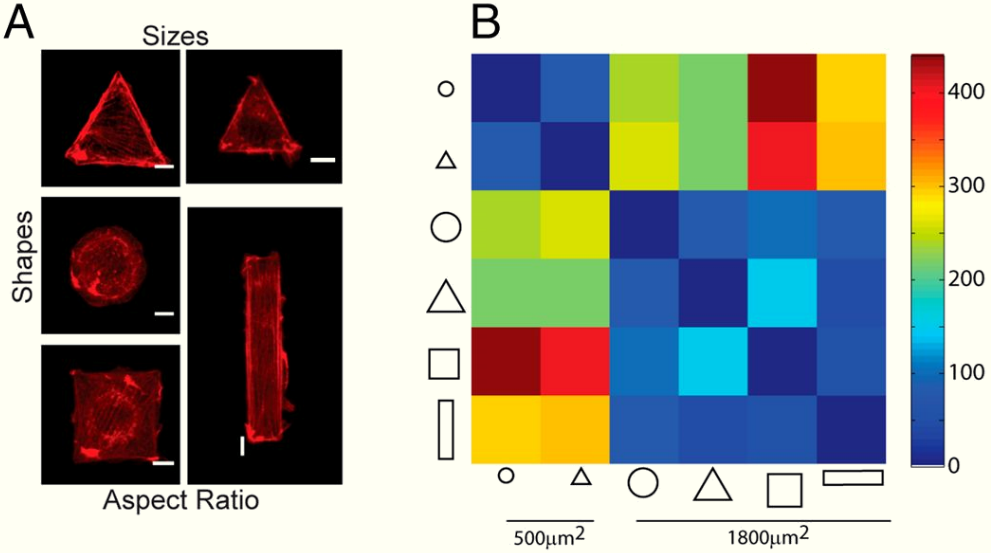

2

Mechanical forces may affect cell function

- Biological function depends on geometry.
- Physical forces may alter the geometry.

→ External forces may change gene expression, development and function.

Input Signals
(Cell geometry) ⟷ Output Signals
(Gene Expression)



differentially regulated genes among cells of different geometries (size, shape, aspect ratio)

Jain Iyer Kumar & Shivashankar, PNAS 2013

Large-scale organization of DNA in the nucleus

- The packing problem:

What are the basic scales and geometry of cellular DNA?

- What is the underlying physics?
- What are the building blocks of chromosome organization?
- How can physical forces affect biological function?