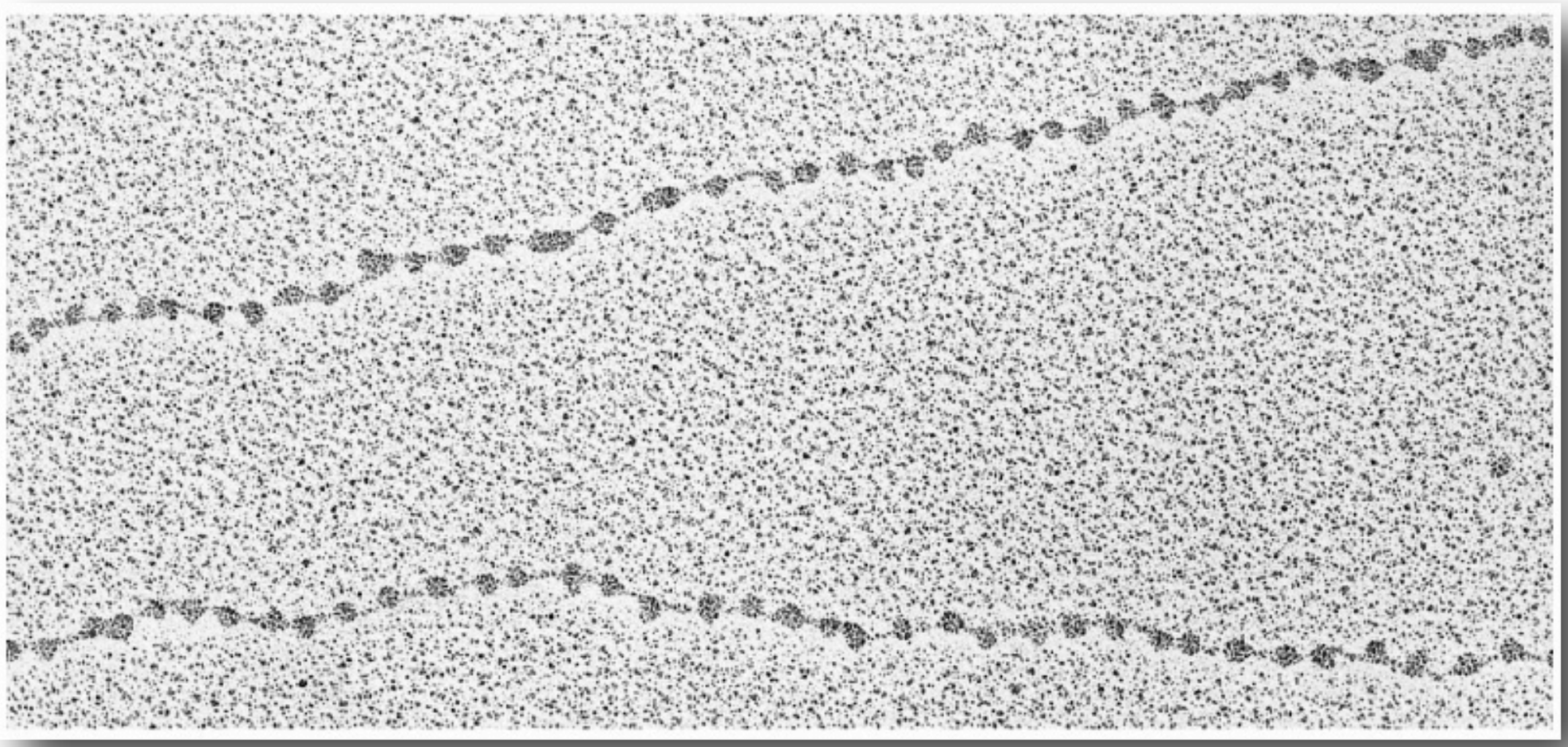
Chromatin organization and dynamics

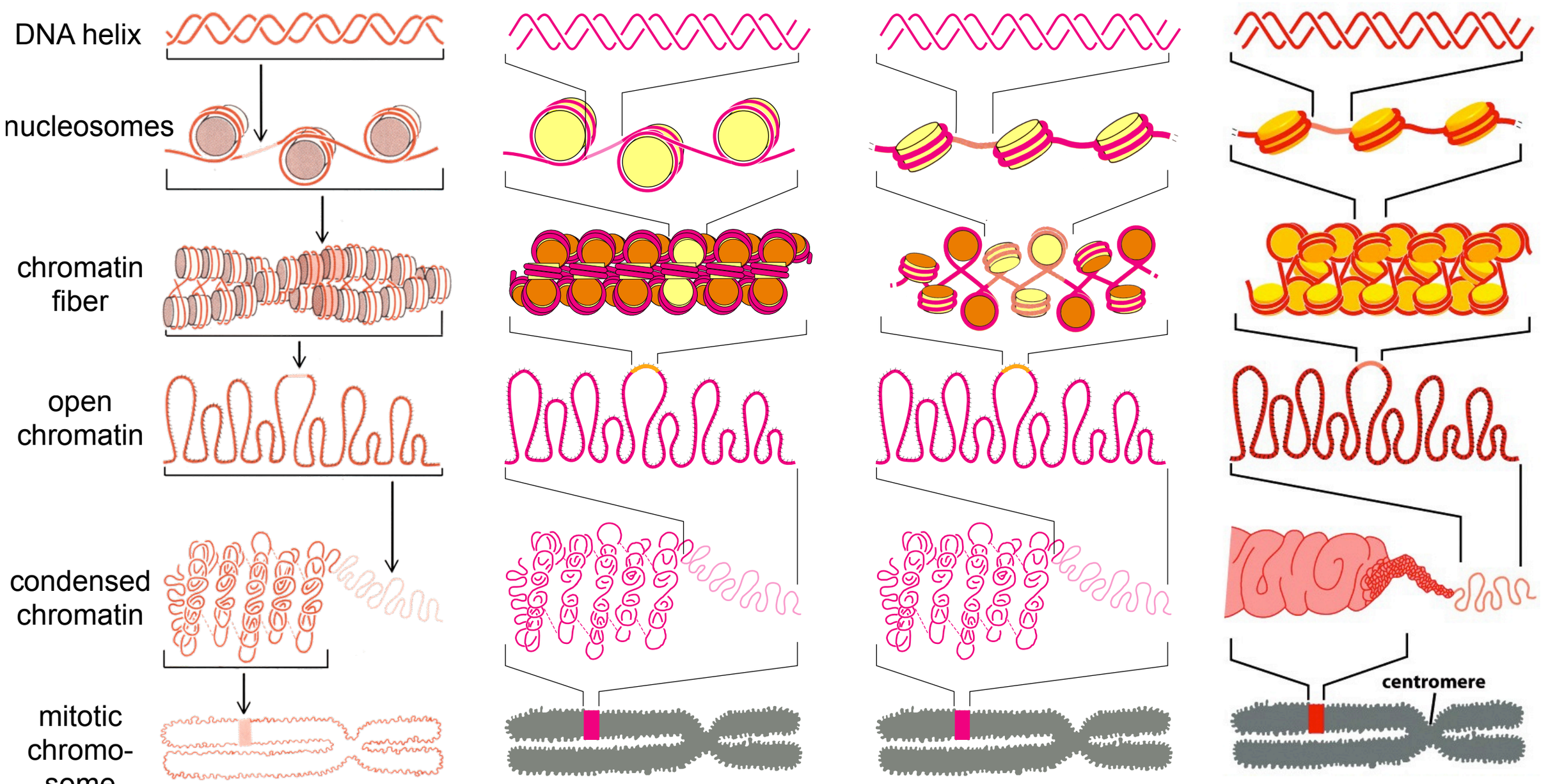
1973: The first visualization of nucleosome chains by electron microscopy



Olins, A. L. and Olins, D. E. (1974) Science 183, 330-332.

Reviewer's comment on the paper Chris Woodcock submitted in 1973:
"I have never read such a naive paper purporting to be of such fundamental significance. Definitly it should not be published anywhere!"

The last 25 years of chromatin structure research from the “Molecular Biology of the Cell” textbook

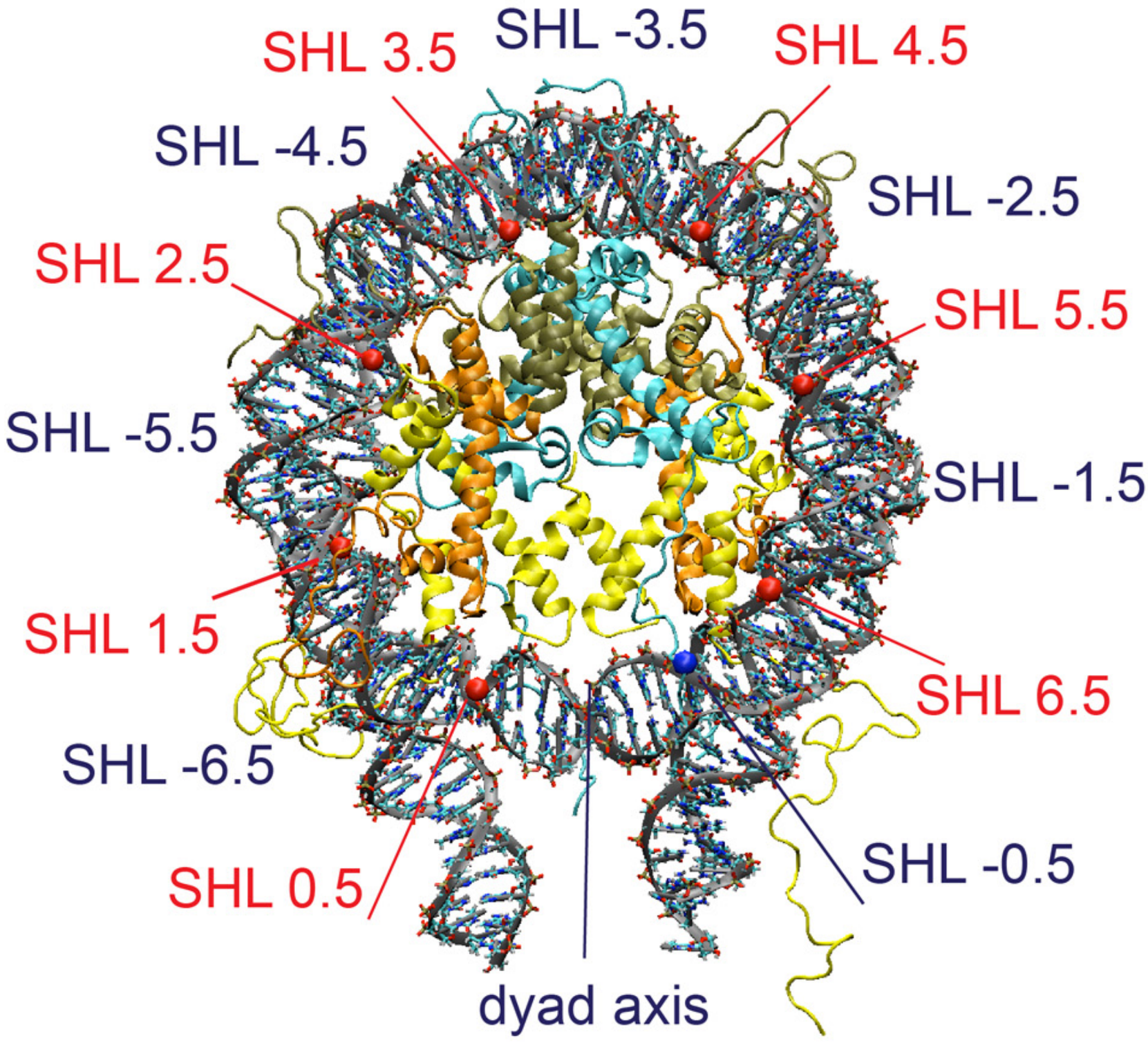


Alberts et al. 1983 | Alberts et al. 1994 | Alberts et al. 2002 | Alberts et al. 2007

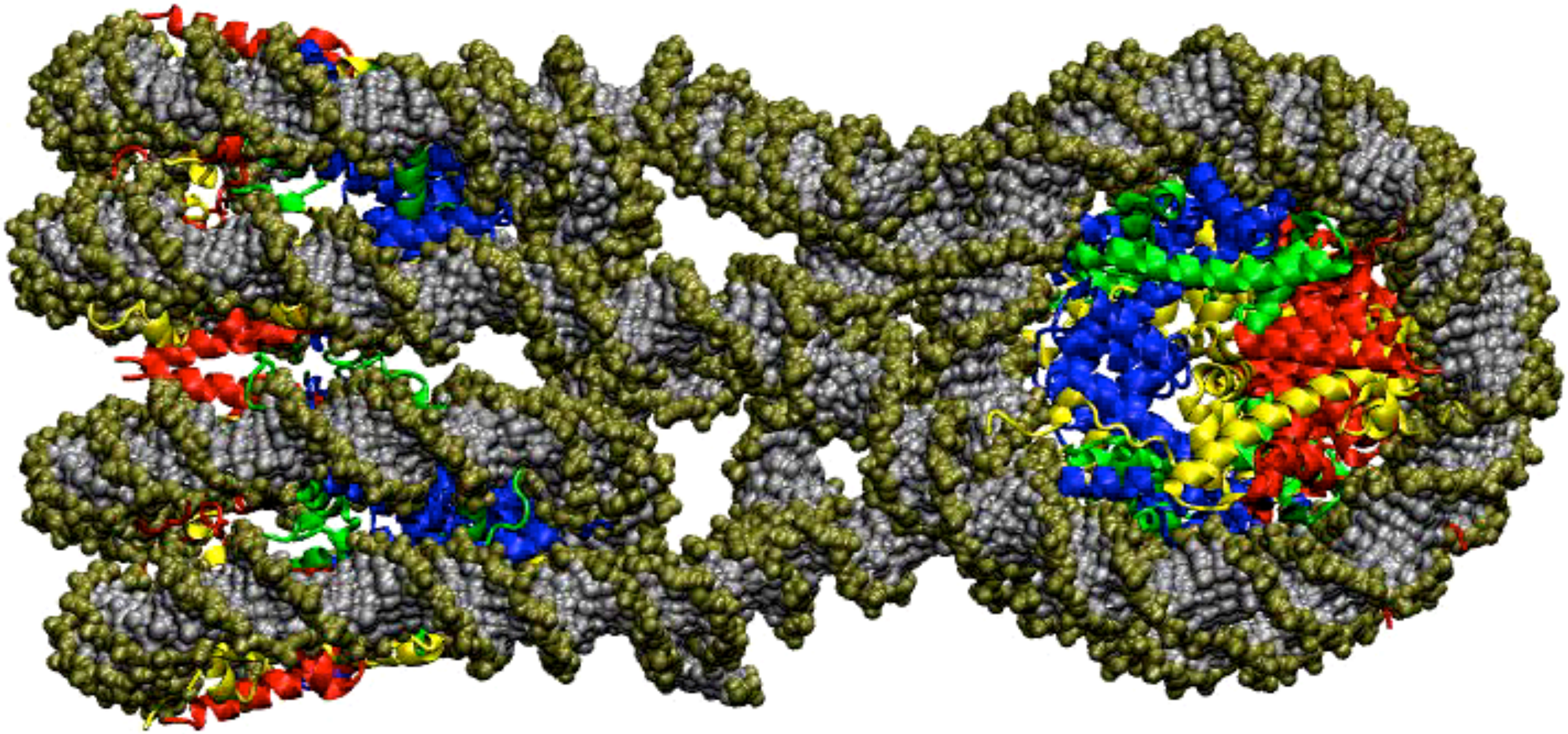
Coarse-grained nucleosome and chromatin models

(Rippe et al., unpublished)

We know a lot about the nucleosome

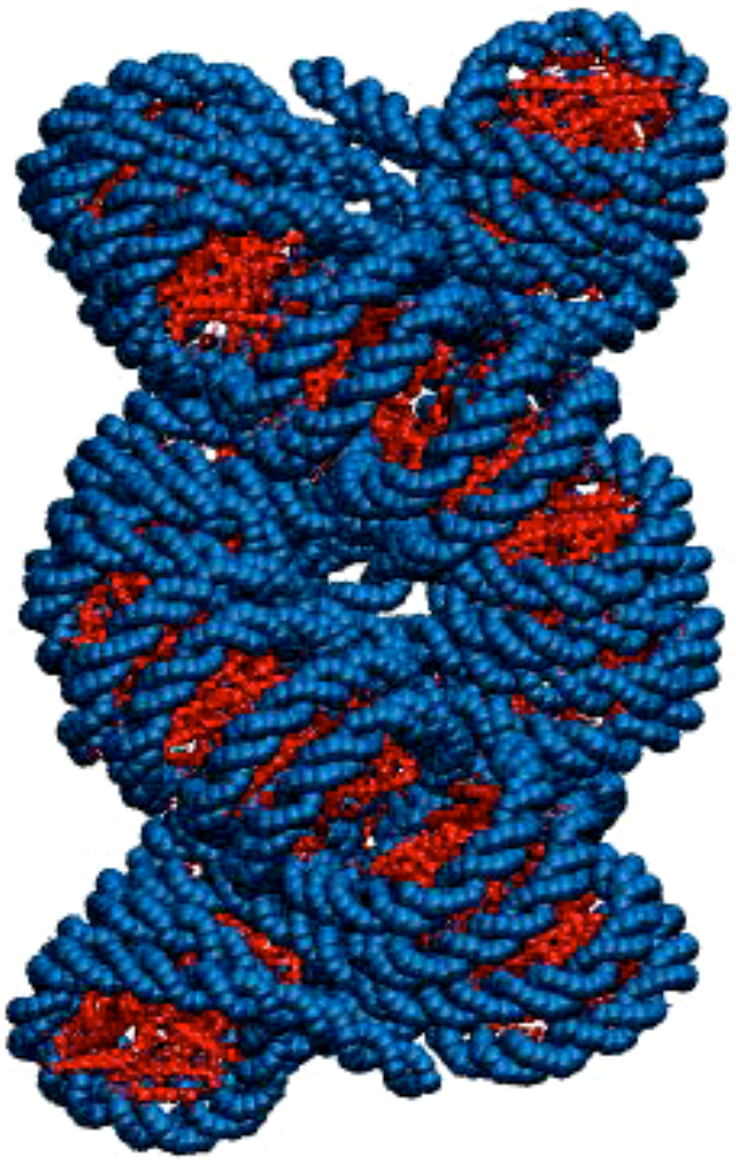


A tetranucleosome structure without H1 and a repeat length of 169 base pairs



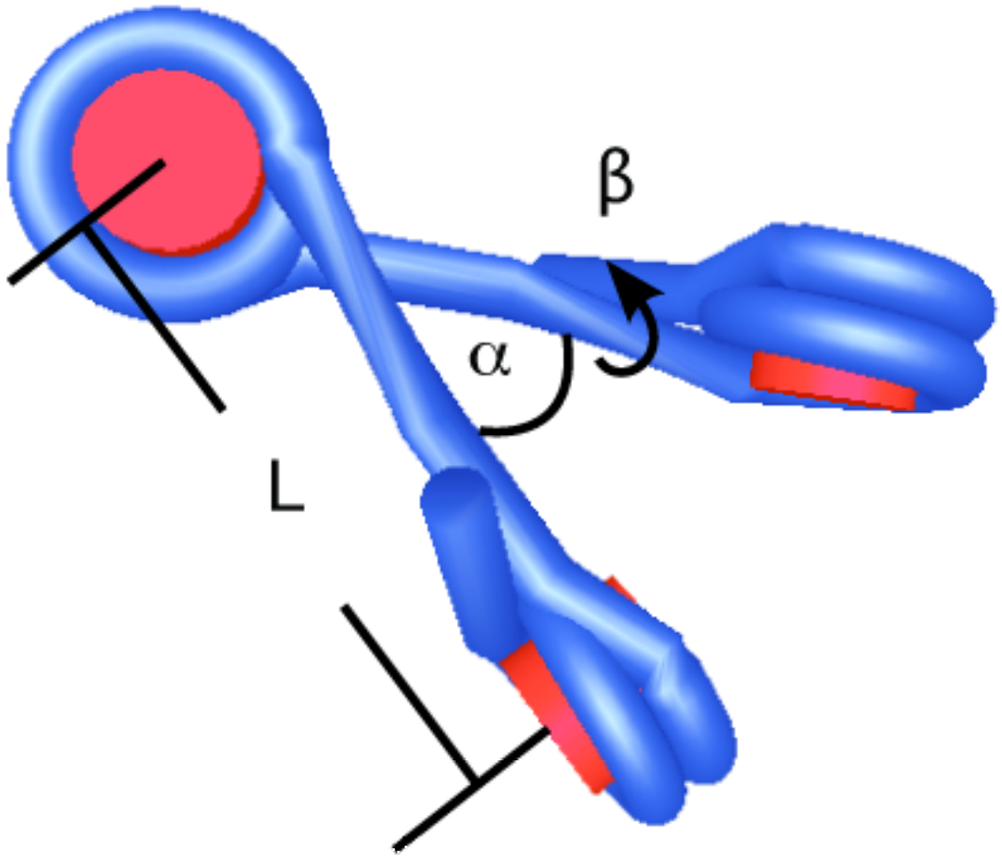
Schalch, T., Duda, S., Sargent, D.F., and Richmond, T.J., Nature, 2005

Can the tetra-nucleosome structure be extended into a stable chromatin fiber? And what about other repeat lengths?

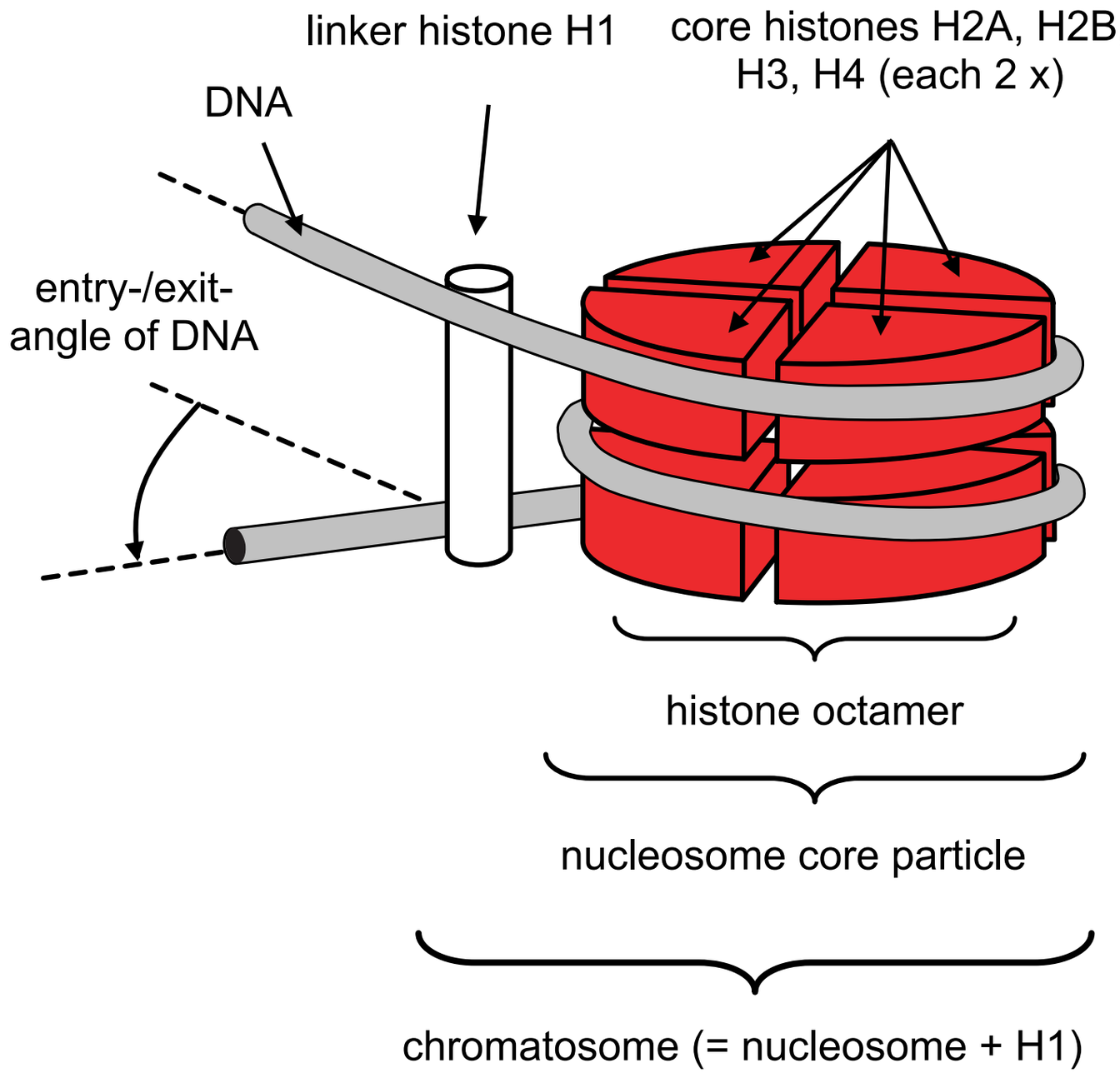


Geometric fiber model from Schalch, T., Duda, S., Sargent, D.F., and Richmond, T.J., Nature, 2005

The two-angle description of the nucleosome geometry



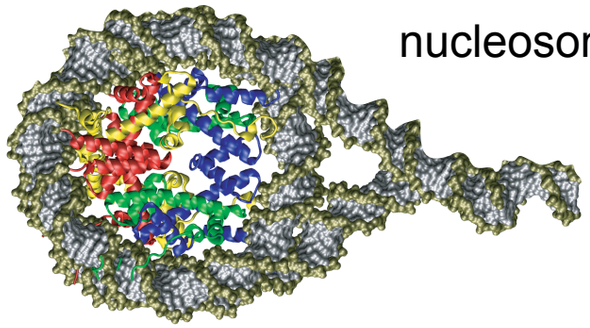
Woodcock, C.L. et al., PNAS, 1993



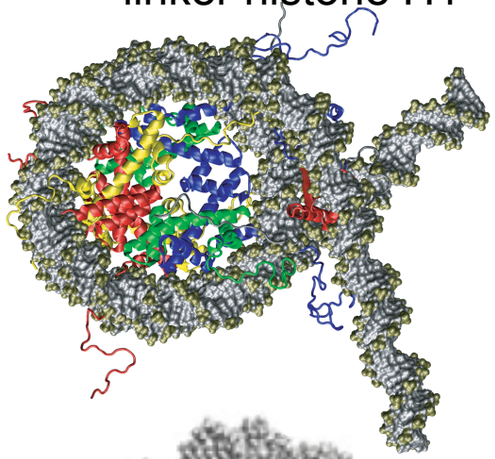
linker length L: length of DNA between nucleosomes
repeat length NRL: linker length + DNA around the nucleosome

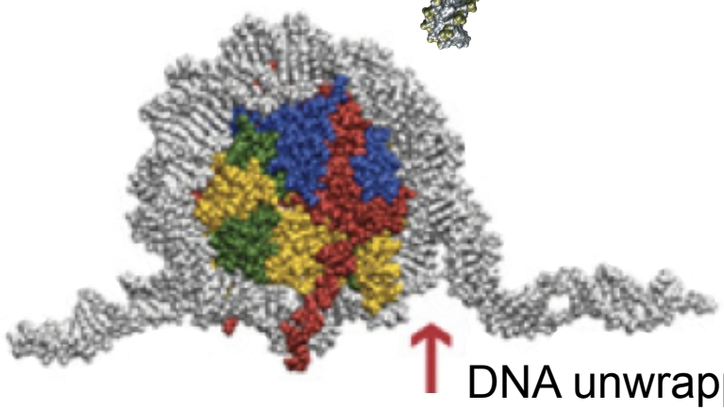
The nucleosome chain conformation regulates DNA access

All atom models

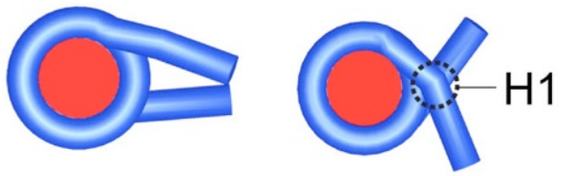


+ linker histone H1

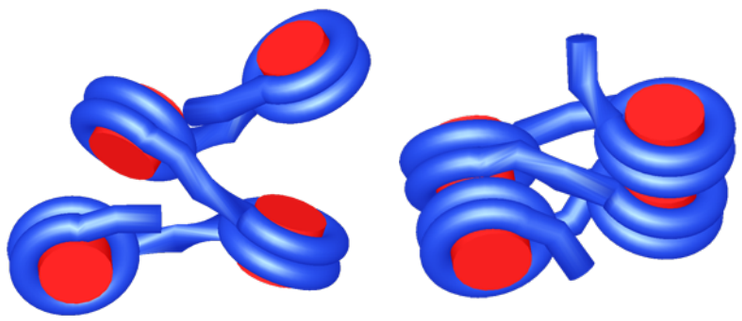




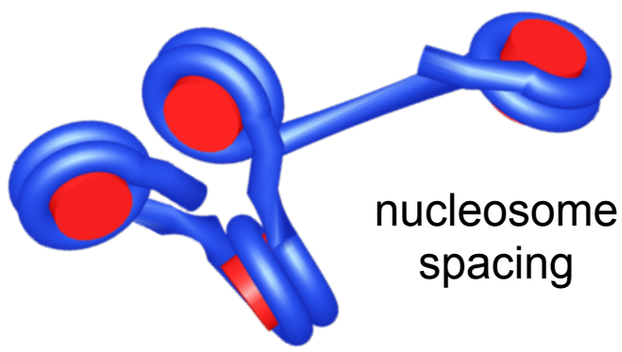
Coarse-grained representation



local geometry

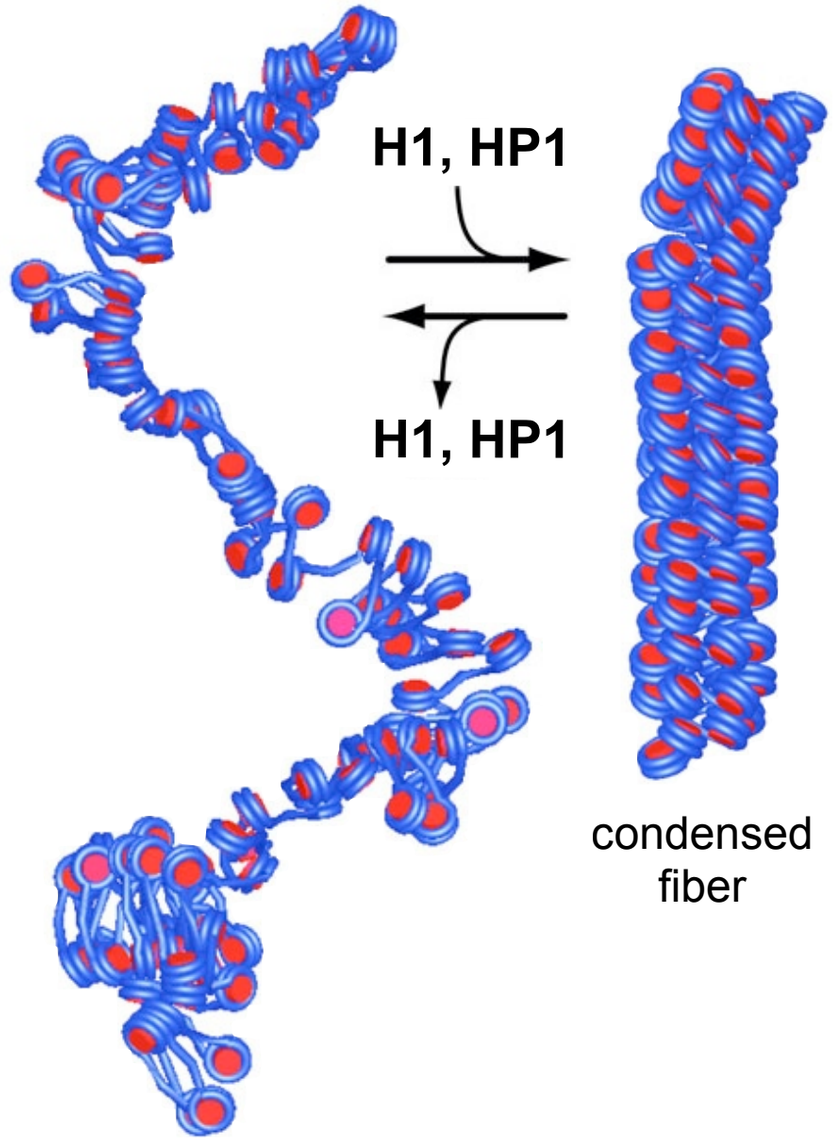


nucleosome interactions



nucleosome spacing

Fiber conformation



condensed fiber

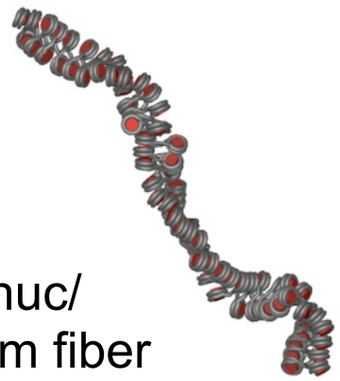
open fiber

Kepper, Foethke, Stehr, Wedemann & Rippe. *Biophys. J.* (2008)
Teif, Ettig & Rippe, *Biophys. J.* (2010)
Ettig, Kepper, Stehr, Wedemann & Rippe, *Biophys. J.* (2011)
Kepper, N., Ettig, R., Stehr, R., Wedemann, G. & Rippe, K. *Biopolymers* (2011).

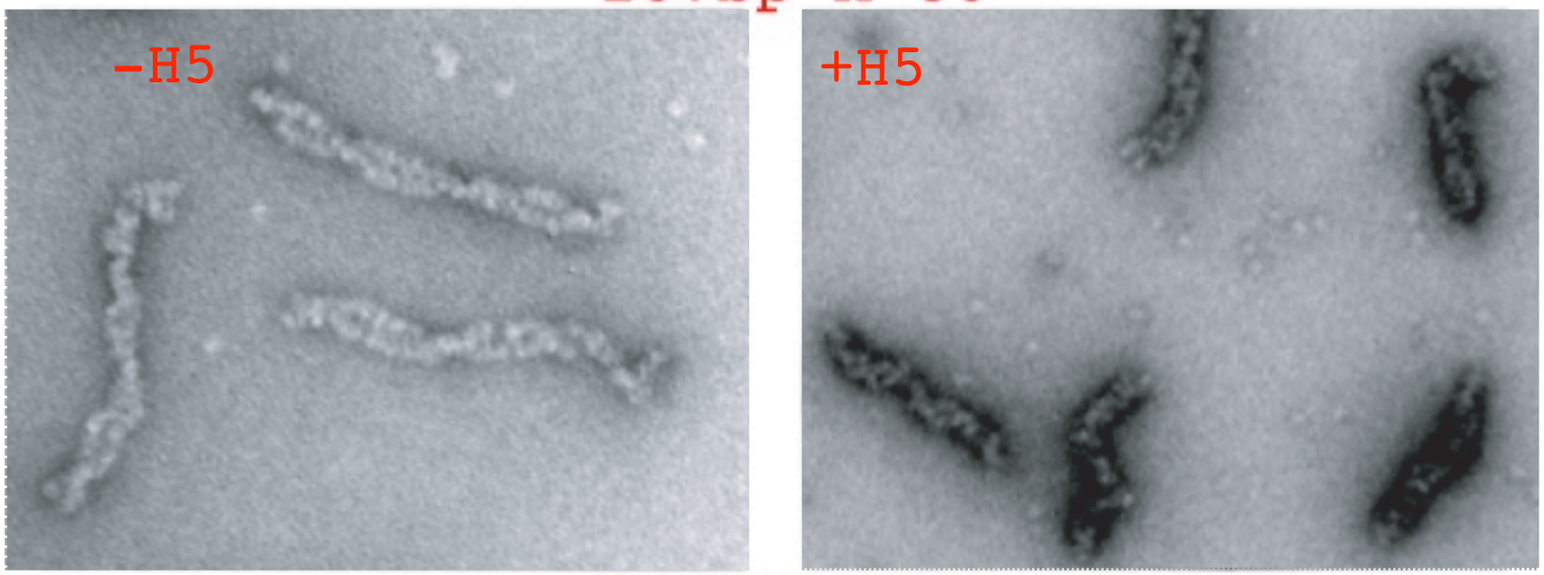
We understand some things about reconstituted nucleosome chains in vitro

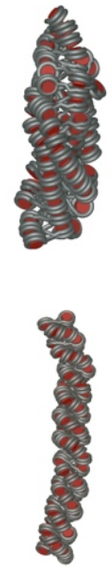
MC simulations

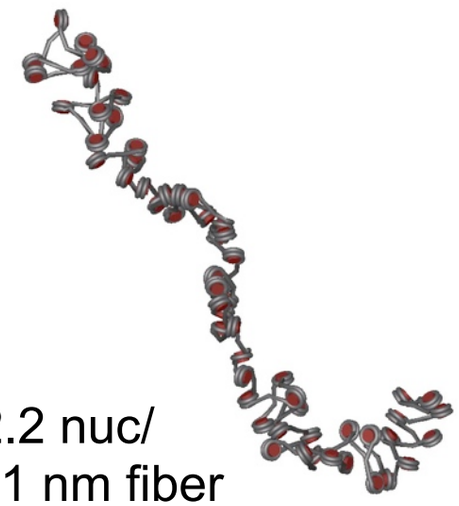
EM images of chromatin



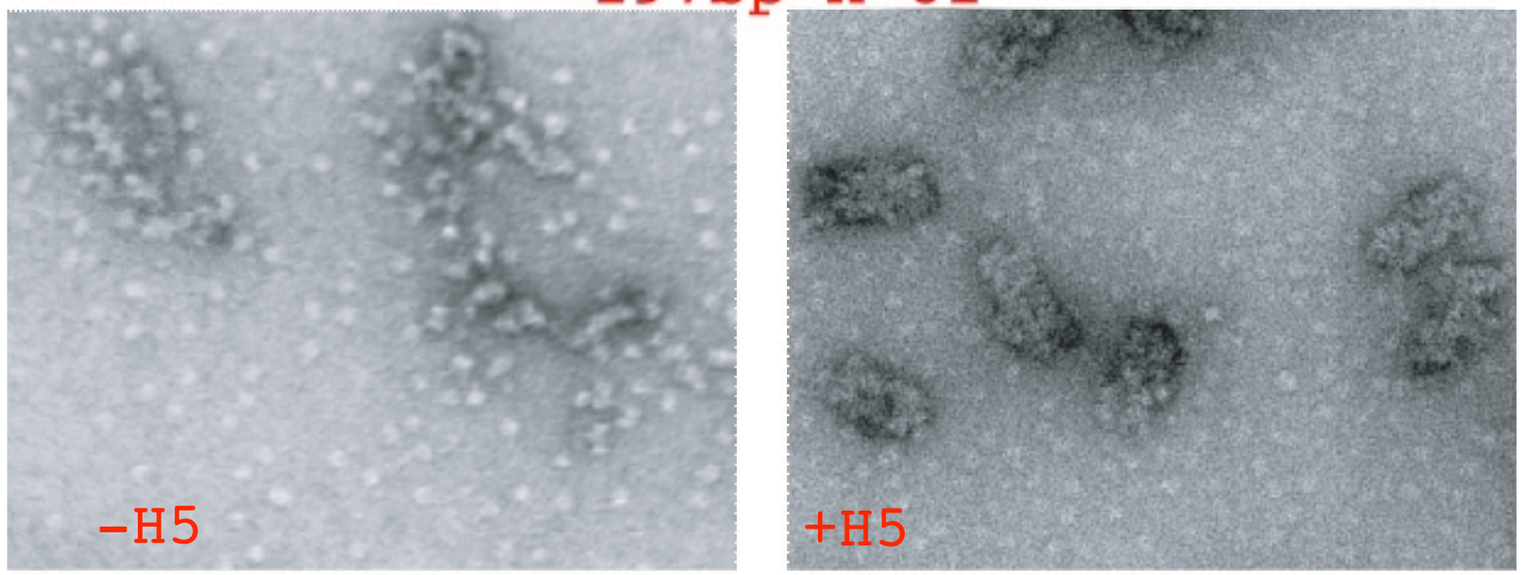
3.1 nuc/
11 nm fiber







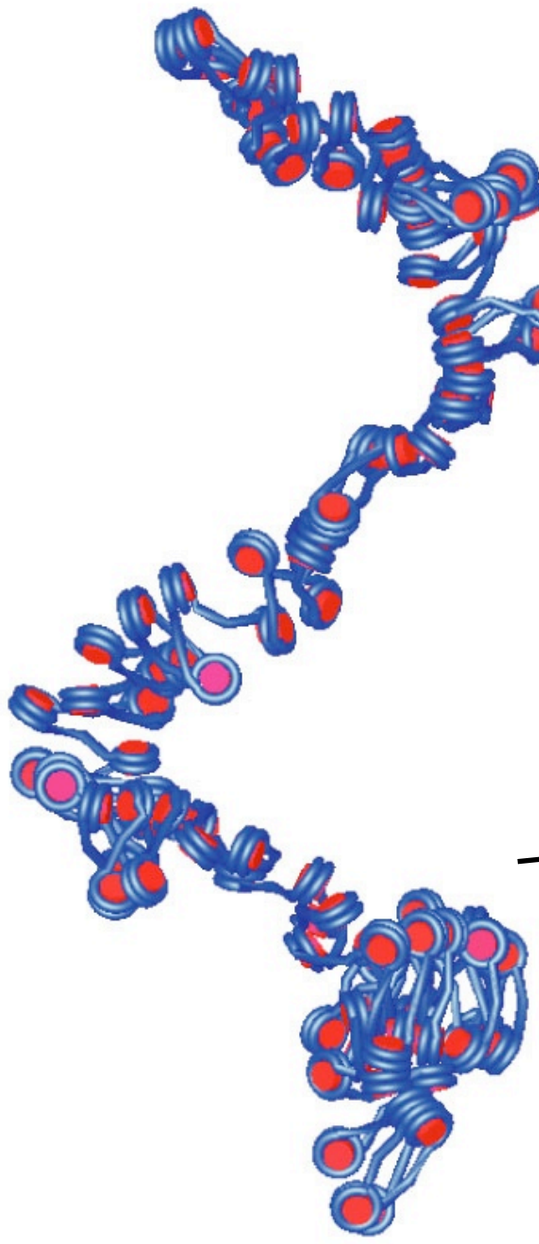
2.2 nuc/
11 nm fiber



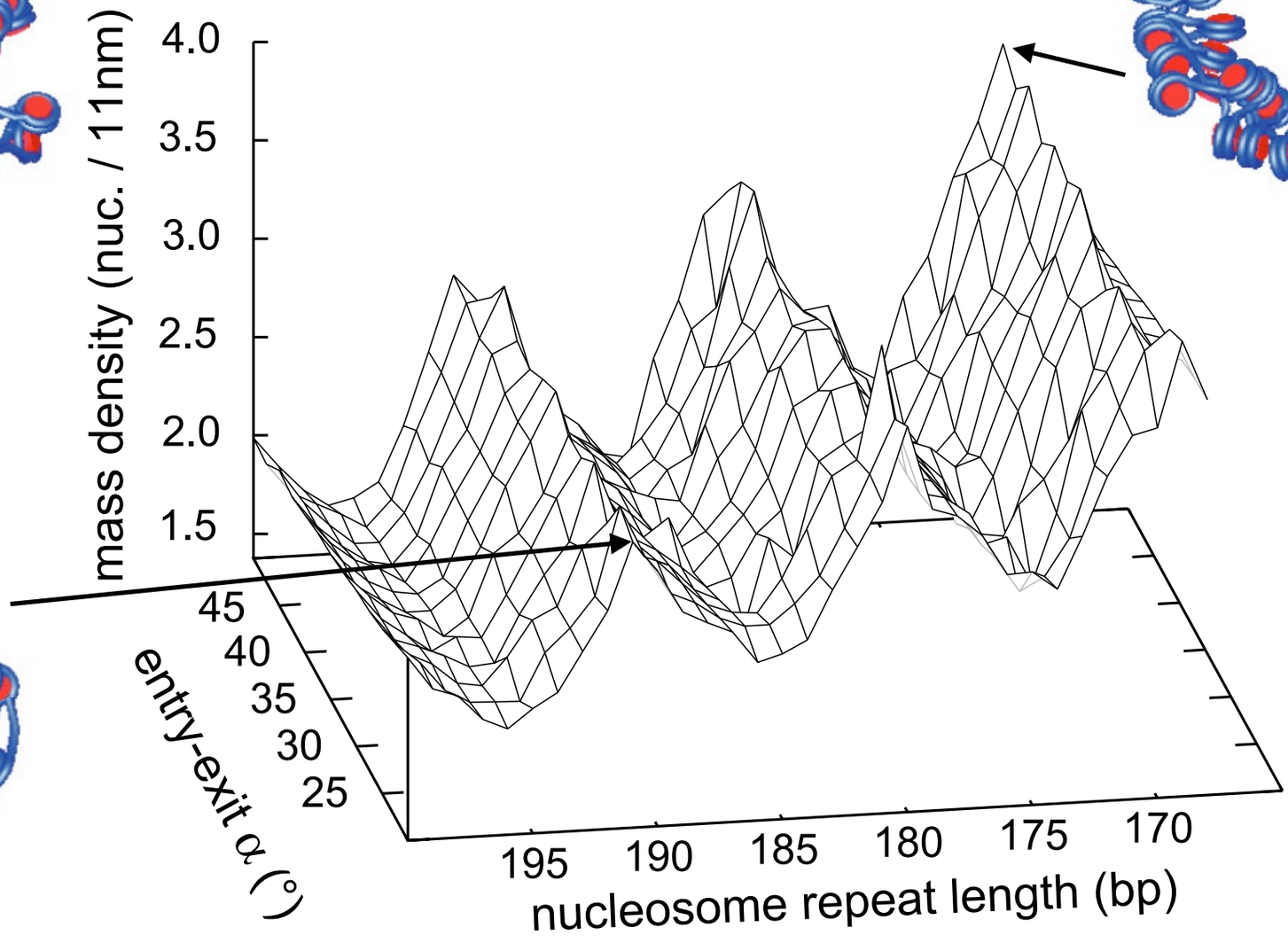
11.1 nuc/
11 nm fiber

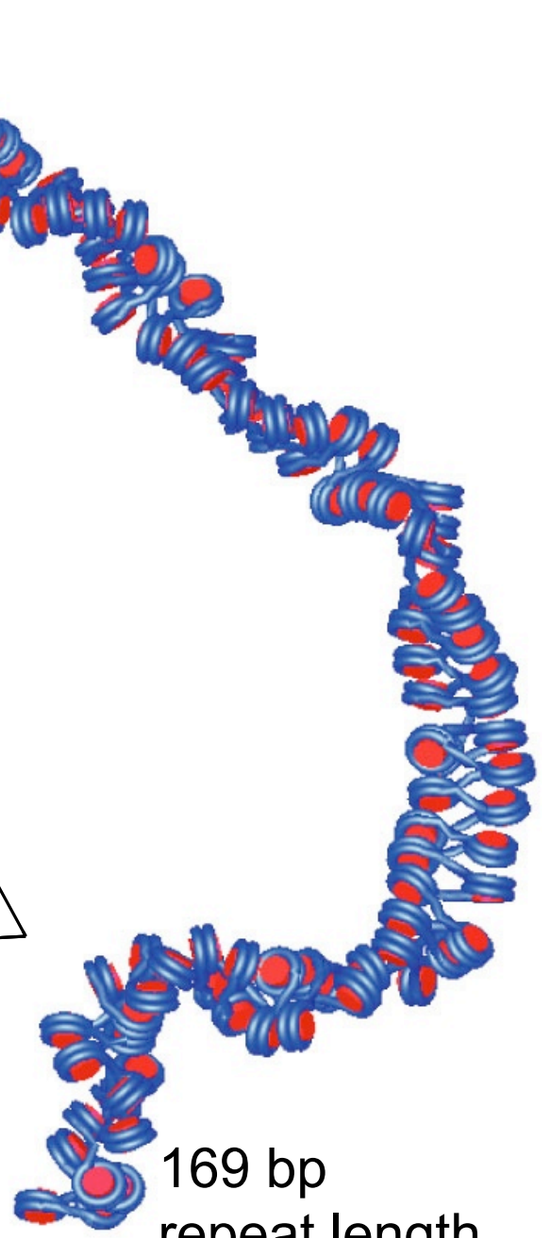
A. Routh, S. Sandin, and D. Rhodes (2008) PNAS, 105: 8872

CL fibers form at different repeat length with 10 bp periodicity and a mass density of 2.4-4 nucleosomes/11 nm fiber



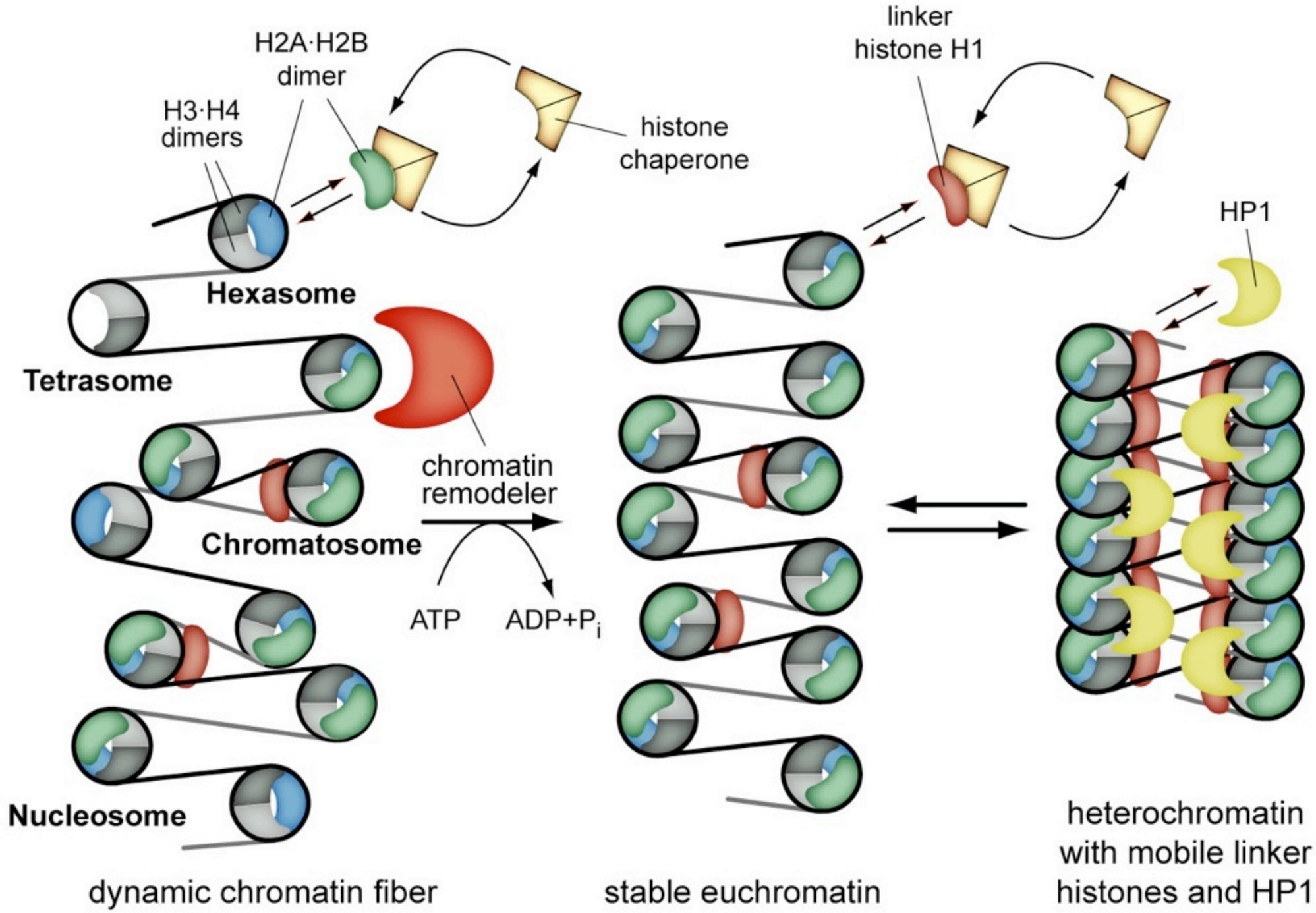
189 bp repeat length, α = 27°





169 bp repeat length α = 50°

Chromatin dynamics: histones and chromosomal proteins

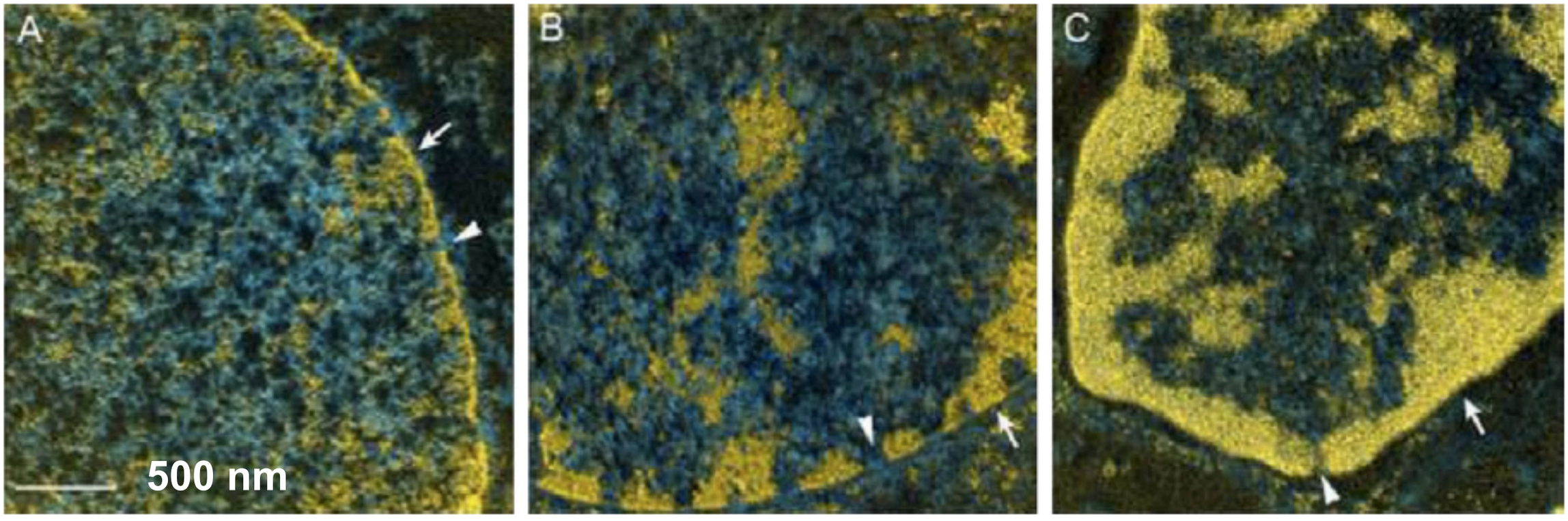


We know little about chromatin in the cell

Embryonic stem cell,
open chromatin

Hepatocyte cell,
intermediate compaction

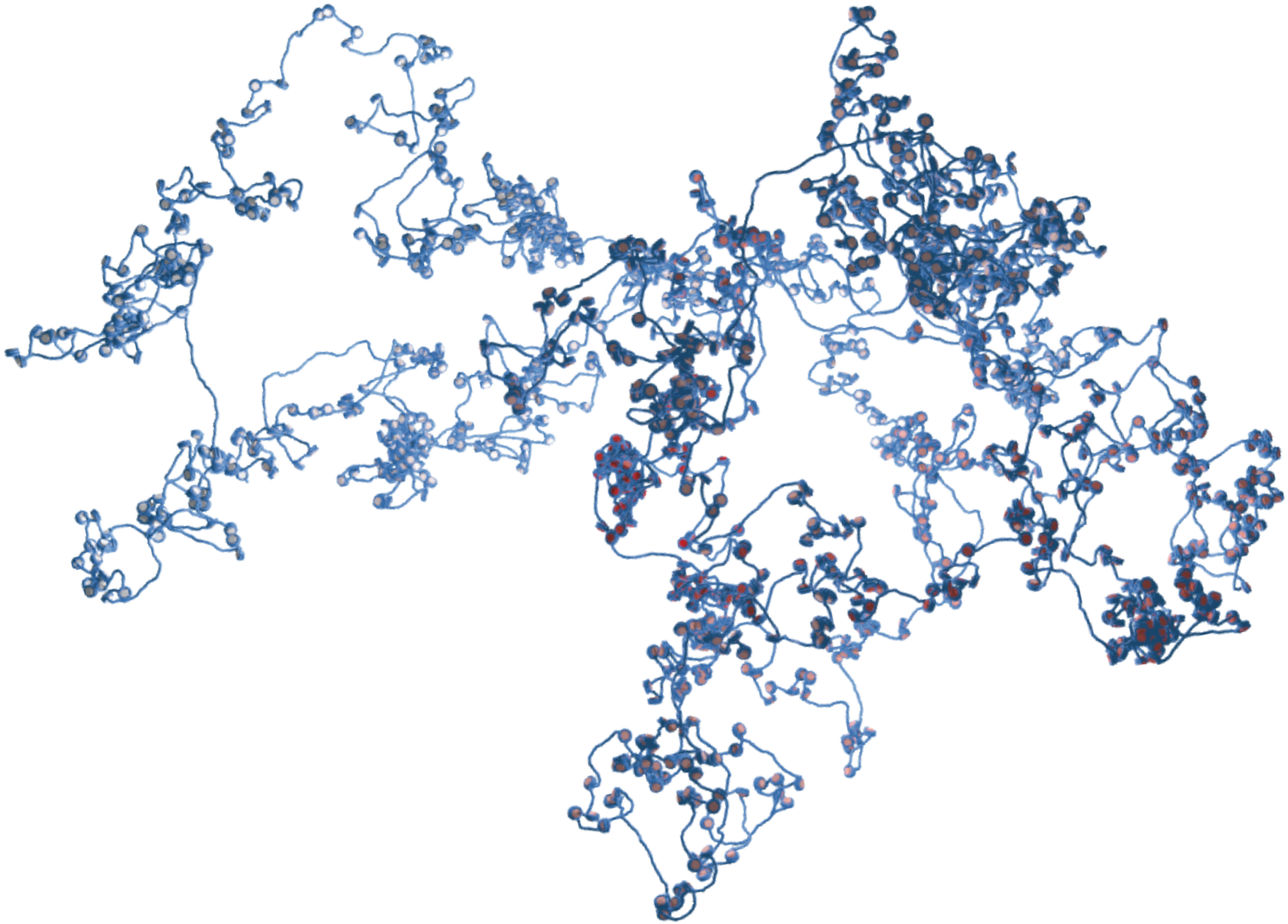
Lymphocyte,
compact chromatin



ESI micrographs. In all images, chromatin is pseudo-colored yellow and protein-based structures cyan.

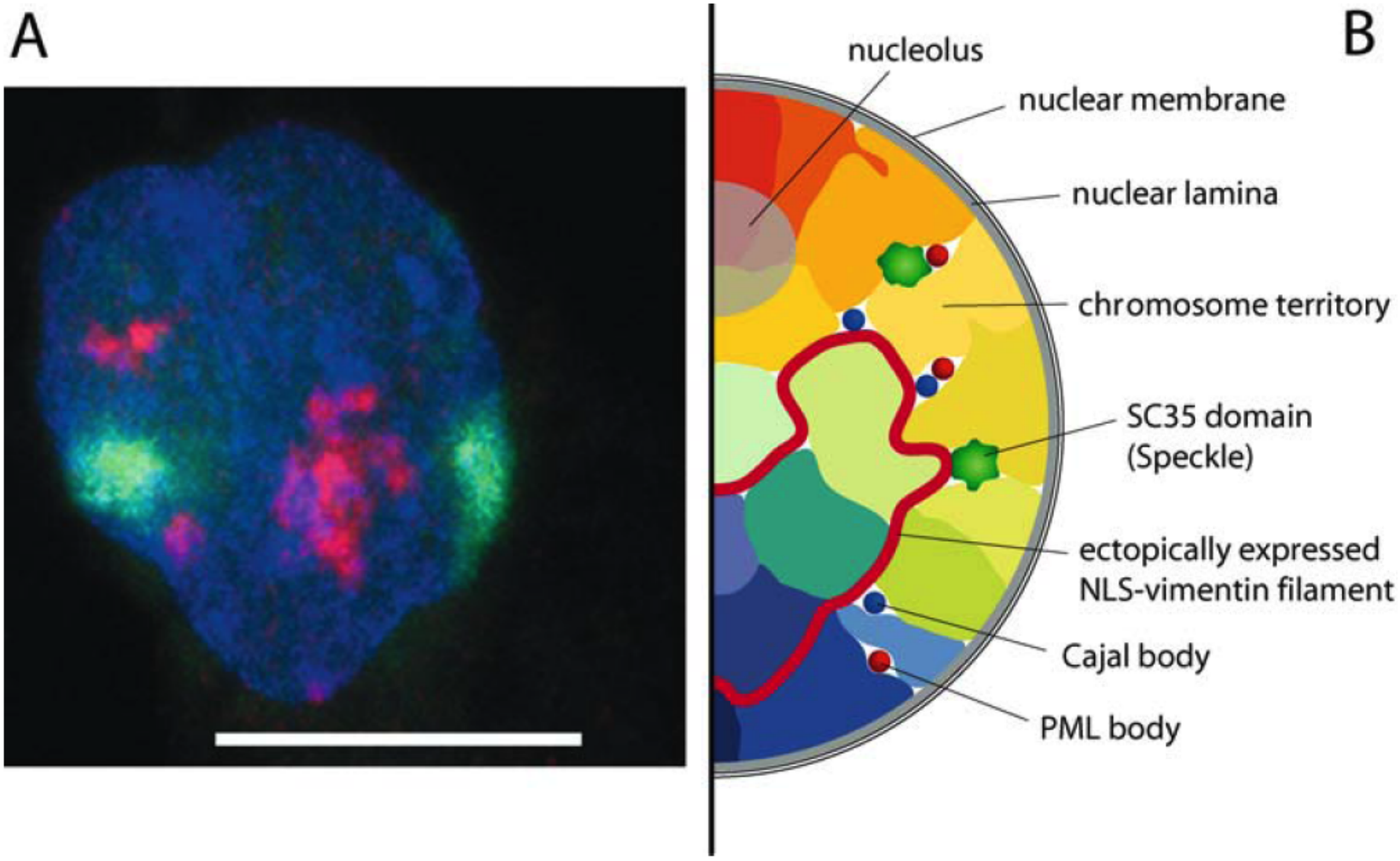
Rapkin, Anchel, Li & Bazett-Jones. *Micron* **43,** 150–158 (2012).

The nucleosome chain could be in a much more irregular conformation than concluded from in vitro experiments

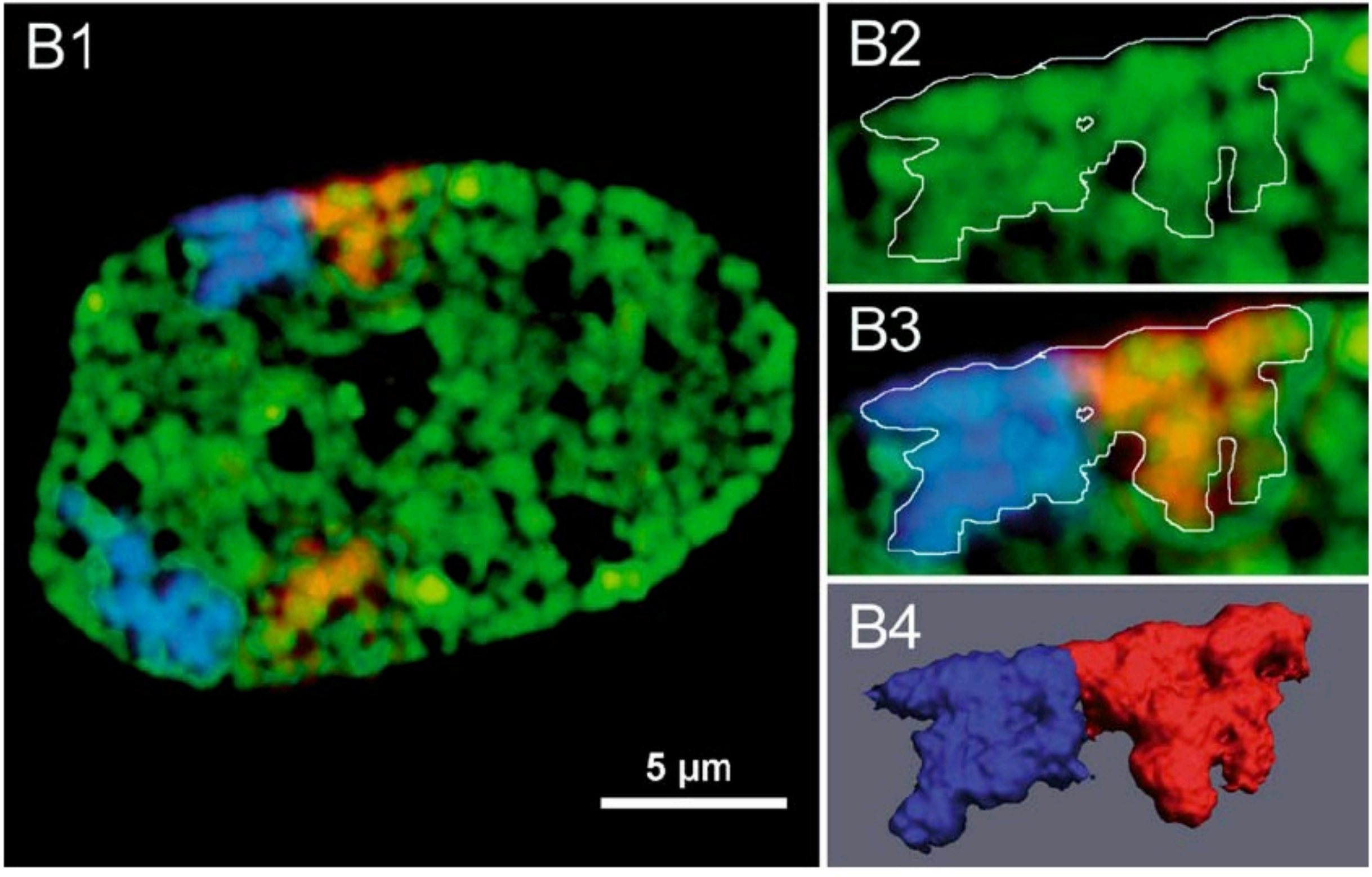


irregular spacing of nucleosomes, local heterogeneity, high concentration...

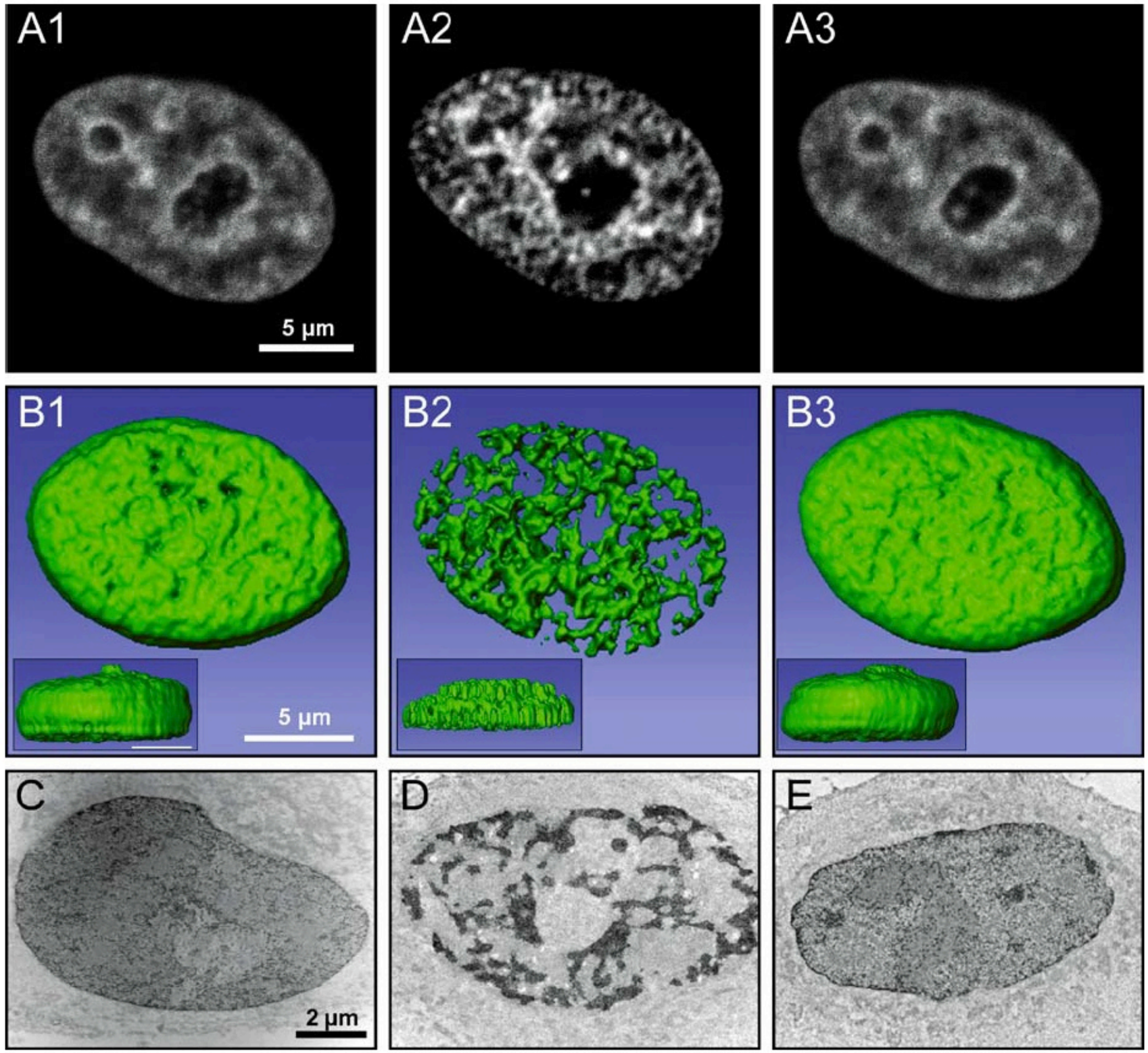
Chromosome territories



Chromosome territories (Albiez et al.)



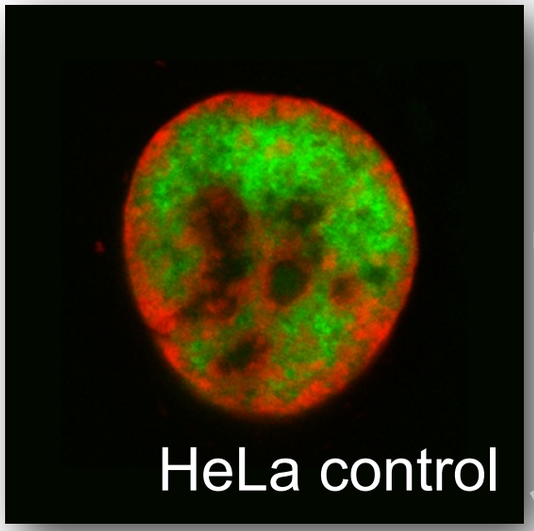
Chromatin can be reversibility condensed by increasing salt concentration in the medium or ATP depletion



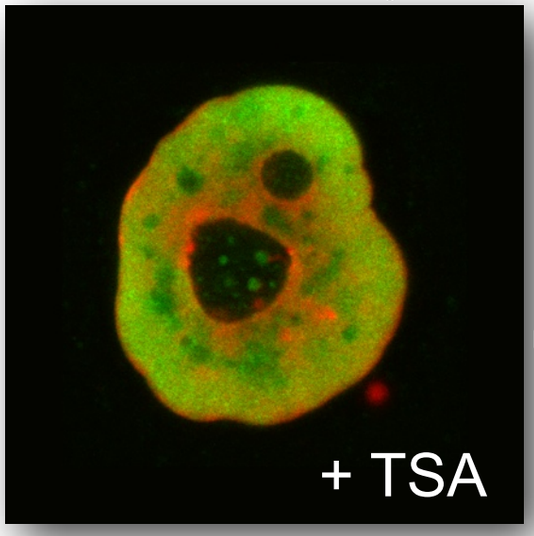
Albiez et al.

Injection of fluorescent dextrans (green) into the cell nucleus reveals chromatin (red) dependend accessibility

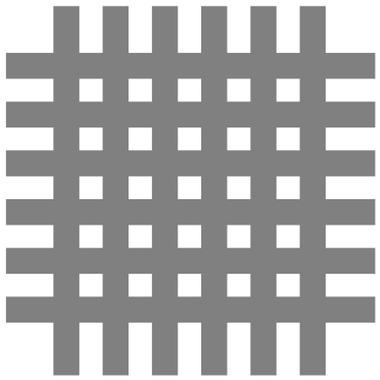
464 kDa dextran (green)
excluded from chromatin (red)



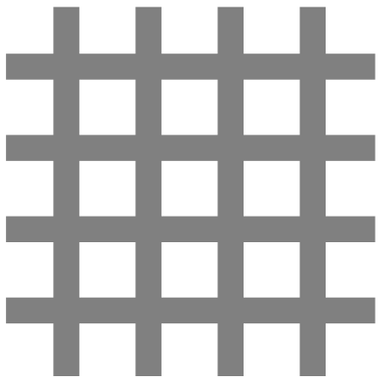
homogeneous
accessibility



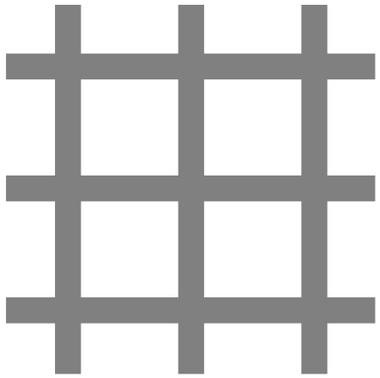
Results from analyzing the distribution of
42, 77, 148, 282, 464 and 2500 kDa dextrans



dense heterochromatin
- 16-20 nm pore size
- 0.4-0.5 mM nucleosomes
- accessible to proteins up to ~1 MDa



“bulk” heterochromatin
- 36-56 nm pore size
- 0.14-0.24 mM nucleosomes
- no restictions to MDa mobile protein complexes but possibly to the formation active chromatin domains like 40-80 nm “transcription factories”



euchromatin or acetylated chromatin
- 60-100 nm pore size
- 0.06-0.13 mM nucleosomes
- excludes only large structure as PML/Cajal bodies (0.1-1 µm) into “interchromatin space”

Fejes Tóth et al. (2004) *J. Cell Sci.,* **117**, 4277-4287
Görisch, S., Lichter, P. and Rippe, K. (2005). *Histochem. Cell Biol.* **123**, 217-228.
Görisch, S., Wachsmuth, M., Fejes Tóth K., Lichter, P. and Rippe, K. (2005), *J. Cell Sci.* **118**, 5825-5834.

What's up next?

- Some basics on fluorescence to understand the techniques applied
- Looking at the inside of a cell from a protein's point of view
- Understanding how mobility and interactions of proteins are measure in living cells
- Dissecting epigentic networks in living cells