

DNA unlinking in bacteria

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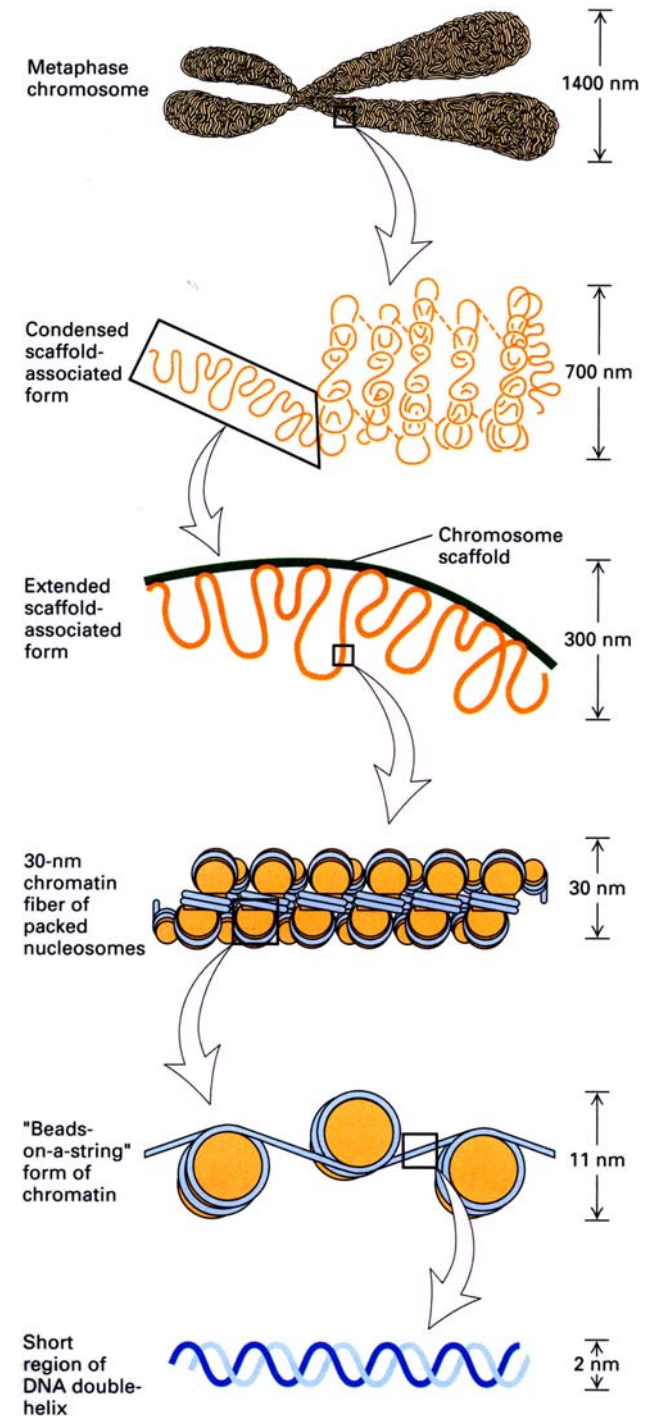
New York University

DNA in chromosomes is organized into several levels of compaction

- Total length of human DNA – 1 meter
- Diameter of a human cell nucleus – $10\ \mu\text{m}$

(Random coil ~ $150\ \mu\text{m}$)

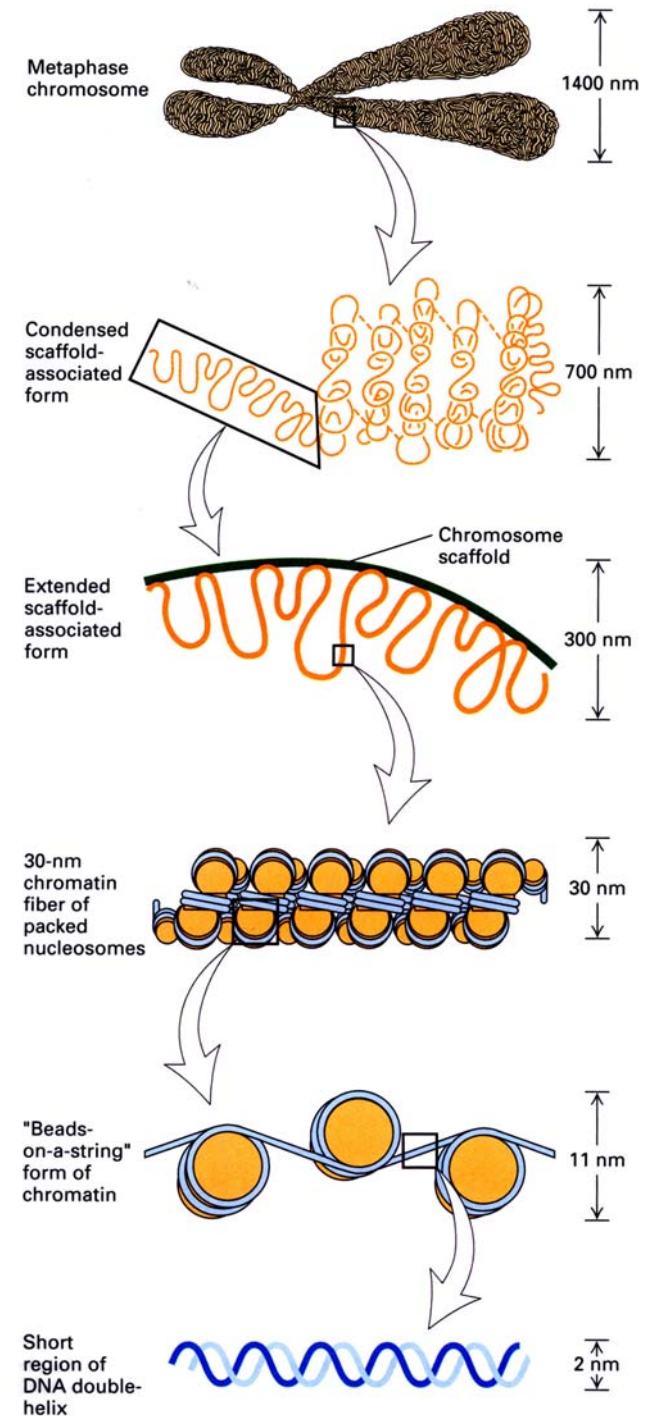
How does the cell package DNA into its tiny confines?



Chromatin is organized into several levels of compaction

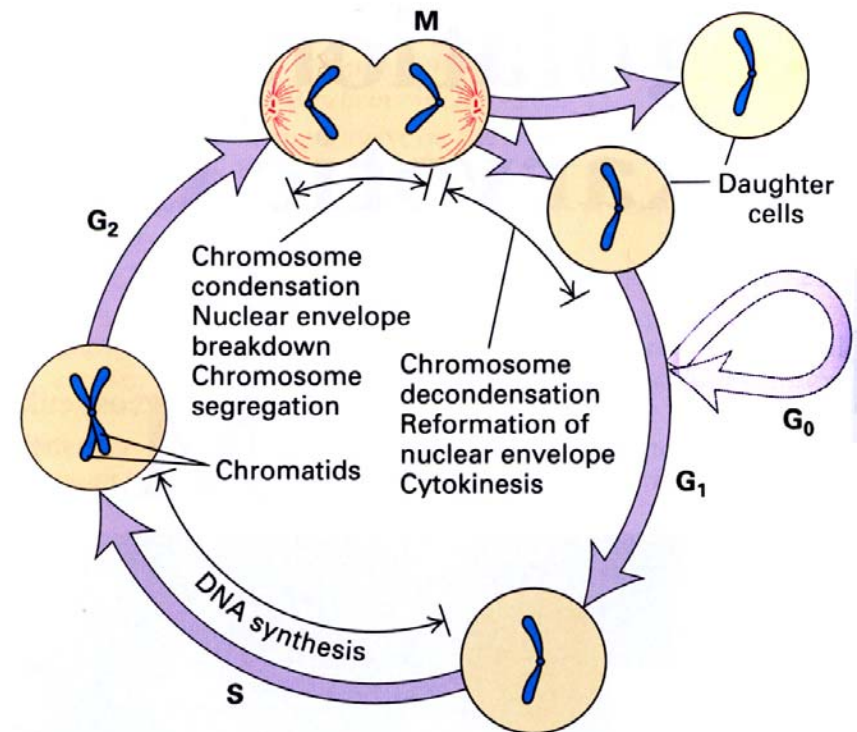
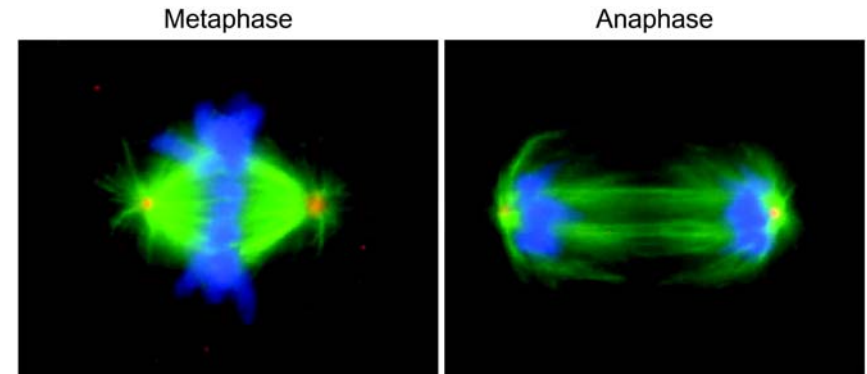
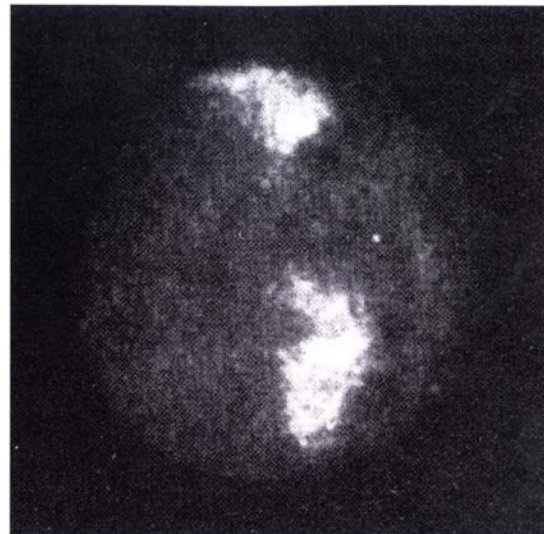
1. Wrapping around nucleosomes
packing ratio ~ 7 : $\sim 160\text{bp}/6\text{nm}$
2. Nucleosome compaction into 30 nm structure
packing ratio ~ 40 : $\sim 1.2\text{kb}/11\text{nm}$
3. Radial loops $\sim 100\text{ kb}$
4. Association of *anchoring elements* (or *chromosome scaffold*) into chromosome axis

In bacteria: nucleosomes – No;
supercoiling & looping - Yes

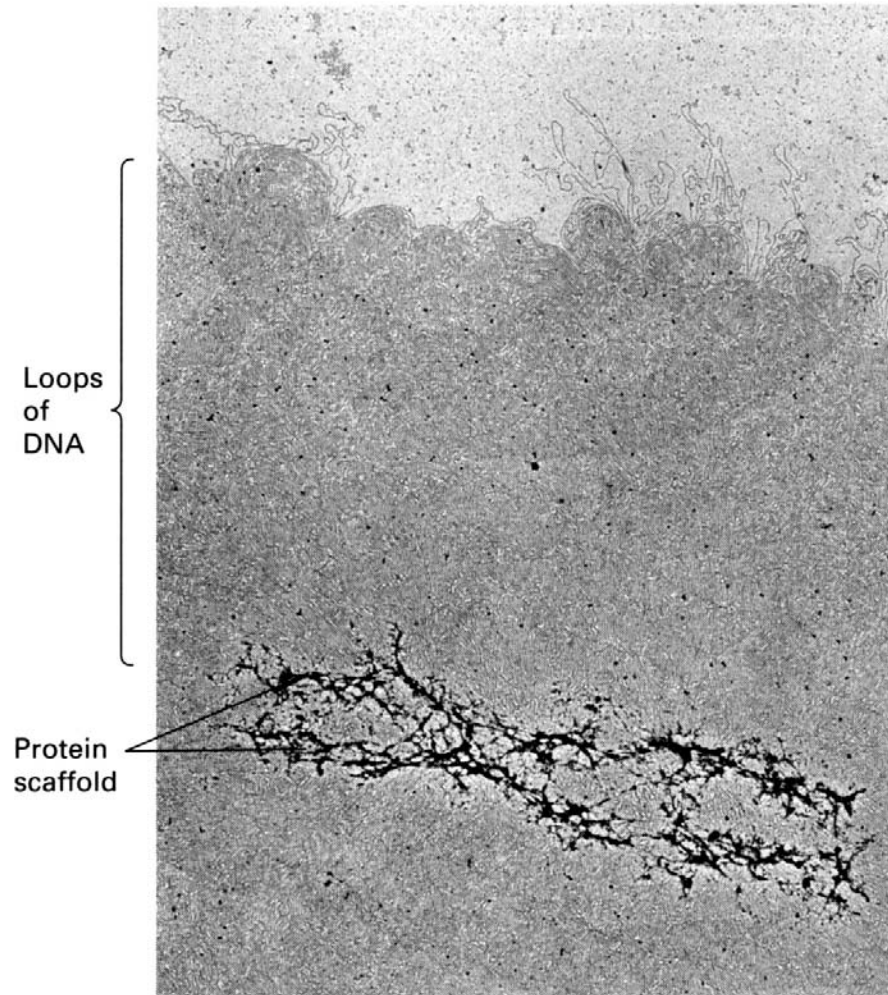


Chromatin structure is highly dynamic

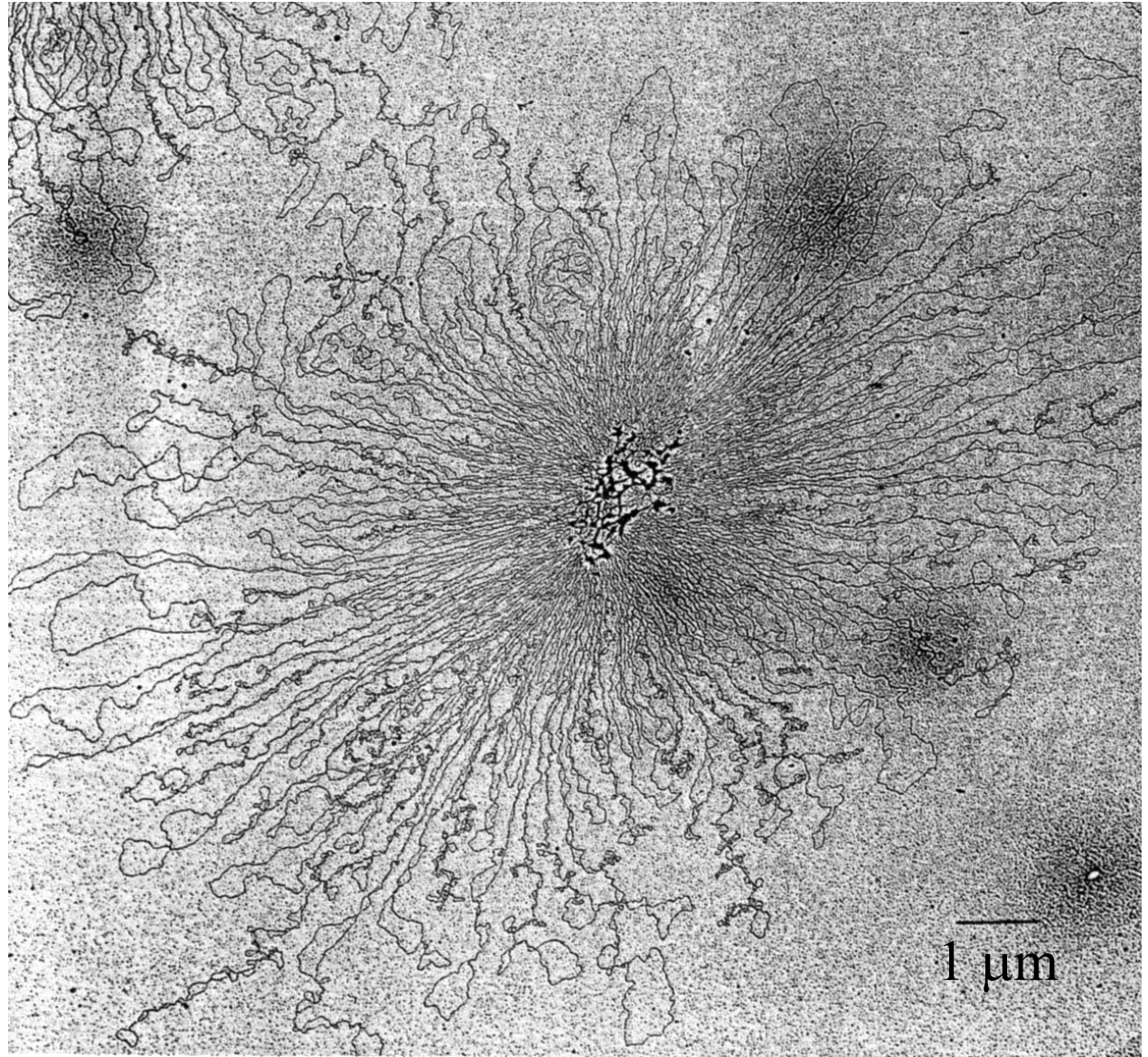
Chromosome condensation during cell division ensures faithful segregation of genetic material

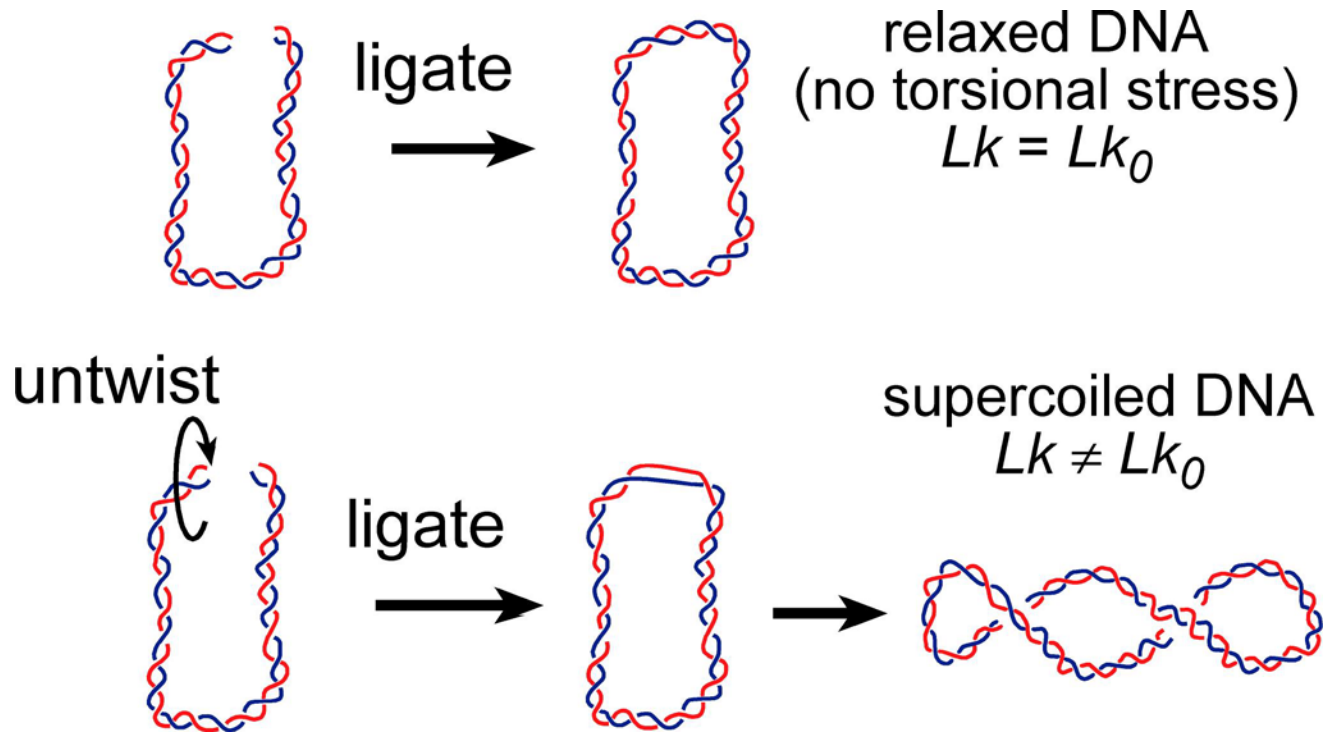


Histone depleted metaphase chromosomes retain their shape



Bacterial
chromosome
is organized
into ~100
supercoiled
loops





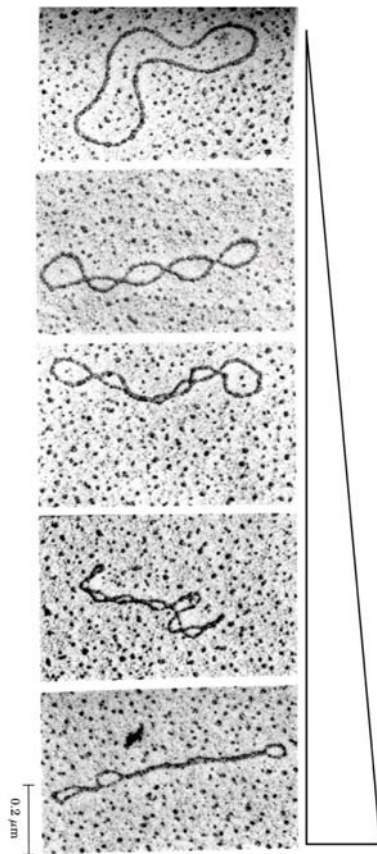
Lk – Linking Number - cannot be changed unless DNA is broken

$\Delta Lk = Lk - Lk_0$ – Linking Number Deficit – a measure of supercoiling

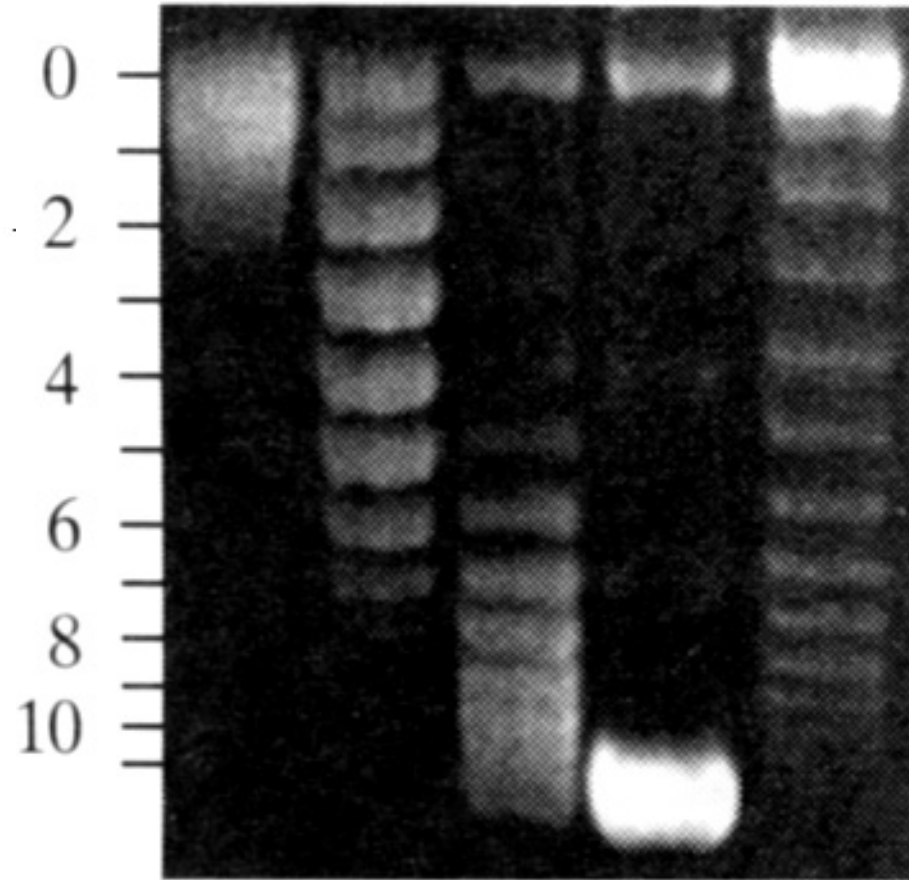
$\sigma = \Delta Lk / Lk_0$ – Degree of Supercoiling

Inside the cell, $\sigma = -0.04$ to -0.08

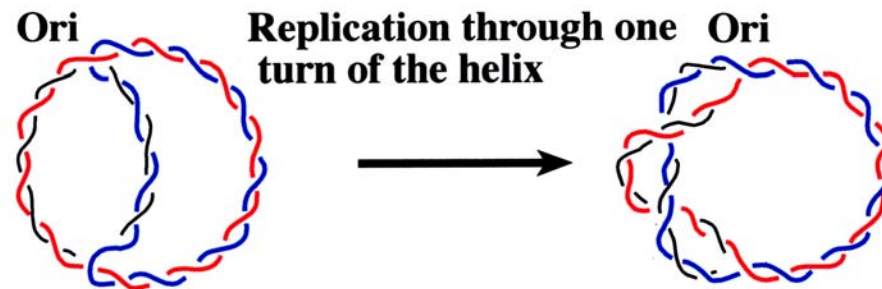
Gel mobility of supercoiled DNA



supercoiling



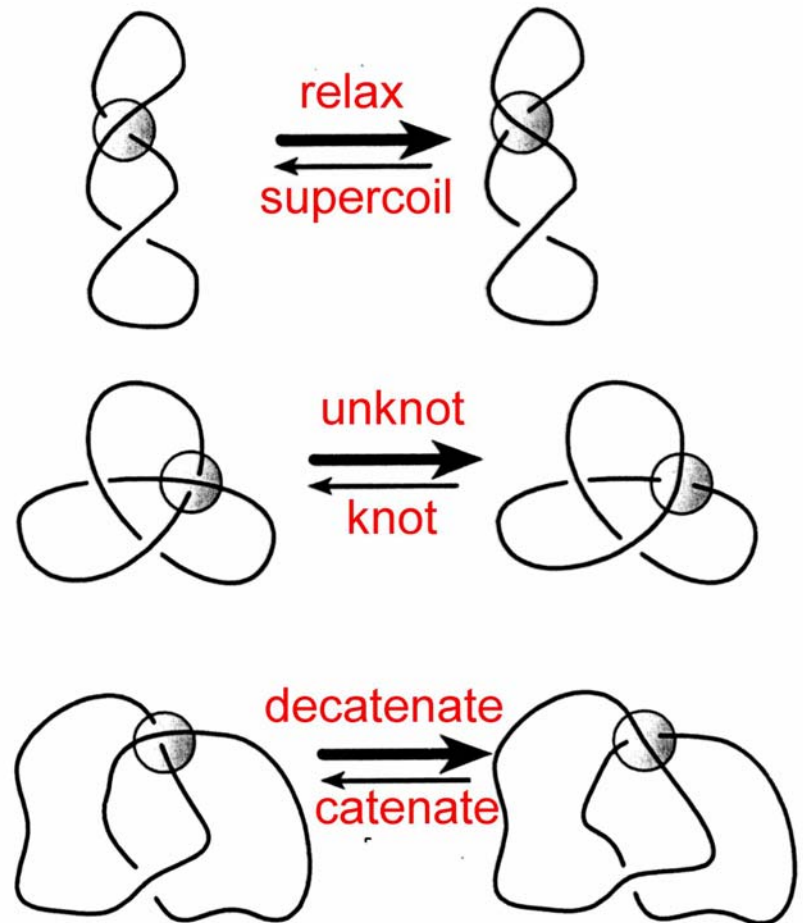
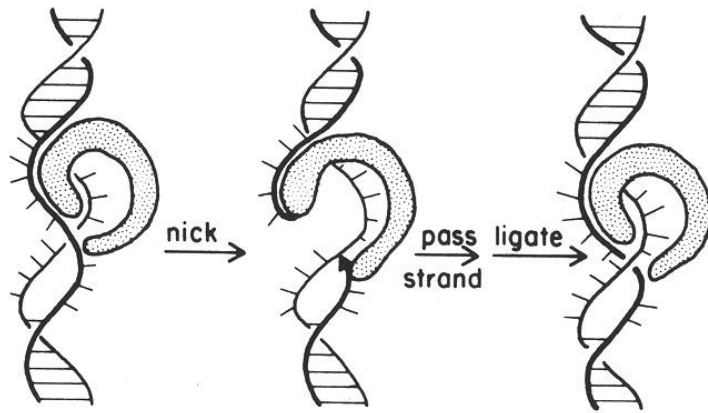
Topological challenges to DNA replication



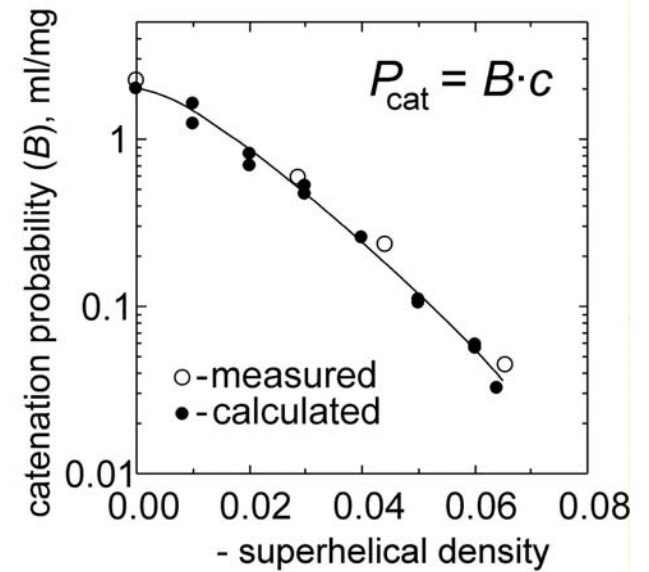
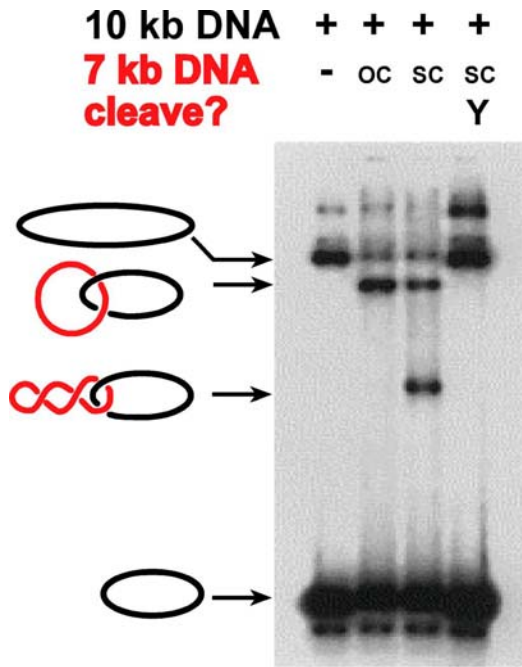
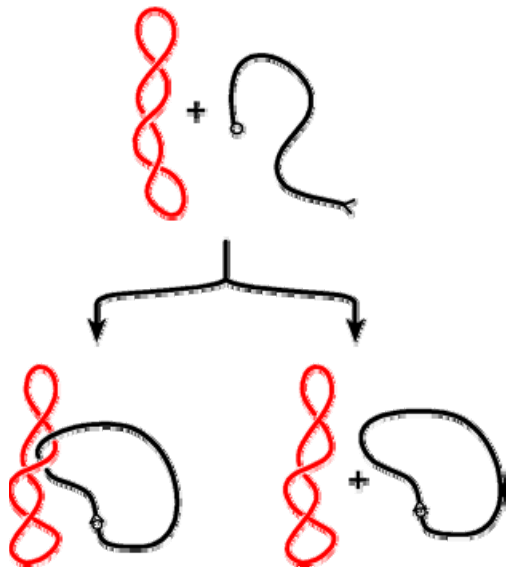
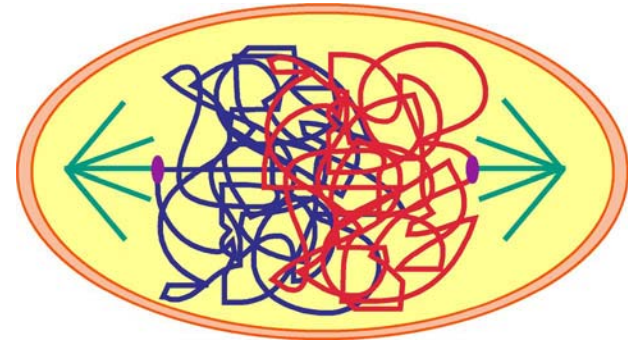
Topological links must be removed:

- Fast
 - to support replication
 - to maintain supercoiling elsewhere
- Completely
 - Catenation between daughter chromosomes results in ds breaks

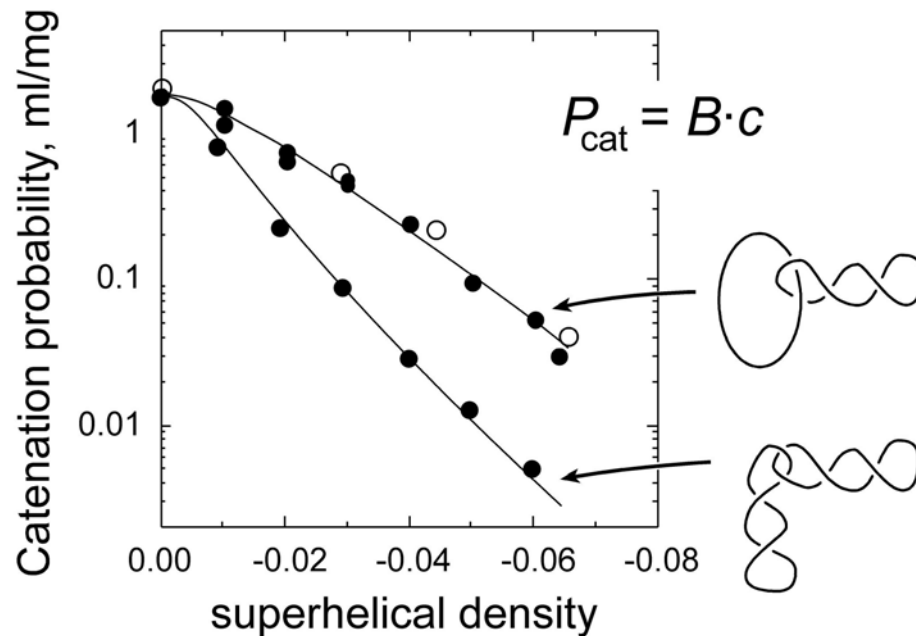
Topoisomerases untangle DNA



Measure of DNA compactness:
 number of links between sister
 chromosomes



Computer modeling can be used to estimate length dependence of catenation and catenation between two supercoiled DNAs



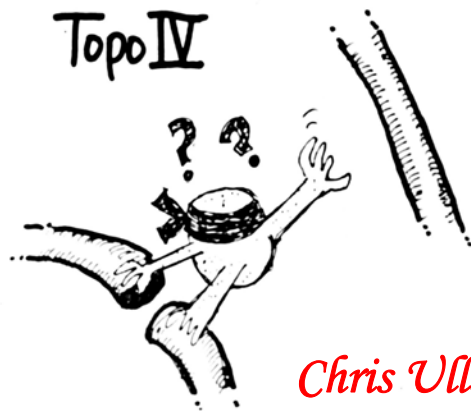
Relaxed Random coil	10^6
Relaxed Loops	10^4
Supercoiled Loops	10^1
Chromosome missegregation	10^{-5}

DNA length dependence:

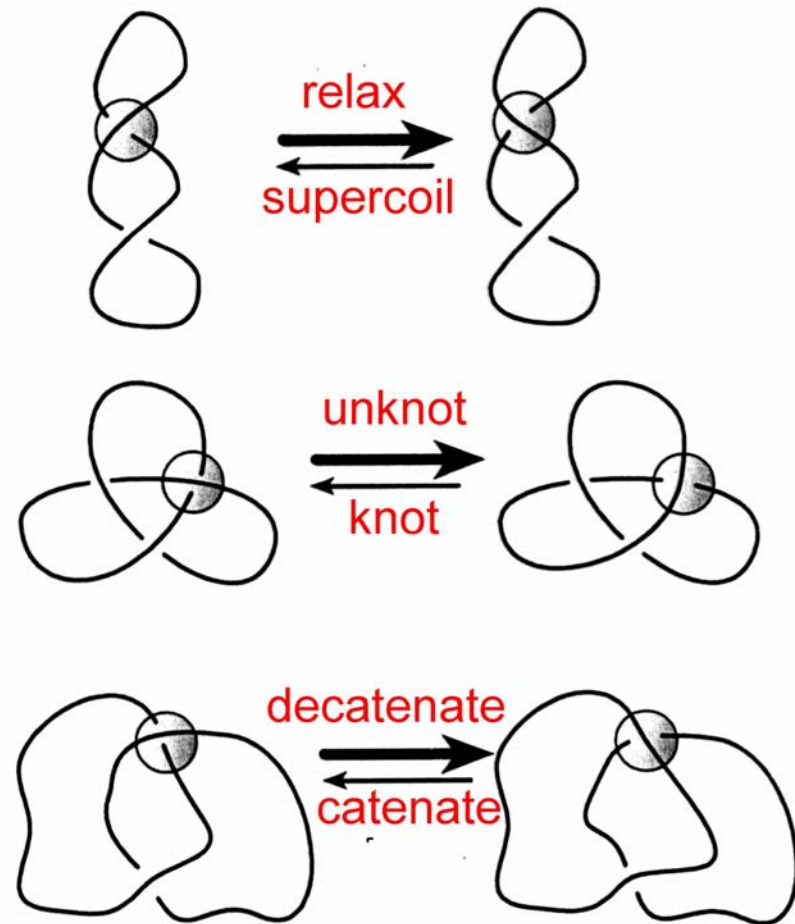
$$B \sim L^{1.7}$$

Alex Vologodskii, unpublished

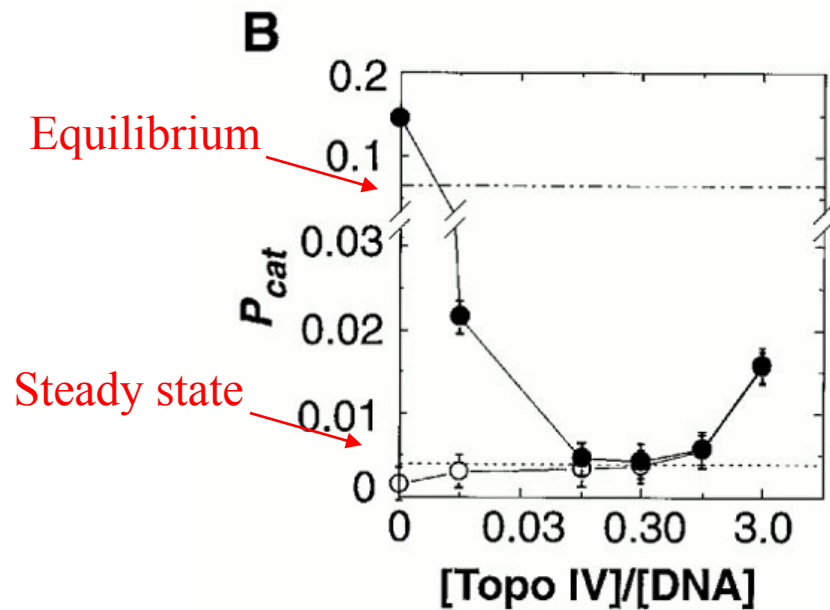
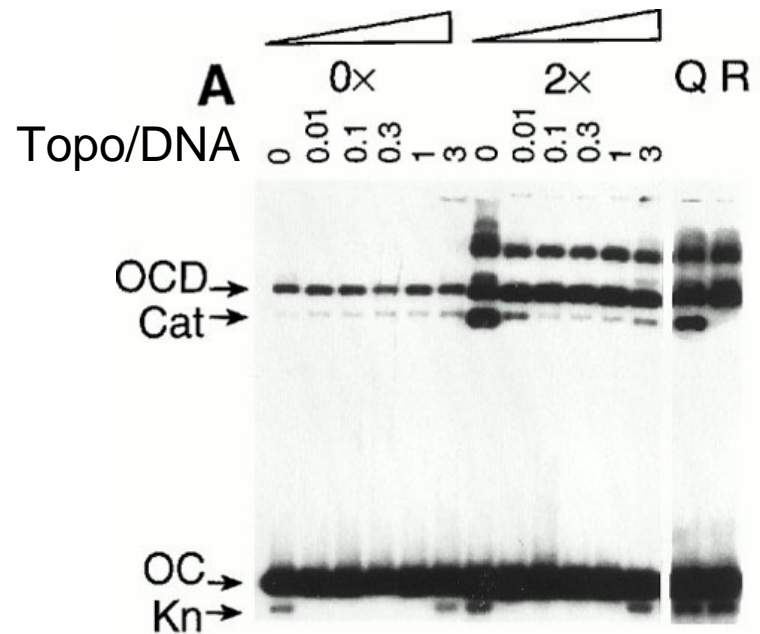
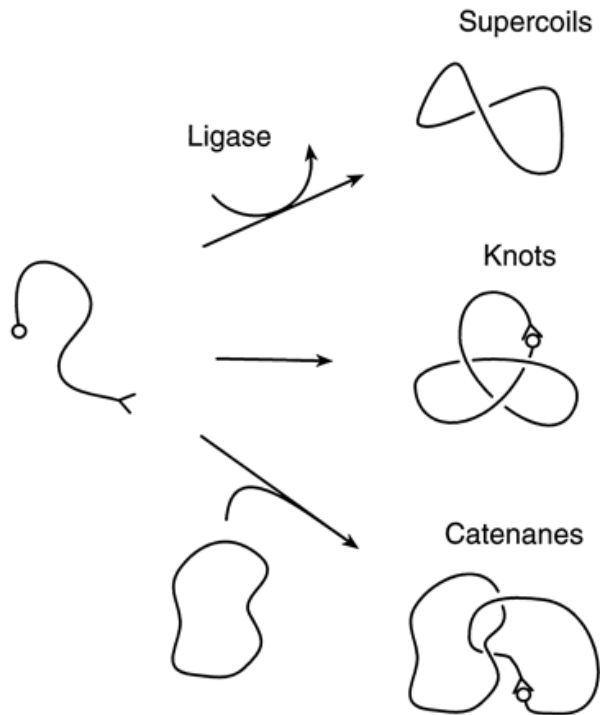
Topoisomerases simplify topological equilibrium in DNA



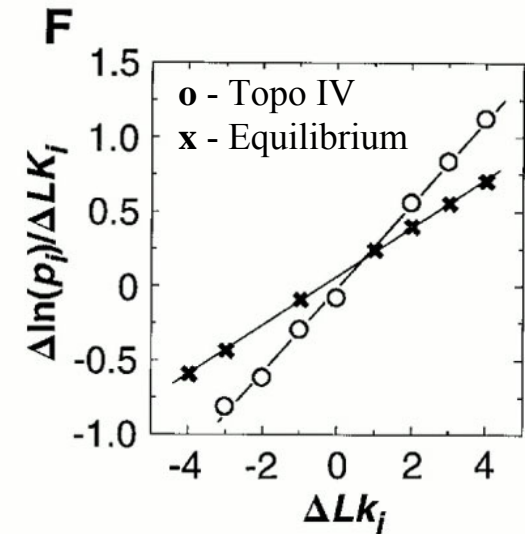
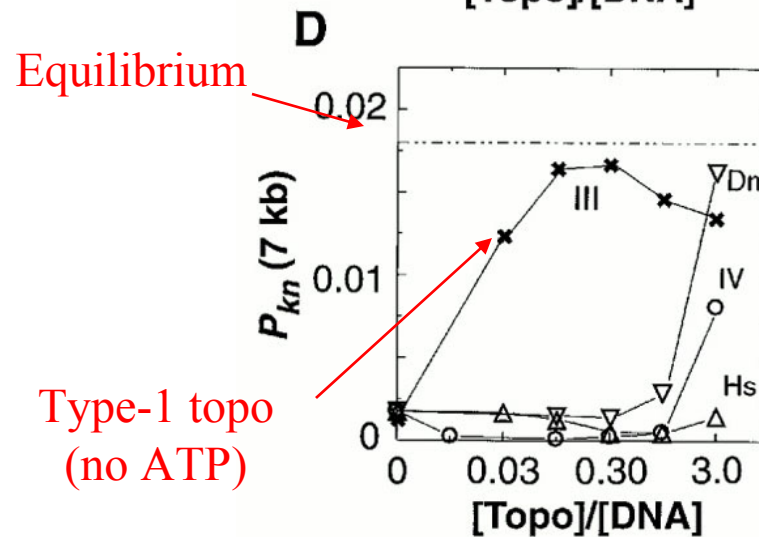
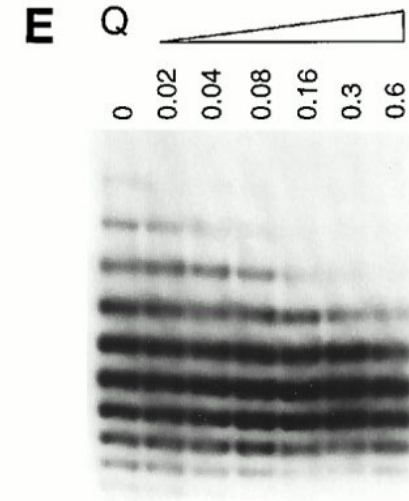
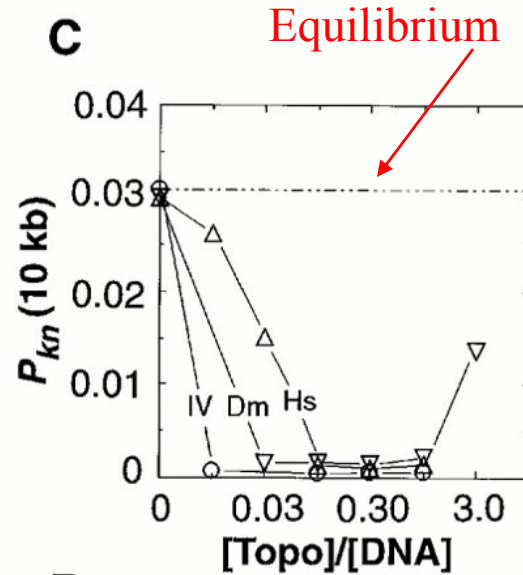
Chris Ullsperger



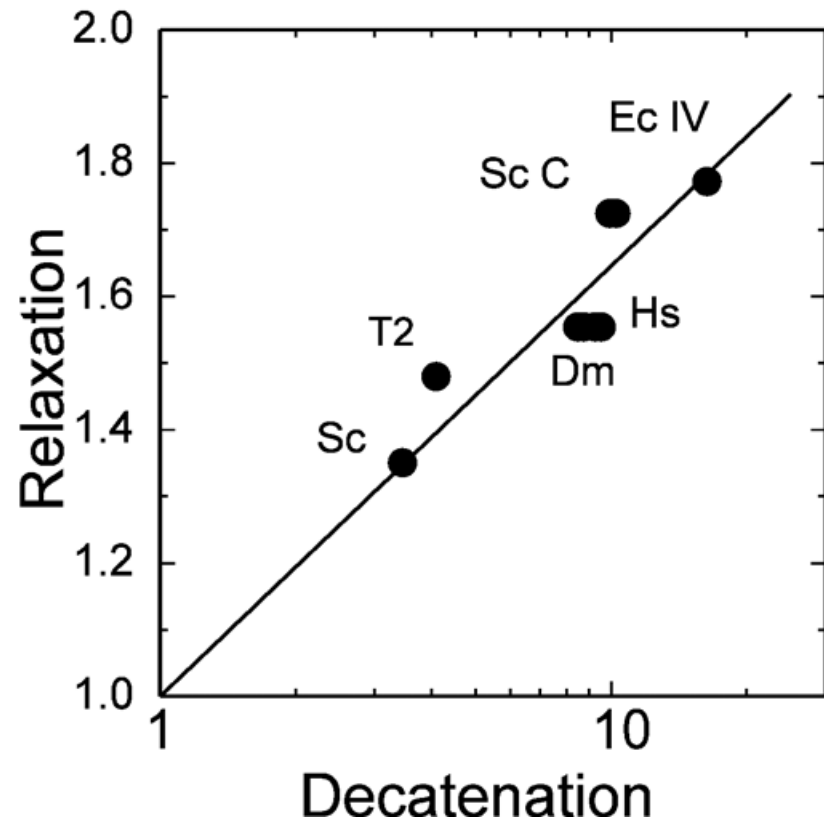
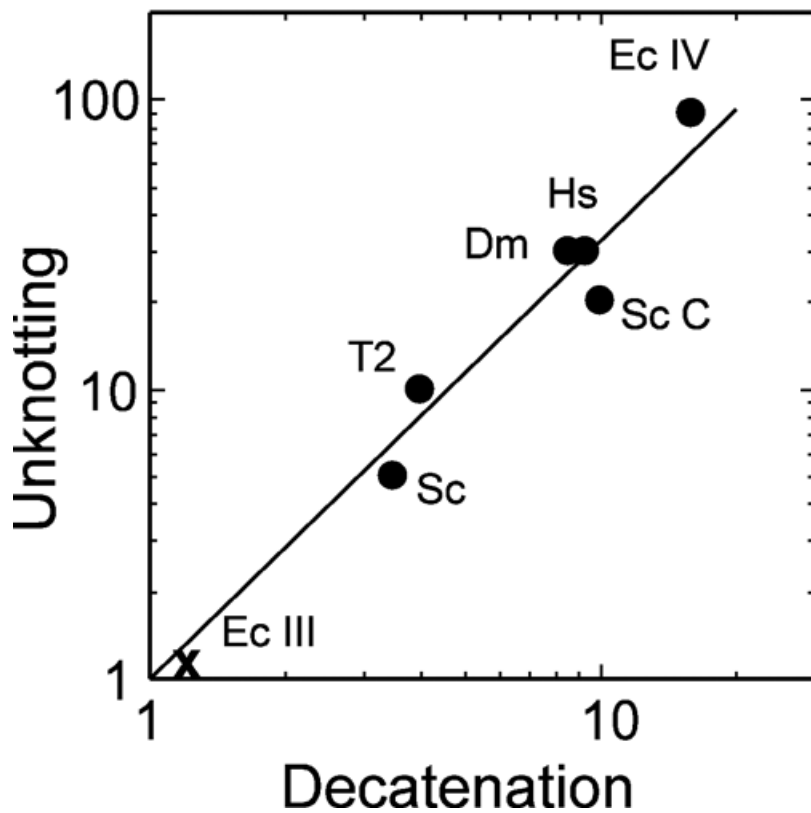
Topo IV is an efficient decatenase



Topo-2s simplify
all aspects of
topological
equilibrium

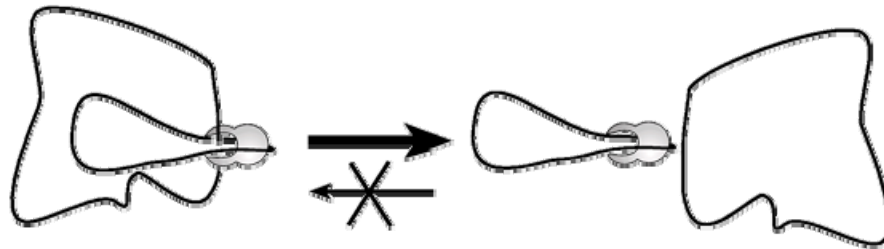


Correlation between preferential decatenation, unknotting and relaxation

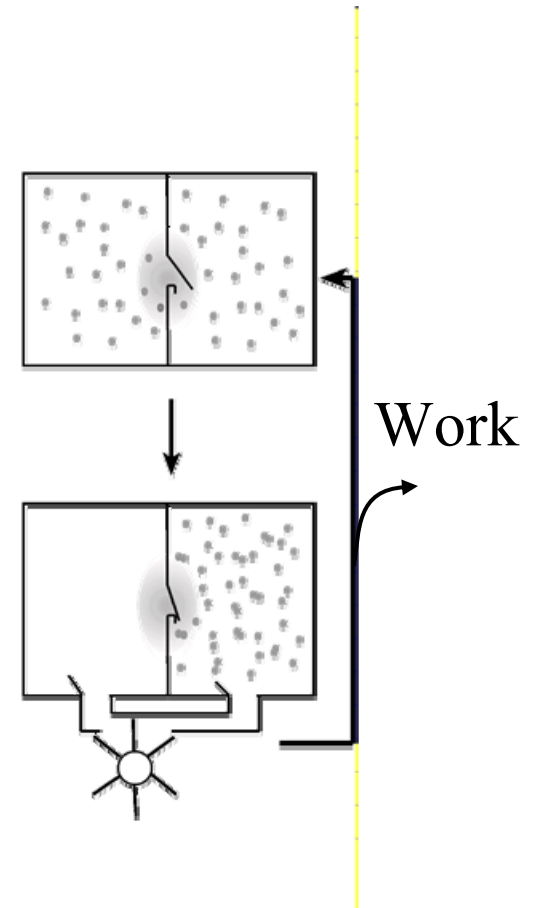
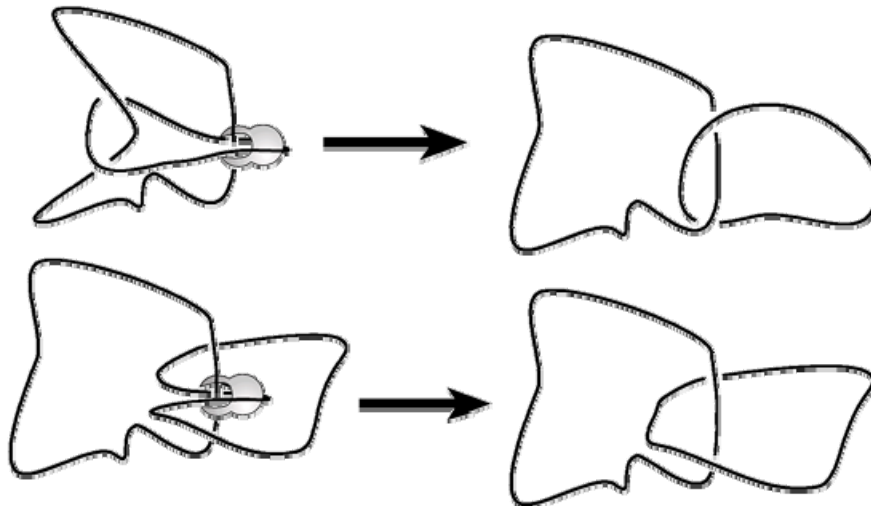


Maxwell's Demon model for topology recognition

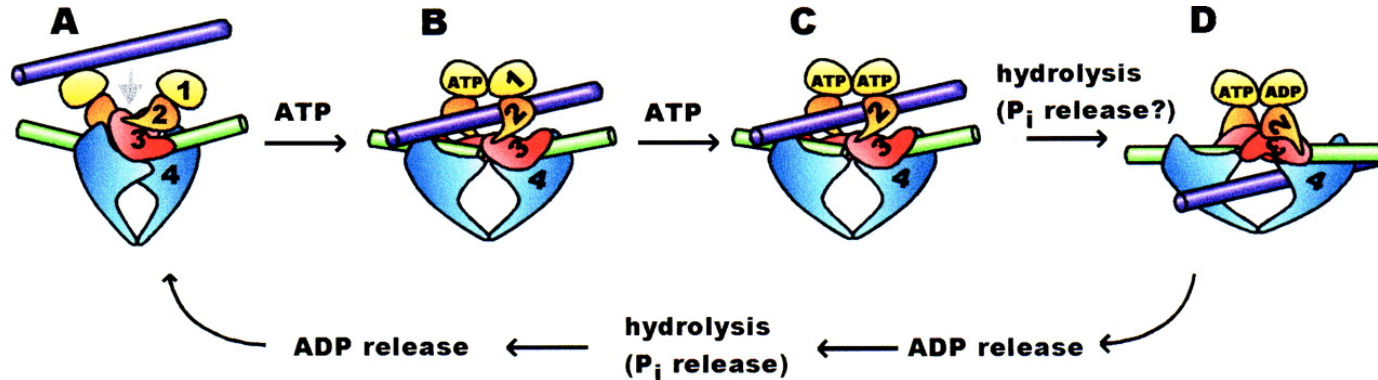
Typical (frequent) DNA conformations



Atypical (rare) DNA conformations



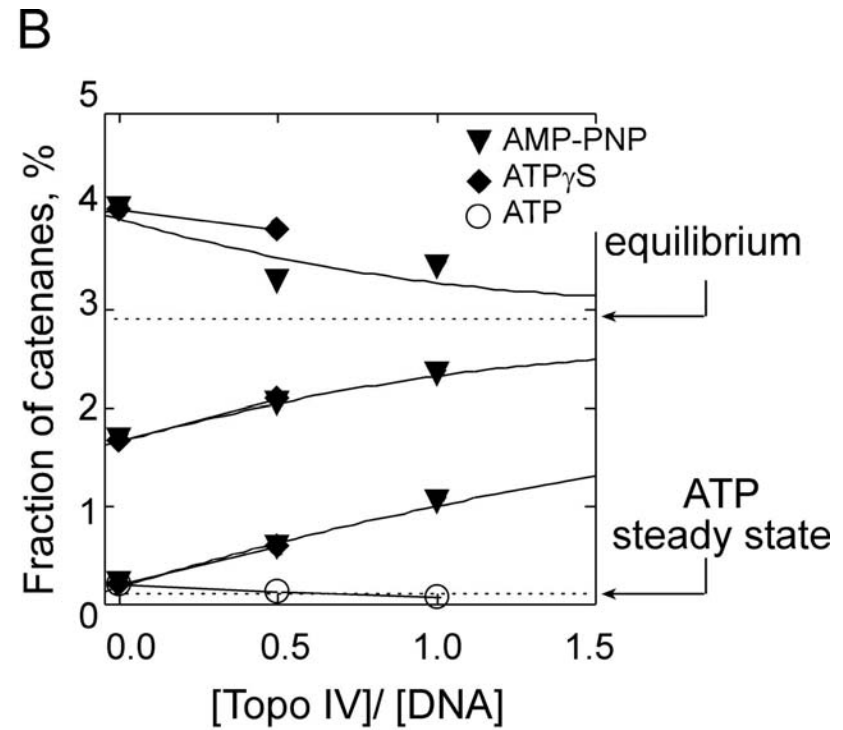
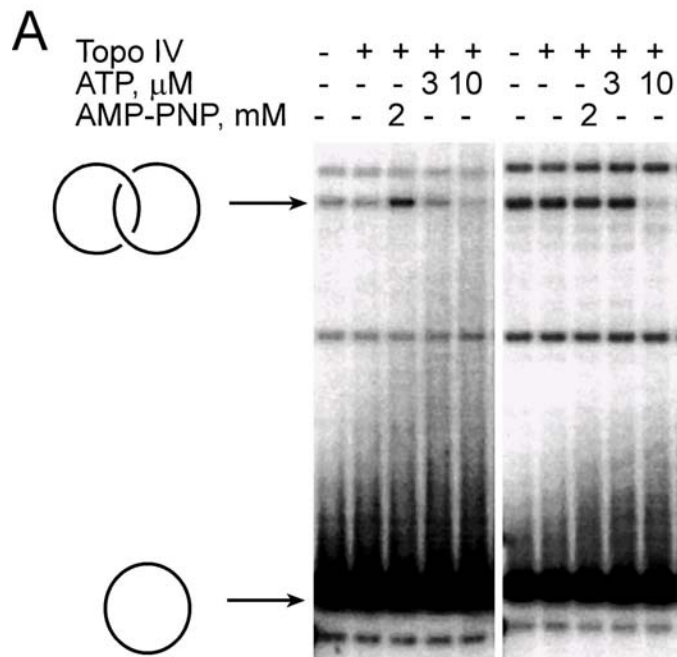
Topo-2s hydrolyze two ATPs per strand transport



Baird, Cheryl L. et al. (1999) Proc. Natl. Acad. Sci. USA 96, 13685-13690

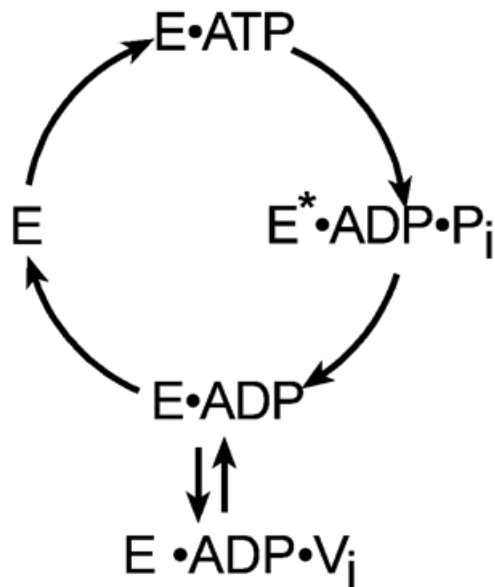
First ATP is hydrolyzed fast; the second ATP hydrolysis is slow.

Non-hydrolyzable ATP analogs promote equilibrium catenation



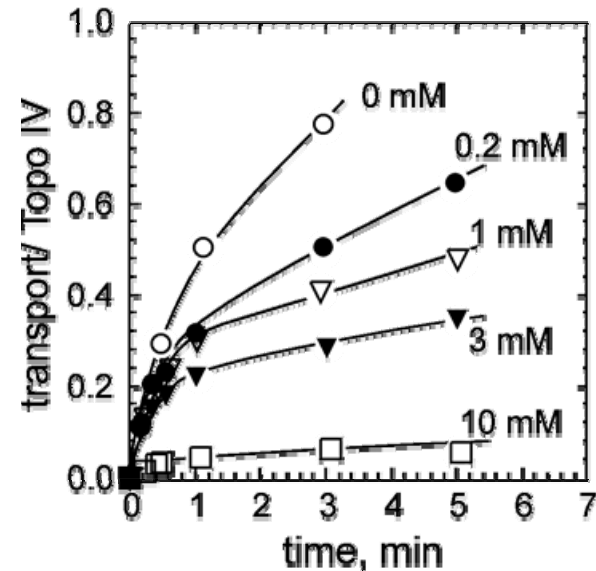
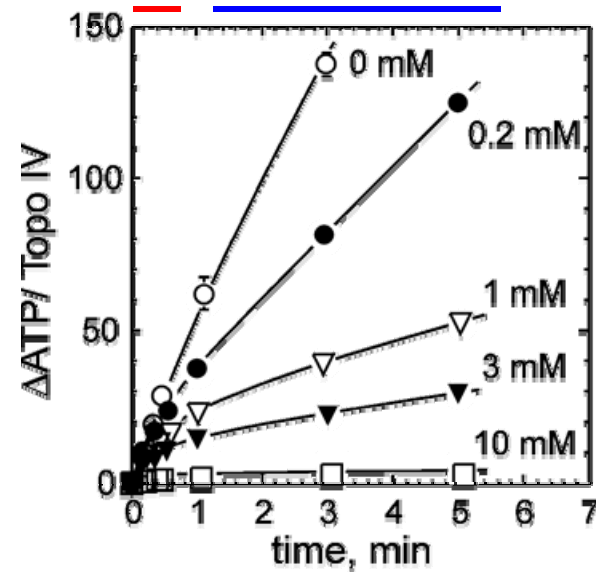
Does Topo IV hydrolyze only 2 ATPs per strand transport?

Single turnover kinetics:
vanadate, phosphate
analog, traps Topo IV in
ADP-bound form

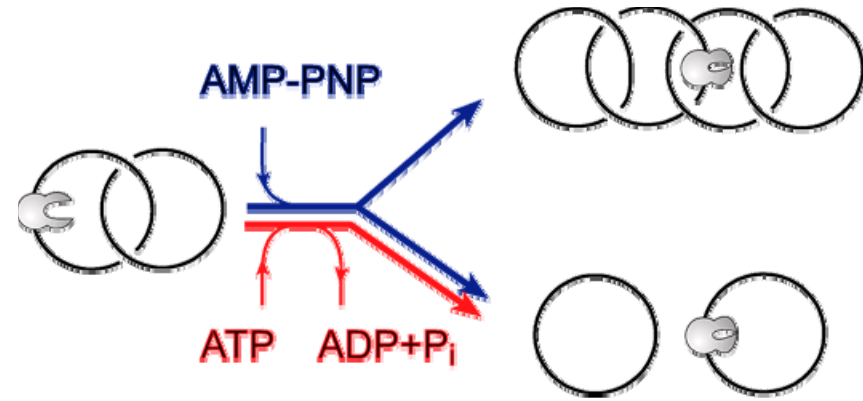


Burst phase kinetics: first enzyme
turnover is faster than the rest

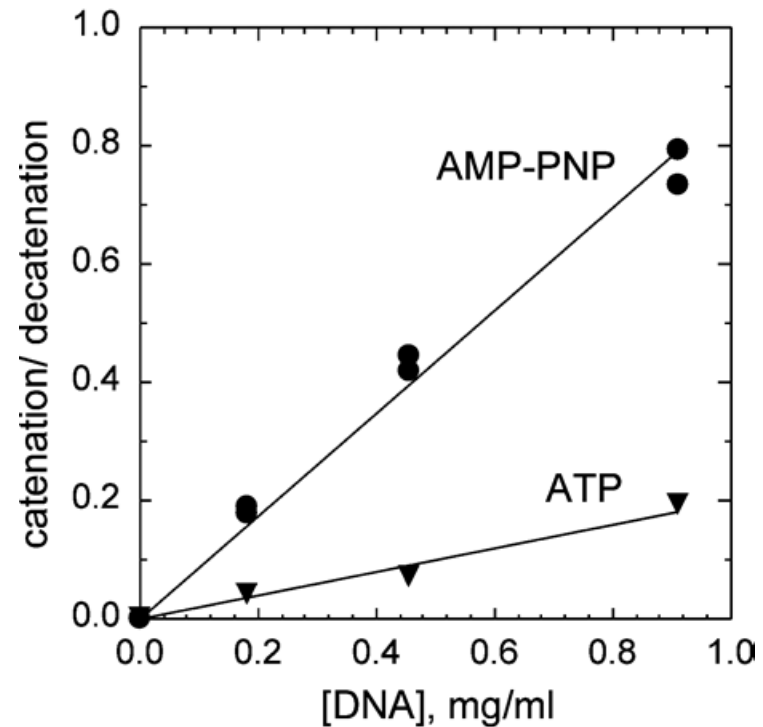
Open clamp (active) Closed clamp (inactive)



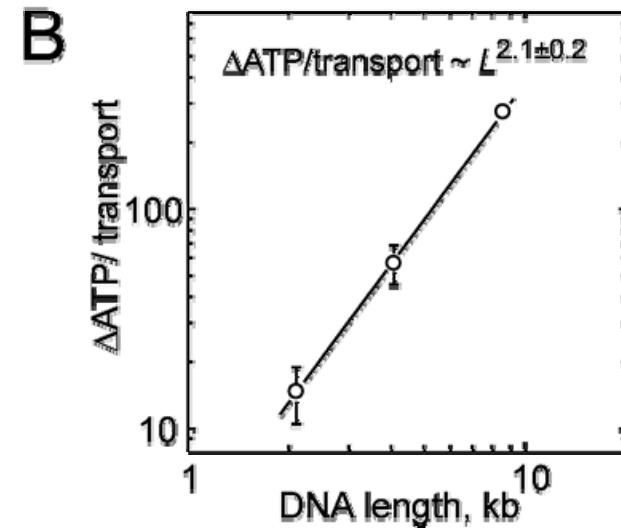
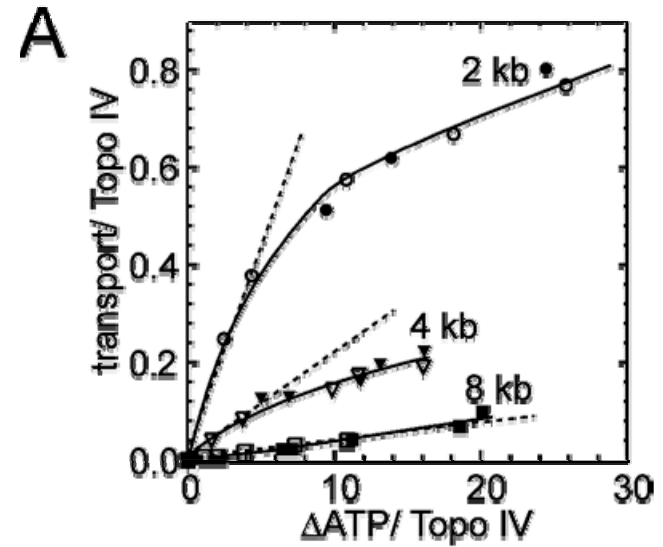
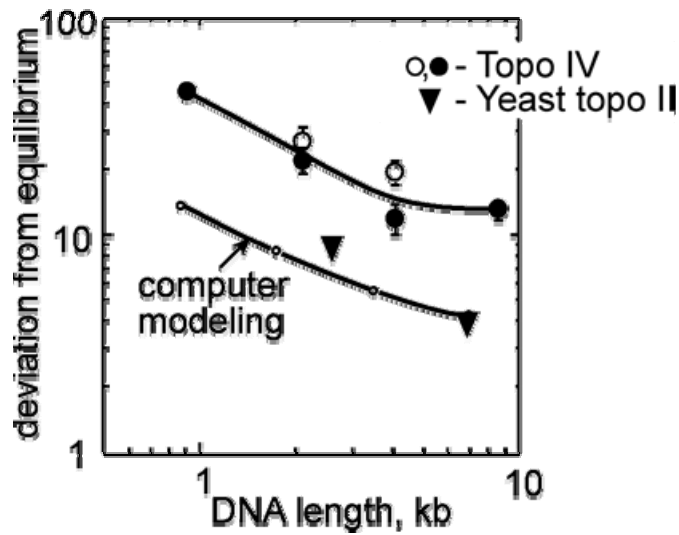
ATP, but not AMP-
PNP supports
selective transport



Two roles of ATP hydrolysis:
- topology recognition (before
strand transport)
- reactivate enzyme (after strand
transport)

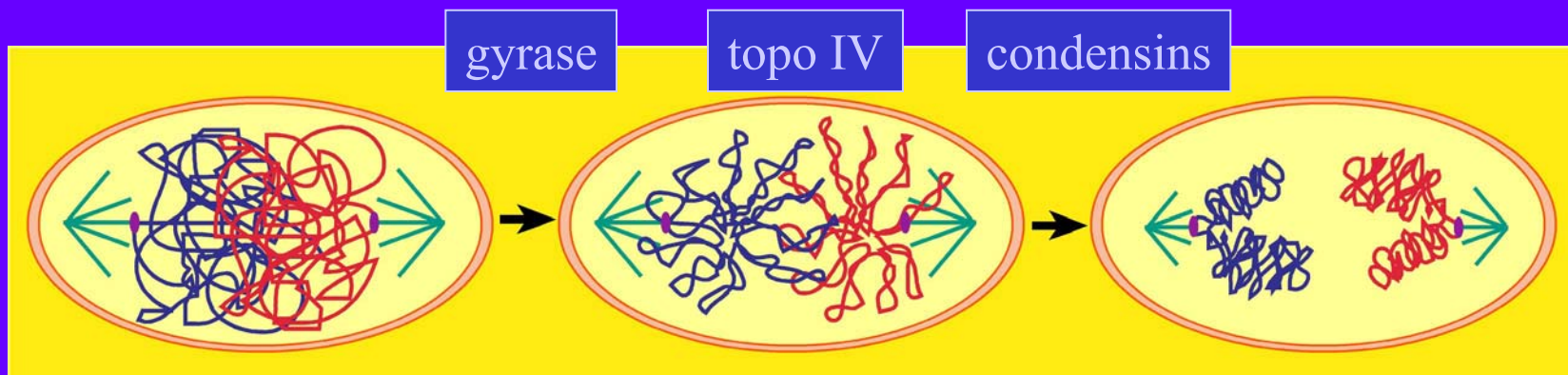


Length dependence of Topo IV efficiency supports “local” models of topology recognition



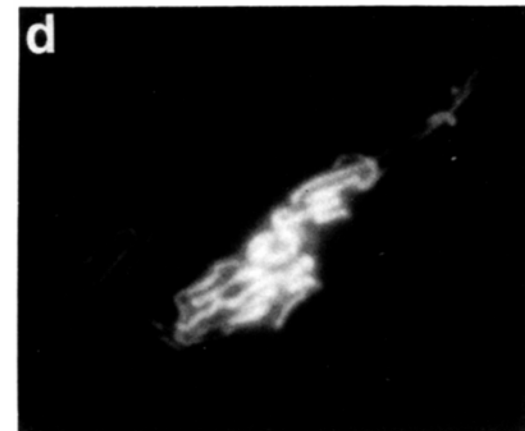
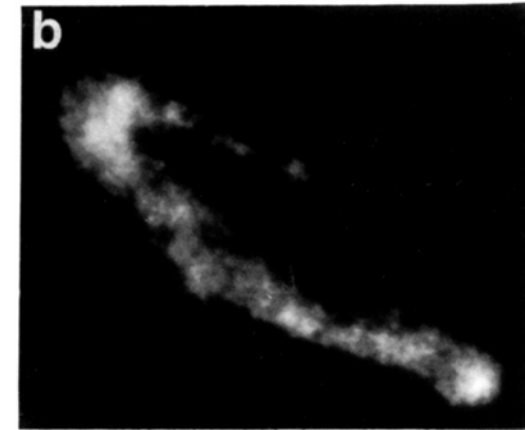
Expected number of links between sister *E.coli* chromosomes

Relaxed random coil	Relaxed loops	Supercoiled; equilibrium	Supercoiled; steady state	Chromosome missegregation
10^6	10^4	10^1	10^0	10^{-5}

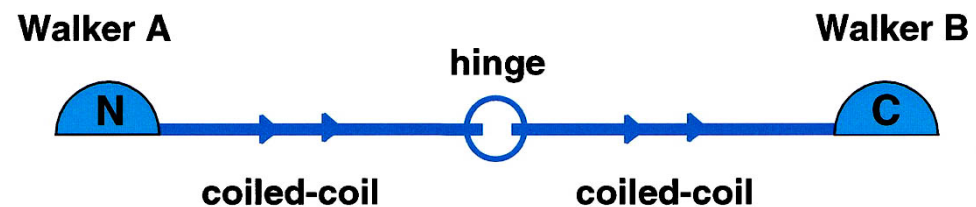
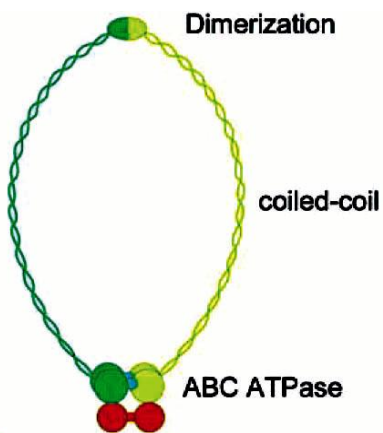


SMC (Structural Maintenance of Chromosome) proteins:

- Are found in all kingdoms of life
- Have distinctive structure
- Are required for diverse global chromatin functions
- Act in complex with non-SMC subunits
- Alter DNA shape in vitro and in situ
- Require ATP for function and activity

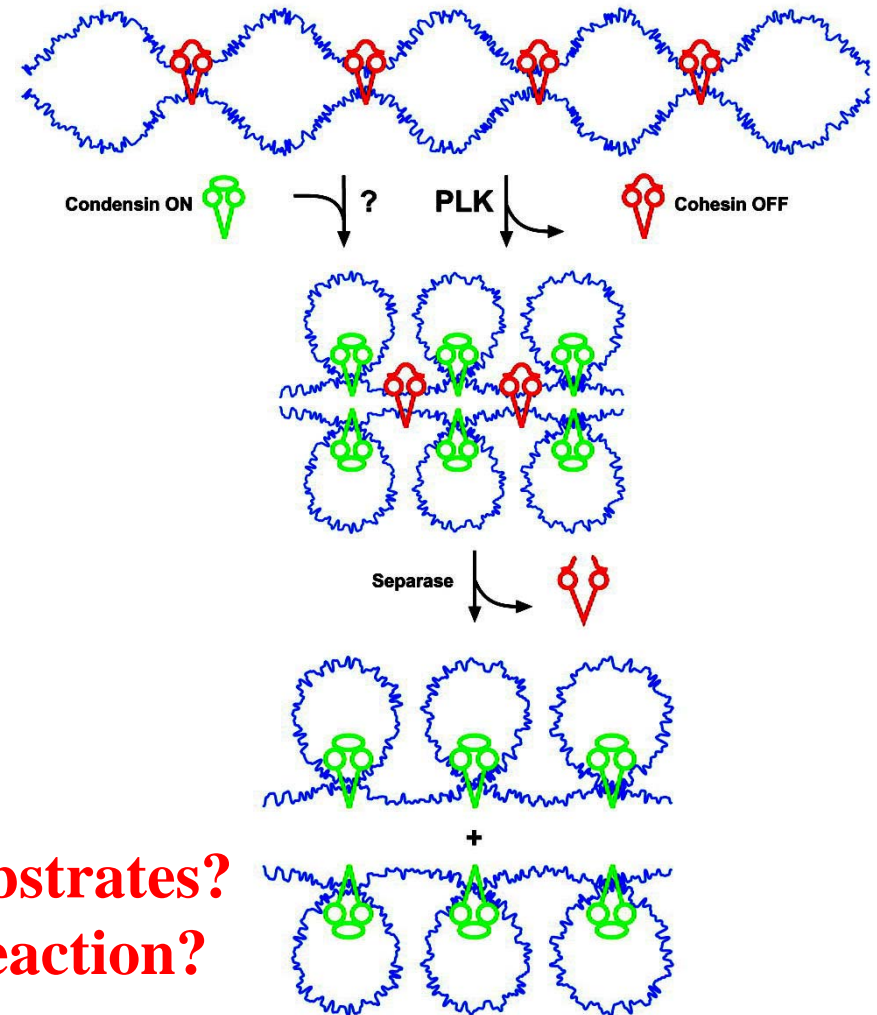


Kimura et al, 1997



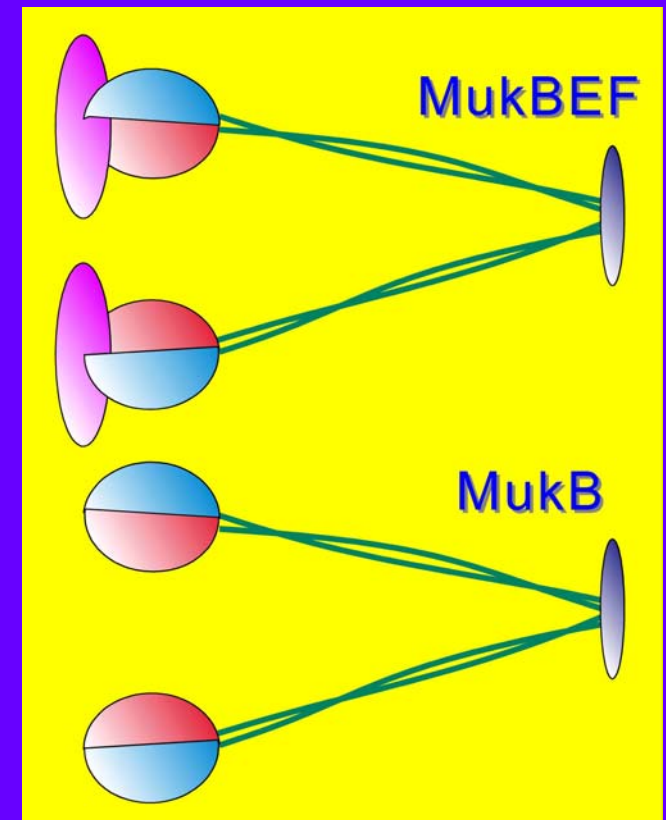
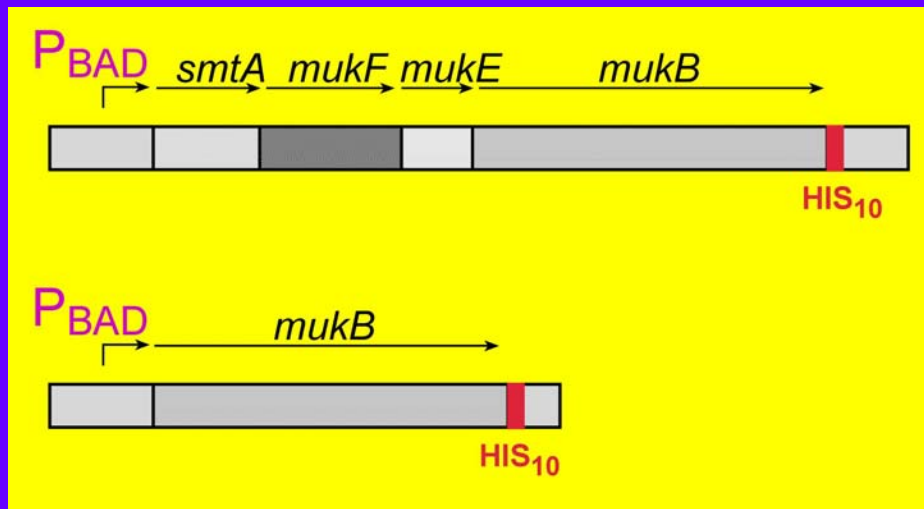
Several kinds of SMC complexes

- Chromosome condensation (Condensins): intramolecular DNA condensation
- Chromosome cohesion (cohesins): intermolecular DNA condensation
- Recombinational repair (e.g. Rad50, SbcC): DNA end binding
- Dosage compensation (compensins): intramolecular DNA condensation?



**How do SMCs recognize DNA substrates?
What determines specificity of reaction?**

DNA reshaping by bacterial condensin MukBEF



Inactivation of MukB, MukE or MukF produces the same phenotype: chromosome decondensation and cutting, anucleate cells

MukB recognizes global DNA shape

Predominant formation of 3- and 5-noded knots suggests solenoidal DNA supercoiling.

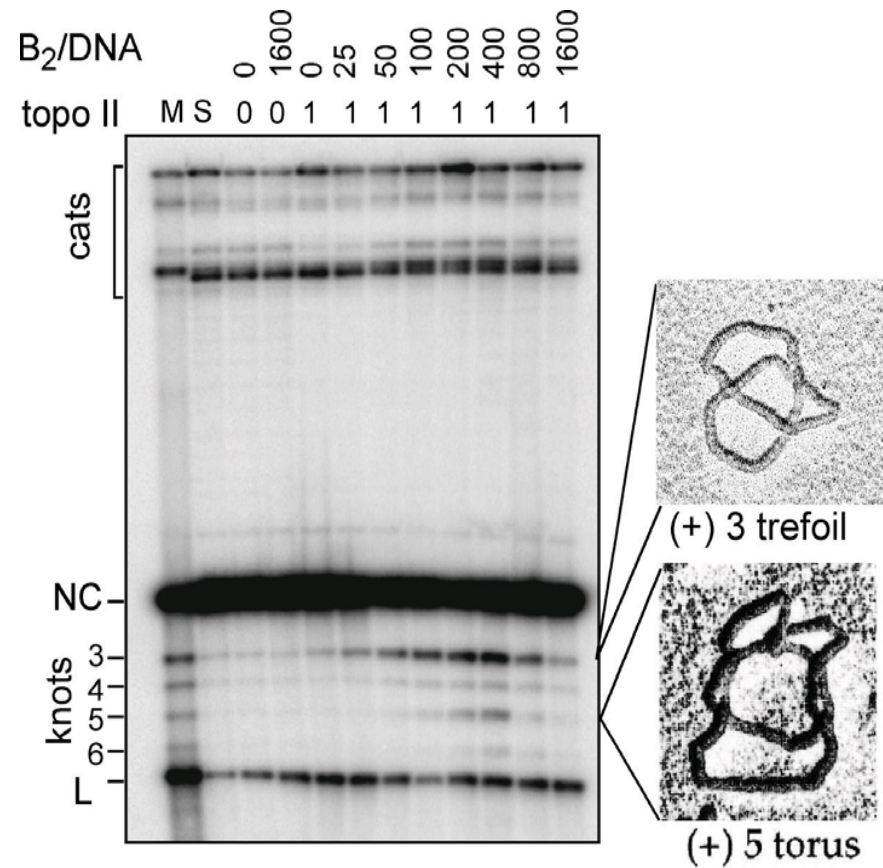
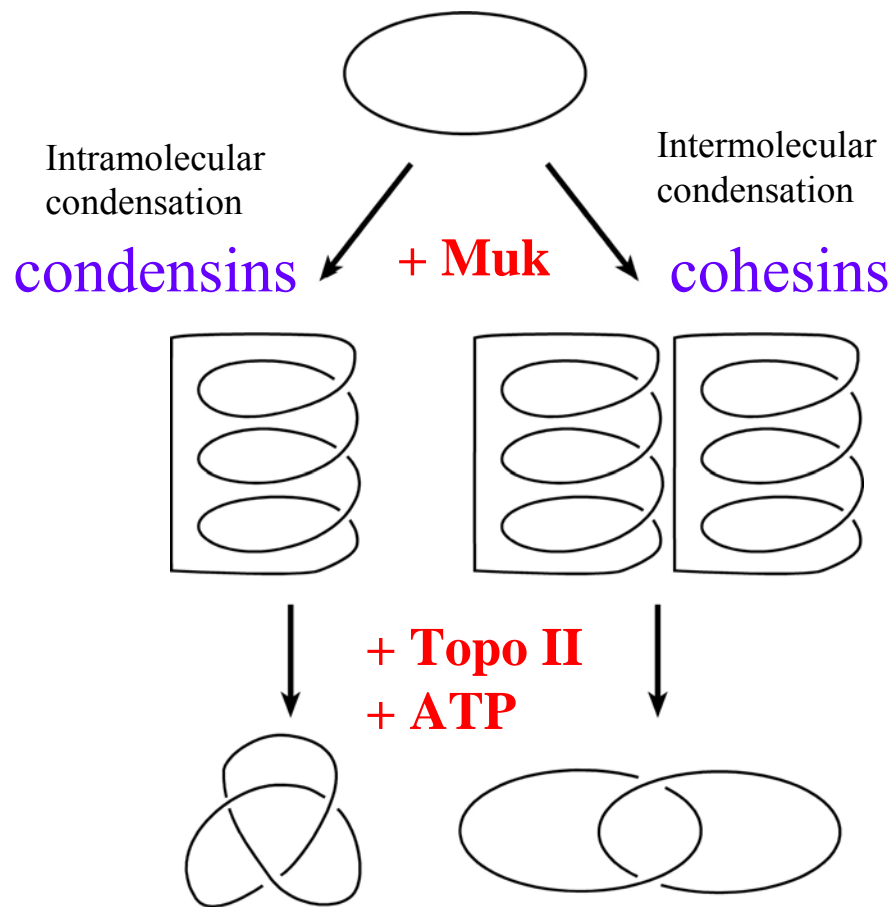
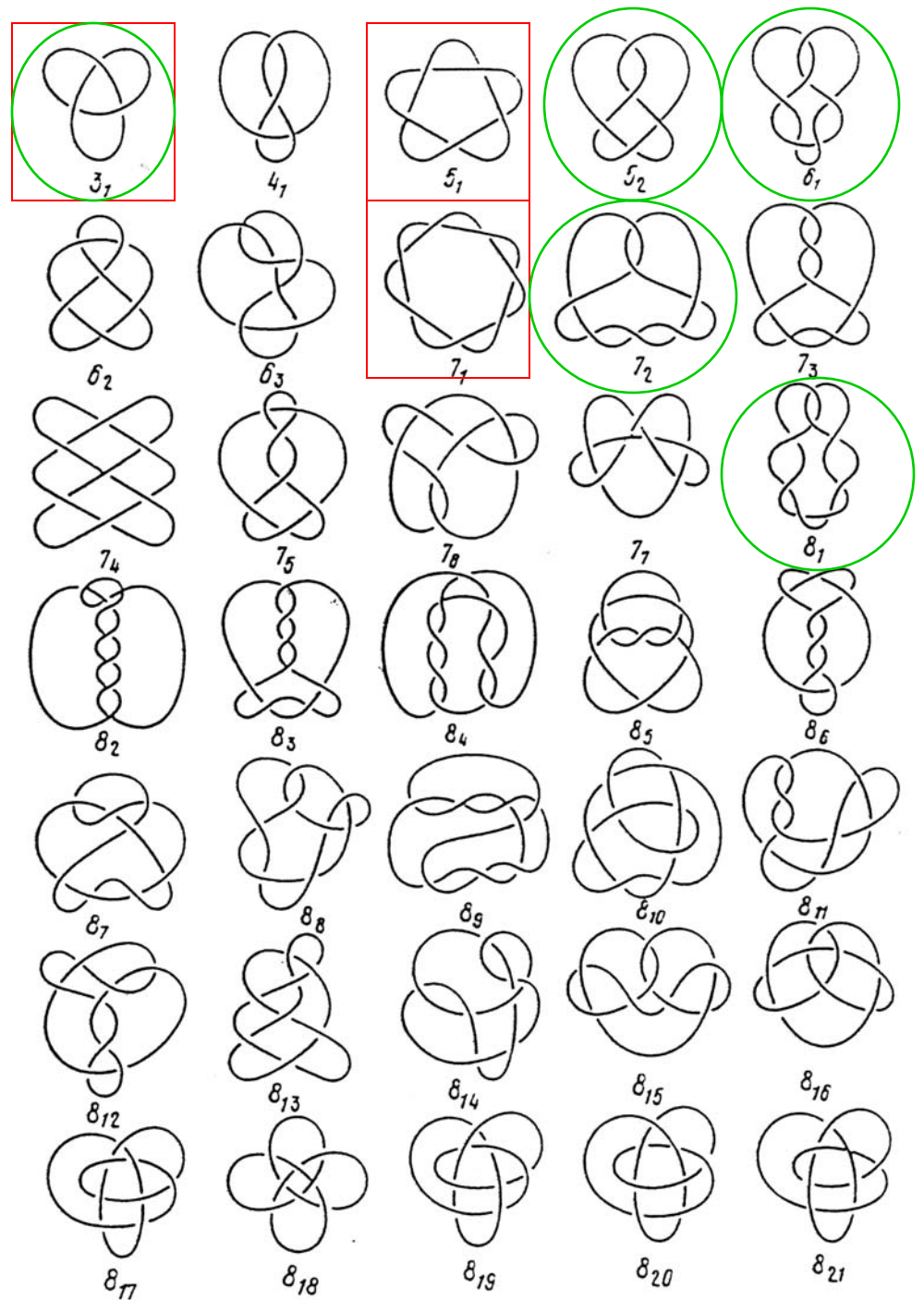


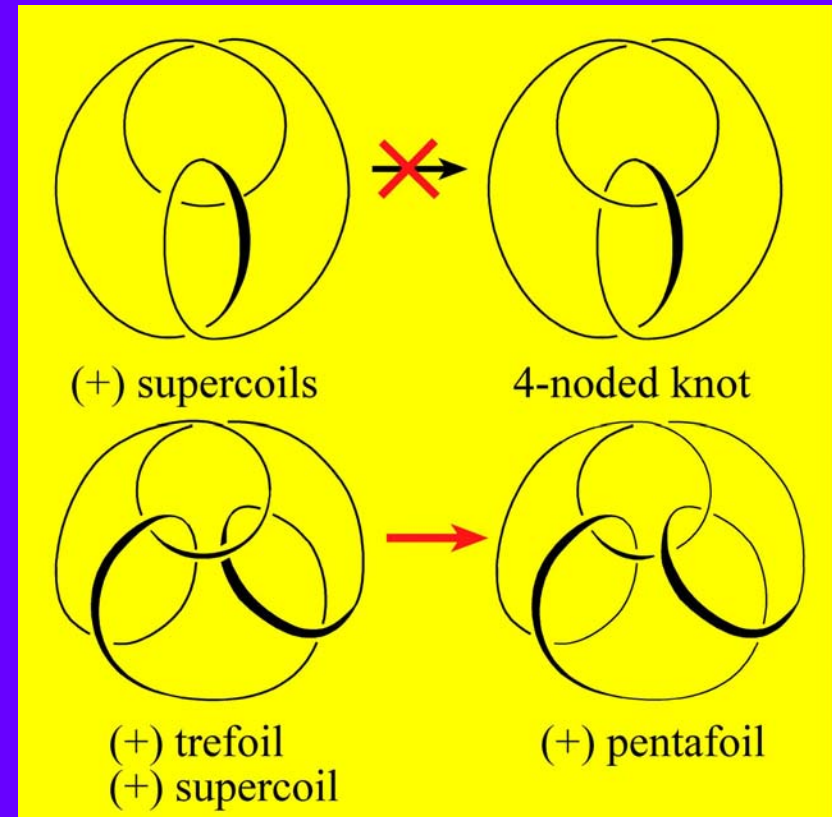
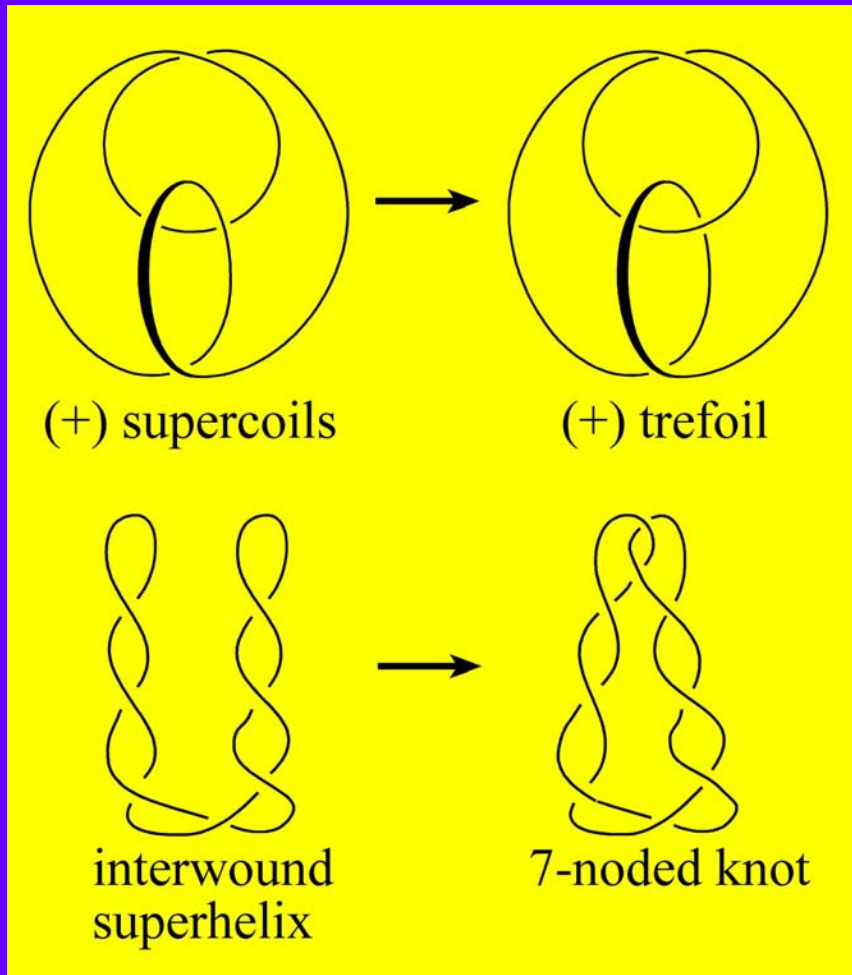
Table of knots with less than 9 minimal crossings

Knots can be classified according to the minimal number of crossings in a projection

Note *twist* (circled; step of 1) and *torus* (boxed; step of 2) families of knots



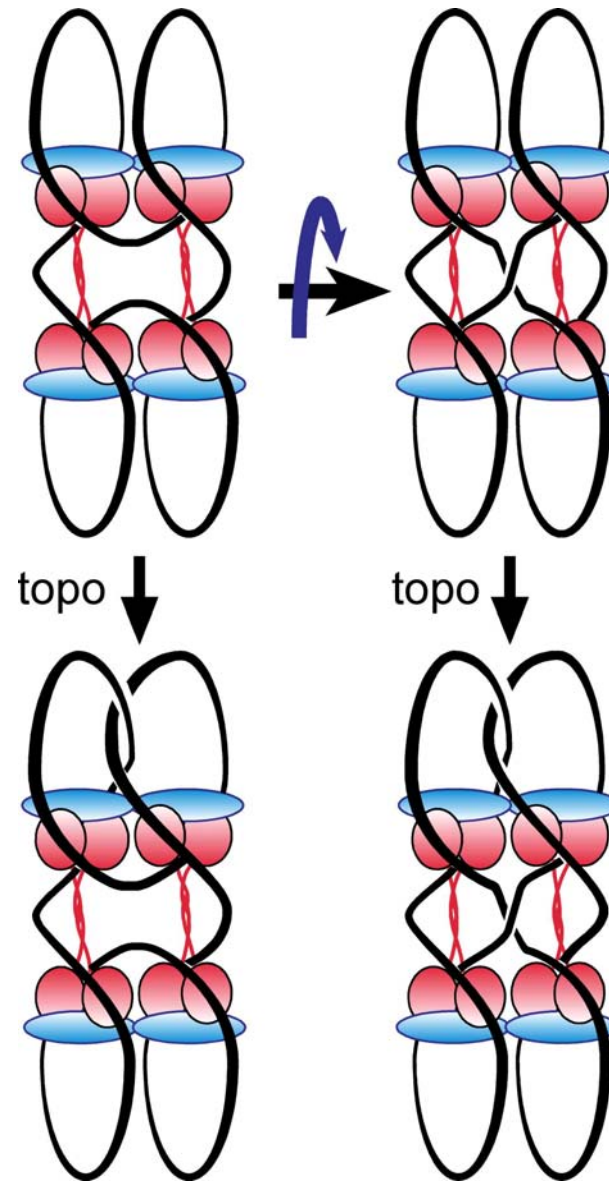
Chiral trefoil knots suggest solenoidal, looped DNA



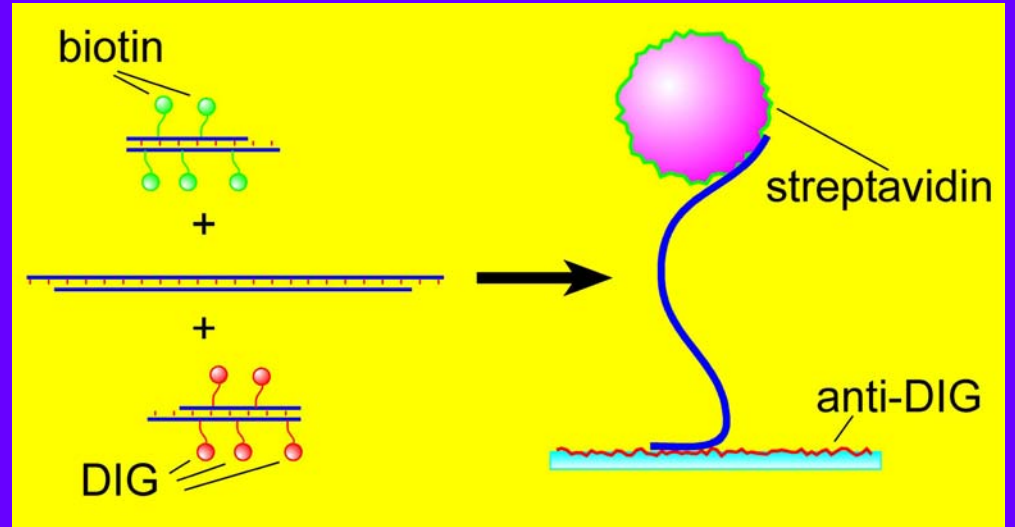
Topology of more complex knots is
also consistent with looped DNA

DNA reshaping by MukB

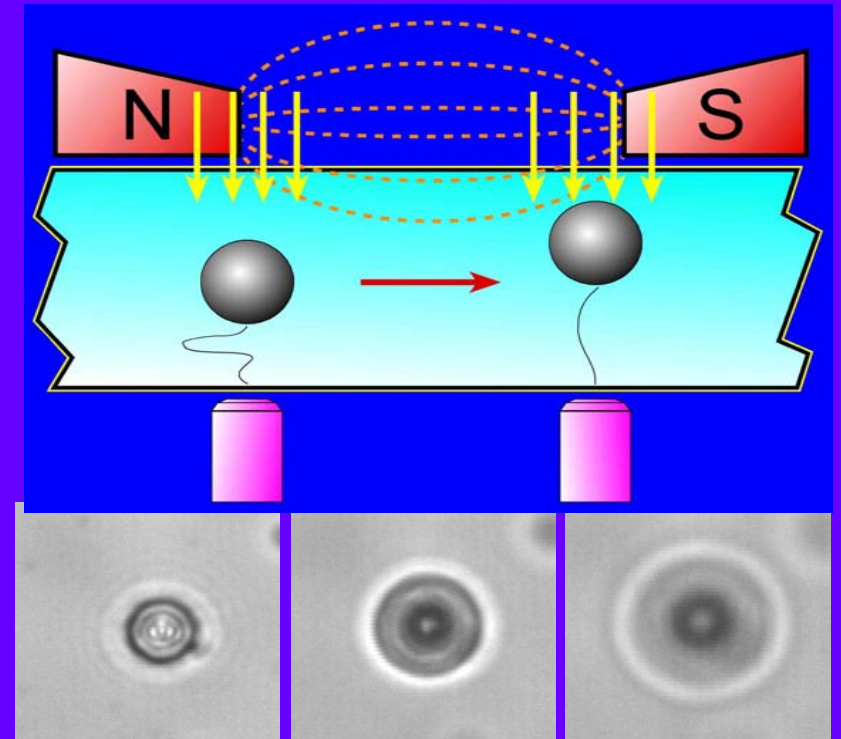
- MukB stabilizes right handed coils in DNA
- DNA coils are arranged in space - and thereby limit excessive knotting – *protein-DNA filament?*



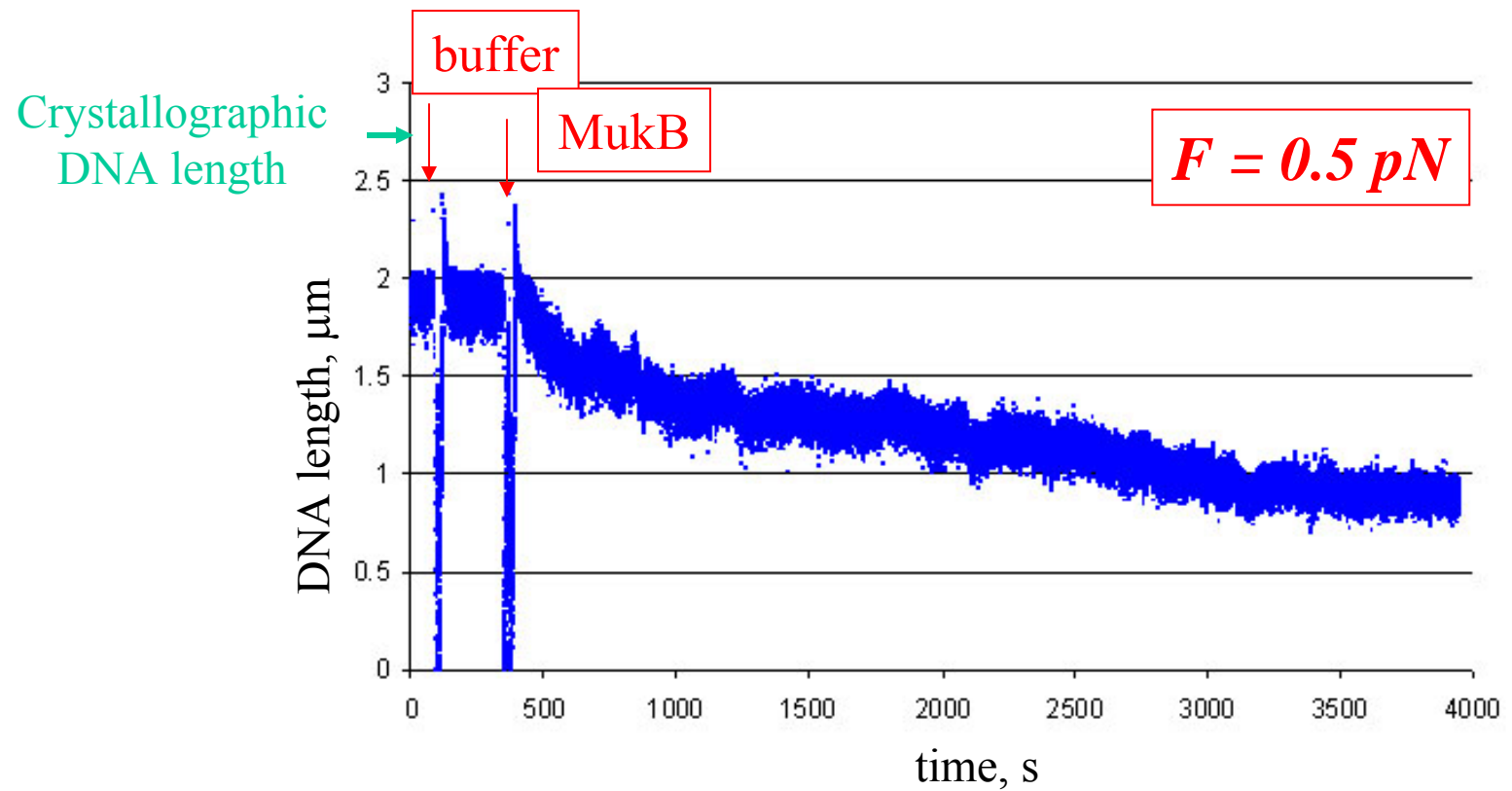
DNA with modified
extremities can be
attached to a bead and
a surface



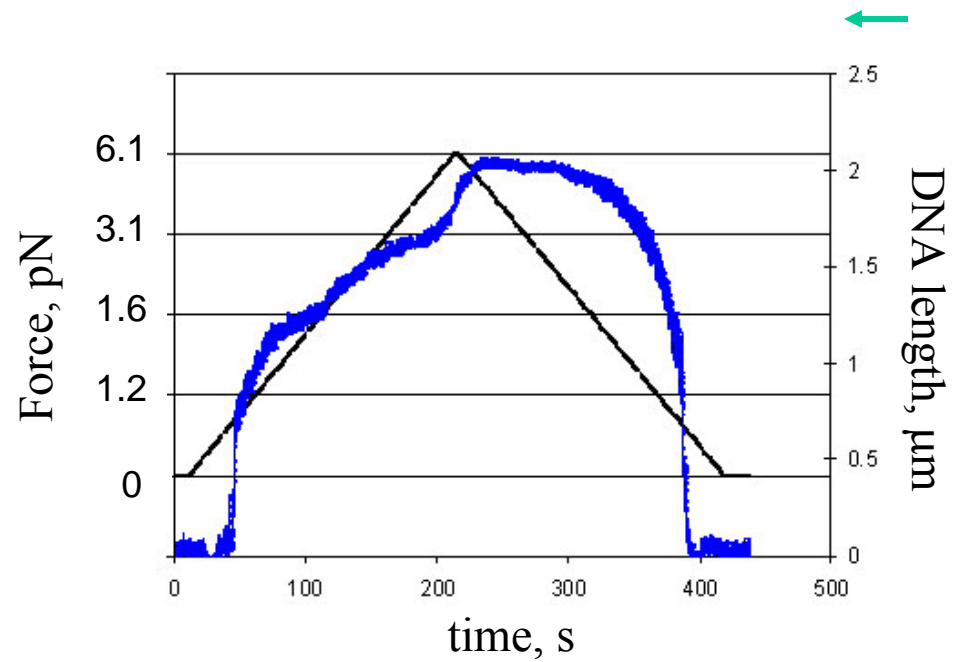
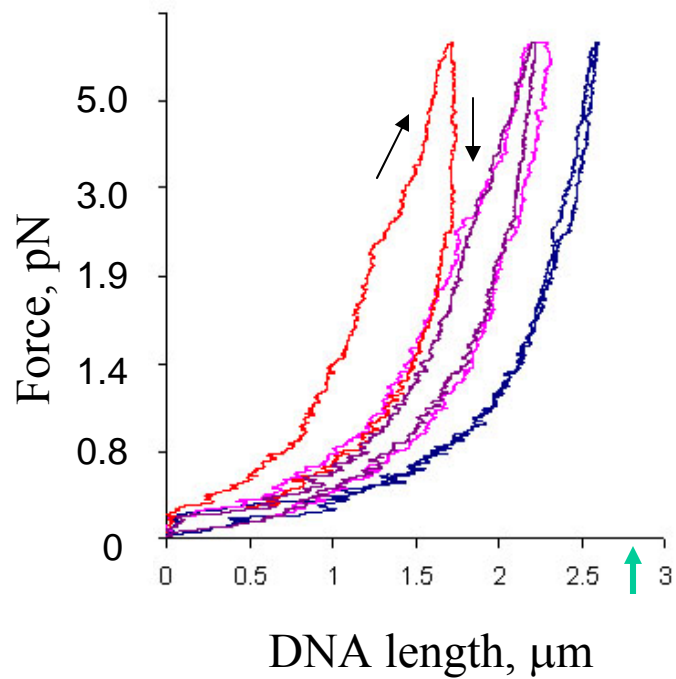
XYZ position of the bead
can be followed ± 10 nm



DNA condensation by MukB in real time



DNA stretching confirms multiprotein MukB-DNA complex

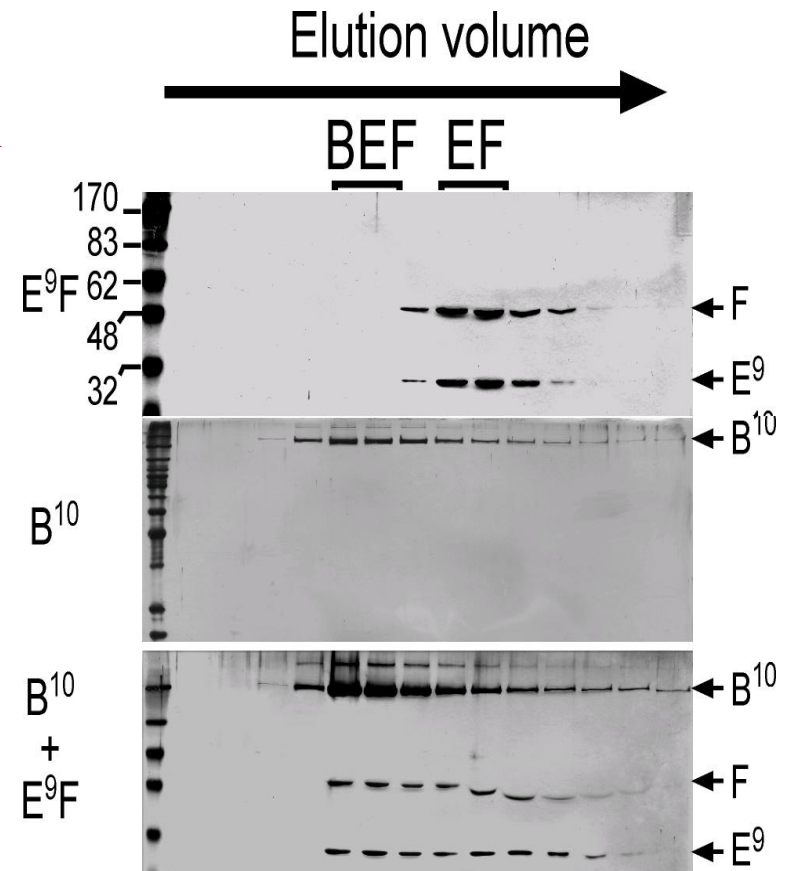
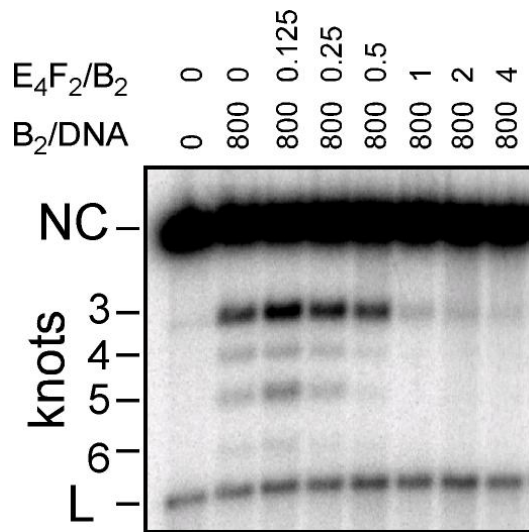
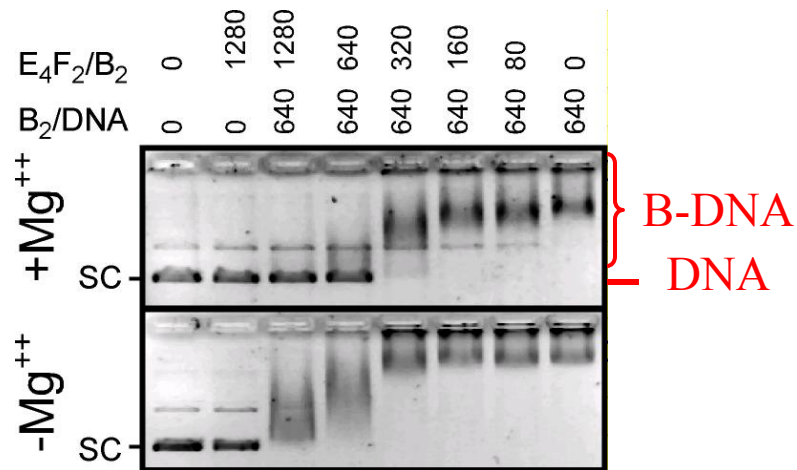


Summary of DNA reshaping by MukB (E.coli) vs. 13S condensin (frogs)

	13S condensin	MukBEF
DNA knotting	(+)trefoils	(+)trefoils
Right handed DNA looping	Yes	Yes
Supercoiling	(+)	(-)
ATP dependence of DNA reshaping	Yes	No
Non-SMC subunits	Yes	No

Why do we need MukEF?

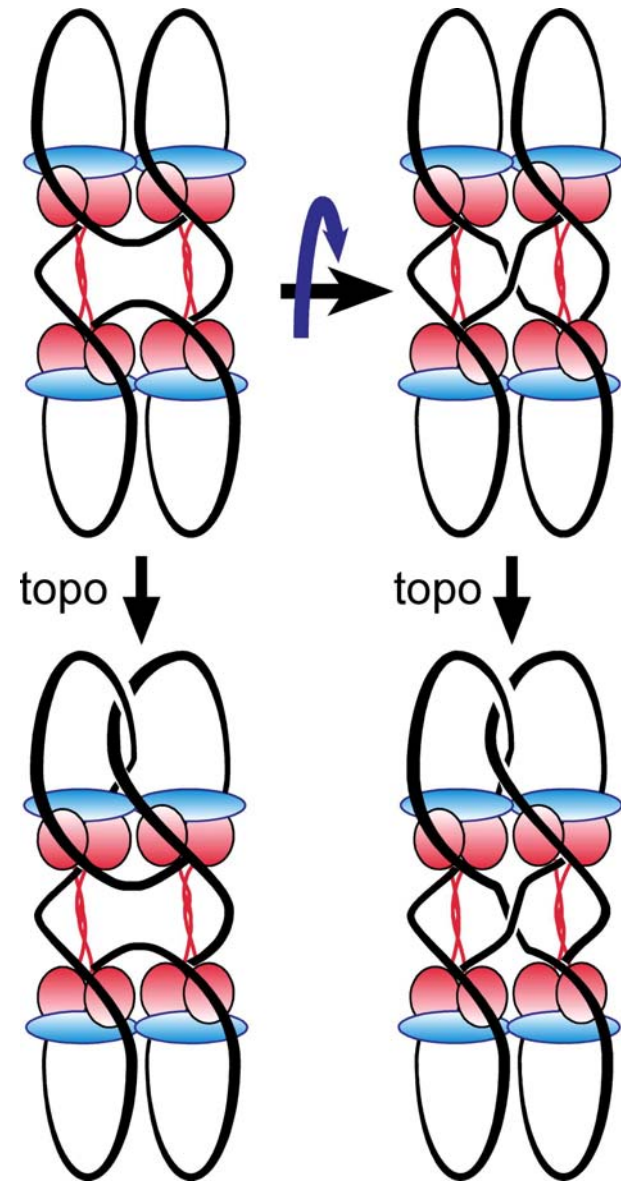
MukEF inhibits DNA binding by MukB



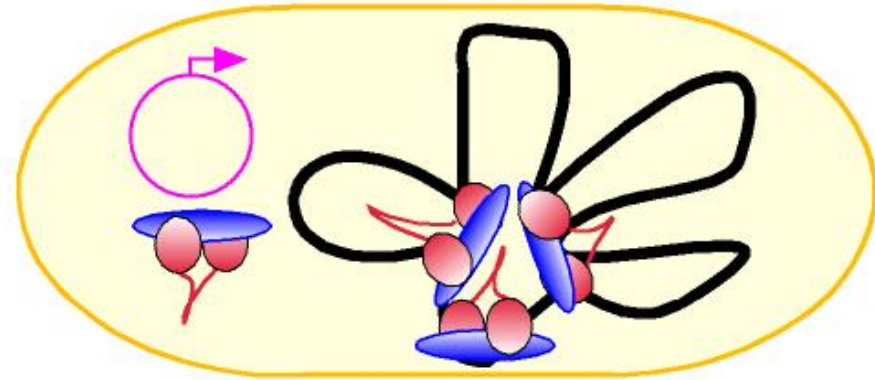
Summary of DNA reshaping by MukBEF

- MukBEF reshapes DNA in vitro by introducing right-handed loops
- MukB is the DNA reshaping end of MukBEF
- MukEF inhibits MukB

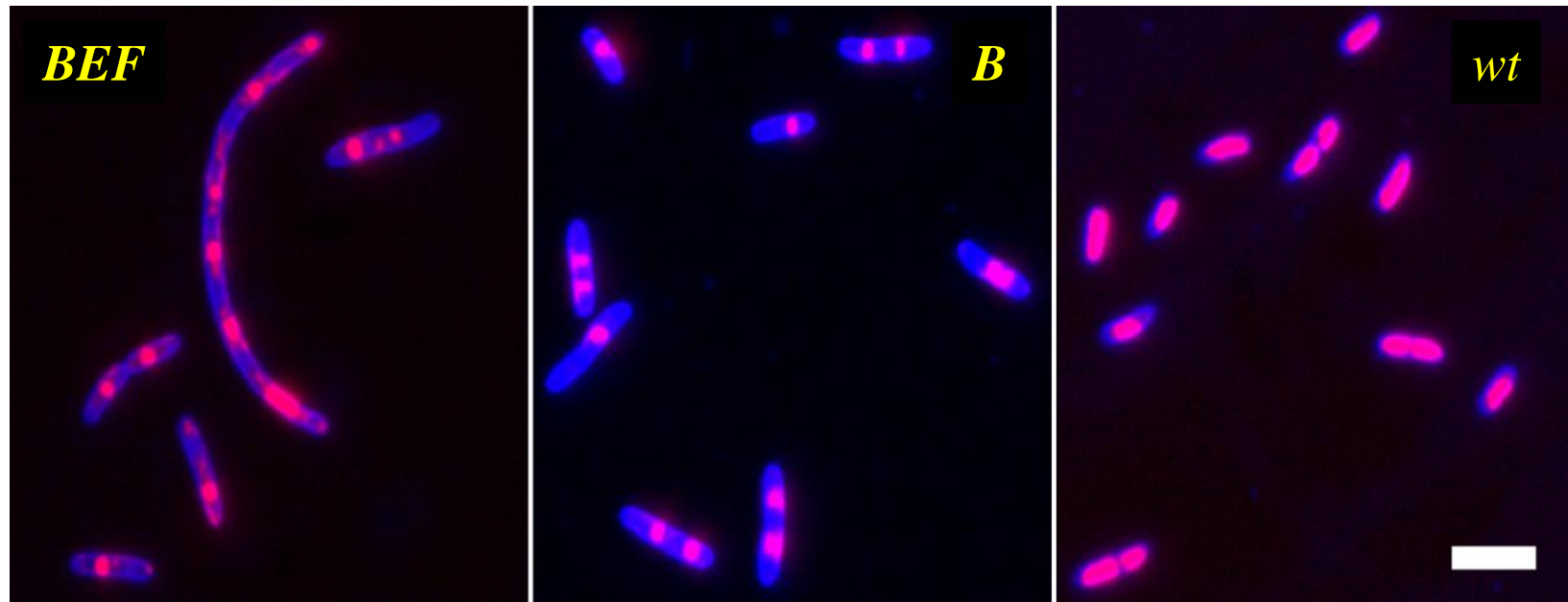
In vitro artifact?



Biochemistry in vivo:
Overproduction of
either MukBEF or
MukB condenses
nucleoids of live cells

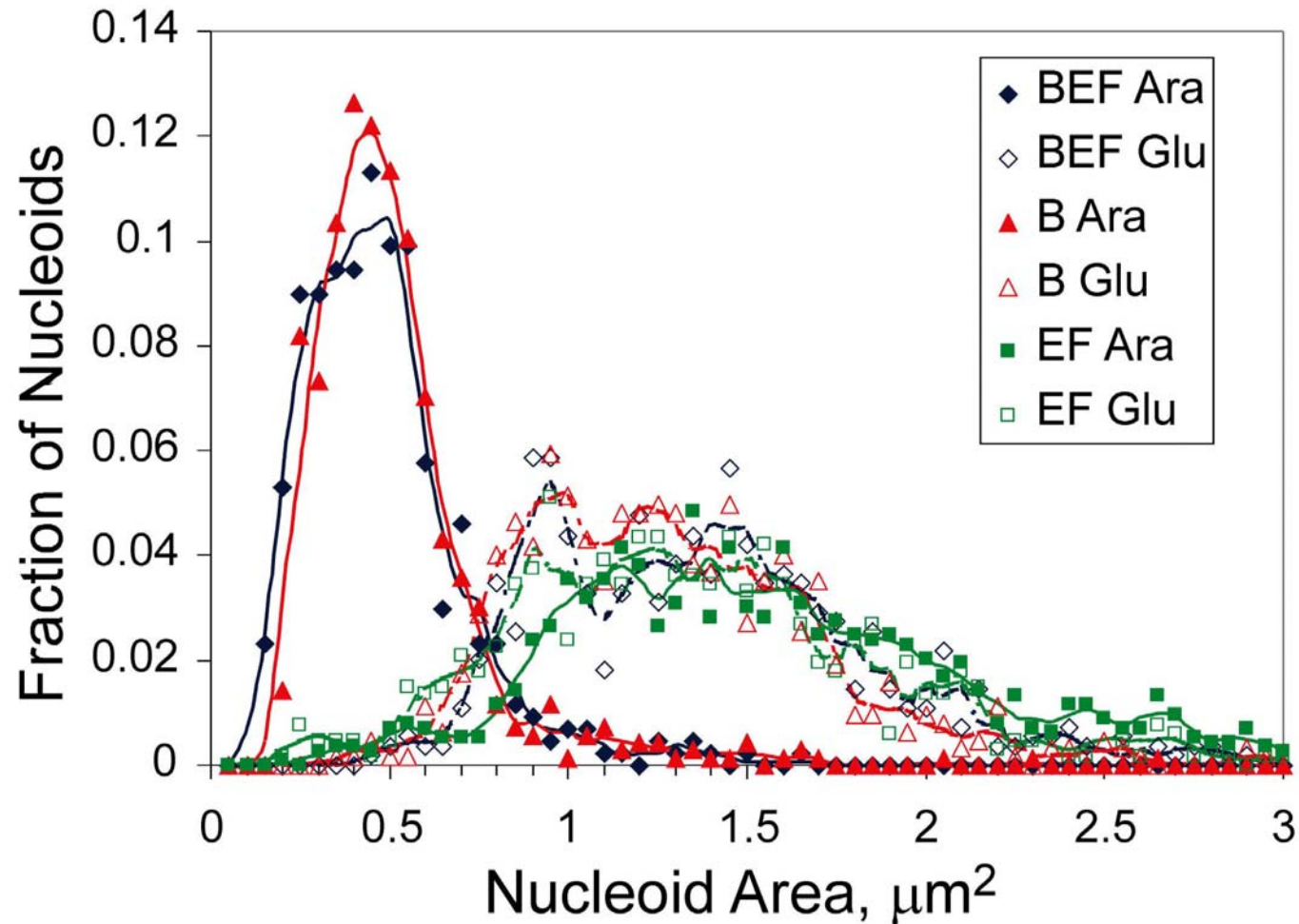


Blue – SyproOrange (cells) *Red* – DAPI (DNA)

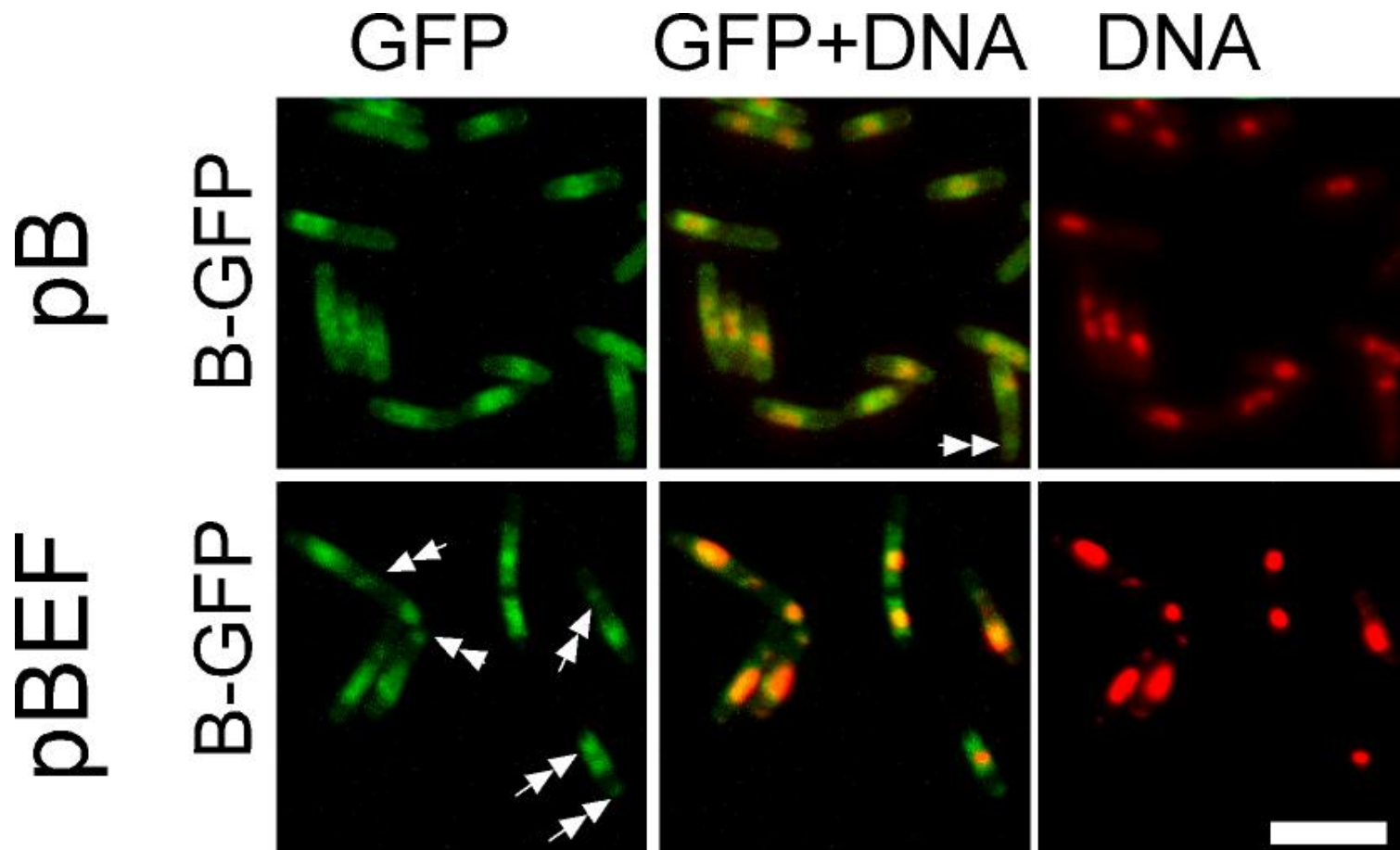


Wang *et al.*, *J. Bacteriol.* 2006

Overproduction of either ***B*** or ***BEF*** results in ~ 3 fold reduction of nucleoid *area*

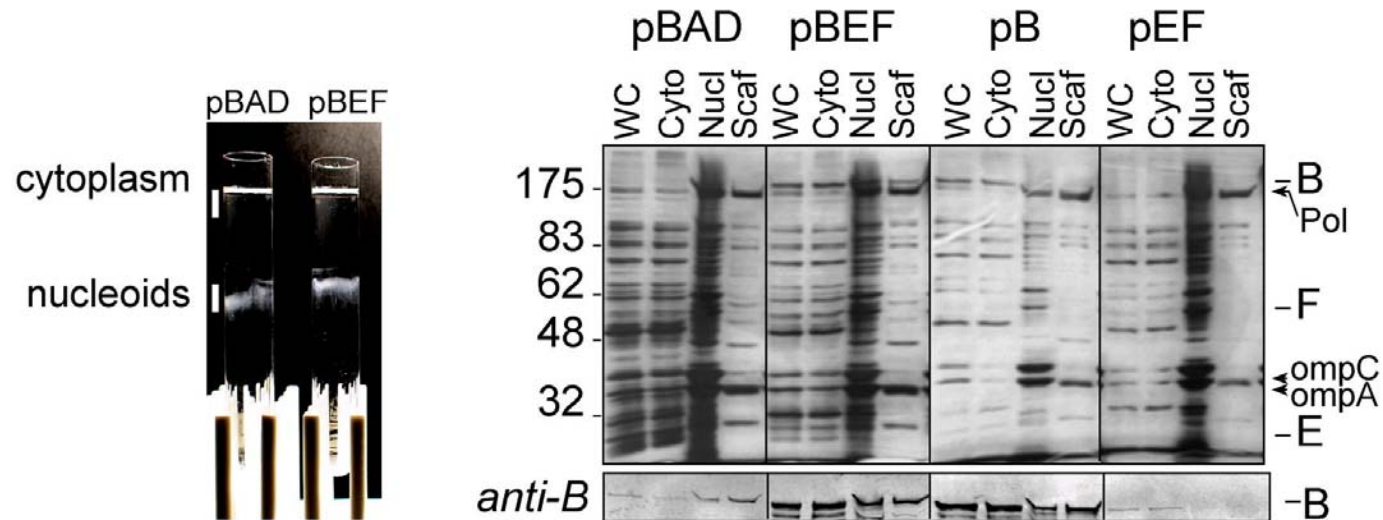


MukBEF has additional attachment sites within the cell



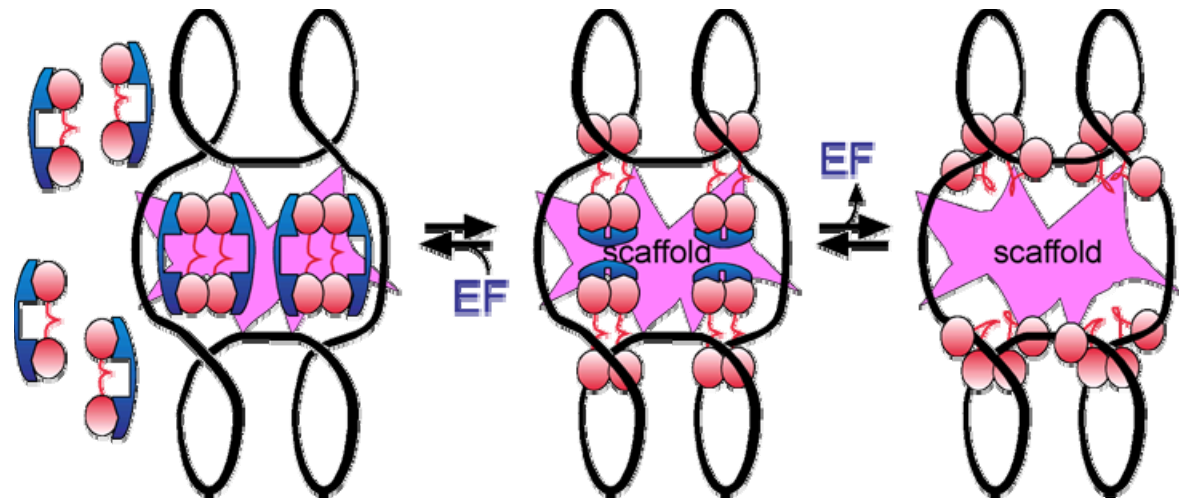
Wang et al. *J. Bacteriol.* 2006

MukBEF copurifies with chromatin scaffold



- Only a subset of proteins co-purifies with the Scaffold fraction of nucleoids.
- These proteins are believed to be a part of chromatin scaffold.
- Most of endogenous but not of overproduced MukB is in Scaffold.
- Excess (or absence) of MukEF disrupts association of MukB with scaffold.

Our current view of condensins



- MukBEF is a condensin: in vitro and in vivo
- Right-handed DNA looping is highly conserved between species
- MukB is the DNA reshaping end
- MukEF is needed for chromosome organization and association with chromatin scaffold.

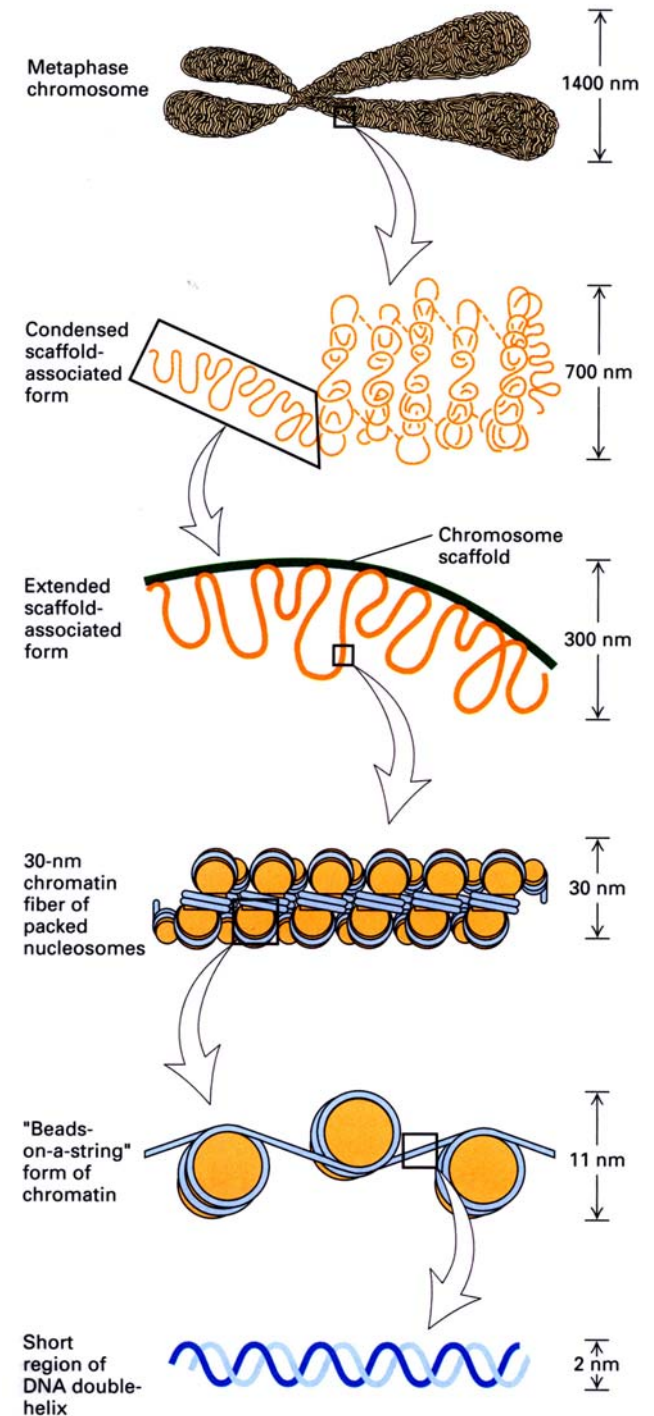
Summary

Chromatin organization in Eukaryota:

- Chromatin scaffold
- Loops
- Nucleosome packing (30-nm fiber)
- Wrapping around nucleosomes

Chromatin organization in Bacteria:

- Chromatin scaffold
- Loops
- DNA supercoiling



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