2015 Summer School on Polymers in Biology

DNA mechanics and structural diversity of DNA

@ KIAS, 22 Jun – 3 July

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Lecture 1

- Hierarchy of biological organization
- Biomolecules: 1D polymers
- Examples of Polymers in Biology: DNA, RNA, Proteins, and Polysaccharides
- DNA: genetic material; double helix
- Central Dogma
- DNA thermodynamics

Lecture 2

- Watson-Crick base pair
- Effects of chemical factors on DNA stability
- DNA sequence vs. charged polymer
- Mechanical models: Freely Jointed Chain model
- Persistence length, end-to-end extension, radius of gyration, force response

Lecture 3

- Mechanical models: Worm Like Chain model
- DNA supercoils: definition (sign, magnitude)
- Linking number, twist, writhe
- Călugăreanu-White-Fuller theorem
- Energy associated with DNA supercoiling
- Non-canonical DNA structures (induced by SC)

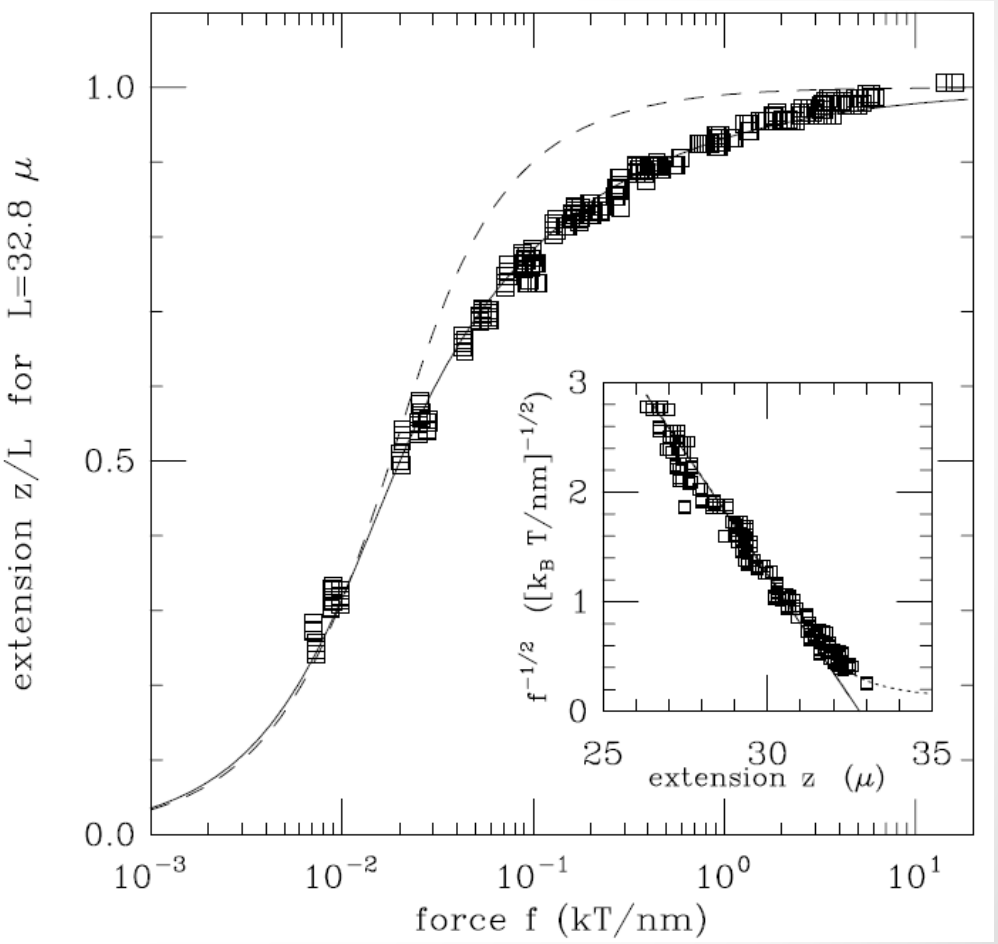
DNA is a semi-flexible chain: Worm-Like-Chain model

- Force-vs.-extension relation

  (Smith & Bustamante; Marko & Siggia)

$$f = \frac{k_B T}{\xi_p}\left[\frac{z}{L} + \frac{1}{4}\left(\frac{1}{(1-z/L)^2} - 1\right)\right]$$

$$\xi_p = 50 \text{ nm}$$



DNA is torsionally stiff

- DNA is a right-handed double helical polymer.
- This helical conformation is a state in a minimum torsional stress.
- It has a pitch of 10.5 bp/turn.
- The pitch depends on T.

- If DNA is twisted away from it, its energy is elevated.
- In order to reduce torsional stress, DNA can adopt a secondary coiling conformation called DNA supercoils.

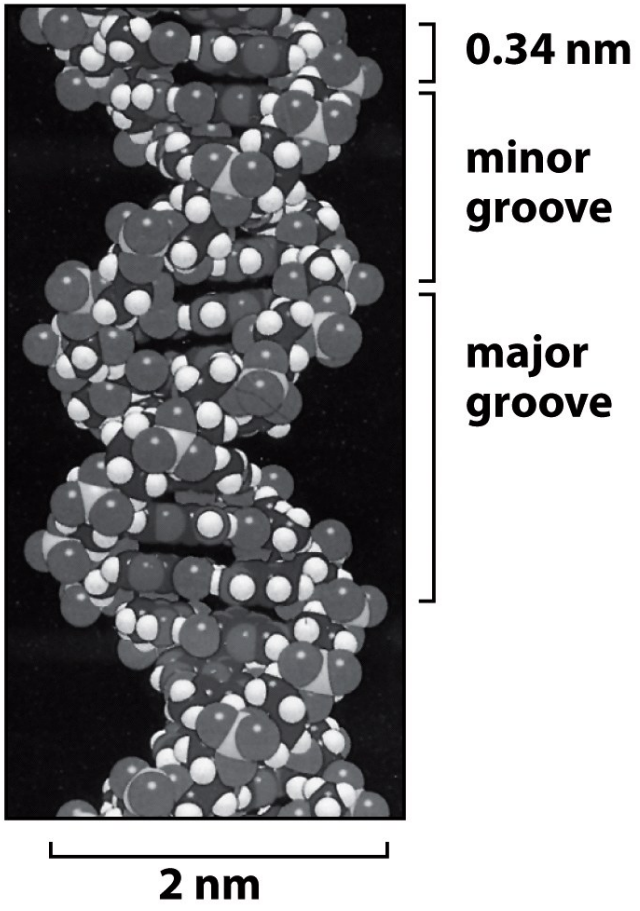
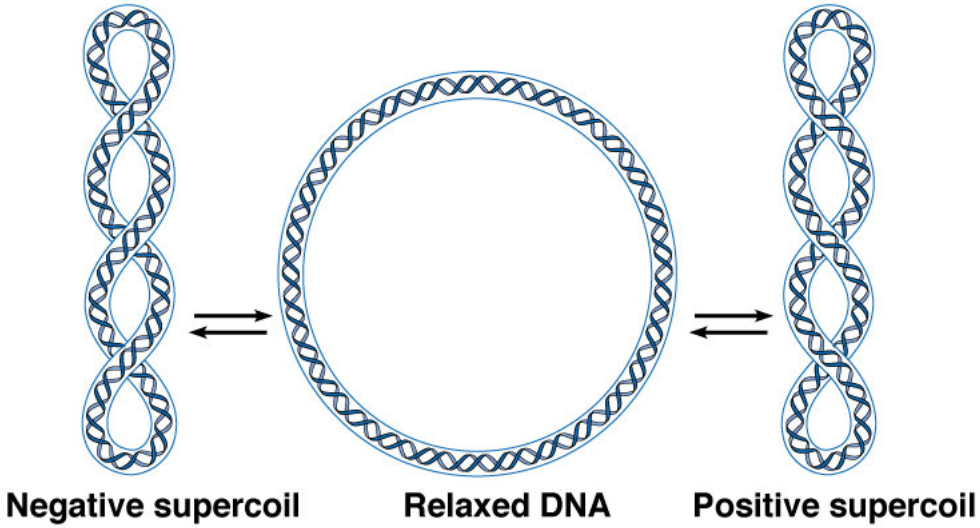
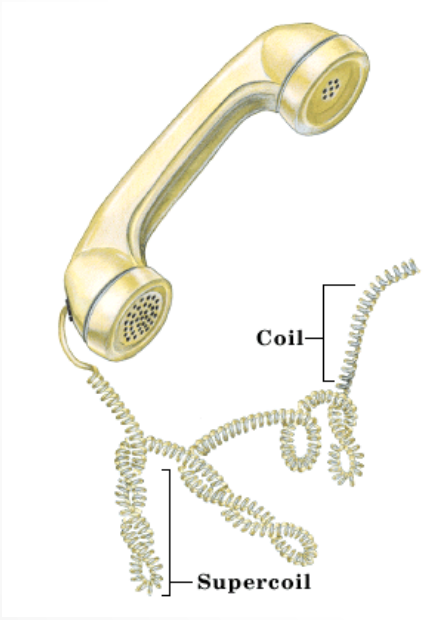
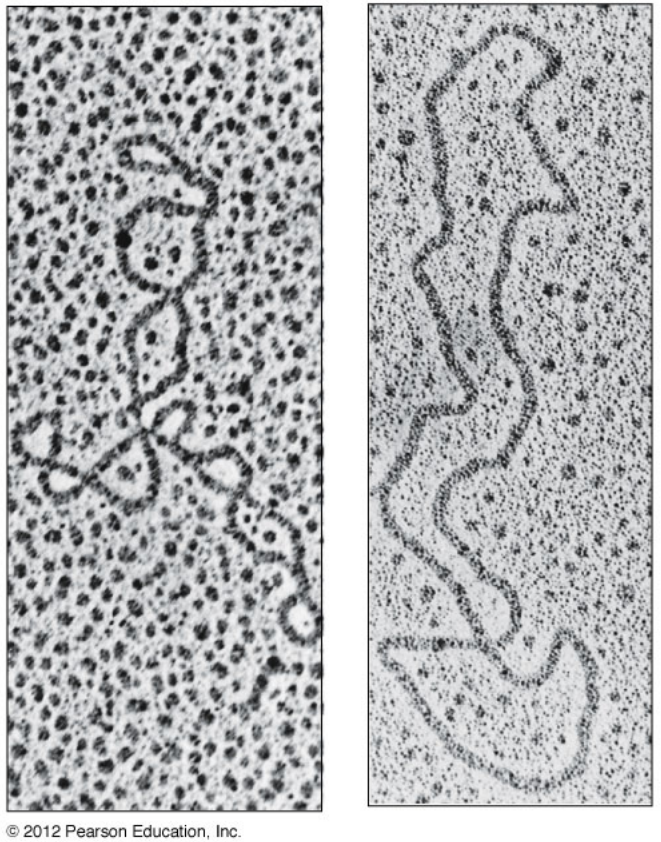


Figure 1.3c Physical Biology of the Cell (© Garland Science 2009)

DNA supercoiling

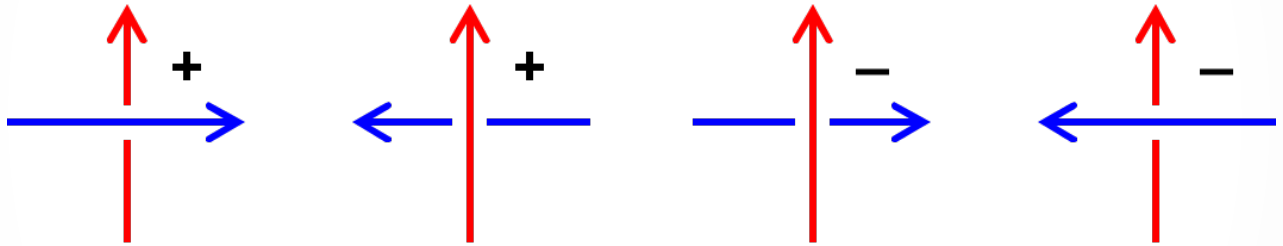






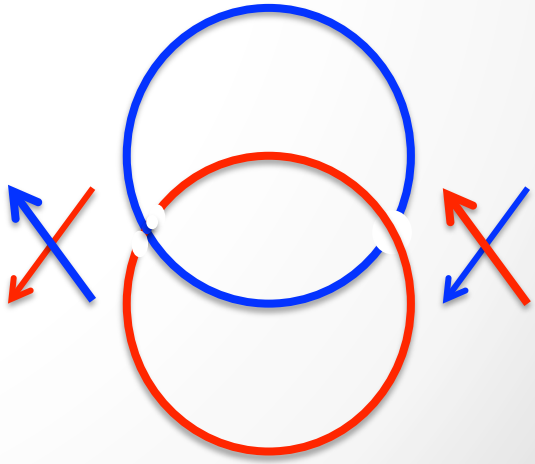
Linking number

- Linking number: number of times that the strands intertwine with each other.



$$Lk = \frac{n_1 + n_2 - n_3 - n_4}{2}$$

$$Lk = \frac{1}{4\pi} \oint_{\gamma_1} \oint_{\gamma_2} \frac{\vec{r}_1 - \vec{r}_2}{\left|\vec{r}_1 - \vec{r}_2\right|^3} (d\vec{r}_1 \times d\vec{r}_2)$$



Twist, Tw

(from NA by Bloomfield, Crothers, Tinoco)

- Consider a cross-sectional plane P perpendicular to A at some point a. The plane will intersect C at some point c. Let v_{ac} be the unit vector from a to c. As P moves along A, v_{ac} will turn. Twist (Tw) is defined as the number of turns that v_{ac} makes around A.

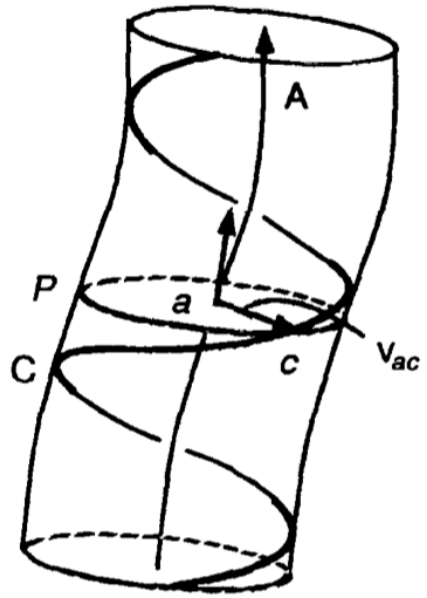


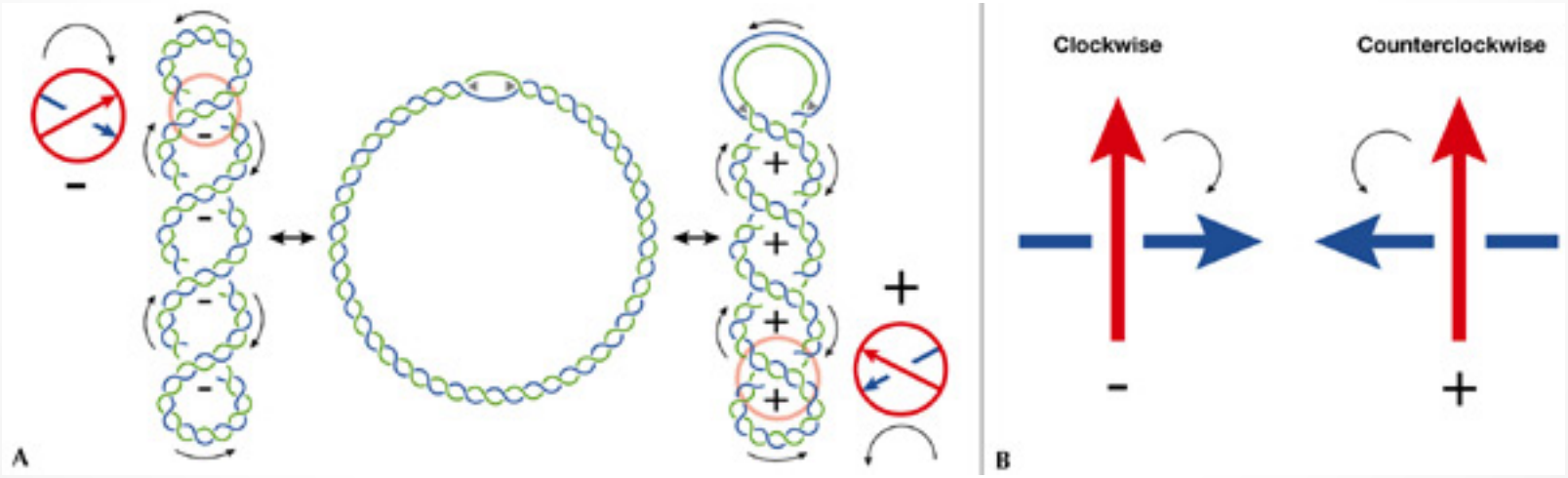
Figure 10-6
Diagram illustrating how to calculate Tw. See text for explanation. [Reprinted with permission from Cozzarelli et al., 1990, Fig. 8, p. 150].

What is Writhe (Wr)?

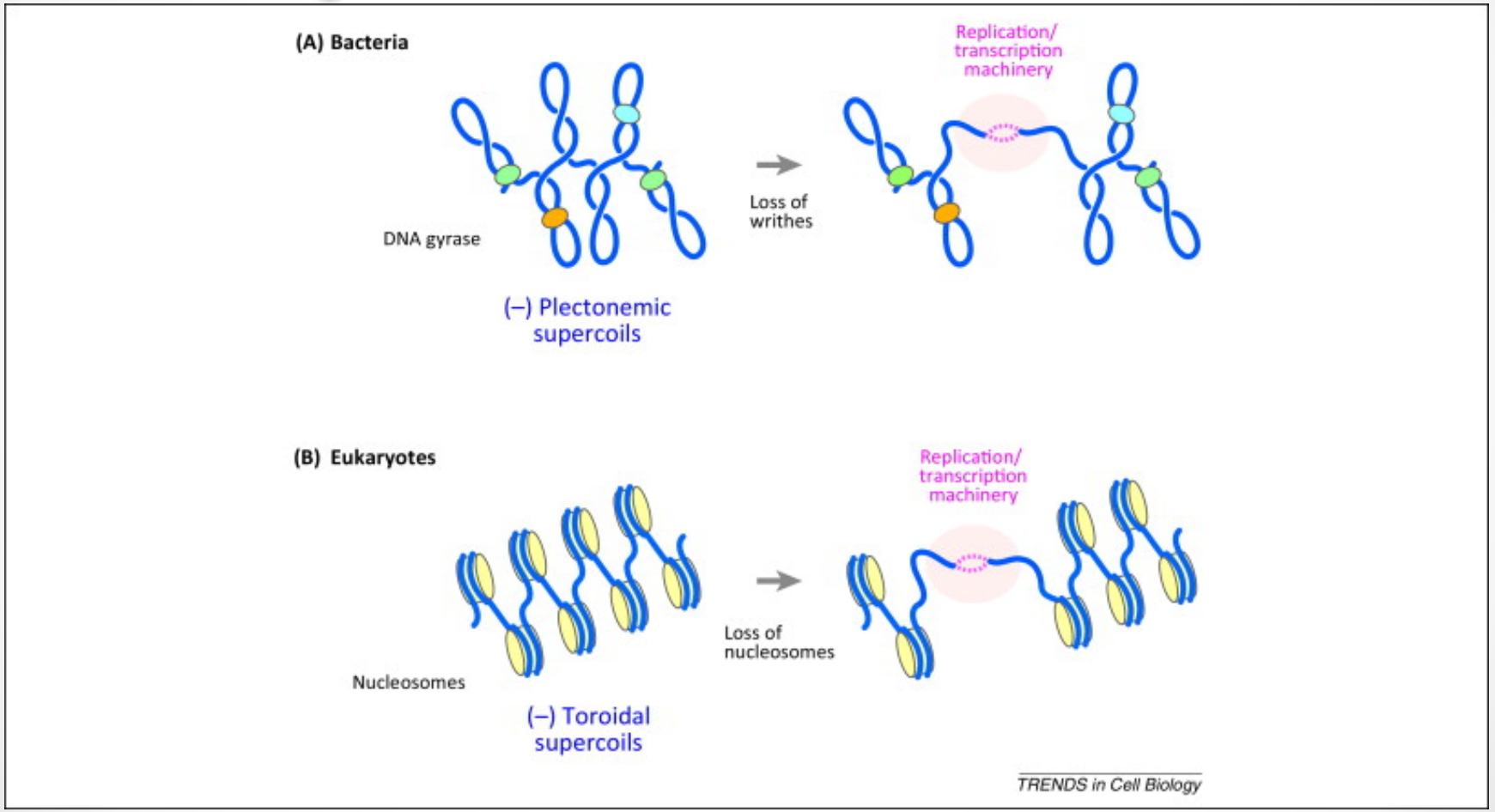
- The Mock Turtle in Alice's Adventures in Wonderland studied Writhing.

Writhe

- Writhe is calculated by counting the intersection of DNA axis with itself in a plane projection. Each clockwise node contributes -1 to Wr.



Two types of DNA supercoils: plectoneme, solenoid



Călugăreanu-White-Fuller theorem

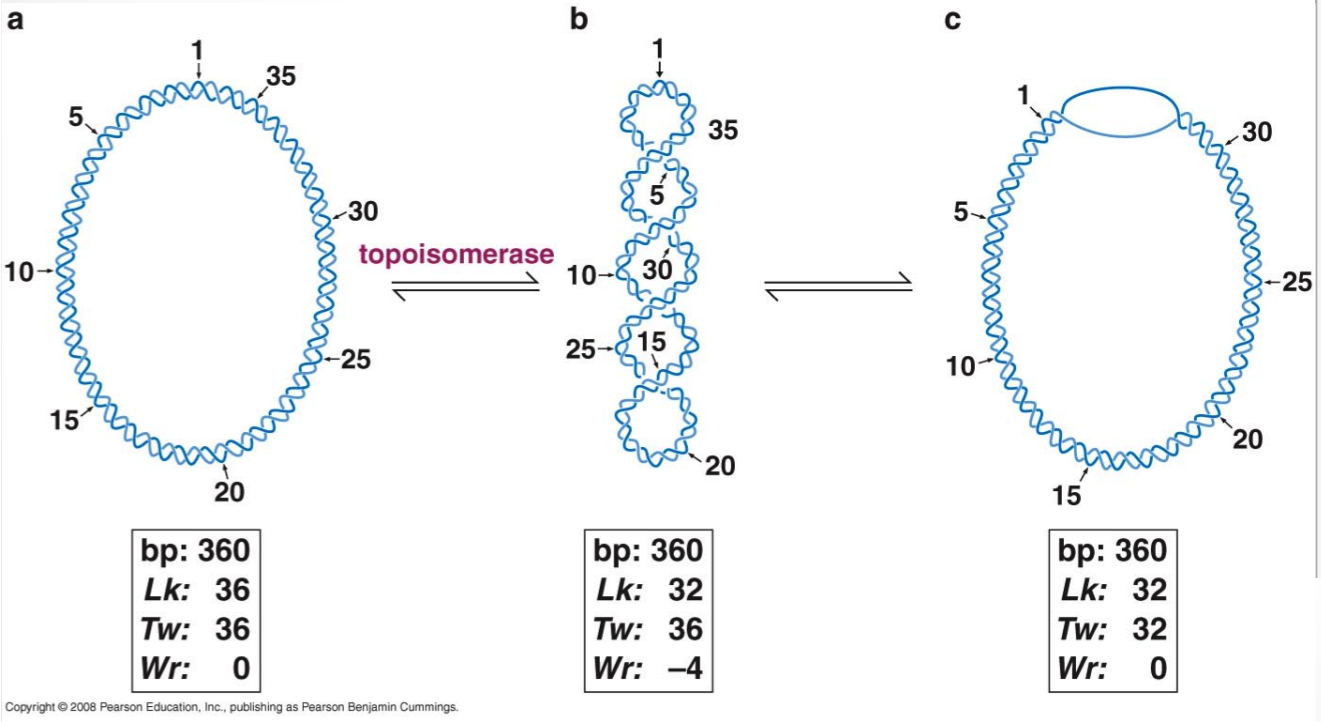
$$Lk = Tw + Wr$$

$$\Delta Lk = \Delta Tw + Wr$$

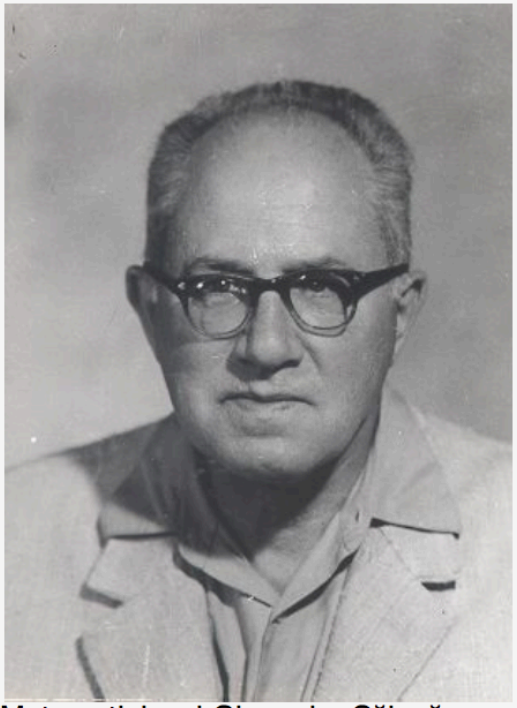
(if Wr is initially 0)

$$\Delta Lk = Lk - Lk_0$$

$$\sigma = \frac{\Delta Lk}{Lk_0}$$



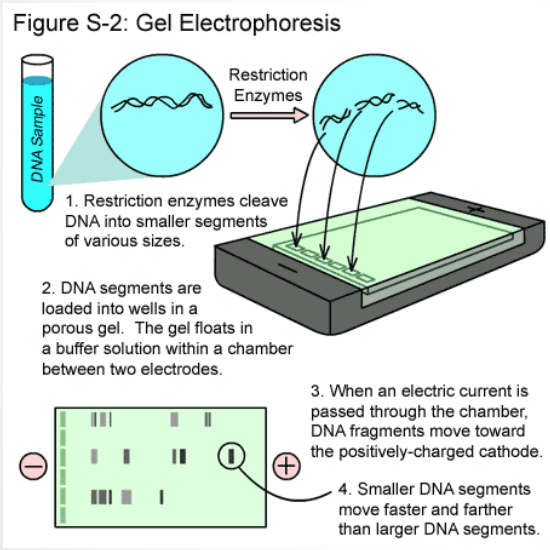
Gheorghe Călugăreanu

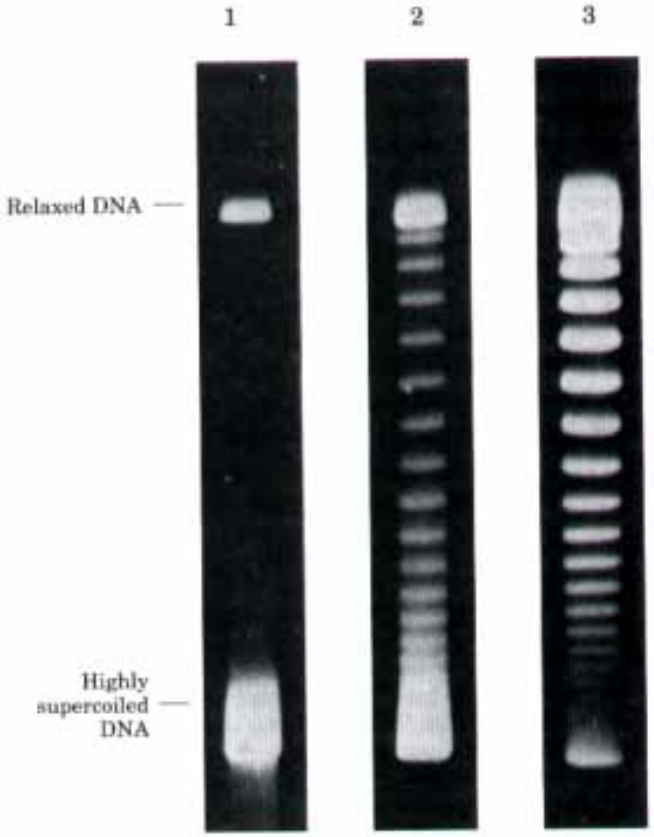


Matematicianul Gheorghe Călugăreanu

Născut(ă)	16 iulie 1902 Iași, România
Deces	15 noiembrie 1976, (74 de ani) Cluj-Napoca, Republica Socialistă România
Naționalitate	România
Ocupație	matematician

How to measure DNA supercoiling





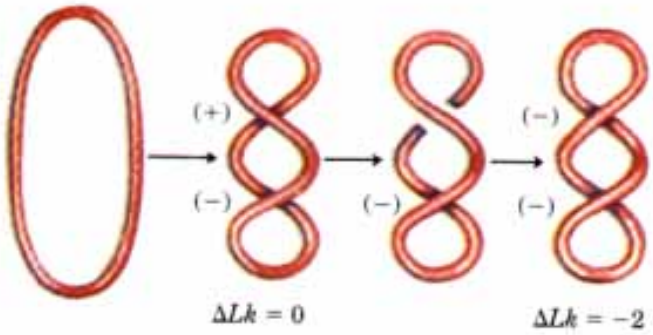
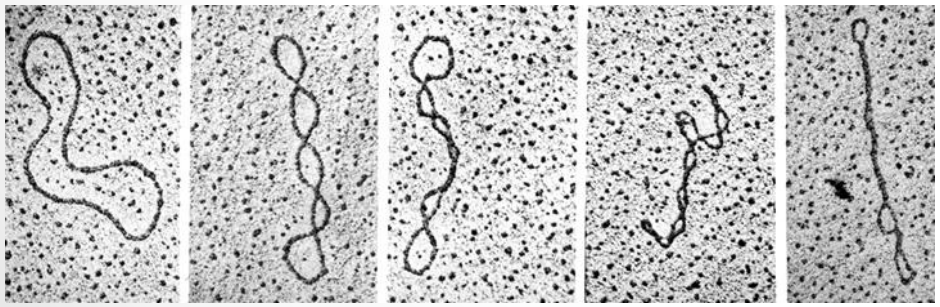


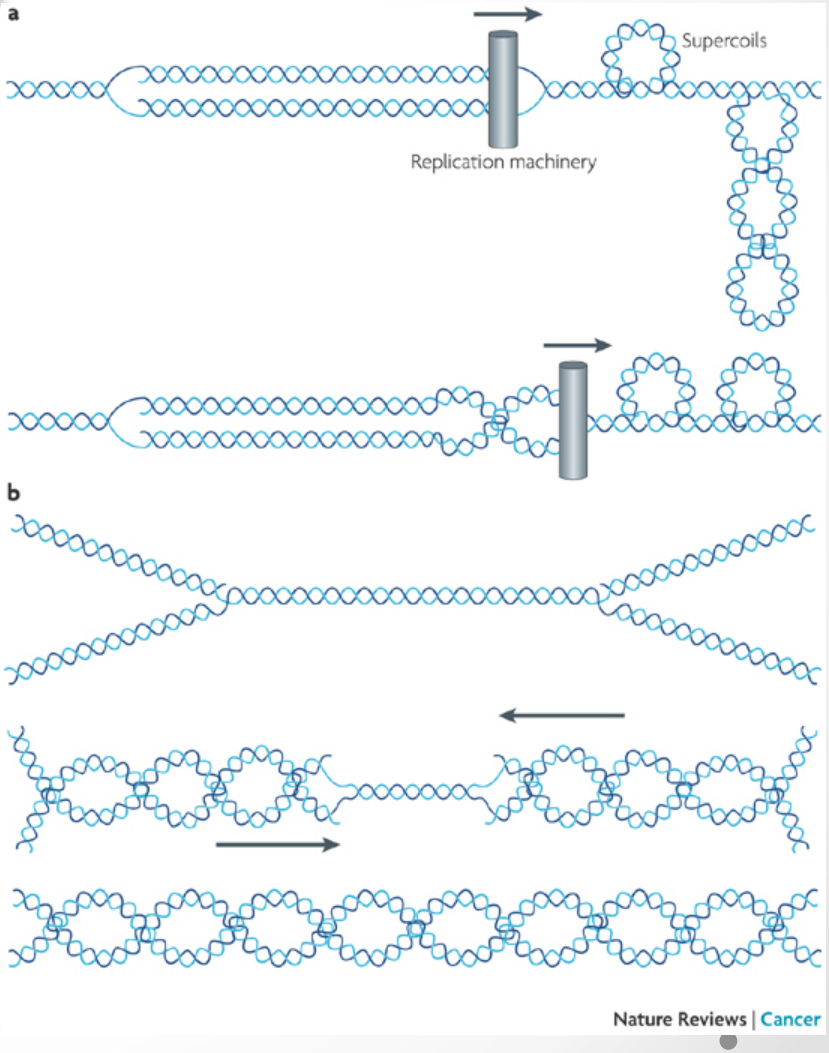
Figure 23–20 *E. coli* topoisomerase II (DNA gyrase) alters the linking number of circular DNA molecules by an unusual mechanism. Two regions of a DNA molecule are overlaid in a specific configuration in the bound complex (a positive (+) node). A compensating (−) node forms spontaneously elsewhere in the DNA molecule. As shown, both strands of one DNA segment are broken, the other segment is passed through the break, and the break is then resealed. The product now contains two minus nodes, and a comparison with Fig. 23–16 shows that the DNA now contains two negative supercoils. The change in structure reflects a change in Lk of −2.

When is DNA supercoil formed?

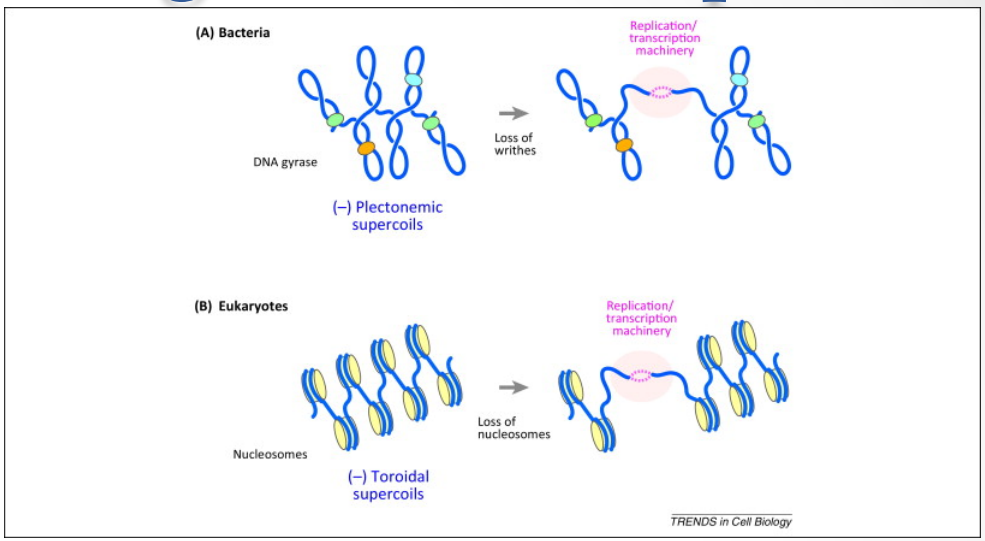
Why is it important?

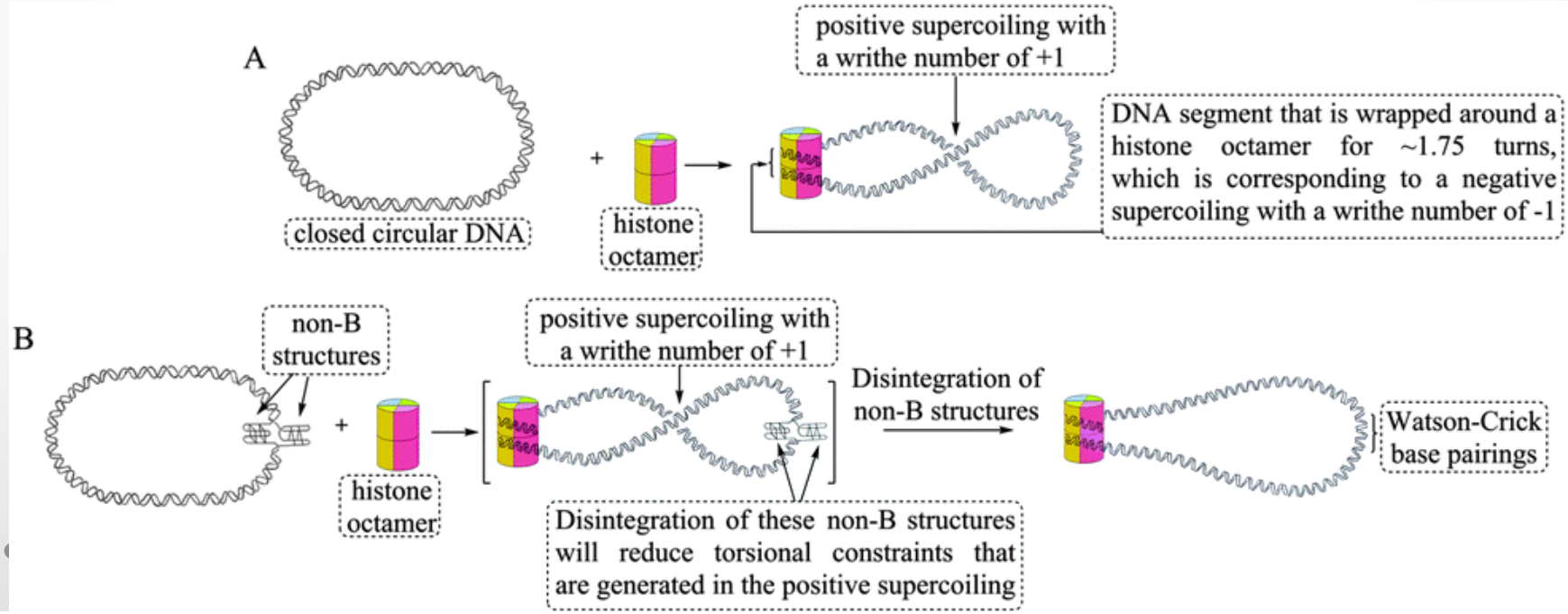
- For compact genome
  - nucleosome
- During DNA replication





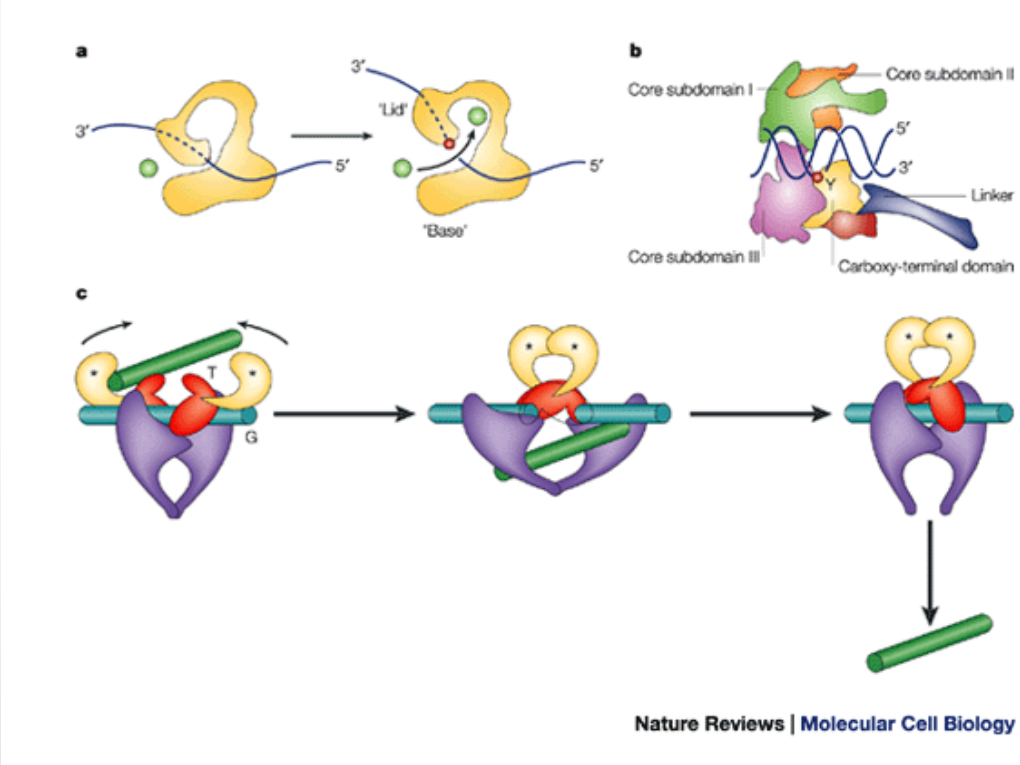
SC is induced in the genome compaction

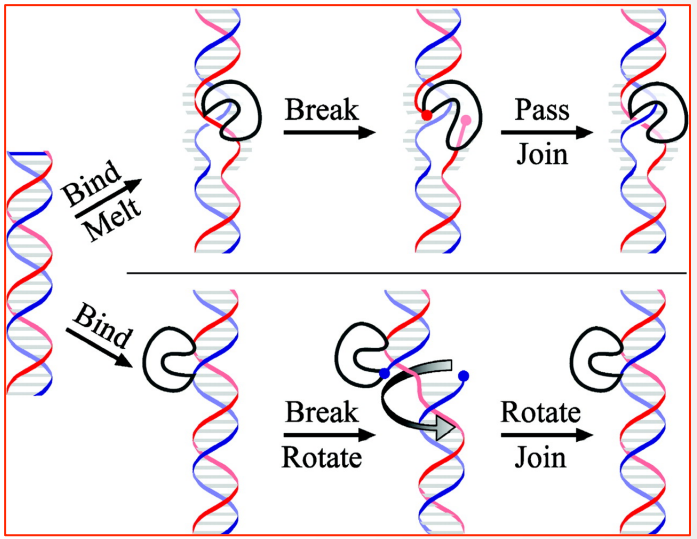




What regulates DNA supercoils?

- Topoisomerases





DNA gel electrophoresis

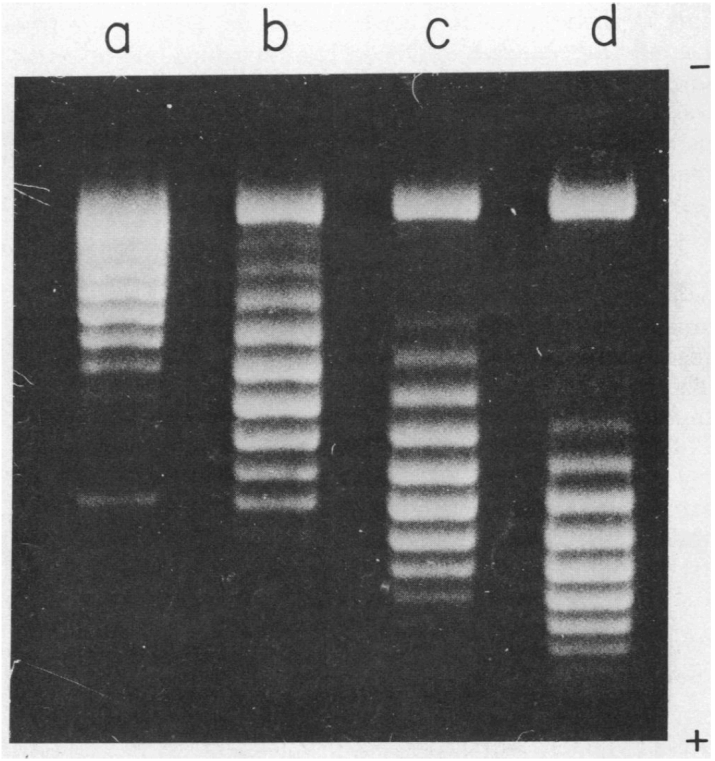


FIG. 1. Electrophoretic patterns of PM2 DNA samples covalently closed by ligase at different temperatures. Approximately 0.2 μg of a DNA sample was placed in each well of a 0.7% agarose slab gel and electrophoresed at room temperature for 17 hr at 2.5 V/cm. The temperatures of ligase reaction for the four samples a–d were 37, 29, 21, and 14°C, respectively.

Nick circular

DNA supercoils

Energy associated with ΔLk

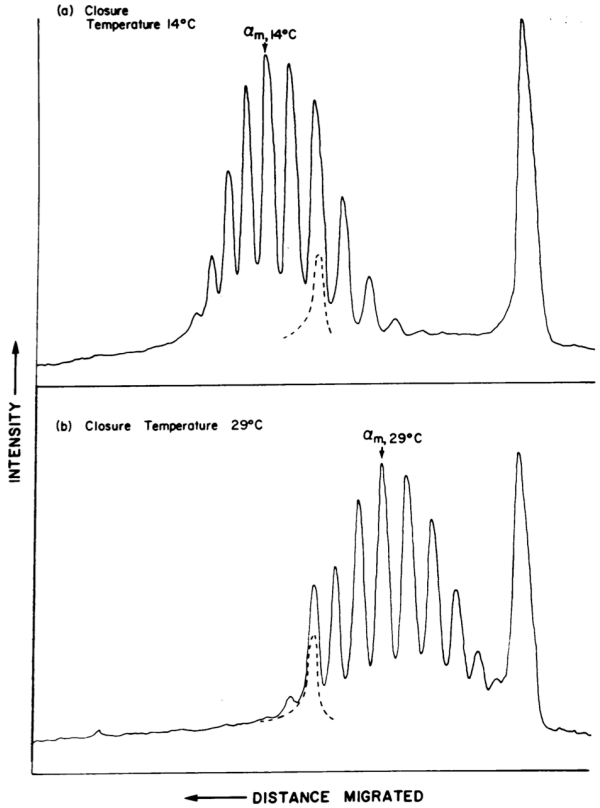


FIG. 2. Microdensitometer tracings of the electrophoretic patterns of two PM2 DNA samples covalently closed by ligase at (a) 14°C and (b) 29°C, respectively. The dotted line in each tracing indicates the position of linear PM2 DNA, which was present in small amounts in the PM2 DNA used.

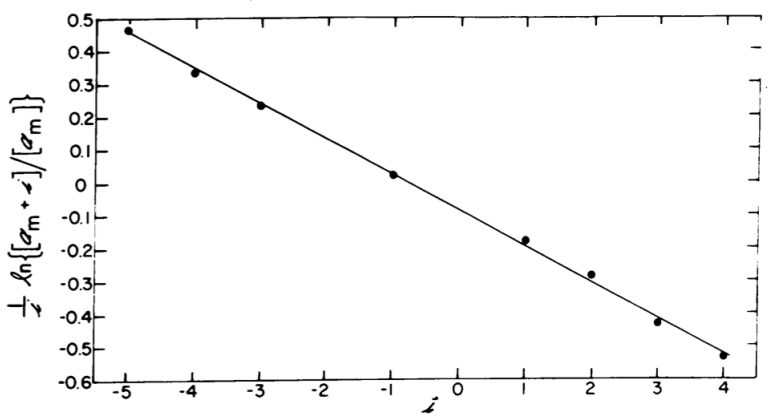


FIG. 3. A typical plot for the evaluation of constants K and ω_T. Data were obtained from the tracing shown in Fig. 2a.

$$G(\alpha_{m,T}) = K\omega_T^2; \quad G(\alpha_{m,T}+i) = K(i+\omega_T)^2$$

$$-RT\ln\left\{\left[\alpha_{m,T}+i\right]/\left[\alpha_{m,T}\right]\right\}$$

$$= G(\alpha_{m,T}+i) - G(\alpha_{m,T}) = K(i+\omega_T)^2 - K\omega_T^2$$

$$-\frac{RT}{i}\ln\frac{\left[\alpha_{m,T}+i\right]}{\left[\alpha_{m,T}\right]} = K(i+2\omega_T)$$

$$\rightarrow K = 0.11RT$$

- Ligate nicked circular DNA @ different temperatures.
- DNA pitch depends on T: $\Delta\theta/\Delta T$ ~ -0.012°/K

$$\Delta g_\tau \equiv \frac{\Delta G_\tau}{N} = NK(i/N)^2$$

$$\leftarrow \Delta G_\tau = -RT \ln \frac{\left[\alpha_{m,T} + i\right]}{\left[\alpha_{m,T}\right]} = Ki^2$$

$$NK \sim 1100RT$$

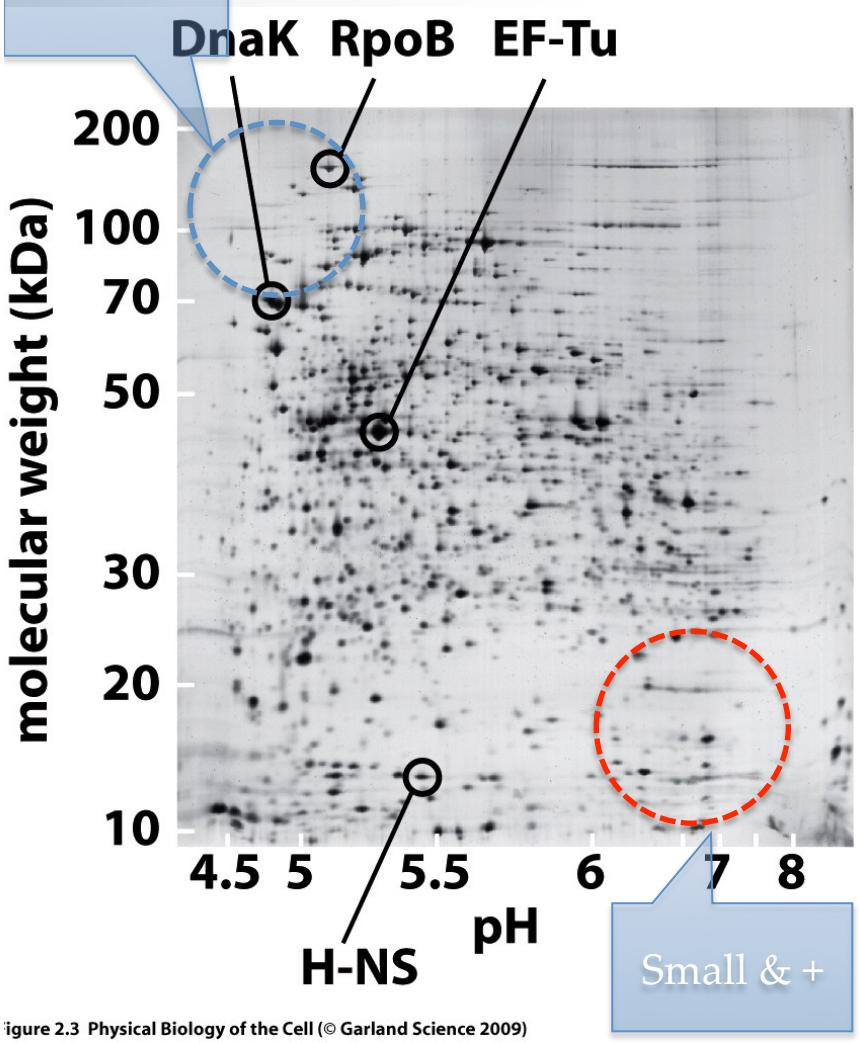
$$E = \frac{1100RT}{N} \Delta Lk^2$$

Proc. Nat. Acad. Sci. USA 72 (1975)

Table 1. Parameters for the Gaussian distributions in the topological winding number α

DNA	*N*	Temperature of closure by ligase, °C	*K*	$-\omega_T$	*NK*
PM2	9850	14	0.106	−0.39	1040
		21	0.102	+0.35	1000
		29	0.097	−0.32	955
fd, double-stranded	5750	10	0.18	+0.25	1030
		20	0.16	+0.25	920
SV40	5300	0	0.23	+0.1	1220
		4	0.19	+0.3	1010
		15	0.23	−0.4	1220
		18	0.21	0	1110
		26	0.19	−0.3	1010
E. coli 15 plasmid	2200	0	0.65	+0.3	1430
		4	0.59	0	1300
		15	0.63	+0.3	1390
		18	0.71	+0.1	1560
		26	0.63	+0.5	1390

Protein census of the cell



igure 2.3 Physical Biology of the Cell (© Garland Science 2009)

A protein mixture is loaded at one end of the gel and an electric field is applied across the gel. $F = qE = \zeta v$

- Separating proteins according to their net charge.

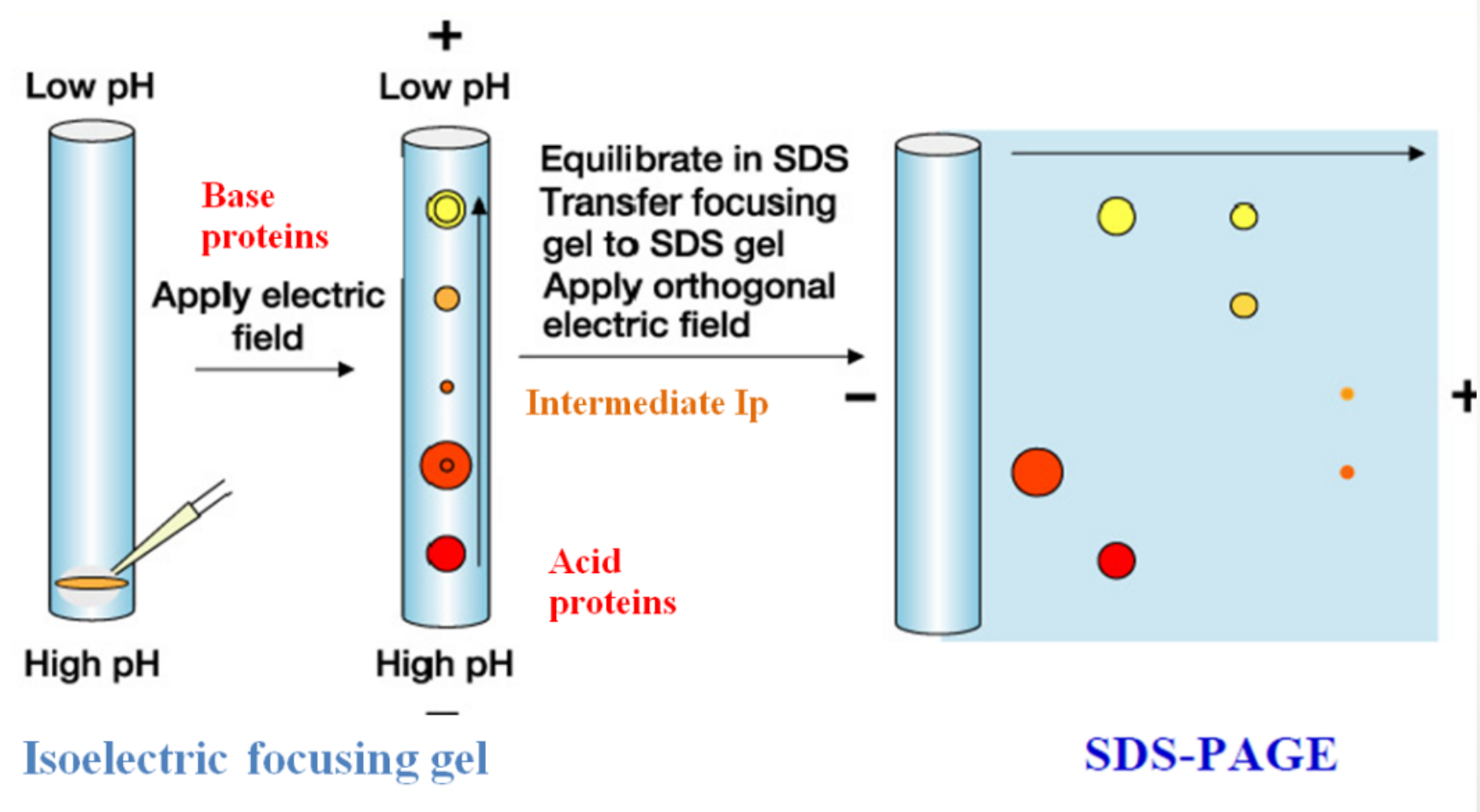
A charged detergent that binds to all proteins is added. The total number of detergent molecules associated with an individual protein ~ the protein's overall size and an electric field is applied along the gel. The net charge on the detergent >> net charge of the protein.

- Separating proteins according to their size.

Proteins are stained using a non-specific dye. The amount of proteins ~ the intensity of the spot.

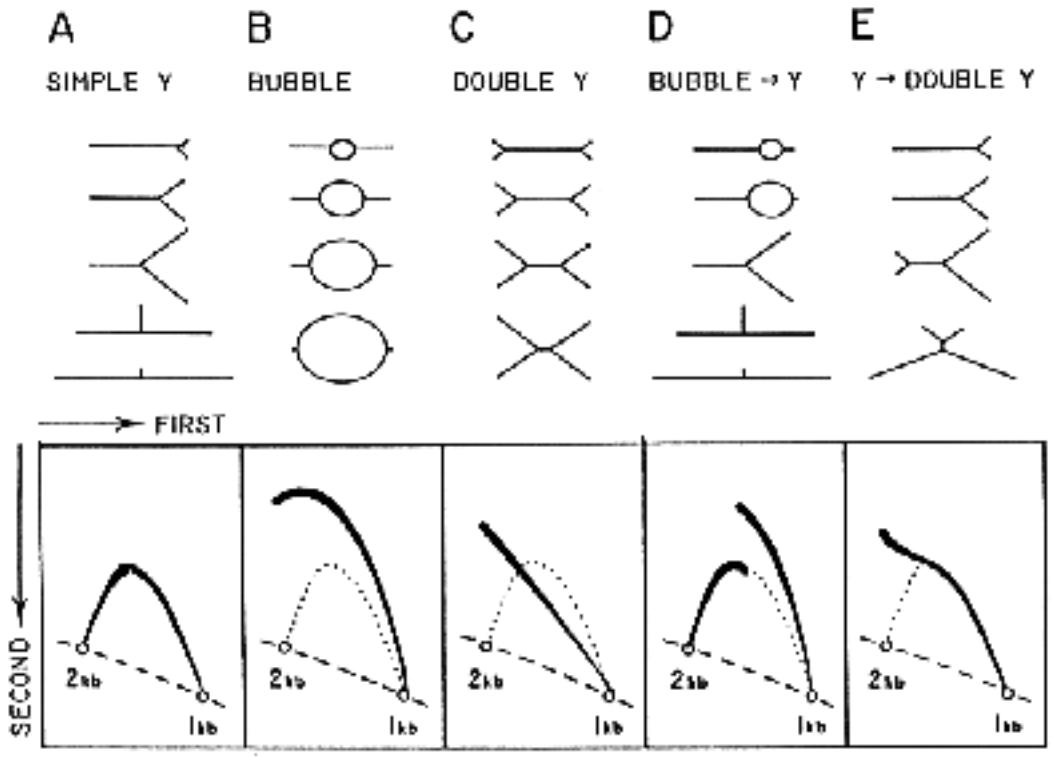
Cut each spot, elute the proteins and determine the size and amino acid content using mass spectroscopy.

2D gel electrophoresis (for proteins)



2D gel electrophoresis for DNA replication intermediate (http://fangman-brewer.genetics.washington.edu/2Dgel.html)

- 2-D gel electrophoresis is adapted from the procedure in Anal. Biochem. 130:527, 1983.
- The 1st dimension gel is intentionally run at low V in low % agarose to separate DNA molecules in proportion to their mass.
- The 2nd dimension is run at high V in a gel of higher [agarose] in the presence of EtBr so that the mobility of a non-linear molecule is drastically influenced by its shape.



2D gel electrophoresis to study non-canonical DNA

1983 Proc. Natl. Acad. Sci. USA 80 6206–6210 Energetics of B-to-Z tr

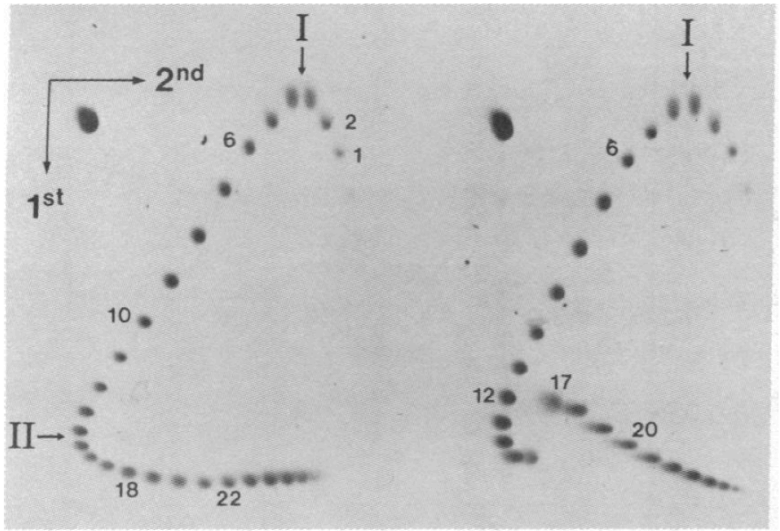
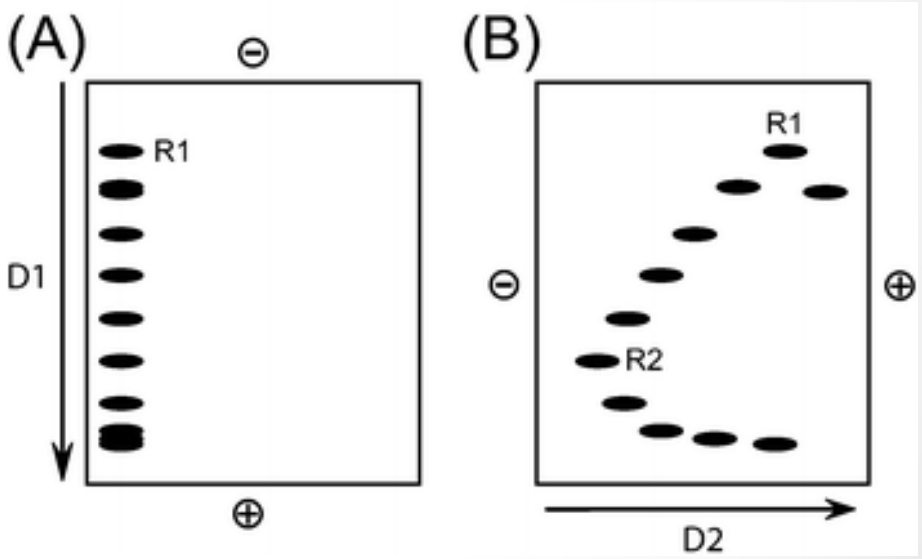


FIG. 1. Superhelicity dependence of the right- to left-handed conformational transition of cloned $d(pCpG)_{16}$·$d(pCpG)_{16}$ as revealed by two-dimensional agarose gel electrophoresis. The electrophoretic pattern of a mixture of topoisomers of plasmid pTR161 is shown in *Left* and that of the $d(pCpG)_{16}$-insert-containing plasmid pLP332 in *Right*. Both samples were electrophoresed in the same gel. Within a panel the topoisomers differ only in their linking number. The directions of electrophoresis are indicated in the figure. The buffer for the first dimension was 90 mM Tris·boric acid, pH 8.3/2.5 mM EDTA (TBE buffer). Prior to electrophoresis in the second dimension equilibration with TBE buffer containing 1.3 μM chloroquine unwound the plasmids about 12 turns. The dark spot in the upper left-hand corner of each panel corresponds to the nicked circular DNA.



2nd dimension:
chloroquine – unwind DNA

Energy associated with supercoiled DNA: in the presence of tension

$$\frac{E}{k_B T} = \frac{2\pi^2 C}{L} \Delta T w^2$$

Lecture 4

- Single molecule methods (revisit)
- Hybrid single molecule technique of smFRET & MT
- Case studies: DNA mechanics via single-molecule methods
- Case studies: Non-canonical DNA and its dynamics via single-molecule methods