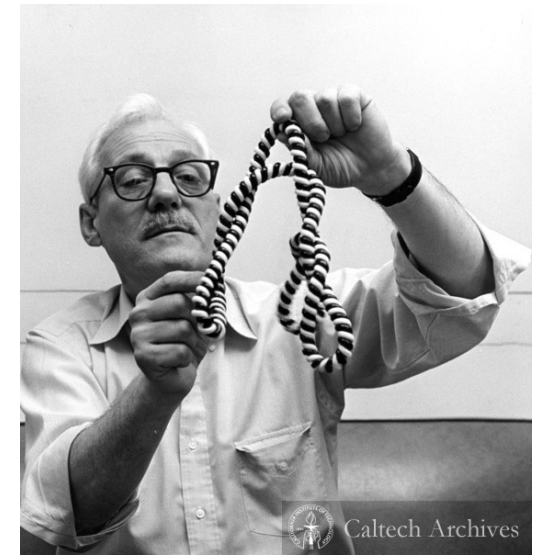


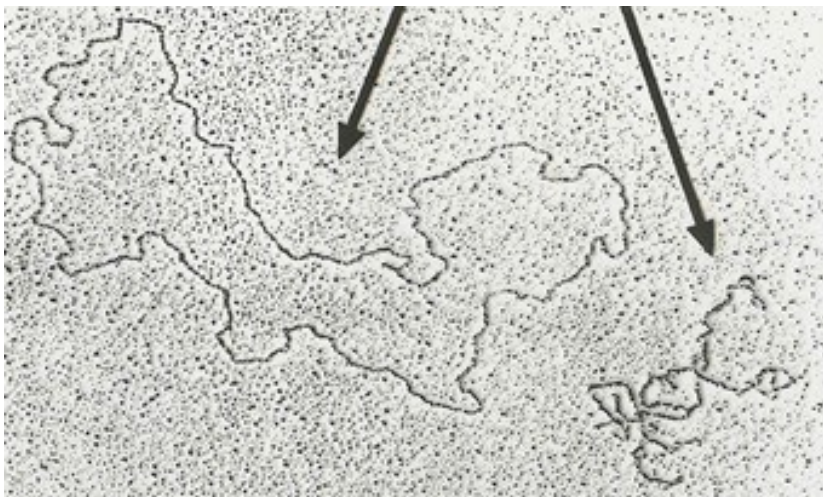
DNA supercoiling

Jerome Vinograd, 1965

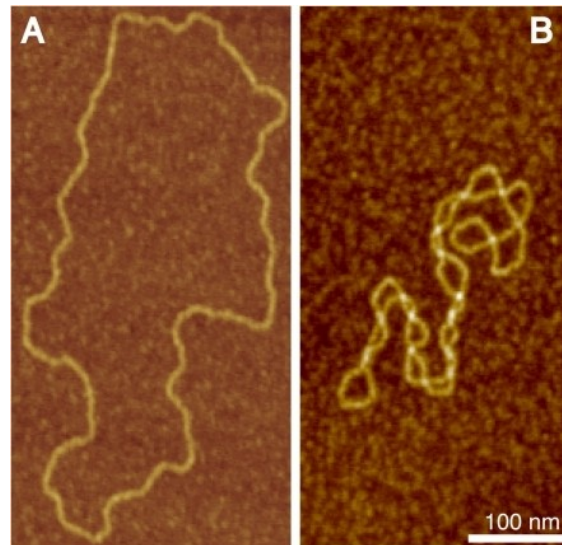
- sedimentation equilibrium experiments with viral DNA
- circular DNAs can exist in two distinct forms differing in buoyant density
- supercoiled (compact) and relaxed (loose)



electron microscopy



AFM

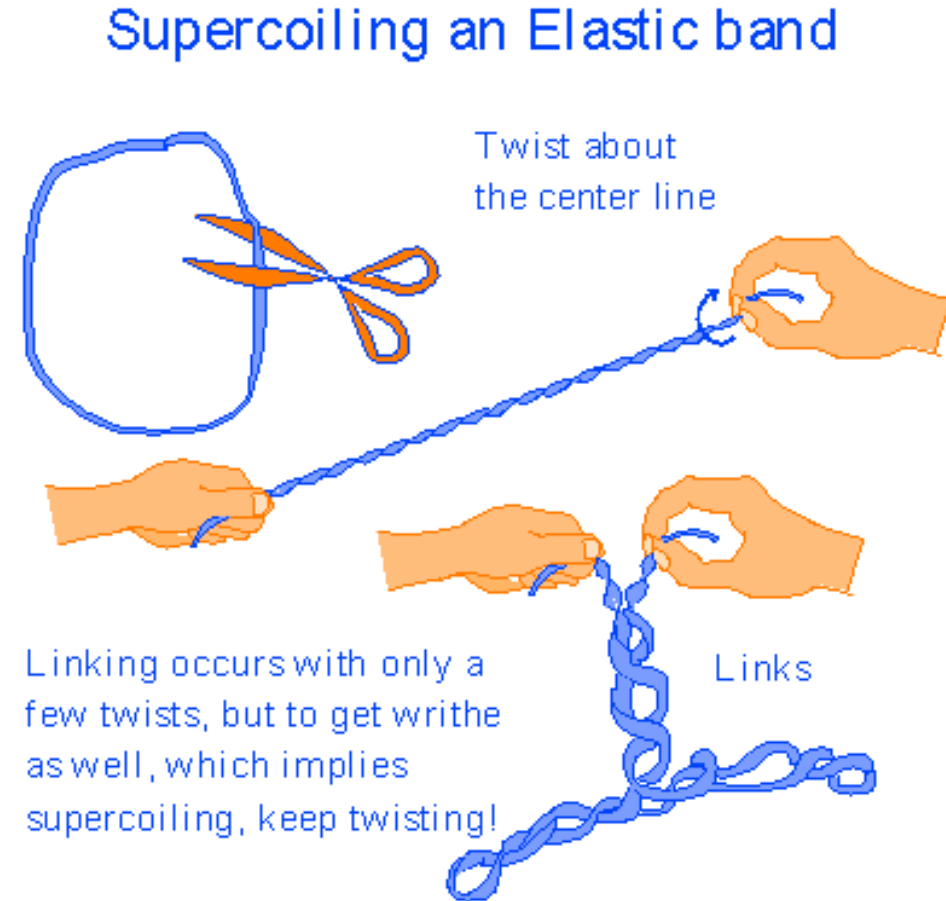


agarose gel elfo



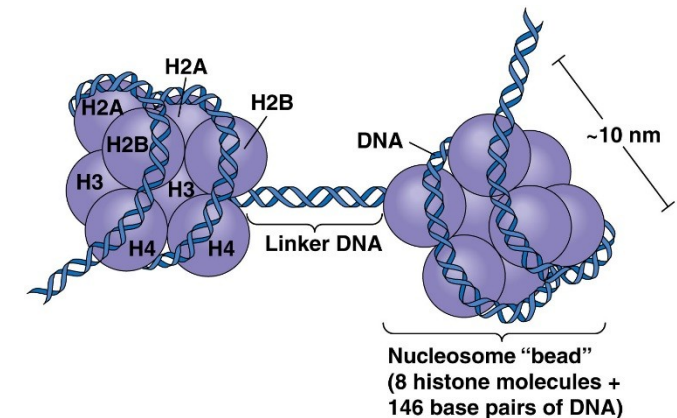
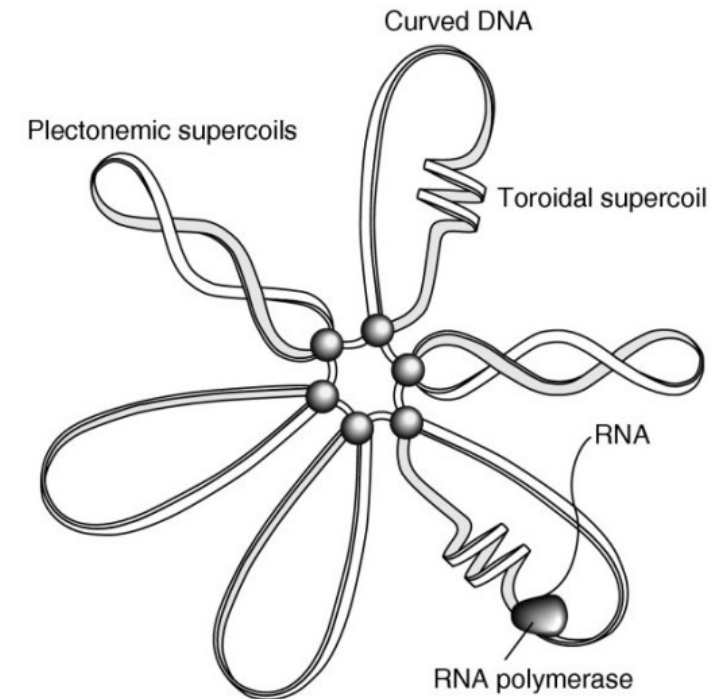
Double helical DNA behaves like a rubber rod

- some torsional and bending elasticity
- „shape memory“
- tendency to keep B-conformation
- tendency to keep the axis straight
- Interplay between twisting and bending deformations



Superhelicity is a property of DNA without free ends

- circular duplex DNA (plasmids)
- linear molecules with constrained (anchored) ends (chromatin loops)
- linear and circular nicked DNAs are inherently relaxed
- plectonemic and toroidal supercoils
- superhelicity absorbed in nucleosomes and other protein-DNA complexes



Linking number

Twist

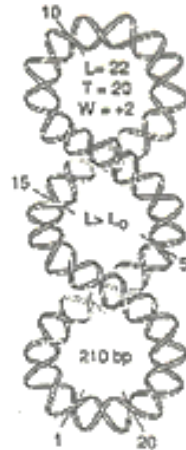
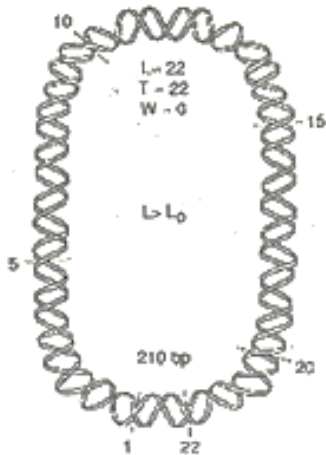
Writhe

$$Lk = Tw + Wr$$

How many times a strand is crossing a strand

Number of crossing points in duplex DNA (=number of double helix turns)

Number of superturns



relaxed DNA: $Lk = N/10.5 = Lk_0$, where N=number of base pairs

equation $Lk = Tw + Wr$ is valid even for relaxed DNA;

when relaxed DNA lies on a plane, $Wr=0$, $Lk_0 = Tw = N/10.5$ (definition of relaxed DNA; Wr may be $\neq 0$)

supercoiled DNA: $Lk \neq Lk_0$

(definition of supercoiled DNA)

negatively scDNA: $Lk < Lk_0$ (linking deficit)

positively scDNA: $Lk > Lk_0$ (linking extent)

$Lk - Lk_0 = \Delta Lk$ (superhelicity level)

$\Delta Lk / Lk_0 = \sigma$ (superhelix density: superhelicity level normalized on DNA molecule size)

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

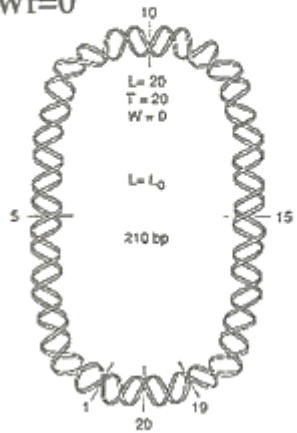
Lk cannot be changed without interrupting at least one DNA strand

relaxed

$$Lk = Lk_0 = 210/10.5 = 20$$

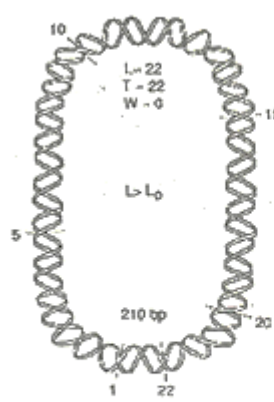
$$Tw = 20$$

$$Wr = 0$$



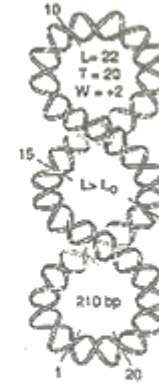
positively supercoiled
(with linking excess)

$$Lk = 22, \Delta Lk = 2$$



$$Tw = 22, \Delta Tw = 2$$

$$Wr = 0, \Delta Wr = 0$$



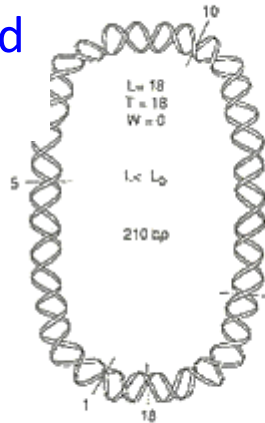
$$Tw = 20, \Delta Tw = 0$$

$$Wr = 2, \Delta Wr = 2$$

positive superhelical stress forces the right-handed double helix to close

negatively supercoiled
(with linking deficit)

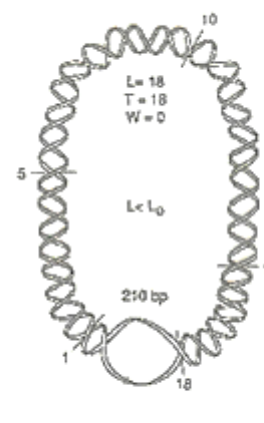
$$Lk = 18, \Delta Lk = -2$$



$$Tw = 18, \Delta Tw = -2$$

$$Wr = 0, \Delta Wr = 0$$

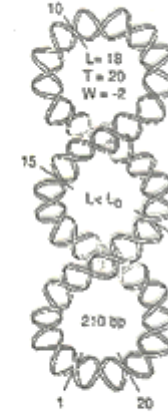
duplex
globally untwisted



$$Tw = 18, \Delta Tw = -2$$

$$Wr = 0, \Delta Wr = 0$$

duplex
locally untwisted



$$Tw = 20, \Delta Tw = 0$$

$$Wr = -2, \Delta Wr = -2$$

duplex
twisted normally,
supercoils formed

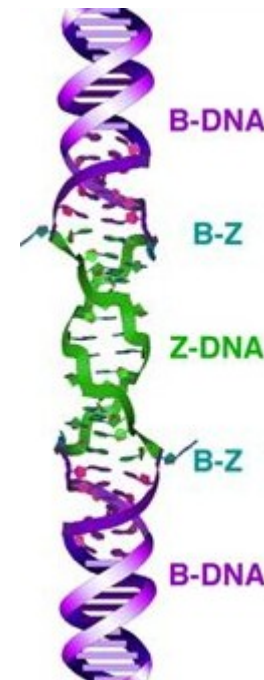
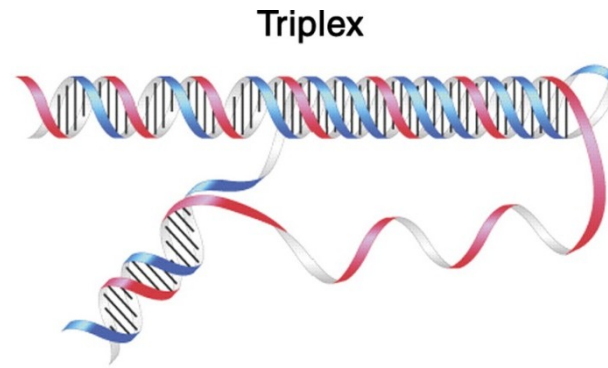
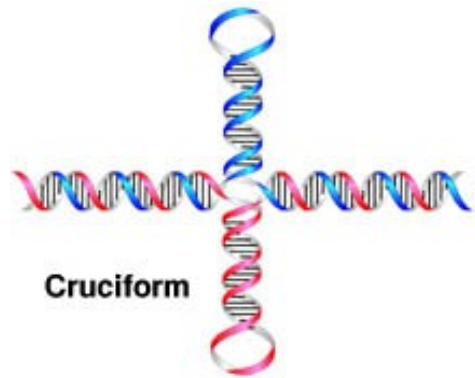
negative superhelical stress forces the right-handed double helix to open

In real situation, superhelicity distributed between ΔTw (locally or globally) and ΔWr

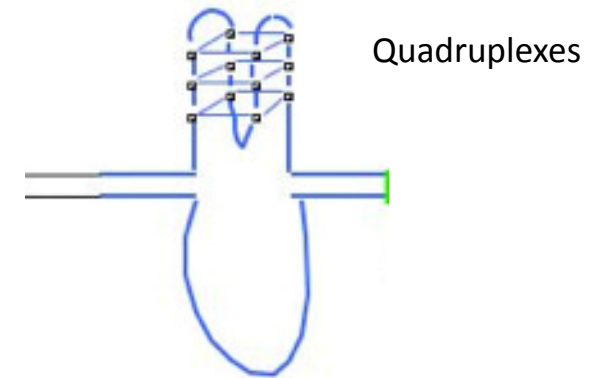


Open local structures

- formed in appropriate sequence motifs
- characterized by locally reduced twist, compared to B-DNA
- paranemic: possible to form/abolish without mutual rotation of opposite strands



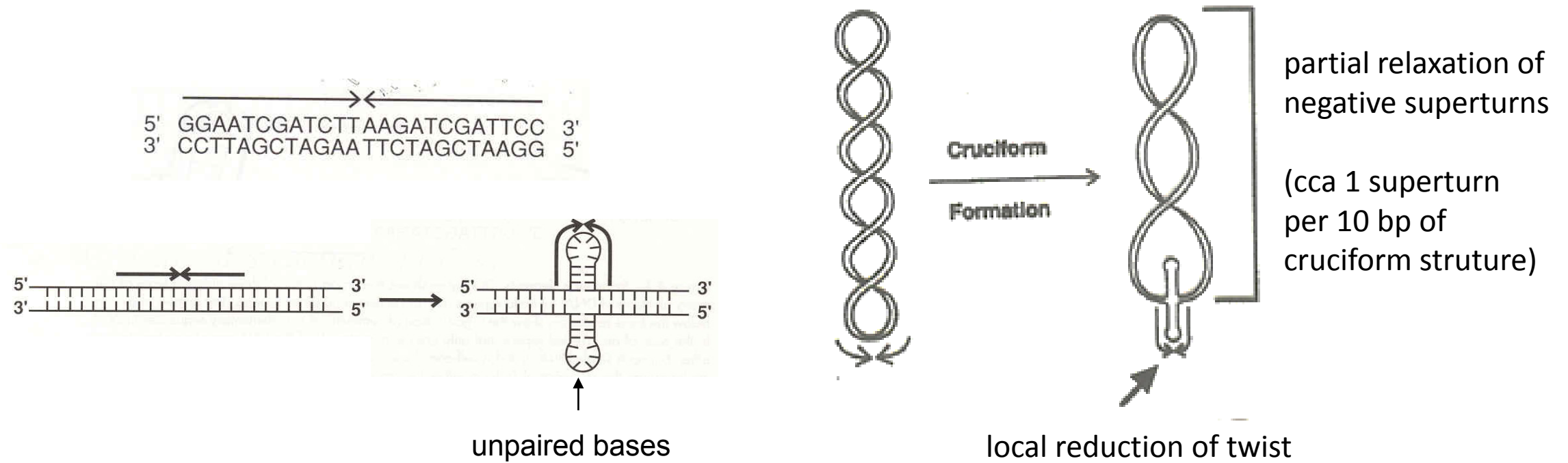
Left-handed (Z-form) duplex



Open local structures in negatively supercoiled DNA

cruciform DNA

inverted repeat (sequences with dyad symmetry)

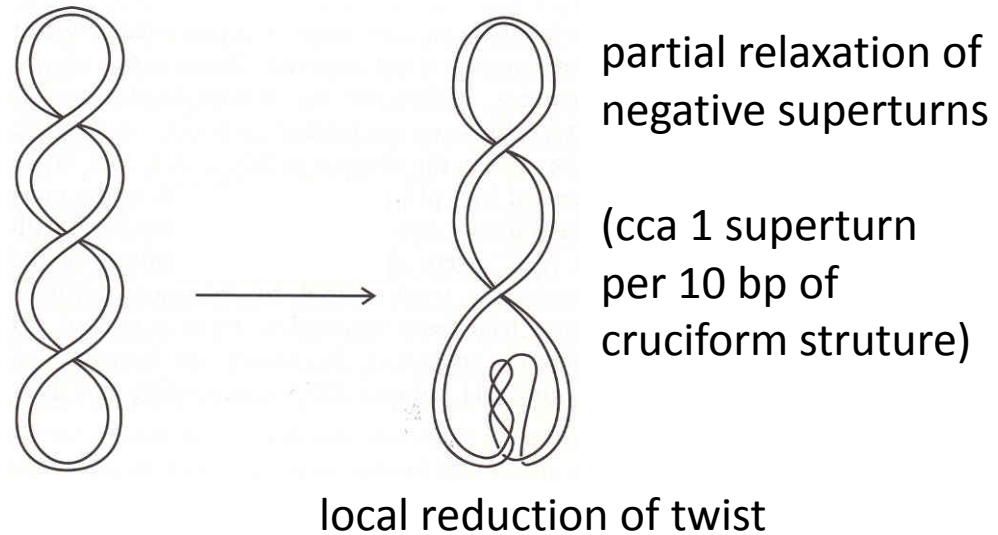
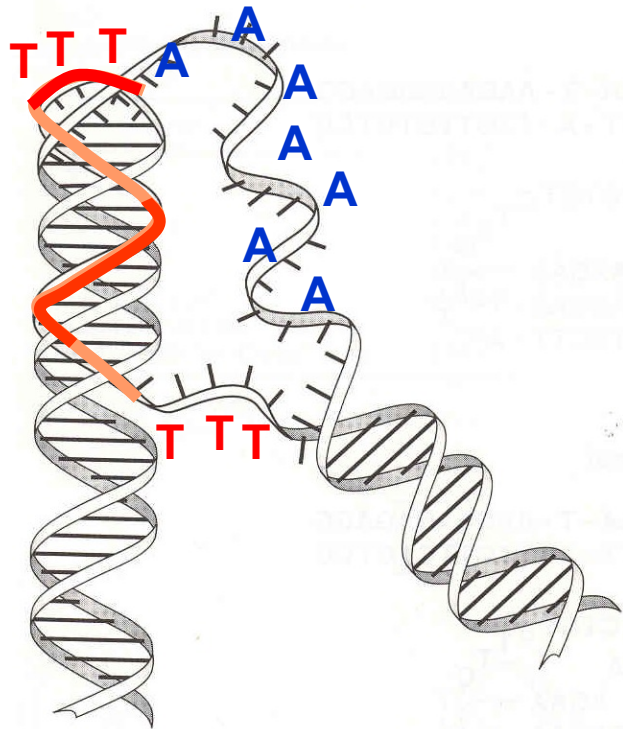


Open local structures in negatively supercoiled DNA

Intramolecular triplex

homoPu•homoPy segment with mirror symmetry

TAT (H^{*}-DNA) – stabilized by Mg²⁺; C⁺GC (H-DNA) – stabilized in weakly acidic media)

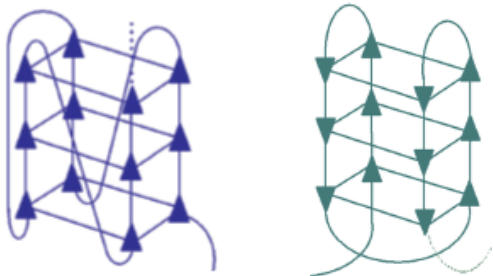


Open local structures in negatively supercoiled DNA

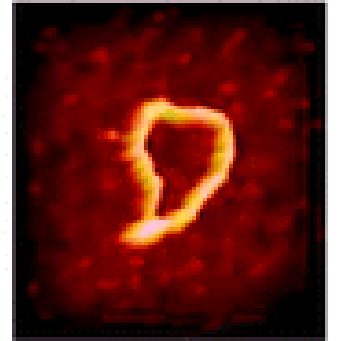
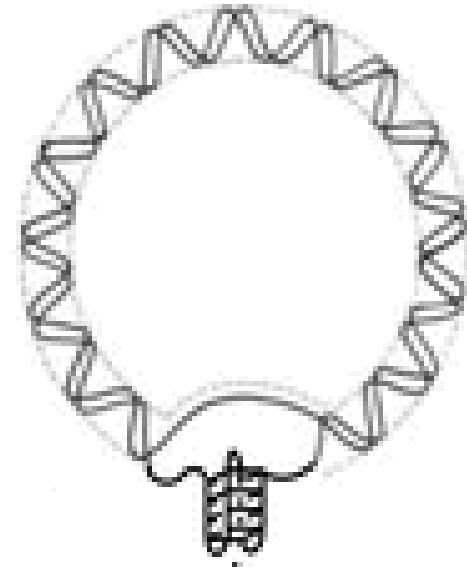
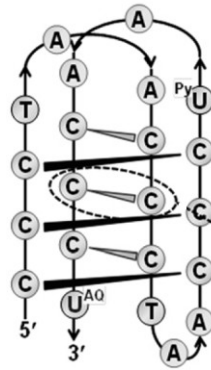
Intramolecular quadruplexes

G:C-rich motifs

G-quadruplexes
(K⁺-stabilized)



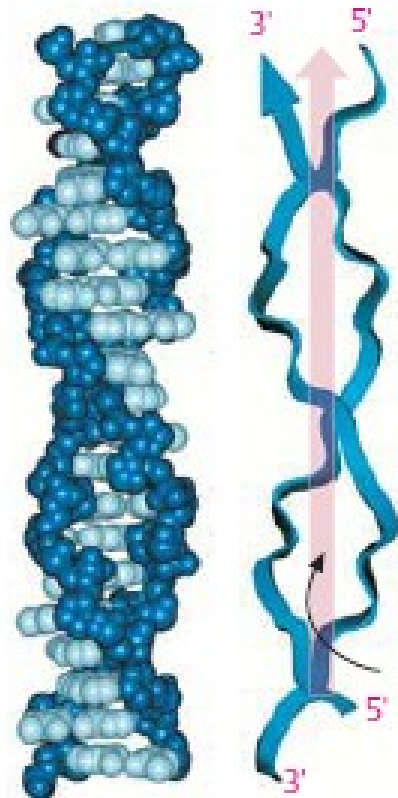
C-quadruplexes (i-motifs)
(weakly acidic pH)



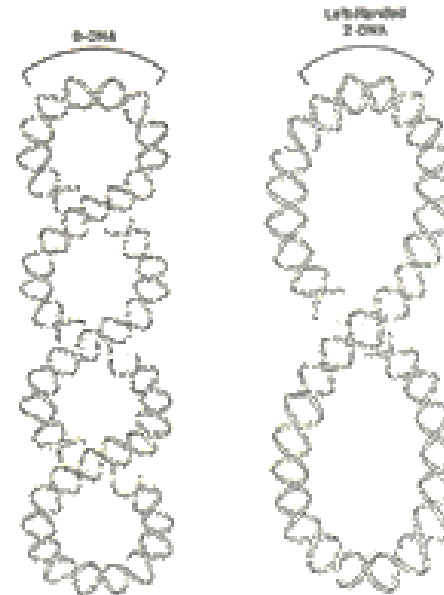
Open local structures in negatively supercoiled DNA

Lef-handed Z-DNA

(Pu-Py)_n segment within negatively supercoiled DNA)



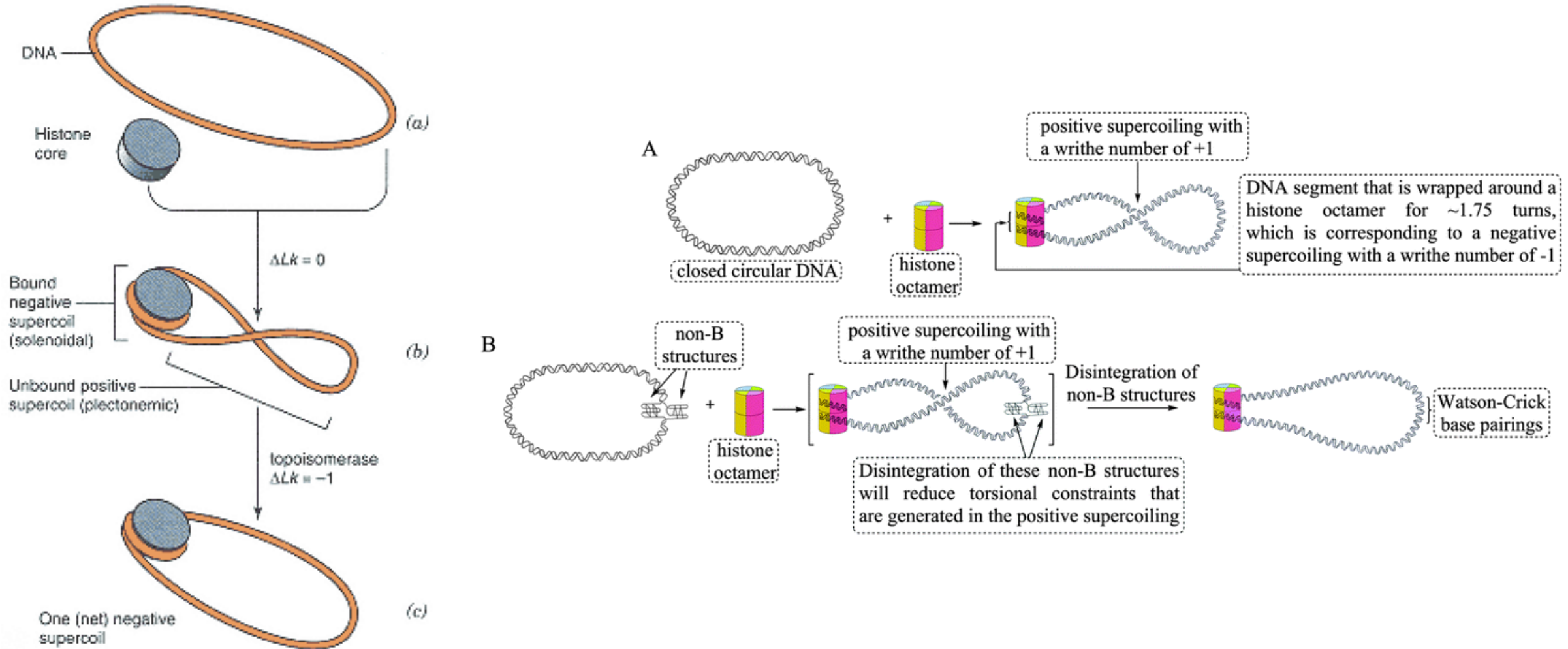
local reduction of twist
(to negative values)



partial relaxation of
negative superturns

(cca 2 superturns
per 14 bp of Z-DNA
structure)

DNA supercoiling and nucleosome formation



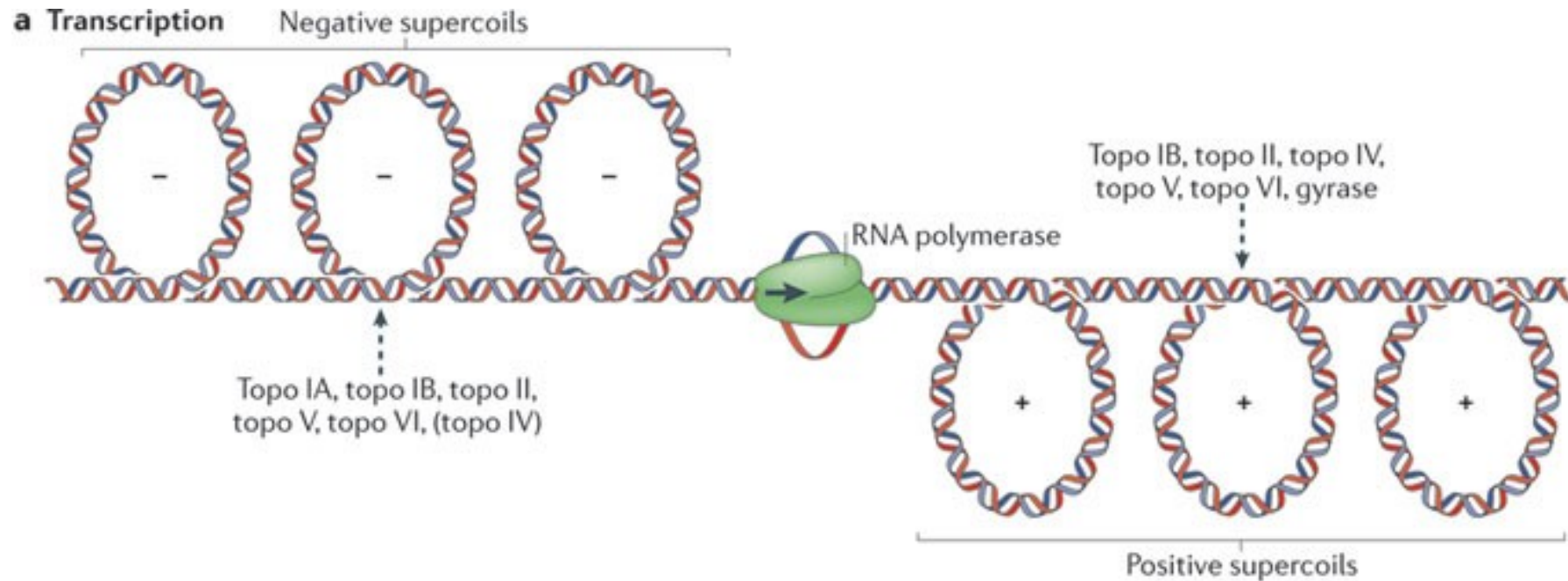
DNA supercoiling and replication/transcription

- local untwisting of duplex in replication fork/transcription complex induces formation of superturns



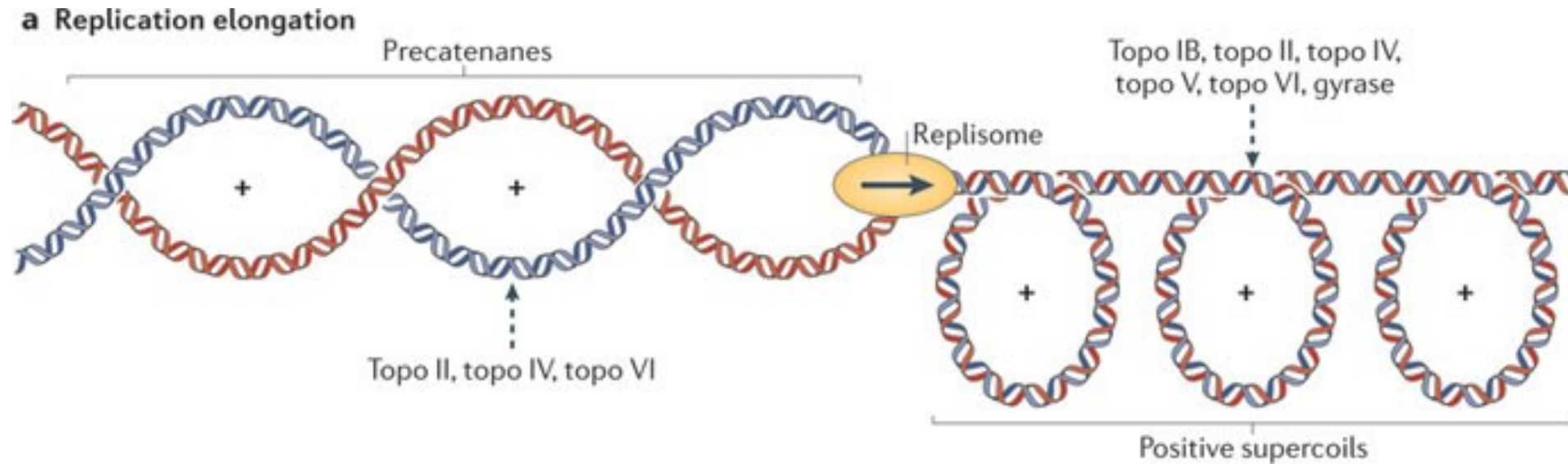
DNA supercoiling and replication/transcription

- local untwisting of duplex in replication fork/transcription complex induces formation of supercoils



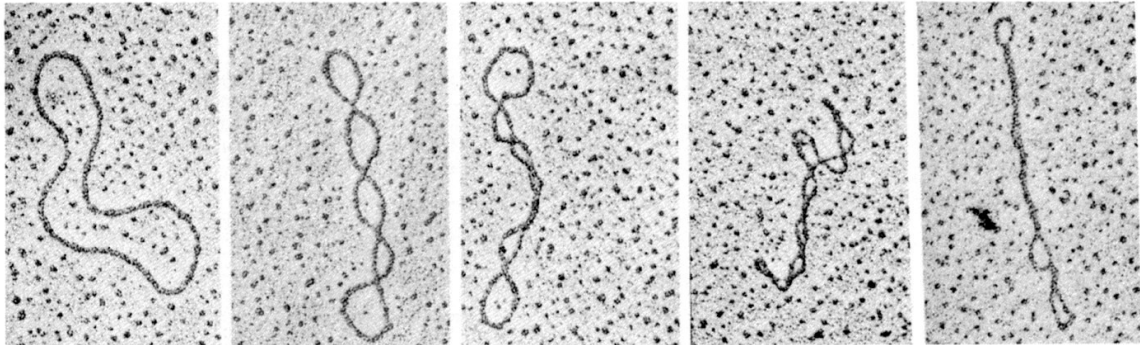
DNA supercoiling and replication/transcription

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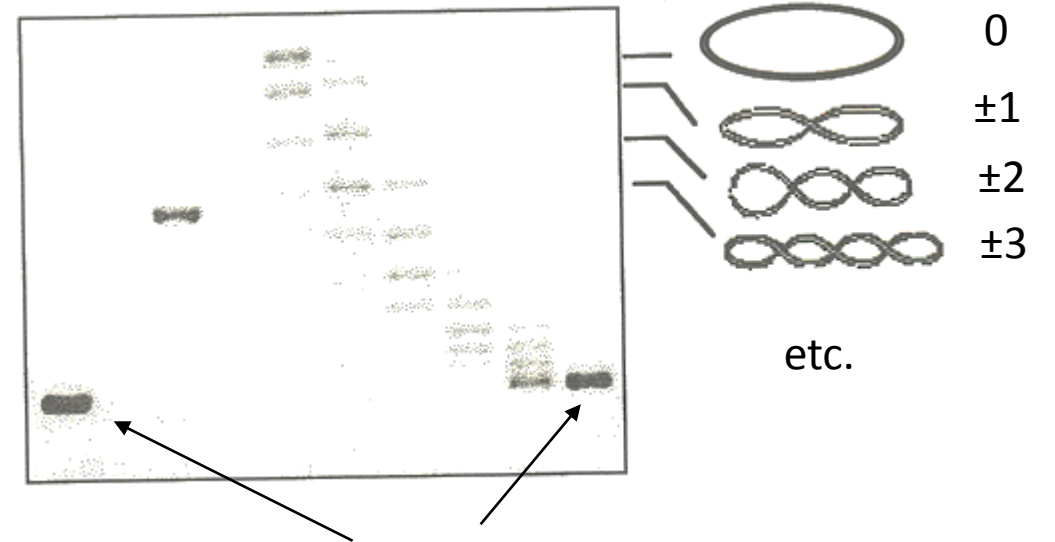
Topoisomers

- molecules of circular duplex DNA differing in Lk value



electron microscopy

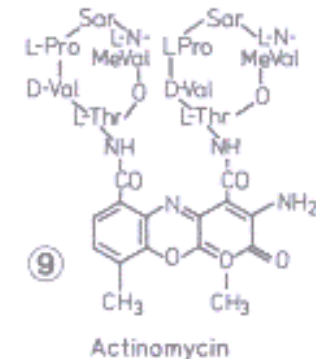
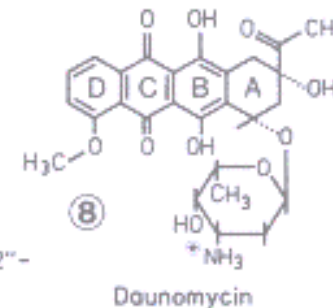
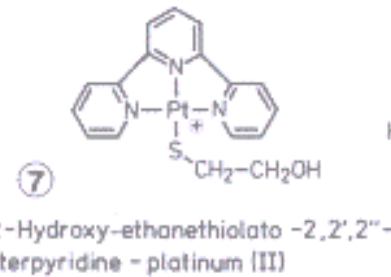
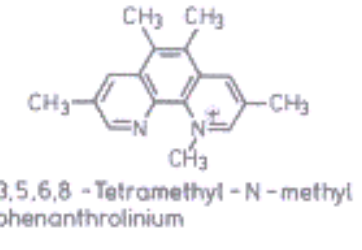
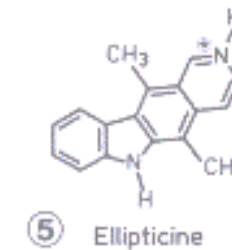
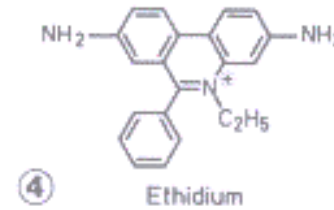
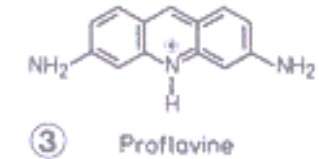
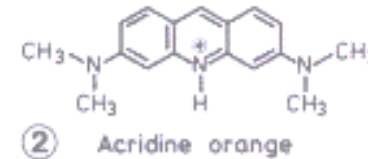
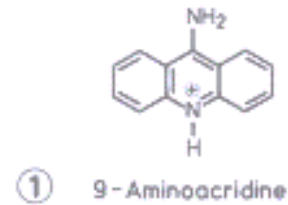
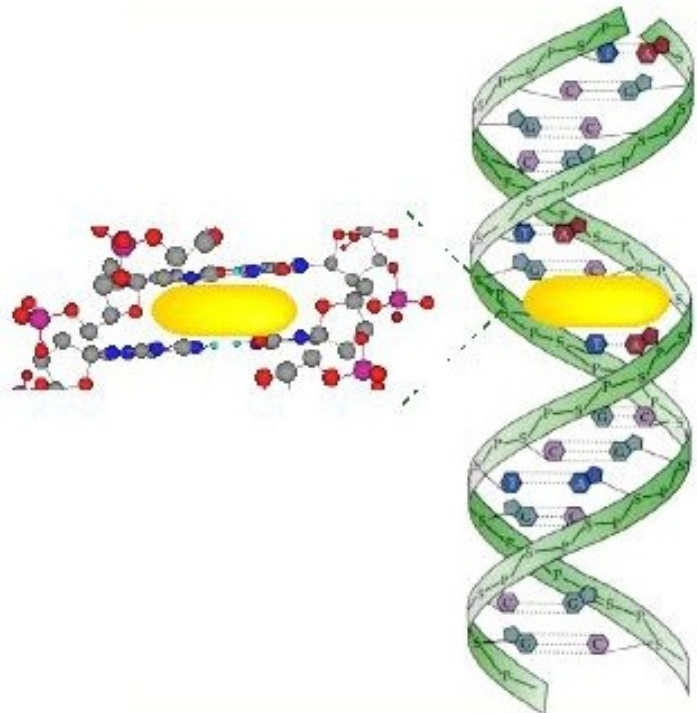
separation of plasmid molecules differing in $|Wr|$ in agarose gel



bundle of unresolved topoisomers of high $|Wr|$

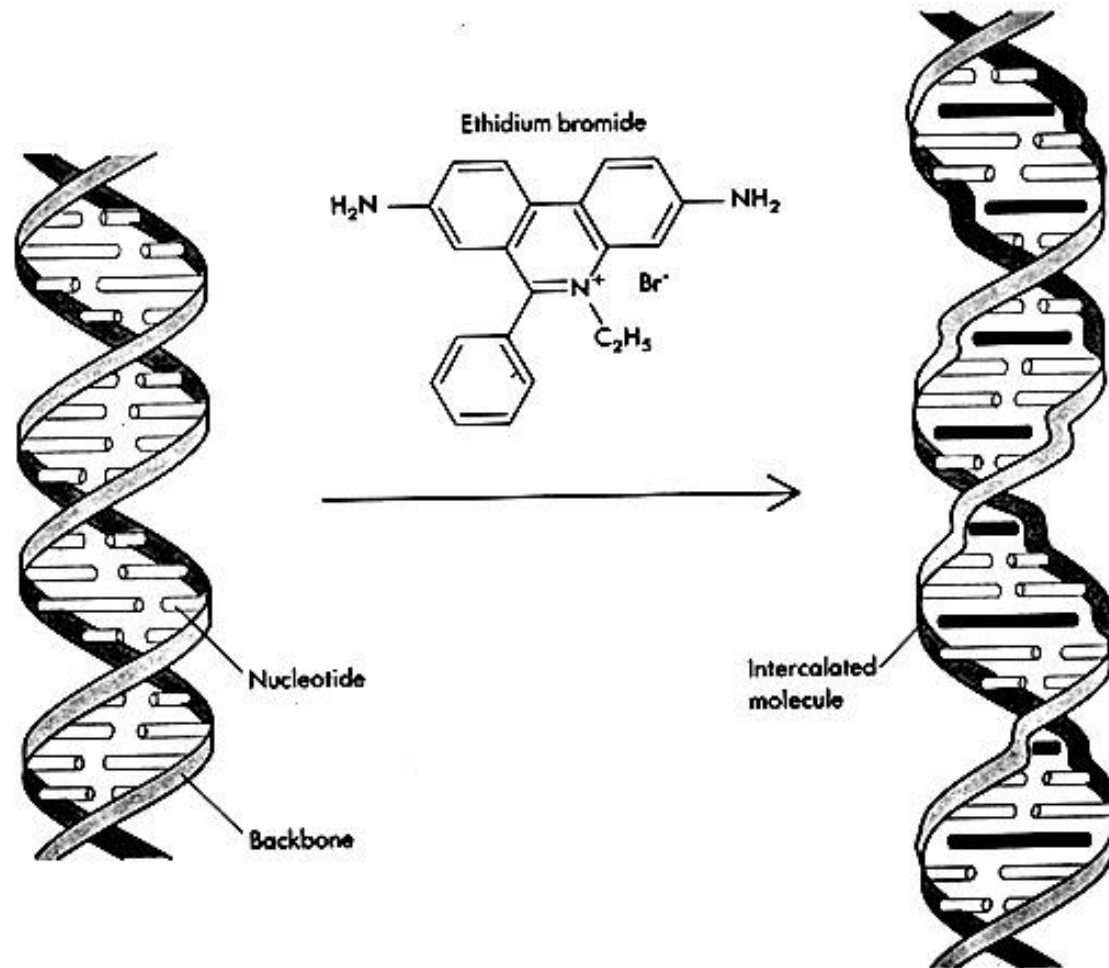
Superhelicity and intercalation

- intercalators: planar ligands intercalating between base pairs in duplex DNA
- stacking interaction



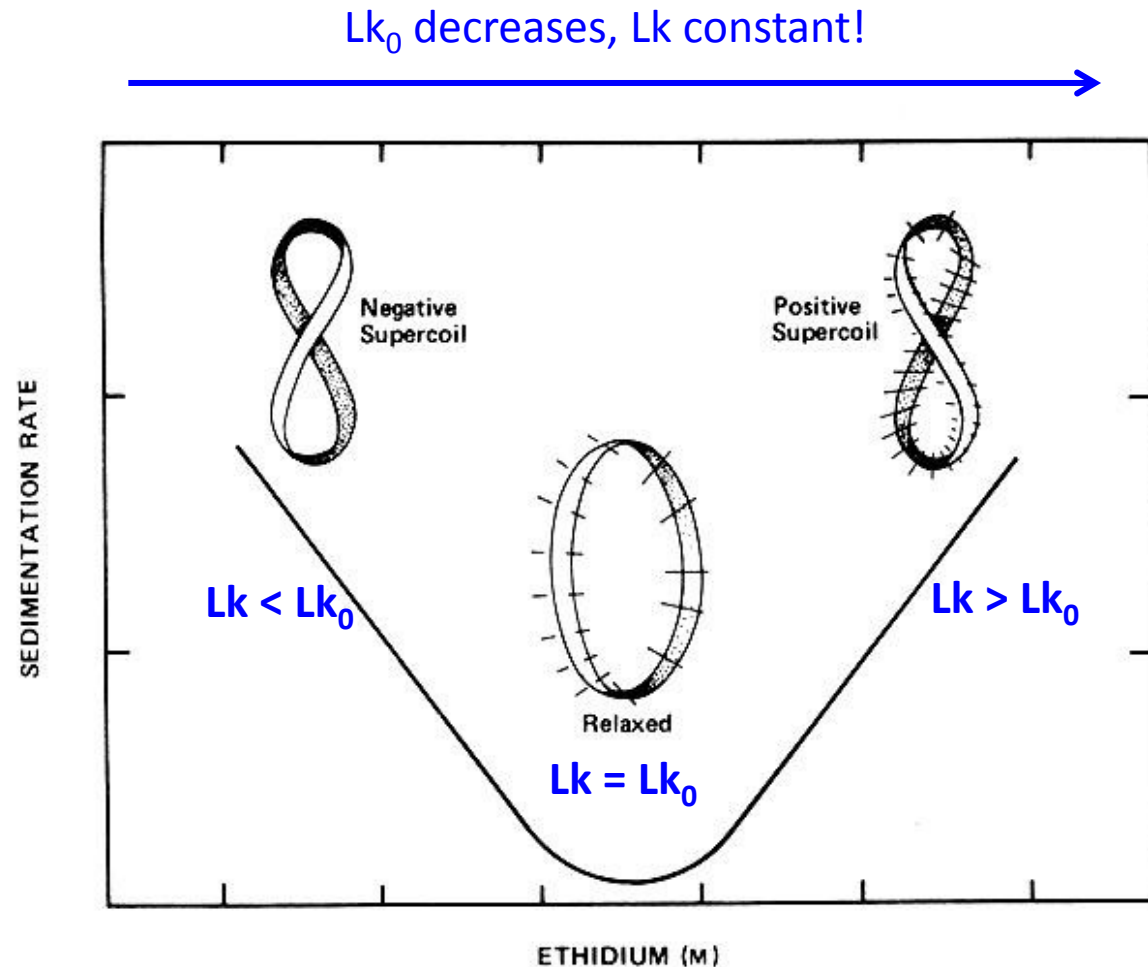
Superhelicity and intercalation

- characteristic changes in DNA conformation:
- extension in length
- **untwisting**



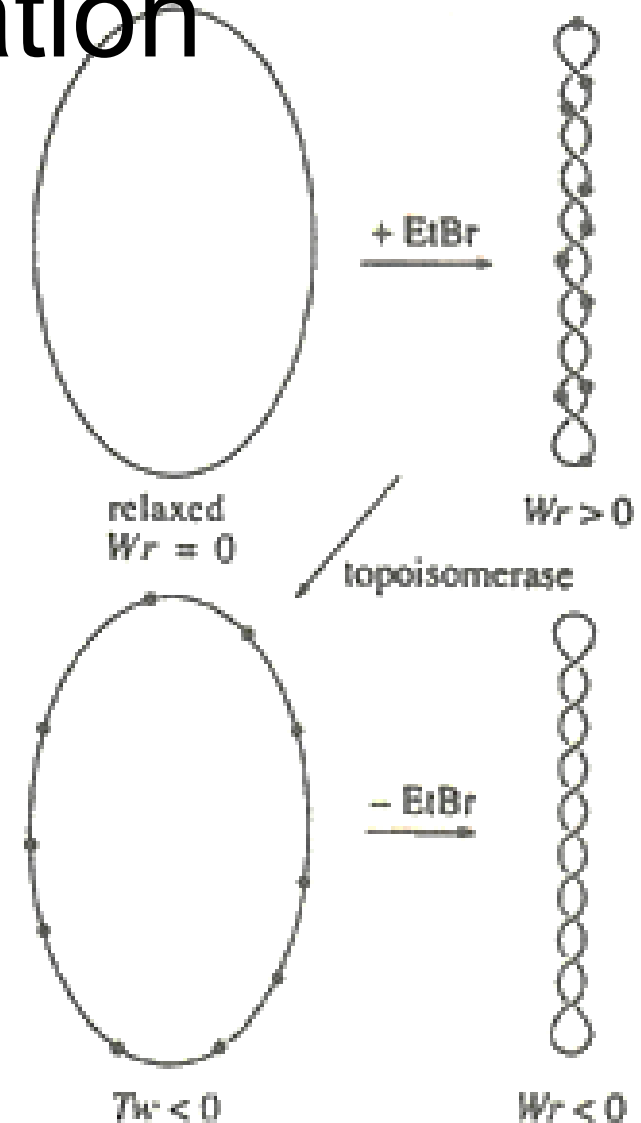
Superhelicity and intercalation

- increasing concentration of an intercalator:
- gradual relaxation of negative superturns
- formation of positive superturns
- **intercalation reduces Lk_0 value!**
- (even in unconstrained relaxed DNA, number of double helix turns is reduced)



Superhelicity and intercalation

- **preparation of topoisomers:**
- an intercalator is used to modulate superhelicity level
- topoisomerase removes superturns existing at the given intercalator concentration
- negative superturns which were absorbed by intercalation are restored after the intercalator removal

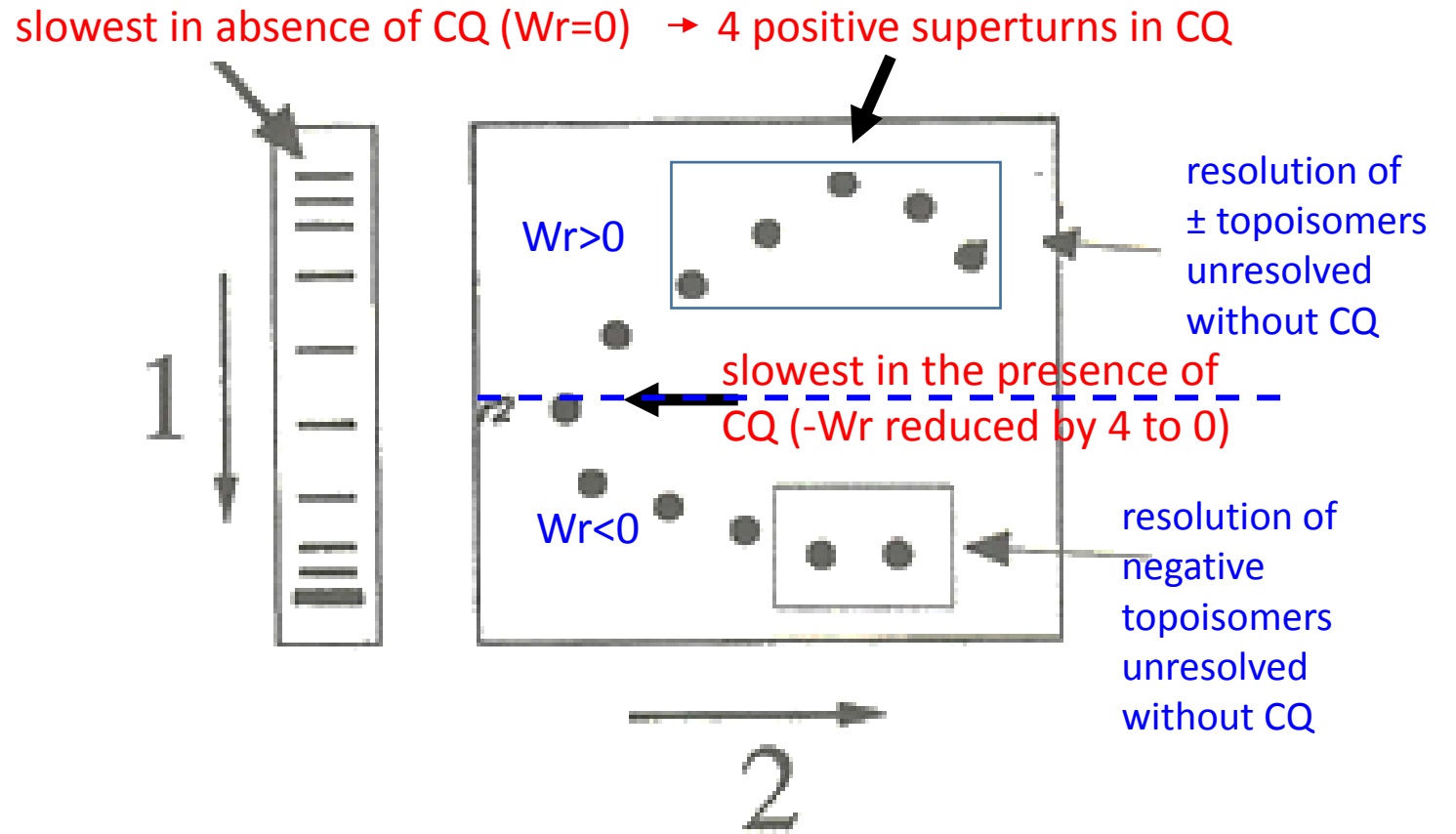
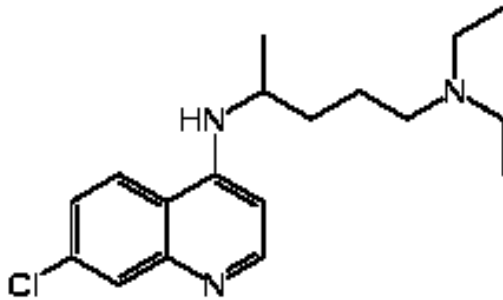


2D electrophoresis of topoisomers and detection of structural transitions

- open local structures are formed in scDNA with sufficiently negative superhelix density
- they absorb a part of the superhelical stress, which is reflected in reduction of W_r (number of superturns)
- decrease of the negative superhelicity causes the open structures to disintegrate and B-DNA duplex to reform
- negative superhelicity reduction can be attained by intercalation

2D electrophoresis of topoisomers and detection of structural transitions

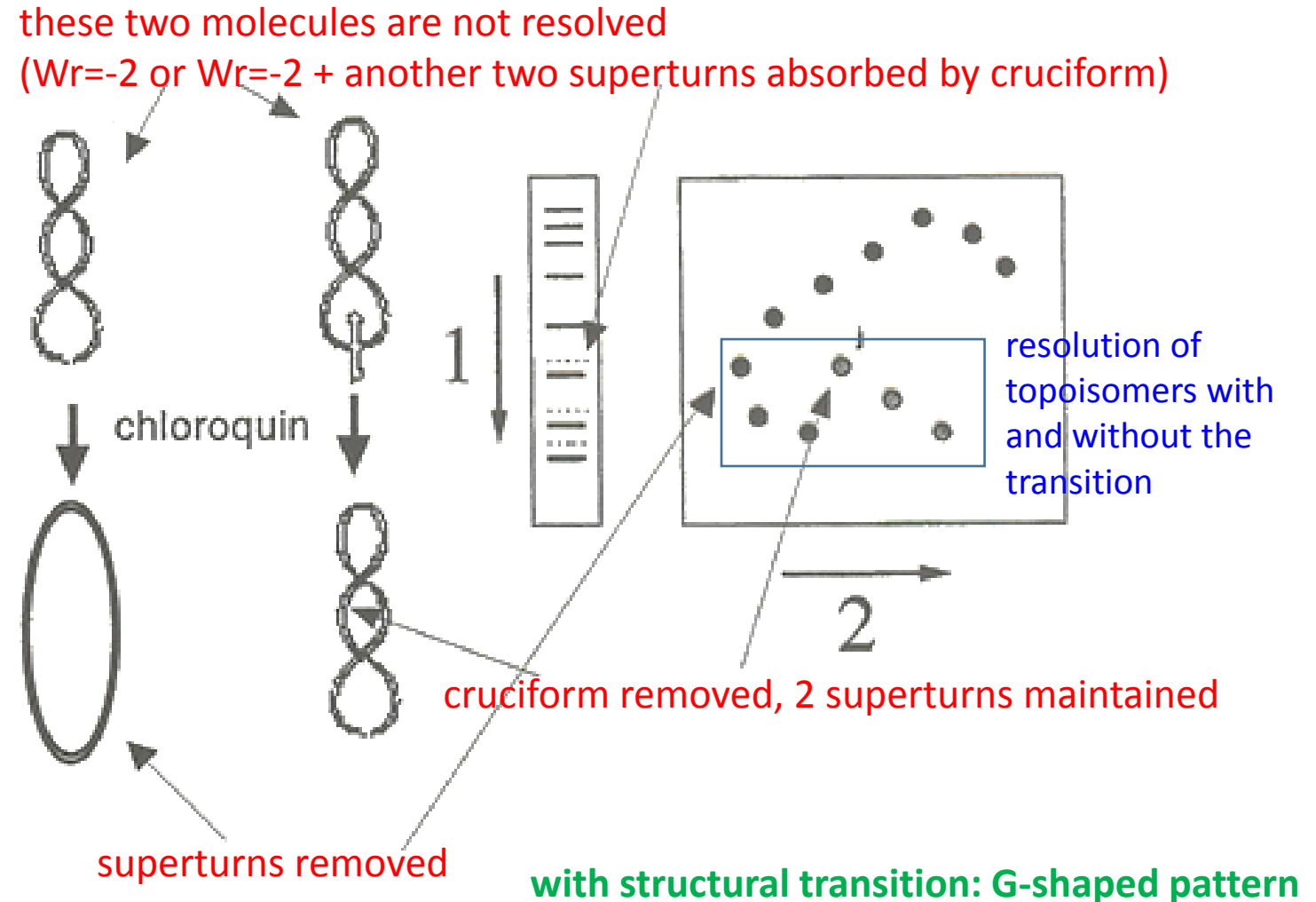
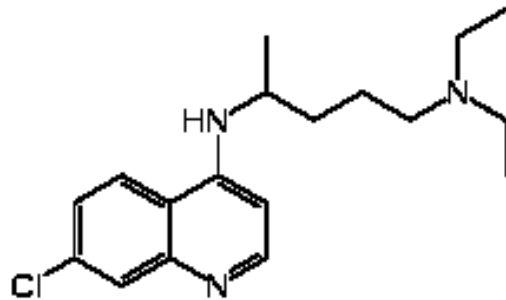
- topoisomers are prepared and separated in first dimension
- then the gel is soaked with chloroquine (CQ) to remove certain number of superturns (e.g., 4) and second dimension is run



without structural transition: C-shaped pattern

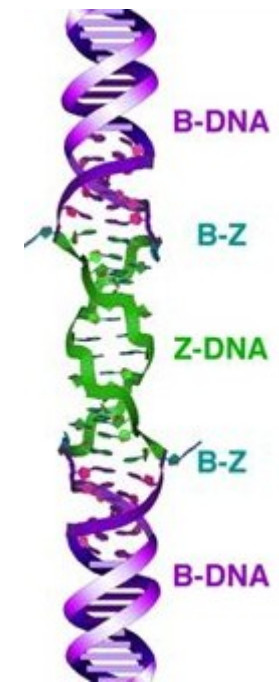
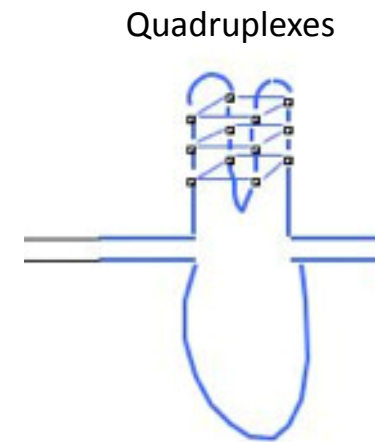
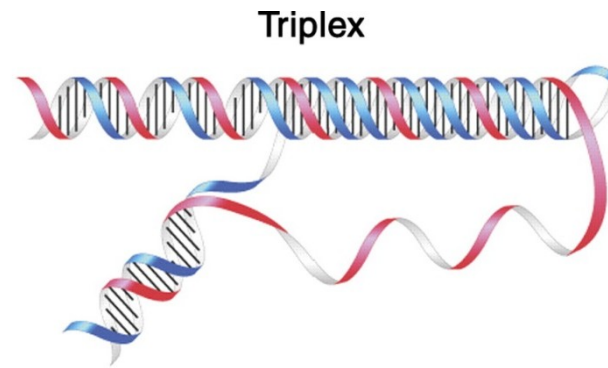
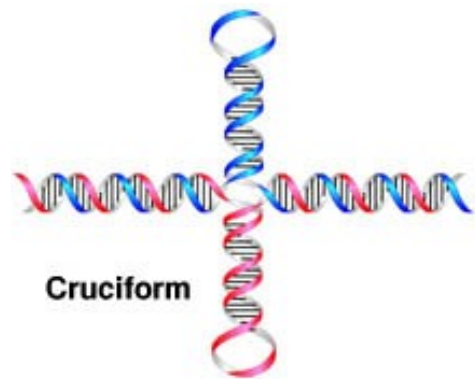
2D electrophoresis of topoisomers and detection of structural transitions

- topoisomers are prepared and separated in first dimension
- then the gel is soaked with chloroquine (CQ) to remove certain number of superturns (e.g., 4) and second dimension is run



Chemical probing of non-B structures (to recall)

- the open local structures contain unpaired bases, unstacked base pairs or otherwise distorted sites

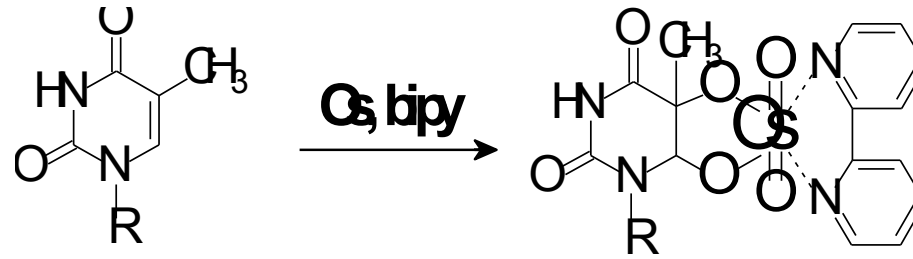


- loops, junctions...
- increased chemical reactivity of the nucleobases

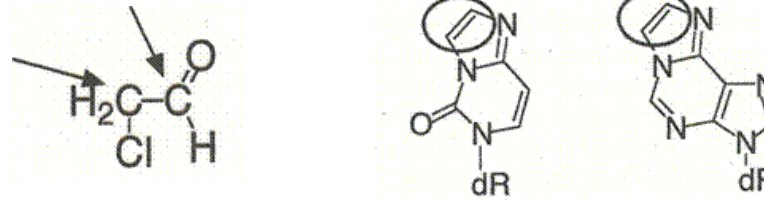
Left-handed (Z-form) duplex

Chemicals selectively reacting with unpaired bases:

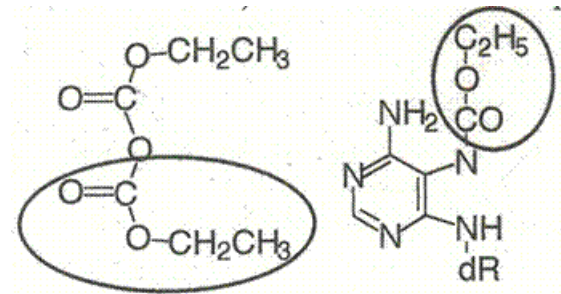
osmium tetroxide complexes
(Os,L)
(T, more slowly C)



chloroacetaldehyde
(CAA)
(A, C)

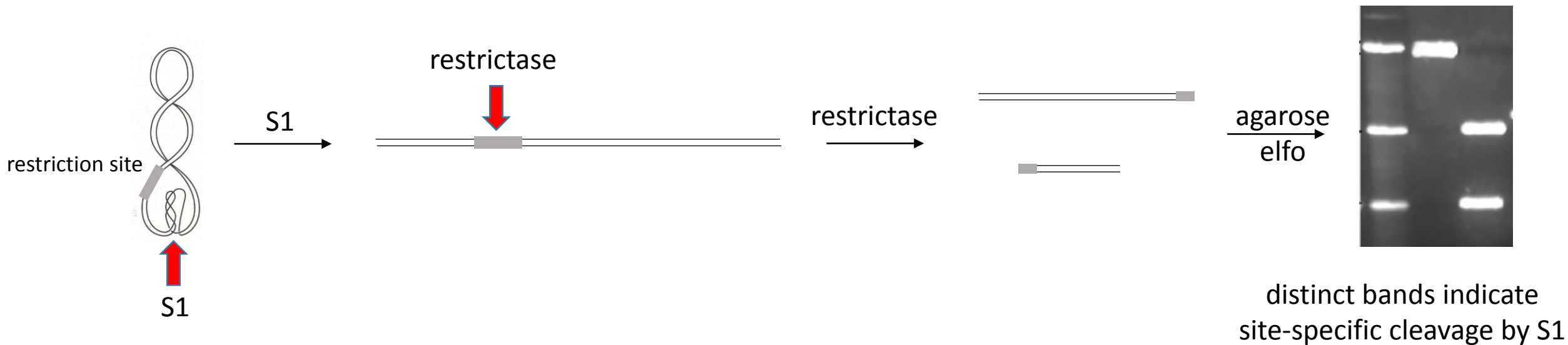


diethyl pyrocarbonate
(DEPC)
(A, G)



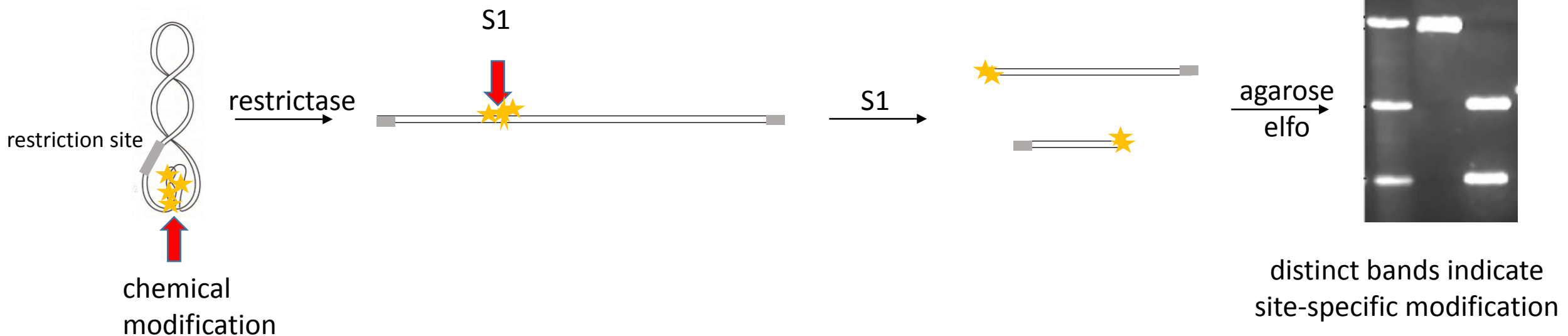
Single-strand selective enzymes

- only detection of a open structure, not identification at the sequence level
- often sufficient: evidence of formation of a expected structure
- nucleases S1, P1, mung bean... cleave ss DNA (or RNA)
- scDNA cleaved by S1, then restriction cleavage to map S1 cleavage site



Combination of chemical probes with S1 nuclease

- chemical probes work within wider range of conditions than enzymes
- modification of scDNA
- then restrictase cleavage
- chemical modification of bases in structure that existed in scDNA prevent formation of B-DNA
- then S1 cleavage in the modified site

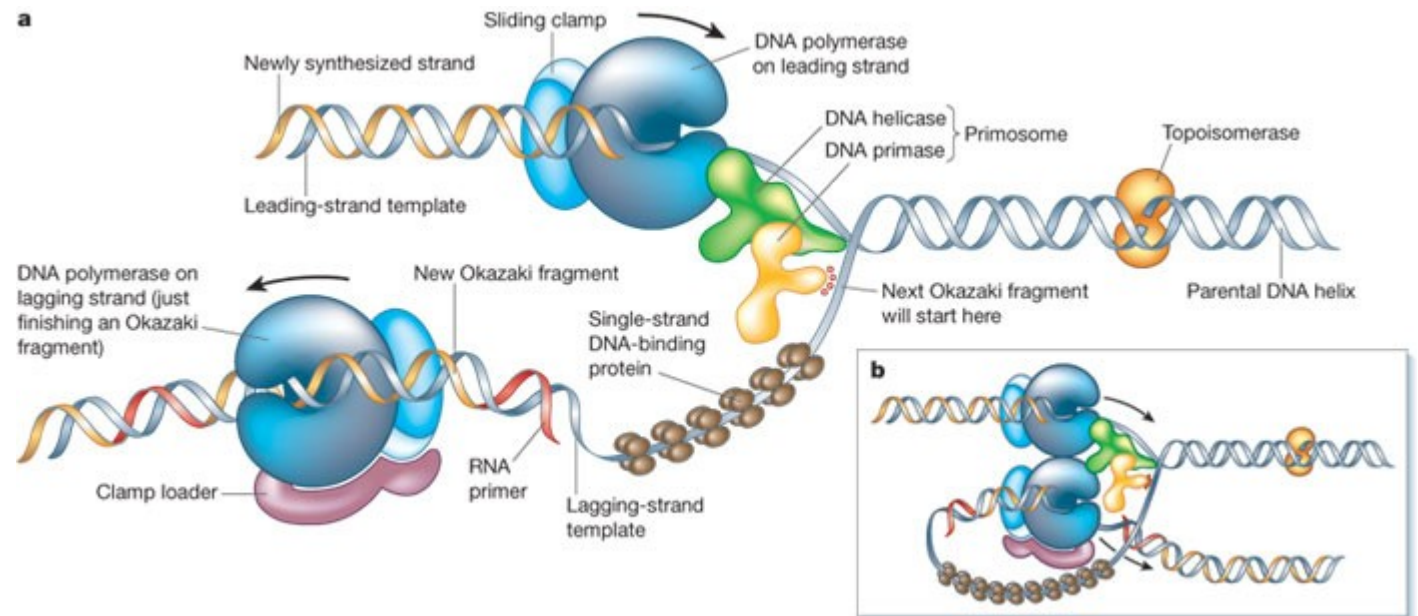


Topoisomerases

- enzymes relaxing (or introducing) superhelical stress in DNA: changing Lk
- solving the „knotty problem“ in replication, transcription

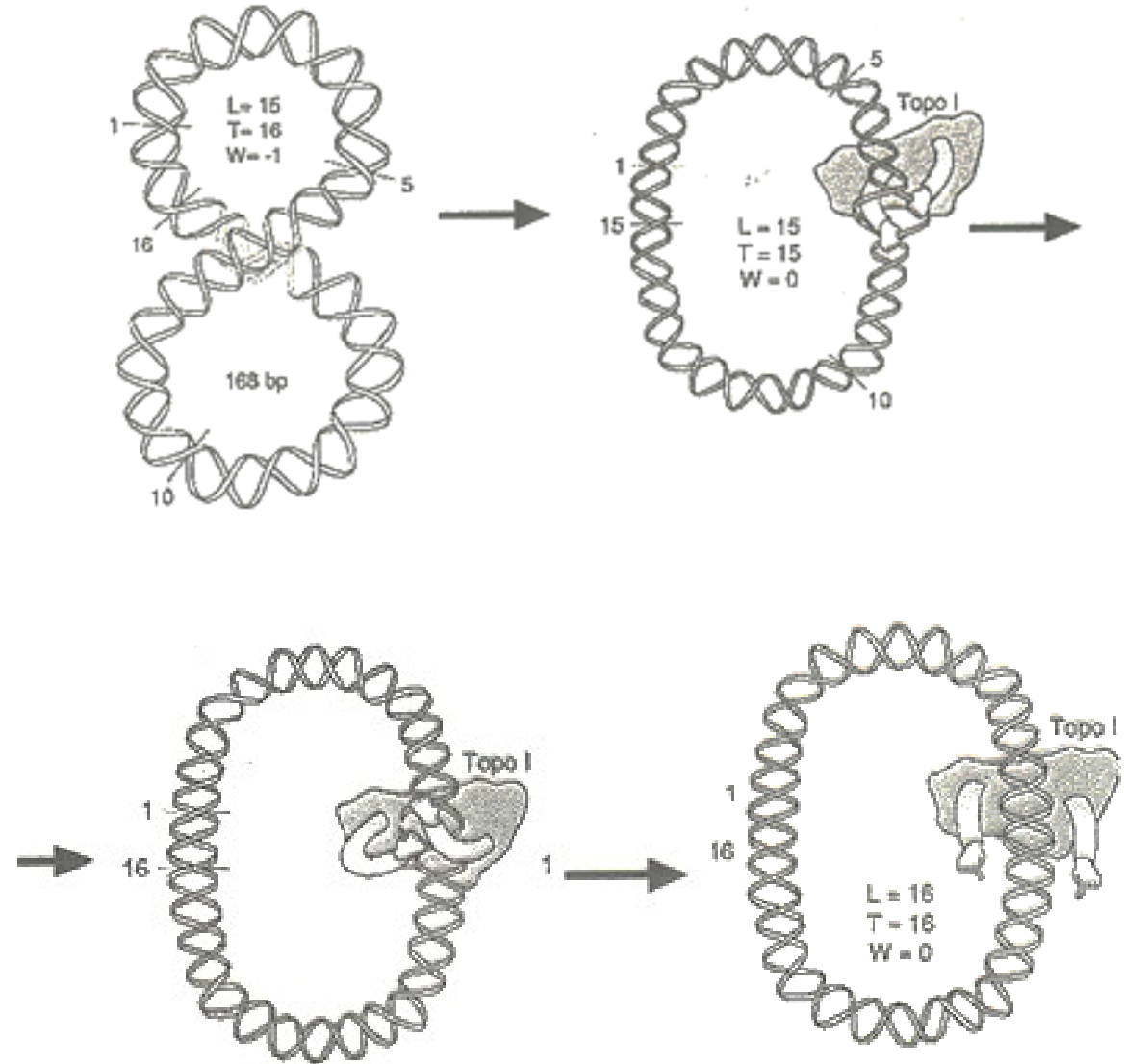


(video)



Topoisomerase I

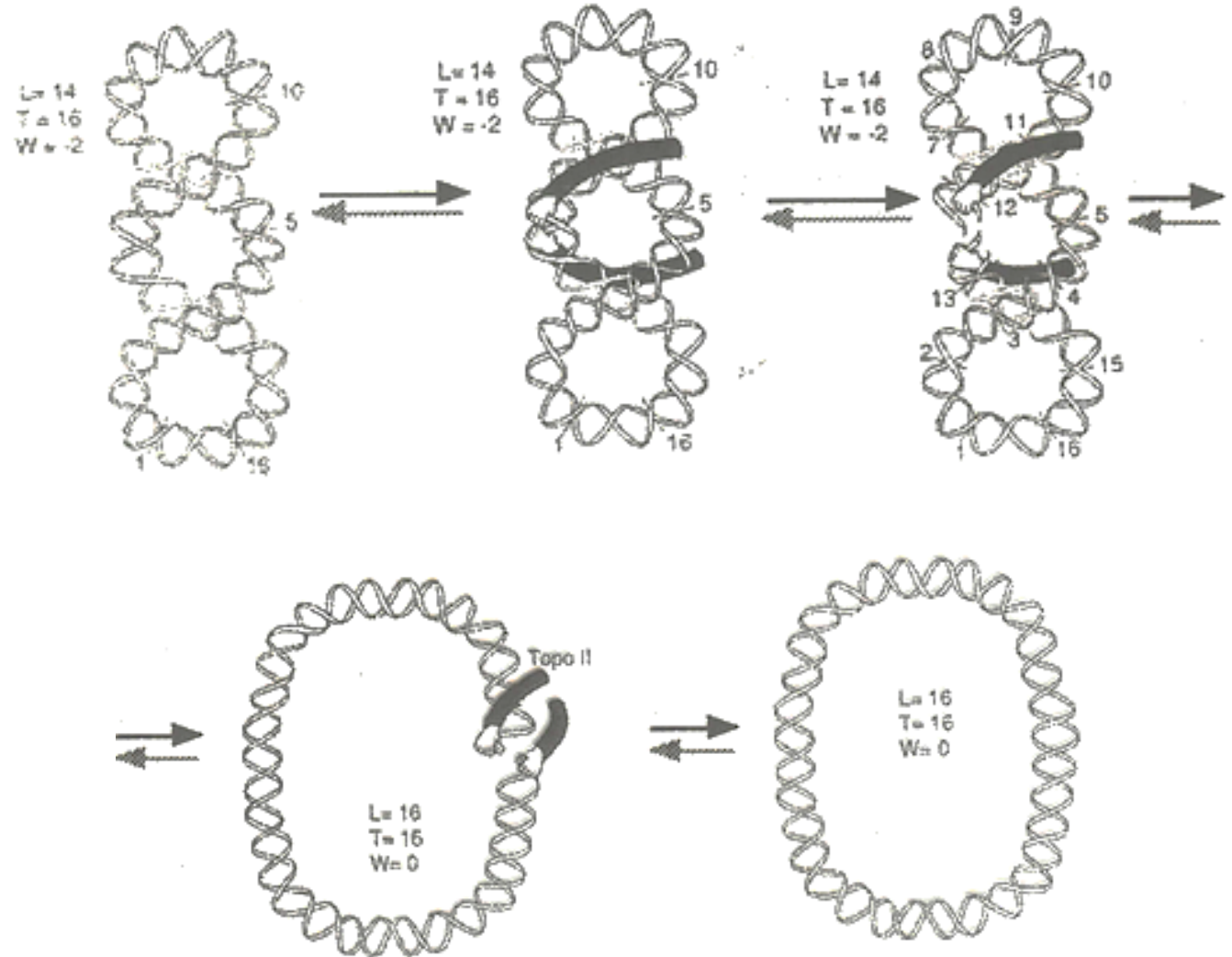
- creating and sealing a single-strand break
- only relaxation
- no ATP needed:
transesterification, covalent binding of the enzyme to DNA (phosphoester of a Tyr residue)
- relax either only negative superturns (*E. coli* topo I), or both positive and negative (topo I from wheat germ)



Lk changed by 1 (one strand threaded through a ssb)

Topoisomerase II

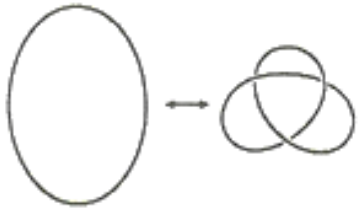
- creating and sealing a double-strand break
- relaxing or introducing superhelicity (DNA gyrase)
- ATP consumption (conformational changes of the protein)



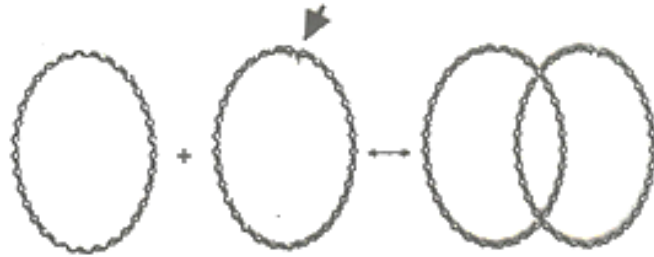
Lk changed by 2 (double helix threaded through a dsb)

Other processes catalyzed by topoisomerases

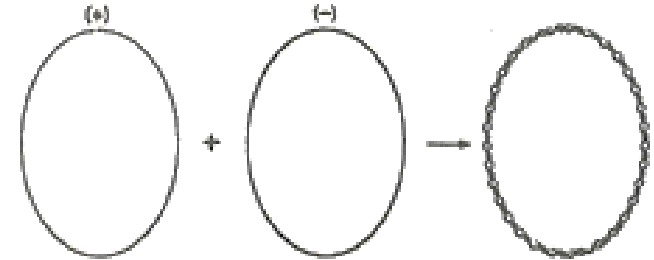
Topoisomerase I:



knotting/unknotting of ss circles

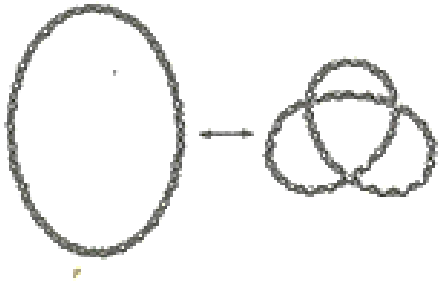


catenation/decatenation of nicked circles

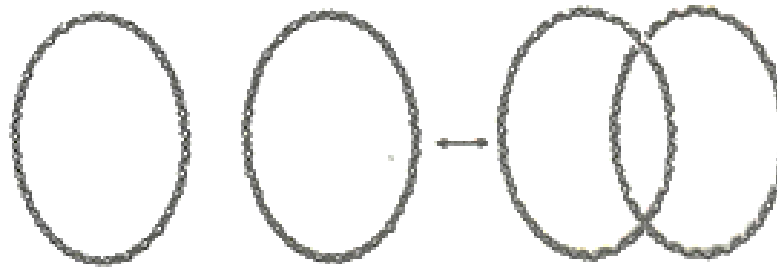


circular duplex formation of two complementary ss circles
(=relaxation of negatively scDNA!)

Topoisomerase II:



knotting/unknotting of duplex circles



catenation/decatenation of duplex circles

Importance of decatenation activity in replication

