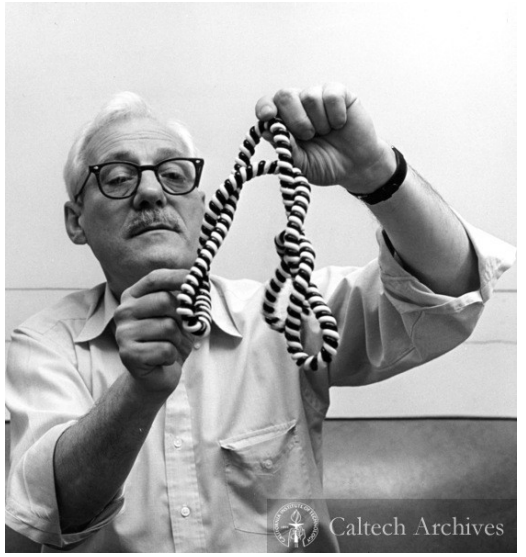
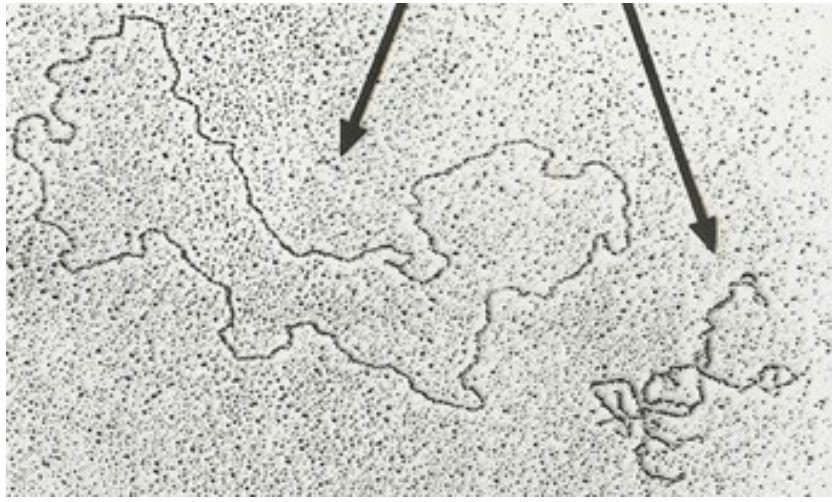
DNA supercoiling

Jerome Vinograd, 1965

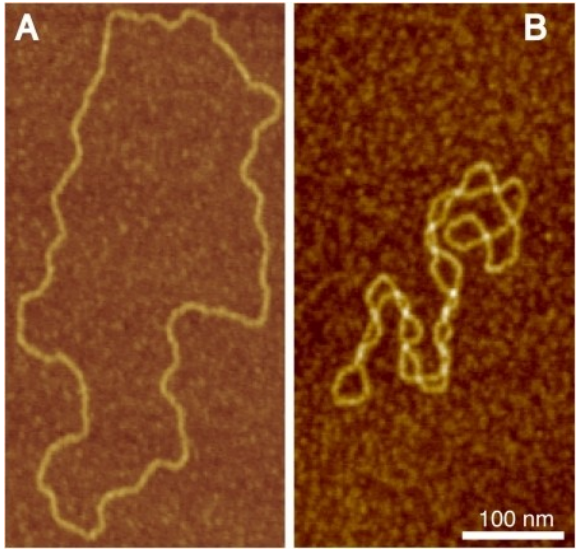


- sedimentation equilibrium experiments with viral DNA
- circular DNAs can exist in two distinct forms differing in buoyant density
- supercoiled (compact) and relaxed (loose)

electron microscopy



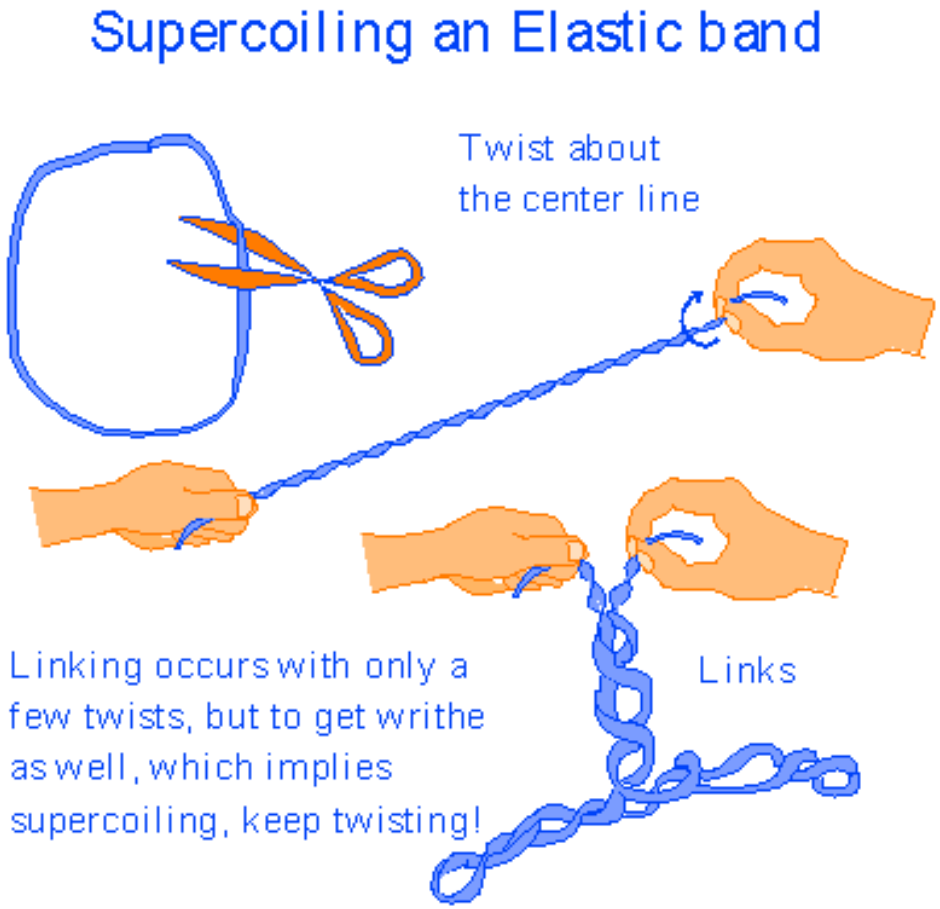
AFM



agarose gel elfo

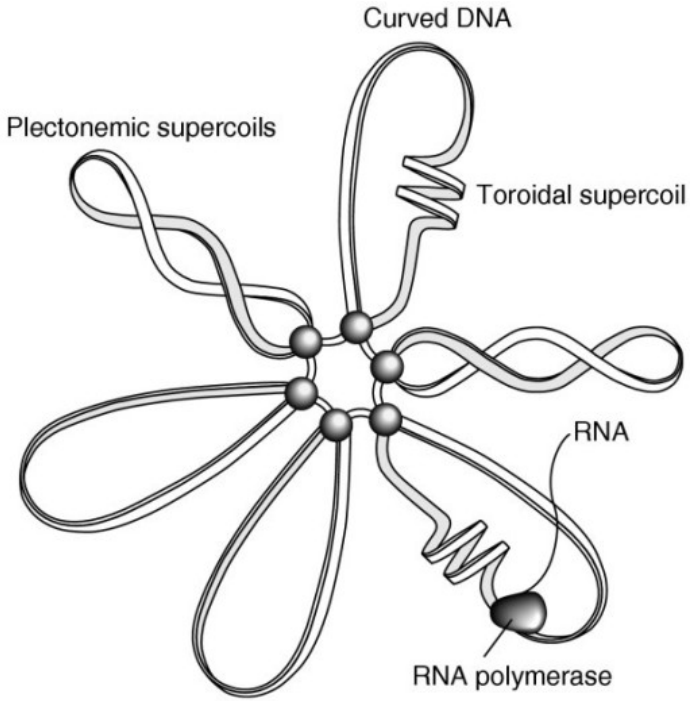
Double helical DNA behaves like a rubber rod

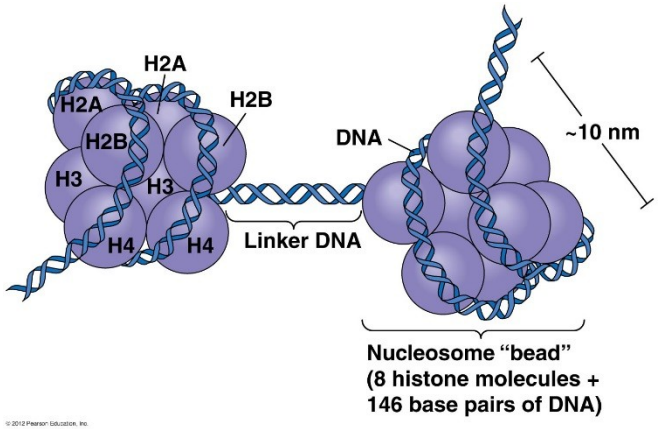
- some torsional and bending elasticity
- „shape memory“
- tendency to keep B-conformation
- tendency to keep the axis straight
- Interplay between twisting and bending deformations



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





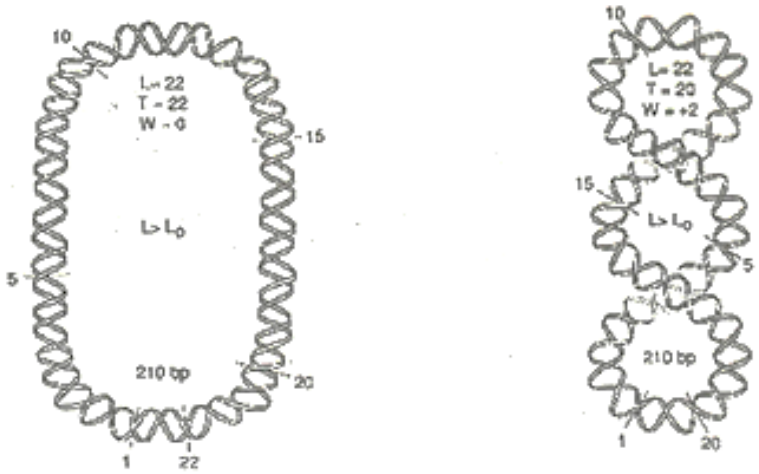
Linking number Twist Writhe

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 **≠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)

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

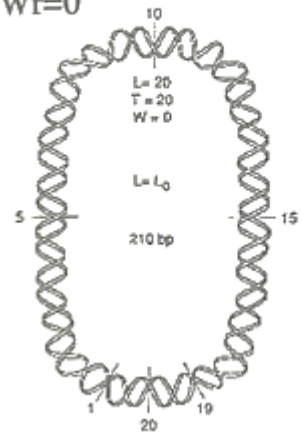
Lk cannot be changed without interrupting at least one DNA strand

relaxed

$Lk = Lk_0 = 210/10.5 = 20$

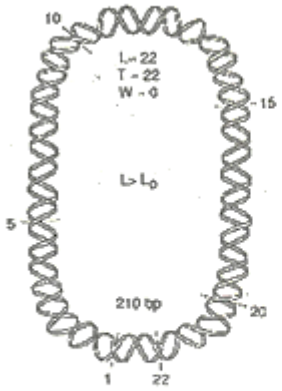
$Tw = 20$

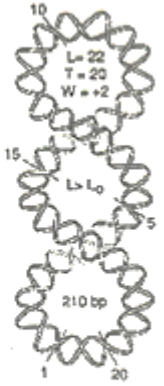
$Wr = 0$



positively supercoiled
(with linking excess)

$Lk = 22, \Delta Lk = 2$





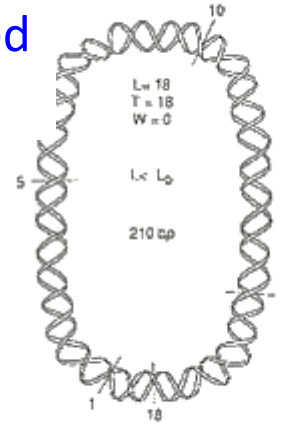
$Tw = 22, \Delta Tw = 2$
$Wr = 0, \Delta Wr = 0$

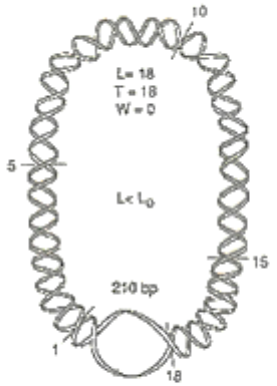
$Tw = 20, \Delta Tw = 0$
$Wr = 2, \Delta Wr = 2$

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

$Tw = 18, \Delta Tw = -2$
$Wr = 0, \Delta Wr = 0$

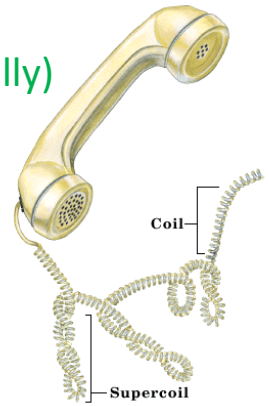
duplex
locally untwisted

$Tw = 20, \Delta Tw = 0$
$Wr = -2, \Delta Wr = -2$

duplex
twisted normally,
supercoils formed

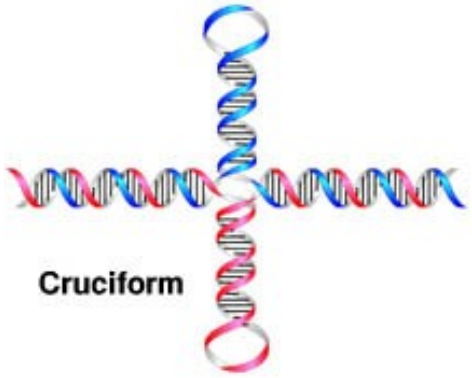
negative superhelical stress forces the right-handed double helix to open

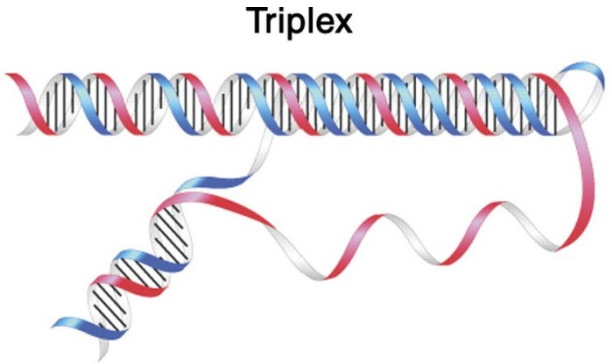
In real situation, superhelicity distributed between ΔTw (locally or globally) and ΔWr

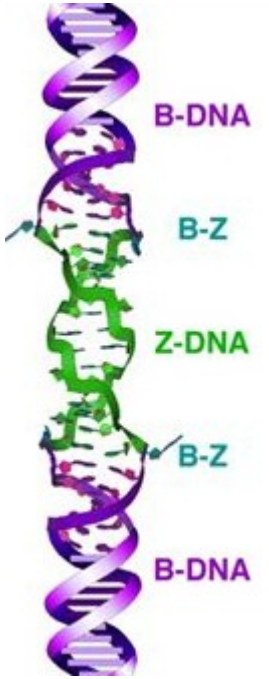


Open local structures

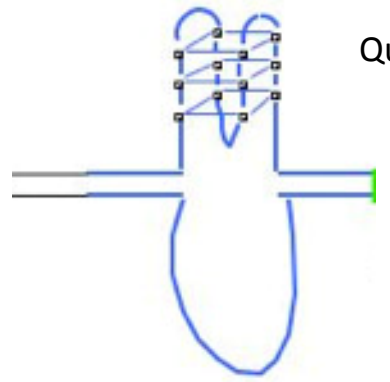
- formed in appropriate sequence motifs
- characterized by locally reduced twist, compared to B-DNA
- paranemic: possible to form/abolish without mutual rotation of opposite strands







Left-handed (Z-form) duplex

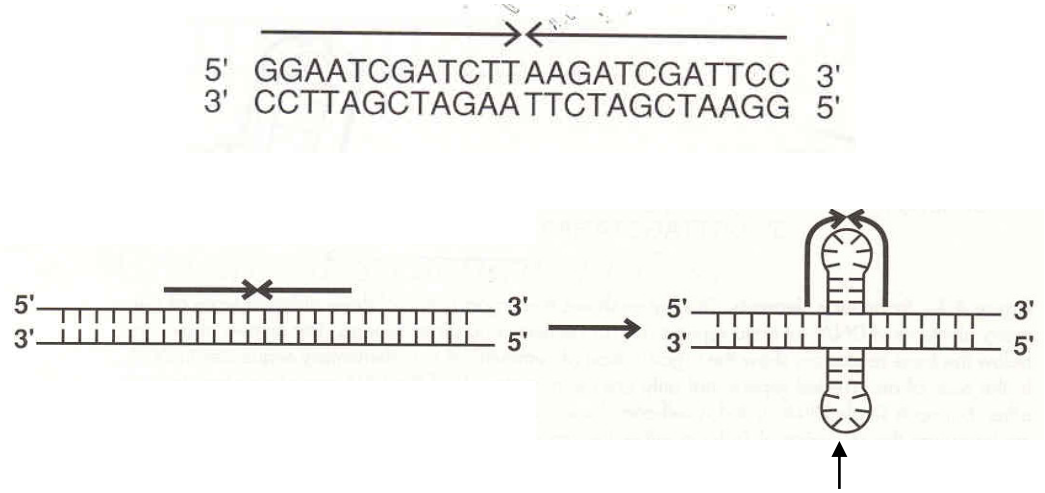


Quadruplexes

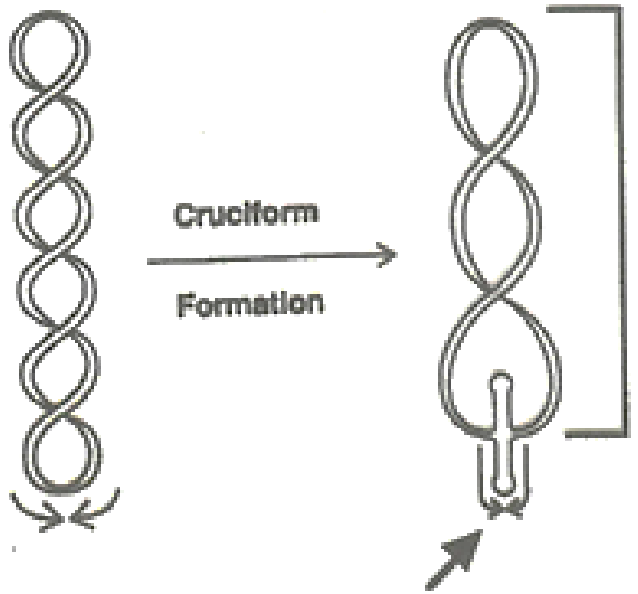
Open local structures in negatively supercoiled DNA

cruciform DNA

inverted repeat (sequences with dyad symmetry)



unpaired bases



local reduction of twist

partial relaxation of negative superturns

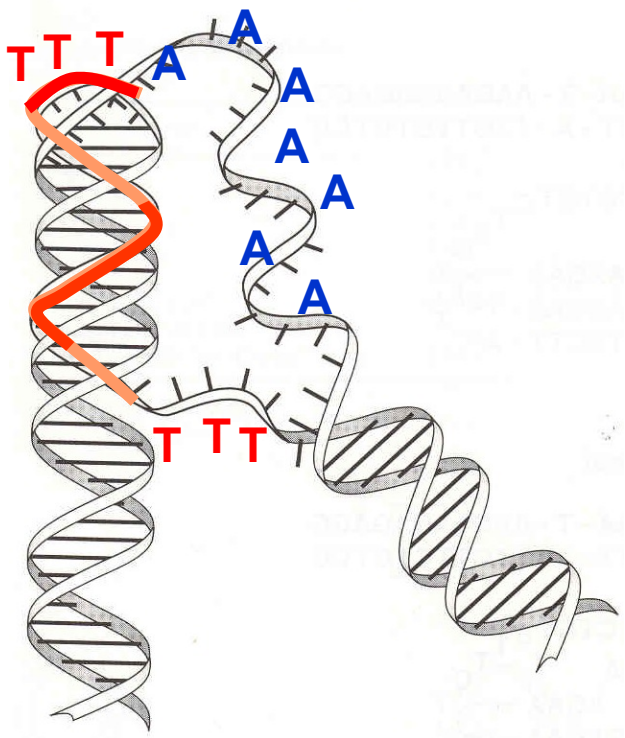
(cca 1 superturn per 10 bp of cruciform struture)

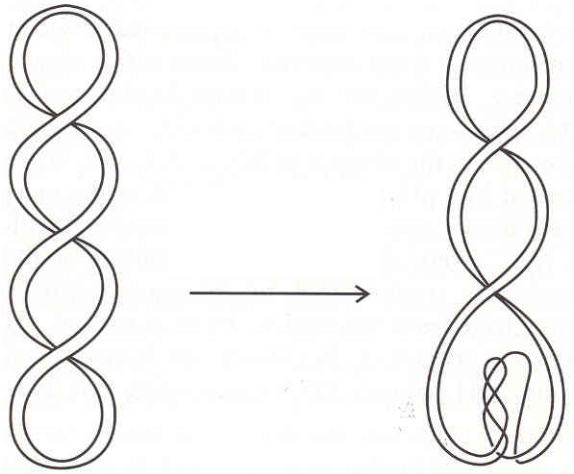
Open local structures in negatively supercoiled DNA

Intramolecular triplex

homoPu•homoPy segment with mirror symmetry

TAT (H*-DNA) – stabilized by Mg^{2+}; $C^{+}GC$ (H-DNA) – stabilized in weakly acidic media)





partial relaxation of negative superturns

(cca 1 superturn per 10 bp of cruciform struture)

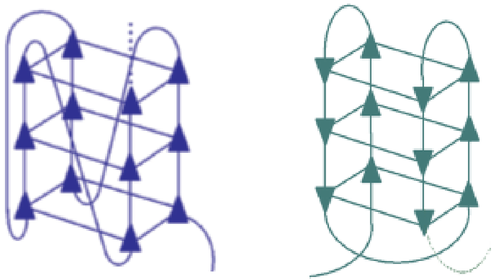
local reduction of twist

Open local structures in negatively supercoiled DNA

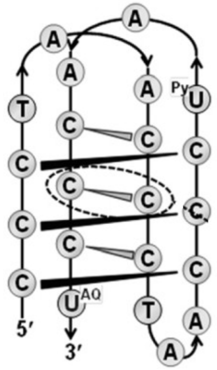
Intramolecular quadruplexes

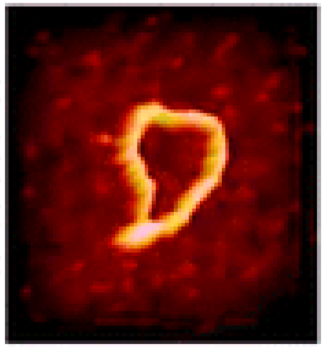
G:C-rich motifs

G-quadruplexes
(K^+-stabilized)



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

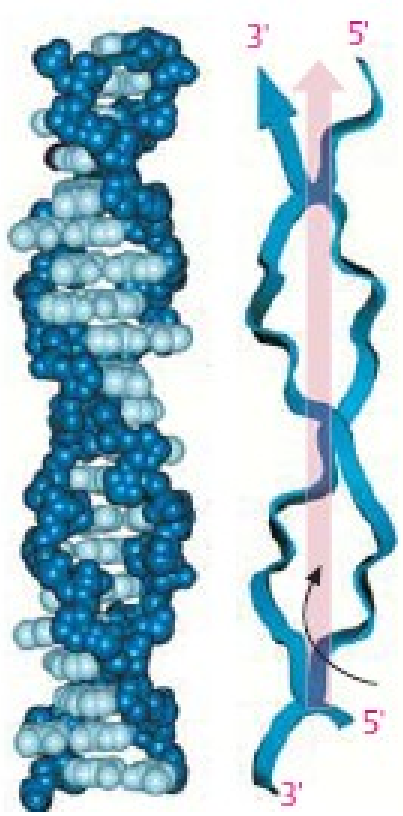




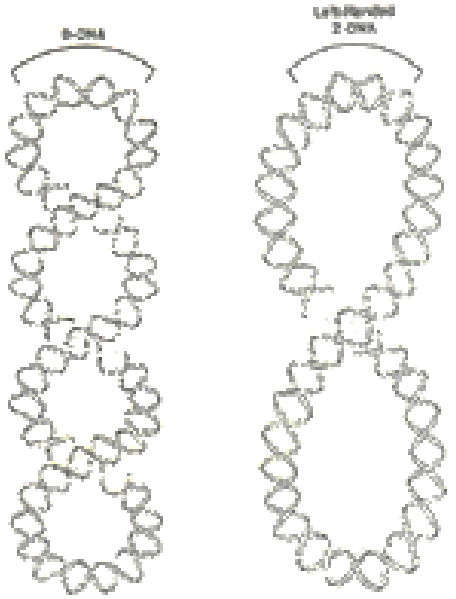
Open local structures in negatively supercoiled DNA

Lef-handed Z-DNA

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



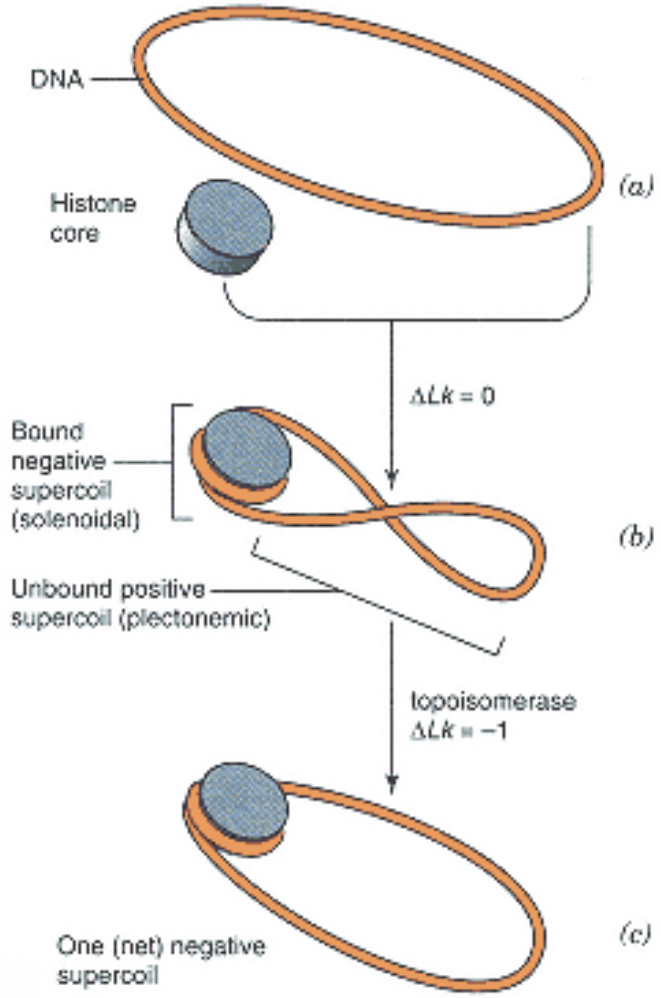
local reduction of twist
(to negative values)

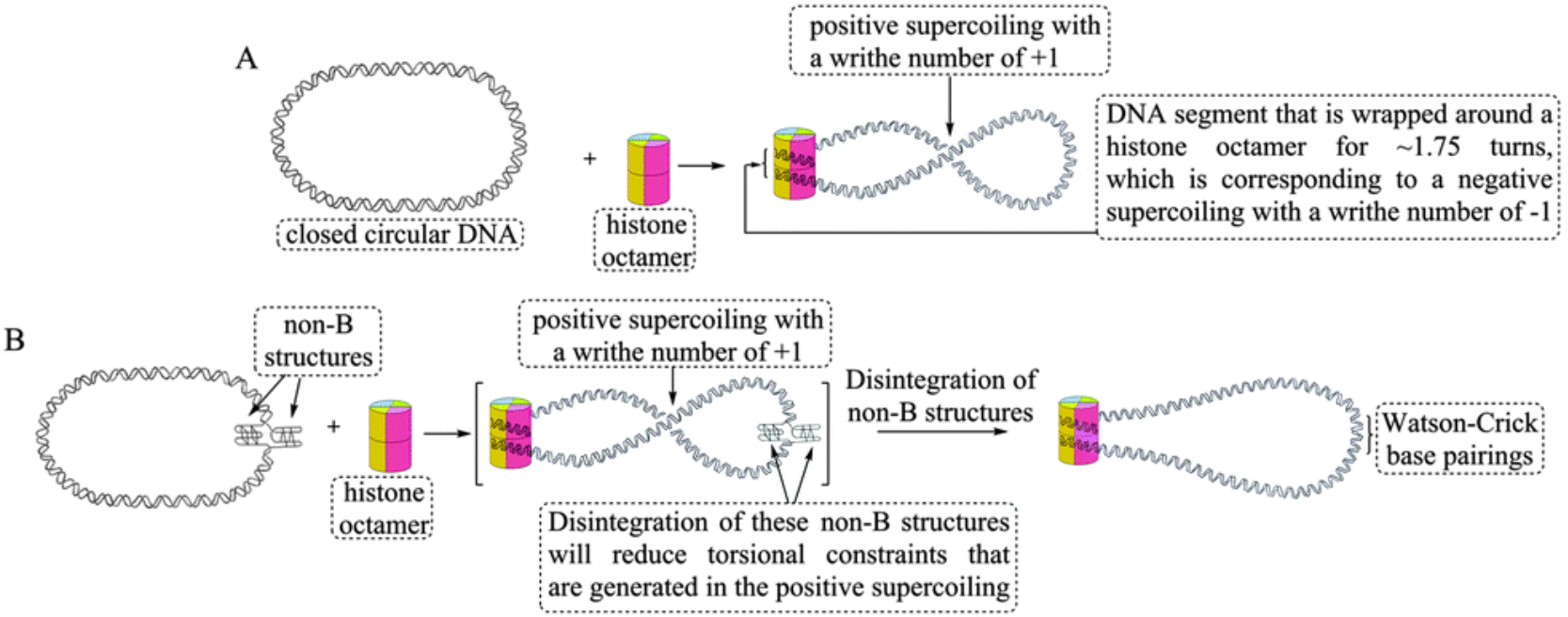


partial relaxation of negative superturns

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

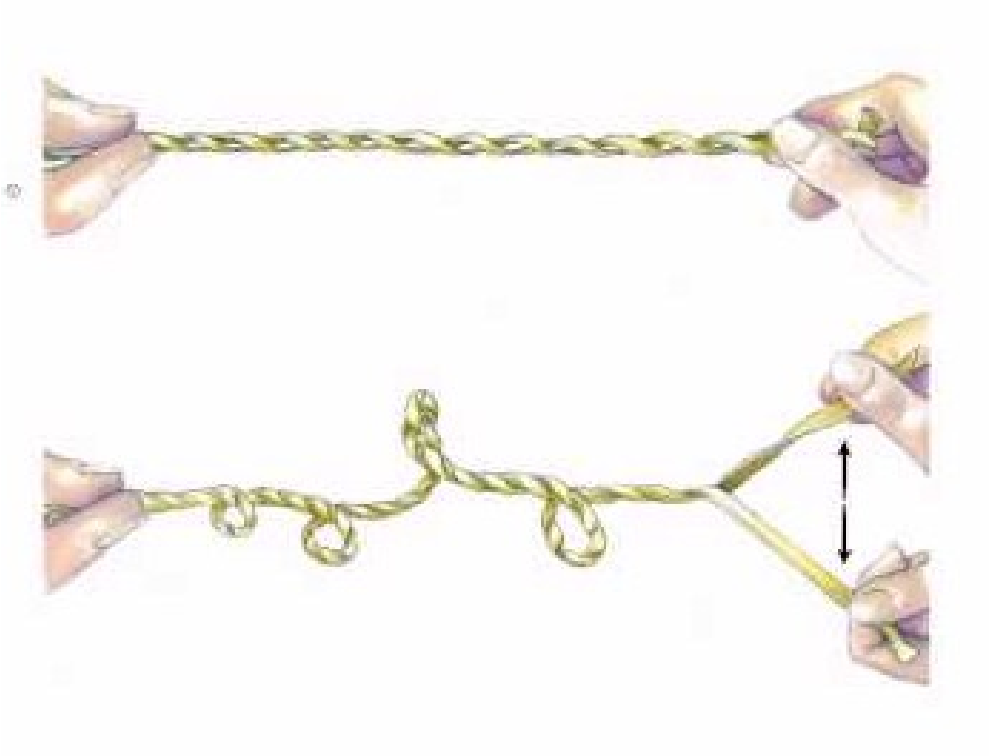
DNA supercoiling and nucleosome formation





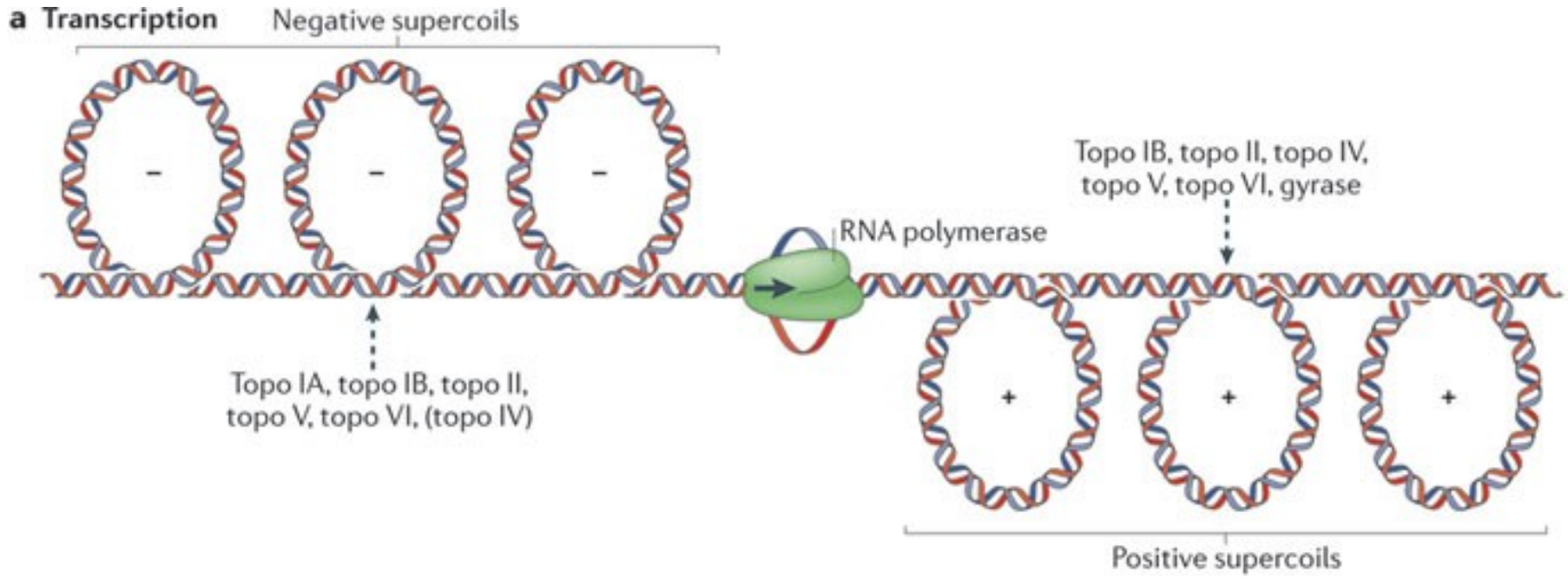
DNA supercoiling and replication/transcription

- 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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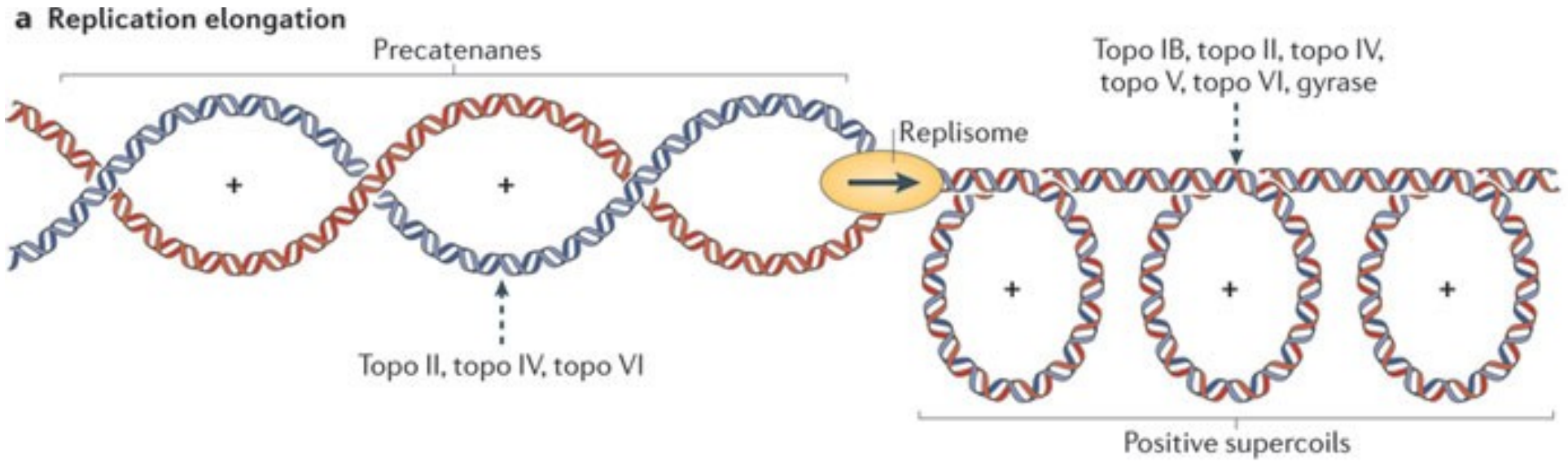
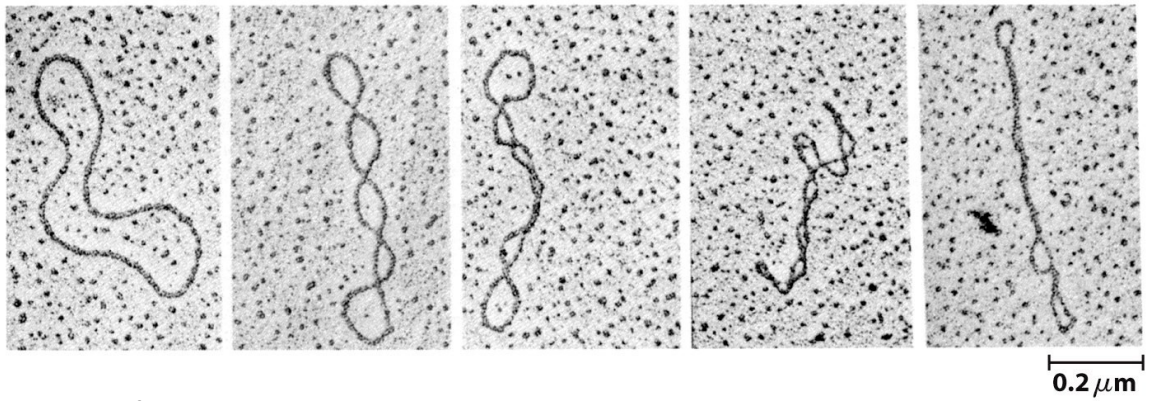


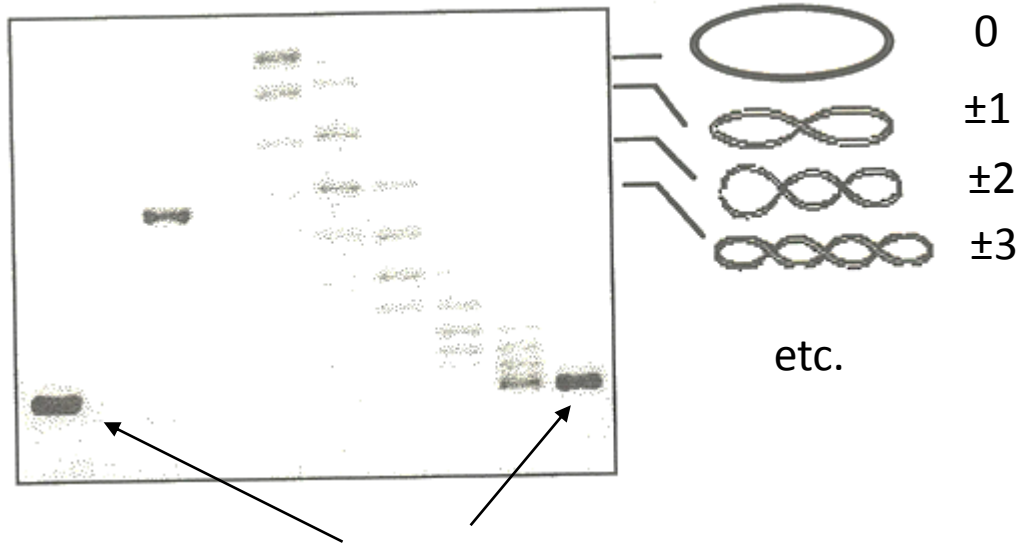
Topoisomers

- molecules of circular duplex DNA differing in Lk value



electron microscopy

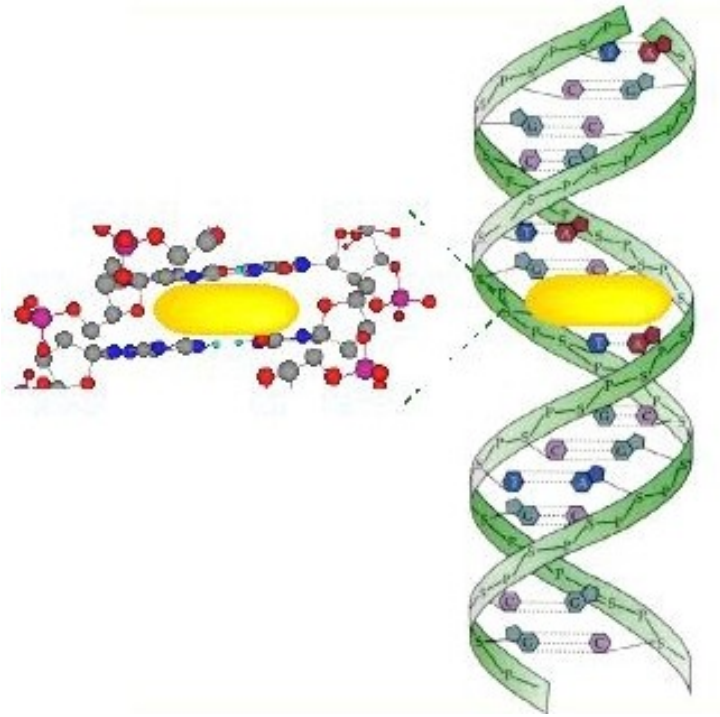
separation of palsmid molecules differing in |Wr| in agarose gel

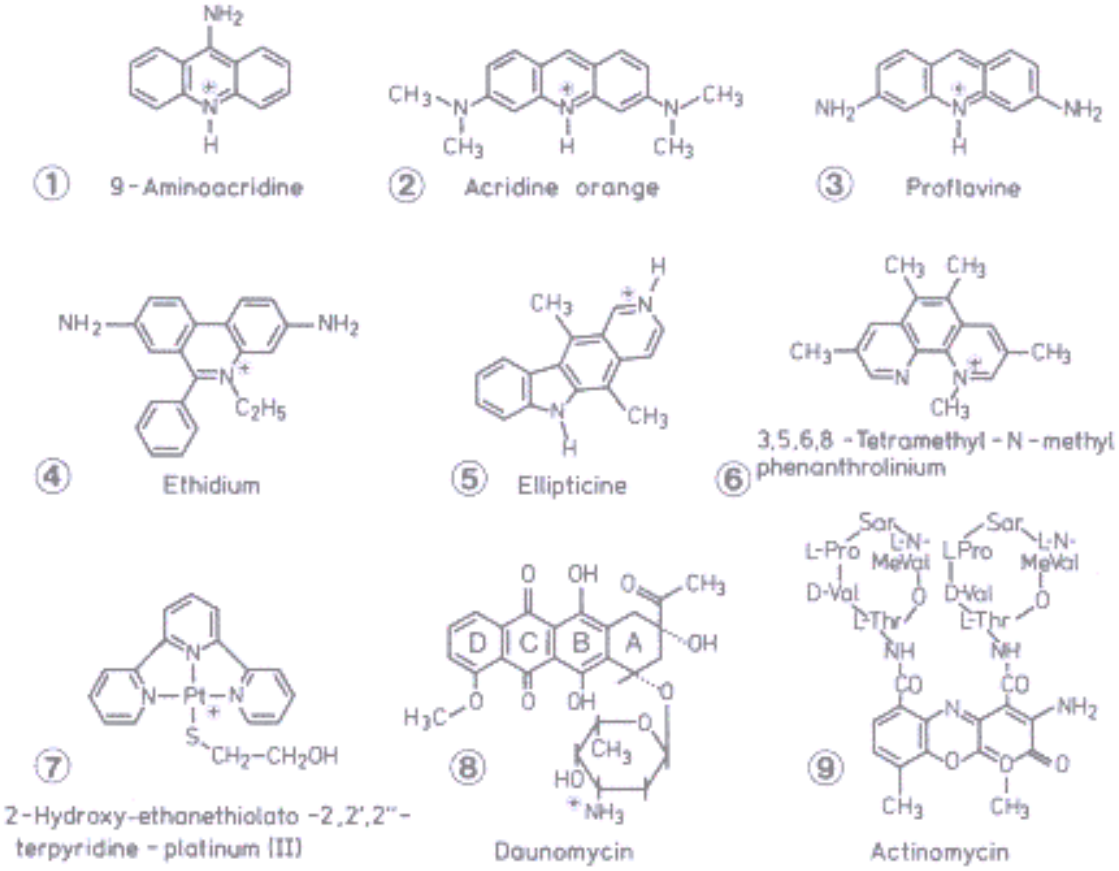


bundle of unresolved topoisomers of high |Wr|

Superhelicity and intercalation

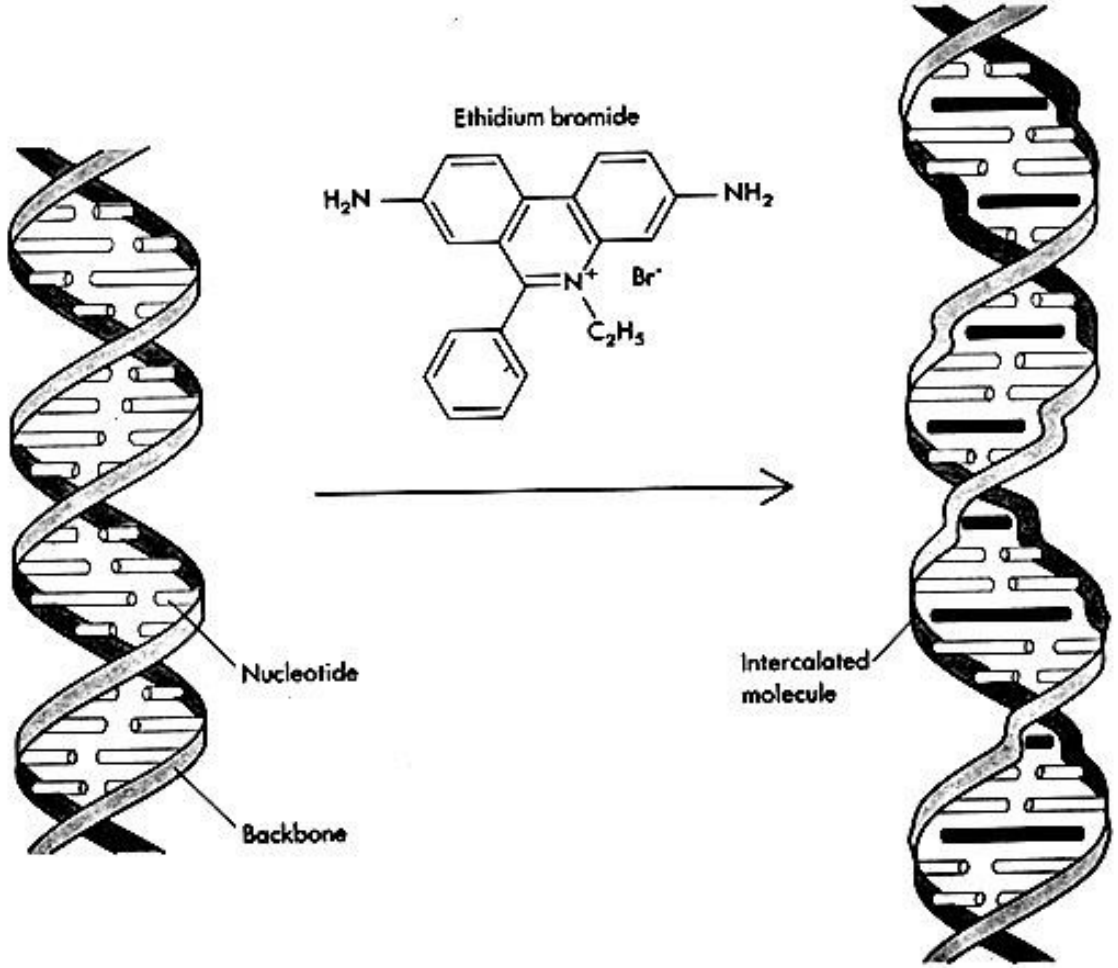
- intercalators: planar ligands intercalating between base pairs in duplex DNA
- stacking interaction





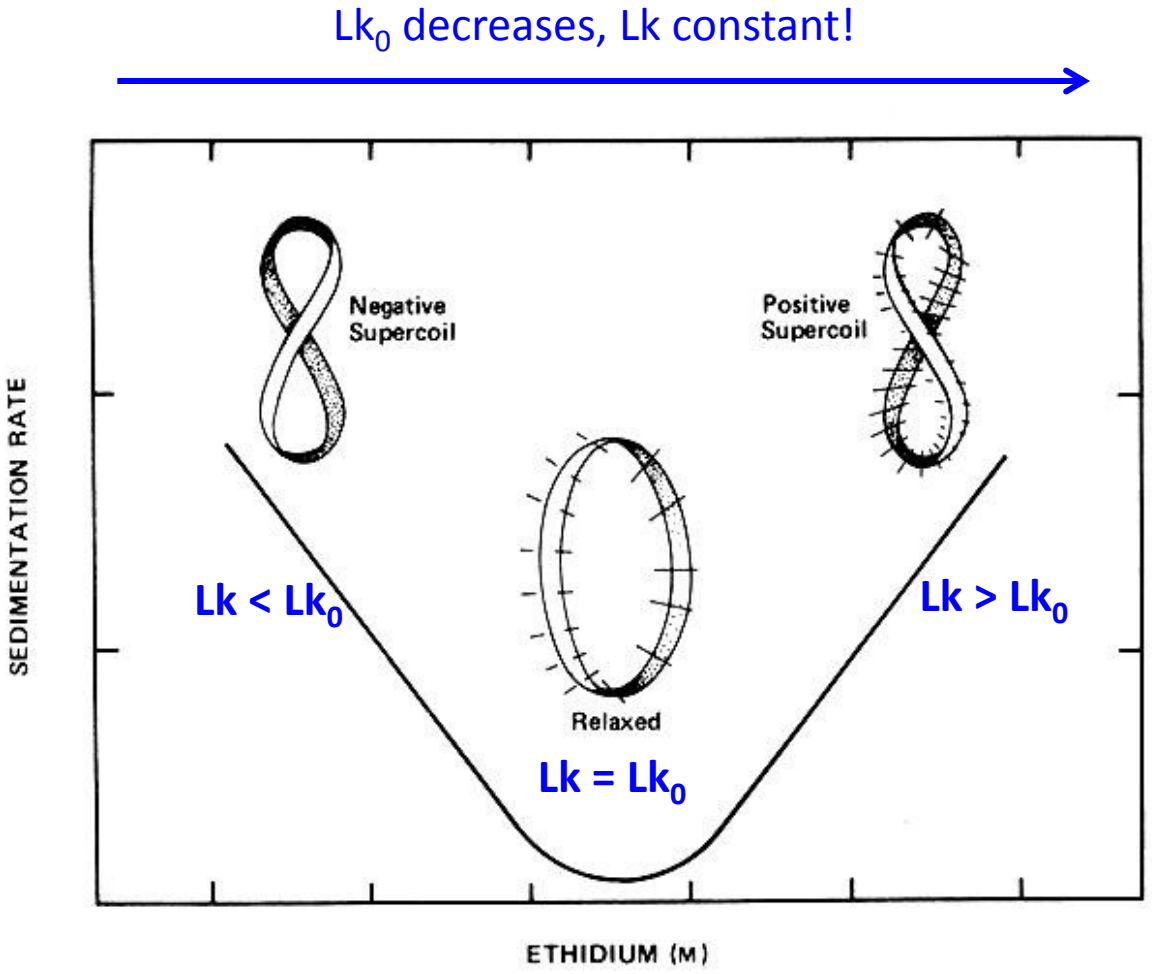
Superhelicity and intercalation

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



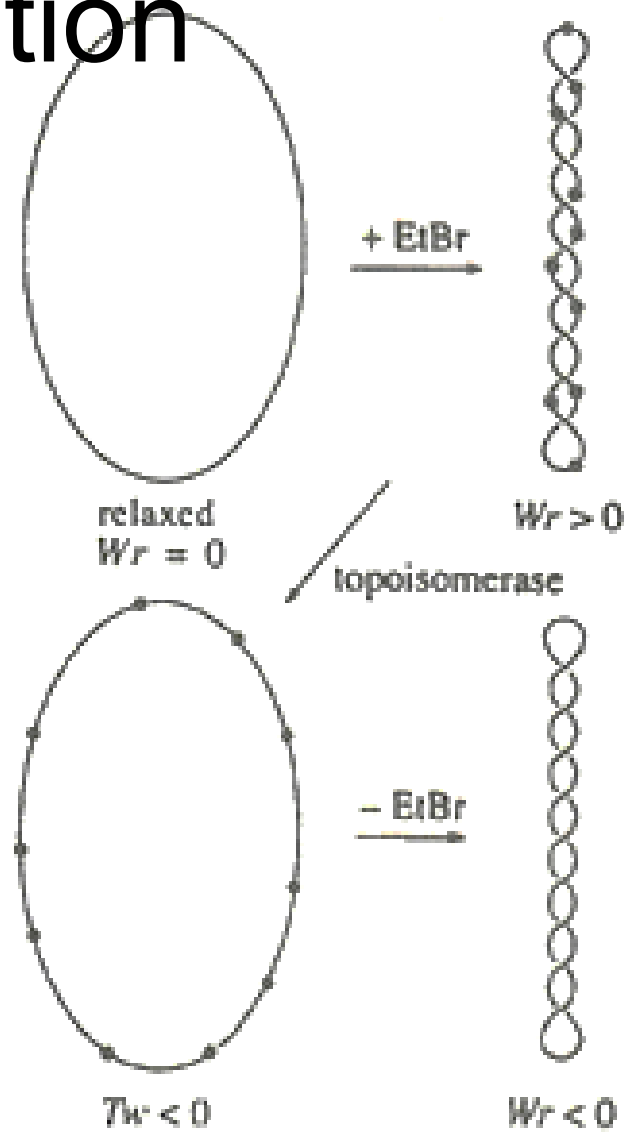
Superhelicity and intercalation

- 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

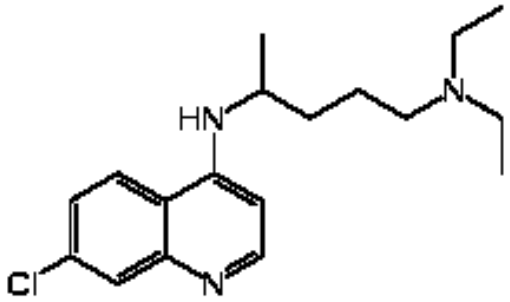


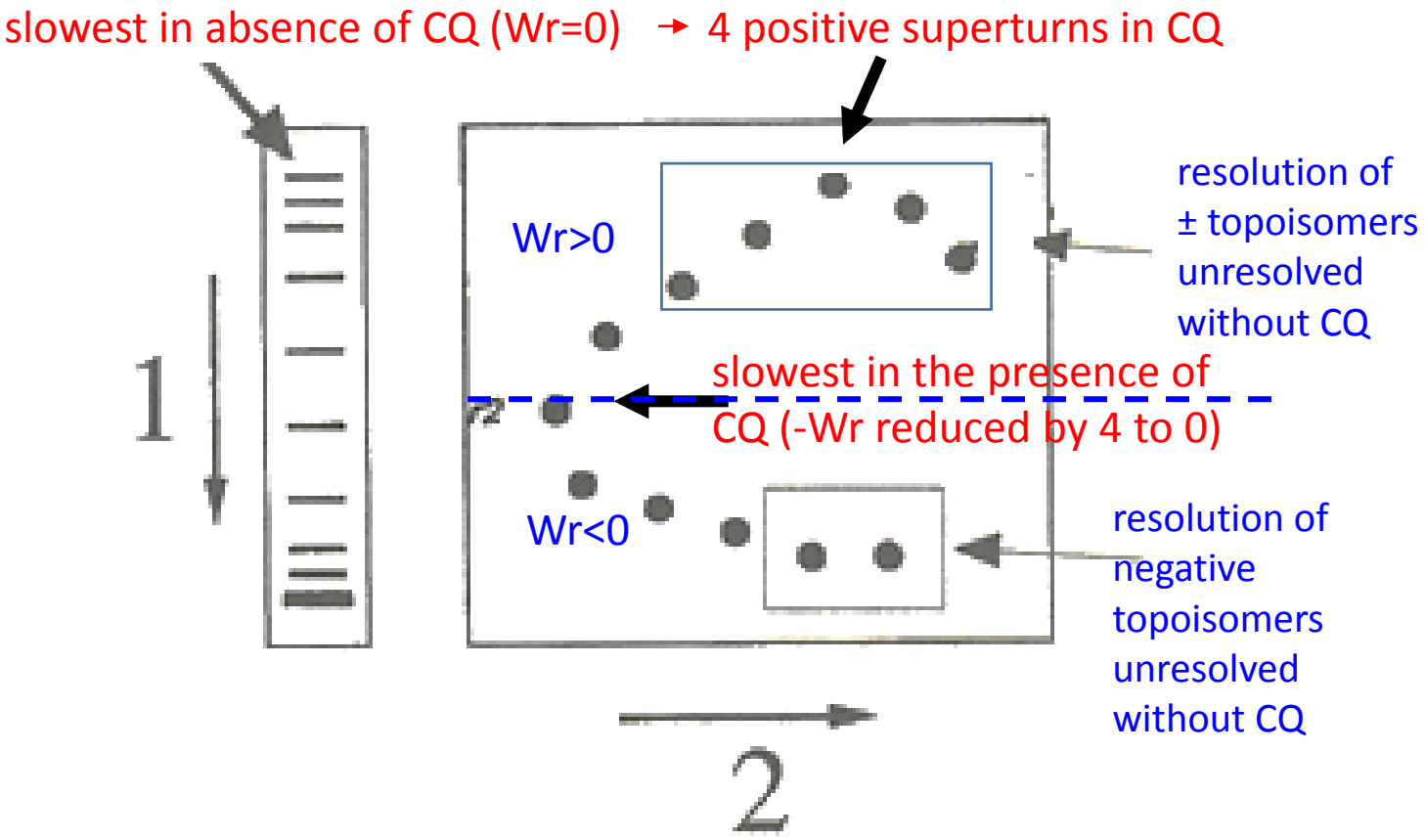
2D electrophoresis of topoisomers and detection of structural transitions

- 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 Wr (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

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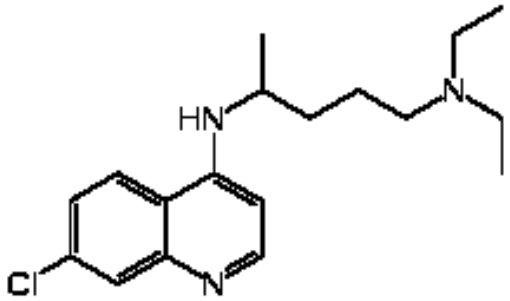
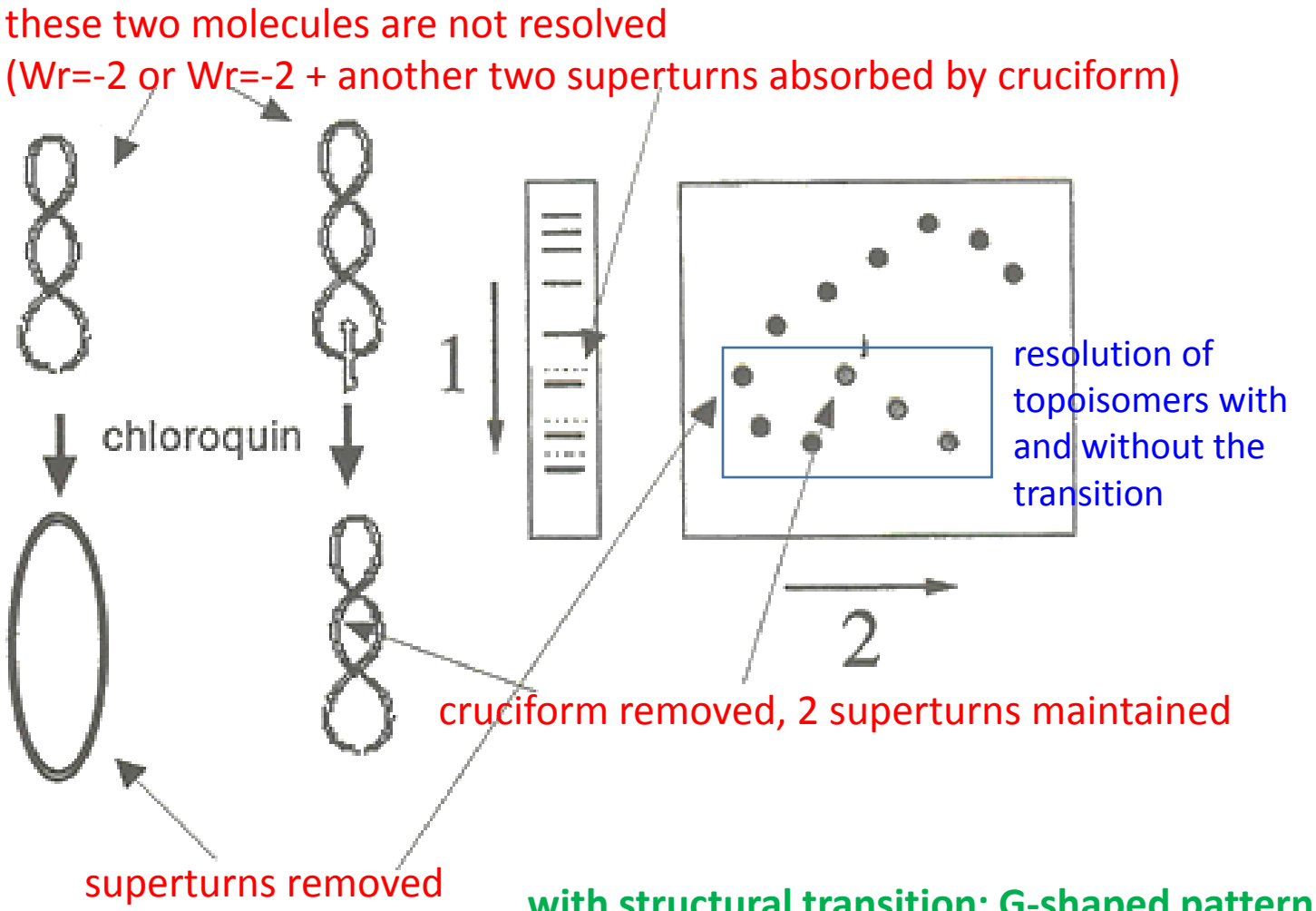
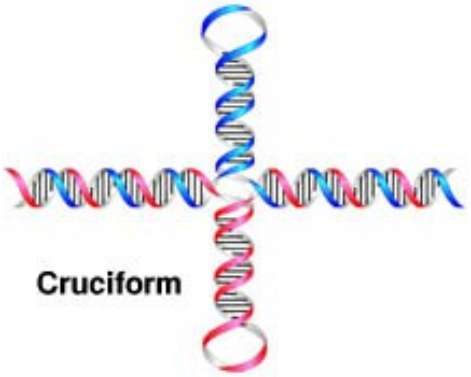
- then the gel is soaked with chloroquine (CQ) to remove certain number of superturns (e.g., 4) and second dimension is run

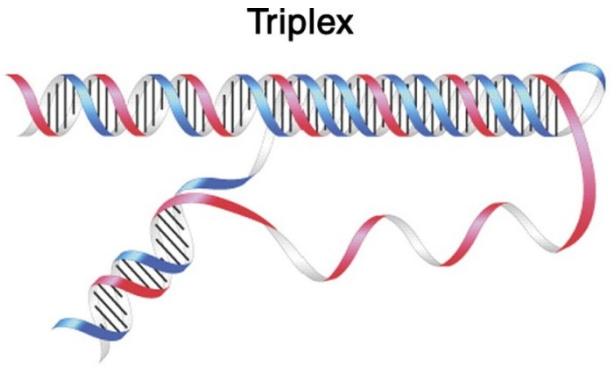




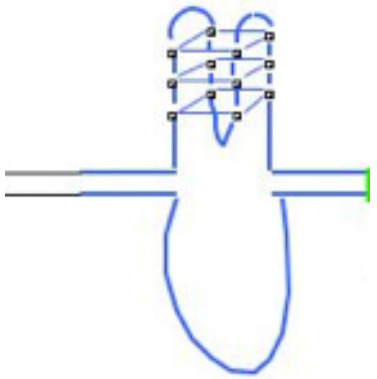
Chemical probing of non-B structures (to recall)

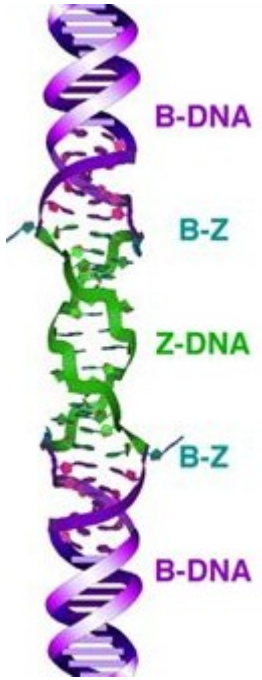
- the open local structures contain unpaired bases, unstacked base pairs or otherwise distorted sites





Quadruplexes



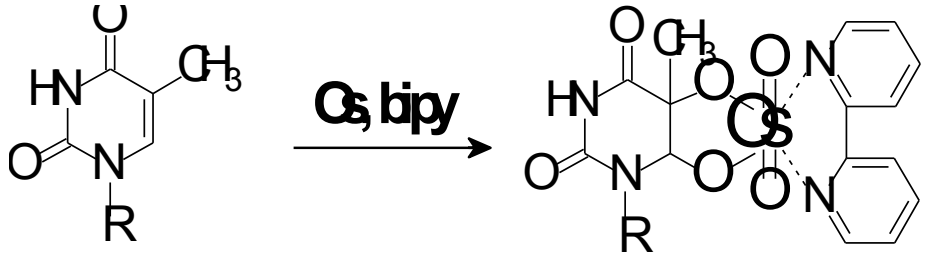


Left-handed (Z-form) duplex

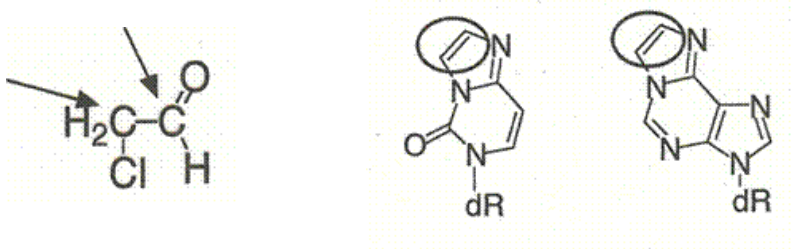
- loops, junctions...
- increased chemical reactivity of the nucleobases

Chemicals selectively reacting with unpaired bases:

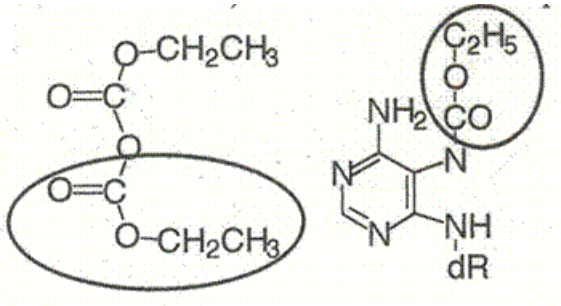
osmium tetroxide complexes
(Os,L)
(T, more slowly C)



chloroacetaldehyde
(CAA)
(A, C)



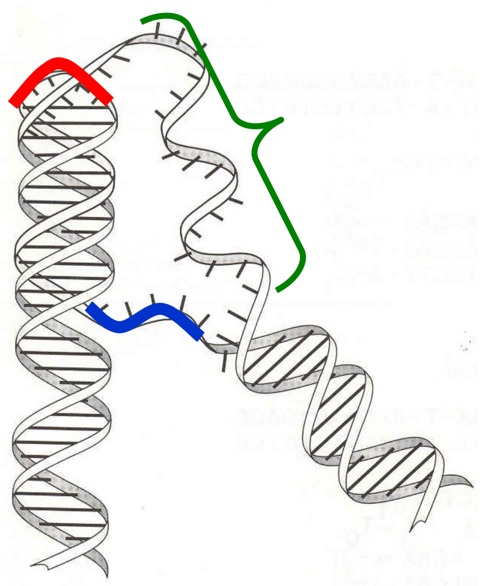
diethyl pyrocarbonate
(DEPC)
(A, G)



Using the Maxam-Gilbert technique, it is possible to determine with a high preciseness which nucleotides are forming the local structure

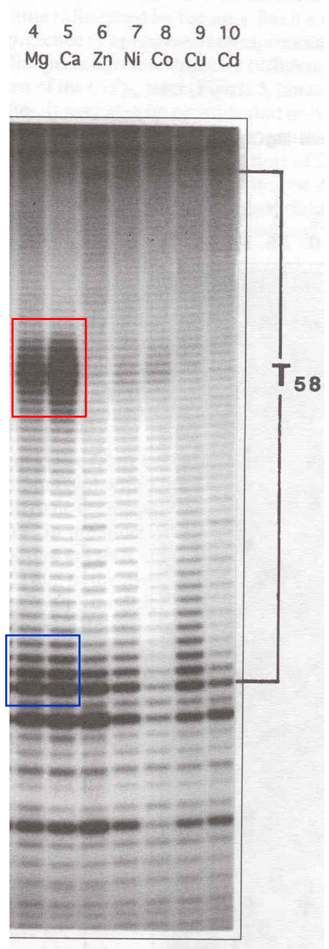
- modification of supercoiled DNA
- restriction cleavage, radiactive labeling
- hot piperidine
- sequencing PAGE

the structure can be deduced from the modification pattern



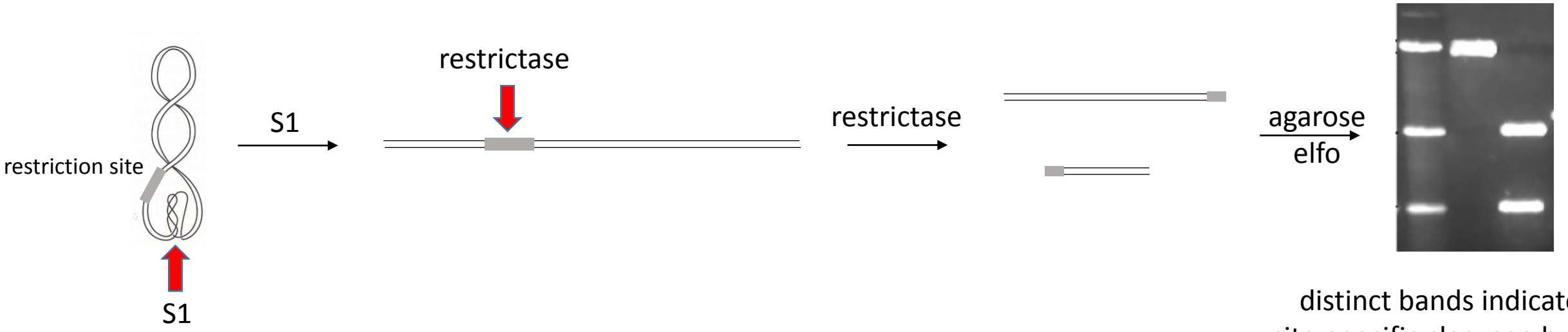
TTTTTTTTTTTTTTTTTT**TTTTTTT**TTTTTTTTTTTTT**TTTT**

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA



Single-strand selective enzymes

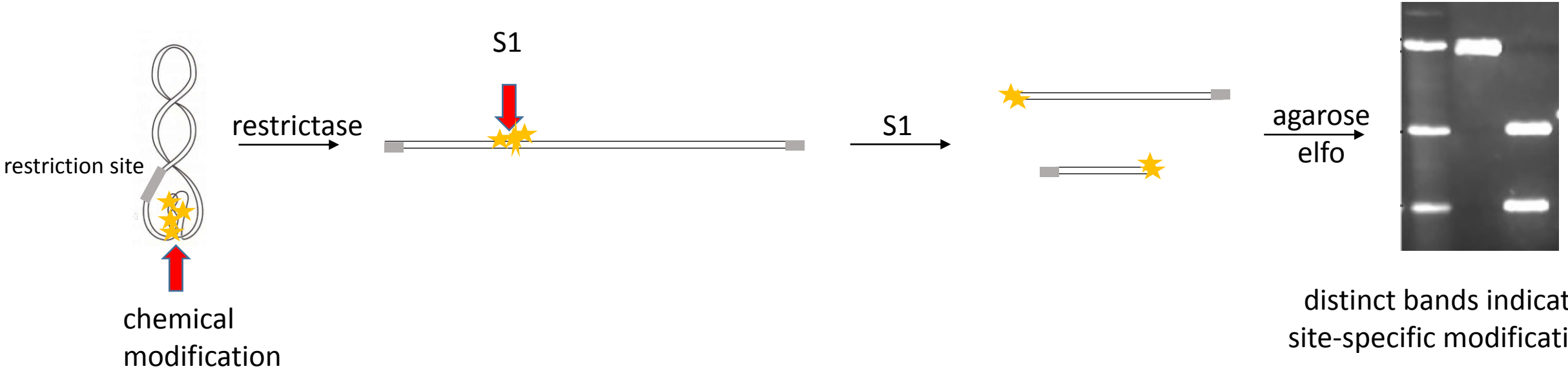
- only detection of a open structure, not identification at the sequence level
- often sufficient: evidence of formation of a expected structure
- nucleases S1, P1, mung bean... cleave ss DNA (or RNA)
- scDNA cleaved by S1, then restriction cleavage to map S1 celavage site



distinct bands indicate
site-specific cleavage by S1

Combination of chemical probes with S1 nuclease

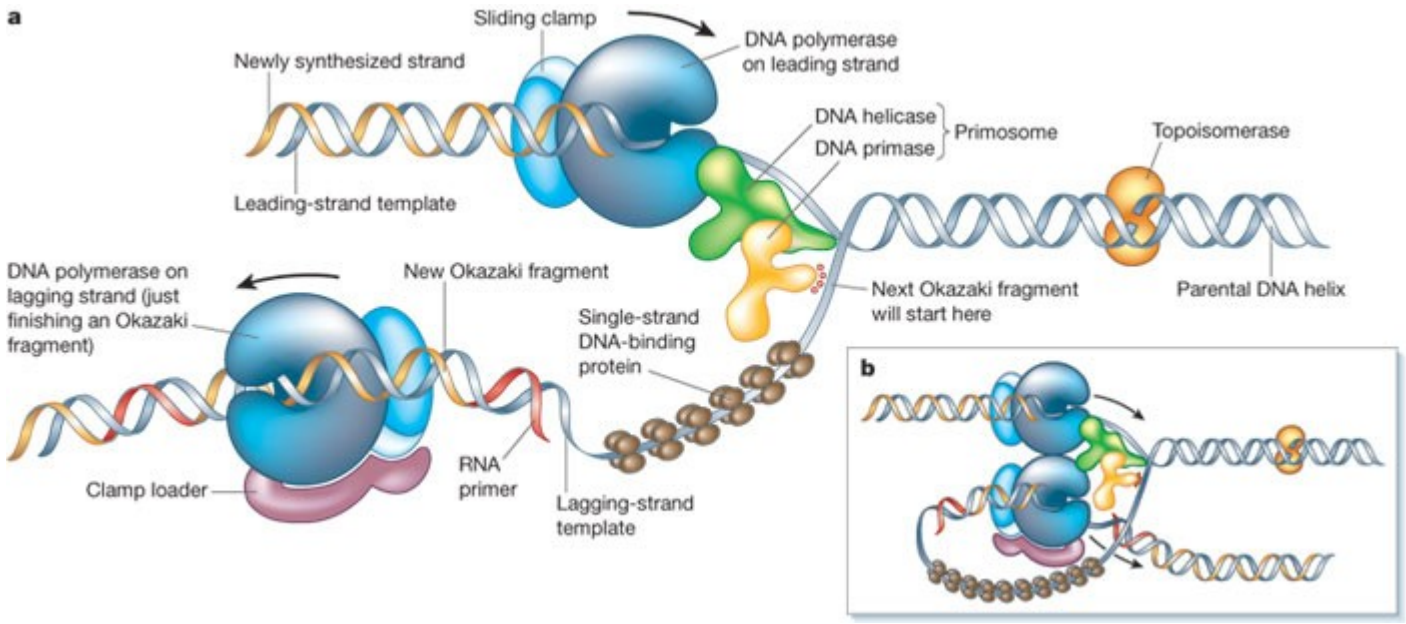
- chemical probes work within wider range of conditions than enzymes
- modification of scDNA
- then restrictase cleavage
- chemical modification of bases in structure that existed in scDNA prevent formation of B-DNA
- then S1 cleavage in the modified site



Topoisomerases

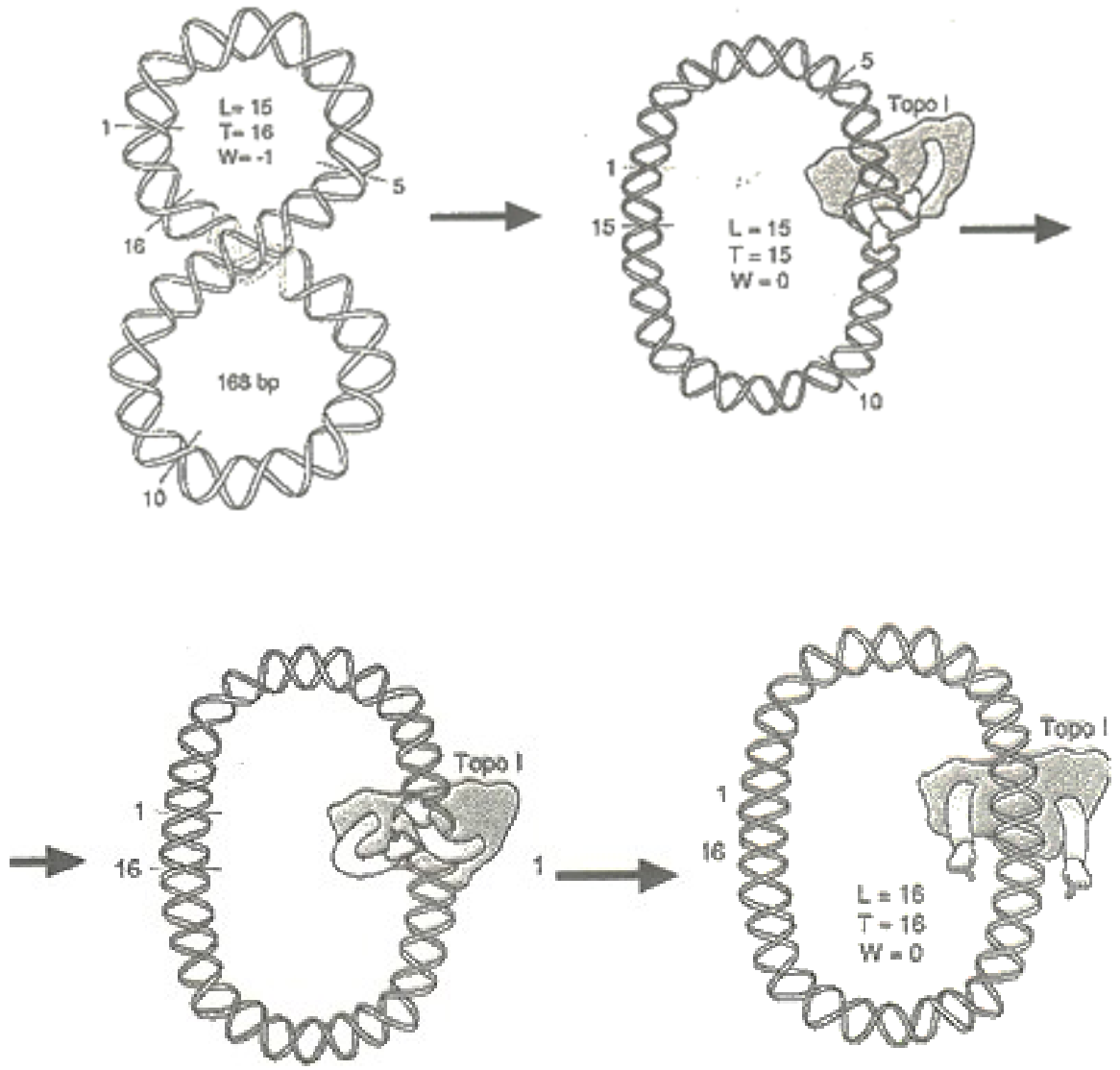
- enzymes relaxing (or introducing) superhelical stress in DNA: changing Lk
- solving the „knotty problem“ in replication, transcription

(video)



Topoisomerase I

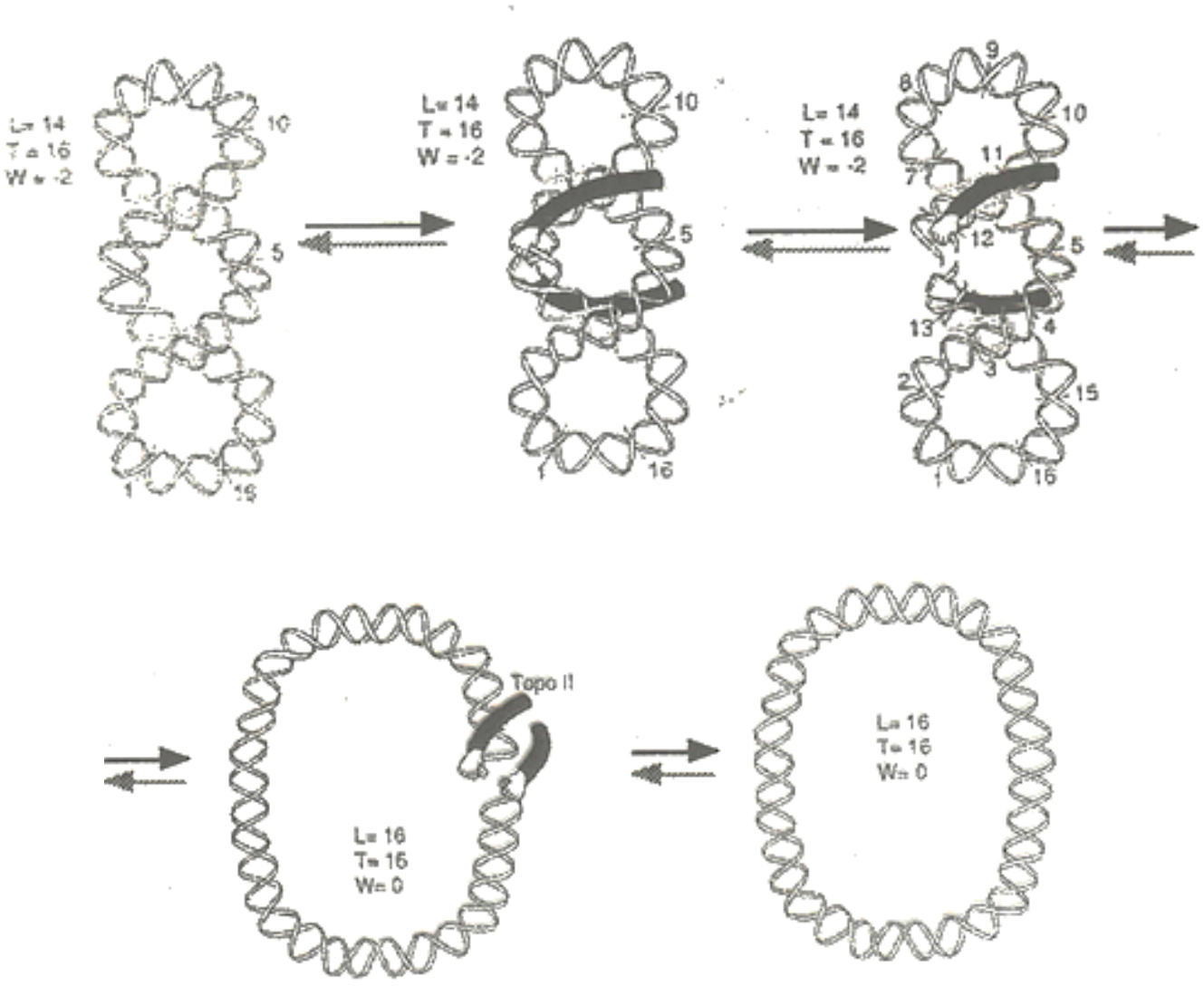
- creating and sealing a single-strand break
- only relaxation
- no ATP needed: transesterification, covalent binding of the enzyme to DNA (phosphoester of a Tyr residue)
- relax either only negative superturns (*E. coli* topo I), or both positive and negative (topo I from wheat germ)



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

Topoisomerase II

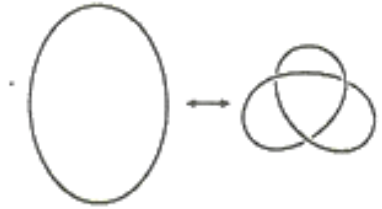
- creating and sealing a double-strand break
- relaxing or introducing superhelicity (DNA gyrase)
- ATP consumption (conformational changes of the protein)



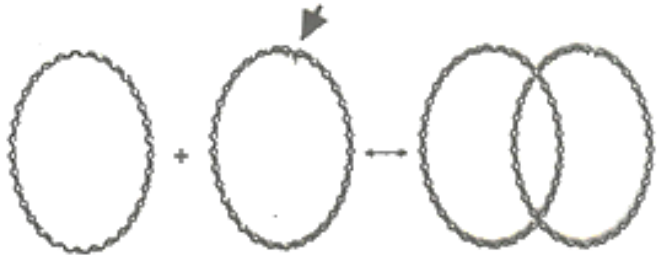
Lk changed by 2 (double helix threaded through a dsb)

Other processes catalyezd by topoisomerases

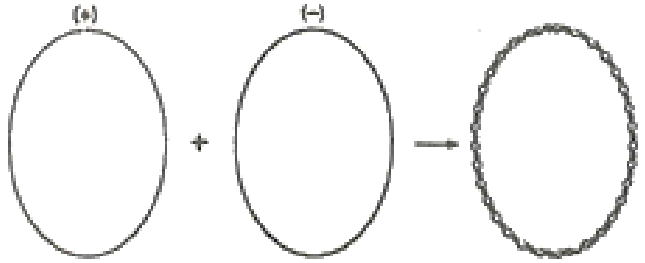
Topoisomerase I:



knotting/unknotting of ss circles

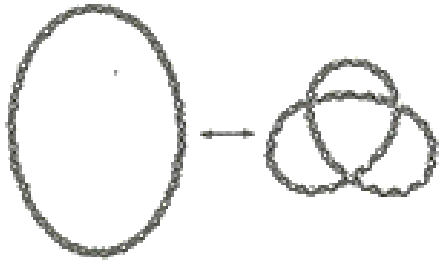


catenation/decatenation
of nicked circles

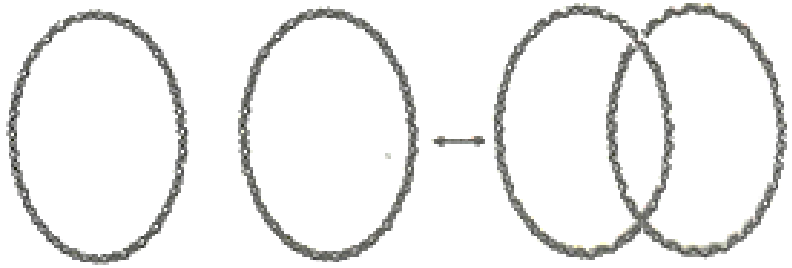


circular duplex formation of two complementary ss circles
(=relaxation of negatively scDNA!)

Topoisomerase II:



knotting/unknotting of duplex circles



catenation/decatenation of duplex circles

Importance of decatenation activity in replication