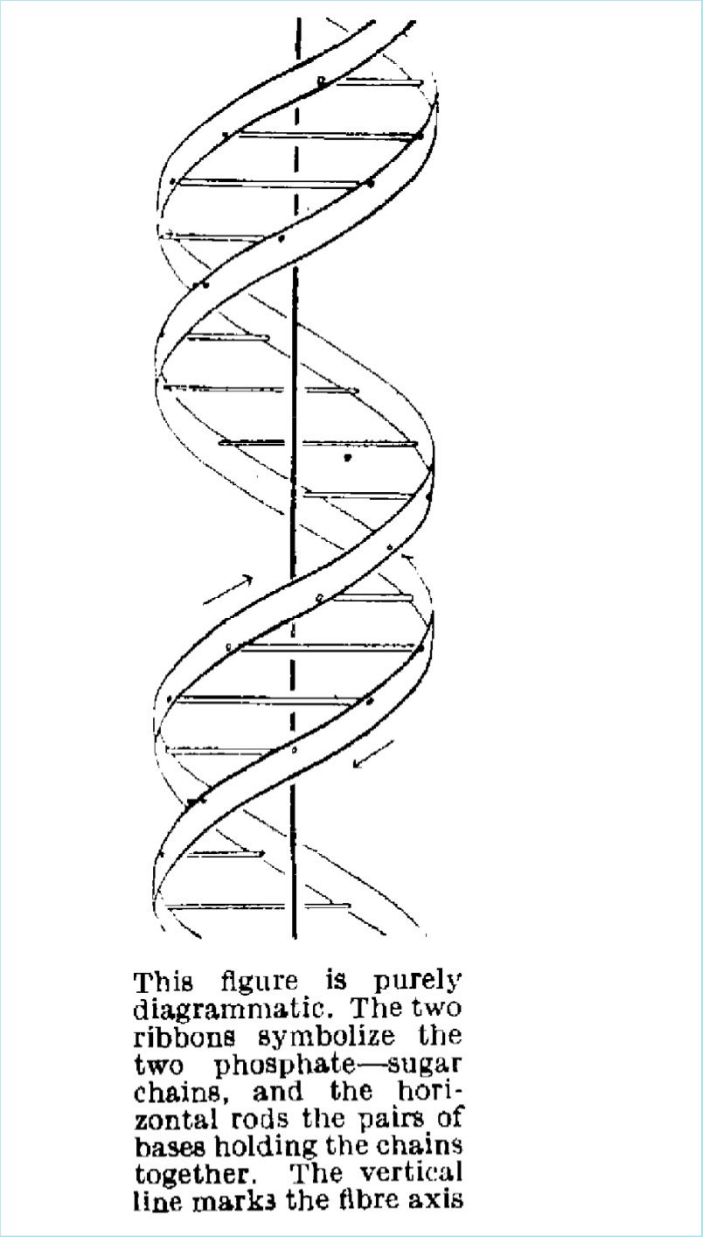
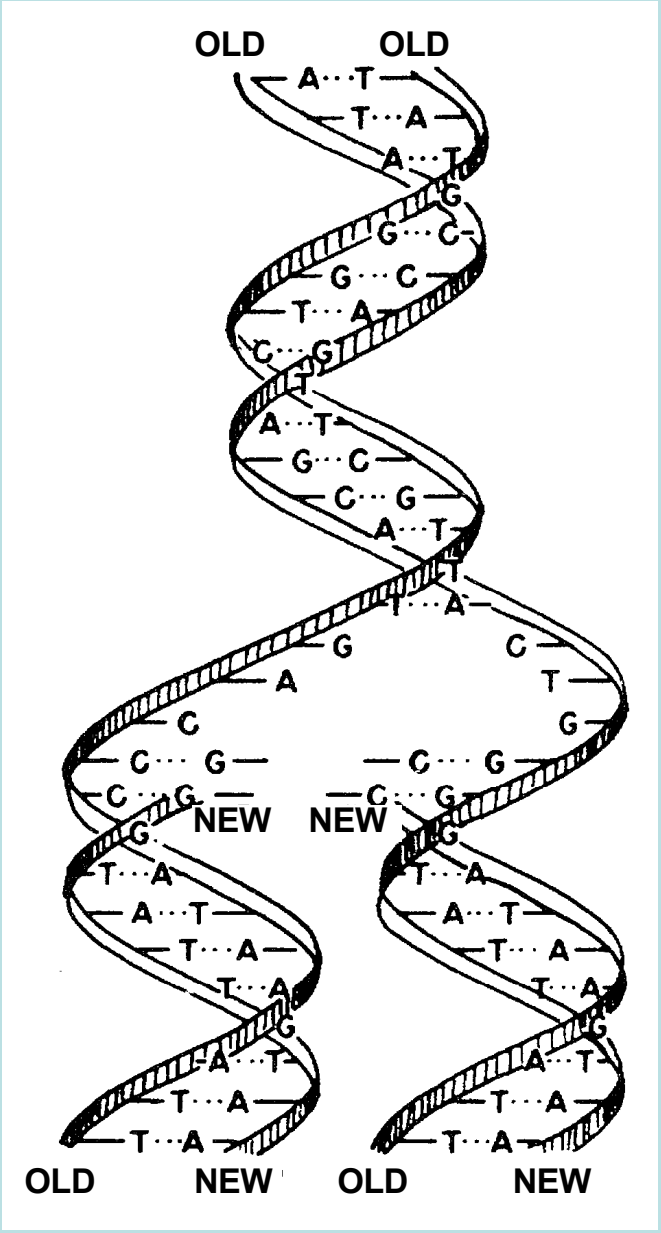
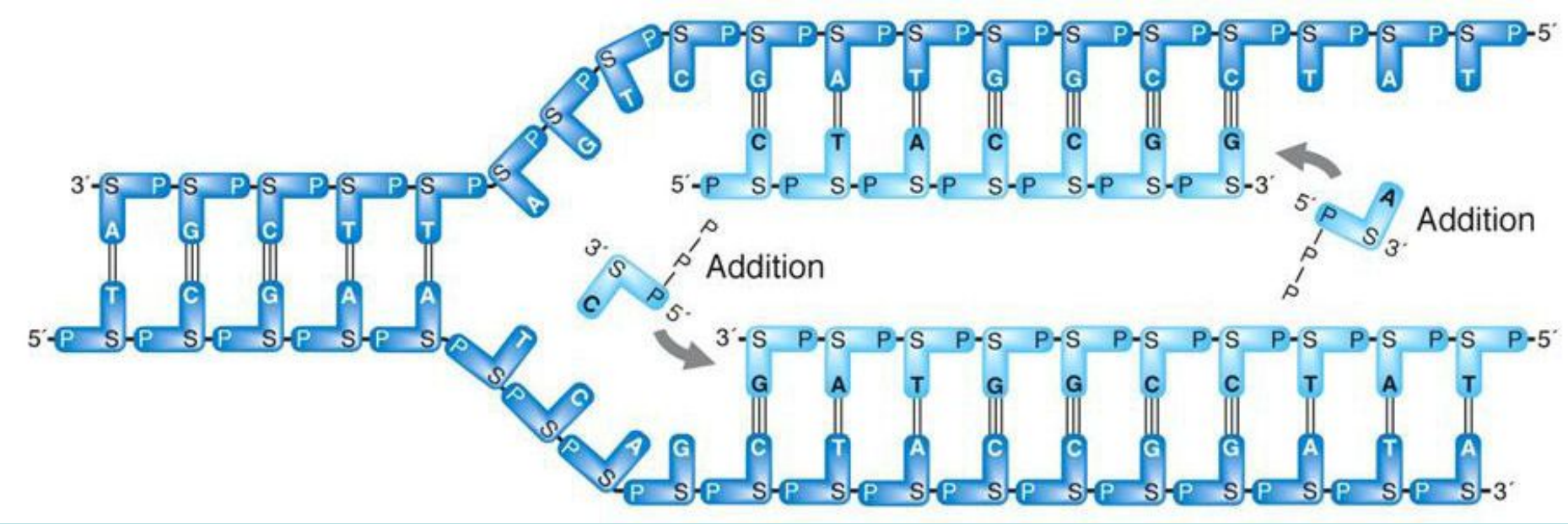
"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

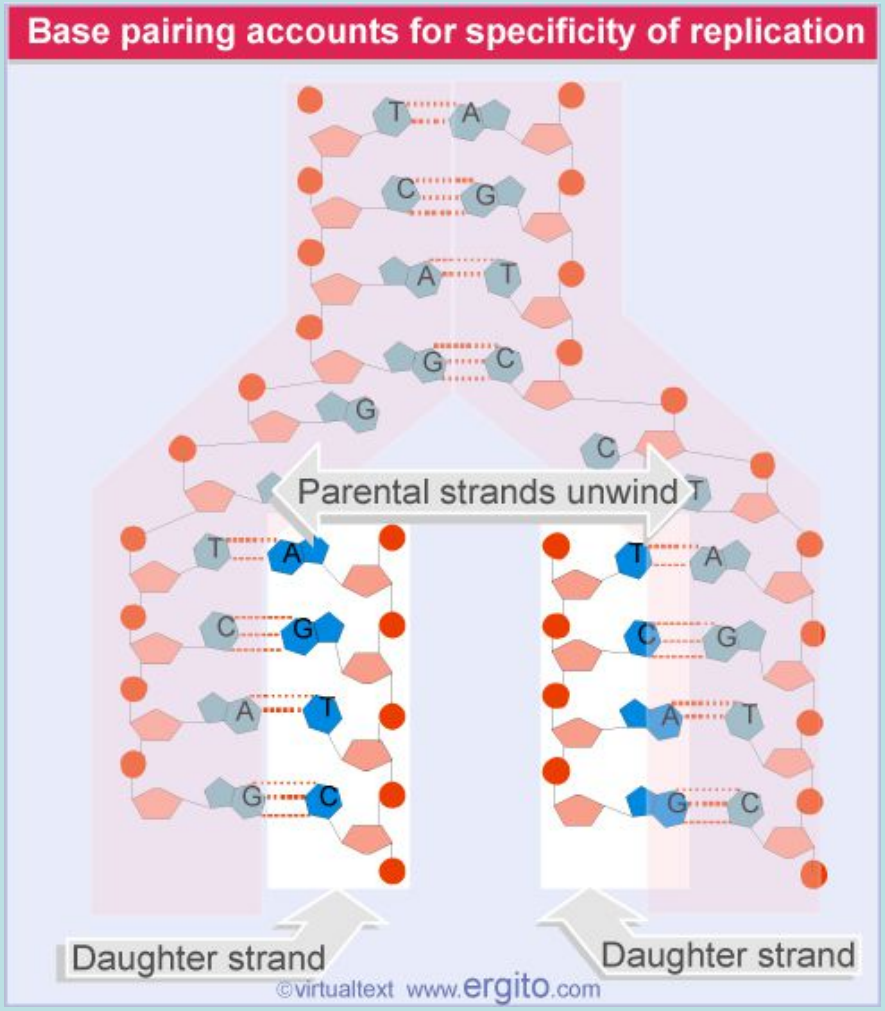
Watson and Crick double helix model suggests a replication mechanism



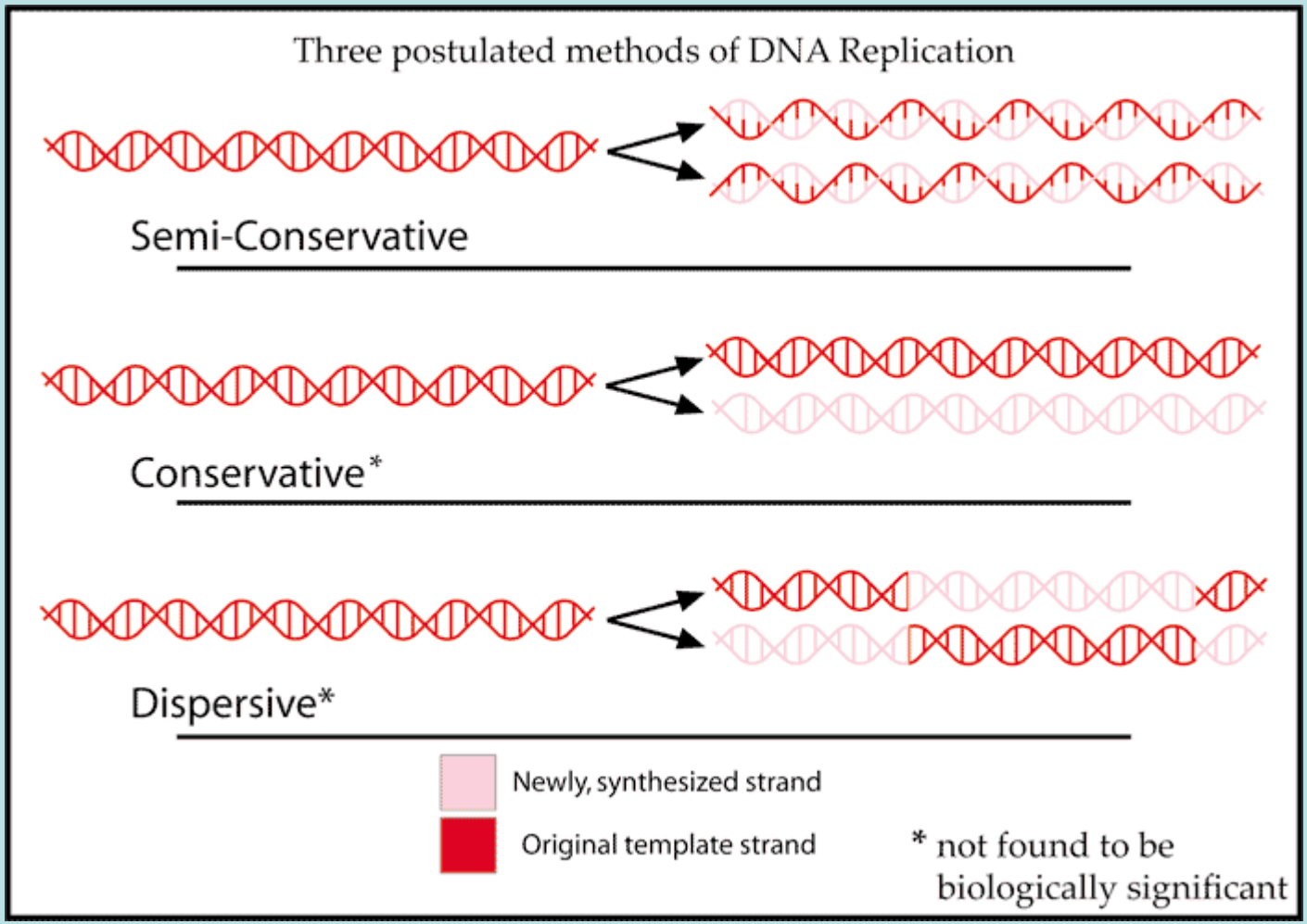
This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis



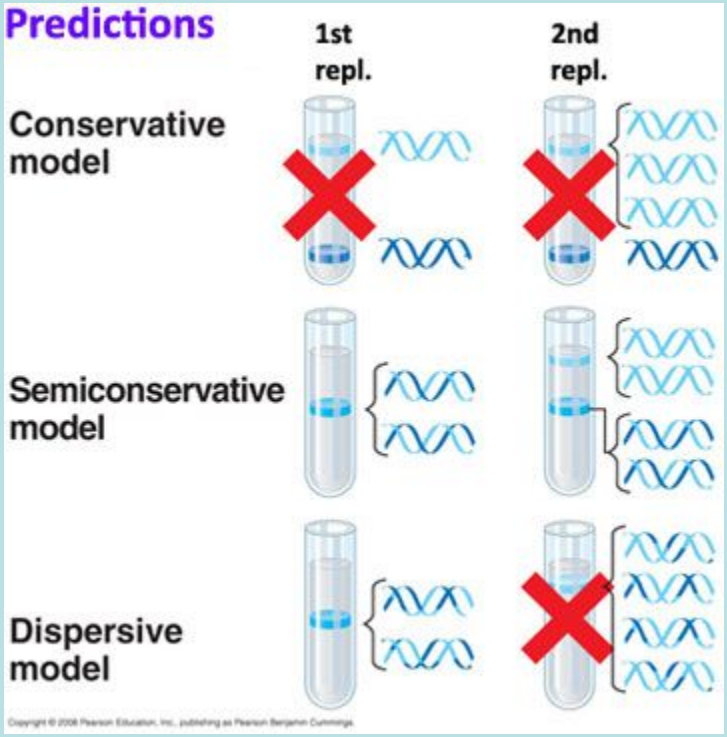

Addition
Addition

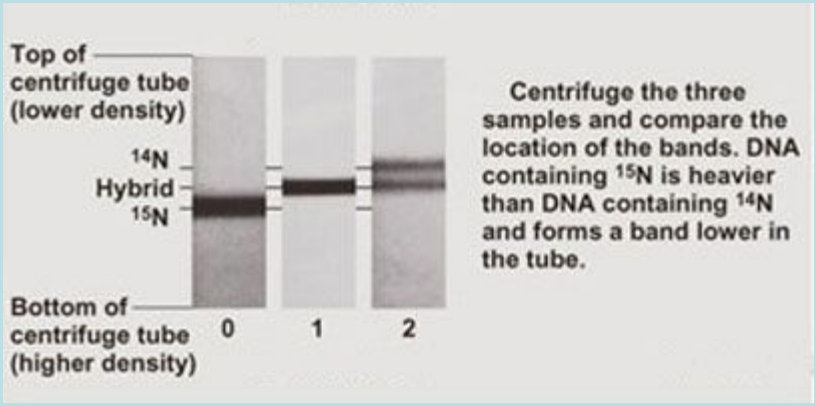


Replication fork moves down the DNA


Three postulated methods of DNA Replication
Semi-Conservative
Conservative*
Dispersive*
Newly, synthesized strand
Original template strand
* not found to be biologically significant

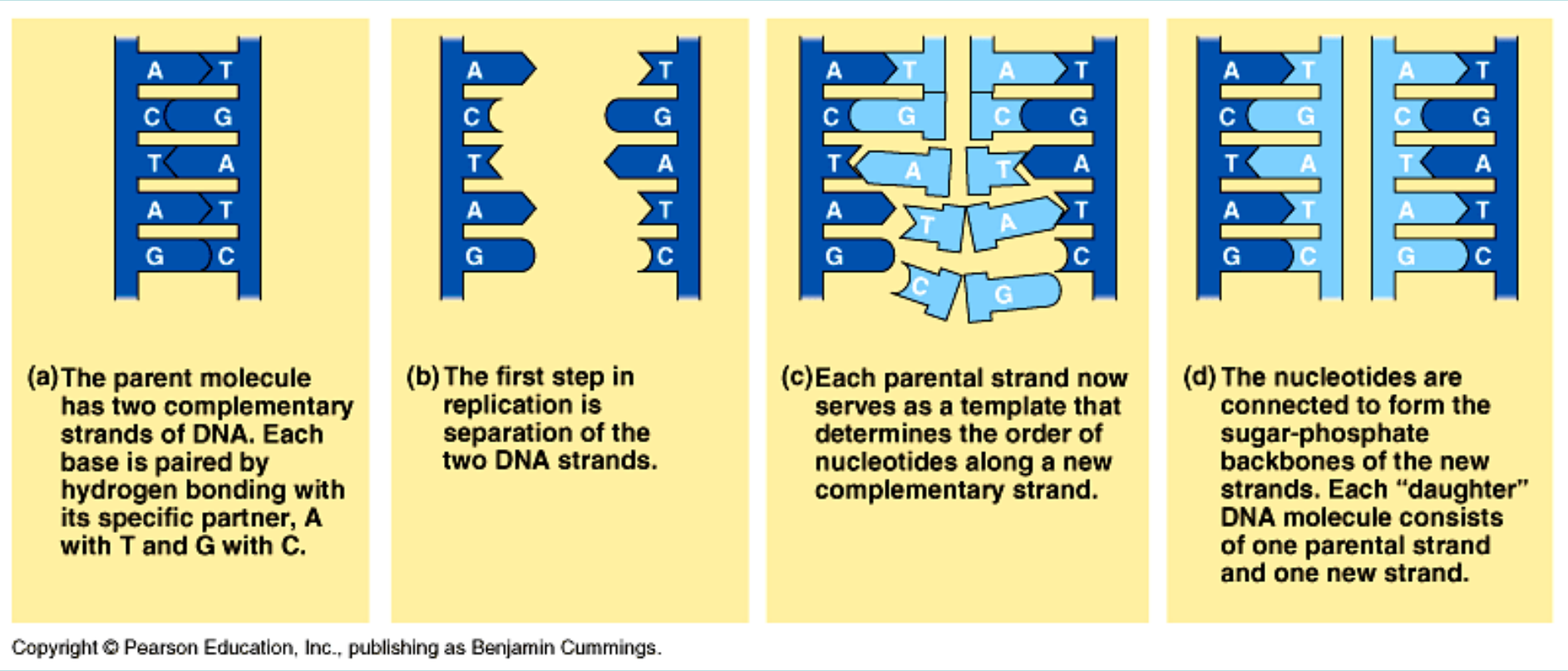
Meselson-Stahl experiment (1958)





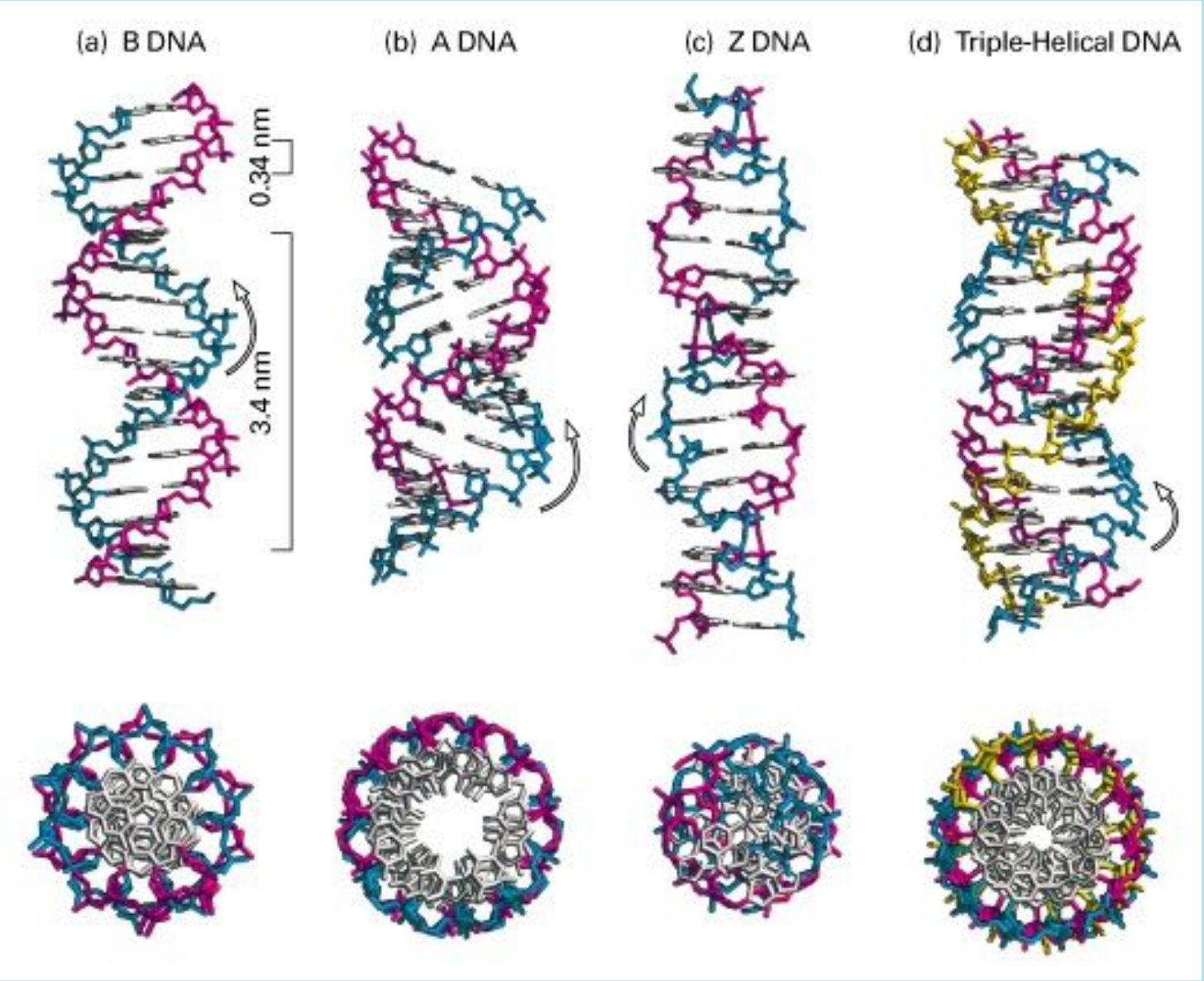
http://highered.mcgraw-hill.com/olc/dl/120076/bio22.swf

A model for DNA replication: the basic concept



DNA alternative structures

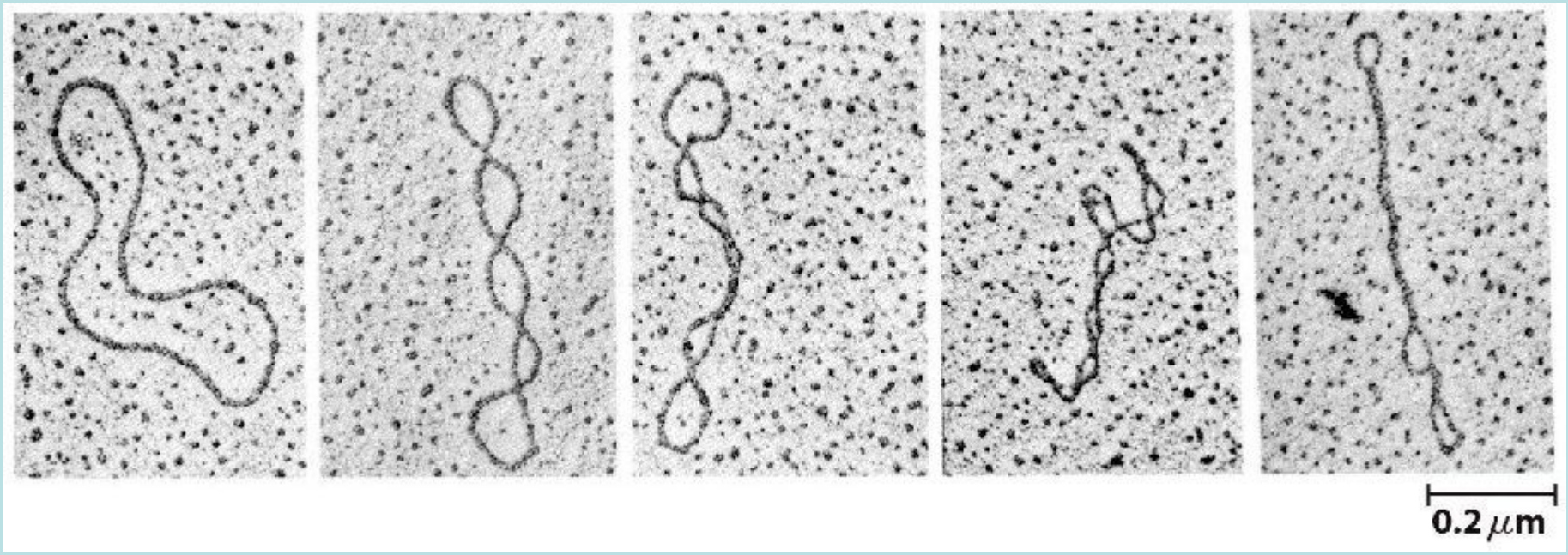
Nucleic acids can form different double helices



Nucleic acids can form different double helix types; three of them are naturally occurring in biological systems

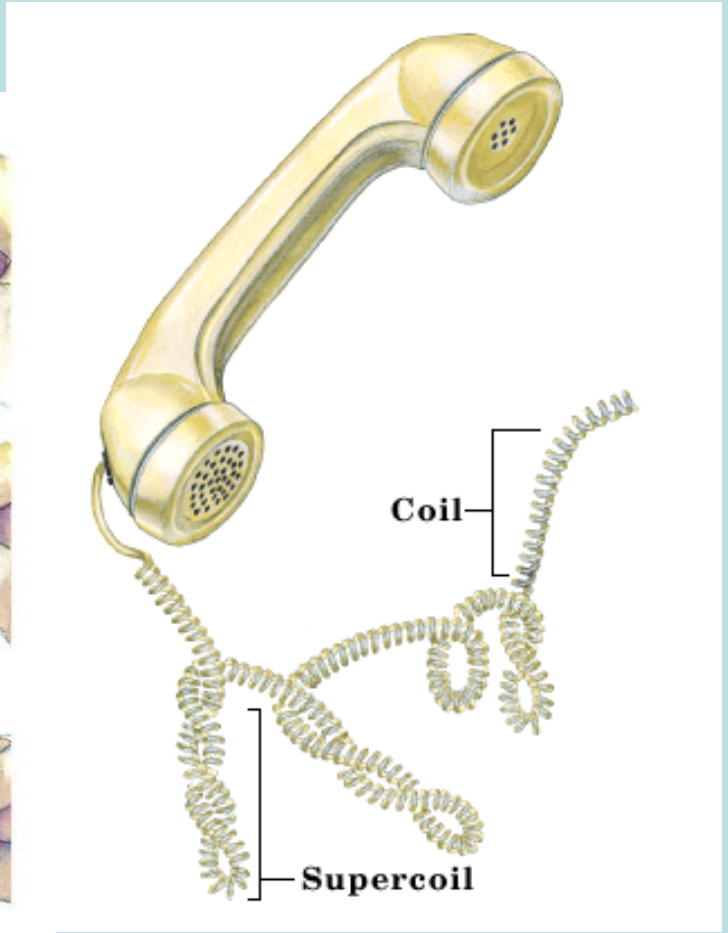
Helix	Base pairs in Helix turn	Rotation between two adiacent bases	Diameter
B	10 (10,4)	36 (34,6)	20 (19)
A	11	32,7	23
Z	12	-30	18

DNA Topology



Relaxed circular DNA

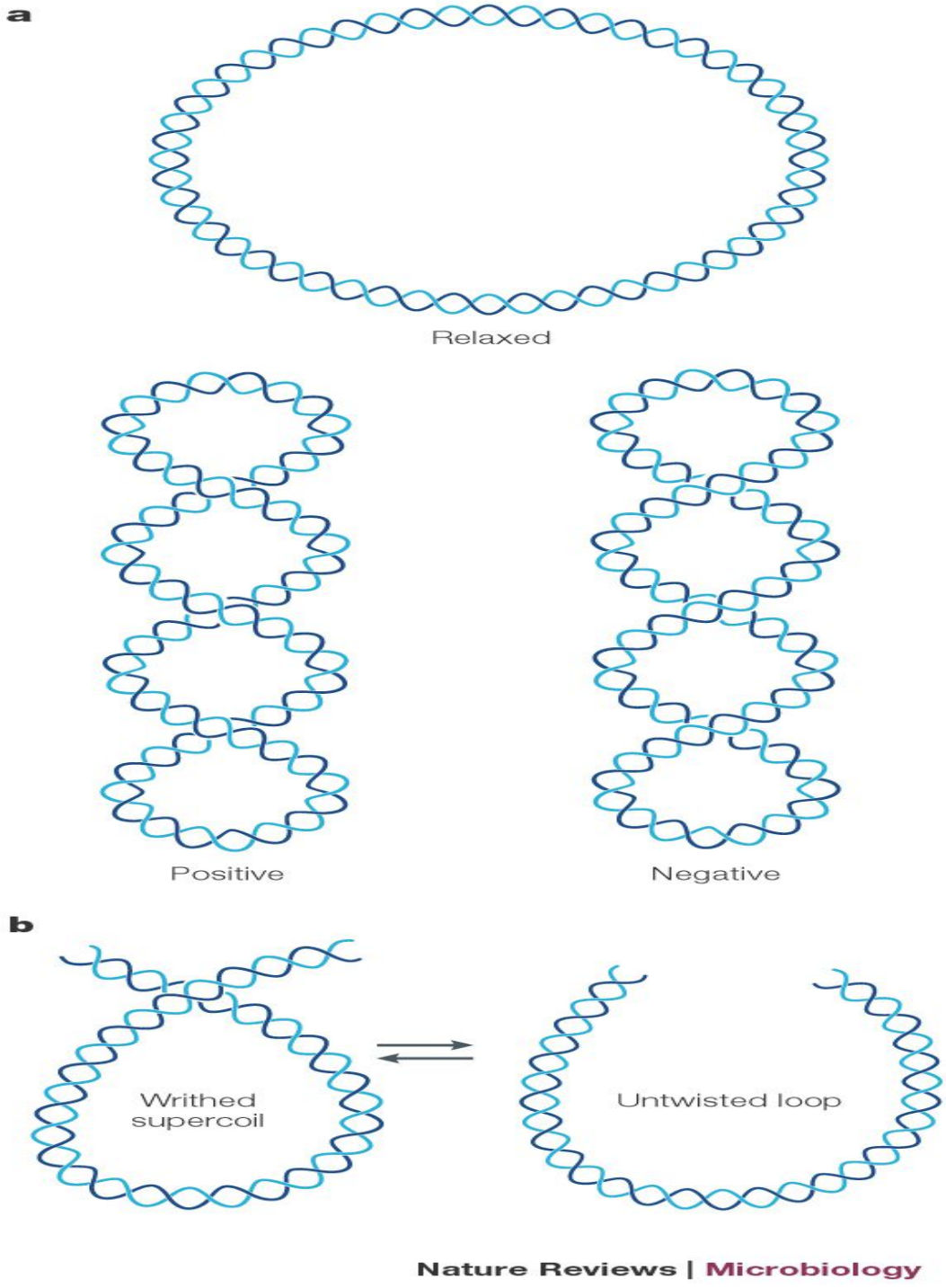
Supercoiled DNA

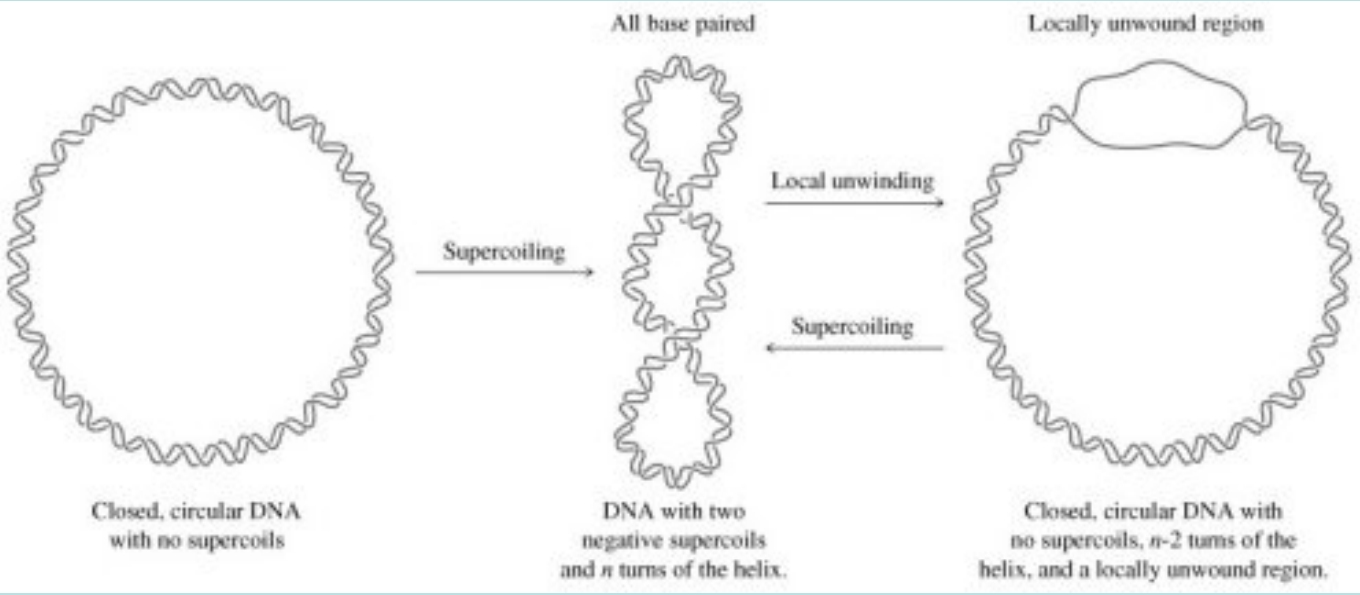

Coil
Supercoil

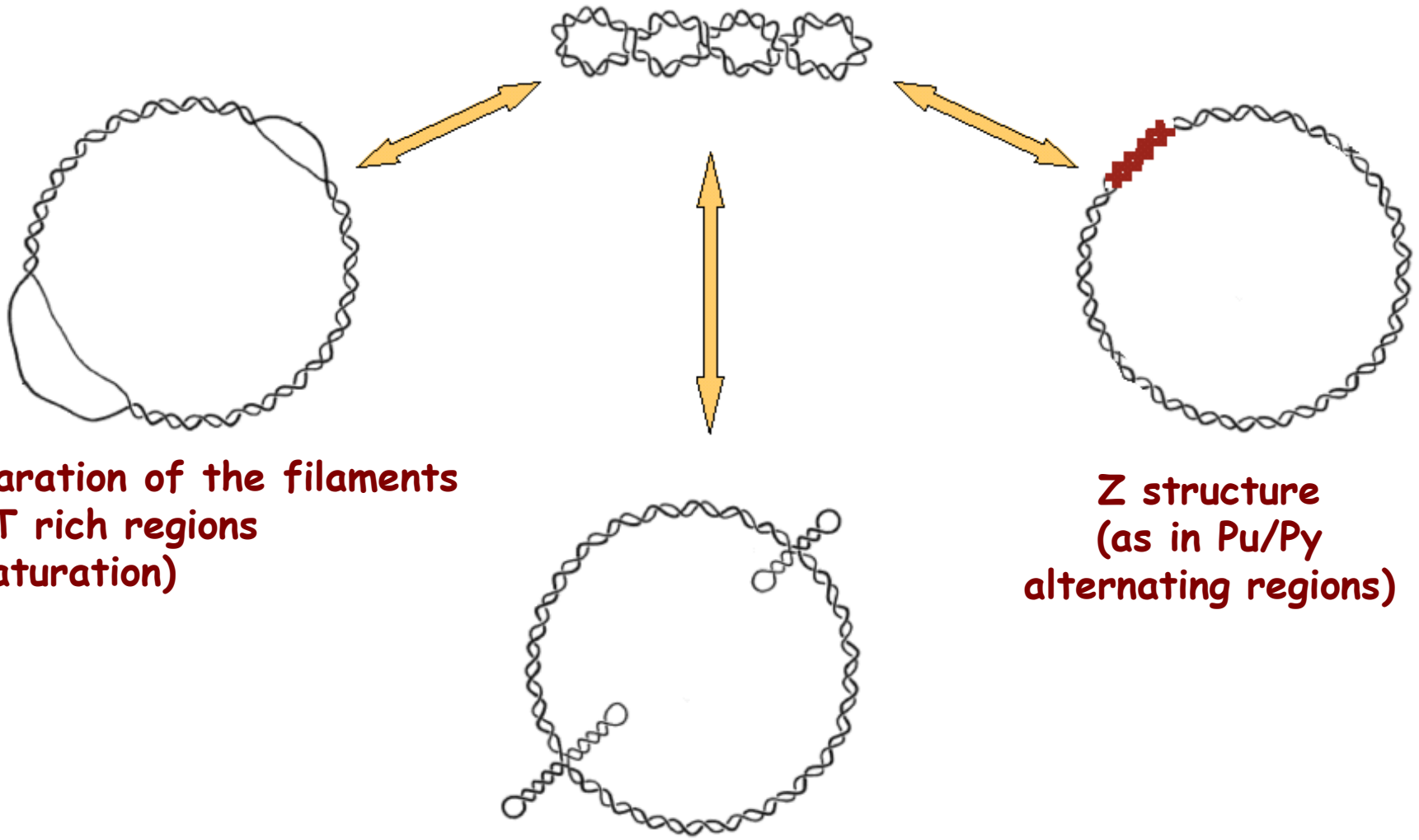
DNA supercoil

Positive= DNA is overwound

Negative= DNA is underwound


a
Relaxed
Positive
Negative
b
Writhed
supercoil
Untwisted loop
Nature Reviews | Microbiology


All base paired
Locally unwound region
Local unwinding
Supercoiling
Supercoiling
Closed, circular DNA with no supercoils
DNA with two negative supercoils and n turns of the helix.
Closed, circular DNA with no supercoils, n-2 turns of the helix, and a locally unwound region.



Separation of the filaments
(A+T rich regions
denaturation)

Z structure
(as in Pu/Py
alternating regions)

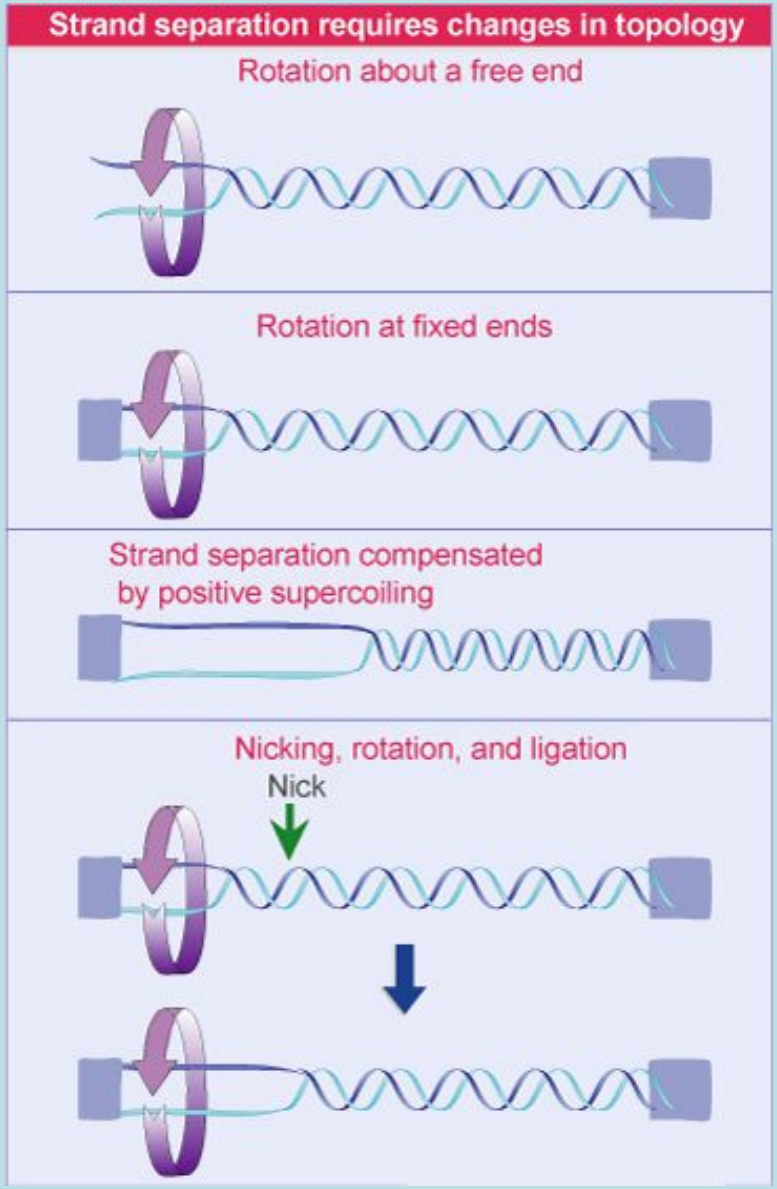
Cruciform structures
(as in palindromic sequences)

Structure is changed by nucleic acid synthesis

Strands must separate for replication

Transcription creates positive supercoiling

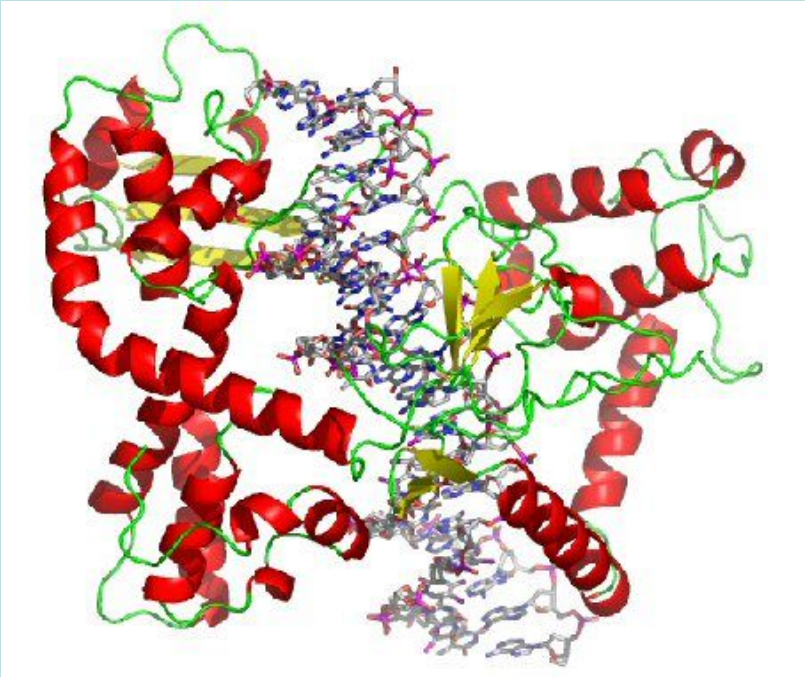
Replication produces catenated DNAs

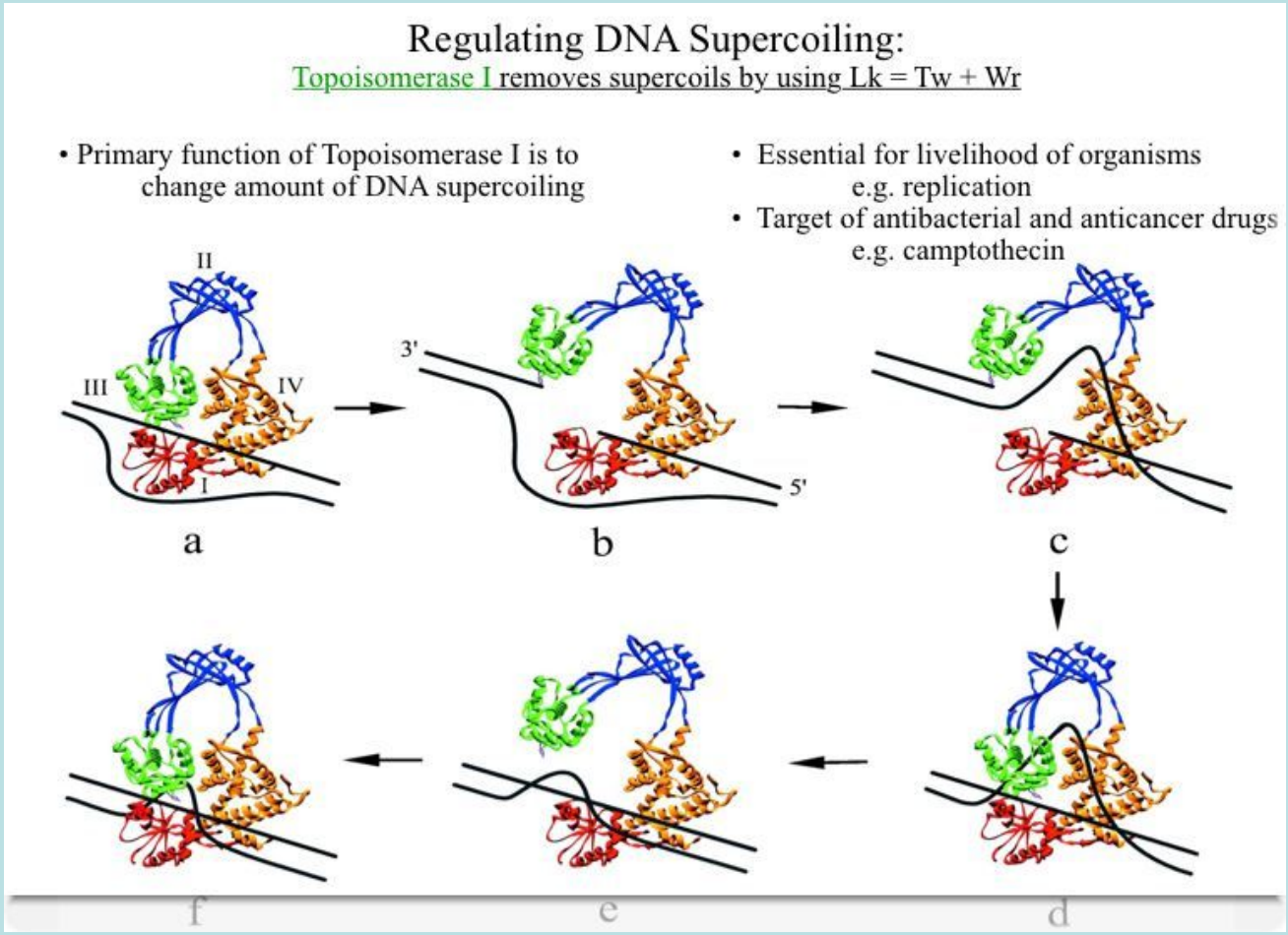

Strand separation requires changes in topology
Rotation about a free end
Rotation at fixed ends
Strand separation compensated
by positive supercoiling
Nicking, rotation, and ligation
Nick

Enzymes changing the DNA topology

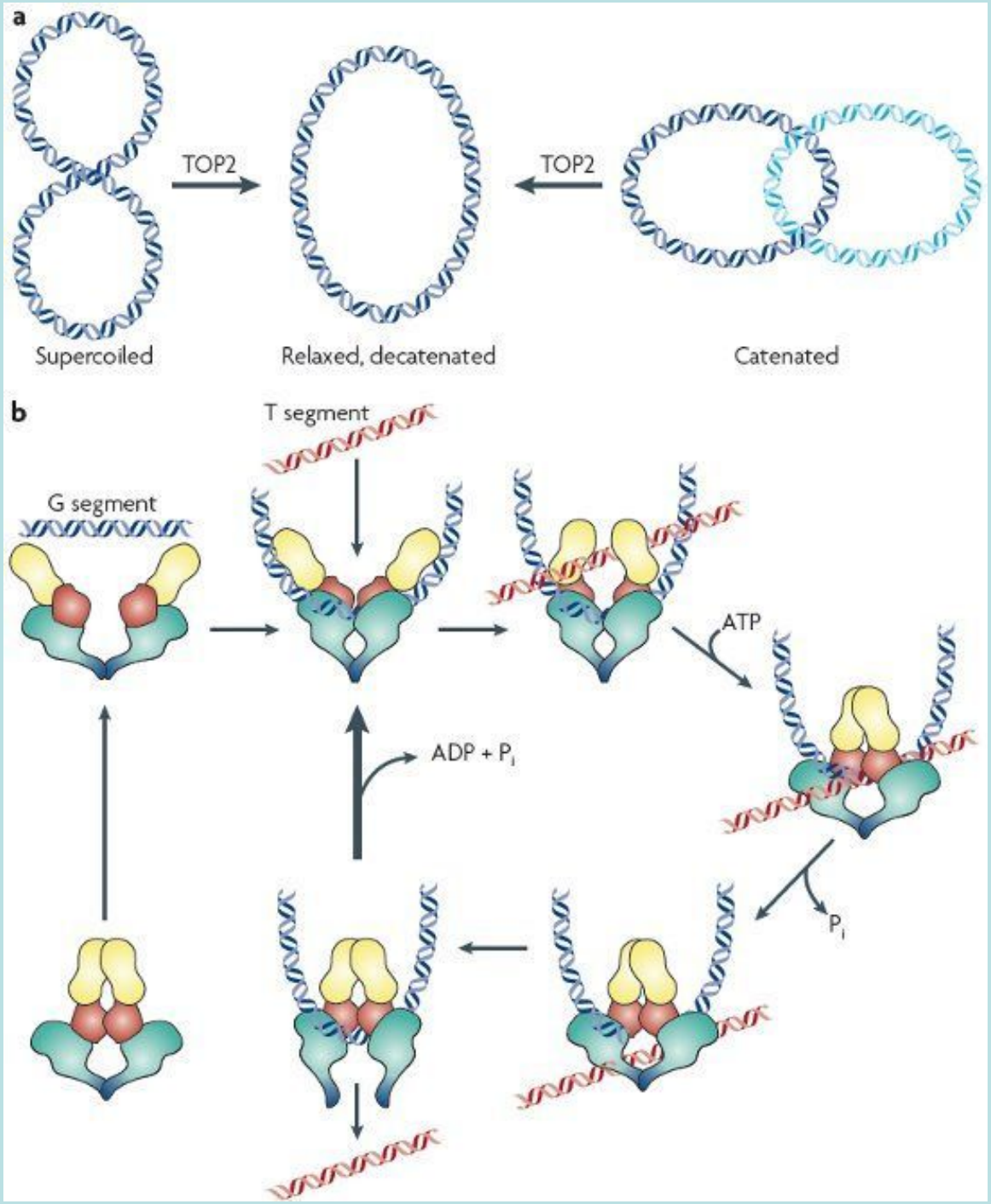
Topoisomerase: type I

typo II (girase)



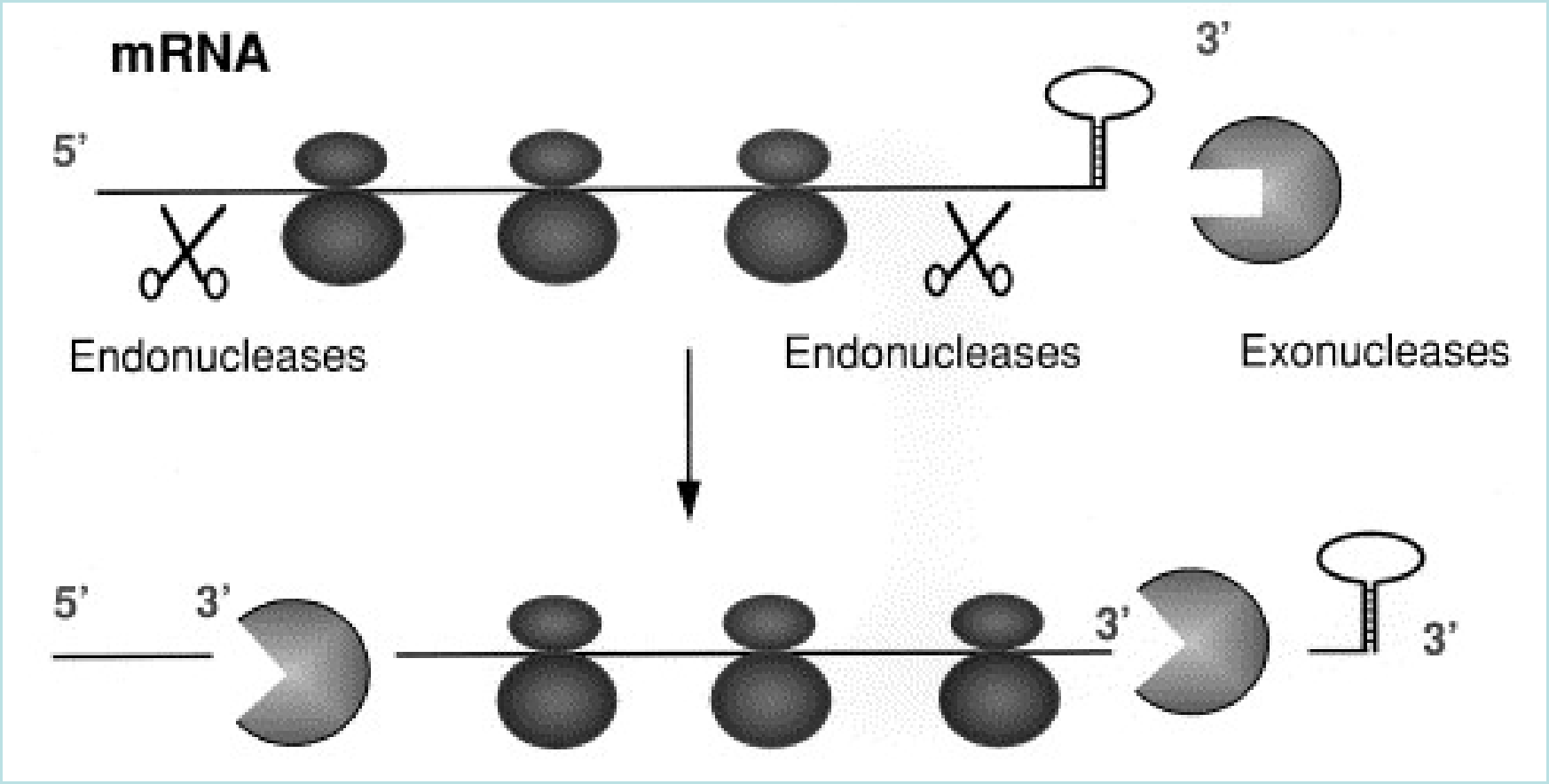


The proposed mechanism of topoisomerase I is:
- The enzyme binds to a locally unwound segment
- A 5' phosphate is transesterified to a tyrosine in the enzyme, breaking the strand
- The other strand is passed through the break
- Reverse transesterification seals the break


a
TOP2
TOP2
Supercoiled
Relaxed, decatenated
Catenated
b
T segment
G segment
ATP
ADP + P$_i$
P$_i$

Enzymes acting on nucleic acids

- Polimerases: DNA polymerase
  RNA polymerase
- Nucleases: esonuclease
  endonuclease


mRNA
3'
5'
Endonucleases
Endonucleases
Exonucleases
5'
3'
3'
3'

Physicochemical properties of DNA

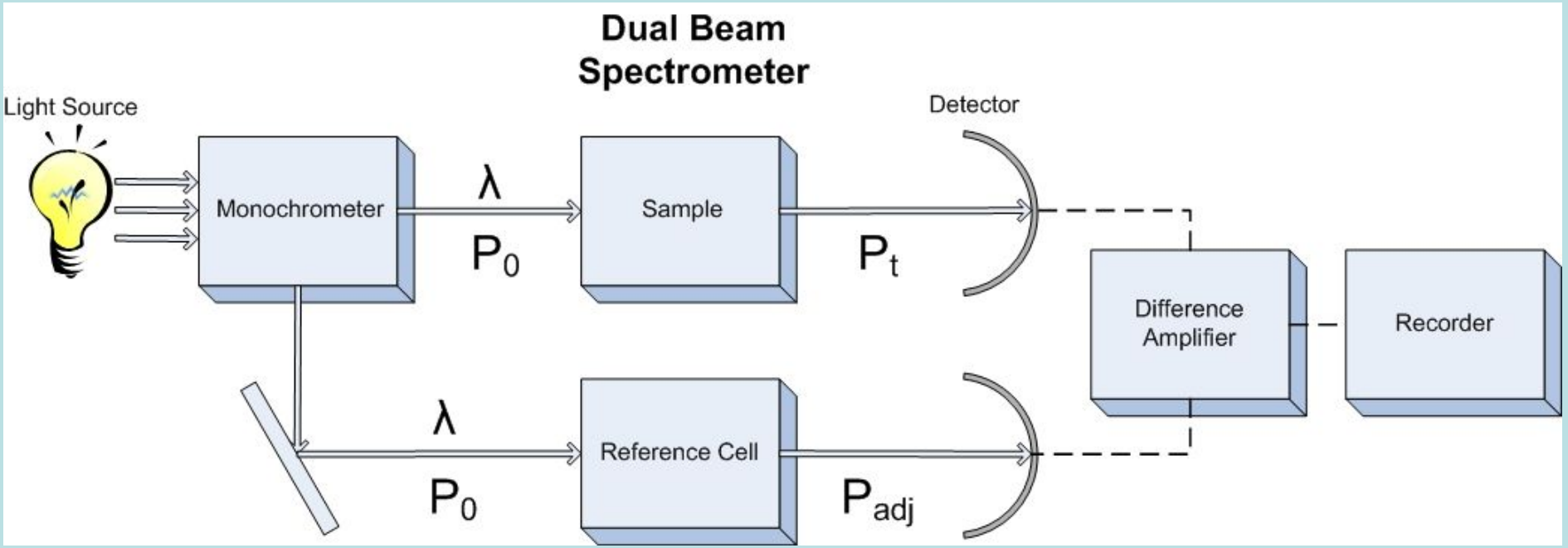
- Denaturation: DNAds → DNAss
- Renaturation (annealing): DNAss → DNAds
- Hybridization: DNAss + RNA → DNA-RNA hybrid

Absorption spectroscopy

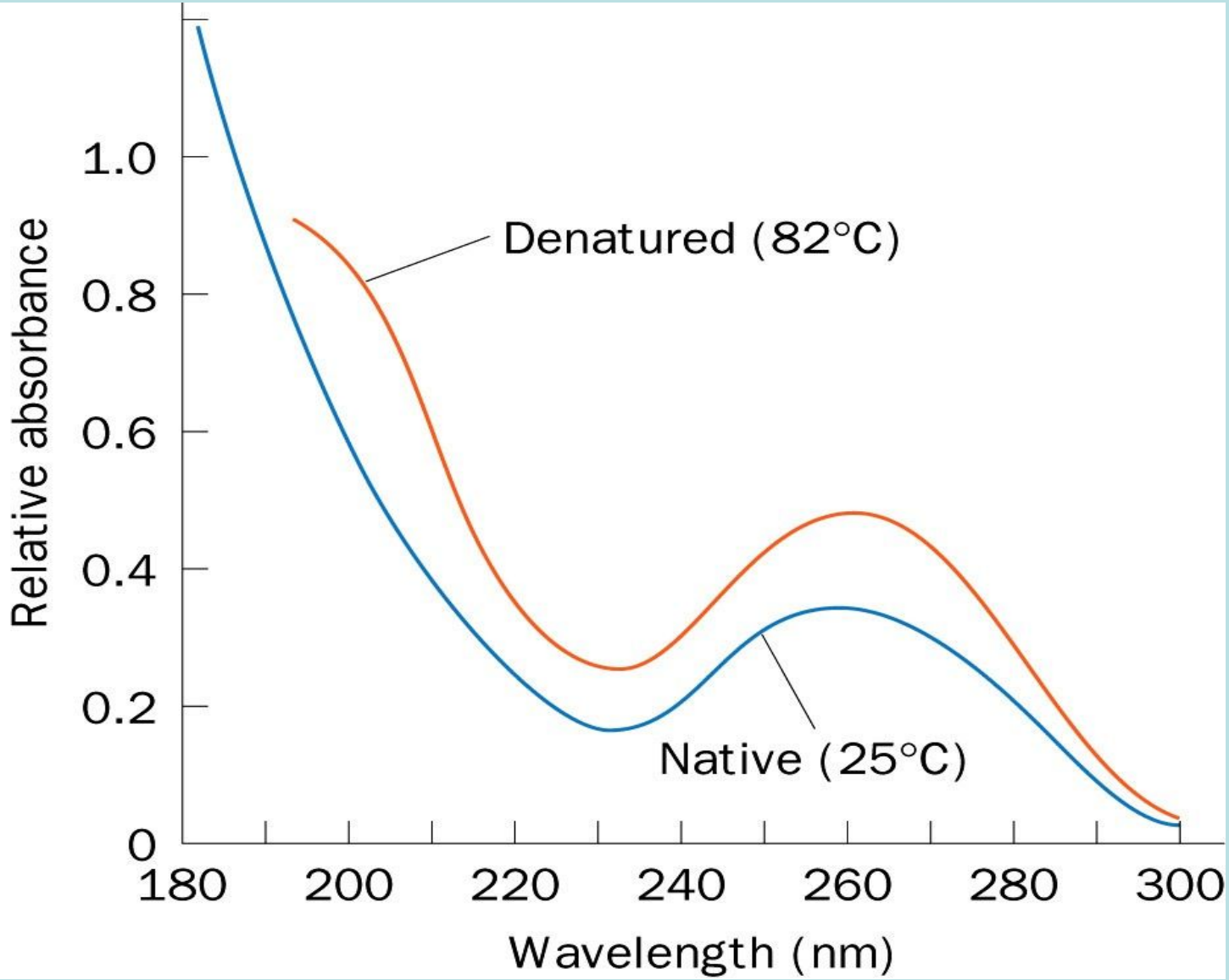
Many molecules absorb ultraviolet or visible light. Different molecules absorb radiation of different wavelengths.

An absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule.

The UV-Vis spectral range is approximately 190 to 900 nm, as defined by the working range of typical commercial UV-Vis spectrophotometers.


Dual Beam
Spectrometer
Light Source
Monochrometer
λ
P0
Sample
Pt
Detector
Difference
Amplifier
Recorder
λ
P0
Reference Cell
Padj

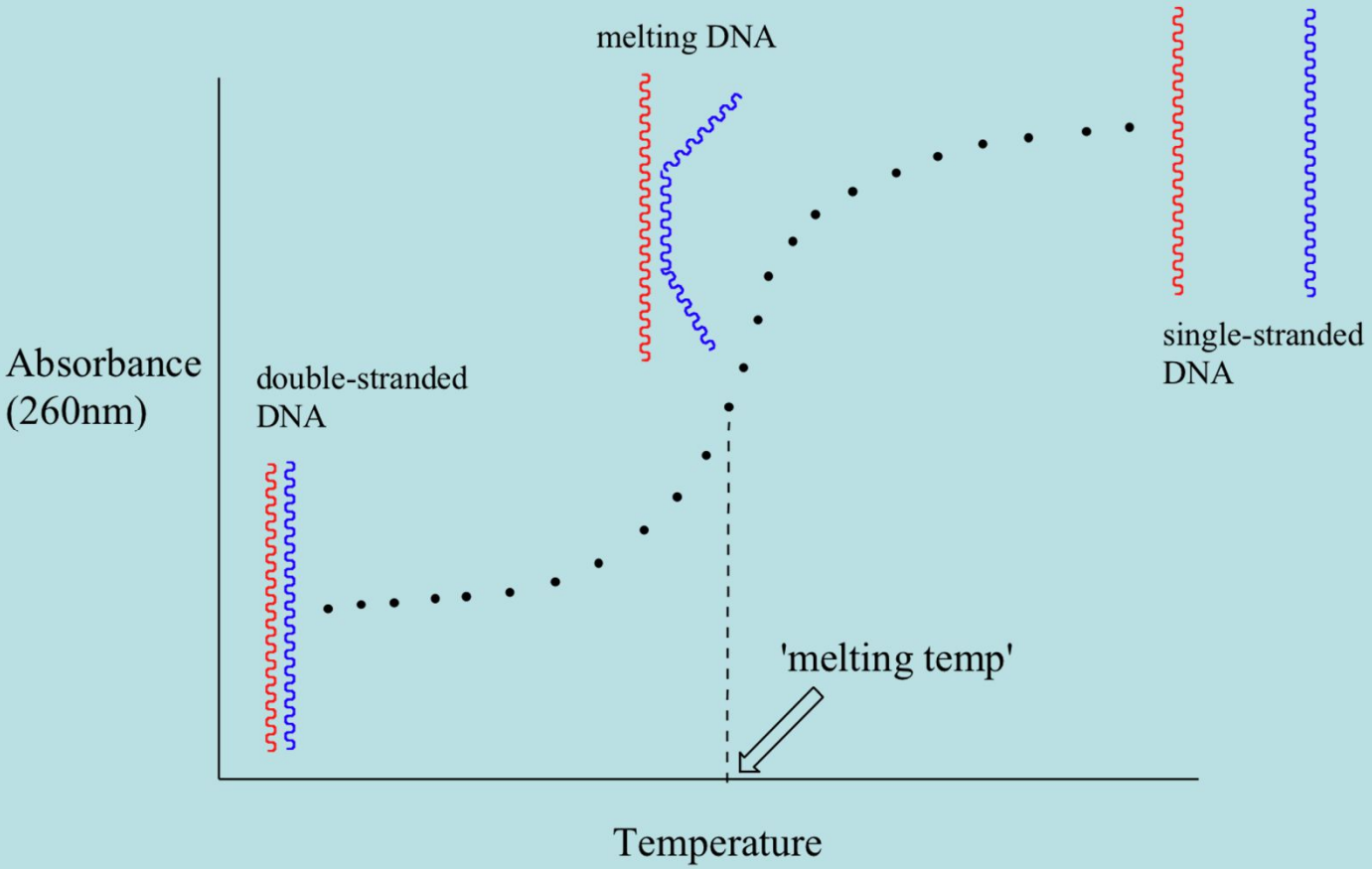
UV absorbance spectra of native and heat-denatured *E. coli* DNA.



DNA's *HyperChromic Effect*:

The double stranded DNA can be dissociated ("melted") into single strands by heat, which breaks the hydrogen bonds between complementary bases. The denaturation of double stranded DNA is easily followed spectroscopically. The purine and pyrimidine bases in DNA absorb UV light maximally at a wavelength of approximately 260 nm.
In double-stranded DNA, however the absorption is decreased due to base-stacking interactions. When DNA is denatured, these interactions are disrupted and an increase in absorbance is seen. This change is called the hyperchromic effect.

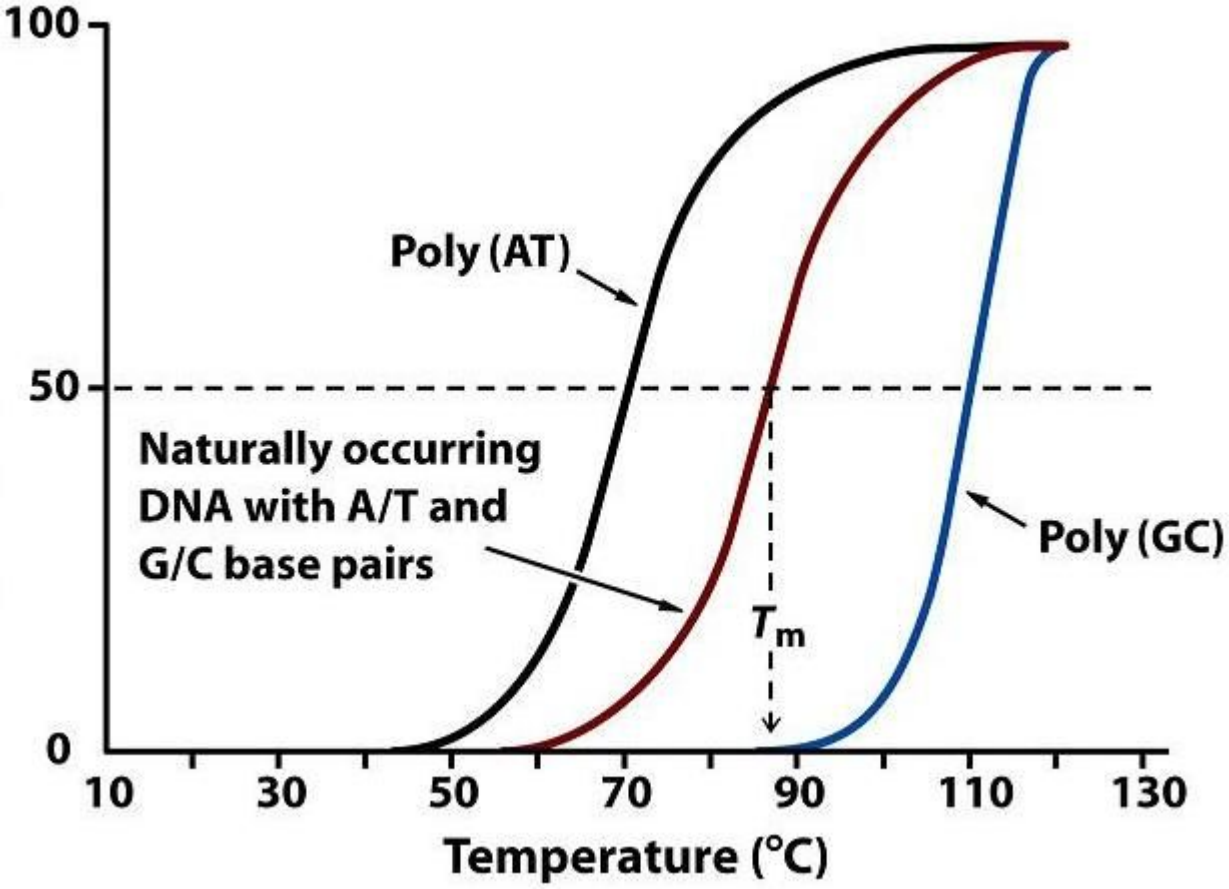
Double helix melting and measurement of Tm (Temperature of melting)

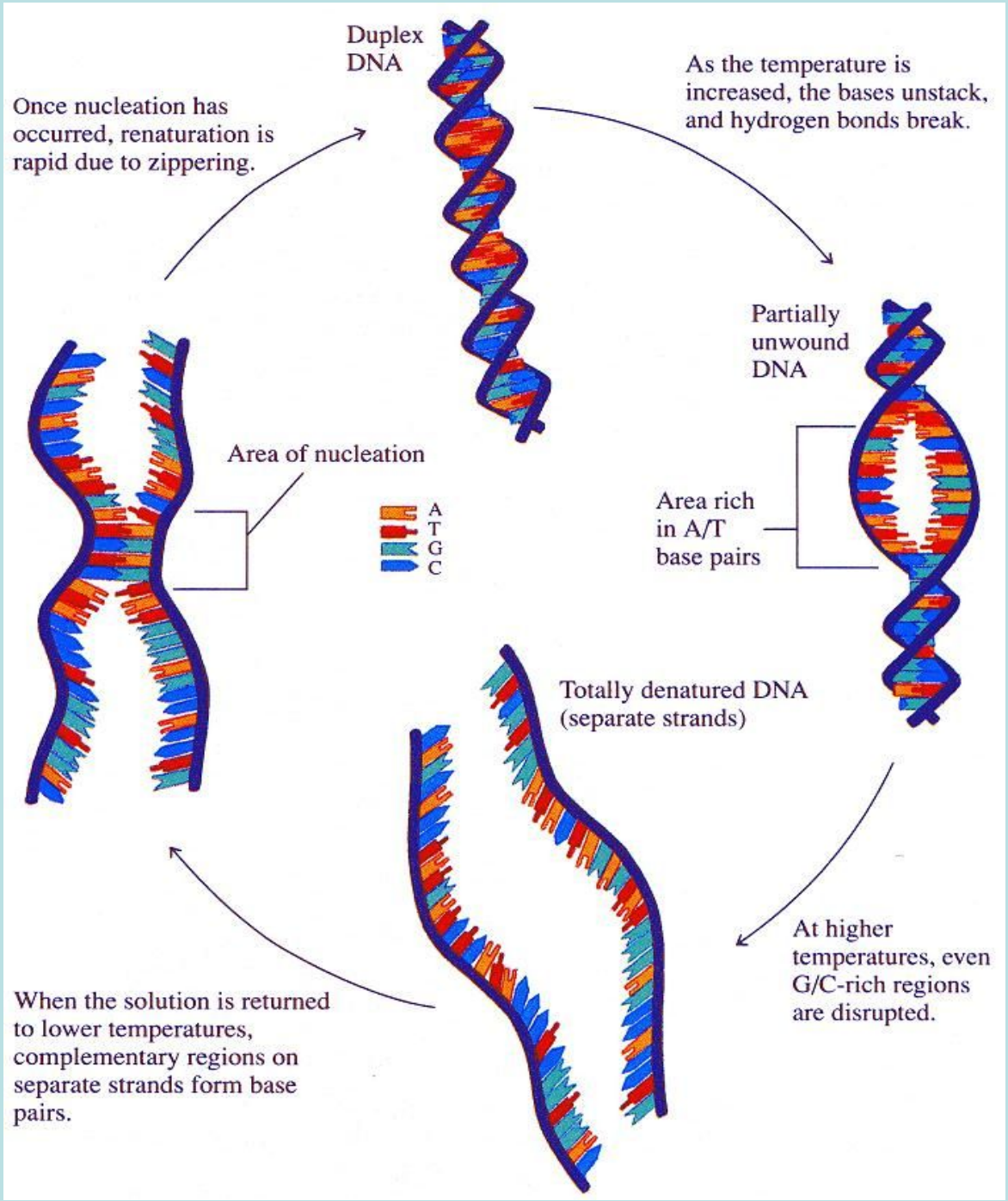


Factors that affect the Tm

- Temperature → H bonds breaking
- Ionic strength→ The concentration of ions affects Tm because DNA is electrically charged. Therefore it interacts with ions, which can compensate this charge.
- DNA sequence → bases composition (AT-GC), formation of internal loops, stacking effect.

DNA Tm dependence from G+C content




Duplex DNA
As the temperature is increased, the bases unstack, and hydrogen bonds break.
Partially unwound DNA
Area rich in A/T base pairs
Totally denatured DNA (separate strands)
At higher temperatures, even G/C-rich regions are disrupted.
When the solution is returned to lower temperatures, complementary regions on separate strands form base pairs.
Once nucleation has occurred, renaturation is rapid due to zippering.
Area of nucleation
A
T
G
C

Denatured DNA can be renatured

- If a solution of DNA is *rapidly* cooled below the T_m, the resulting DNA is only partially base paired.
- However, if the temperature is maintained at 25 ºC belowe the T_m, the base paired regions will rearrange until DNA completely renatures.
- These are called **annealing conditions** and are important for hybridization of complementary strands of DNA or RNA-DNA hybrid double helices.

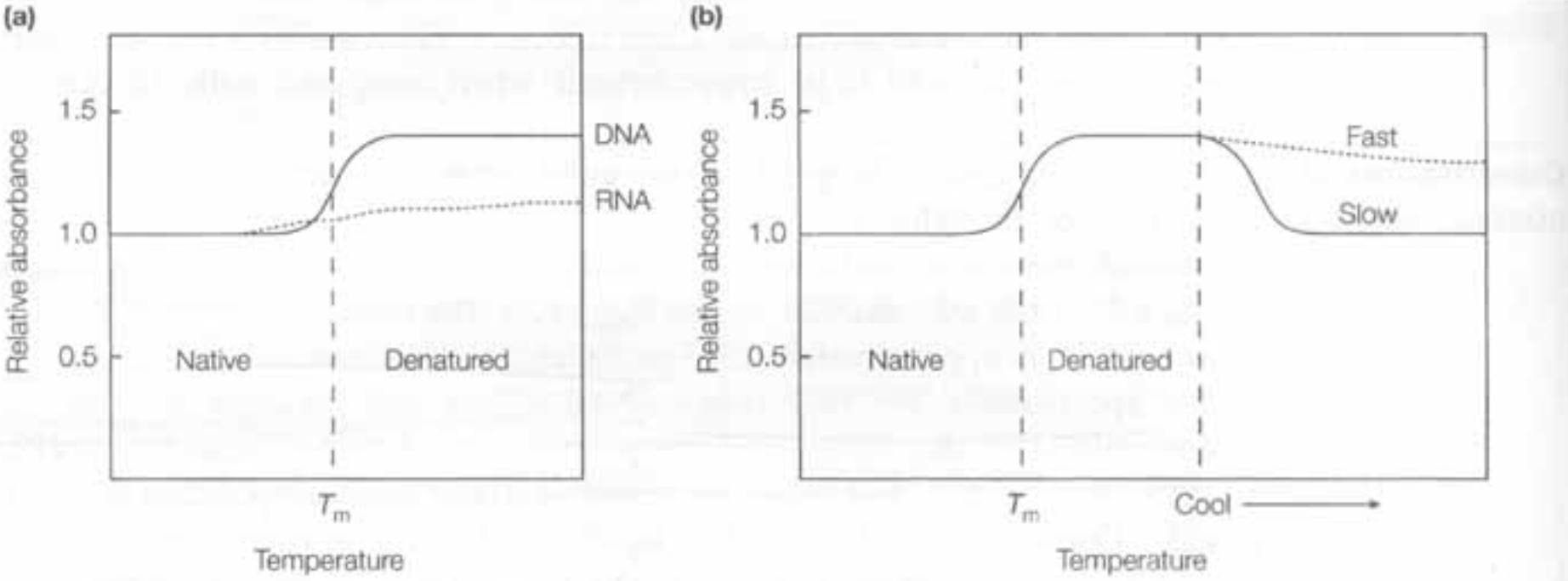
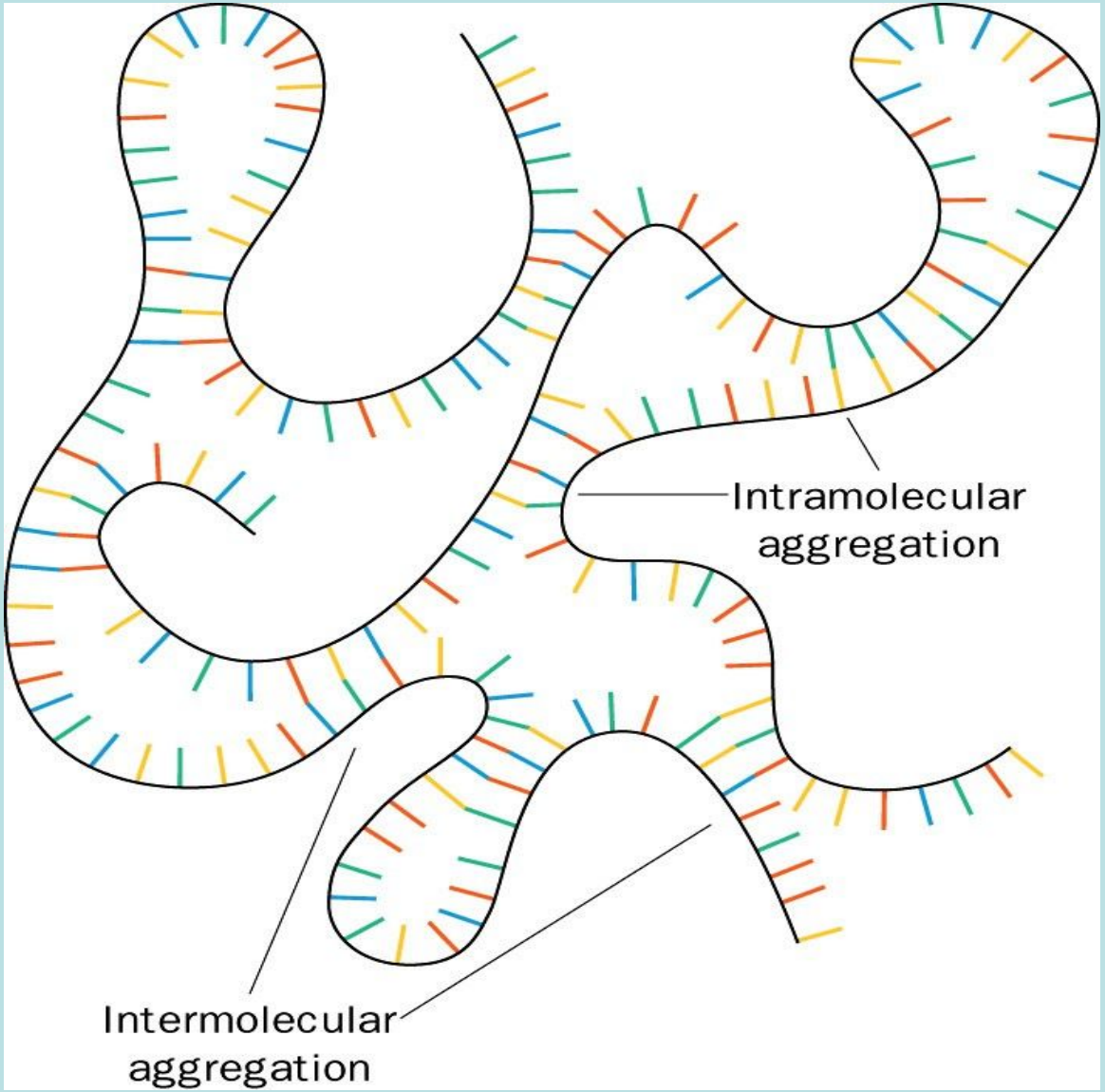


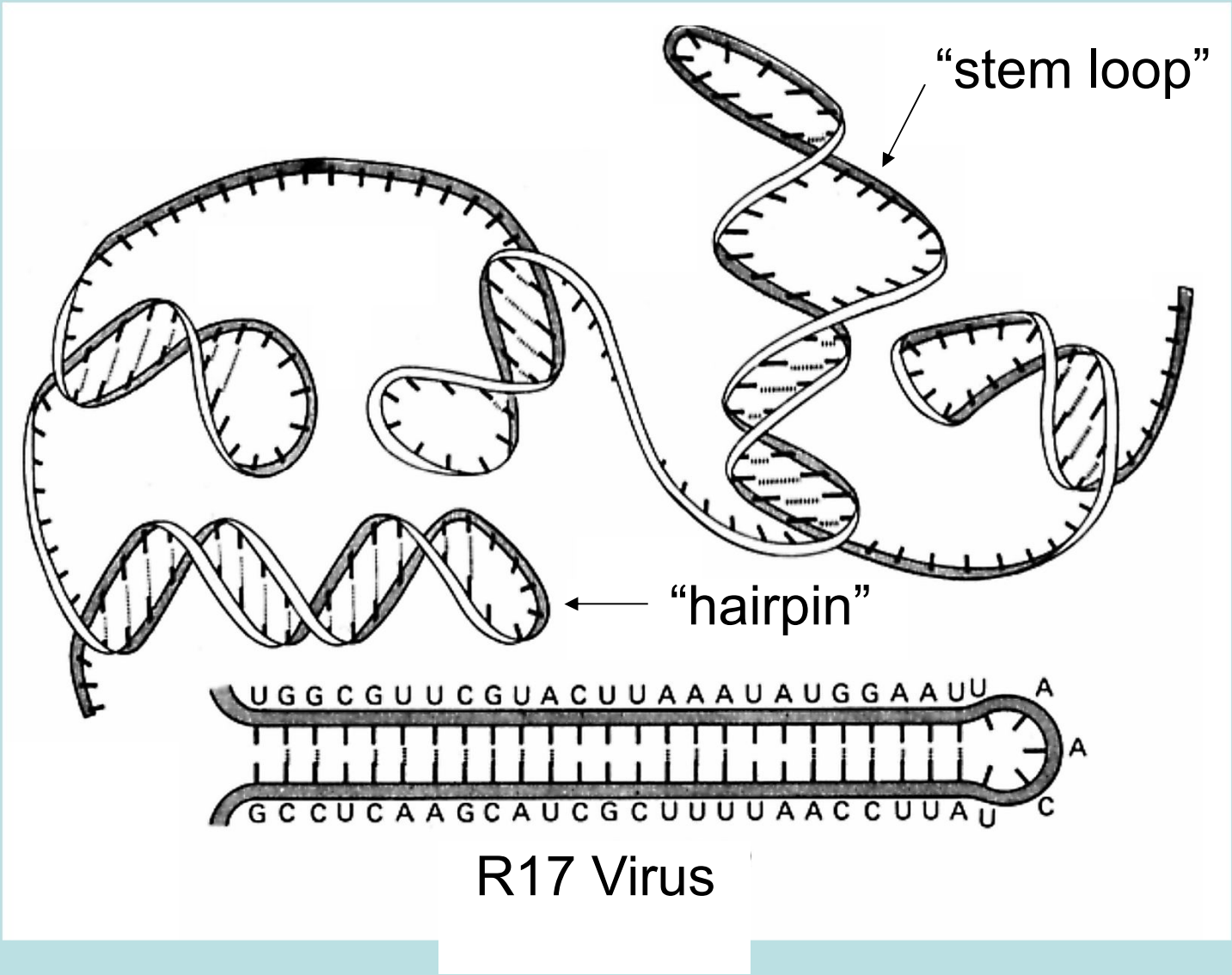
Fig. 2. (a) Thermal denaturation of double-stranded DNA (co-operative) and RNA; (b) renaturation of DNA by fast and slow cooling.

Partially renatured DNA.



RNA

- Although RNA is usually single stranded, short complementary regions within a nucleotide strand can pair and form secondary structures.
- These RNA secondary structures are often called hairpin-loops or stem-loop structures.
- When two regions within a single RNA molecule pair up, the strands in those regions must be antiparallel, with pairing between cytosine and guanine and between adenine and uracil


“stem loop”
“hairpin”
UGGCGUUCGUACUUAAAUAUGGAAUU
A
A
GCCUCAAGCAUCGCUUUUAACCUUAU
C

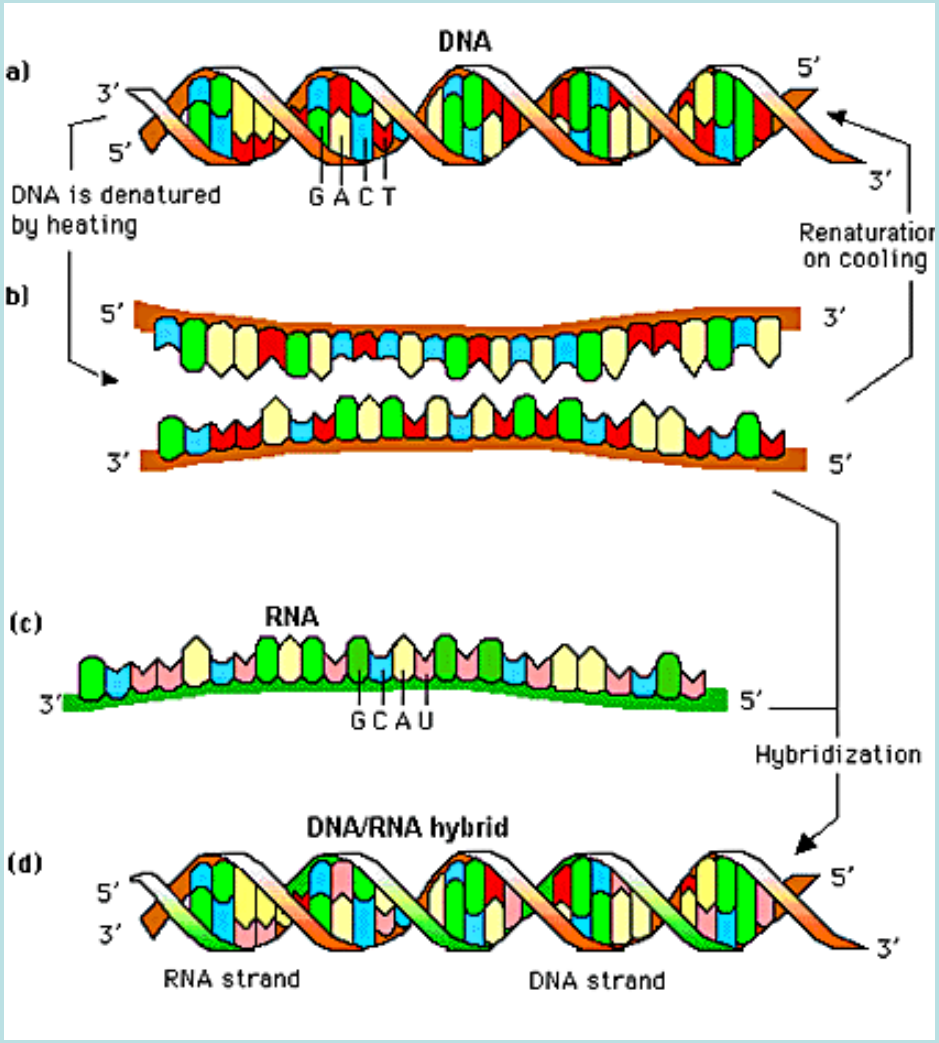
R17 Virus

Hybridization

- Single-stranded DNA or RNA will naturally bind to complementary strands.

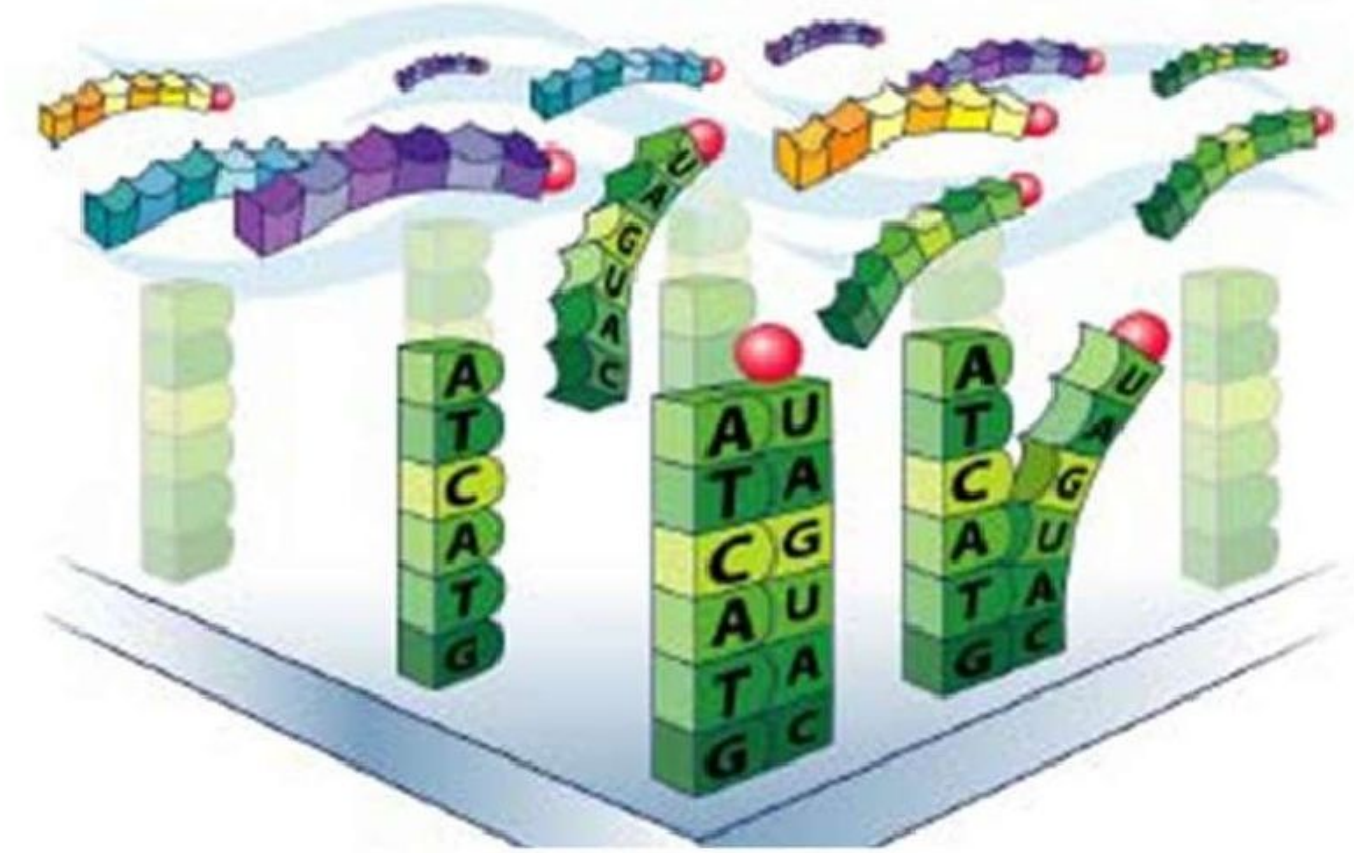
DNA ATGAGTAACGCG
TACTCATTGCGC

RNA ATGAGTAACGCG
UACUCAUUGCGC



Hybridization

RNA fragments with fluorescent tags from sample to be tested



RNA fragments hybridizes with DNA on GeneChip