DNA as a genetic material

DNA is the genetic material

- **DNA its role as carrier of genetic information was established only in 1943.**
- **Previously it was believed that genetic information is transmitted through the proteins.**
- **For molecule to carry genetic information from one generation to another generation, it was necessary that compound should be**

i. **Be relatively stable and be able to replicate itself with a high degree of fidelity.**
ii. **It should have enough flexibility so that it can get altered and can account for evolution. DNA fulfill these both requirements.**

DNA is the genetic material

- **1928, Frederick Griffith did an experiment in which he injected a encapsulated virulent strain of streptococcus pneumonia to a mouse and found that mouse developed pneumonia and died.**
- **The injection of virulent strain result in either in manifestation of the disease or death of animal.**
- **Later, heat killed virulent strain and injected these dead bacteria to mouse. These were non-pathogenic and mouse remain healthy.**
- **however, when injected a mixture of heat killed organisms of virulent strain along with living organisms of non virulent strain-mouse sick and died.**

DNA is the genetic material

- Later, Oswald Avery, Colin, Macleod and Maclyn McCarty in 1943 extracted material from virulent pneumococcus which are responsible for transformation of non-virulent strain.
- The extracted material later identified to be DNA by fact that it had physical characteristic and elemental analysis of DNA molecule.
- Further, its transforming property was not lost upon the extraction of lipids and polysaccharides from this material.
- Further its transforming property was not lost by its complete enzymatic digestion with protases. Similarly, upon treatment with Rnases, transforming activity was not lost but treatment with Dnase resulted in loss of transformation property.

DNA is the genetic material

- **They also found that addition of DNA to non-virulent strain resulted in its permanent transformation into virulent strain.**
- **However, some scientist still expressed doubts and believed the transformation of non virulent strain as seen by avery et al due to small amounts of proteins which might have co-purified with DNA during isolation procedure and may have been present as contaminant in their DNA preparation.**
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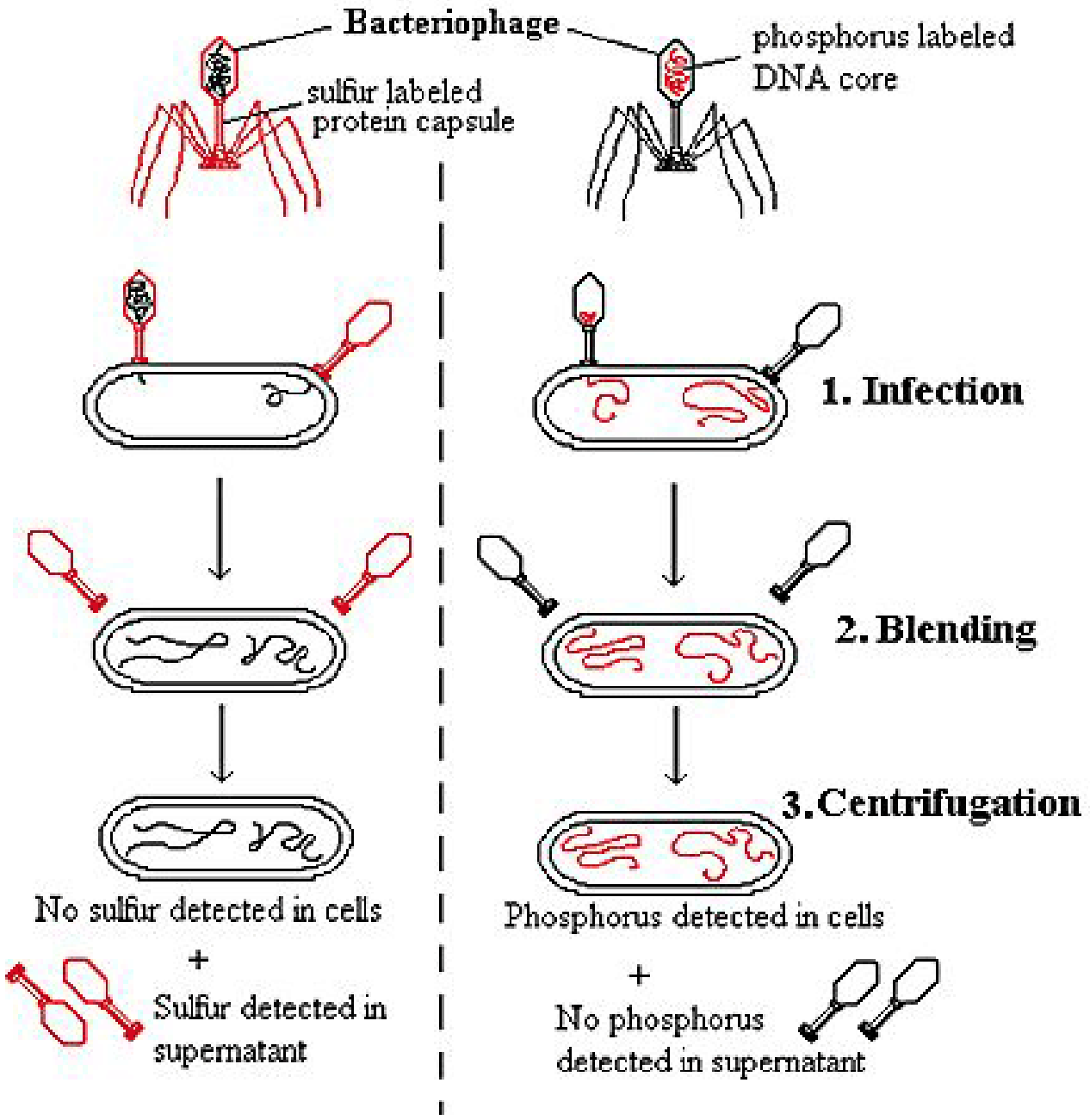
DNA is the genetic material

- **Later a direct double experiment was carried out by Hershey and Martha Chase in 1952.**
- **They incorporated two separate radioactive precursors into bacteriophage T2 using the radiolabelled nucleotides, the phage DNA was labelled with ^{32}P and using radiolabelled methionine, proteins were labelled with ^{35}S.**
- **This doubly labelled phage was used to infect the *E.coli* and the fate of radioactivity was followed.**
- **It was found that the ^{32}P has entered inside host cells and can be recovered from there. The ^{35}S did not enter host cell and could be isolated from cell supernatant.**
- **Confirmed DNA is carrier of genetic information.**


Bacteriophage
sulfur labeled protein capsule
phosphorus labeled DNA core
1. Infection
2. Blending
3. Centrifugation
No sulfur detected in cells
+
Sulfur detected in supernatant
Phosphorus detected in cells
+
No phosphorus detected in supernatant

DNAs with Unusual Structures

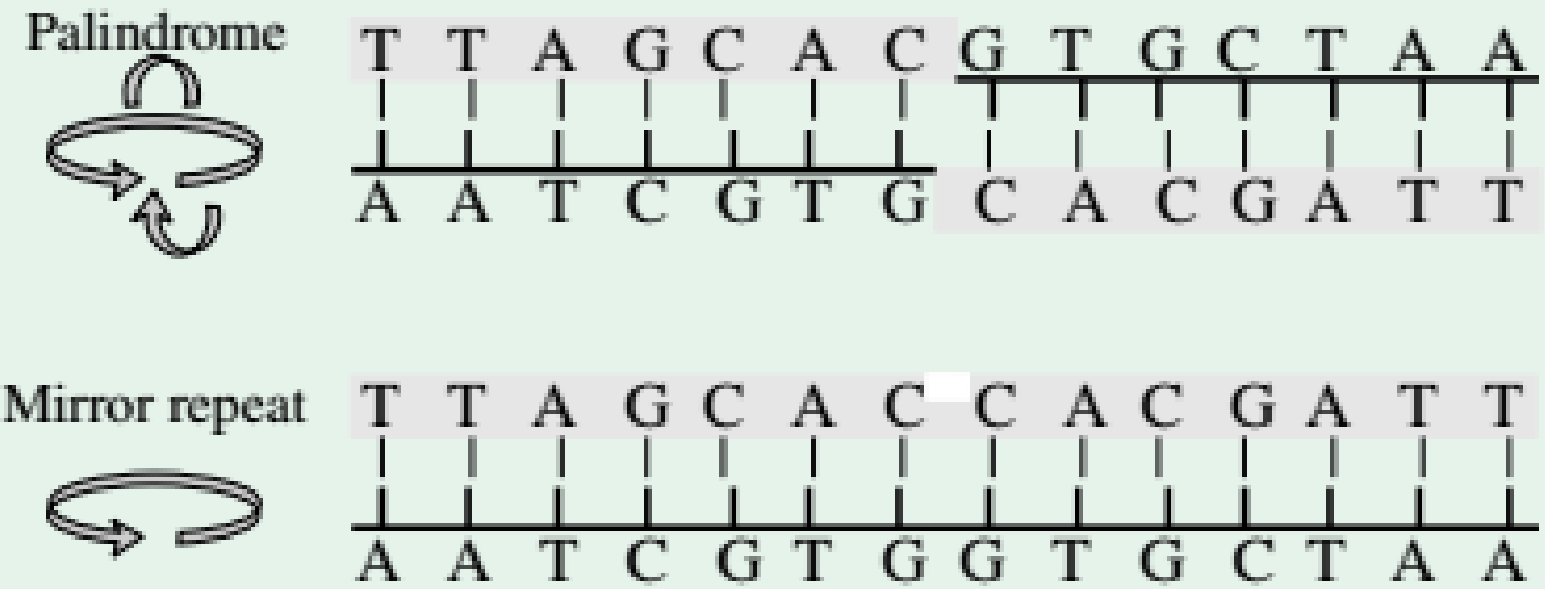
- **A number of other sequence dependent structural variations in DNA have been detected that may serve locally important functions in DNA metabolism. These are:**

A. Palindromic DNA:

It has sequences with twofold symmetry. In order to superimpose one repeat on the other, it must be rotated 180^{O} around the horizontal axis and then again about the vertical axis.

B. Mirror repeat:

It has a symmetric sequence on each strand. Superimposing one repeat on the other repeat only a single 180^{O} rotation about the vertical axis.


Palindrome
T T A G C A C G T G C T A A
A A T C G T G C A C G A T T
Mirror repeat
T T A G C A C C A C G A T T
A A T C G T G G T G C T A A

DNAs with Unusual Structures

➢ Palindromic sequences:

The term palindromic DNA is applied to regions of DNA in which there are inverted repetitions of base sequence with twofold symmetry occurring over two strands. Such sequences are self complementary within each of the strands and therefore have potential to form hairpin or cruciform.

Mirror repeats: Do not have complementary sequences within same strand and cannot form hairpin or cruciform structures. Sequences of these types are found in virtually every large DNA molecule and can involve a few or up to thousands of base pairs.

DNAs with Unusual Structures

Bend DNA:

- **Some sequence cause bends in the DNA helix. Bends are produced whenever 4 or more adenine residues appear sequentially in one of two strands. Six adenines in a row produce a bend of about 18^{O}. Bending may be important in the binding of some proteins to DNA.**
- **Hairpin: When only a single strand of palindromic DNA (or RNA) is involved, a hairpin is formed.**
- **Cruciform: When both the strands of a double helical DNA are involved, a cruciform is formed.**

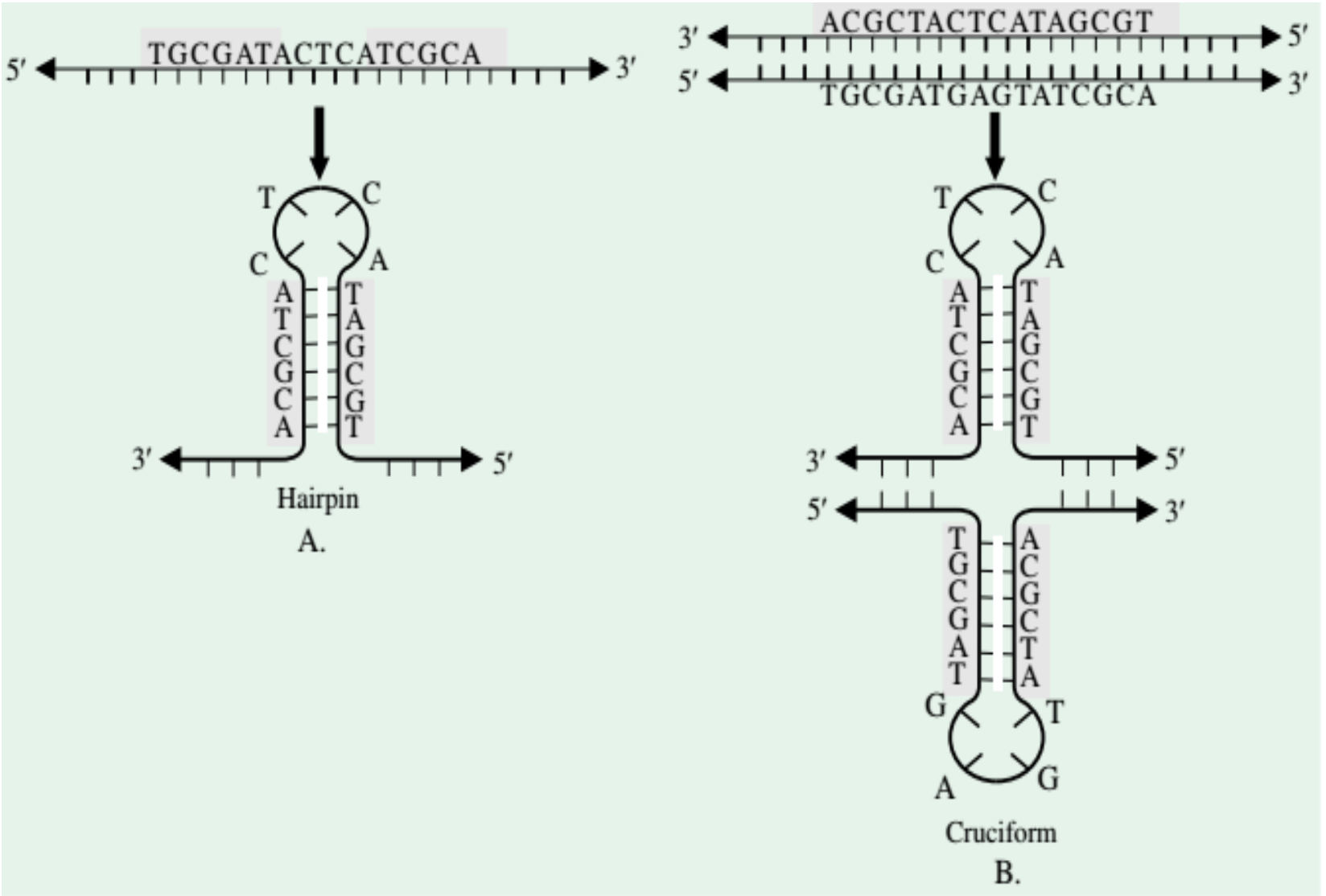
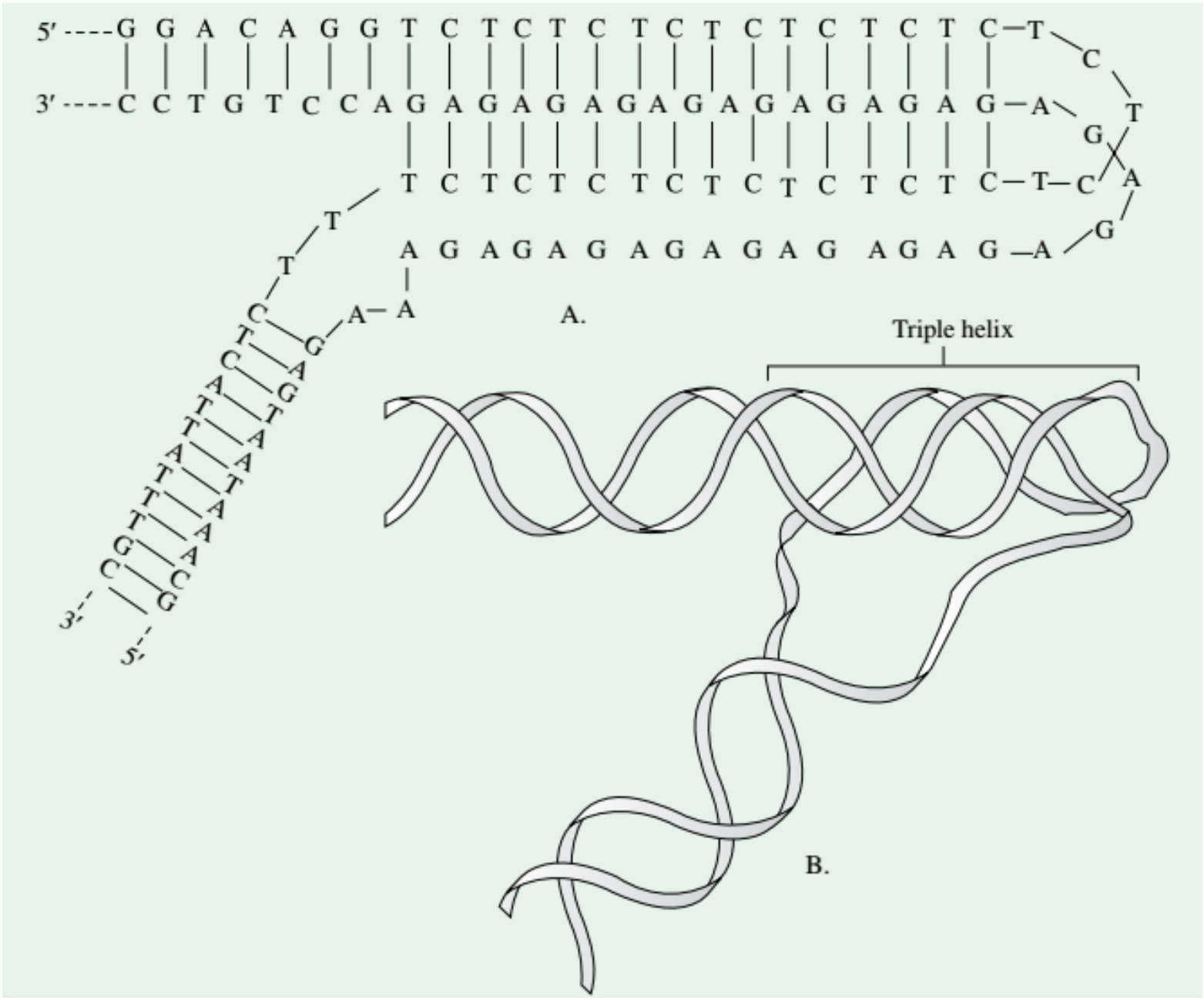


Fig. 15–32. Hairpins and cruciforms

DNAs with Unusual Structures

H- DNA:

- **H-DNA is usually found in polypyrimidine or polypurine segments that contain within themselves a mirror repeat. One example is a long stretch of alternating T and C residues. A striking feature of H-DNA is the pairing and interwinding of 3 strands of DNA to form a triple helix.**
- **Two of three strands in the H-DNA triple helix contain pyrimidines and the third contains purines.**


A.
Triple helix
B.

Denaturation and Renaturation of DNA helix

Denaturation:

- **Loss of biological activity by cleavage of hydrogen bonds.**
- **Separation of the double helix into two constituent polynucleotide chains.**
- **the firm, helical, two stranded native structure of DNA is converted to a flexible, single stranded denatured state.**
- **The denatured form is usually very abrupt and is accelerated by reagents such as urea and formamide, which enhance the aqueous solubility of the purine and pyrimidine groups.**

Denaturation and Renaturation of DNA helix

Denaturation involves the following changes.

1. Increase in absorption of ultraviolet light(hyperchromic effect).

2. Decrease in specific optical rotation.

3.Decrease in viscosity.

Denaturation and Renaturation of DNA helix

Effect of pH on denaturation:

- **Denaturation of DNA helix also occurs at acidic and alkaline pH values at which ionic changes of the substituents on purine and pyrimidine bases can occur.**
- **In acidic solutions near pH 2 or 3, at which amino groups bind protons, the DNA helix is disrupted.**
- **In alkaline solutions near pH 12, the enolic hydroxyle groups ionize, thus preventing the keto-amino group hydrogen bonding.**

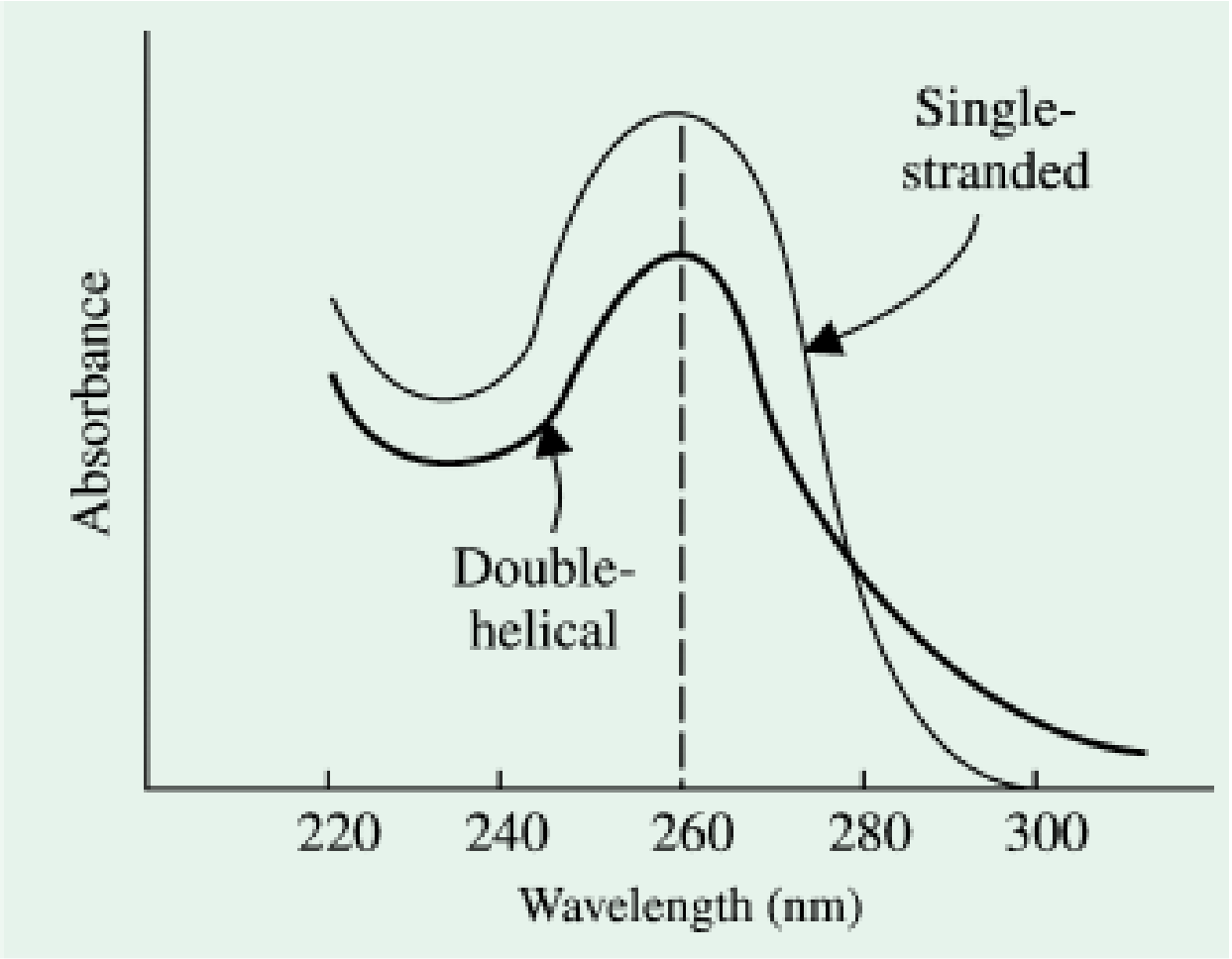


Fig. 15–25. The absorbance spectra of a DNA solution at 260 nm and pH 7.0

Effect of temperature on denaturation:

- **Melting**
- **melting temperature(Tm)**
- **the abruptness of the transition indicates that the DNA double helix is highly cooperative structure, held together by many reinforcing bonds; it is stabilized by the stacking of bases as well as by pairing.**
- **the melting of DNA is readily monitored by measuring its absorbance of light at wavelength near 260nm**
- **Tm is analogous to the melting point of a crystal. It can be lowered by the addition of urea which disrupts hydrogen bonds.**
- **In 8M urea, Tm is decreased by nearly 20^{O}C. DNA can completely denatured by 95% formamide at room temperature only.**

Effect of temperature on denaturation:

- **DNAs with high G and C might be more stable and have a higher Tm.**

- **Tm of DNA from many species varies linearly with G-C content, rising from 77 to 100^{O}C as fraction of G-C pairs increases from 20% to 78%. This be used as an index of heterogeneity of nucleic acid molecules.**

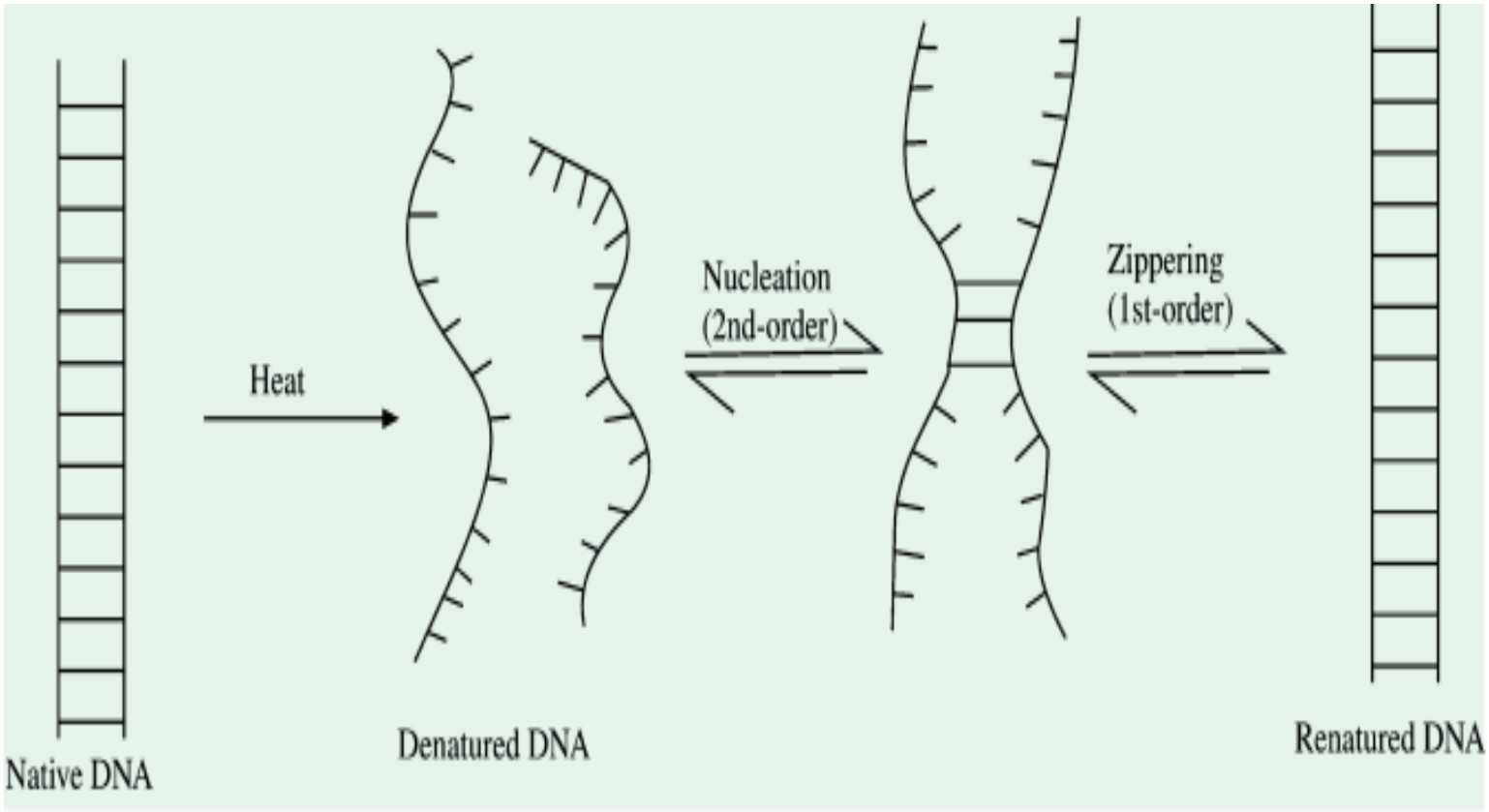
- **Viral DNA has a much sharper thermal transition than that prepared from animal sources.**

- **Complete rupture of two stranded helix by heating is not a readily reversible process.**

Effect of temperature on denaturation:

- **Maximum reversibility (50-60%) usually attained by annealing the denatured DNA, ie. Holding the solution at a temperature about 25^{O}C below Tm and above a conc of 0.4M Na+ for several hours.**

- **Fast cooling will not reverse denaturation but if cooled solution again heated and cooled-renaturation will take place.**
- **Renaturation of complementary single strands to produce fully double stranded molecule requires 2 separate reactions.**
- **Nucleation reaction: In this hydrogen bonds form b/t two complementary single strands; bimolecular**
- **Zipper reaction. In this hydrogen bonds form b/t all the bases in the complementary strands; this is unimolecular**


Heat
Nucleation
(2nd-order)
Zippering
(1st-order)
Native DNA
Denatured DNA
Renatured DNA