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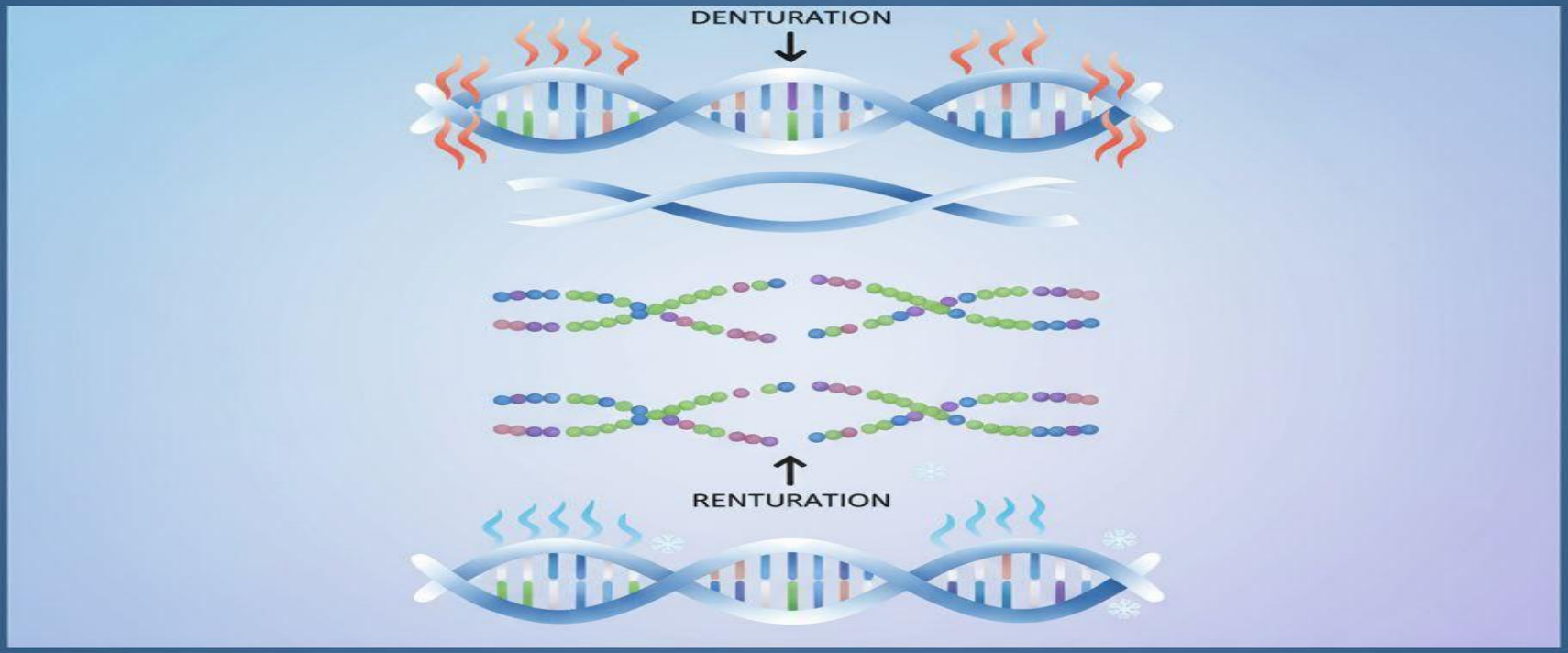
DNA Denaturation and Renaturation

Lec:3 For the third stage



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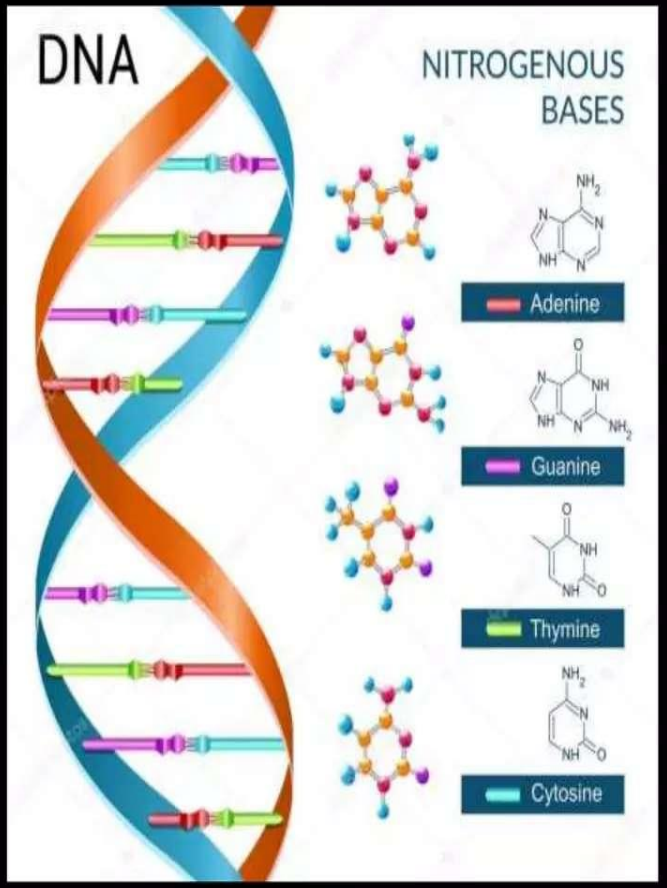
SYNOPSIS

- Introduction:- What is DNA
- DNA denaturation
- Causes
  - ✓ Elevated temperature
  - ✓ Extremes of pH
  - ✓ Non physiological salts
- Characters of denatured DNA
- DNA renaturation
- Applications
- Conclusion
- References

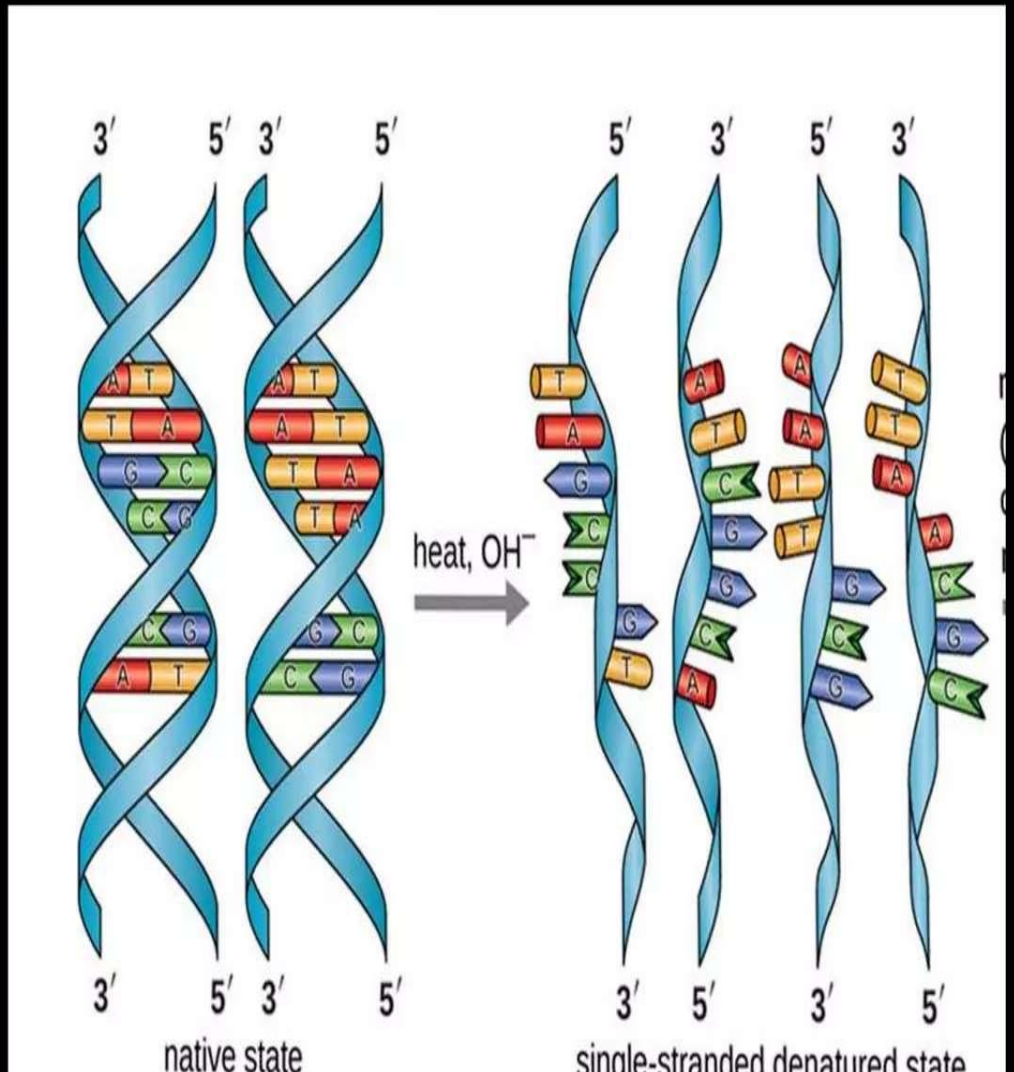
INTRODUCTION

- DNA is a molecule composed of 2 strands running in opposite directions to form a double helix structure carrying genetic information.
- Each strands composed of de-oxy ribose sugar molecules linked together by phosphate linkage.
- The two strands are held together along their length with hydrogen bonds between the bases.



DNA DENATURATION

DNA denaturation refers to the melting of doubled stranded DNA to generate two single strands by breaking the hydrogen bonds.



CAUSES OF DENATURATION

Denaturation can occur when nucleic acids are subjected to:

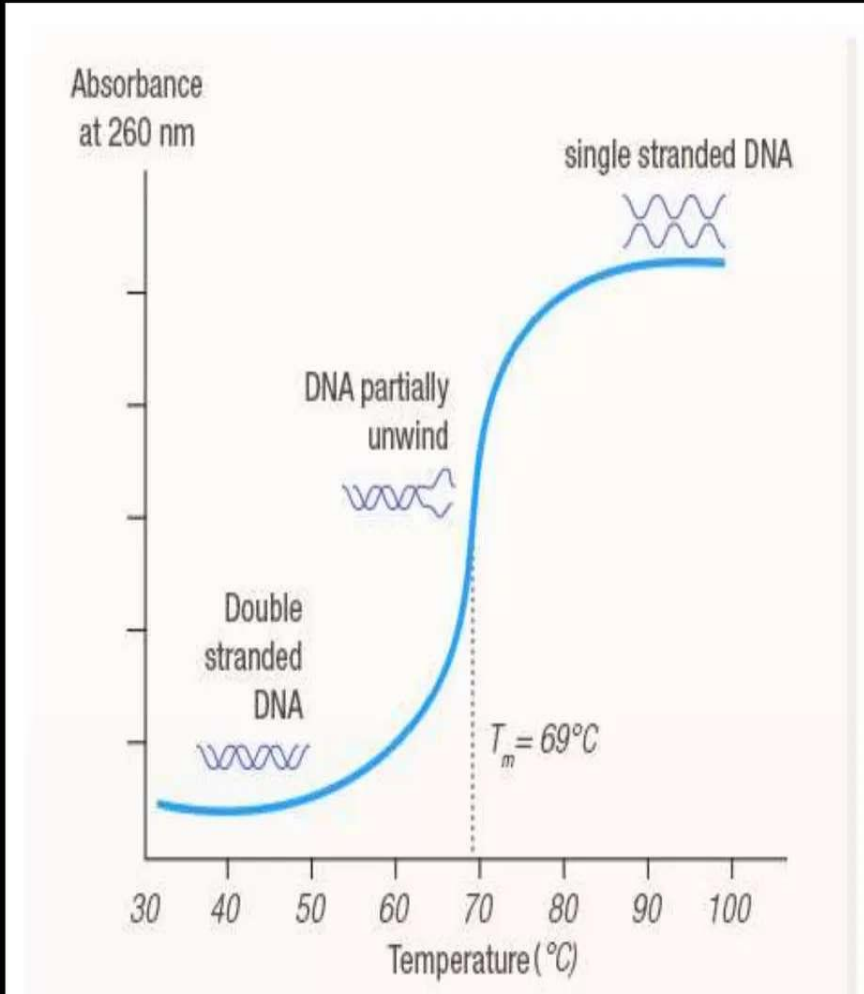
- elevated temperature
- extremes of pH
- non-physiological concentrations of salt, organic solvents, urea, or other chemical agents.

Elevated temperature

- The most common type of denaturation is thermal denaturation

Tm: Temperature at which DNA is half denatured.

- Above Tm DNA is single stranded
- Below Tm DNA is double stranded



Extremes of pH

- At high pH, the hydroxide ions (negatively charged ions) can pull hydrogen ions from base pairs (forming H bond between two strands) causing them to separate.
- High pH will alter the charged groups that are involved in H-bond
- H-bond cannot occur at pH>11.3 and DNA denatures

Non physiological salts

- Low salt concentration could also denature DNA double strands by removing ions that stabilize the negative charges on the two strands from each other.
- The Tm value is lowered by the addition of Urea. In 8M urea, Tm is decreased by nearly 20 degree celsius
- DNA can be denatured with 95% formamide at room temperature only.

Characters of Denatured DNA

- Hyperchromic: increase the absorbance(A260) upon denaturation
- The rate of increase in absorbance is directly proportional to the rate of denaturation
- Viscosity decreases upon denaturation

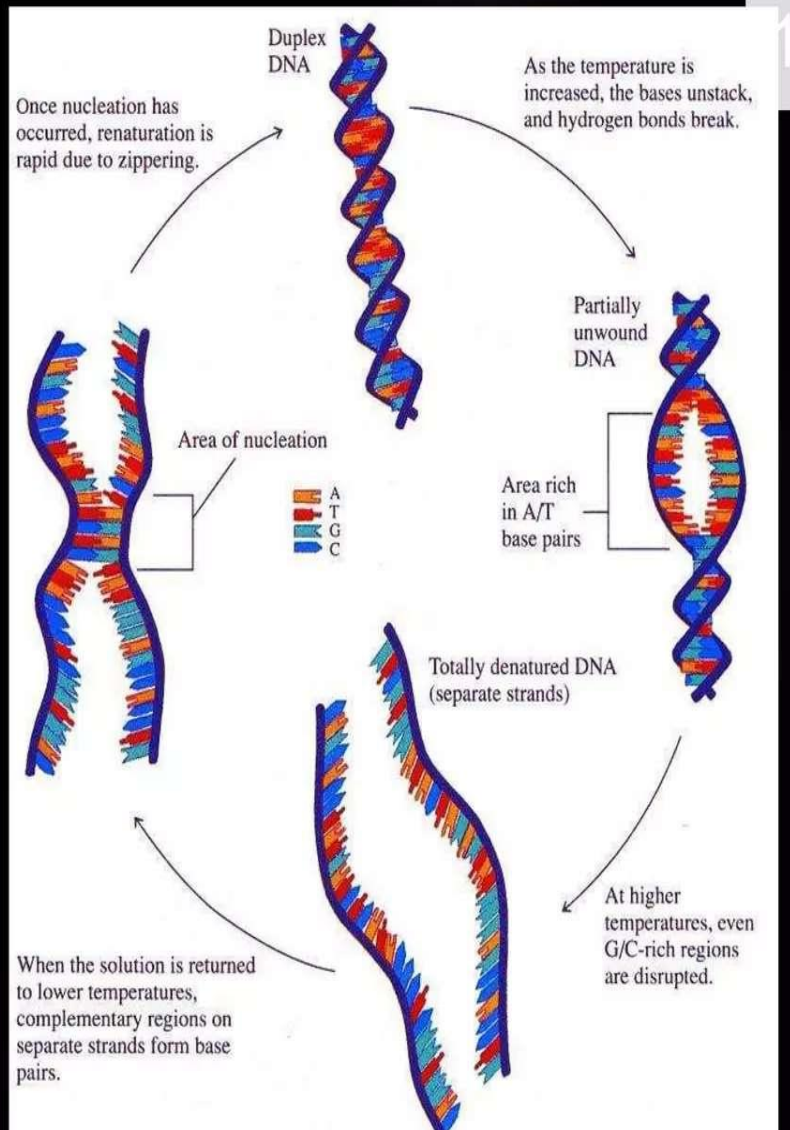
DNA Renaturation

- Renaturation or annealing:

  is the formation of base repairs and complementary strands of DNA come back together.

- Renaturation occurs if double stranded DNA is heated above Tm then the temperature is slowly decreased under appropriate conditions

DENATURATION AND RENATURATION OF DNA



Applications :

- Understanding the genome size and complexity.
- Understanding genetic relatedness.
- Understanding relative proportion of repetitive sequences.

Conclusions

- These processes form the basis of many important techniques for DNA manipulating. Though there are many techniques associated with DNA denaturation, the end result is the same that is to break the bonds between the strands and new molecules are formed which can then be compared as desired. The ideal process of DNA denaturation depends on what the DNA needs to be use for, how accurate and specific the comparisons need to be and the volume of the material that has to be processed.

REFERENCES:-

- **Orby's illustrated reviews of biochemistry**
- **Lippincott's illustrated reviews of biochemistry**
- www.biologydiscussion.com

Harper's biochemistry 5th edition

Review lecture on "illustrated biochemistry "

THANK YOU