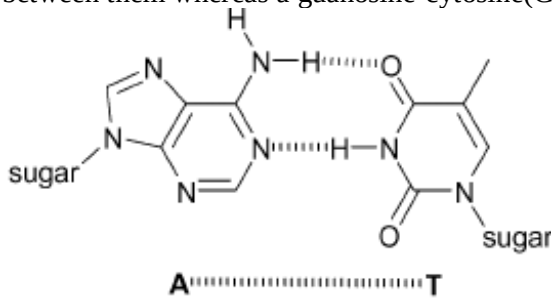
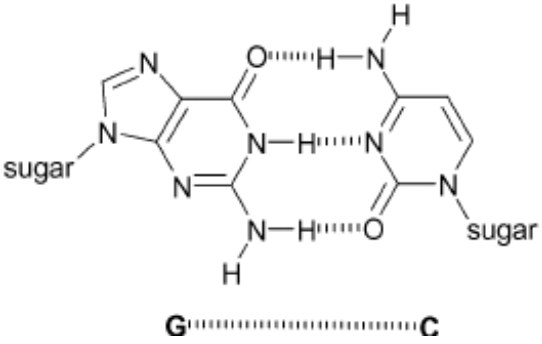
Structure of DNA (The Double Helix)

It was suspected that most naturally occurring DNA did not exist as single chains. Instead two or more chains appear to interact with one another in some way. The resulting macromolecules were known to be long, thin and rigid such that they formed highly viscous solution in water. The double Helix structure of DNA was proposed by James Watson and Francis Crick in 1953 . It was based primarily on model building and X-ray diffraction pattern. Information from X-ray patterns was added to information from chemical analysis that showed that the amount of ***A*** was always same as the amount of ***T*** and that the amount of ***G*** always equal to the amount of ***C***. Both of these lines of evidence were used to conclude that DNA consist of polynucleotide chains wrapped around each other to form in helix . Hydrogen bonds between bases on opposite chains determine the alignment of helix. The paired bases lie in a planes perpendicular to the Helix . The sugar phosphate backbone in the outer part of the Helix . The chains run in antiparallel directions one 3'to 5' and other 5'to3'. Important features of the Watson and Crick model are :

1.The base pairing is complementary, meaning that adenine pairs with thymine and guanine pairs with cytosine . This complementary base pairing occurs along entire double Helix, the two chains are also referred as complimentary chains. And adenine-thymine(A-T) base pair has to hydrogen bonds between them whereas a guanosine-cytosine(G-C) pair has three.

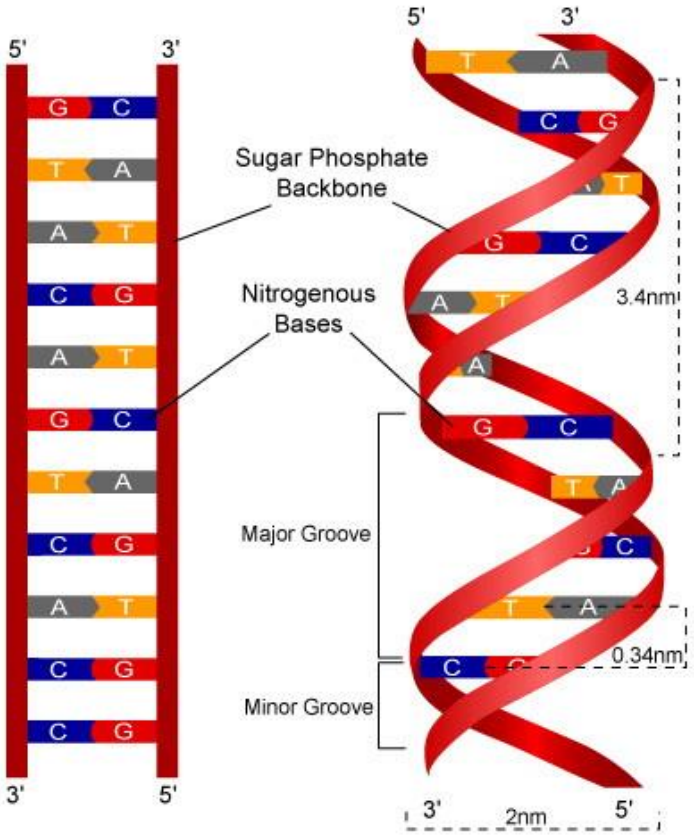




2.The inside diameter of sugar phosphate backbone of the double Helix is about 1.1 nm. The distance between the points of attachment of the bases of the 2 strands of the sugar phosphate backbone is the same for two base pairs(A-T and G-C),about 1.1 nm. This gives DNA an extremely ordered structure.

3.The outside diameter of the Helix is 20 A^o(2nm). The length of one complete turn of the helix along its axis is 34A^o(3.4nm) and contains ten base pairs.

4.The double Helix has empty spaces known as grooves. There is a major groove and a smaller minor groove in the double helix. Both can be sites at which drugs or polypeptides bind to DNA.



Important features of Watson and Cricks model:

1. The double Helix comprises of two complementary polynucleotide chains .
2. The two polynucleotide chains are wrapped clockwise around central access to form circular staircase type structure .
3. The two strands of double Helix are antiparallel i.e., they run in opposite direction one chain is aligned in 5'→ 3′direction and the other chain is aligned in 3'→ 5′ direction.
4. Each polynucleotide chain has sugar phosphate backbone and nitrogenous bases are directed inside the Helix i.e., towards the central axis .the sugar phosphate backbone is formed of covalently bonded deoxyribose and phosphate groups and therefore, occur on the outside of Helix.
5. The phosphate groups give the molecule negative charge
6. The nitrogenous bases are hydrophobic in nature. They avoid water and therefore remain inside the Helix.
7. The diameter of double Helix is 2nm.
8. The nitrogenous bases lie almost perpendicular to the axis of molecule. Therefore these are stacked one on top of another. The hydrophobic interaction between the bases provide stability to DNA.
9. The nitrogenous bases of two antiparallel polynucleotide strands are linked through hydrogen bonds. there are two hydrogen bonds between adenine and thymine and three between guanine and cytosine .The hydrogen bonds are the only attractive forces between two polynucleotide chains of the double Helix these serve to hold the strands together .
10. There are ten base pairs in one turn Helix therefore each turn of the Helix is 3.4nm.
11. The double Helix has two helical groups a major groove which is deep and wide and minor groove which is shallow and narrow.

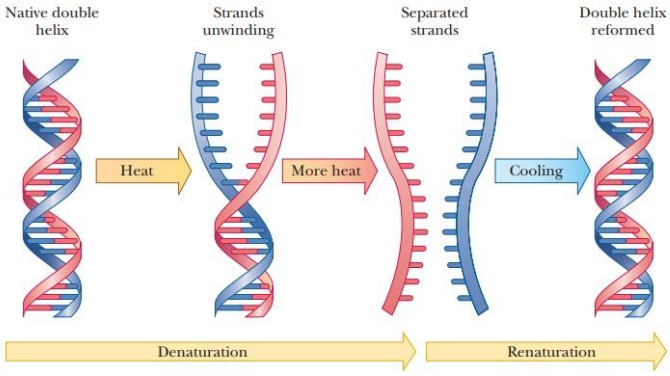
The double helical structure described by Watson and Crick was also B-DNA. The other secondary structures can occur depending on the condition such as nature of the positive ion associated with the DNA and the specific sequence of bases . Thus, we have three important forms of DNA. These are A, B &Z forms . B and Z confirmations occur as cellular DNA .

1.***B-DNA***: This form of DNA occurs all living beings under normal conditions i.e., this is the naturally occurring form. it occurs under low salt concentration and high degree of hydration .It's each turn measures 3.4 nm and each turn has ten base pairs. Each base pair occupies 3.4A^o. Its two strands are wounded in right-handed coils and each strand follows a clockwise path.

2.***A-DNA***: It is the dehydrated form of DNA. It occurs under conditions of decrease hydration and increase concentration of sodium ions. Its polynucleotide chains are coiled clockwise and have right handed configuration it has 11 base pairs per turn of the helix and a diameter of 2.3 nm. A-DNA is more compact and bulky as distance between base pairs is 2.7A^o instead of 3.4A^o.the orientation of bases is also different. It is pre assumed that A-DNA is derived from B-DNA as a result of hydrophobic molecules and dry conditions.

3.***Z-DNA***: Another variant of double Helix is Z-DNA which is left handed it winds in the direction of fingers of the left hand. it occurs in small proportion in cellular DNA. it is called Z-DNA because the phosphodiester backbone of its polynucleotide strands follow zigzag course . Z-DNA was discovered by Alexander rich in 1979 . It has 12 base pairs per turn . One complete Helix is 45A^o as against 34A^o in B-DNA. The diameter of Z-DNA is 18A^o. It has only one groove. Z-DNA is less stable than B-DNA. It is formed only when purine and pyrimidine are present alternatively in the chain . Z-DNA is more compact . The Z confirmation is stabilized by high salt concentration.

DENATURATION AND RENATURATION OF DNA



■ FIGURE 9.19 **Helix unwinding in DNA denaturation.** The double helix unwinds when DNA is denatured, with eventual separation of the strands. The double helix is re-formed on renaturation with slow cooling and annealing.

Hydrogen bonds between the base pairs are responsible for holding double helix together. The stacking of the bases contribute the largest part of stabilisation energy.
Energy must be added two a sample of DNA to break the hydrogen bonds and to disrupt the stacking interactions. if we heat a DNA sample in a solution the two strands of DNA molecules separate. The phenomena of separation of the two strands of DNA molecule by heating is known as denaturation or melting. The denatured DNA becomes single stranded.

(If a DNA solution is heated to approximately 90°C or above there will be enough kinetic energy to denature the DNA i.e., The hydrogen bonds between bases of two anti parallel polynucleotide strands break and two polynucleotide chains to separate into single strands. This denaturation is very abrupt and is accelerated by chemical reagents like urea and formamide. The chemicals enhance the aqueous solubility of the purine and pyrimidine groups. This separation of double helix is called melting as it occurs abruptly at a certain

characteristic temperature called denaturation temperature or melting temperature (T_m). It is defined as temperature at which 50% of the DNA is melted. The abruptness of the transition indicates that the DNA double helix is highly cooperative structure, held together by many reinforcing bonds.)

The strands of DNA molecules separate over at temperature range. the midpoint of this range is called melting temperature or transition temperature and is represented by Tm(the temperature at which DNA is half denatured, meaning half double stranded ,half single stranded).

The melting of DNA can be followed spectrophotometrically by monitoring the absorbance of DNA at 260 nm. T_m is analogous to the melting point of crystal. The T_m value depends on the nature of the DNA.

Several samples of DNA are melted, it is found that the T_m is highest for those DNAs that contain the highest proportion of G—C. Actually the value is used to estimate the percentage of G—C in a DNA sample. In fact, the T_m of DNA from many species varies linearly with G—C content.

This relationship between T_m and G—C content arises due to guanine and cytosine form three hydrogen bonds when base paired, whereas adenine and thymine form only two.

If denaturation is followed spectrophotometrically by monitoring the absorbance of light at 260 nm, it is observed that the absorbance at 260 nm increases as the DNA become denatured, a phenomenon known as the hyperchromatic effect or hyperchromicity or hyperchromism. This is due to un-stacking of base pairs.

A plot of the absorbance at 260 nm against the temperature of a DNA solution indicates that little denaturation occurs below approximately 70°C, but further increases in temperature result in a marked increase in the extent of denaturation.

If melted DNA is cooled it is possible to reassociate the separated strands, a process known as renaturation. However, a stable double-stranded molecule may be formed only if the complementary strands collide in such a way that their bases are paired precisely. But renaturation may not be precise if the DNA is very long and complex. Thus the rate of renaturation (renaturation kinetics) can give information about the complexity of a DNA molecule. Complete denaturation is not a readily reversible process. If a heat-denatured DNA solution is cooled slowly (annealing) and hold the solution at about 25°C below T_m and above a concentration of 0.4M Na^+ for several hours, some amount of
DNA (50-60%) is renatured. Rapid cooling does not reverse denaturation, but if the cooled solution is again heated and then cooled slowly, renaturation takes place.