Lecture 5:

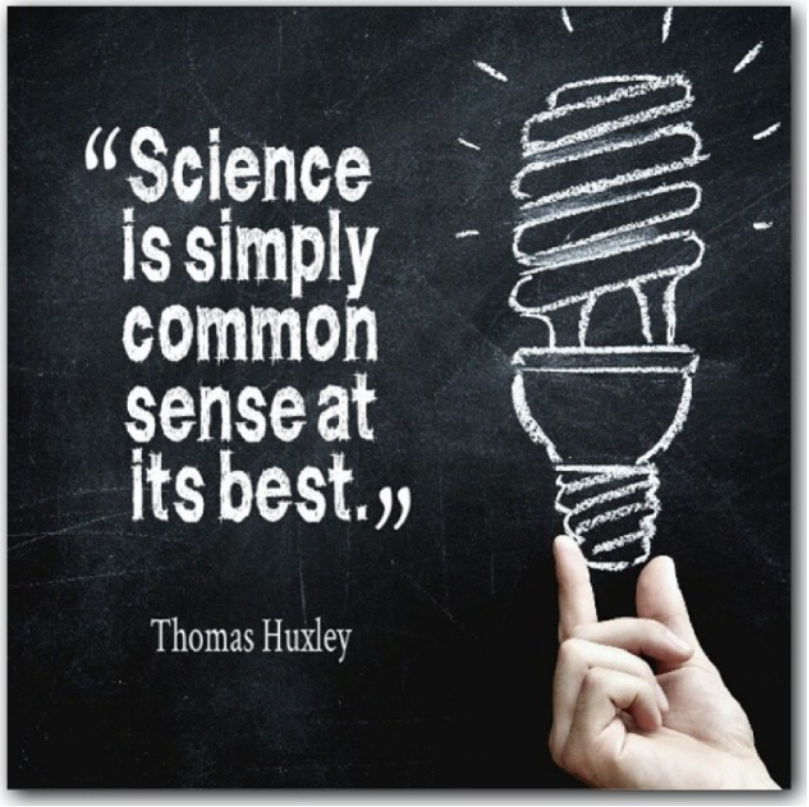
DNA:

The double helix structure

Readings (chapter 2)

Course 281

Lessons for life



AIMS

- Understand the molecular structure of DNA (the double helix).
- Learn the elements that led to the discovery of DNA structure.
- Enjoy the fact that what you are learning few people around the world know and enjoy studying ☺

Structure?

Now the chemical composition is understood

What about the structure?

Experiments and findings

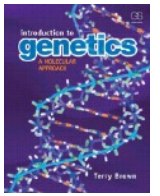
RESEARCH BRIEFING 2.1 THE DISCOVERY OF THE DOUBLE HELIX

How did Chargaff's experiments contribute to the discovering the DNA structure?

- Chargaff's base ratios paved the way for the correct structure.

What is the method used to discover the DNA structure?

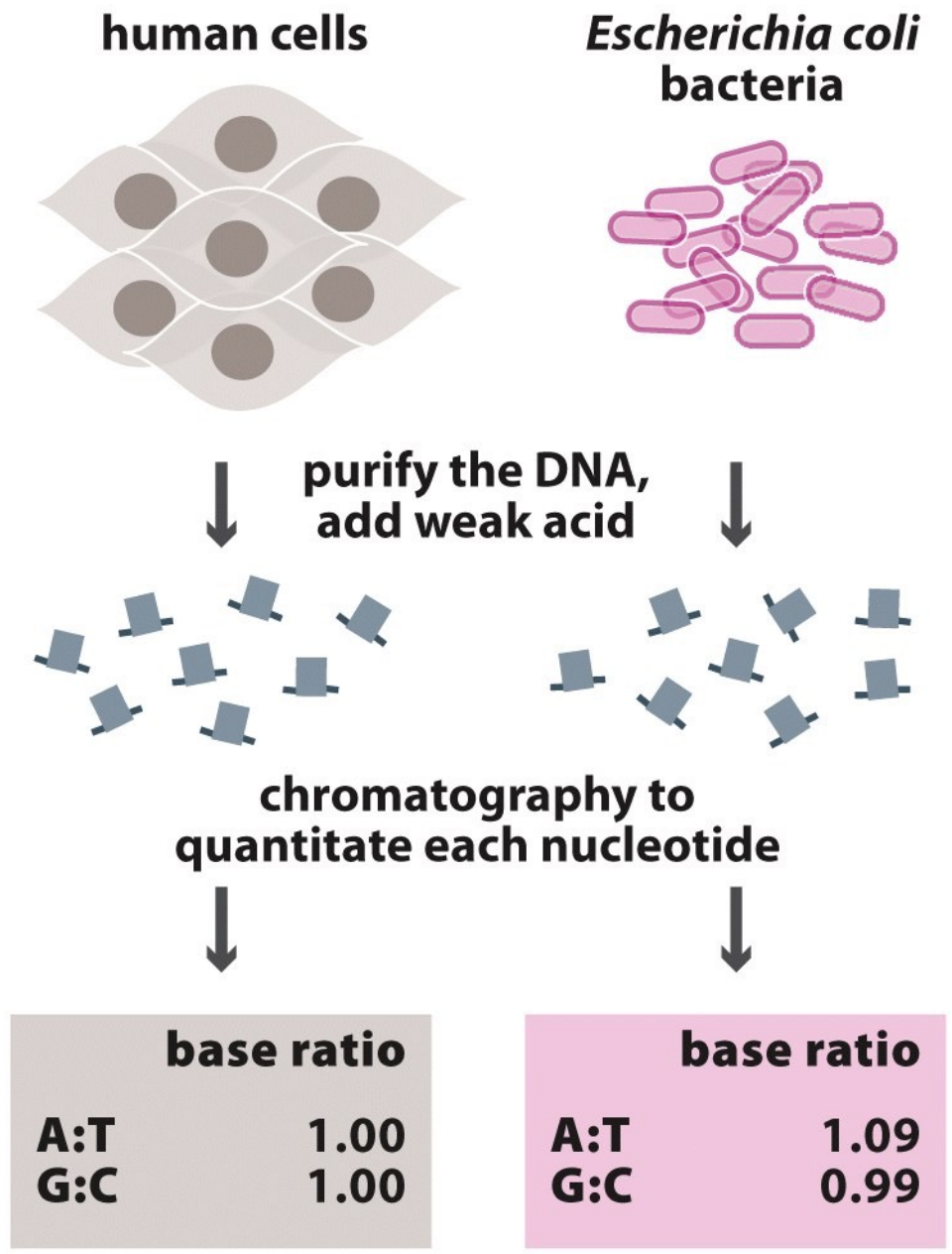
- X-ray diffraction analysis indicates that DNA is a helical molecule
- Pulling together the evidence



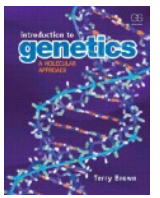
Chargaff's rule

- In a chemical study E. Chargaff found that pyrimidines and purines have equal ratios.
- 50% of nucleotides were purines and 50% were pyrimidines.

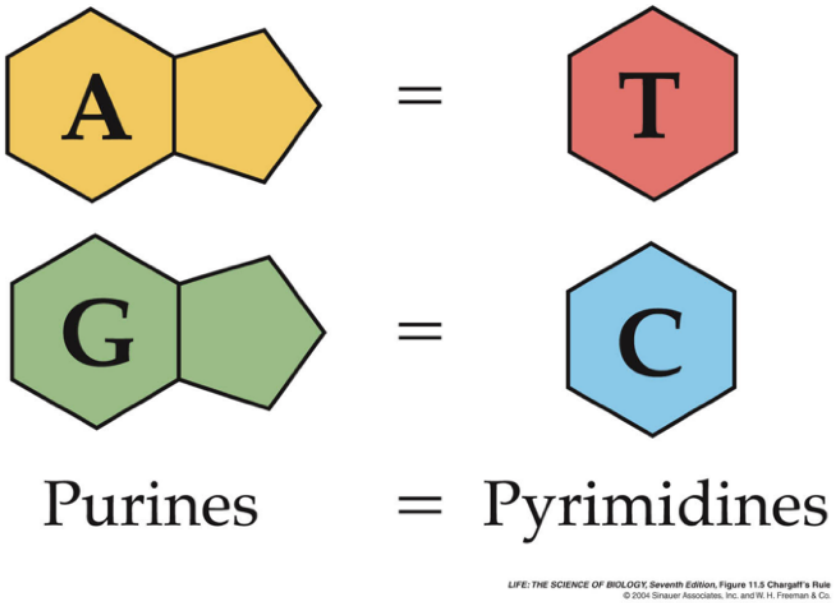
Erwin Chargaff



Research Briefing 2.1 Figure 2 Introduction to Genetics (© Garland Science 2012)



Chargaff's rule



LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 11.5 Chargaff's Rule
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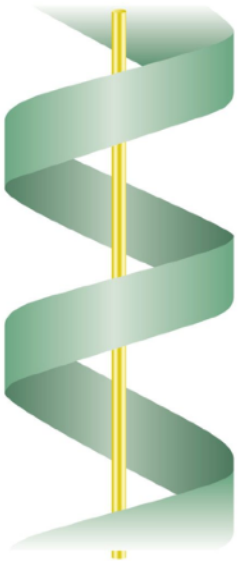
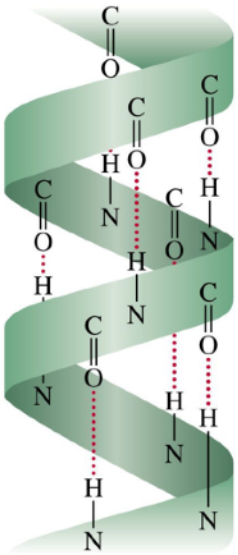
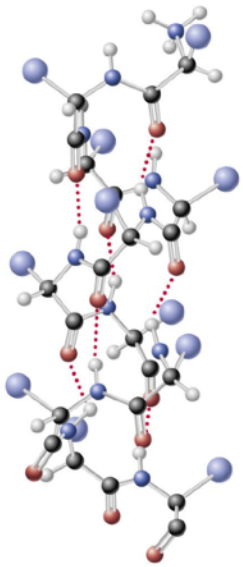
- The amount of Adenine = the amount of Thymine.
- The amount of Guanine = the amount of Cytosine.
- He failed to make a connection to the structure of DNA.
- Indicated that DNA is symmetrical.

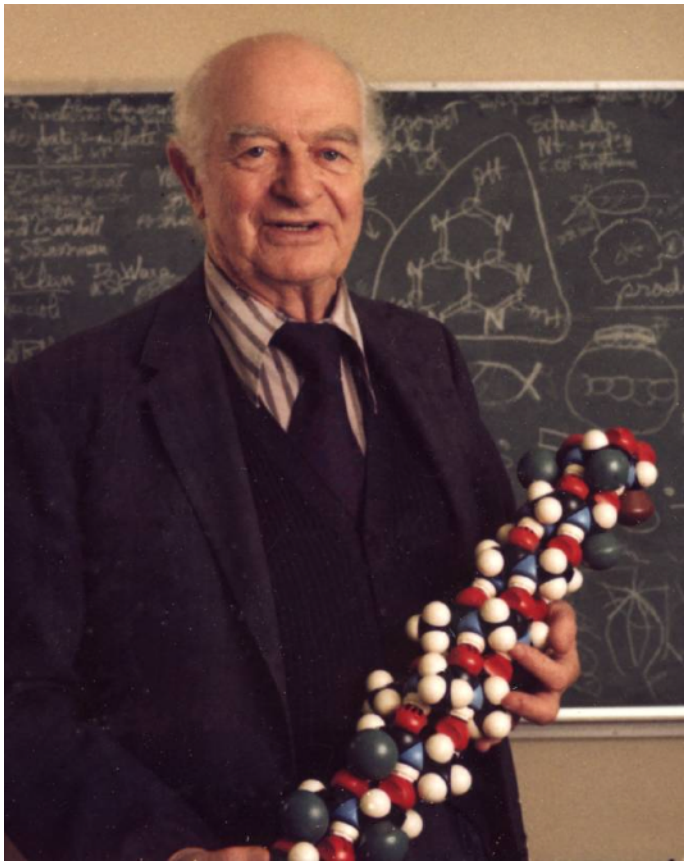
Pauling protein modeling

Linus Pauling discovered the alpha helix protein structure through modeling and x-ray diffraction.





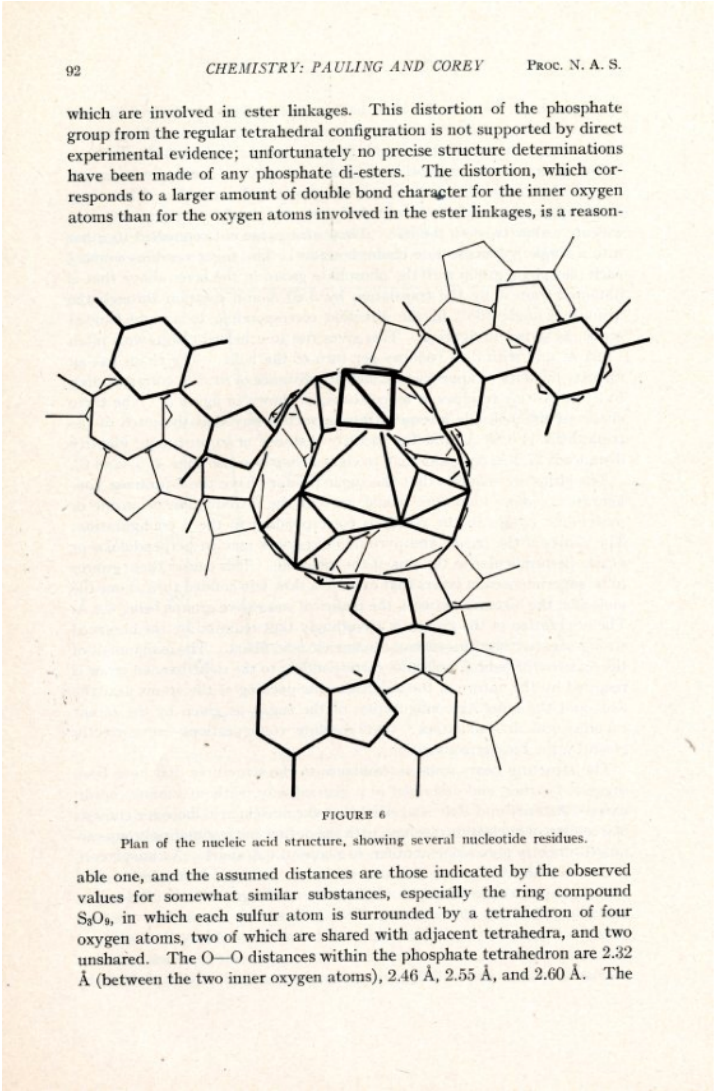




Pauling protein modeling

- Worked on the DNA structure but not very smart findings.

- Proposed three helices with bases pointing outside and phosphate-sugar backbone pointing inside.



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which are involved in ester linkages. This distortion of the phosphate group from the regular tetrahedral configuration is not supported by direct experimental evidence; unfortunately no precise structure determinations have been made of any phosphate di-esters. The distortion, which corresponds to a larger amount of double bond character for the inner oxygen atoms than for the oxygen atoms involved in the ester linkages, is a reason-

FIGURE 6

Plan of the nucleic acid structure, showing several nucleotide residues.

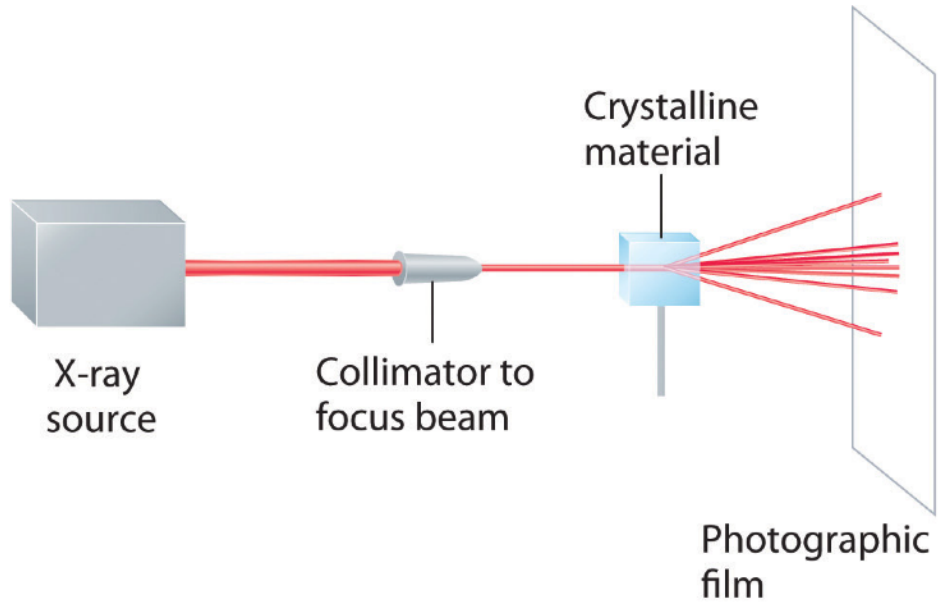
able one, and the assumed distances are those indicated by the observed values for somewhat similar substances, especially the ring compound S_3O_9, in which each sulfur atom is surrounded by a tetrahedron of four oxygen atoms, two of which are shared with adjacent tetrahedra, and two unshared. The O—O distances within the phosphate tetrahedron are 2.32 Å (between the two inner oxygen atoms), 2.46 Å, 2.55 Å, and 2.60 Å. The

Pauling protein modeling

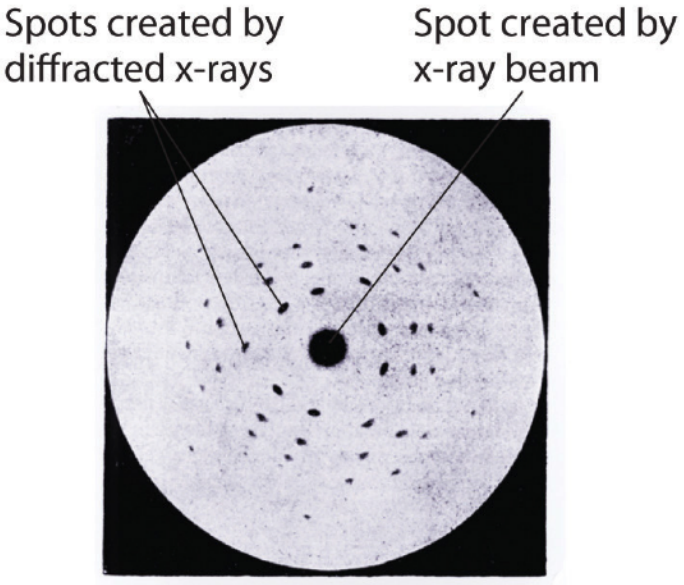
- Entering the race to find the structure of DNA was a blessing.
- His alpha helix is similar to that of DNA.
- His son Peter was a friend of James Watson and Francis Crick.
- They knew Pauling was after the structure so they wanted to win.

X-ray diffraction

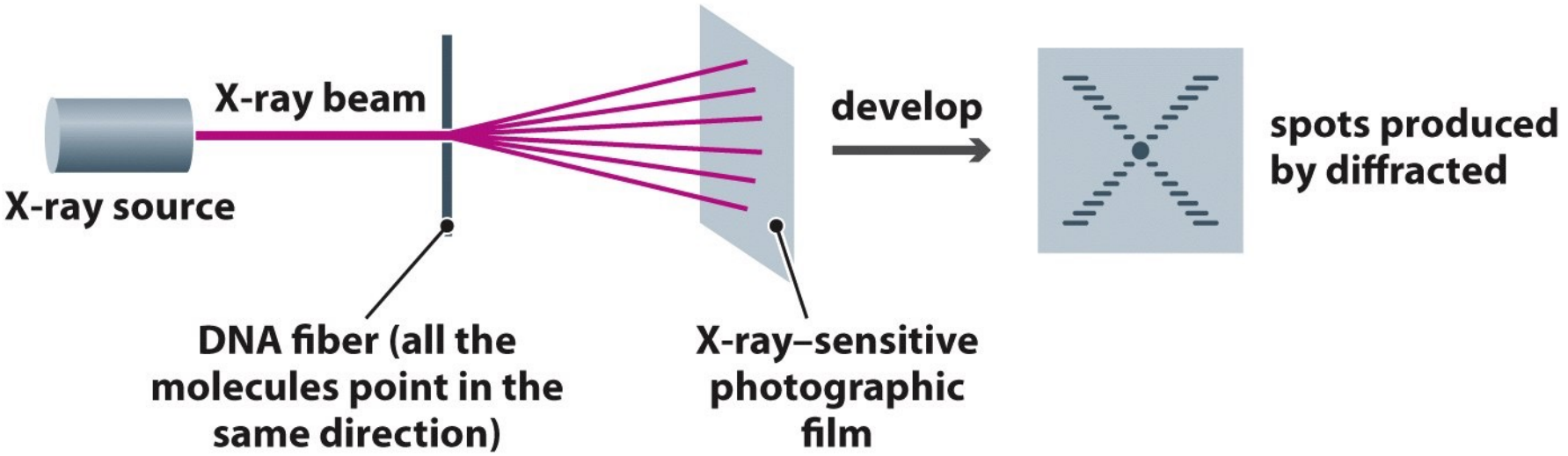
X-ray diffraction was the method to study the fine structure of molecules. DNA was no different!



(a) X-ray diffraction



(b) X-ray diffraction pattern captured on photographic film



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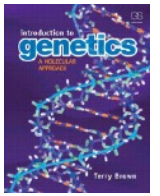
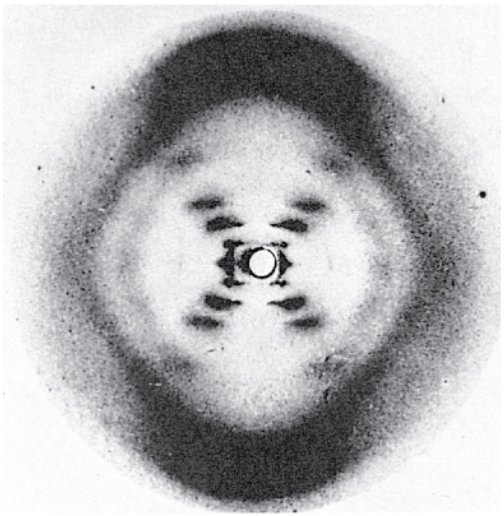
Chapter2



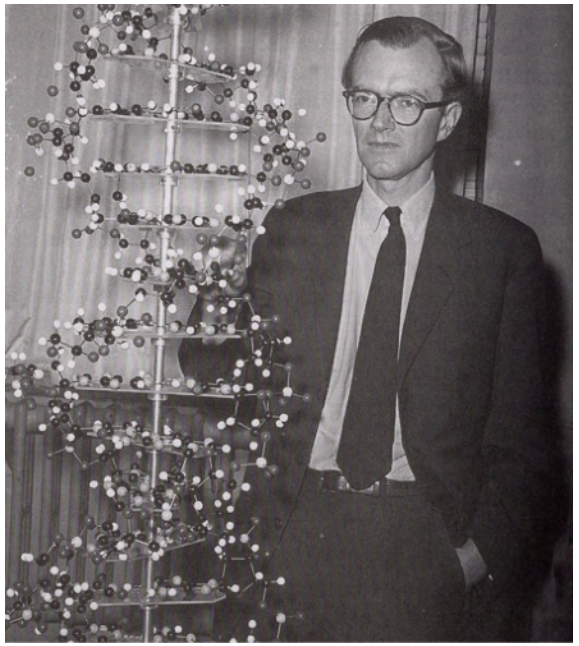
DNA X-ray diffraction

• At King's College London, Rosalind Franklin and Maurice Wilkin were working on X-ray diffraction of DNA.

(a) Rosalind Franklin

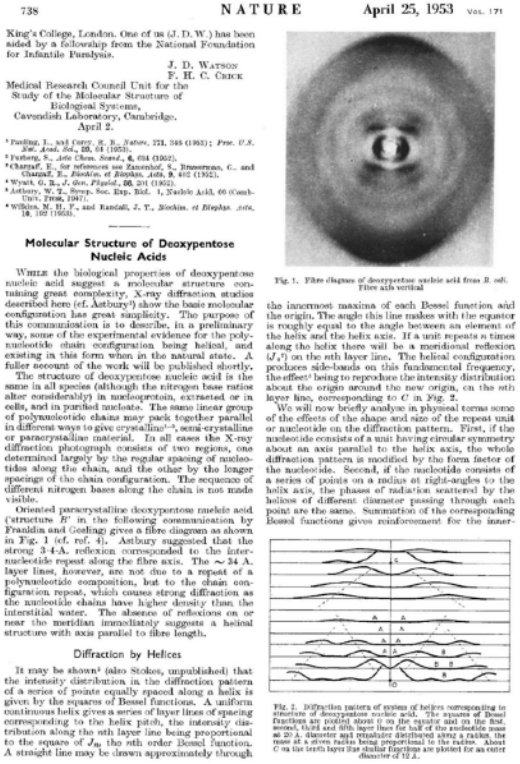


(b) Franklin's X-ray diffraction photograph of DNA



DNA X-ray diffraction

- R. Franklin produced the best diffraction photo (called photo 51).
- Her findings were shared (with or without her approval) Watson and Crick by Wilkin.



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King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. WATSON
F. H. C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge. April 2.

[1] Pauling, L., and Corey, R. B., *Nature*, **171**, 346 (1953); *Proc. U.S. Nat. Acad. Sci.*, **39**, 84 (1953).
[2] Furberg, S., *Acta Chem. Scand.*, **6**, 634 (1952).
[3] Charpaf, E., for references see Zamenhof, S., Brawerman, G., and Chargaf, E., *Biochim. et Biophys. Acta*, **9**, 402 (1952).
[4] Wyatt, G. R., *J. Gen. Physiol.*, **35**, 201 (1952).
[5] Astbury, W. T., Symp. Soc. Exp. Biol. 1, Nucleic Acid, 66 (Cambridge Univ. Press, 1947).
[6] Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta*, **10**, 192 (1953).

Molecular Structure of Deoxypentose Nucleic Acids

WHILE the biological properties of deoxypentose nucleic acid suggest a molecular structure containing great complexity, X-ray diffraction studies described here (cf. Astbury[5]) show the basic molecular configuration has great simplicity. The purpose of this communication is to describe, in a preliminary way, some of the experimental evidence for the polynucleotide chain configuration being helical, and existing in this form when in the natural state. A fuller account of the work will be published shortly.

The structure of deoxypentose nucleic acid is the same in all species (although the nitrogen base ratios alter considerably) in nucleoprotein, extracted or in cells, and in purified nucleate. The same linear group of polynucleotide chains may pack together parallel in different ways to give crystalline[1–5], semi-crystalline or paracrystalline material. In all cases the X-ray diffraction photograph consists of two regions, one determined largely by the regular spacing of nucleotides along the chain, and the other by the longer spacings of the chain configuration. The sequence of different nitrogen bases along the chain is not made visible.

Oriented paracrystalline deoxypentose nucleic acid ('structure B' in the following communication by Franklin and Gosling) gives a fibre diagram as shown in Fig. 1 (cf. ref. 4). Astbury suggested that the strong 3·4-A. reflexion corresponded to the inter-nucleotide repeat along the fibre axis. The ~ 34 A. layer lines, however, are not due to a repeat of a polynucleotide composition, but to the chain configuration repeat, which causes strong diffraction as the nucleotide chains have higher density than the interstitial water. The absence of reflexions on or near the meridian immediately suggests a helical structure with axis parallel to fibre length.

Diffraction by Helices

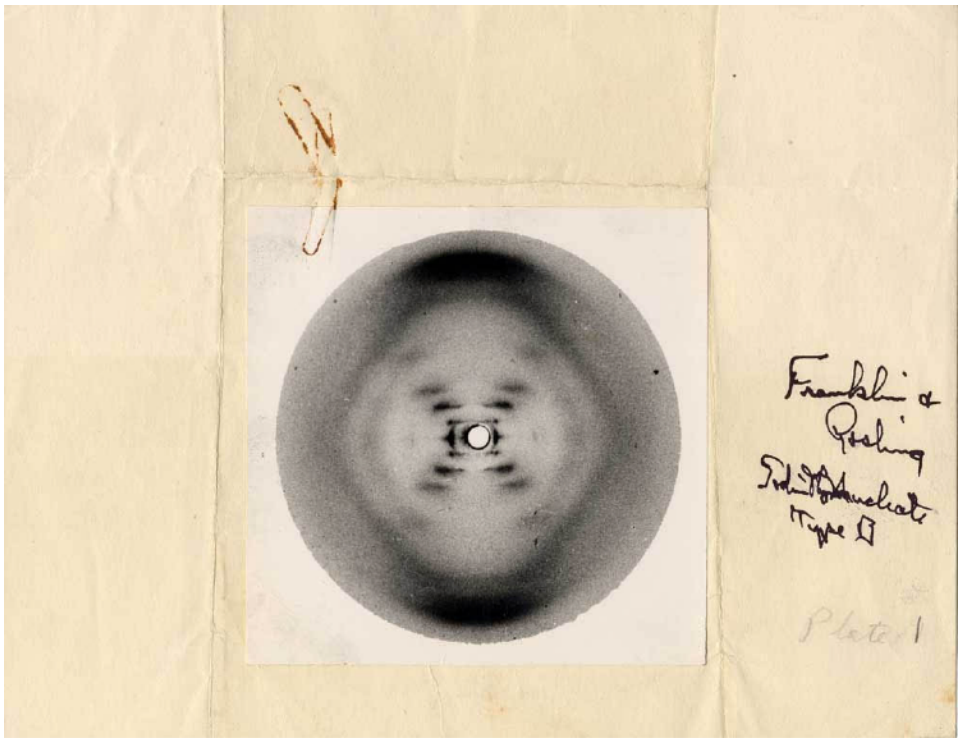
It may be shown[3] (also Stokes, unpublished) that the intensity distribution in the diffraction pattern of a series of points equally spaced along a helix is given by the squares of Bessel functions. A uniform continuous helix gives a series of layer lines of spacing corresponding to the helix pitch, the intensity distribution along the nth layer line being proportional to the square of J_n, the nth order Bessel function. A straight line may be drawn approximately through

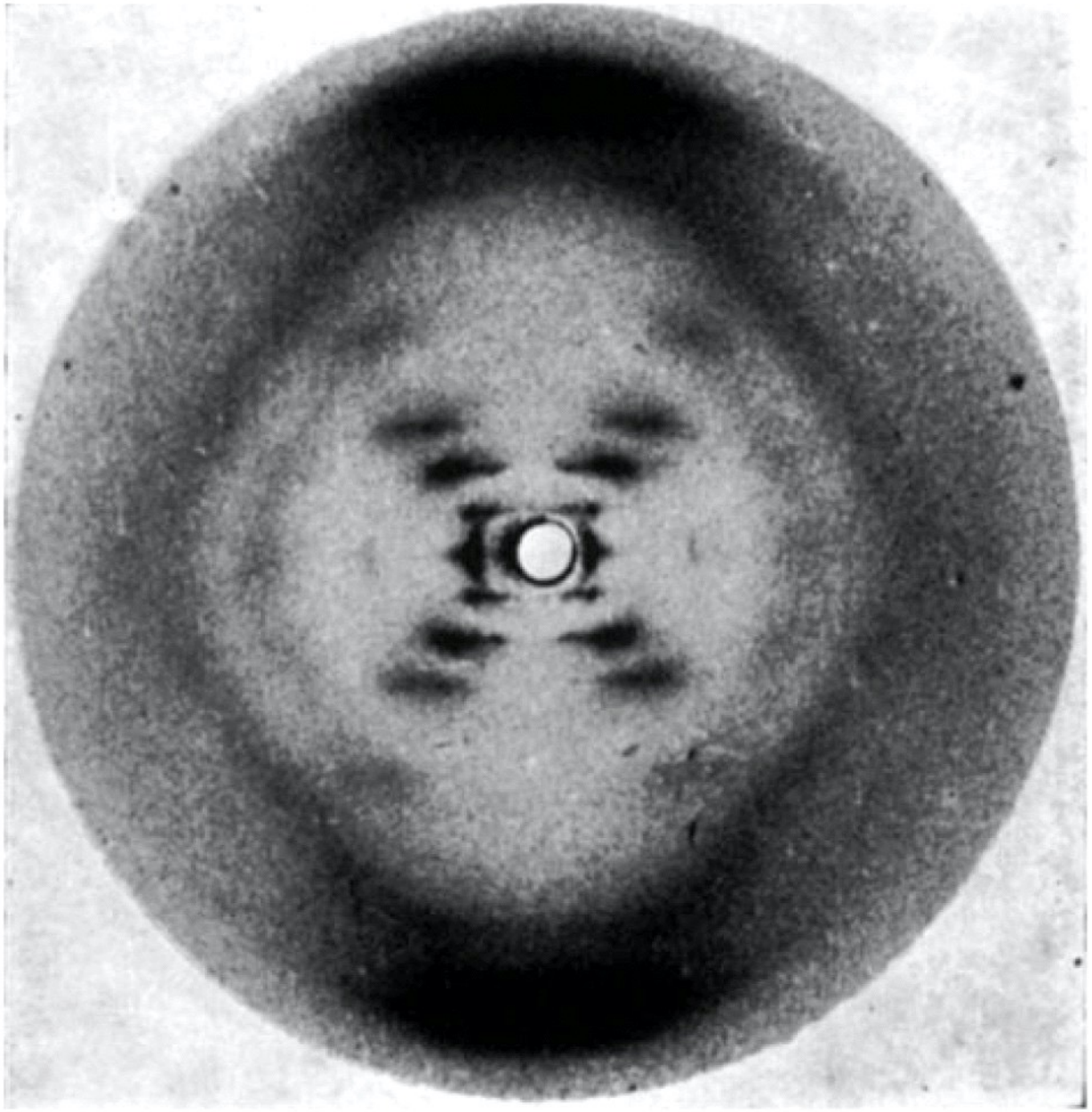
Fig. 1. Fibre diagram of deoxypentose nucleic acid from *B. coli*. Fibre axis vertical

the innermost maxima of each Bessel function and the origin. The angle this line makes with the equator is roughly equal to the angle between an element of the helix and the helix axis. If a unit repeats n times along the helix there will be a meridional reflexion (J_n[2]) on the nth layer line. The helical configuration produces side-bands on this fundamental frequency, the effect being to reproduce the intensity distribution about the origin around the new origin, on the nth layer line, corresponding to C in Fig. 2.

We will now briefly analyse in physical terms some of the effects of the shape and size of the repeat unit or nucleotide on the diffraction pattern. First, if the nucleotide consists of a unit having circular symmetry about an axis parallel to the helix axis, the whole diffraction pattern is modified by the form factor of the nucleotide. Second, if the nucleotide consists of a series of points on a radius at right-angles to the helix axis, the phases of radiation scattered by the helices of different diameter passing through each point are the same. Summation of the corresponding Bessel functions gives reinforcement for the inner-

Fig. 2. Diffraction pattern of system of helices corresponding to structure of deoxypentose nucleic acid. The squares of Bessel functions are plotted about 0 on the equator and on the first, second, third and fifth layer lines for half of the nucleotide mass at 20 A. diameter and remainder distributed along a radius, the mass at a given radius being proportional to the radius. About C on the tenth layer line similar functions are plotted for an outer diameter of 12 A.





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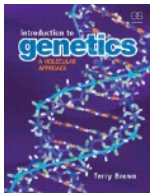
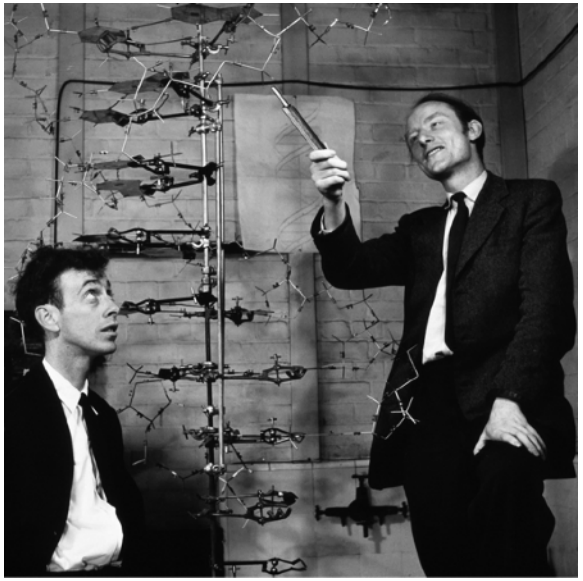
Chapter2



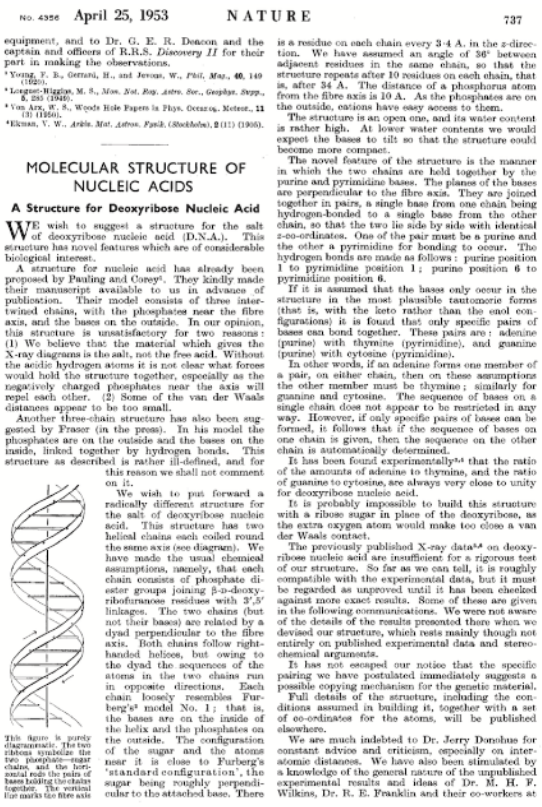
Watson and Crick

- Watson and Crick used the empirical data of Franklin, Wilkin, and Chargaff to come up with a model of the DNA structure.
- It was an important finding to the field of molecular biology and genetics.



Watson and Crick

- They published a 900 words paper and Franklin and Wilkin also published on the same issue of Nature.



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equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

¹ Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1925).
² Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).
³ Van Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).
⁴ Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

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

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

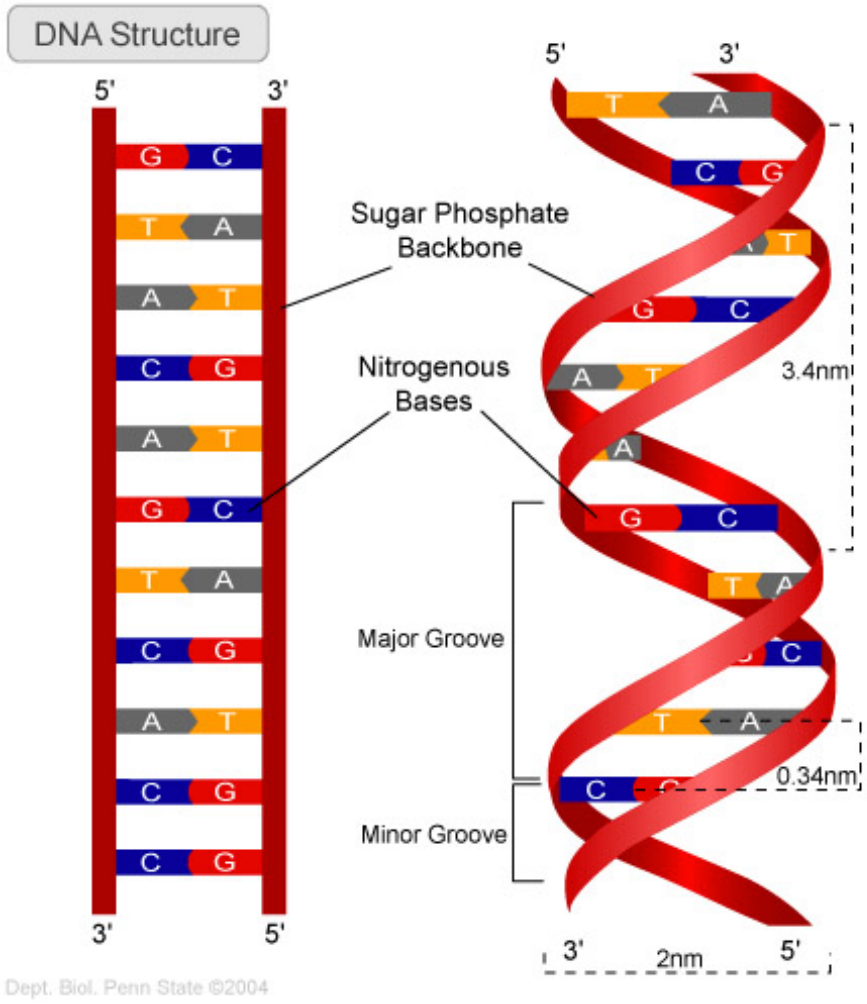
This figure is purely diagrammatic. The two ribbons symbolize the two phosphate–sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

DNA structure

1) DNA is a double helix.

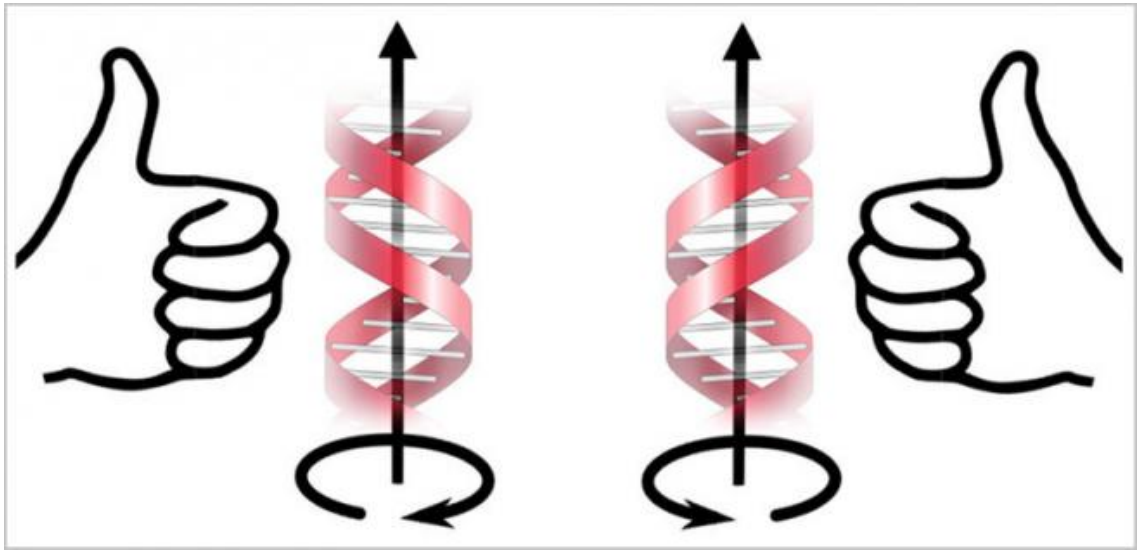
DNA structure

2) Two polynucleotides chains.



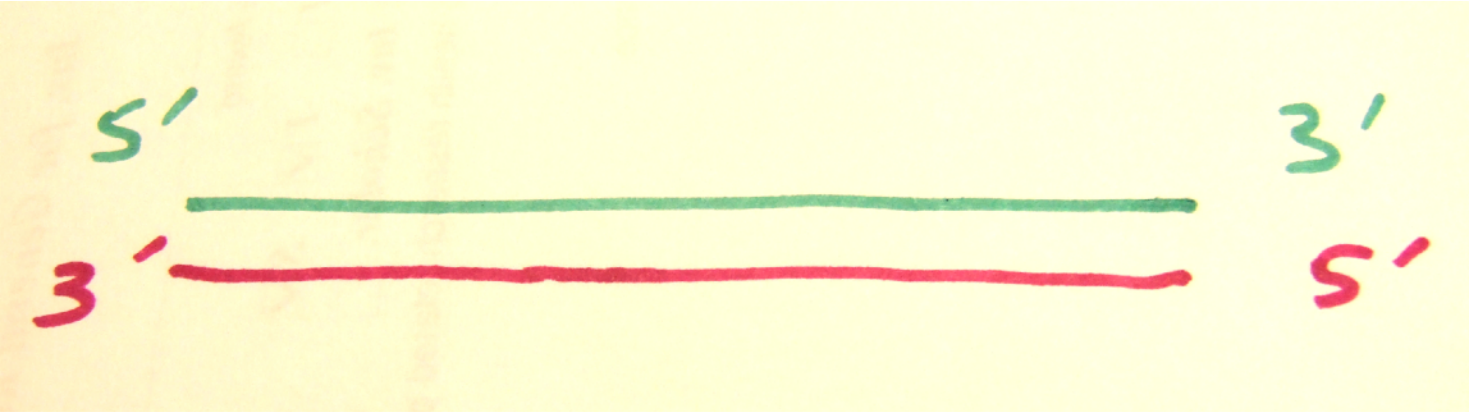
DNA structure

3) The two chains wind around right handedly - right handed double helix.



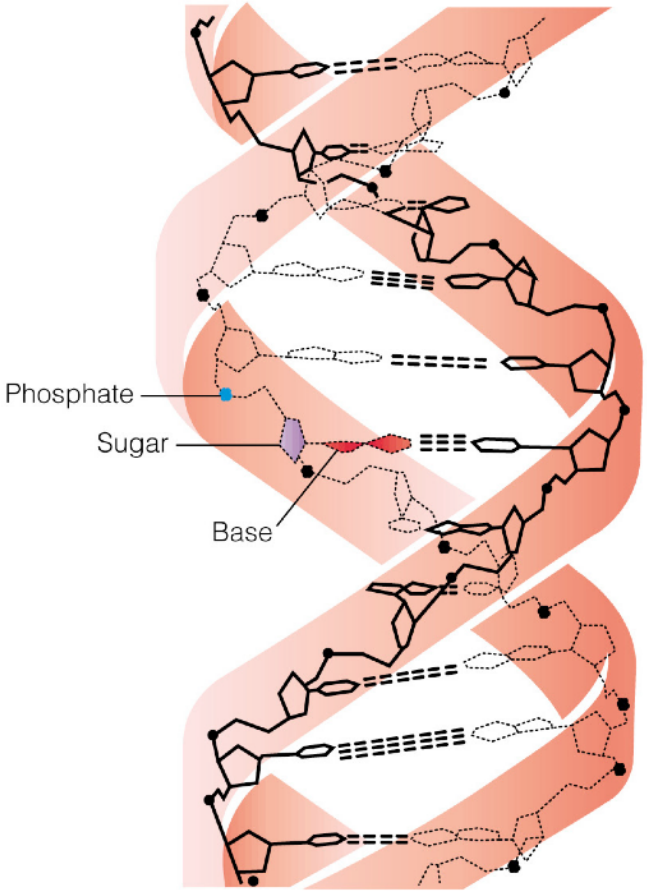
DNA structure

4) The two chains are in an anti-parallel orientation. (one strand 5’ – 3’ orientation and the other 3’ – 5’).



DNA structure

5) Sugar-phosphate backbone is located on the outside of the helix.

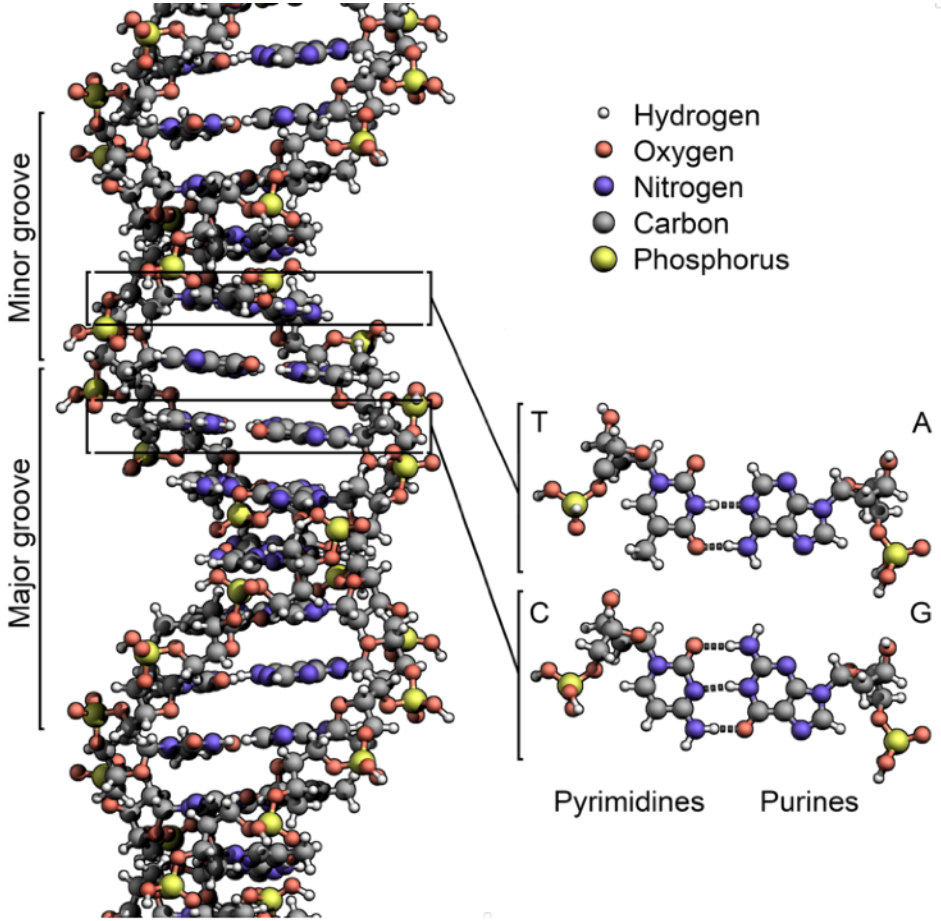


(a)

(b)

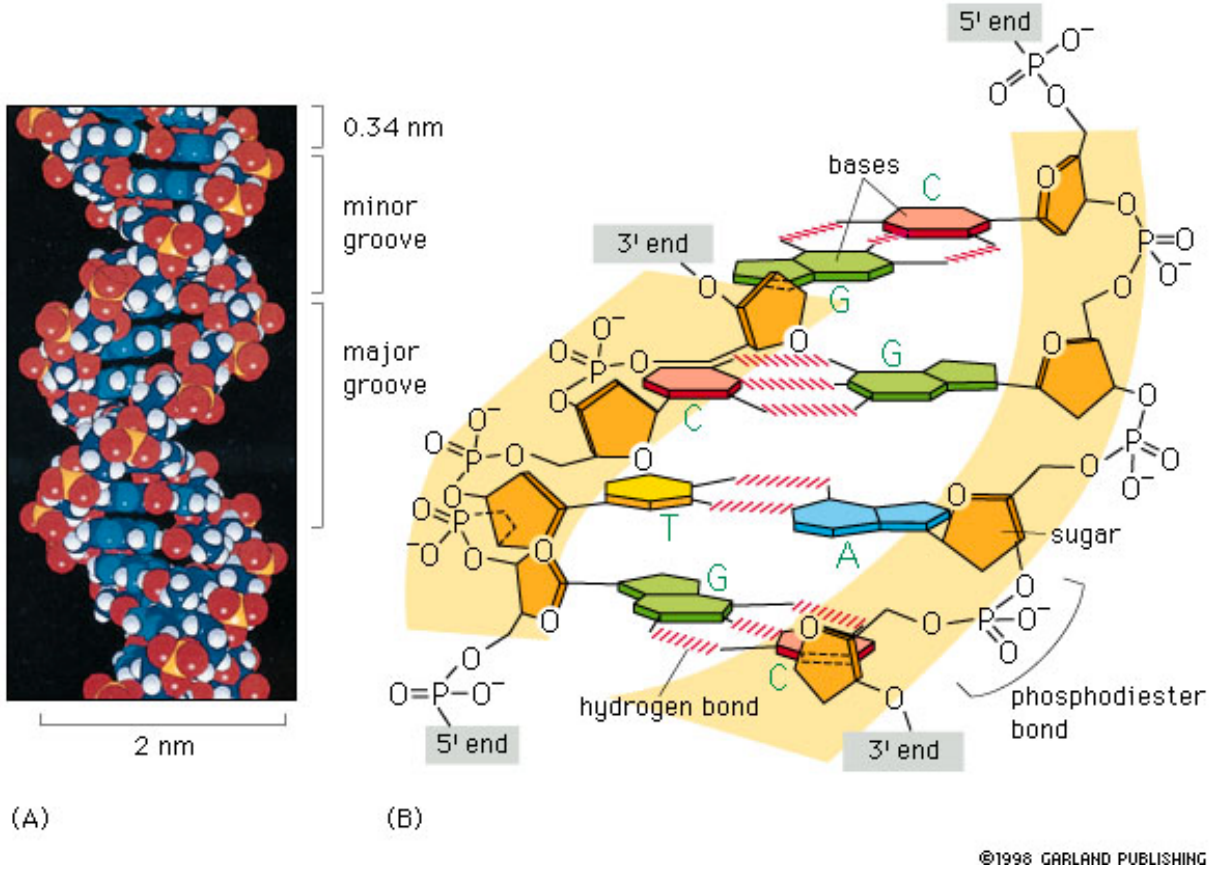
DNA structure

6) The nitrogenous bases located on the inside of the helix.



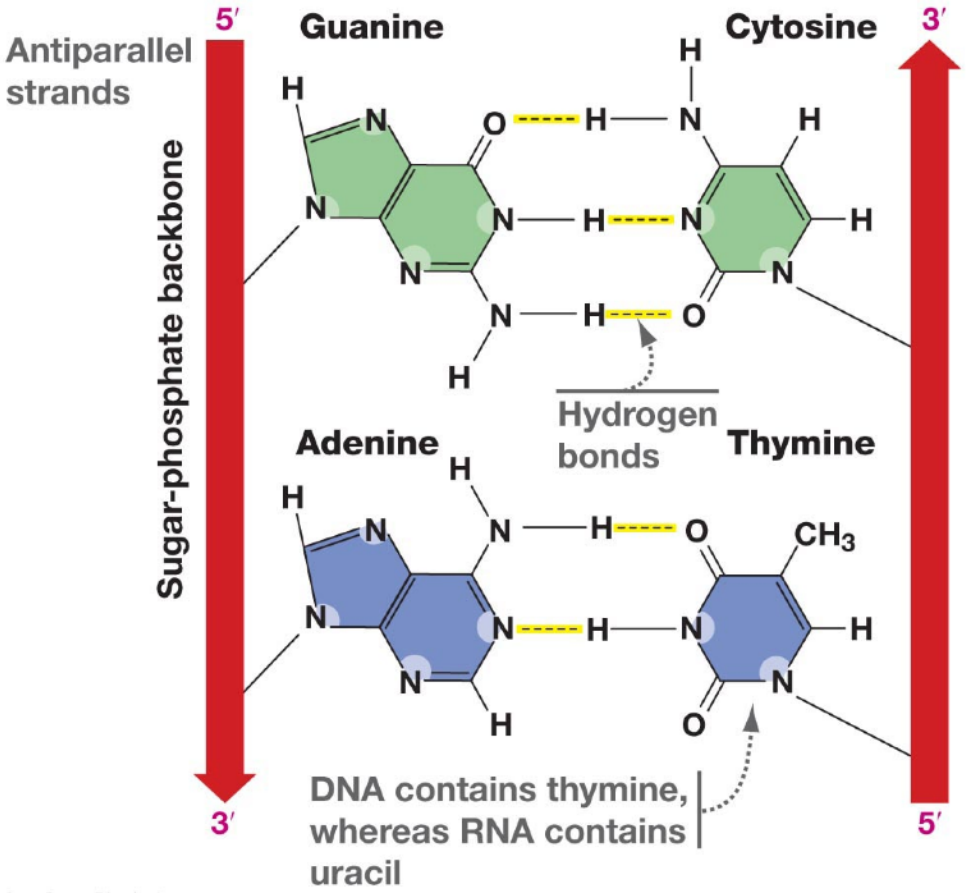
DNA structure

7) The bases are stacked flat and perpendicular to the axis of the helix. The bases are on top of each other following the twist of the helix.



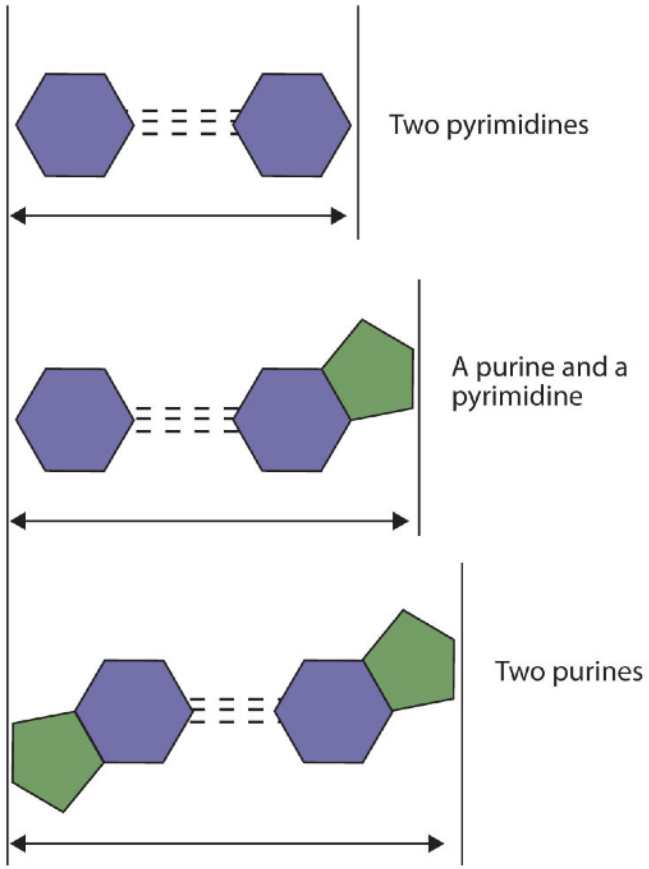
DNA structure

8) The bases of the two polynucleotides are bonded together via hydrogen bonds on the inside of the helix.



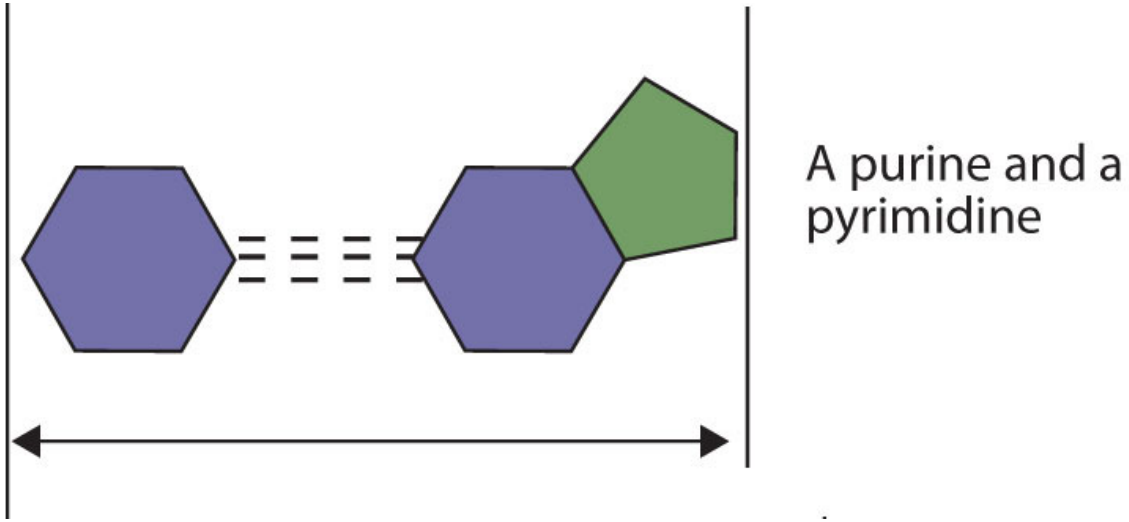
DNA structure

9) Bases of the two polynucleotide chains are base-pairing to maintain similar diameter of the double helix.



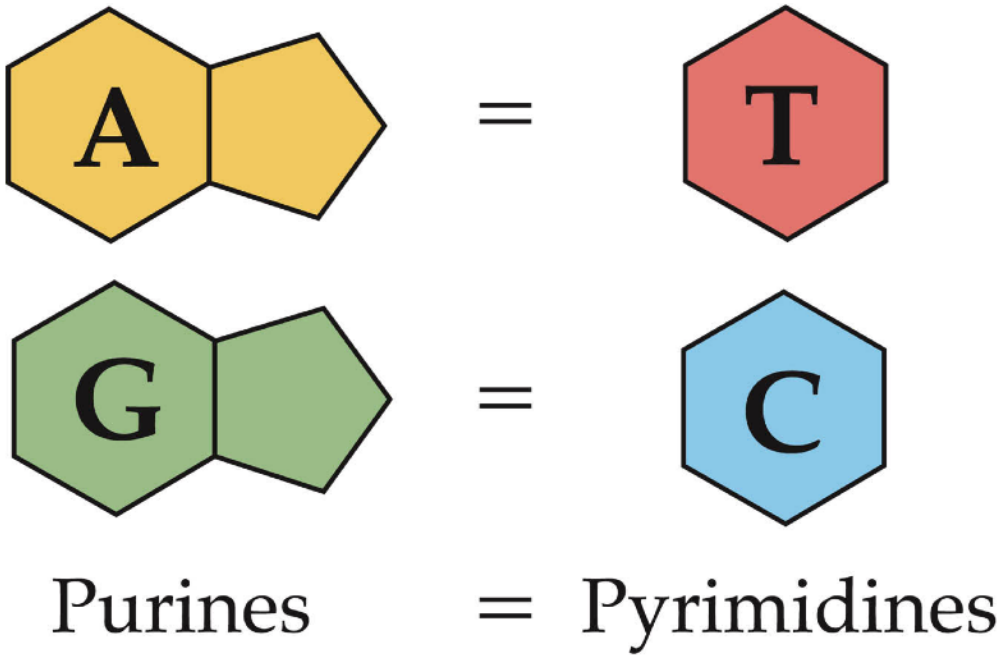
DNA structure

10) A Pyrimidine always basepair with Purine forming **complementary base pairs.**



DNA structure

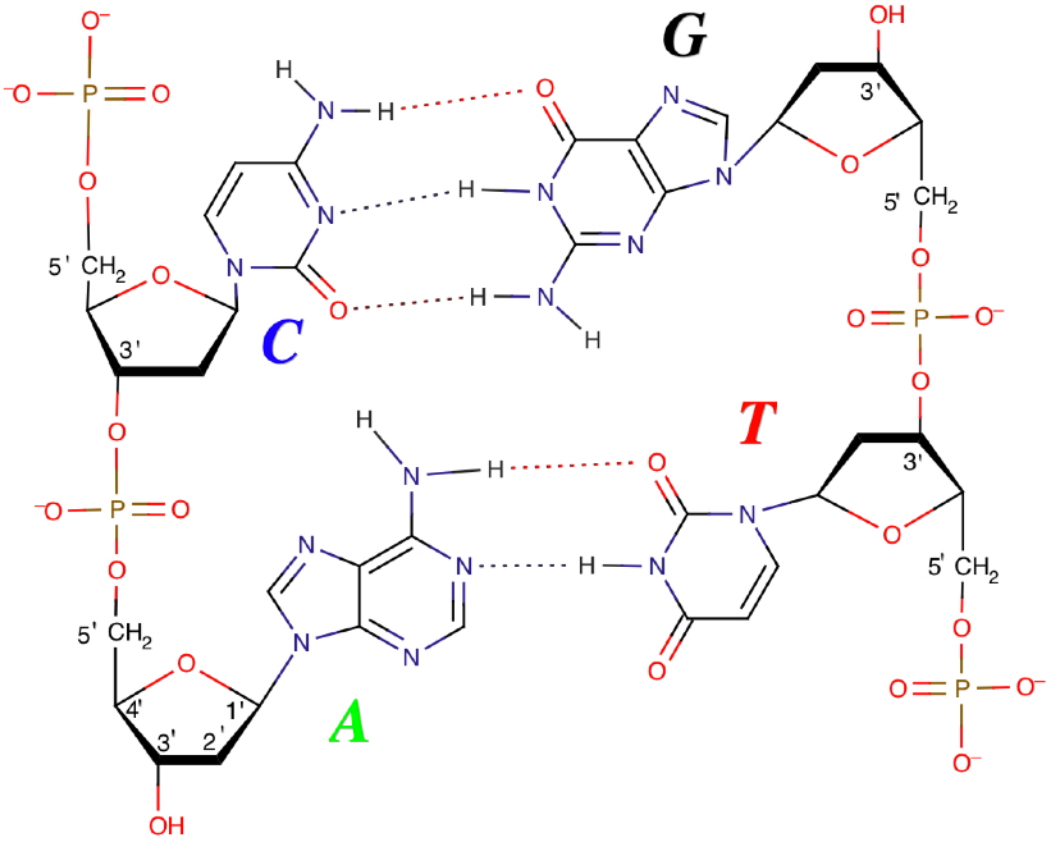
11) Thymine (T) basepair with Adenine (A), and Cytosine basepair with Guanine (G). Chargaff rule !!!!

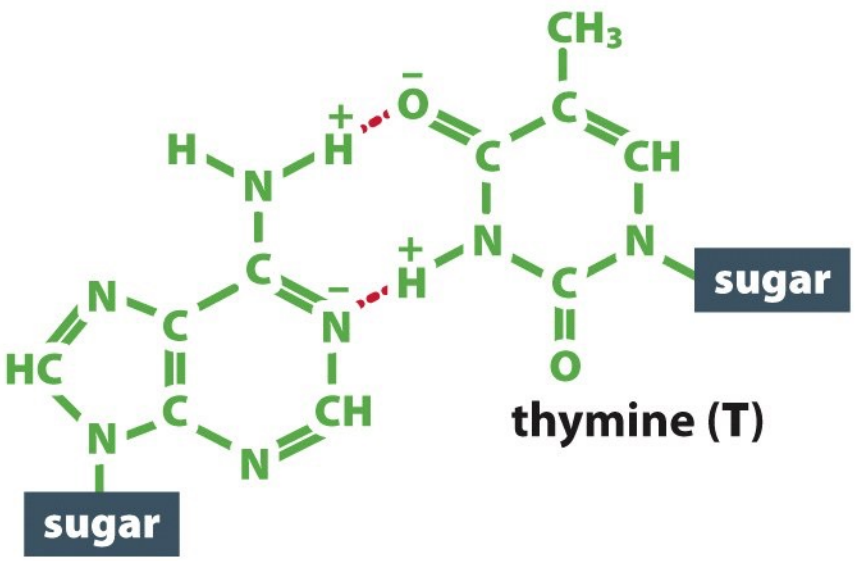


LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 11.5 Chargaff's Rule

DNA structure

12) Two hydrogen bonds involve the base-pairing of (A and T) and three hydrogen bonds between (G and C).





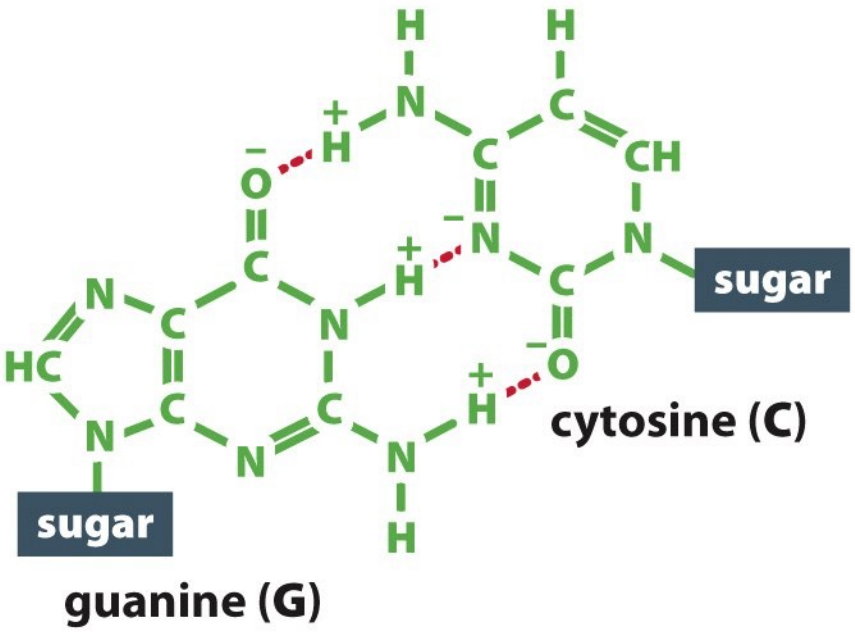
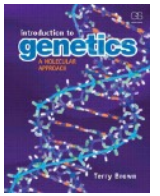


Figure 2.7 Introduction to Genetics (© Garland Science 2012)

Chapter2

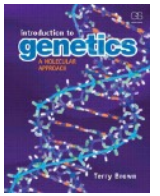


What are hydrogen bonds?

Are hydrogen bonds temporary or permanent bonds?

How are hydrogen bonds formed or deformed?

Chapter2



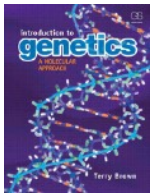
2.2 THE MOLECULAR EXPLANATION OF THE BIOLOGICAL ROLE OF DNA

How and where is biological information contained?

- Biological information is contained in the nucleotide sequence of a DNA molecule

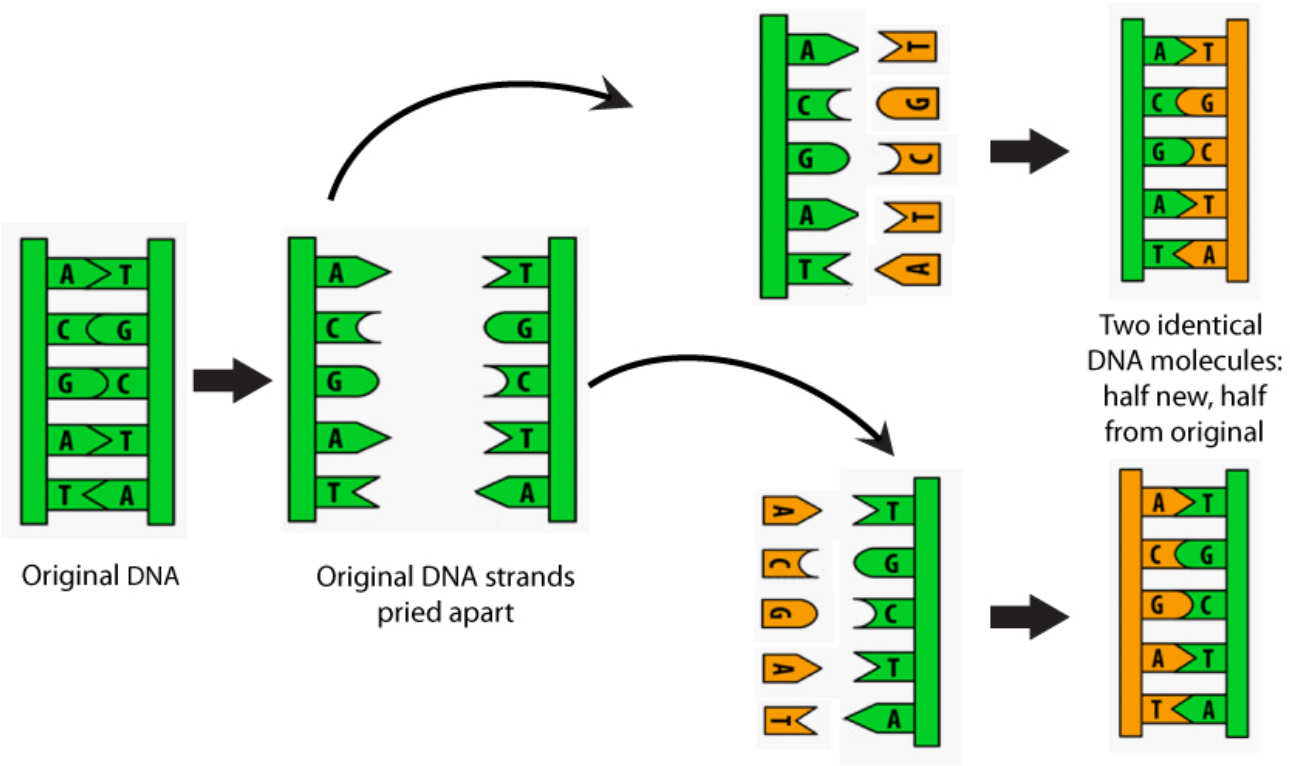
What is complementary base-pairing and its significance to DNA replication?

- Complementary base pairing enables DNA molecules to replicate



DNA structure

13) The sequence of one chain (strand) is enough to predict the complementary one in the other orientation.



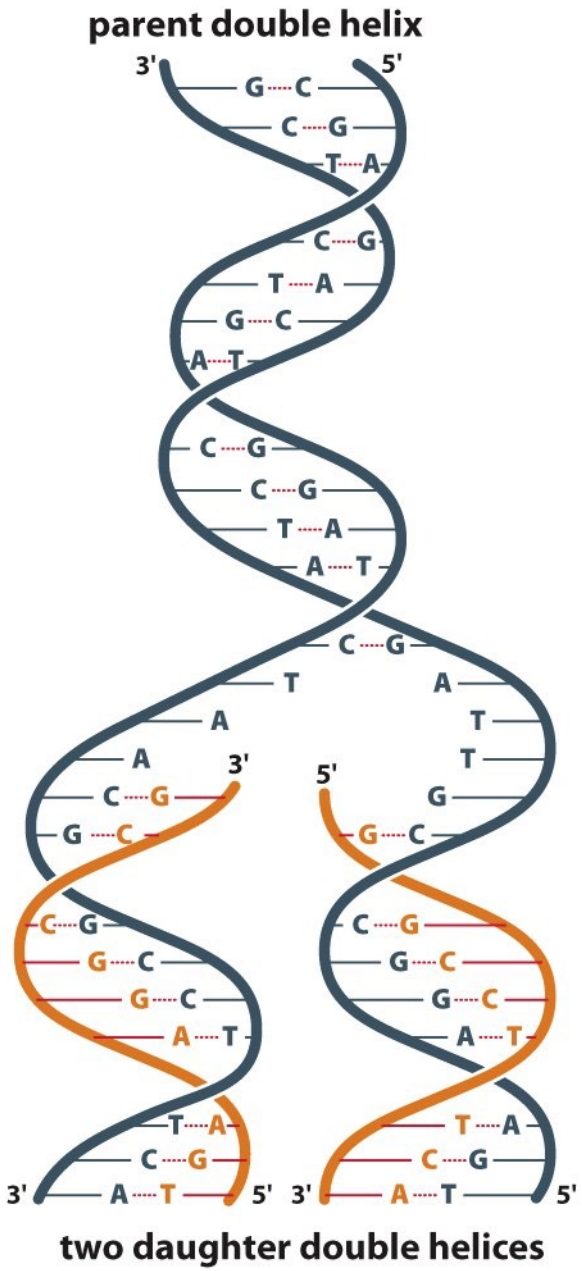


Figure 2.14 Introduction to Genetics (© Garland Science 2012)

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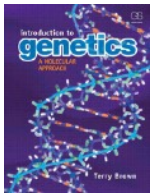


Quiz

What is the complementary sequence of the following?

5’ A-T-G-C-G-G-G-A-A-A-T-T-T-C-C-C ‘3

a) 5’ A-T-G-C-G-G-G-A-A-A-T-T-T-C-C-C ‘3

b) 5’ T-A-C-G-C-C-C-T-T-T-A-A-A-G-G-G ‘3

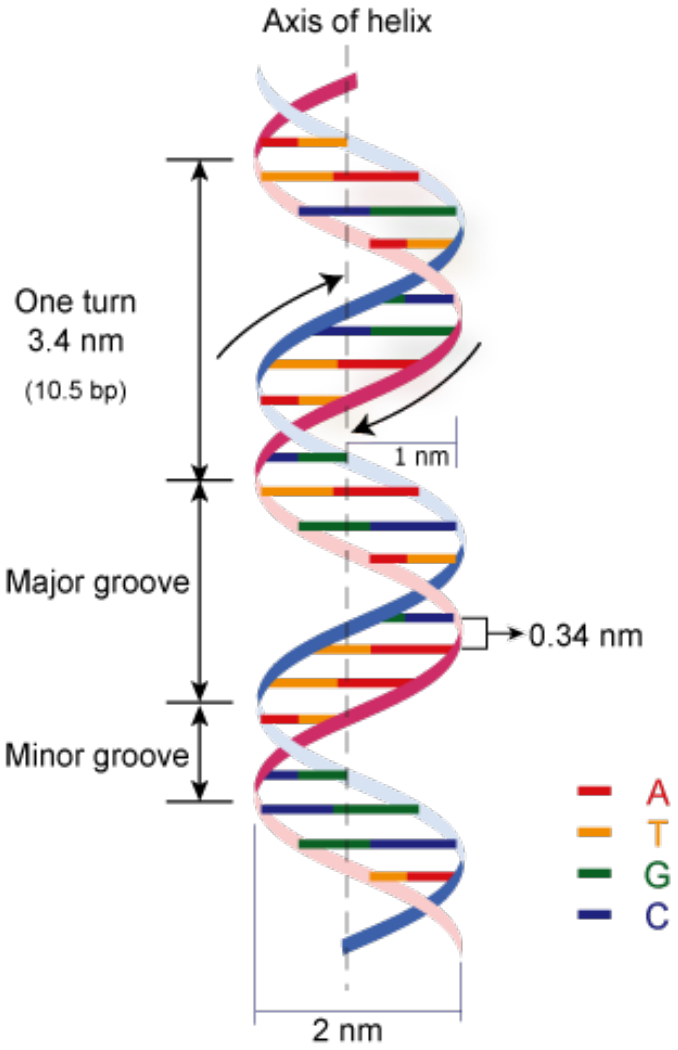
c) 5’ G-G-G-A-A-A-T-T-T-C-C-C-G-C-A-T ‘3

d) a and b

e) b and c

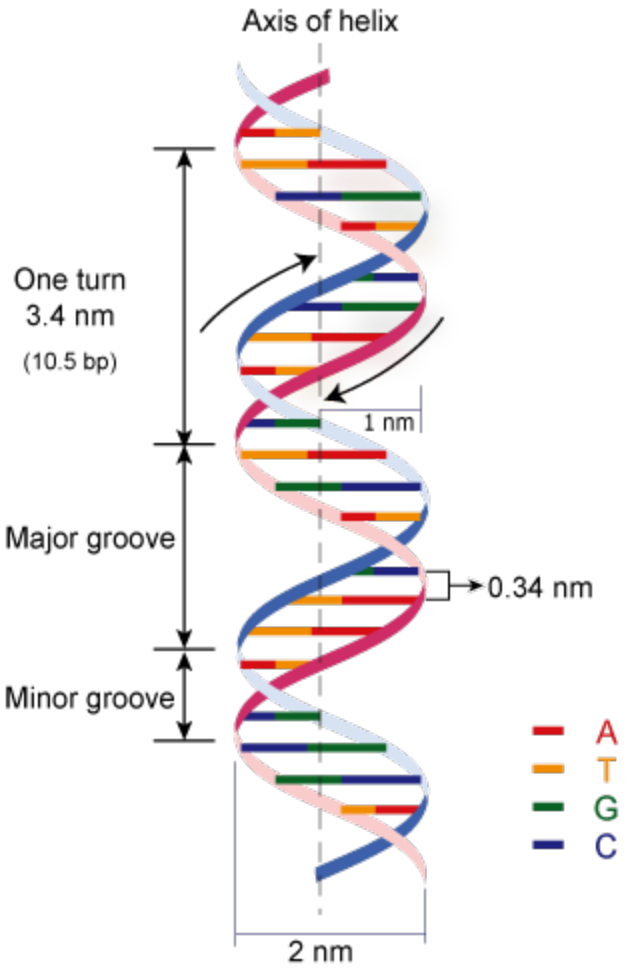
DNA structure

14) The bases are 0.34 nm apart (nm = 10^{-9}).



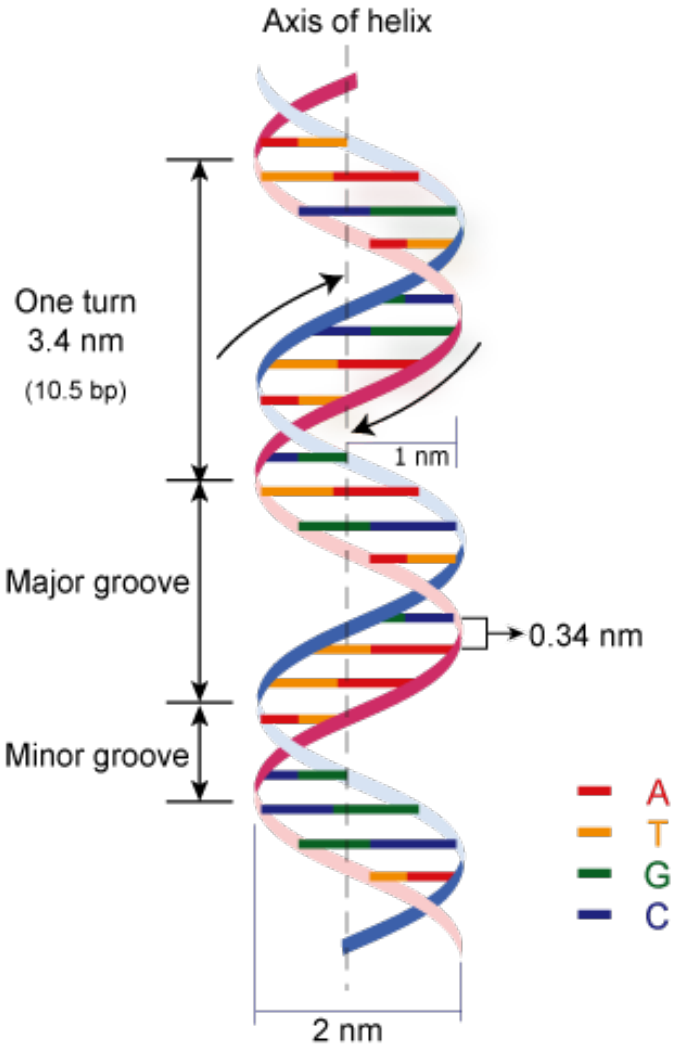
DNA structure

15) One turn of the helix is achieved (360°) every 10 basepairs or 3.4 nm.



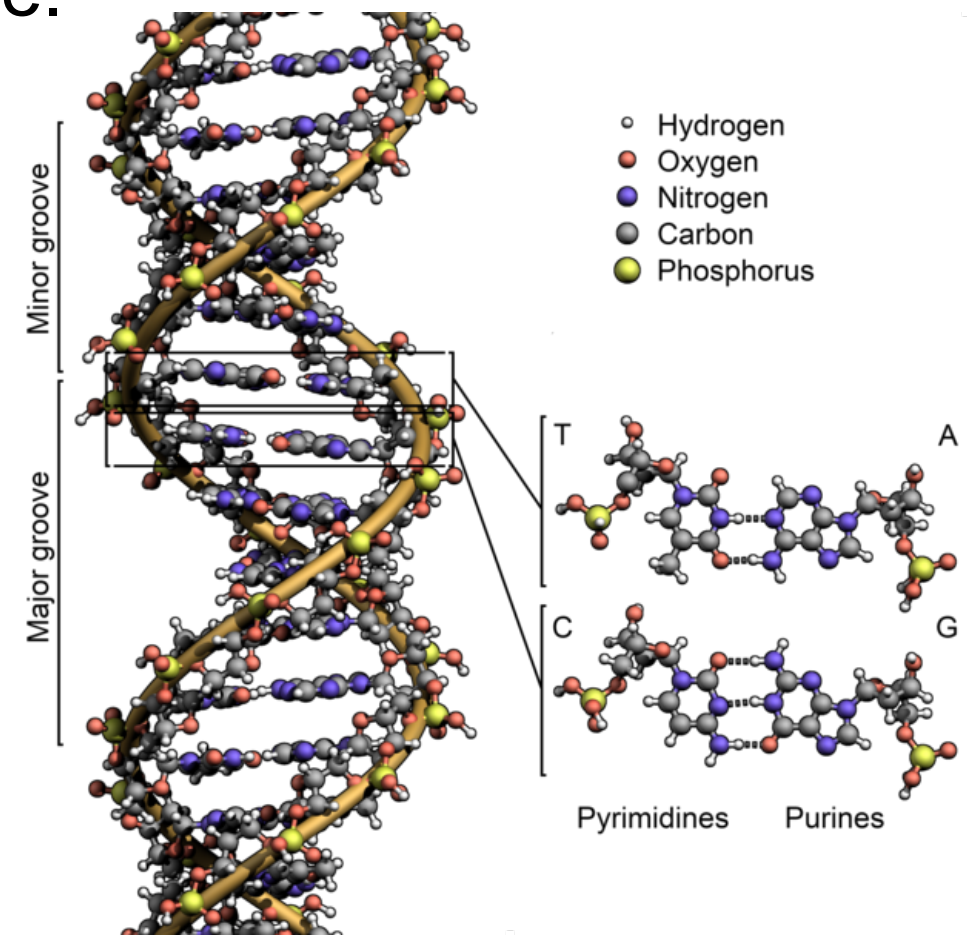
DNA structure

16) The double helix external diameter is 2nm.



DNA structure

17) A major and minor groove result from the unequal spacing of the phosphate-sugar backbone.



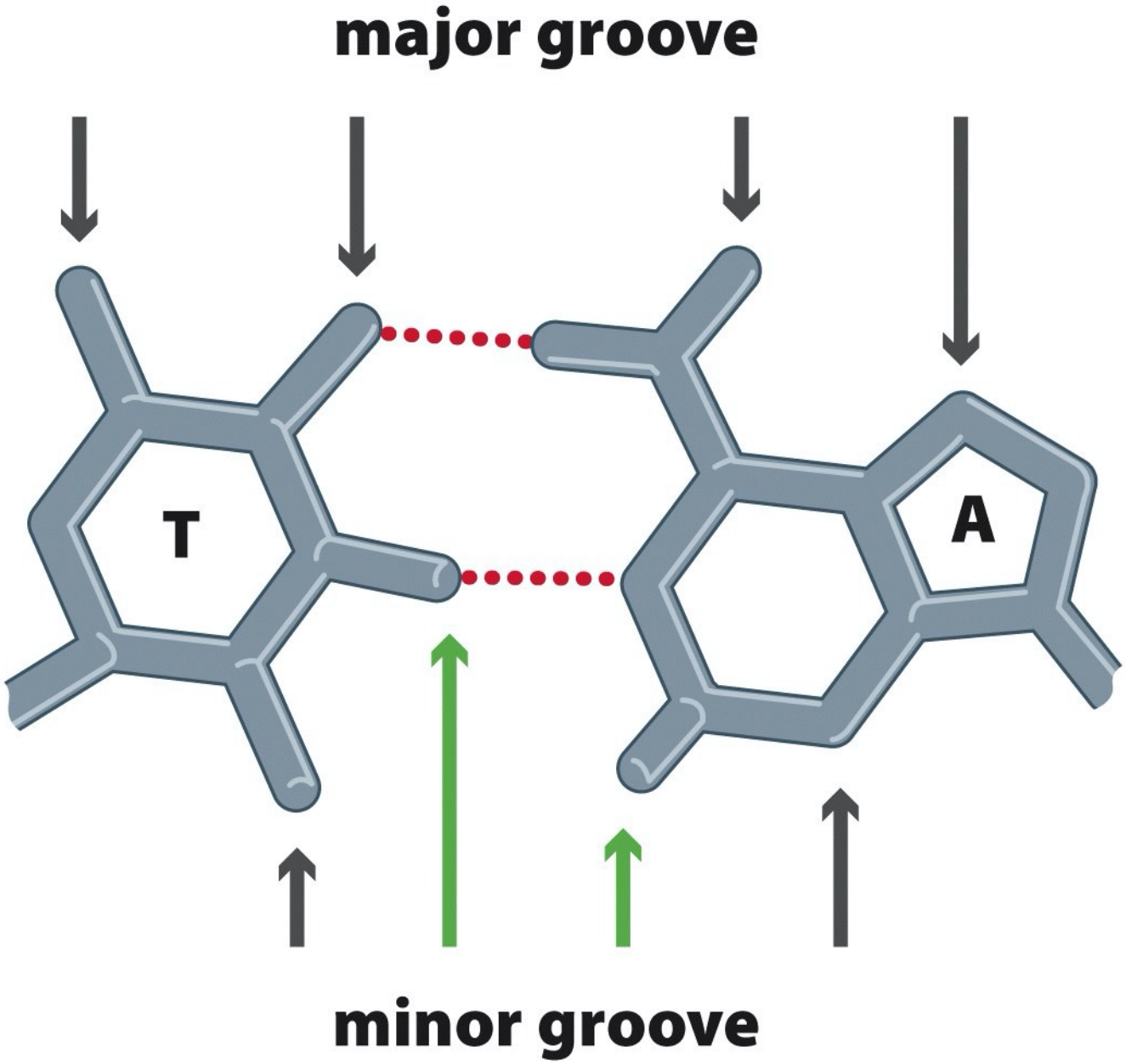
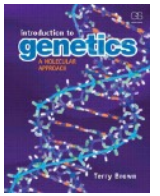


Figure 2.10 Introduction to Genetics (© Garland Science 2012)



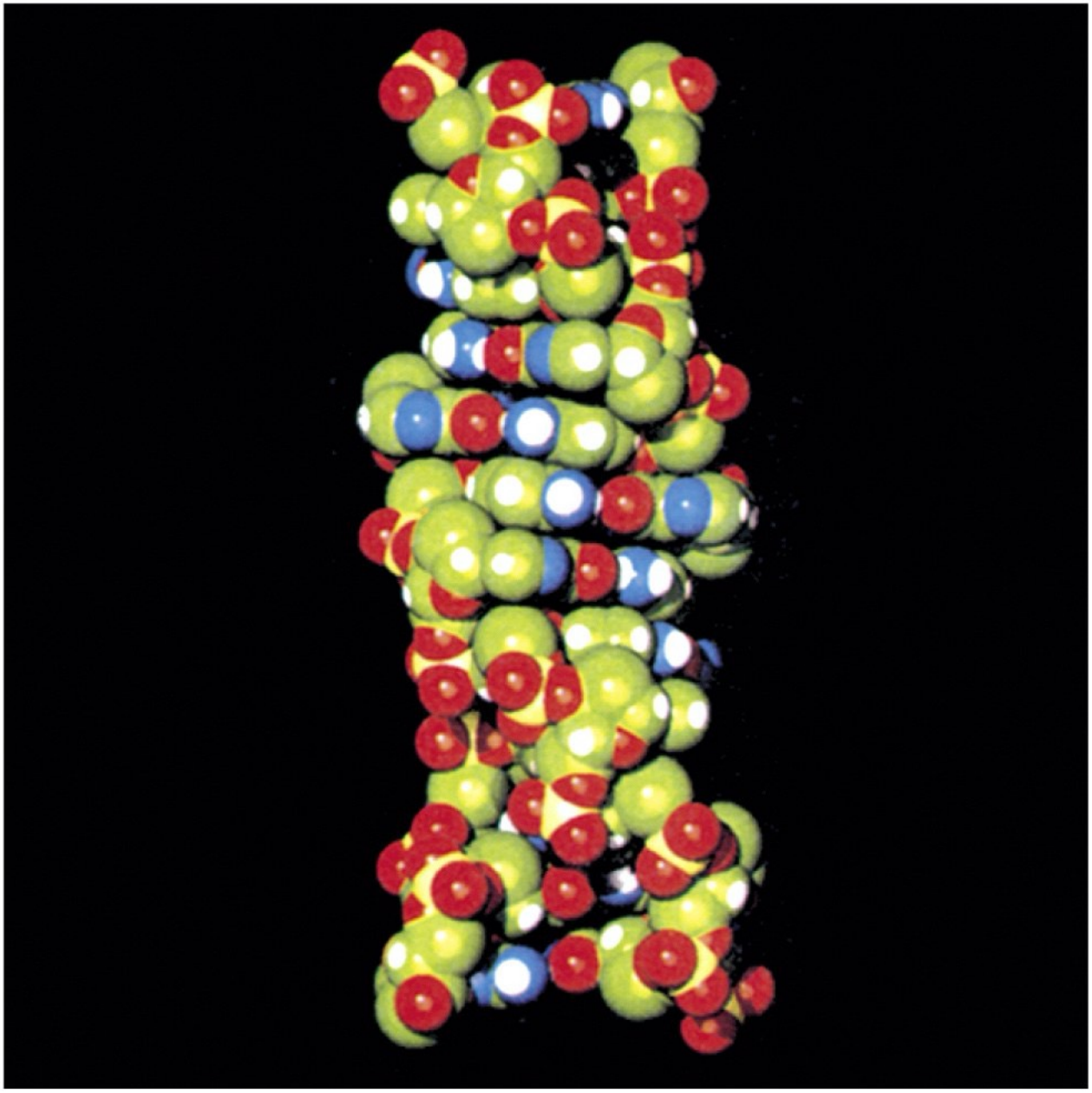
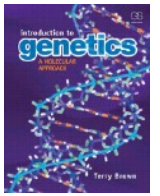
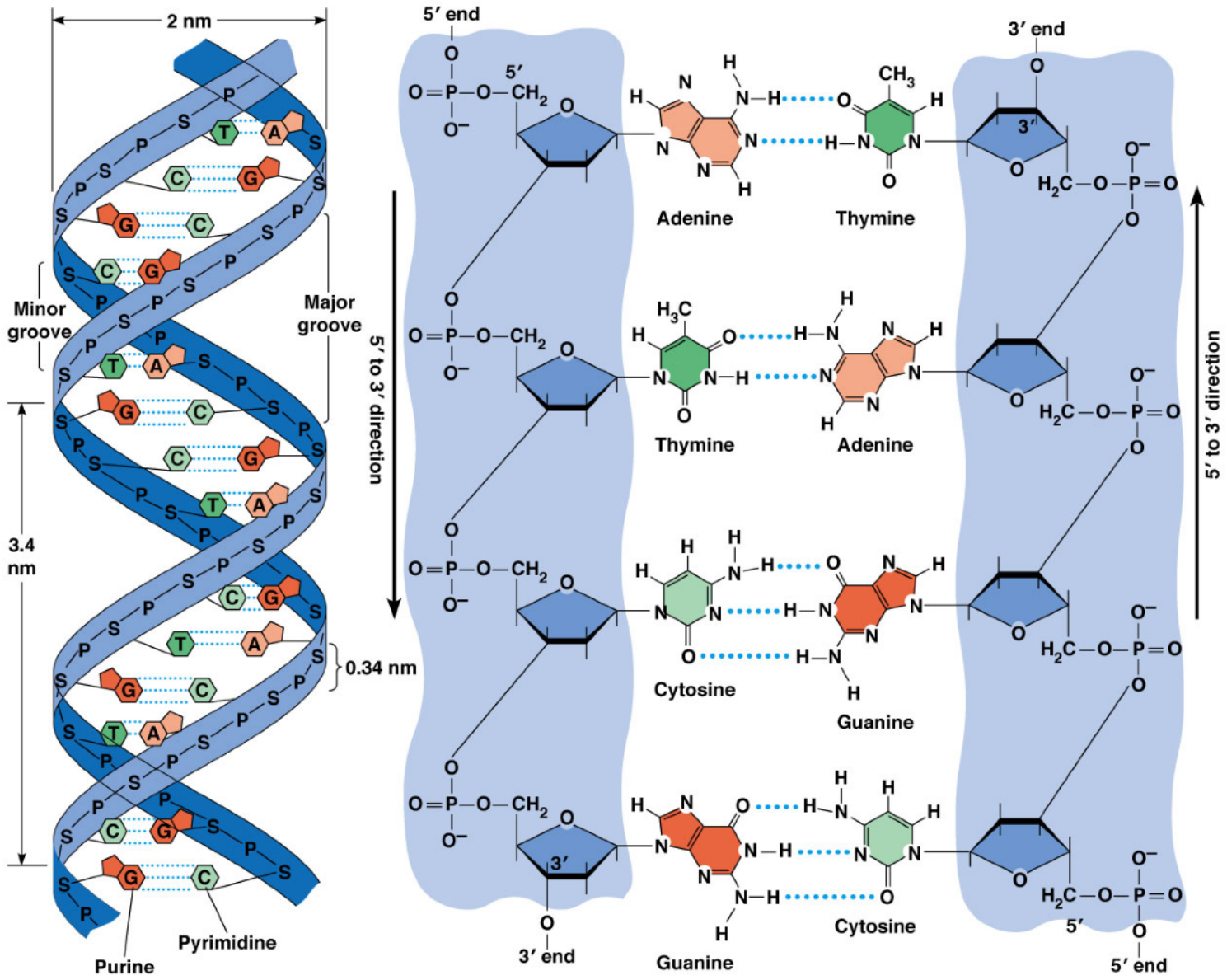


Figure 2.11 Introduction to Genetics (© Garland Science 2012)

Chapter2



Summary



(a) Double helix

(b) Antiparallel orientation of strands

To study

Thymine

2nm

Nucleotide

DNA backbone

Polynucleotide

Adenine

5’ carbon

Hydrogen bond

Phosphodiester bond

0.34nm

DNA

Uracil

2’ carbon

deoxyribose

3’ carbon

Minor groove

ribose

Cytosine

Guanine

Chargaff rule

RNA

antiparallel

3.4nm

RNA backbone

Pyrimidine

2-deoxyribose

Major groove

basepair

Expectations

- You know the structure of DNA.
- You know the story behind the discovery of the structure.
- Study the terms (They are my source for exam questions).
- Share your knowledge with people around you. Try to make simple for them.

For a smile