base-pairs remain exactly parallel to each other and horizontal, and exactly 10 steps are needed (with $T = 36°$) to complete one helical turn of 360°. Hence, the side views of the top and bottom blocks in Fig. 3.14(a) are just the same. For these two particular blocks, numbered 1 and 11, we get a full-frontal view of the minor-groove edge, which is here colored black. If we go along five steps from either end to base pair 6, we find a block which is seen with a full-frontal view of the white, major-groove edge; in between you should be able to make out the different edges of the blocks as they rotate about the vertical axis.

Next, let us start from the simple helix shown in Fig. 3.14(a), having $R = 0°$, $S = 0\,\text{Å}$, and $T = 36°$, and then introduce a slide of $S = -2\,\text{Å}$ at each step. The resulting helical geometry is shown in Fig. 3.14(b), where the base pairs spiral outwards from a central helix axis. To understand this motion more clearly, we can study a top view of the structure, as shown in Fig. 3.15. This diagram shows a half-turn of a helix in which the center of every block has moved outwards, radially from the axis by a distance r. By looking at the sideways displacement of the centers of the blocks, we can see that the outward motion is, in fact, associated with a negative slide at each step. Applying trigonometry to Fig. 3.15, we find that

$$r = (-S/2)/\sin(T/2).$$

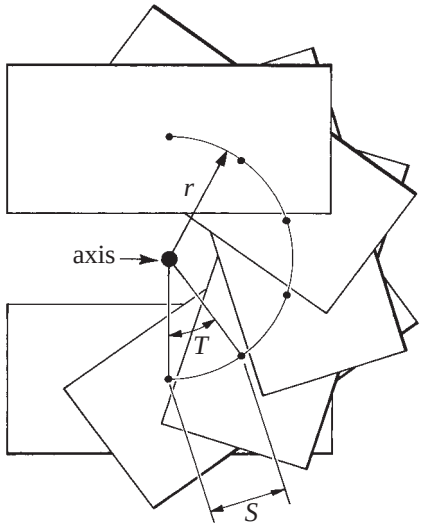

Figure 3.15 A top view of part of the helix shown in Fig. 3.14(b) illustrating the geometry by which radial displacement r is related to the magnitude of slide S and twist T. The small spots mark the centres of the blocks: to find which belongs to which, trace the full outline of a given block, and see which spot is at its centre.

Thus, given $S = -2$ Å while $T = 36°$, the base-pairs move outwards by

$$r = (2/2)/\sin(36°/2) = 3.2 \text{ Å}.$$

In summary, the top view of Fig. 3.15 shows that negative slide S makes a big hole in the middle of the helix, while the side-view of Fig. 3.14(b) shows that the stack of bases becomes wider than it was before.

Next, what happens if the base-pairs roll apart from each other by some angle R, while $S = 0$ Å and $T = 36°$? How will the picture of Fig. 3.14(a) change on account of the introduction of roll R? The resulting helical geometry is shown in Fig. 3.14(c), where each base pair tilts from the horizontal relative to its position in diagram (a). (In fact, each block rotates through a few degrees about its own 'front–back' axis, Fig. 3.7.) This kind of motion may well be a surprise to you, but if you look carefully at (c), you will see that, at every step, the gap between the minor-groove edges is larger than that between the major-groove edges, corresponding to the positive roll of $R = 12°$ which has been used for the drawing. The cumulative effect of many roll angles is to cause a *tilt* of the base-pairs with respect to the vertical axis. When the roll angles are small, it can be shown that

$$\text{tilt} \quad (R/2)/\sin(T/2).$$

Thus, when $R = 0°$, tilt $= 0°$; but when $R = 12°$ and $T = 36°$, then the tilt becomes

$$(12°/2)/\sin(36°/2) = 19°,$$

as shown in (c).

Also, roll and tilt cause the base-pairs to move out slightly from the axis. The best way to understand this is to think about the thin black 'rod' which connects the base-pairs in pictures Fig. 3.14(a) and (c), and passes through them at right-angles. In (a) it is straight but in (c), the roll at each step curves the rod; and since the direction of curvature is different for each step, the rod ends up as a gentle spiral. Calculations show that here the outward movement of base-pairs along the rod is approximately $3.3 \sin(\text{tilt})/2 \sin(T/2) = 1.7$ Å here, where 3.3 Å is the distance between base-pairs along the stack locally.

So far we have considered combinations of just two parameters, S and T, to give distance from the axis as in Fig. 3.14(b), or R and T to give both distance from the axis and also tilt from the axis as in (c). But when all three parameters R, S, and T act simultaneously, something new happens: the helix gets shorter, because the negative sliding motion goes 'downhill' on account of the tilt. The relevant

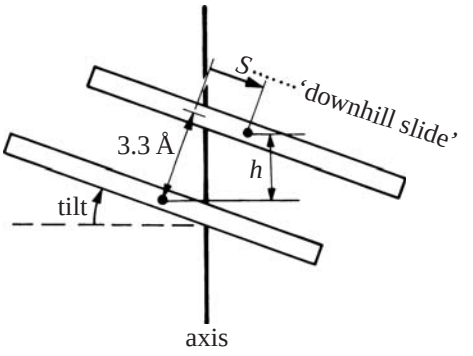

Figure 3.16 For base-pairs at a standard perpendicular separation of 3.3 Å, the 'rise' h in the direction of the helix axis depends both on tilt and slide S. This picture has been drawn with a negative value of slide, as in Figs 3.14 and 3.15.

geometry is drawn in Fig. 3.16, and its effect on the overall structure may be seen in Fig. 3.14(d). The vertical stacking distance at each step remains constant at 3.3 Å measured perpendicular to the blocks, but the rise h along the helix axis from the center of one base-pair to the next is changed to

$$h = 3.3\cos(\text{tilt}) + S\sin(\text{tilt}).$$

For $R = 12°$, we know that tilt = 19°; and so for $S = -2$ Å, we have

$$\begin{aligned} h &= 3.3\cos(19°) - 2\sin(19°) \\ &= 3.1 - 0.6 = 2.5 \text{ Å}. \end{aligned}$$

To summarize, one can calculate approximately the overall shape of a long helix made out of many uniform (R, S, and T) steps according to three formulas:

(i) tilt from axis $= (R/2)/\sin(T/2)$,

(ii) distance from axis $= (-S/2)\cos(\text{tilt})/\sin(T/2) + 3.3\sin(\text{tilt})/2\sin(T/2)$,

(iii) length along axis $= 3.3\cos(\text{tilt}) + S\sin(\text{tilt})$.

We are now ready to relate different values of R, S, and T, as determined by the base sequence of DNA, to the overall shape of a double helix. For example, in Fig. 3.6, we showed that AA/TT prefers to stack so that it can form an extra hydrogen bond in the major groove; values of (R, S and T) for an AA/TT step in its preferred arrangement are (0°, 0 Å, 36°). Therefore, a double helix containing only AA/TT steps will resemble Fig. 3.14(a), except, of course, that the base pairs will be highly propeller twisted: remember that the drawings of Fig. 3.14 do not show the propeller twist.

As another example, consider some DNA consisting entirely of GG/CC steps, all having uniform slide $S = -2$ Å and roll $R = 6°$, as

shown earlier on the left-hand side of Fig. 3.12(c). This DNA will resemble the models shown in Fig. 3.14(b) and (d); and will be intermediate in structure between them, since the roll angle of 6° is halfway between the roll angles of 0° and 12° used to construct those two models.

Finally, note that the models shown in Fig. 3.14(a) and (d) correspond broadly to the 'B' and 'A' forms of DNA as shown earlier in Fig. 2.7. Look carefully at the pictures in Fig. 2.7, and locate the base pairs. Then compare the locations of the base pairs with those shown in Fig. 3.14(a) and (d), especially with regard to tilt and distance from the axis.

Almost all of the external features of the 'B' and 'A' helices, such as the distance of base-pairs from an axis, the tilt of pairs with respect to an axis, and the rise along the axis, can be calculated from their (*R*, *S*, and *T*) values according to the formulas given above.

Why then do different sequences of DNA prefer either the 'B' or 'A' forms in crystals? As shown earlier in Fig. 3.12, AA/TT steps do not appear at all in the 'A'-form region, whereas CA/TG or GG/CC steps may be found in either the 'B'-form or 'A'-form region, with some space in the center between them near slide $S = 0$ Å. Hence, the preference of DNA for two general forms, whether 'B' or 'A', may derive in part from the tendency of steps CA/TG or GG/CC with two G–C base pairs, to favor either of two separate values for roll *R* and slide *S*, rather than a continuous range. In fact, the classification of a long double helix into either of the two categories, 'B' or 'A', becomes somewhat arbitrary and ambiguous, when many successive steps having different roll *R* and slide *S* are considered as a broad average.

These few examples of the use of (*R*, *S*, and *T*) values in understanding DNA have related mainly to physical measurements of DNA structure from X-ray studies, rather than to the role of DNA in biology. But we must start somewhere! In the following chapters, we shall explain how the roll–slide–twist model is indispensable for understanding many of the roles of DNA in biology, such as how promoters work, how DNA coils around proteins in a chromosome, and how DNA binds gene-regulatory proteins such as 'repressors' or 'activators'. We do not need to bother about the other three of Euler's six degrees of freedom, unless we are dealing with DNA that has been severely distorted by contact with a protein or drug: see Appendix 2. Do not worry too much about the details of this chapter, such as the various formulas and constructions, so long as you grasp the meanings of propeller twist, and of roll, slide, and twist.

You may be puzzled that we have not shown the sugar–-phosphate chains in any of the pictures of this chapter, except in Fig. 3.13. The chains are there, of course, but they have not been

shown in the drawings. This is analogous to the way in which we have not shown propeller twist in some of the diagrams, either. The really important point in the present chapter is that the outward features of DNA *all* depend strongly on base-stacking arrangements at the inner core of the molecule.

Notes

1. In this book, we regard the left-handed sense of propeller twist, as shown in Fig. 3.4, as 'positive'. This is opposite from the sign convention given in the 'Cambridge Accord' (Dickerson *et al.* (1989) *EMBO Journal* **8**, 1–4), but it should not lead to confusion, since almost all propeller twists are seen to be of the same sense as that shown in Fig. 3.4, which is positive according to our convention.
2. See Appendix 1.
3. We have used here the symbols R, S, and T for roll, slide, and twist, respectively, with the sign conventions shown in Fig. 3.8. In fact, many different symbols and sign conventions have been used for those quantities by different scientists, and we have chosen the present set for the sake of simplicity in a textbook. Our symbols can be translated into those of the 'Cambridge Accord' of the X-ray diffraction workers (see Note 1, above) as follows: $R = \rho$, $S = D_y$, and $T = \Omega$, without any change of sign.

Further Reading

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Dickerson, R.E. *et al*. (1989) Definitions and nomenclature of nucleic acid structure parameters. *EMBO Journal* **8**, 1–4. A comprehensive listing of possible structural parameters for DNA. Note that propeller twist is reversed in sign there, as compared with many papers in the literature and in this book.

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Wing, R.M., Drew, H.R., Takano, T., Broka, C., Tanaka, S., Itakura, K., and Dickerson, R.E. (1980) Crystal structure analysis of a complete turn of B-DNA. *Nature* **287**, 755–8. First direct structural evidence for propeller twist in DNA base-pairs, by X-ray diffraction.

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Nelson, H.C.M., Finch, J.T., Luisi, B.F., and Klug, A. (1987) The structure of an oligo(dA).oligo(dT) tract and its biological implications. *Nature* **330**, 221–6. Direct observation of very high propeller twist in a series of A–T base pairs, and a postulate of an additional hydrogen bond in the major groove between adjacent pairs (see Fig. 3.6).

Exercises

3.1 Normally the DNA double helix is right handed, as shown schematically in Fig. 3.1. In this case, the provision of left-handed or counter-clockwise propeller twist (when looking along the long axis of any base pair), as shown in Fig. 3.4, can reduce the access of water to the bases, as shown in Fig. 3.3.

For a hypothetical *left-handed* double helix of DNA, with $T = -32°$, what sense of propeller twist would be required to reduce likewise the access of water to the bases?

(Note: the left-handed 'Z'- DNA shown in Fig. 2.7 has almost no propeller twist, because the bases there stack not only onto neighboring bases, but also onto neighboring sugars.)

3.2 In the models of the 'A' and 'B' forms of DNA shown in Fig. 2.7, the *major*-groove edges of the bases are shaded heavily – a convention which is opposite from that used elsewhere in this book. Identify the major and minor grooves which lie between the sugar and phosphate chains in these two models. In the 'B' form, which groove has the larger width? In the 'A' form, which groove is wider? Or are the widths about the same? In the 'A' form, which groove is *deeper*?

3.3 Using Fig. 3.5 as a guide, identify the major- and minor-groove edges of the base-pairs shown with atomic detail in Fig. 2.11(a) and (b).

3.4 Leonard Euler explained long ago that any one rigid block has six degrees of freedom of motion with respect to another rigid block. Each of these may be described in terms of a translation along, or a rotation about, any of the three axes which are labeled in Fig. 3.7. In practice, three of these six degrees of freedom are *not* mobilized significantly in the base-pair steps of DNA.

By use of a simple model involving blocks of wood, or cardboard boxes, confirm that translation or shortening along the 'twist axis', and rotation about the 'front–back' axis, are inhibited because of the close surface-to-surface stacking of the bases.

Also, examine the third unused degree of freedom, which involves translation along the front–back axis. It is not clear whether this motion is inhibited in real DNA by the chemical forces which influence base stacking, or by the action of sugar–phosphate chains, or both. The front–back motion could conceivably be favored for certain sequences in DNA, but we have at present few good examples, apart from a few steps which include two G–C base pairs, and so repel due to partial electric charge along their short axes, and a few CA/TG steps in DNA wrapped around protein spools.

Finally, confirm that the three allowed motions of roll, slide, and twist in DNA, as shown in Fig. 3.8, are also allowed in the wooden-block models.

3.5a Make a simple physical model to illustrate the linkage between slide and twist shown in Fig. 3.13. (Expanded polystyrene foam can be cut easily into suitable blocks using a bread knife, and the sugar–phosphate chain links may be made from wires or paper clips, with their ends pushed into the blocks.)

b Make a simple physical model of two adjacent propeller-twisted base-pairs, with the bases of unequal size as in Fig. 3.6. (Expanded polystyrene foam blocks may be held apart conveniently, in a propeller-twisted arrangement, by means of cocktail sticks.) Use these model base pairs, without any sugar–phosphate chains, to study the linked slide–roll motion which is shown in Figs 3.10 and 3.11.

c Use the model of part (b) to investigate the 'locking' of both slide *S* and helical twist *T* in AA/TT steps by the additional hydrogen bond shown in Fig. 3.6 and the associated high propeller twist. Also demonstrate that the absence of these effects allows *S* and *T* to vary without hindrance.

3.6 The table below gives approximate, uniform values for roll, slide, and twist at the base-pair steps in three different well-known forms of DNA – 'A', 'B', and 'C' – which have been studied by X-ray diffraction of fibers:

	'A'	'B'	'C'
R(°)	+12	0	−6
S(Å)	−1.5	0	+1
T(°)	32	36	40

Use the equations on p. 58 to calculate approximate values of the following parameters for each of the three forms 'A', 'B', and 'C':

a the distance of the centers of base-pairs from the axis of the double helix;

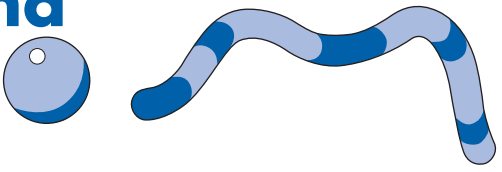
b the angle by which base-pairs are tilted from planes normal to this axis;

c the length, or 'rise', of the molecule per base-pair along the axis.

(Note: calculate the tilt angle first.)

CHAPTER 4

Twisting and Curving



In the last two chapters we have learned some rudimentary things about DNA. We have learned: (a) why DNA forms a helix, (b) how the bases make ordered pairs at the center of the helix, (c) how the bases twist like a propeller within any base-pair, and (d) how the overall shape of a helix depends on the local parameters roll, slide, and twist over a series of base-pair steps. This is the stuff of chemistry, not biology. When are we going to start talking about DNA in biology? That is exactly what we shall be doing in this chapter.

The two most fundamental actions of DNA in biology involve either the *twisting* or *curving* of a DNA double helix. First, we consider the twisting of DNA – or, to be more precise, its *un*twisting. There are two main instances where DNA has to untwist as it carries out its duties in a cell: first, when DNA is copied into the messenger-RNA that tells the cell how to make protein; and second, when DNA is copied into another DNA strand just before a single cell divides into two cells. The first of these processes is called 'transcription', and the second is called 'replication': see Fig. 4.1. In each case, the DNA unwinds into two separate sugar–phosphate strands. These pictures provide, of course, only a static representation of a brief instant in the life of a cell. You have to imagine that the unwound regions of DNA in pictures (a) and (b) are moving rapidly across the page, from right to left, in order to grasp the dynamic nature of DNA unwinding in living systems.

Often DNA unwinds only over a short region, say 15 to 20 base-pairs, when making RNA as in (a), because it takes a lot of energy to pull the base-pairs apart and expose them to water. The 'bubble' of unpaired bases can travel along the length of the DNA very rapidly, at about 100 base-pairs per second; but then, time doesn't mean

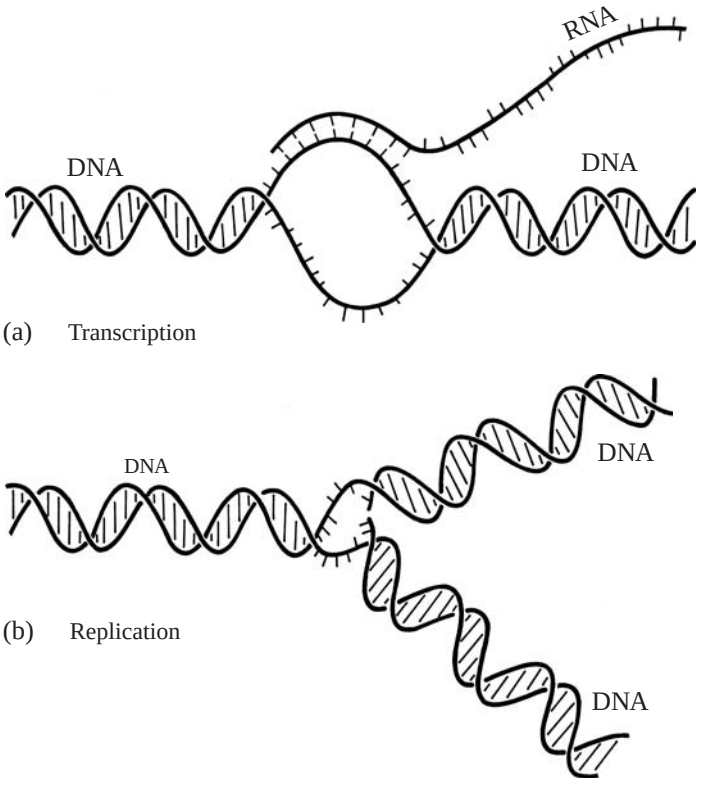


Figure 4.1 Schematic representations of transcription and replication of DNA. In each case, the DNA must unwind locally to let one strand serve as a template for the synthesis of a new strand, either of (a) RNA or (b) DNA.

much to these tiny molecules that we can hardly see by using a light microscope. When DNA gets copied into RNA, a copying protein or enzyme called 'RNA polymerase' attaches itself to one of the two DNA strands: see Fig. 4.2. Then the polymerase pulls nucleotides out of solution to match the bases it finds within the DNA chain. In Fig. 4.2 the enzyme is just about to add an RNA base C to a DNA base G. Another somewhat similar enzyme carries out the process of copying DNA into DNA (Fig. 4.1(b)); it is called 'DNA polymerase'. Any cell contains several varieties of RNA polymerase, and several varieties of DNA polymerase, to do different kinds of copying tasks.

These RNA polymerases in the cell always try to put C with G, G with C, A with T, and U with A to make Watson–Crick base-pairs, as shown inside the 'bubble' of Fig. 4.2. The DNA polymerases do the same, but add T instead of U to A. Some of the copying enzymes, in a test-tube, will incorrectly put T with G if you feed them a lot of T nucleotides and no C at all; but they add T to G much more slowly than C to G. These kinds of copying error do not happen very often