that its minor edge approaches the helix surface, making the minor groove very shallow and the major groove cavernously deep. In Z-DNA, it is the major edge of each base pair that is pushed toward the surface, so that the minor groove is deep and the major groove is so shallow as hardly to be characterized as a groove at all. It is sometimes stated that 'Z-DNA has no major groove', but space-filling stereos, such as Fig. 1 of reference Z6 or Fig. 3 of Z23 reveal the shallowest of major grooves running around the helix cylinder, flanked by very slightly higher phosphate backbones.

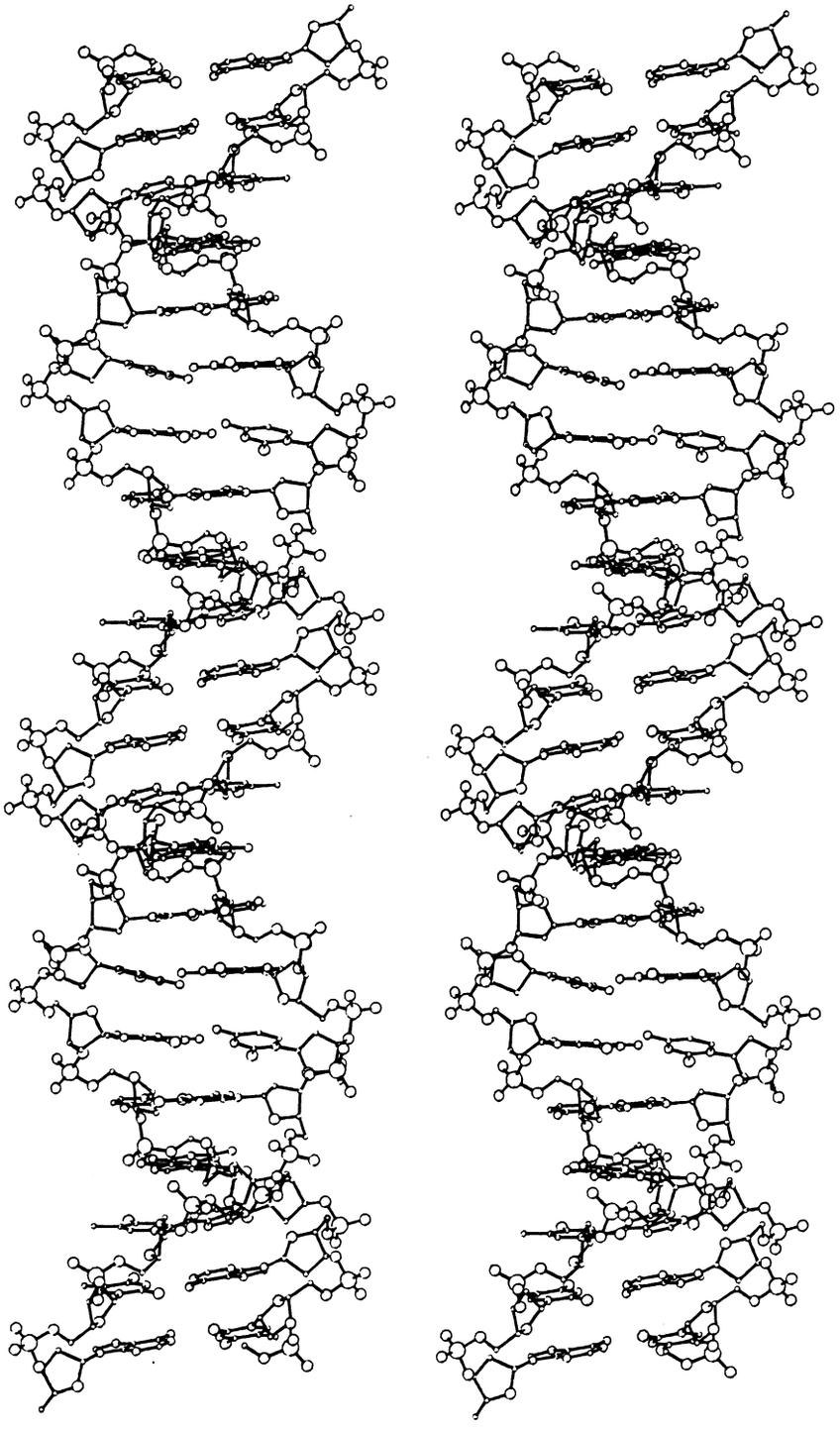

Fig. 23.3.3.2. The B-DNA stereo pair drawing from which Fig. 23.3.1.3 was derived, with repeating sequence -(G-C-G-A-A-T-T-C-G-C)$_n$-. The variation of minor groove widths on the front and back sides of the helix is striking. (From Dickerson, 1983.)

23.3.3.2. *Glycosyl bond geometry*

In both A- and B-DNA, all glycosydic bonds are *anti*, with sugar rings swung to either side away from the minor groove, as in Fig. 23.3.3.4(*a*). As mentioned earlier, when viewed into the minor groove, the backbone chains describe a clockwise rotation, with the chain on the right running downward, and that on the left upward, as in Fig. 23.3.2.1. In Z-DNA, both chains run in the *opposite* direction, leading to a counterclockwise rotation sense viewed into the minor groove. But Z-DNA has yet another striking (and defining) feature. Purines and pyrimidines alternate along each chain. G and C are most strongly favoured by far, but A and T can substitute intermittently at a price in stability. Breaking the strict alternation of purines and pyrimidines is even more unfavourable and is rarely encountered in crystal structures (Table A23.3.1.3). At each purine base, the glycosyl bond is rotated into the minor groove to the *syn* position, as in Fig. 23.3.3.4(*c*). This causes the local backbone directions, defined by sugar ring atoms C4′ and C3′, to be parallel in the two strands. Z-DNA avoids becoming a parallel-chain helix by performing a local chain reversal at each pyrimidine. In Fig. 23.3.3.4(*c*), although the local C4′–C3′ chain direction at the cytosine sugar is downward, the double loop in backbone chain gives it a net upward orientation. In stereo Fig. 23.3.3.3, the ascending backbone chain rises smoothly past each guanine, with a chain path parallel to the helix axis. However, the chain bends abruptly at right angles when passing a cytosine, in a direction tangential to the helix cylinder. Guanine sugar rings point their O4′ oxygen atoms in the backward chain direction (as is also true for all bases in A- and B-DNA), but cytosine sugars point their oxygens in the forward direction. This 'up at G, across at C' pathway and inversion of sugar rings is what produces the zigzag backbone pathway that leads to the name Z-DNA. The O4′ atom of each cytosine sugar is stacked on top of the guanine ring of the subsequent nucleotide, and this stacking of a polar O (or N) on top of a polarizable aromatic ring contributes to the stability of the Z helix, as it does to many other base–base interactions to be discussed later (Bugg *et al.*, 1971; Thomas *et al.*, 1982; B32).

23.3.3.3. *Sugar ring conformations*

Sugar ring conformations in A- and B-DNA have a logical structural basis. The B-DNA backbone is more extended than the A-DNA backbone, with P–P distances of *ca* 6.6 Å along one chain, compared with *ca* 5.5 Å in A-DNA. In turn, C2′-*endo* is a more extended ring conformation than C3′-endo, demonstrable in Fig. 23.3.2.4 by a greater distance between C5′ and O3′ atoms. Hence, it is logical that

the more extended ring conformation should be associated with the more extended backbone chain. In Z-DNA, the extended C2′-*endo* form is adopted at cytosine, where a zigzag double chain reversal must be accommodated, while the more compact C3′-*endo* occurs at the straight backbone segment running past a guanine.

The cramped *syn* glycosyl conformation is strongly disfavoured, although not absolutely forbidden, at pyrimidines, most probably because of steric clash between the pyrimidine O2 and the *syn* ring (Haschmeyer & Rich, 1967; Davies, 1978; Ho & Mooers, 1996; Basham *et al.*, 1998). Hence, the Z-DNA helix is effectively limited to alternating pyrimidine/purine sequences, with a price that must be paid for intermittent substitution of A and T for G and C, and an even higher price paid for breaking the pyrimidine/purine alternation. This is reflected in the X-ray crystal structures listed in Table A23.3.1.3. Only one non-alternating sequence has been completely solved and published: *C-G-G-G-*C-G (Z40), where adoption of the Z form has been forced by 5-methylation of cytosines (*C). A second non-alternating sequence that includes AT base pairs, *C-G-A-T-*C-G (Z13), was solved in 1985, but its coordinates have never been made public. It, too, required methylation of cytosines to induce the Z form. A third sequence, C-C-G-C-G-G (Z42), opens its terminal base pairs to make intermolecular base pairs with crystal neighbours. The 52 remaining Z-DNA structures in Table A23.3.1.3 all have strict alternation of pyrimidines and purines.

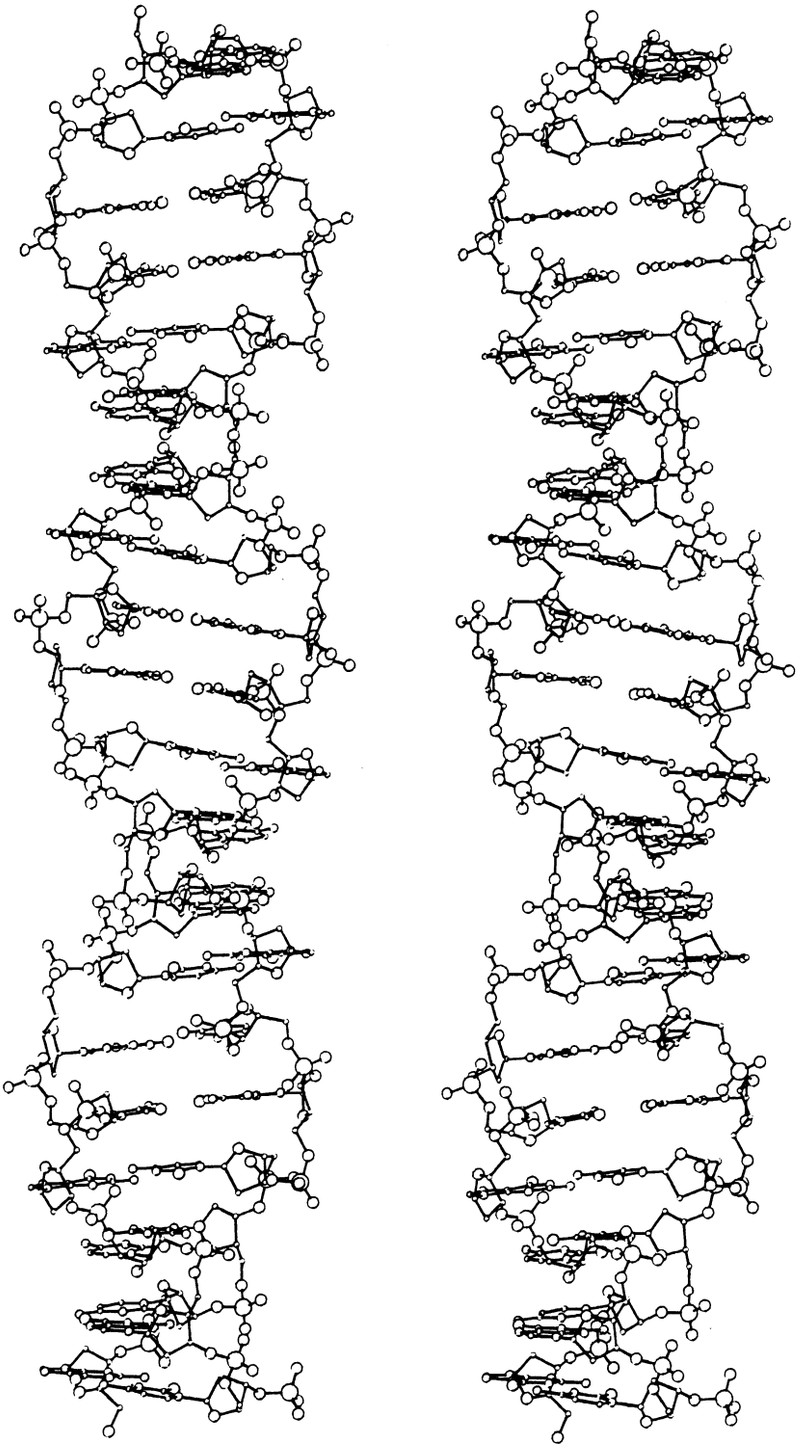

Fig. 23.3.3.3. The Z-DNA stereo pair drawing from which Fig. 23.3.1.4 was derived, with repeating sequence -(G-C-G-C)$_n$-. Note the left-handed zigzag path of the sugar–phosphate backbone, which led to its designation as the Z helix. (From Dickerson, 1983.)

23.3.3.4. *Helical twist and rise, and propeller twist*

The helical repeat unit in Z-DNA is therefore two successive base pairs, rather than the single base pair of A- and B-DNA. Ho & Mooers (1996) propose that the C-G or $^{5'}$pyrimidine-P-purine$^{3'}$ step be considered the fundamental unit of the Z-helical structure, because of the tight overlap between the two base pairs. As can be seen in Fig. 23.3.3.3, in a C-G step the pyrimidine rings from the two base pairs actually stack over one another, whereas the purine rings are packed against neighbouring sugar O4′ atoms. Helix-axis rotation at this step is only −8°, whereas the preceding and following G-C steps have a mammoth −52° twist. Hence, although Z-DNA has 12 base pairs per turn, it technically is not a dodecamer helix, but a hexamer with a two-base-pair repeating unit and a total rotation of −60° per unit.

This virtual restriction to purine/pyrimidine alternation means that Z-DNA cannot be involved in the coding of genetic information. A and B helices have no such restriction; their structures can accommodate a random sequence of bases. Average twist angles are as shown in Table 23.3.3.1, although extreme variation in twist is observed at individual steps in single-crystal structure analyses, from as little as 16° to as much as 55°. Base-sequence preferences for local helix parameters are discussed below.

In both B and Z helices, base pairs are very nearly perpendicular to the helix axis, whereas in the winding double ribbon of A-DNA, the long axis of each base pair is inclined by 10 to 20° away from perpendicularity to the axis. Hence, the rise per base pair for all B-helical steps and for G-C steps of Z-DNA is equal to the thickness of a base pair, 3.4 Å. The rise at a C-G step of

references