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.

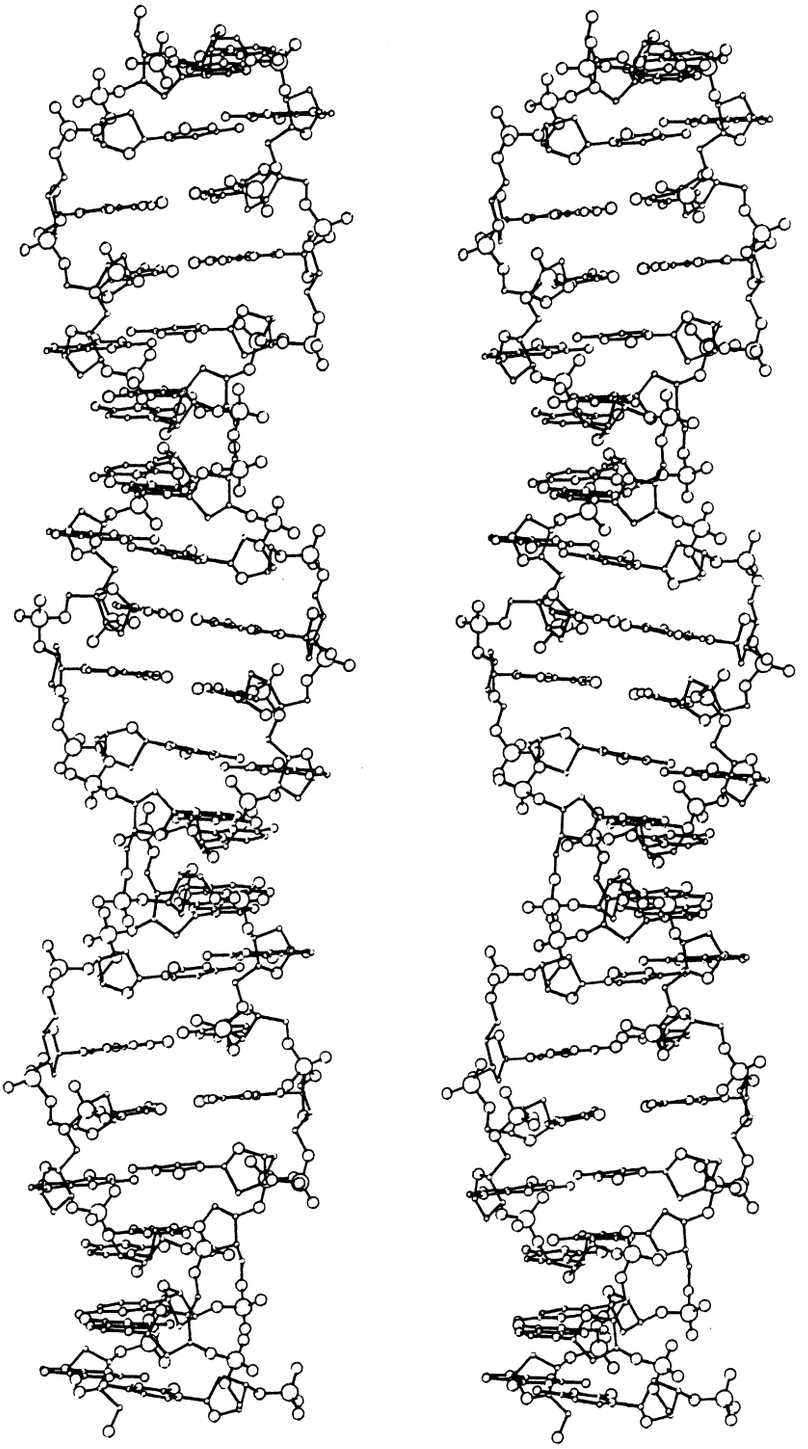

Fig. 23.3.3.3. The Z-DNA stereo pair drawing from which Fig. 23.3.1.4 was derived, with repeating sequence -(G-C-G-C)$_n$-. Note the left-handed zigzag path of the sugar–phosphate backbone, which led to its designation as the Z helix. (From Dickerson, 1983.)

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

Table 23.3.3.1. *Comparison of structures of A, B and Z helices*

	A	B	Z
Handedness	Right	Right	Left
Helix axis relative to base pairs	Major groove side	Through centre of base pair	Minor groove side
Major groove	Very deep and narrow	Wide, same depth as minor	Very shallow and broad
Minor groove	Shallow and broad	Variable, same depth as major	Very deep and narrow
Glycosydic bonds	*anti*	*anti*	C: *anti* G: *syn*
Minor groove backbone chain sense *	Clockwise	Clockwise	Counterclockwise
Sugar conformation	C3′-*endo* (narrow range)	C1′-*exo*/C2′-*endo* (broad range)	C: C2′-*endo* G: C3′-*endo*
Base pairs per helix repeat	1	1	2
Base sequence limitations	None	None	Alternating $(C\text{-}G)_n$ or close variants
Rise per base pair (average)	2.9 Å	3.4 Å	C-G: 4.1 Å G-C: 3.5 Å
Base pair inclination	10–20°	*ca* 0°	*ca* 0°
Mean twist angle	30–33°	34–36°	C-G: −8° G-C: −52°
Helix repeats per turn	11–12	10–10.5	6 (2 base pairs)
Propeller twist	Often substantial, 0–25°	Often substantial, 0–25°	Usually small
Common biological occurrence	RNA	DNA	None?

* Relative 5′-to-3′ directions of the two backbone chains, when viewed into the minor groove.

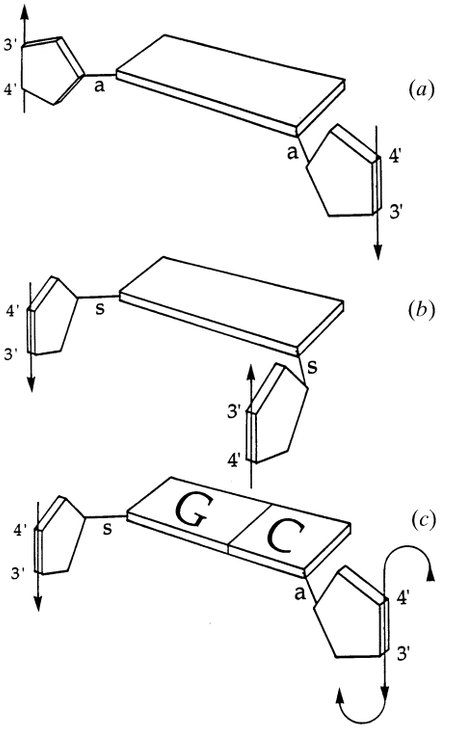

Fig. 23.3.3.4. Glycosyl conformation and chain sense. (*a*) Glycosyl conformations *anti/anti*, backbone chains antiparallel, with clockwise sense when viewed into the minor groove, as here. This is typical for A- and B-DNA. (*b*) Glycosyl conformation *syn/syn*, backbone chains antiparallel, with counterclockwise sense viewed into minor groove. This is not known for any nucleic acid duplex. (*c*) Glycosyl conformation *syn* at G and *anti* at C, with the C4′—C3′ edge of the sugar pointing downward in both strands, which would seem to imply a parallel-stranded helix. However, in Z-DNA, antiparallel strands are achieved by a local reversal of chain direction at each C, as shown here. This produces the zigzag backbone pathway that is characteristic of the Z helix, visible in Fig. 23.3.3.3.

Z-DNA is larger because it involves stacking of a sugar oxygen on each purine ring, not ring stacked on ring. For A-DNA, the rise along the helix axis can actually be less than the thickness of a base pair, because adjacent base pairs are stacked at an incline. The perpendicular distance from one base pair to the next in A-DNA is still 3.4 Å. Both A- and B-DNA exhibit considerable base pair propeller twist, especially at A·T pairs with only two hydrogen bonds rather than three. In contrast, Z-DNA, with predominately G·C pairs, shows only a small propeller twist.

The stacking of base pairs has immediate consequences for crystal growth. For Z-DNA, four base pairs are one-third of a helical turn, and six base pairs are a half turn. Hexamers are the most common crystal form in Table A23.3.1.3 by a large majority. In contrast, octamers and decamers are not simple fractions of a turn, and they stack in a disordered manner. One would predict that dodecamers of Z-DNA might crystallize well if the oligomers were not so long as to fall prey to cylindrical disorder.

By the same principles, B-DNA decamers stack easily and well to build pseudo-infinite helices through the crystal, with ordered cylindrical rods packed in six different space groups. The other common crystallization mode for B-DNA, the dodecamer, has a two-base-pair overlap of ends that both stablizes the crystals and yields a functional ten-base-pair repeat. (See Fig. 2 of Dickerson *et al.*, 1987.) Because the dodecamers are held by their outer two base pairs, the central eight pairs are unobstructed and accessible in the crystal, making dodecamers particularly good subjects for the study of minor-groove binding drugs.

A-RNA duplexes [Table A23.3.1.1, part (*k*)] also stack end-for-end in a manner simulating an infinite A helix, even though the end base pairs are inclined and are not perpendicular to the helix axis. This behaviour has been seen for octamers with roughly two-thirds of a helical turn, for nonamers, and for dodecamers with roughly a full turn.

In contrast, crystals of A-DNA behave quite differently. Regardless of chain length, A-DNA helices crystallize with the outer base pair of one helix packed against one wall of the broad, open and relatively hydrophobic minor groove of another helix.

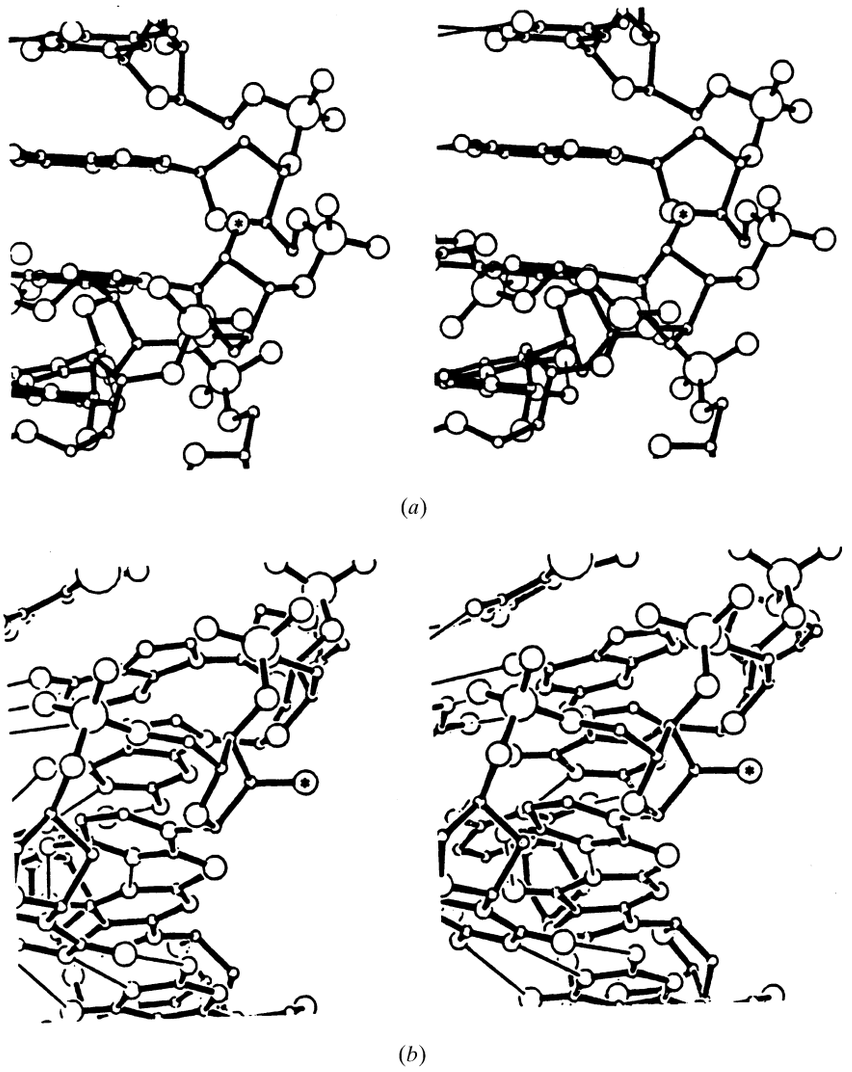

Fig. 23.3.3.5. The role of the C2′-OH in RNA helix geometry. (*a*) Addition of a C2′-OH group (*) to the B-DNA helix leads to close contacts and unallowable steric hindrance with the following O5′ and O4′ atoms and, to a lesser degree, the subsequent base itself. (*b*) A C2′-OH group added to the A-DNA helix extends outward radially from the helix cylinder surface and produces no steric clashes. Hence, A-RNA is quite possible, whereas B-RNA is disallowed.

This packing mode is sufficiently adaptable to accommodate duplexes of lengths four, six, eight, nine, ten and 12 base pairs. Hence, A-DNA does not simulate infinite helices through the crystal lattice, as A-RNA and B- and Z-DNA do.

23.3.3.5. *Allowable RNA helices*

So far this discussion has only been concerned with DNA. Which of the three helix types can be adopted by RNA? Fig. 23.3.3.5 shows that addition of a 2′-OH group to a B-DNA helix [part (*a*)] creates severe steric clash with the phosphate group and sugar ring of the following nucleotide, whereas in an A helix [part (*b*)], the added hydroxyl group extends radially outward from the helix cylinder and causes no steric problems. Hence, the natural helical form for RNA is the A helix, not the B helix. Table A23.3.1.1 shows several single-crystal analyses of A-RNA and RNA/DNA hybrids; Table A23.3.1.2 shows no B-RNA structures. One RNA/DNA hybrid is known as a Z helix: C-G-c-g-C-G (Z24), in which the two central nucleotides are RNA. If one mentally adds an —OH to each C2′ atom in Fig. 23.3.3.3, on the same side of the ring as O3′, it is apparent that the C2′-OH is not inherently incompatible with the Z helix, as it is with the B helix. At guanine sugars, the C2-OH points out and away from the helix, while at cytosine sugars it points away from the base into the spacious minor groove.

23.3.3.6. *Biological applications of A, B and Z helices*

The B helix is the biologically relevant structure for DNA. The A form might logically be adopted at the stage of transient DNA–RNA duplexes during transcription, but elsewhere the B form holds sway. It was once thought that binding of DNA to a protein surface, most particularly nucleosomal winding, might constitute a sufficient dehydration of bound water molecules from the DNA duplex to shift it to the A form. This proved to be false; nucleosomal DNA clearly retains the B conformation. The closest that one comes to biological A-DNA is local deformations upon binding of B-DNA to a few proteins that have been described as ‘A-like distortions’. On the other hand, the A helix has been found repeatedly in RNA duplexes, including tRNA and ribozymes.

The situation is even more restrictive with the Z helix. Although its alternating purine/pyrimidine sequence makes it unusable for genetic *coding*, the suggestion has been made on many occasions that Z-DNA might be an important element in genetic *control* by being involved in negative supercoiling (Herbert & Rich, 1996). It has been shown that a left-handed DNA conformation can be induced by negative superhelical stress, but it is not absolutely clear that this induced, left-handed conformation is the same as the Z helix seen in crystal structures of small oligomers. As noted by Herbert & Rich (1996), after nearly twenty years of enquiry, it is still far from certain that Z-DNA itself has any demonstrable biological role.*

A major stumbling block is the cumbersome mechanism that must be invoked to explain a B-to-Z interconversion. As mentioned previously, a simple twisting of the helix from right to left is not sufficient, because the backbone chains run in opposite directions in the two forms. Fig. 23.3.3.6 demonstrates the steps that must still be undertaken after both B and Z helices have been unwound so as to remove all of their helical character. Note the opposite sense of the backbone strands in B [part (*a*)] and Z [part (*e*)]. In order to accomplish the interconversion, base pairs of B-DNA must be

* Rich and co-workers (Schwartz *et al.*, 1999) have recently solved the crystal structure of the Zα domain of the human editing enzyme ADAR1 in a complex with a six-base-pair Z-DNA helix of sequence CGCGCG. This left-handed hexamer may suffer from the same length *versus* conformation uncertainty mentioned later in this chapter in connection with oligonucleotide crystals, especially since protein–DNA contacts in the Zα complex occur only with the zigzag phosphate backbone, which is not that dissimilar in Z-DNA (Fig. 23.3.3.3) and Z(WC)-DNA (Fig. 23.3.3.7). Nevertheless, it is encouraging to see a short segment of Z actually making contact with its protein in a presumably biologically relevant context.

references