Variety in DNA secondary structure

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Double helix models for DNA suffer from unsolved difficulties. These include: (i) the conservation of angular momentum in the unravelling necessary for semi-conservative replication, (ii) the regular phasing of nuclease cutting of nucleosomal DNA, and (iii) the failure of all electron microscope height measurements of duplex DNA to date to confirm the expected value of 2.02.2 nm. Variously obviating such difficulties are the side-by-side model proposed in 1976 by groups led by Rodley and Bates[1] and Sasisekharan[2,3] and the paranaemic SBS model proposed by one of us (C.S.D.)[4] in 1991.

VARIETY in DNA secondary structure is a topic of growing interest. While retaining base-pairing, many researchers, e.g. (see refs 5–11) continue to explain the properties of DNA by conformations outside the confines of the double helix first defined by Watson and Crick[12].

There can be no doubting the profound revolution in biochemistry precipitated by the 1953 Watson and Crick (W–C) DNA structure commonly called 'the double helix'. Their main contribution, the structural concept of base-pairing, promptly became central in theories of not only the structures of nucleic acids (DNA and RNA), but also their functions in genetics, cellular differentiation and viral action. By the time they were awarded a Nobel Prize (shared with Wilkins) in 1962, the status of their base-paired double-helix model for DNA was already iconic. No derogation of base-pairing or of the seminal effects of two-strand models for DNA is implied in reviewing the evidence for the ravelled (i.e. plectonaemic) structure of 'the' double helix.

During the five decades since the W–C model for B-DNA was proposed, various workers have analysed X-ray diffraction by the B-form and other liquid-crystal fibres of aqueous DNA to envisage nearly two dozen other helical DNA duplexes (some of left-handed helicity, whereas the W–C model is right-handed) – see for example, the comprehensive review in Leslie *et al.*[13]. Mainstream experts in the W–C tradition are thus in agreement that the particular conformation envisaged in the W–C model is certainly not the only form of secondary structure (i.e. short-range folding) that occurs in DNA duplexes.

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But, more fundamentally, from 1953 onwards, the plectonaemic concept has also been queried. In science, it is continually necessary to review the paradigms as new evidence is discovered or as old evidence is re-assembled in new patterns. Even in 1953, Max Delbrück[14] (1969 Nobel Prize-winner in Medicine) was able to write to his former student James Watson:

> 'These [the two DNA strands] would have to be untwiddled to separate the threads . . . one must postulate that the DNA opens up in some manner, both for replication and for doing its business otherwise. In the structure you describe (the double helix) this opening up is opposed . . . by the interlocking of the helices, and it becomes a very important consideration to find a way out of this dilemma, or to think of a modification of the structure that does not involve interlocking.'

Mainly as a response to that argument, in 1976 groups from both New Zealand[1] and, independently, India[2] put forward non-ravelled (side-by-side, SBS) models for two-strand DNA. More recently, Rodley[11] elaborated modifications of the duplex structure, viz. those SBS models known to him, precisely so as to escape from what came to be known after 1953 as the 'unwinding problem'.

The unwinding problem of DNA

Ready strand separation ('melting') has been reported *in vitro* in the absence of enzymes. No explanation has ever been offered, using any double-helical model, to account for the finding of Alexander and Stacey[15] in 1956 that duplex DNA will denature – separate into individual strands – at constant room temperature in dilute aqueous solution of urea. (Aqueous urea is known to break the hydrogen bonds in base-pairs.)

There is substantial literature, for example, Porschke[16] and references therein, seeking to explain how, in solution, thermal energy, which is a scalar, can generate the angular momentum, which is a vector and furthermore a conserved entity, needed to unravel the long, folded and twisted DNA strands as observed *in vitro*.

Nucleic acids have been reported by many workers to denature spontaneously at room temperature in non-aqueous solvents[16]. The reduced dielectric constants of non-aqueous solvents seem to be sufficient to allow the phosphate charges on the strands to effect strand separation purely by coulombic repulsion of like charges.

Similar evidence occurs in the closely-studied kinetics[17] of the reverse process *in vitro* – renaturing ('annealing') in simple fully-defined solutions. Indeed, a textbook account of this process shows only 2D ladder structures[18].

Without offering any explanation of their results, Arnott and coworkers[13,19,20] report the supposed unwinding of duplexes, and subsequent rewinding into triplexes, inside solid fibres.

In theorizing on disruption and reformation of secondary structures in DNA, intertwined 'rope' models such as the W–C thus encounter severe difficulties with the conservation of angular momentum, one of the best-established laws of science. This is perhaps the most neglected doubt about what have become standard models for DNA secondary structures.

SBS models escape the winding/unwinding problem because they do not have to wind or unwind as their strands are not ravelled.

The corresponding processes *in vivo* are of course more complex, but similar reasoning can be applied. In bacterial DNA replication, the two strands of a DNA molecule containing 10^6 base-pairs can separate within a few minutes (each acting in the process as the template for enzymic synthesis on itself, of the complementary strand defined by base-pairing – a mode of replication termed semi-conservative and commonly, but misleadingly said to constitute 'self-replication'). '[T]he unreplicated portion would have to rotate rapidly about its helical axis. A rate of about 10,000 revolutions per minute was estimated based on the rate of replication in bacteria'[21]. No source has been suggested for this angular momentum. If some chiral process in forming the new polymer chains is to transfer angular momentum from some other molecule(s) to the unravelling strands, this warrants a detailed explanation.

Partly in an attempt to solve this difficulty, various enzymes have been interpreted as cutting one or both strands of the DNA approximately every 10 base-pairs, so as to allow one strand to pass through the other. Thus the unwinding difficulty has been acknowledged in the postulating of 'topoisomerases' and 'helicases'; but whether such enzymes operate *in vivo* for every 10 base-pairs or so remains unknown. These enzymes may merely act to remove supercoils. In the replication of eukaryotic DNA, the large numbers of replicating complexes known to act simultaneously on the DNA duplex may not be unwinding DNA at all.

The law of conservation of angular momentum applies on a smaller but still significant scale in transcription: synthesis of mRNA on one strand of DNA requires strand-separation in DNA to the extent of 10^2–10^5 base-pairs, which implies 10^1–10^4 helical turns in a W–C structure. Such rotation would have to take place against any adjacent cellular membranes or other microstructures.

The phasing problem

In addition to physico-chemical studies of the denaturing of duplex DNA, its structure has also been probed using enzymic techniques in solution and on solid substrates.

Many teams have reported their studies of the cutting of linear duplex DNA on its own with the general nuclease deoxyribonuclease-1 in solution (for example, see refs 22 and 23). These researchers report that the cutting is highly regular, and that the recurrent regularity consists of the appearance of fragments forming a ladder, usually in 10 bp steps. This has been interpreted conventionally as cutting somehow concentrated at intervals of one pitch of a W–C structure.

It has been supposed that the preferred cutting sites of general nucleases are related to the base sequence, with sequence-dependent variations in groove widths leading to preferred cutting sites for deoxyribonuclease-1. However, the base sequences in the studies referred to here are highly irregular, whereas the actual preferred cutting sites are just the opposite – they are highly regular.

Were duplex DNA to be a cylindrically symmetrical double helix, it would be equally accessible from every side in solution. No explanation has ever been offered as to how the preferred cutting sites in solution could be highly regular, as multiples of 10 bp, across all these studies and across all the individual molecules with no fragments of intermediate length.

It seems evident that the highly regular, preferred cutting sites cannot be related to the irregular base sequence, but must be related to the overall structure of the duplex. In a double helix of irregular base sequence, deoxyribonuclease-1 would be able to cut at irregular sites, if the base sequence really was of importance to a general nuclease.

But suppose DNA has a SBS structure. Then, the binding site of the nuclease might only allow cutting from a far more restricted geometry. This could explain the observed regularity of cutting as largely determined by the overall geometry of the duplex.

In 1977, Lohr *et al.*[24] also published results which pose a severe test for the double helical model of the structure of DNA. These workers equilibrated histone cores onto DNA, cleaved the complexes with DNase-1 and succeeded in resolving the sizes of the resulting fragments of DNA up to a maximum length of some 300 bp. They reported:

> 'Since the average size of the nucleosomal repeat in yeast is 160 bp . . . the presence of discrete, regularly spaced bands between 160 and 300 bases shows that . . . there is structural regularity extending over regions much larger than one nucleosomal repeat unit. Even when digestion produces fragments so large that they must have arisen from cleavages within two different nucleosomes, the fragments are still of discrete sizes, spaced at intervals of 10 bases.'

The vital result here is that not only does DNase-1 cleave nucleosomal DNA to create fragments with lengths spaced at 10 bp intervals, and the spacer DNA between nucleosomes at 10 bp intervals also, but the two sets of fragments are also 10 bp apart from each other, producing an '. . . extended ladder . . . extremely clear and background free'.

As before, no explanation has ever been offered using the double helical model, as to how DNase-1 can score single-hit cleavages on physically separate and different chromatin sequences, both in the spacer and in the nucleosomal DNA, at random, in such a way that lengths extending across several spacers and nucleosomes always produce length differences of 10 bp, 'extremely clear and background free', when the nuclease can approach the double helix from any side in the linker DNA.

Though these experimental results are inexplicable in terms of the double helix, the result is comprehensible using SBS models, since they are not cylindrically symmetrical to the same extent (Figures 1 and 2)[1–3] and one not at all (Figure 3)[4].

Winding duplex DNA around the histones locks the DNA into one rotational isomer with one face in contact with the histones, according to SBS models. DNase-1 can cleave the DNA along the exposed front face of the spacer and the nucleosomal DNA, because it binds to the outer face and always cuts the duplex DNA at the same geometric contour (a crest or a trough, for example) in the helix of each strand. In this scenario, it becomes easier to understand how only fragments with lengths as a multiple of 10 bp can be produced.

Even *in vitro*, in the absence of nucleosomal proteins, several teams have reported highly regular cutting with DNase-1 (for example, see refs 22 and 23).

In another important experiment, McGhee and Felsenfeld[25] reacted nucleosomal DNA with dimethyl sulphate and recorded their surprise at their results:

> 'We are unable to detect any significant difference between the reactivity of the . . . guanines in nucleosomal DNA and of that in naked DNA . . . Contrary to our expectation, there is no detectable periodic modulation

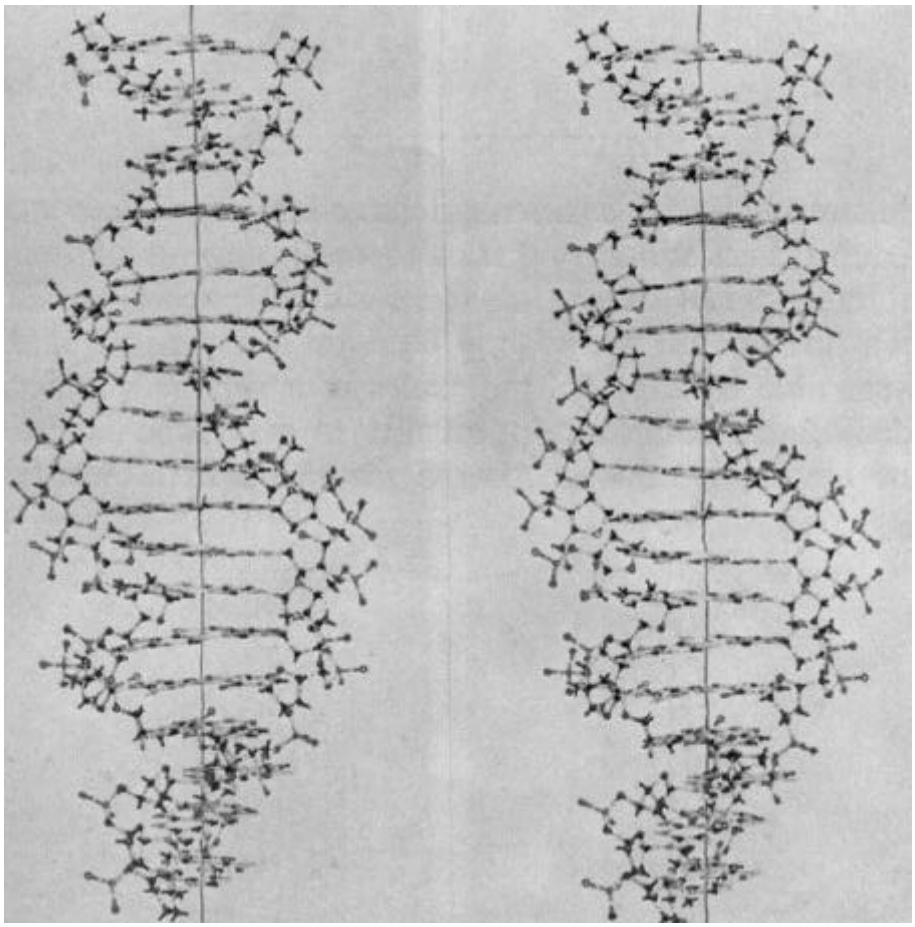

Figure 1. The original New Zealand SBS structure, as published in *PNAS* 1976 (ref. 1) – Courtesy the late Dr G. A. Rodley.

of reactivity corresponding to the twist of DNA on the nucleosome surface . . .'.

For a double helix, about half the DNA wrapped around a nucleosome core should lie on the inside in contact with the histone proteins, or up against adjacent turns of the DNA, and therefore offer reduced accessibility to methylation, contrary to the observed results.

We next outline another (little-noticed) physico-chemical technique that was deployed early on to probe the physical dimensions of duplex DNA.

DNA dimensions measured in monolayers

The strikingly direct 1953 Langmuir trough studies of James and Mazia[26] found that 1 mg of dry calf thymus DNA could be spread on aqueous salt solution in a monolayer to cover 0.28 m^2. From this measurement, it is immediately possible to calculate the diameter of the DNA as it lies across the film surface. Calf thymus DNA, with 40% C + G base-pairs, has an average mass of 330 Da per deoxyribonucleotide, and a base repeat distance of 0.334 nm, giving a diameter, measured across the surface film, of 1.2 nm. (The full calculation is available[4,27].)

James and Mazia measured the film height as 2.16 nm by optical interferometry. So, in this single paper there are independent, direct determinations of the width and height of the duplex DNA as 1.2 nm × 2.2 nm, i.e. duplex DNA would have an oval cross-section with a major and a minor axis.

The minor axis of the DNA molecule in the spread film of 1.2 nm is close to that of 1.3 nm found by Lee *et al*.[28] using scanning tunnelling microscope (STM).

Atomic force and scanning tunnelling microscopy

These relatively new techniques are applied, in this context, usually to the study of individual biomolecules. The results to date have illuminated various aspects of DNA structure not evident before.

In 1989, Lee *et al*.[28] published STM images of duplex DNA. Figure 3 *b* shows lengthy stretches of at least four well-resolved, linear DNA duplexes, each comprising several hundred base-pairs. The vital feature of these images of DNA is that no ravelling of the strands can be discerned; i.e. they show no sign of being double helical. The duplexes consist of long, paired helices with a consistent, uninterrupted co-parallel contour running between the two strands of each of the four duplexes, whose

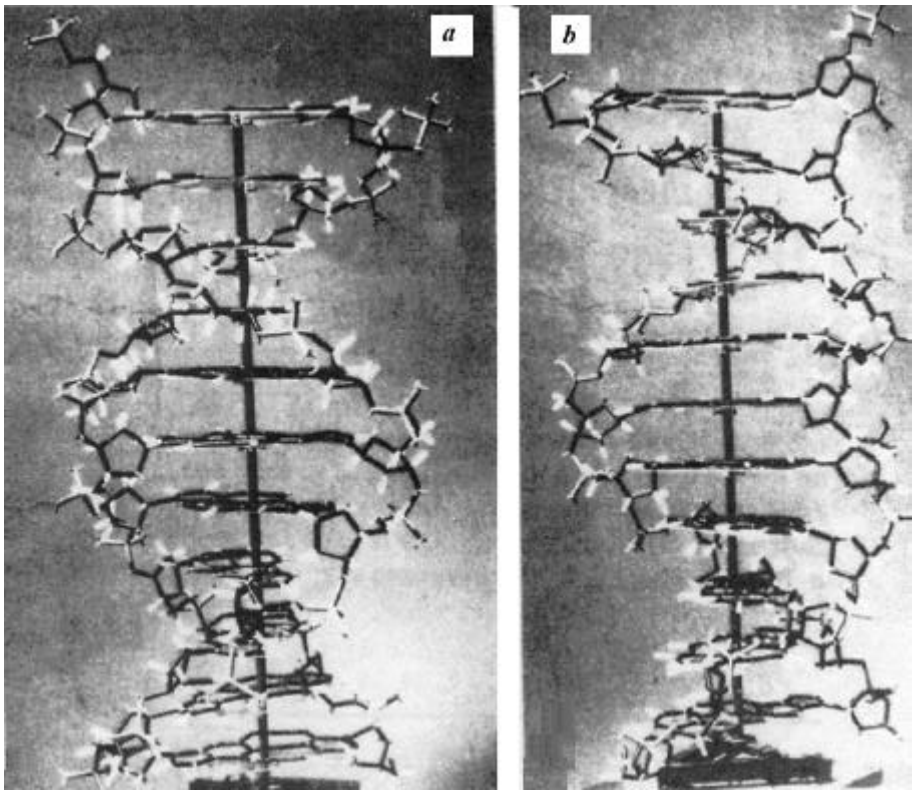

Figure 2. The original Indian SBS structure, as published in *Current Science* 1976 (ref. 2).

highlighted features remain exactly in phase with each other over their whole lengths. The highlighted, prominent features in the paired strands, which are strictly in phase with each other in Figure 3 *b*, are the turns of the SBS sugar–phosphate chains.

The measured heights – presumably the minor axis – of DNA duplexes have been reviewed[27]. In atomic force microscopy (AFM) and STM work, the preponderance of the measured strand heights for isolated duplex DNA falls in the range 1.6 to 1.2 nm, or less. None of the workers has reported the expected double-helix diameter of 2.0 to 2.2 nm for an isolated duplex.

Minor-axis measurements in the range 1.2 to 1.6 nm accord with SBS models (for example see ref. 4). Using lateral compression, Mou *et al.*[29] record AFM heights of up to 2 nm, values to be expected for the major axis of a duplex of oval section pushed up onto its narrow edge by the lateral compression.

Driscoll *et al.*[30] have published STM images of DNA which allow a ready interpretation in terms of the Indian, New Zealand and paranaemic SBS models.

The four-stranded polyguanosine nanostructure of Marsh *et al.*[31] is found by AFM to have both a height and a width of some 2.1 to 2.5 nm, corresponding to two duplexes of oval section lying upon each other (but see also ref. 32).

X-ray diffraction studies of DNA fibres and oligodeoxynucleotide fragments

Crystallographic techniques and X-ray diffraction from fibres continue to shed new light on aspects of duplex DNA structure which have traditionally been interpreted in favour of the double helix. However, the oligonucleotide diffraction data now available, taken with a careful review of older data from fibres, cast doubt on the early, perhaps precipitate choice of plectonaemic double-helical models as the only type of model to match the data.

X-ray diffraction studies pose severe challenges to the double helix. It is not widely understood that X-ray diffraction was never capable of playing a dominant role in the inference of DNA conformations, because DNA liquid crystals in wet fibres give only a few dozen diffuse X-ray reflections rather than the thousands of sharp reflections scattered by highly-ordered crystals.

Crick (with Cochran and Vand)[33] showed mathematically that a whole-molecule regular helical conformation can diffract X-rays mainly in a characteristic 'X' pattern. But other secondary structures too can give that pattern of scattering, since SBS models are also helical. This aspect of DNA structure is discussed by Stokes[34,35].

Bates wrote in 1978, 'if we have done one useful thing, it is to emphasize that the available X-ray diffraction data for DNA is of such poor quality that it is difficult to have confidence in any quantitative conclusion drawn from it'. But, insofar as X-ray analysis is relevant, Bates, Rodley and coworkers[36,37] showed that the SBS model fits the data for B-DNA better, when examined by the Patterson method which is unbiased in that it does not assume a helix or any other shape.

Stokes[38] showed in 1955 that the 'X' structure applied to fibres with axial ordering such as DNA, could be used to calculate the helical diameter of the macromolecules. Using Franklin's scattering pattern of B-DNA, this gives

Figure 3. A paranaemic structure for the B form of DNA (ref. 4).

a diameter of 1.2 nm, far removed from the expected double helix value of 2.0 to 2.2 nm. A value of 1.2 nm accords with AFM and STM results, with the monolayer studies of James and Mazia, and with a SBS model (Figure 3)[4].

A severe objection to fibre X-ray diffraction studies of DNA can be seen, for example, in the approach of Wonacott[39] and Arnott and Wonacott[40], where it is made clear that their work is based upon regressing their X-ray reflections upon an algorithm that was a model double helix. Thus their work could never produce an objective *ab initio* structural solution, but only double-helical outcomes.

More recently, true crystals have been made from fragments of DNA, up to a dozen or so base-pairs per molecule, known as oligodeoxynucleotides, (cf. the millions of base-pairs in typical DNA molecules *in vivo*). These oligodeoxynucleotides are claimed to have 'rope' structures, said to be beyond challenge. Scrutiny of the Rutgers Protein Database[41] shows that a number of the chosen unit cells are, in fact, supercells. Thus, for the structure factor files ar0002, bd0018, bd0019, bd0020, bdj039, ud0001 and zdd014 (an orthogonal unit cell), for example, all but one show patterns of systematic absence in a hexagonal net which would allow the choice of transformed, smaller unit cells reduced in size by the divisor root 3 on each side.

By an unfortunate coincidence, the W–C base-pair width at 1.1 nm, which determines the diameter of the helices in the paranaemic DNA duplex[4] at some 1.2 nm, is reduced also by the divisor root 3 compared with a typical W–C double helix at 2.0 nm. Therefore, irrespective of the high resolution of the X-ray diffraction from such oligoeoxynucleotide crystals, it seems that the structure in the larger unit cell housing a double helix could be refined equally well as the smaller-diameter helix in the smaller unit cell accommodating helices in a paranaemic SBS model[4], rather than the chosen double helix, as a preliminary starting model prior to further refinement.

Luger *et al.*[42] have reported a detailed X-ray study at 0.28 nm resolution of DNA wrapped around a nucleosome. Though this is claimed to show a double-helix structure for DNA *in vivo*, all the heavy atoms, offering the most intense diffraction reflections, are placed in the histones and none lie in the DNA.

While much of this article seeks to demonstrate the variety of alternative DNA structures which may be necessary to invoke to explain a wide range of experimental results, it is equally important to note that plectonaemic winding has been securely identified by X-ray diffraction from a number of crystal studies, for example, from crystals of a four-stranded hexanucleotide, $d(TGGGGT)_4$, at the very high resolution of 0.095 nm[32]. Moreover, the Rutgers Protein Database records a number of double-helical oligodeoxynucleotide structures deduced from the use of heavy atoms to improve the X-ray diffraction data. Therefore, there are oligodeoxynucleotide crystal structures that are double-helical and not SBS.

SBS structures in more detail

SBS models continue the well-established idea that the two chains run in opposite directions (are 'antiparallel'). Not surprisingly, several SBS models, (Figures 1 and 2)[1–3] are similar to the W–C: the phosphate–sugar backbones on the outside, the base-pairing inside, the stacking of the base-pairs reminiscent of graphite, and the 3.4 nm repeat distance along the two-stranded structure.

The first SBS models envisaged the strands as wrapped around each other – but only for short distances. The improvement is that each strand, having been helical in the right-handed sense for five base pairs, is bent in its phosphate–sugar backbone to become left-handed, and then after only a further five base-pairs reverses its helical sense again, and so on.

The 3.4 nm axial repeat distance, containing 10 base-pairs (at least in the fibre), thus consists in this model not of one whole (right-handed) helical turn but of two opposite-sense half-helix turns. *In vivo*, it is possible that the number of base-pairs in each alternating sequence is not exactly five, and is not necessarily exactly the same in the right- and left-handed stretches. An interesting variant by the Indian group includes 'upside-down' base pairs[3]. SBS models obviate the need to unravel; since the two strands are not intertwined but are instead topologically independent, they can simply 'unzip'. Arnott[43] and Stokes[34,35] have attempted to popularize the name 'warped zipper' instead of SBS.

A further SBS model is that of one of us (C.S.D. Figure 3)[4,27,44]. This paranaemic model, a term first used by Watson and Crick[45], is a structure for the B-form of DNA. It is claimed to resolve the structural problems faced by the double helix. There are two antiparallel, right-handed sugar–phosphate helices lying side by side with the phosphate charges around the outer edge of each helix, and in phase in the B-form, with the W–C base-pairs stacked on the same face of the duplex. Each individual helix has a diameter of 1.2 nm, giving the structure a minor axis of 1.2 nm, and a major axis of some 2.1 nm – the dimensions reported by James and Mazia[26] – with a pitch of 3.4 nm as in the double helix. This paranaemic structure for B-DNA conforms to standard bond lengths and bond angles, and has 10 W–C base-pairs per full helical turn with a pitch of 3.4 nm, as in the double helix.

Conclusion

The very idea that DNA *in vivo* has but one form of secondary structure is belied in the results of many workers. As Blackburn and Gait[46] remarked in 1996: 'Since 1980, there has been a rapid expansion in our awareness of the heterogeneity of DNA structures'.

The Canterbury group has reminded us, as they put it in 1980, that we should consider whether 'this important

molecule can be said with certainty to possess a single large-scale conformation'. They have not suggested that SBS models are ubiquitous; they merely point out that SBS models could well be part of the structural repertoire of duplex DNA.

Present researches by one of us (C.S.D.) are directed towards the re-indexing of the oligonucleotide supercells listed earlier, and the re-working of the original diffraction intensities against predictions deduced from members of the paranaemic family of structures. This should show how closely the paranaemic models[4], reproduced here as one example in Figure 3, match the crystal diffraction data held in the Rutgers Protein Database.

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