RNA-RNA, DNA-DNA, AND DNA-RNA POLYMORPHISM

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Most of our information on the detailed structures of nucleic acids has been derived from x-ray diffraction analyses of fibrous specimens. We have used restrained and constrained linked atom least-squares refinement of molecular and crystal models and, more recently, new automatic ways of processing diffraction patterns, to elucidate the structures of many nucleic acids.

Native and synthetic RNA and DNA double helices can be assigned to four genera: A, B, Z, and H. Each of the duplex species assigned to the first three has two conformationally identical, antiparallel chains (1). So far, all the RNA double helices observed in fibers and single crystals have been A type with C3′-*endo* furanose rings, average rotations per residue (t) of 30° to 33°, and axial rise per residue (h) of 0.26 to 0.33 nm (Fig. 1). This variability is accompanied by dramatic changes in the width of the major groove. DNA double helices in fibers can be A, B, Z, or H but are mainly B type with C2′-*endo* rings, average t = 36° to 45° and h = 0.30 to 0.34 nm (Fig. 2). No B-type RNA-containing duplex has been observed, presumably because the somewhat deep and narrow minor grooves of this genus are not compatible with the presence of a quasi-equatorial 2′-OH on ribose.

Z-DNAs are underwound (left-handed) double helices with a dinucleotide containing a C3′-*endo* furanose, *syn* purine and a C2′-*endo* furanose, *anti* pyrimidine as the repeated structural motif. Poly d(GC)·poly d(GC), poly d(AC)·poly d(GT), poly d(As^{4}T)·poly d(As^{4}T) and a DNA duplex complexed with an intercalating drug have been trapped in fibers in the Z form; they are also polymorphic, with t = 0° to −60° and h = 0.73–1.02 nm for a dinucleotide (Fig. 3). A spectroscopic study of poly r(GC)·poly r(GC) (2) indicates that this RNA duplex may be Z-like. One therefore expects that the DNA-RNA hybrid, poly d(GC)·poly r(GC), in appropriate milieu may also be Z-like.

Previous x-ray fiber studies of an f1 phage DNA-RNA

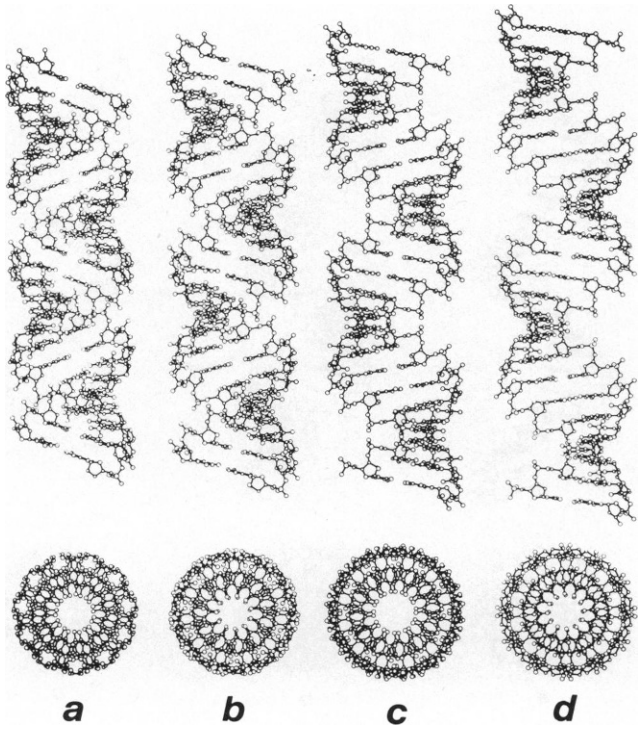

FIGURE 1 Mutually perpendicular views of four members of the A family of polynucleotide duplexes. The axial rise (h) and rotation per residue (t) are (*a*) 0.26 nm, 32.7°; (*b*) 0.28 nm, 32.7°; (*c*) 0.30 nm, 30.0°; and (*d*) 0.33 nm, 30.0°.

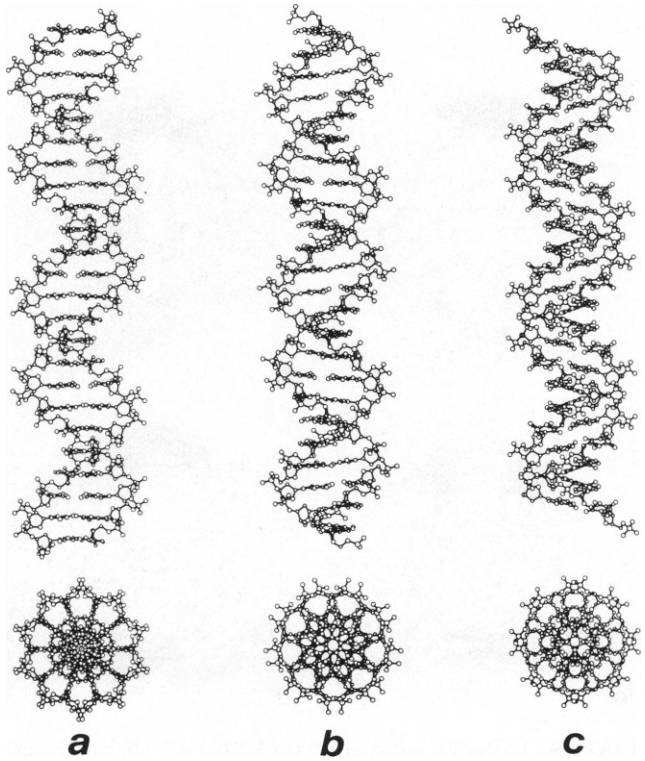

FIGURE 2 Mutually perpendicular views of three members of the B family of DNA duplexes. h and t are (*a*) 0.34 nm, 36.0°; (*b*) 0.32 nm, 40.0°; and (*c*) 0.30 nm, 45.0°.

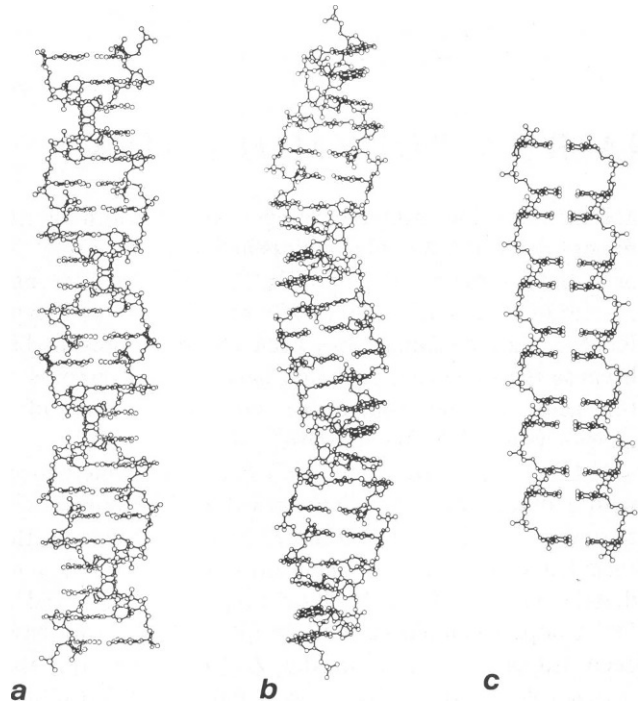

FIGURE 3 Structures of (*a, b*) two Z-like DNA duplexes and (*c*) an achiral DNA duplex. *h* and *t* (for a dinucleotide) are (*a*) 0.73 nm, −60.0°; (*b*) 0.76 nm, −51.4°; and (*c*) 1.02 nm, 0.0°.

hybrid (3) showed an A-DNA–like structure, and of poly r(I)·poly d(C) (4) revealed one like A′-RNA. Also, a more recent x-ray single-crystal structure analysis (5) showed that the duplex formed by r(GCG) d(TATACGC) is like A-DNA. A systematic fiber diffraction study (1) of synthetic homopolymer DNA-RNA hybrids has confirmed the preponderance of A-like structures but, in addition, has turned up structures of poly d(A)·poly r(U) and poly d(I)·poly r(C) with diffraction fingerprints (Fig. 4) not assignable to known double helices. We have established recently that these structures belong to the H genus which, uniquely, has two chains with very different conformations emphasized by C3′-*endo* furanoses in one chain and C2′-*endo* furanose rings in the other. The new 11-fold helix of poly d(A)·poly r(U) (Fig. 5) and 10-fold helix of poly d(I)·poly r(C) both have C2′-*endo* rings in the DNA strand and C3′-*endo* rings in the RNA strand. These heteromerous structures have the interesting property of being bidirectional and hence may be relevant to the fundamental biological roles of transcription and replication in which DNA-RNA hybrids are involved. Fibrous poly d(A)·poly d(T) forms a 10-fold double helix of this type (6). The model proposed for the gel form of poly r(A)·poly d(T) (7) is a 10-fold helix of this kind but the poorly resolved diffraction pattern could also be interpreted in terms of an 11-fold helix.

This work was supported by a National Institutes of Health grant GM17371.

Received for publication 30 April 1985.

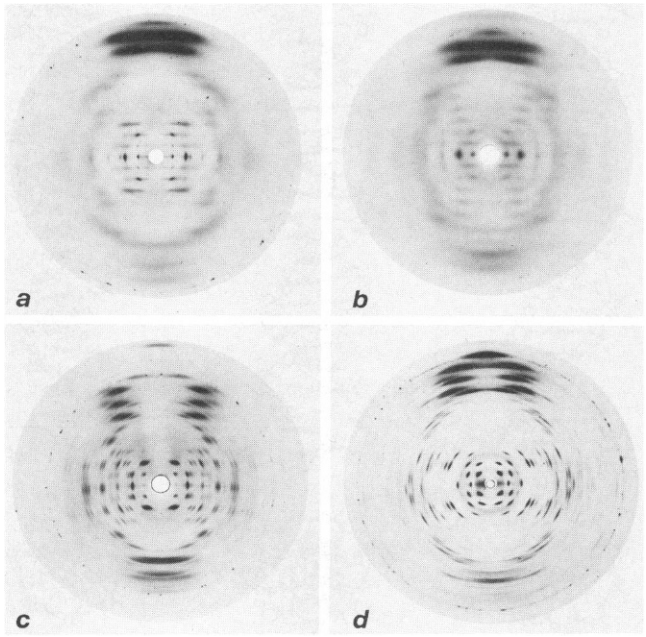

FIGURE 4 Diffraction patterns from DNA-RNA hybrids (with symmetries and helical pitches): (*a*) poly d(A)·poly r(U) (11_1, 3.37 nm), (*b*) poly d(I)·poly r(C) (10_1, 3.13 nm). These may be compared with the patterns of classical A-type structures (*c*) A-DNA (2 11_1, 2.8 nm) and (*d*) A′-RNA (2 2 12_1, 3.6 nm).

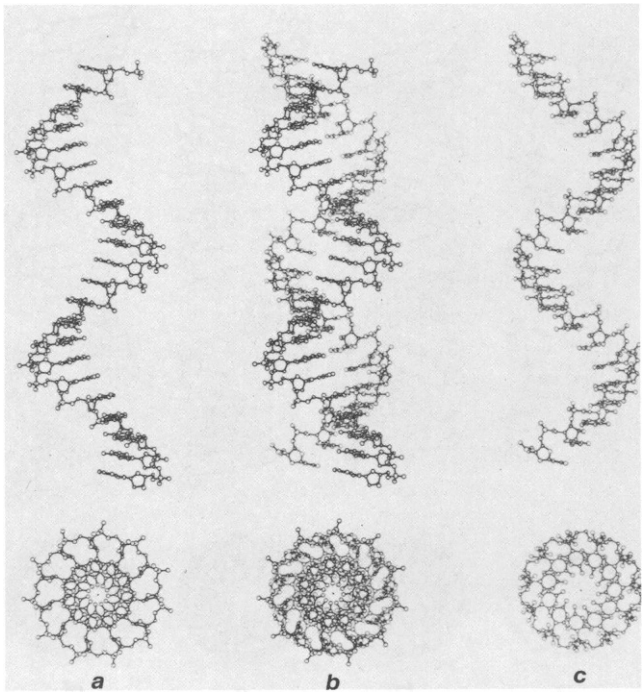

FIGURE 5 Mutually perpendicular projections of heteromerous poly d(A)·poly r(U) are shown in *b* flanked by its component strands: (*a*) B-type poly d(A) and (*c*) A-type poly r(U). Please refer to the color figure section at the back of this book. Please refer to the color figure section at the back of this book.

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STRUCTURE AND DYNAMICS OF RECA PROTEIN-DNA COMPLEXES AS DETERMINED BY IMAGE ANALYSIS OF ELECTRON MICROGRAPHS

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The recA protein of *Escherichia coli* mediates genetic recombination; regulates its own synthesis; controls the expression of other genes; acts as a specific protease; forms a helical polymer; and has an ATPase activity, among other observed properties (1–3). The binding of recA to DNA has been shown to alter dramatically the conformation of DNA (4). A key question in understanding DNA recombination, protein-nucleic acid interactions, and protein function in general is how a 38k mol wt molecule can perform all of these activities. Many of the activities of the recA protein are dependent on an ATPase, and previous electron microscopic work has shown that the recA polymer can be seen in different conformations as a function of the presence or absence of ATP and an ATP analogue (5–7). We have used computed image analysis of electron micrographs of recA-DNA complexes to gain more understanding of this fascinating system.

METHODS

Several different complexes have been examined in the electron microscope: pure recA polymers formed with a nonhydrolyzable ATP analogue, ATP-γ-S; recA-double stranded DNA (dsDNA) complexes formed with ATP-γ-S (8); recA-single stranded DNA (ssDNA) complexes formed with ATP (9); and recA-ssDNA complexes formed in the absence of nucleotide cofactors. Negatively stained specimens were used for three-dimensional reconstruction, while platinum-shadowed specimens were used for the determination of the hand of the helices.

The greatest difficulty in processing images of the polymer is the large disorder within the filaments. Large numbers of filaments were examined to find a few which gave rise to useful transforms. In the ATP and ATP-γ-S state, the most striking feature of the transforms is that the pitch of the nominally 95 Å helix can vary by over 10%. Further, the recA polymer is very flexible, and most filaments examined had to be corrected for curvature.

RESULTS

At the lowest resolution, the recA polymer seems to exist in one of two states: a very "open" structure, characterized by a 95 Å-pitch helix, and a compact "closed" filament, characterized by a 64 Å-pitch helix. Both helices are right handed. The 95 Å-pitch helix is formed in the presence of ATP and ATP-γ-S, and the 64 Å-pitch structure is formed in the absence of nucleotide cofactors. Fig. 1 shows the striking differences between these two states as seen in the helically averaged densities. Because of the large disorder, layer lines extracted from individual filaments did not generally average together well. However, the layer lines arising only from the right-handed one-start helix (of either 64 or 95 Å nominal pitch) did average together better, and the resulting views that are shown are equivalent to averaging the detailed density of the polymer along the one-start helix. The effect of removing the nucleotide cofactor is to dramatically compress the deep grooves of the filament.

At this level of resolution the structure can illuminate some of the observed mechanical properties of the filaments. The spring-like conformation of the ATP-γ-S state, with no apparent groove-spanning contacts, should readily permit the structure as a whole to compress and extend axially, as it is observed to do. This structure can also explain the enormous flexibility of the polymer, since the grooves can be easily compressed and extended during bending. For purposes of comparison, recA polymers in the ATP-γ-S state are ~10 times more flexible than actin, even though they have a greater mass/unit length.

A few filaments in the ATP-γ-S state generated additional layer lines arising from the modulation of continuous