Models of hypothetical recombination junctions

S McGavin

Chemistry Department, University of Dundee, Dundee, Scotland, UK

A range of molecular models has been built of suggested recombination junctions and intermediates using the computer graphics system at EMBL (Heidelberg). These are based on the idea that there may be specific pairing between duplexes to form a four-strand structure.

Keywords: recombination models, DNA, structure, computer graphics

received 2 October 1984, revised 31 May 1985

Some time ago, a molecular model (McGavin [1-3]) was described in which two duplexes of the Watson–Crick type are paired specifically to each other about a common long molecular axis with their major grooves in continuous and specific contact. It was suggested that this kind of structure might occur in recombination. The model was discussed in relation to recombination (McGavin[4]) and from other points of view (McGavin [5-7]).

Interest has been shown by others in the application of such four-strand models to recombination. In particular Nash *et al*[8]. (see also Kikuchi and Nash[9] and Nash and Pollock[10]) have used the idea of four-strand structure as the basis for models of the site-specific recombination of lambda bacteriophage and have pointed out the interest which attaches to the topological consequences of models of this kind. Following Nash *et al*[8] a simple model was described in which duplexes are replaced by rubber strips which helps in visualising and following the topological consequences of these and related models[11]. Interest has also been shown in four strand models in relation to general recombination (see for example West *et al*.[12,13]).

Such applications of four-strand models to recombination, as they are presently formulated, do not depend on any one particular detailed molecular model but depend only on some kind of four-strand association, that is on a close, specific and restricting juxtaposition of duplexes. In this paper the consequences of the detailed model are considered. This is now possible using the computer graphics system at EMBL (Heidelberg).

Recombination models are not reviewed here (for recent reviews see Potter and Dreessler[14] and Nash[15]). It should however be noted that models arrived at by consideration of four-strand structures can be formally similar to other types of model, and can lead to intermediates of the Holliday[16,17] type.

The use of the graphics system to study models which relate particularly to recombination is described. A more general account of the use of the graphics system to study four-stranded and other multi-stranded models will be given elsewhere[18,19].

EXPERIMENTAL

General

The aim is to follow the approach and pairing of duplexes, breakage and reunion and the subsequent separation of daughter duplexes.

It seemed intuitively obvious that if the possibility of the four-strand structure is accepted, then junctions with duplexes could be made, if these are simply extended. An aim of this work, therefore, has been to make junctions which were compact and to make them in as few moves as possible.

There are no electron density maps or parameters from fibre diffraction patterns to fit which would focus attention on limited regions of conformational space. Several fairly rigid simplifications have therefore been introduced into the model building.

Simplifications

The main simplifications are as follows. C2′ endo sugars were used normally. Planar and coplanar bases lying perpendicular to the helix axis were used. Regular distribution of glycosyl bonds was assumed. In the models discussed here as essentially square or rectangular distribution of glycosyl bonds was used. Some four-strand models in which base pairs are sheared with respect to each other have now been built to improve bonding schemes and contacts between base pairs. These sheared models have not so far been used in attempts to build recombination junctions.

Some departure from the regularities just mentioned resulted from the use of a least squares refinement program. The glycosyl bond arrangement of the model used as the basis for the junctions described below was not exactly square, for this reason.

In these models of junctions GC base pairs were used throughout, the assumption that CG, AT and TA pairs, but not other pairs, could probably fit into similar structures without too much deformation (see earlier papers), provided they are paired to identical pairs about dyad axes coincident with the long molecular (helix) axis.

Most of the work involved use of duplex which was numbered C1 to C9 and G10 to G18 (i.e. it involved a C and a G chain). Bending was between residues C4 and C5 in the C strand and between residues G14 and G15 in the G strand.

Graphics system

All of the models described in this paper were built using the computer graphics system at EMBL (Heidelberg). The initial coordinates for the basic four-strand model were, however, obtained earlier by use of wire models (see papers cited above).

Hardware

The graphics system at EMBL is based on an Evans and Sutherland Multi Pictures System hosted by a VAX computer (Digital Equipment Corporation VAX-11/780). Most of the work described here involved use of an Evans and Sutherlands CSM scope (i.e. colour was normally used). Interaction was via a digitizing tablet and a normal computer terminal (VT 100).

Full screen stereo was available, in which pictures, alternating rapidly, are viewed by means of a slotted rotating cylinder (Bausch and Lomb) synchronized with the display. This proved to be very useful for interactive work.

Photography

The diagrams reproduced here are photographs of the screen of the Pictures System colour scope. They were taken using a Rolleiflex SLX camera with Kodak Ektachrome Professional Film (daylight). The stereo pairs reproduced here are full screen pictures taken at rotations of +3 and −3° about the screen X axis.

Choice of colours

Choice of colours was made with a view to contrasting the paired duplexes and the joints in the hybrid chains. In the initial paired structures one duplex is a light blue and the other is orange. O4′ atoms of the sugar rings were coloured the conventional red for oxygen to indicate the polarity of the chains (the oxygen atom acts as an arrow indicating the polarity). C1′ atoms were coloured green because these atoms were used as guides in modelling.

Programs

The main programs used were:

- Frodo, written by Jones[20]. The version which is in use at EMBL has been very extensively revised at the laboratory.
- Nuclin and Nuclsq, which are programs for the least squares refinement of nucleic acid structures, modified by Westhof (Strasbourg) from Quigley's Refin (MIT) and Hendrickson's Prolsq, respectively.

CONSTRUCTION OF MODELS AND RESULTS

Basis of models

The models described below are based on a short section of four-strand structure as has been described elsewhere (McGavin, references cited above). A five-layer section was used which had 32° rotation between successive layers of four bases, and a translation of 3.4Å between layers.

A length of normal Watson–Crick B form DNA was read into the same file as the section of four-strand structure. This duplex had been constructed using a program Dnahelix written by Westhof. It had 36° rotation between successive base pairs. An end (top) base pair of one of the constituent duplexes of the four strand section was docked with one end (bottom) of the B form duplex.

The docked sections containing ten base pairs (five from the four strand region and five from the B form duplex) were then read twice into another file and the four-strand structure was regenerated by rotating one of these sets of ten base pairs by 180° about the long axis. The result is a plectonemically wound four-strand region merging into two parallel and interpenetrating B form duplexes.

Initial junctions

Construction of *Y* junctions

The construction of the initial pairing junctions involved first tilting of the normal B form duplexes equally away from the axis of the four-strand region until these duplexes were clear of each other. The minimum angle required for this was found to be about 36° (Y angle twice this). Second correction of the geometry at the junction as much as possible in one of the dyad-related components. Third, least squares refinement, and finally construction of the complete Y and double Y junctions by symmetry (see colour plates 1 and 2). Correction of the geometry at such junctions sometimes involved a considerable amount of work which is not described here.

Junctions with 90° bends (*T* junctions)

Junctions, essentially T junctions (Colour plate 3) were also made with a bend of 90° in each of the constituent duplexes. Little difficulty was found in making these. In an initial attempt a short contact was found between 01P(C7) and 01P(G17), this is improved but remains rather short in the model with the 90° bends shown here.

Details of models of initial junctions

In the Y junction no distances (that is bond length and angle distances) deviated from 'ideality'* by more than two standard deviations. Most single torsion contacts seem reasonable the worst being C4′.....P which are about 3Å at the bends (i.e. between residues C4 and C5 and between C14 and C15).

The only multiple torsion contacts which might be considered short are C2′.....P distances between successive residues. This however is a feature of the helical four-strand model as it stands at present and indeed similar contacts are present in the B form duplex which was used in this work.

After refinement, no distances in the T junction (with 90° bends) deviated from 'ideality' by more than two standard deviations. Torsion contacts are reasonable. One contact at the bend remains short as mentioned above.

*The program NUCLSQ outputs lists of bond lengths and angle distances which differ from 'ideality' by more than two standard deviations, one of which is set at 0.02 Å for bond lengths and at 0.03 Å for angle distances.

Table 1. Torsion angles associated with bends in models

		Initial *Y* Junction			*T* Junction				Final *Y* Junction			
C4′ . . . C3′ . . . O3′ . . . P	alpha	G14	−28	C4	−32	G14	−107	C4	−109	G14	−176	C4	82
C3′ . . . O3′ . . . P . . . O5′	beta	G14	−156	C4	−152	G14	105	C4	113	G14	−93	C4	91
O3′ . . . P . . . O5′ . . . C5′	gamma	G15	−13	C5	−17	G15	92	C5	84	G15	−71	C5	−101
P . . . O5′ . . . C5′ . . . C4′	delta	G15	−85	C5	−84	G15	−87	C5	−90	G15	−73	C5	117
O5′ . . . C4′ . . . C3′ . . . O3′	epsilon	G15	−150	C5	−139	G15	−171	C5	−155	G15	−52	C5	80
O1′ . . . C1′ . . . N1 . . . C6	chi	G14	64	C4	66	G14	74	C4	−75	G14	72	C4	35
O1′ . . . C1′ . . . N9 . . . C8	chi	G15	64	C5	64	G15	59	C5	59	G15	66	C5	64

The torsion angles associated with the bends in these models are shown in Table 1.

Formation of initial junction

It is difficult to see how duplexes could pair to form structures of the kind discussed without some catalysis of the process. One of the reasons for looking at models in which duplexes are extended (see below) was that it seemed that extension might be required to bring about pairing. Once paired an extended structure might collapse into the more compact kind of structure discussed above.

Another possibility, however, is that initial pairing might be over a short region, as is suggested by the work of Nash *et al.*[8–10] If two bends in a duplex are separated by a small number of base pairs (probably six or fewer), then pairing between two such doubly bent duplexes could take place without any further deformation. Reversible pairing of this kind can easily be demonstrated (Colour plate 4). A structure involving such pairing over a short region might act as a nucleus for formation of a more extended four-strand region.

Final junction

The duplexes of the initial junctions just described have approximations to dyad axes coincident with the bisectors of the angles formed by bending. Such symmetry does not occur in the 'final' junction. In the initial junctions the bend is essentially in a normal duplex. In the final junctions the bend is between a normal B form duplex and a different kind of duplex in the four-strand section (this second kind of duplex is half of the four-strand region formed by dividing (splitting) this along its entire Watson–Crick bonding system). The torsion angles of the two strands at the bend would therefore be expected to differ more from each other, than in the initial pairing junction. Some difficulty was in fact met in trying to construct such a final junction.

Construction of the model

The final junction models were also constructed from the junction with parallel interpenetrating duplexes, used as the basis of the initial junctions. The steps involved were similar except that first of all cuts were made in diametrically opposed strands and then strands were rejoined after swivelling essentially as shown in Colour plates 6 and 7. The only unsatisfactory distance (of the bond length and angle distance kind) was that corresponding to the angles at O3′ (in the best models now about 125°). Colour plate 5 shows a model of this junction.

Formation of the final junction

Swivelling could be of 90° or of 270° in the opposite direction. 270° is favoured by Nash *et al.* for topological and steric reasons.

Using the structure with parallel interpenetrating duplexes, these interpenetrating duplexes were swivelled towards each other to a half way (45°) position where overlap is maximum and were then tilted until they were clear of each other. Tilt had to be nearly 90°. Here pivoting was about C1′ atoms. This model is shown in Colour plate 6.

It should be noted that the tilting involved here is different from that involved in the junctions discussed above since it follows strand breakage and therefore involves single strands. Clearly such tilting is not required if swivelling is in the opposite direction[8]. Colour plate 7 shows a model half way through a 270° swivel.

Less effort was put into refining these models than the junctions described above. As they stand, however, these models are reasonable and as they involve single strands could almost certainly be improved further.

Separation of daughter duplexes from final junction

Given final structures of the kind shown in Colour plate 5 separation could be a simple, neat process, involving the exchange of base-pairing partners. The possibility of exchanging base-pairing partners within compact structures is one of the attractive features of four-strand models of recombination, and seems unlikely to require separation into single strands. This was first shown diagramatically (McGavin[4]). Colour plates 8a and b show models of this process in one plane. Here swivelling was about C1′ atoms. For this to occur in the one plane some separation of bases (of about 1Å) is required. Movement out of the plane, as would be allowed in structures of the kind shown in Colour plates 5 and 6, would make this process easier.

Models with parallel strands

Models with parallel extended strands were also constructed because it seemed possible that they would be simpler, and that they might make it easier to see what was involved in a process of exchange of base-pairing partners. It also seemed at first that some extension of duplexes would be required for an initial pairing to occur. It is also known that recA protein is associated with an extension of duplex structures under some conditions[21]. These extended models are not considered further here.

DISCUSSION

Some of the models described still have unsatisfactory features. It is believed, however, that stereochemically reasonable structures are likely to exist fairly close to those described. What seems to be required for a continuation of work of this kind is the development of programs which would allow scanning of a range of structures in which nucleotides are moved relative to each other in a systematic manner. 'Manual' movements of parts of models are too time consuming and least squares refinement is limited to fine detail.

The idea of building *T* junctions was suggested by the model building work of Crick and Klug[22], on 'kinks' in duplex structures. They found that in building kinks in duplexes that the best kink angles were in the range 90–100°. The initial *Y* junctions were based on the minimum angles required to bring the duplex arms of the *Y* clear of each other.

Crick and Klug also point out that where the DNA bends with a large radius of curvature a smooth and continuous curve is likely, but that where a small radius of curvature is required kinks are likely. They suggest that where kinks occur it is better if only one stacking contact is broken. With the compact models involving helices described above it was necessary to use kinks (sharp bends) so that the duplex regions could clear each other.

With the extended models (mentioned above) bands can be smooth and have large radii of curvature. It is unlikely therefore that there need be any difficulty in making junctions of some kind.

All of the models described here depend on the possibility of the four-strand structure. Although the four-strand model as it has been described still has some tight features, these are probably related to the arbitrary simplifications which have been introduced into the models.

ACKNOWLEDGMENTS

Stewart McGavin wishes to thank staff of EMBL (Heidelberg) for their help and hospitality during several visits. Particular thanks are due to H E Bosshard, C Carlson, D Banner and D A Marvin. His work could not have been done at all without the great deal of work which has been carried out by H E Bosshard and C Carlson in developing an excellent graphics system at EMBL. He also wishes to thank the European Molecular Biology Organisation for the award of a short-term fellowship, which supported part of this work.

REFERENCES

1 **McGavin, S** *J. Mol. Biol.* Vol 55 (1971a) pp 293–298
2 **McGavin, S** *Proc. First European Biophysics Congress* (1971b) pp 259–262
3 **McGavin, S** *J. Theor. Biol.* Vol 77 (1979) pp 83–99
4 **McGavin, S** *Heredity* Vol 39 (1977) pp 15–25
5 **McGavin, S** *Nature, Lond.* Vol 242 (1973) p 330
6 **McGavin, S** *J. Theor. Biol.* Vol 85 (1980) pp 665–672
7 **McGavin, S** *J. Theor. Biol.* Vol 91 (1981) pp 33–40
8 **Nash, H A, Mizuuchi, K, Enquist, L M and Weisberg, R A** *Cold Spring Harbor Symp.* Vol 45 (1980) pp 417–428
9 **Kikuchi, Y and Nash, H A** *Proc. Natn. Acad. Sci. USA* Vol 76 (1979) pp 3760–3764
10 **Nash, H A and Pollock, T J** *J. Mol. Biol.* Vol 170 (1983) pp 19–83
11 **McGavin, S** *J. Theor. Biol.* Vol 107 (1984) pp 37–56
12 **West, S C, Cassuto, E and Howard-Flanders, P** *Proc. Natn. Acad. Sci. USA* vol 78 (1981a) pp 6149–6153
13 **West, S C, Cassuto, E and Howard-Flanders, P** *Nature, Lond.* Vol 290 (1981b) pp 29–33
14 **Potter, H and Dressler, D** *Ann. Rev. Biochem.* Vol 51 (1981) pp 727–761
15 **Nash, H A** *Ann. Rev. Genet.* Vol 15 (1981) pp 143–167.
16 **Holliday, R** *Genet. Res.* Vol 5 (1964) pp 282–304
17 **Holliday, R** *Genetics* Vol 78 (1974) pp 273–285
18 **McGavin, S, Bosshard, H E and Carlson, C** in preparation
19 **McGavin, S, Bosshard, H E and Carlson, C** in preparation
20 **Jones, T A** *J. Appl. Cryst.* Vol 11 (1978) pp 268–272
21 **Stasiak, A, Di Capua, E and Koller, Th** *J. Mol. Biol.* Vol 151 (1981) pp 557–564
22 **Crick, F H C and Klug, A** *Nature* Vol 255 (1975) pp 530-533