Nick-free formation of reciprocal heteroduplexes: A simple solution to the topological problem

(genetic recombination/homologous DNA pairing/winding topology/fused heteroduplexes)

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ABSTRACT Because the individual strands of DNA are intertwined, formation of heteroduplex structures between duplexes—as in presumed recombination intermediates—presents a topological puzzle, known as the winding problem. Previous approaches to this problem have assumed that single-strand breaks are required to permit formation of fully coiled heteroduplexes. This paper describes a simple, nick-free solution to the winding problem that satisfies all topological constraints. Homologous duplexes associated by their minor-groove surfaces can switch strand pairing to form reciprocal heteroduplexes that coil together into a compact, four-stranded helix throughout the region of pairing. Model building shows that this fused heteroduplex structure is plausible, being composed entirely of right-handed primary helices with Watson–Crick base pairing throughout. Its simplicity of formation, structural symmetry, and high degree of specificity are suggestive of a natural mechanism for alignment by base pairing between *intact* homologous duplexes. Implications for genetic recombination are discussed.

A central issue in genetic recombination is the mechanism by which two DNA duplexes come to recognize their mutual sequence homology. In 1964, Holliday (1) proposed that this recognition involved base pairing via formation of reciprocal heteroduplexes at regions of homology in the recombining duplexes. Ample genetic and biochemical evidence supports a structure of the general type proposed by Holliday as an intermediate in the recombination process (2, 3). Many models of recombination have been formulated to account for the temporal sequence of the cutting and pairing events required to form a Holliday structure and to resolve it into recombined duplexes (4–6). A *uniform* feature of all these models is that the pairing of complementary strands to form complete heteroduplexes with Watson–Crick structure throughout is *preceded* by breakage of phosphodiester bonds. This requirement for strand breakage arises because of the topological constraint that *net* intercoiling of separate, closed curves be zero. If parental strands separate locally prior to heteroduplex formation, as they do in these models, this topological constraint precludes formation of *fully* right-handed heteroduplexes in the absence of strand breaks. This topological difficulty has been termed the winding problem (7).

In this paper I describe a straightforward, nick-free pathway for formation of reciprocal heteroduplexes that coil together into a four-stranded helix. Winding by this pathway, which occurs in the absence of strand separation, permits all topological constraints to be satisfied by a structure composed *entirely* of right-handed primary helices. This unique topological solution to the winding problem thus eliminates the need for strand breaks that have been considered a prerequisite for complete heteroduplex formation. Model building indicates

that the structure predicted by this winding pathway is reasonable and has some remarkable properties, including a higher degree of specificity than simple heteroduplex formation. This plausible mode of pairing between intact homologous duplexes defines a new pair-first pathway of recombination. As is discussed, presumptive recombination intermediates with the gross topology predicted by the pathway recently have been observed by electron microscopy.

Topology of duplex pairing

It is easiest to think about the topology of duplex pairing initially in the absence of DNA structure. Consider, for example, a pair of ropes each composed of two strands that are intertwined in a right-handed fashion (Fig. 1). Hybrid ropes can be formed in two distinct ways by winding together strands of different parentage. Path I for hybrid rope formation involves winding strands together about axes that are separated in space. Winding about different axes requires that strands in each rope separate and then rewind. If strand separation is confined to the interior of a rope so that the ends do not come apart, the separated strands effectively form a single closed curve. The net intercoiling of two such closed curves is constrained topologically to be zero. Consequently, this winding pathway leads to an equal mixture of right-handed and left-handed helical segments within the hybrid region. This mixed handedness in the hybrid region is the essence of the winding problem (7). To produce hybrid ropes that are entirely right-handed by path I winding requires that one strand in each hybrid be broken and properly rewound. Because all current models of recombination utilize this basic winding pathway, they uniformly require a breakage
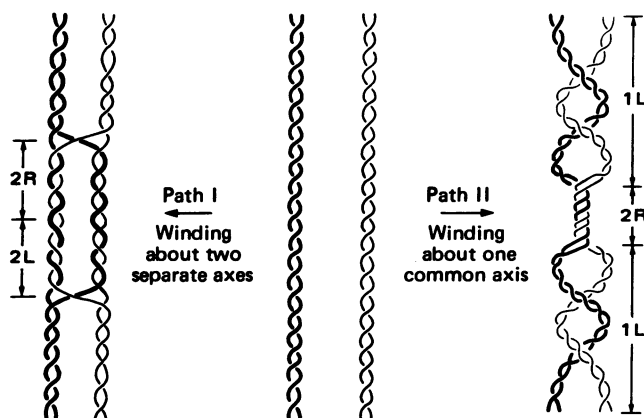

FIG. 1. Two winding pathways for hybrid rope formation. R and L denote right- and left-handed helical segments, respectively, that result from the indicated winding. If a strand on the front surface of a vertically oriented helix points upward to the right, the helix is right-handed; if it points upward to the left, the helix is left-handed.

of phosphodiester bonds before formation of complete heteroduplexes.

The second pathway for hybrid rope formation does not require strand separation (Fig. 1). Path II involves winding parental ropes together about a common axis to produce a four-stranded rope in which each individual strand coils about the others with the same handedness and approximately the same periodicity as in the parental ropes. Because all four strands of rope are equivalent, the four-stranded region can be viewed either as intercoiled parental ropes or as intercoiled hybrid ropes. If the ends of the ropes remain fixed as shown in Fig. 1, each right-handed intercoil in the four-stranded region will be compensated by one left-handed intercoil in the external parental ropes. Strictly speaking, the topological constraint of zero net intercoiling applies in path II winding only if the parental structures are circular; free ends could rotate around each other to remove the left-handed intercoils.

Path II winding permits homologous duplexes to pair by reciprocal heteroduplex formation in the absence of strand breaks. In contrast to strands of rope, the two strands of duplex DNA are not identical. Their intertwining produces a twisted-ribbon structure with two chemically distinct surfaces that form the major and minor grooves of the double helix. Two DNA duplexes can wind together symmetrically about a common axis after initial association either across their major-groove surfaces or across their minor-groove surfaces. Winding across major-groove surfaces leads directly to the structure in Fig. 2a, which has been described by McGavin (8). It is a four-stranded helix consisting of intercoiled *homo*duplexes. A distinctly different structure (Fig. 2b) results from an initial
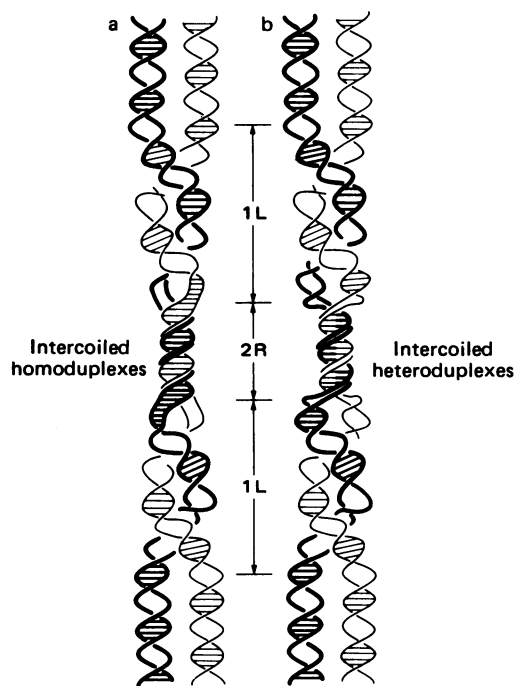
association across minor-groove surfaces followed by a switch in strand pairing as described below. This four-stranded structure is composed of intercoiled *hetero*duplexes. Within the four-stranded region of each structure the two duplexes intercoil once per 10–11 base pairs. The compensatory intercoiling outside the four-stranded regions is required topologically only for circular DNAs, but is a reasonable expectation for long linear DNAs. Although the structures in Fig. 2 are topologically equivalent and structurally similar, they are not interconvertible (except by way of separated duplexes) because of the sidedness of the initial association.

Switch in strand pairing

The lateral shift in base pairing that underlies the topological solution to the winding problem is illustrated schematically in Fig. 3. Base pairs are juxtaposed initially across their minor-groove surfaces. This orientation places complementary strands opposite one another. In this position a switch in strand pairing can form two heteroduplexes simultaneously. The shift in pairing requires that hydrogen bonds between parental base pairs be broken, that each base rotate 90° about bonds in the backbone, and that hydrogen bonds between complementary bases from the two parental duplexes be formed. Note that the heteroduplex base pairs now face each other across their major-groove surfaces. The switch in strand pairing for several base pairs is illustrated in Fig. 4 by using a nonhelical, "railroad track" model of DNA. This model can be converted to a coiled structure like that in Fig. 2b by twisting; no covalent or noncovalent bonds need be broken.

Superficially it would appear equivalent for parental base pairs to associate initially across their major-groove surfaces and then switch pairing partners to form heteroduplex base pairs associated by their minor-groove surfaces. However, examination of space-filling models indicates that, although duplexes can intercoil comfortably across their major-groove surfaces, symmetric intercoiling across their minor-groove surfaces requires untwisting and unstacking, even if it involves only a few

FIG. 2. Duplex pairing by major-groove association (a) and minor-groove association (b). R and L denote right- and left-handed intercoils, respectively. Base pairs have been drawn only in the minor grooves to emphasize the two distinct surfaces of DNA. Note that only minor-groove surfaces are exposed in the four-stranded regions. Because net intercoiling of the paired duplexes is 0, there is no effect of intercoiling *per se* on the supercoiled state of the parental duplexes. However, if the screw of the four-stranded helix differs from that of native DNA, there will be axial rotation of the parental duplexes and a consequent effect on supercoiling.
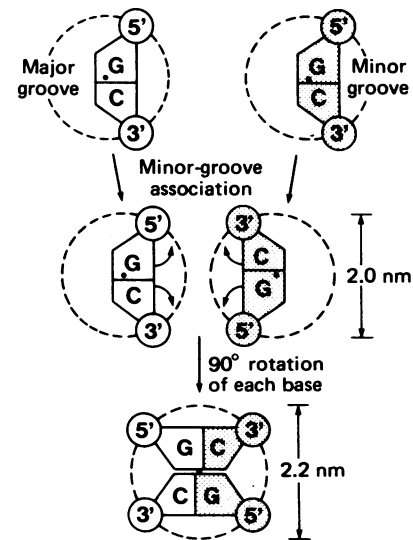

FIG. 3. Lateral shift in base pairing. This viewpoint looks down the helix axis. Identical base pairs initially associate by their minor-groove surfaces. Each base rotates 90°, as indicated by the curved arrows, to form two heteroduplex base pairs simultaneously. Small circles represent sugar-phosphate backbones; 5' and 3' indicate the ends of the sugar that point toward the reader. Black dots represent helix axes, and dashed circles represent the cylinders in which the helices spiral. The helix axis for the fused heteroduplex base pairs is displaced approximately 0.2 nm from the axis of B form DNA.
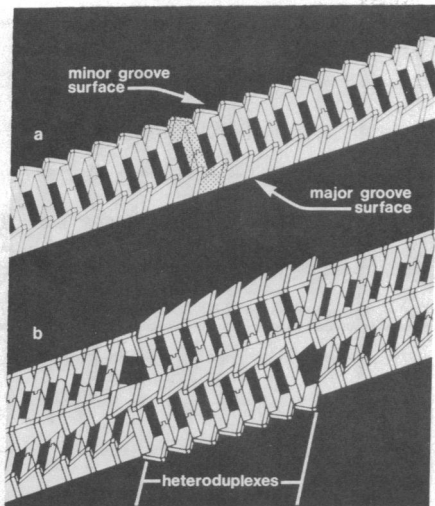
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FIG. 4. Railroad track model of DNA (a) and of fused hetero-duplexes (b). The rails of the model depict the sugar-phosphate (trapezoid-rectangle) backbones of DNA. The small end of the trapezoid represents the 3' end of the sugar. One nucleotide pair is shaded in a. The indicated directions of the rails uniquely define the upper, jagged surface as the minor-groove surface and the lower, flat surface as the major-groove surface. Base pairs are shown as railroad ties. Rotations about backbone bonds in b are shown as occurring about the 3'-sugar-phosphate bonds for aesthetic reasons. For a different view of b, rotate the page 180°.

or by switching pairing partners in regions of identity to form intercoiled heteroduplexes.

Structure and properties of intercoiled heteroduplexes

Intercoiled heteroduplexes are most easily described by comparison with intercoiled homoduplexes. These two structures differ significantly in specificity of interaction, inherent driving force for coiling, and the structure of the junctions between the four-stranded helix and the parental duplexes.

The four-stranded helices in each structure are essentially identical, differing only in strand pairing. McGavin has demonstrated, with space-filling models, that a four-stranded helix can be constructed with about 10 base tetrads per turn. However, each duplex in the helix is slightly distorted because the four-strand axis is not coincident with the helix axis for native DNA (Fig. 6). The magnitude of the resulting distortion can be appreciated when one considers that a duplex as it exists in a McGavin four-stranded helix (8) is closer to native duplex structure than that initially proposed by Crick and Watson (9). As a result of this small distortion, the diameter of a four-stranded helix (2.2 nm) is only slightly larger than the diameter of native DNA (2.0 nm). Thus, a four-stranded helix contains two duplexes in approximately the same cylindrical volume normally occupied by one.

A surprising feature of four-stranded helix formation is that there are specific, interduplex hydrogen-bonding possibilities between bases in the paired duplexes (8). Because these extra hydrogen bonds are optimal between like base pairs related by 2-fold rotational symmetry (Fig. 6), they represent a source of specificity for the pairing of homologous duplexes. They are the sole source of specificity for pairing via intercoiled homoduplexes. However, for pairing via intercoiled heteroduplexes, interduplex hydrogen bonds represent a source of specificity in addition to that provided by heteroduplex base pairing. These two sets of specific hydrogen bonds would provide a particularly sensitive criterion for sequence identity of the interacting duplexes (Fig. 5).

Formation of the structures in Fig. 2 is associated with extensive intercoiling. The only property of formation that might

nucleotides. This difference in intercoiling behavior, which derives ultimately from the asymmetric placement of nucleotide pairs relative to the duplex axis, makes heteroduplexes associated by their minor-groove surfaces an unlikely end product of a switch in strand pairing (Fig. 5). In contrast, mutually untwisted parental duplexes paired along their minor-groove surfaces would be a reasonable transition state in the initial formation of intercoiled heteroduplexes (Fig. 5). Duplexes associated by their minor-groove surfaces could recover stacking interactions by disassociation into separate duplexes
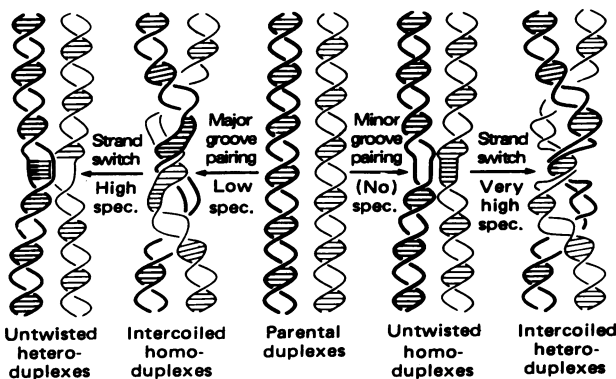

FIG. 5. Formation of symmetric four-stranded structures. Parental duplexes are shown pairing initially by either their major or minor grooves and subsequently undergoing a switch in strand pairing. Pairing by minor grooves requires significant distortion of the duplexes. Formation of intercoiled homoduplexes involves only interduplex hydrogen bonds (low specificity). The transition between intercoiled homoduplexes and untwisted heteroduplexes involves the breaking of interduplex hydrogen bonds but the formation of heteroduplex hydrogen bonds (high specificity). The transition between untwisted homoduplexes and intercoiled heteroduplexes involves formation of both interduplex and heteroduplex hydrogen bonds (very high specificity). Minor-groove association between parental duplexes involves neither set of these hydrogen bonds.
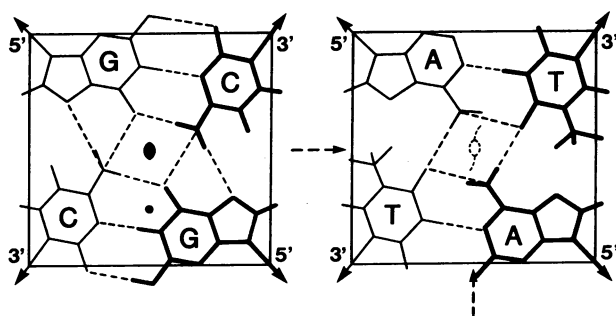

FIG. 6. Base tetrads in intercoiled heteroduplexes. Bases originally derived from the same parental duplex are drawn with the same thickness lines. Solid arrows indicate bonds to the sugar-phosphate backbones. 5' and 3' indicate the ends of the sugars that point toward the reader. The relative positions of the bases and the patterns of hydrogen bonding are according to McGavin (8); however, this pairing is between heteroduplexes rather than between homoduplexes. The four-strand helix axis is a 2-fold rotation axis (●). The B form DNA axis is shown on the lower G·C base pair (●). Rotation axes for backbone symmetry are indicated with dashed symbols on the A·T tetrad: there are two diad axes (− →) for the square arrangement of bases shown and one 10- to 11-fold screw axis (⊹). A coplanar arrangement for the four bases in an A·T tetrad at optimum hydrogen-bonding distances would require a slight overlap in the van der Waals radii of thymine methyl and N7 of adenine (8). This overlap may require a tilting or windmilling of the bases or both.
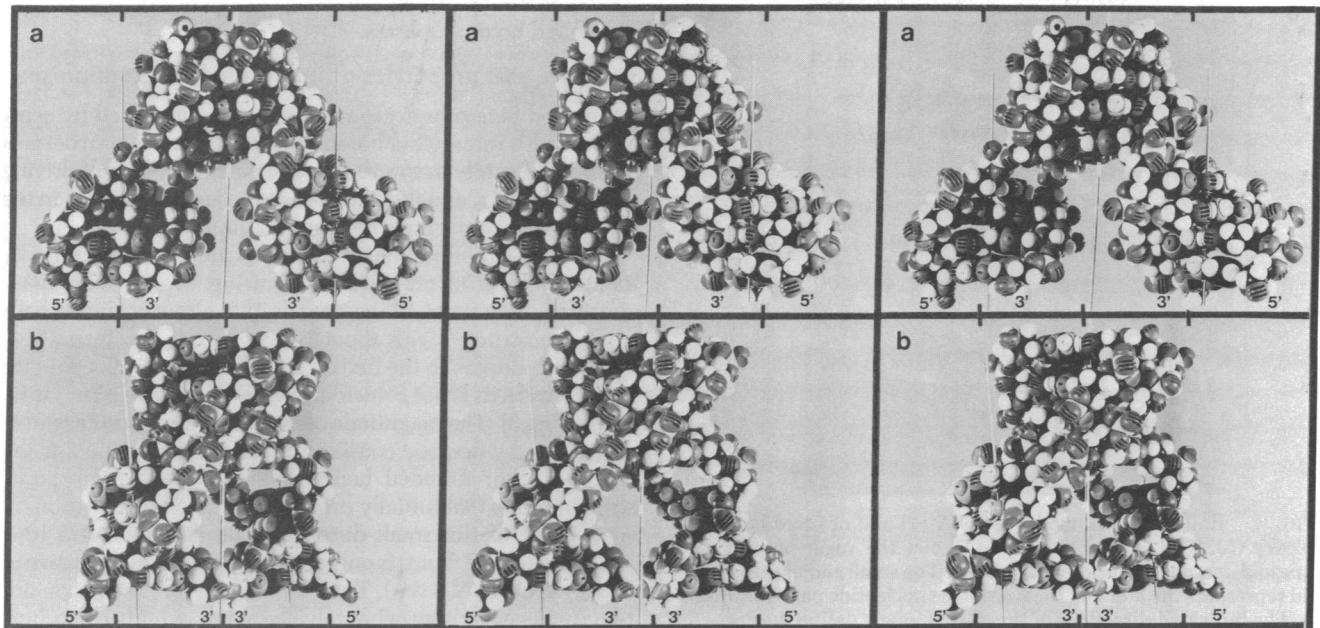
FIG. 7.   Space-filling model of a fused heteroduplex junction. View a looks into the minor grooves of the left parental duplex and one of the fused heteroduplexes and into the major groove of the parental duplex on the right. View b is a 90° rotation of view a, showing how the two strands of the parental duplex on the left come to lie side by side in the fused heteroduplexes. Strand polarities are indicated at the bottom of the structures. At the junction, the two 5' strands are extended and the two 3' strands are kinked. These deviations are achieved readily by acceptable rotations about bonds in the backbone. Each strand, in its path through a fused heteroduplex structure, is extended at one junction and kinked at the other. Notice that the parental base pairs next to the junction face each other across their minor-groove surfaces and are thus in position to switch pairing partners to form heteroduplexes. In principle, each junction can migrate independently in regions of homology by appropriate shifts in base pairing.

These pictures are arranged to encourage stereo viewing with unaided eyes. This visual skill can be acquired relatively easily. The left-hand pair of each view is arranged for viewing with distal convergence; the right-hand pair, for viewing with proximal convergence. Because of its intrinsic advantages I describe briefly here a step-by-step method for learning stereo viewing with *proximal* convergence. (*i*) Look at the tip of your finger about 10 inches in front of your eyes. If you see two images of objects that are beyond your finger, you are capable of learning this viewing method. (*ii*) Initially cover the left-hand picture of each view. Three out-of-focus images of view a will appear when you are looking at a point approximately half of the way from your eyes to the page. (You can use a pencil tip as a guide, or simply cross your eyes slightly.) Keep your attention on the center image, which is an overlay of the other two; you may need to tilt your head toward one shoulder to bring the overlaid images to the same level. (*iii*) Focus the center image. The first few times you try this there will be a natural tendency for the central image to split apart because you have trained yourself since infancy to converge and focus at the same point in space. It may take you a few minutes to become aware of your ability to control convergence and focus independently.

favor such intercoiling for homoduplexes is dehydration of the major-groove surfaces in the four-stranded region. By contrast, intercoiling of heteroduplexes would be driven additionally by heteroduplex base-stacking interactions. If stacking interactions between base pairs in each heteroduplex are similar to those in normal duplexes, each tetrad of bases will be rotated 33°–39° relative to its neighbors. The rotation due to tetrad stacking causes a right-handed intercoiling of the two heteroduplexes and *simultaneously* a compensating left-handed intercoiling of the parental duplexes. Thus, all coiling during formation of intercoiled heteroduplexes follows as a *natural* consequence of the lateral shift in base pairing.

The patterns of hydrogen bonding and base stacking suggest that intercoiled heteroduplexes will form a relatively more stable four-stranded core structure than will intercoiled homoduplexes. The separation of intercoiled homoduplexes into parental duplexes requires only the breaking of interduplex hydrogen bonds. However, the separation of intercoiled heteroduplexes into parental duplexes requires the breaking of interduplex hydrogen bonds, heteroduplex hydrogen bonds, and heteroduplex base-stacking interactions. In this sense intercoiled heteroduplexes are fused together; stacking interactions between heteroduplex base pairs cause the heteroduplexes to intercoil tightly, and interduplex and heteroduplex hydrogen bonds interlock the bases.

Junctions between the four-stranded helix and the parental

duplexes differ substantially in intercoiled homoduplexes and intercoiled heteroduplexes. At both types of junction all bases are properly paired and arranged with 2-fold rotational symmetry about the four-stranded axis. The primary difference between the two junctions is in base stacking. At a homoduplex junction the bases are stacked approximately normally, although the parental duplexes are in contact and must untwist slightly as they wind away from the four-stranded core (Fig. 2a). At a heteroduplex junction the four base pairs that flank the junction are unstacked due to the switch in strand pairing (Figs. 4b and 7). As a result, the parental duplexes are not in contact (and thus present no steric impediment to intercoiling). A second consequence of the strand switch is that the sugar-phosphate backbone of each strand deviates from its normal structure between the last base of a parental duplex and the first base of a heteroduplex—i.e., exactly at the junction (Fig. 7). The distorted backbones and unstacked bases that characterize a fused heteroduplex junction could make it particularly sensitive to the cutting events involved in genetic recombination.

Implications for genetic recombination

Genetic recombination apparently involves several distinct events: the parental Watson strands must be cut and joined together, the parental Crick strands must be cut and joined together, and complementary base pairing must create a heteroduplex region. Cut-first models of recombination generally

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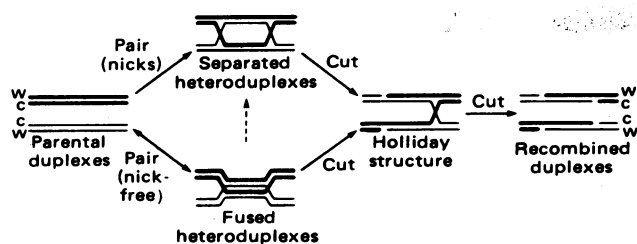
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FIG. 8. Pair-first pathways of recombination. Two distinct reciprocal heteroduplex structures can result from duplex pairing, depending on whether pairing is nick-promoted or nick-free. Formation of fused heteroduplexes is freely reversible and occurs without topological linkage. The dashed arrow indicates that fused heteroduplexes, in principle, could be converted to separated heteroduplexes. This conversion would require breaking of interduplex hydrogen bonds, simultaneous untwisting and unstacking of both heteroduplexes to permit separation, and subsequent action of a nicking-closing enzyme. Whether such a transition is reasonable is not clear. W and C identify strands of like polarity. In this diagram W strands are cut first and C strands second. If all cuts occur in W strands or in C strands, the recombinants will be insertion heteroduplexes that are nonrecombinant for outside markers. If asymmetry is required, as certain nonreciprocal features of recombination suggest (5), it could occur in pair-first pathways during resolution of the Holliday structure into recombined duplexes. Joining events have not been specified.

lead to intermediates with a single point at which one or two strands cross over. Most cut-first models lead more or less directly to a structure similar to the Holliday structure shown in Fig. 8 (4). By contrast, pair-first models of recombination lead to intermediates with two points at which strands cross over. These two points of exchange are most clear in the separated heteroduplexes in Fig. 8, but are present in the fused heteroduplexes as well.

Several pair-first models of recombination that involve strand separation and nicking have been proposed (6, 10, 11). The most recent involves the nicking-closing activity of a DNA topoisomerase (6), which would permit correct winding of heteroduplexes after strand separation (see Fig. 1). All proposed pair-first models that involve nicking lead to separated heteroduplexes. Duplex pairing in the absence of nicks and strand separation, as described here, leads to heteroduplexes that are fused together over their entire length. Breakage of corresponding strands at one site of strand crossover in either fused or separated heteroduplexes would generate a Holliday intermediate directly, as indicated in Fig. 8.

Both pair-first pathways predict a precursor to the Holliday structure in which the recombining duplexes are associated over a longer region than would be expected for the single point of exchange in a Holliday structure. An intermediate containing separated heteroduplexes might be expected to show an "eye" between the two crossover points, whereas an intermediate with fused heteroduplexes would not. Two recent *in vitro* recombination studies have demonstrated presumptive recombination intermediates with variable regions of association extending up to 500 base pairs in length, suggesting a pair-first pathway of formation (12, 13). None of these structures had "eyes." However, the data do not yet distinguish between the two pathways. That is because pairing via separated heteroduplexes could lead to "eyeless" association if the heteroduplexes were constrained to lie side by side due to spreading forces encountered in preparation for electron microscopy (13).

Summary

Fused heteroduplexes represent a straightforward topological solution to the winding problem of recombination. The symmetry of the structure, along with its high degree of specificity, simplicity of formation, coiling properties, and "cocked" junctions, is aesthetically appealing. Although a fused heteroduplex structure is probably less stable than the separated parental duplexes, its formation and stabilization could plausibly be accomplished by appropriate proteins. In this paper I have focused on its possible relevance to general genetic recombination. However, because formation of the structure is freely reversible, fused heteroduplexes do not represent a commitment to recombination and thus potentially could serve other functions as well.

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