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Genetics

A mechanism for initiation of genetic recombination

(palindrome/crossed strand connection)

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ABSTRACT **A mechanism for the initiation of genetic recombination is proposed. Its key features are the pairing, nicking, and cross-annealing of palindromic loops, i.e., structures formed by DNA with sequences of inverted complementary repeats. This mechanism may provide a simple, yet specific means of producing crossed strand connections between homologous DNA duplexes to form structures which can be intermediates in the process of genetic recombination.**

Genetic and physical studies indicate that genetic recombination in both prokaryotes and eukaryotes involves the breakage and rejoining of homologous DNA molecules, which is initiated with, or at least accompanied by, the formation of regions of hybrid DNA (for review see refs. 1–3). These hybrid or heteroduplex regions are made up of one strand from each parental molecule and are the presumed sites of gene conversion, i.e., recombination by mismatch repair (4). Analysis of unselected tetrads in yeast (5) suggests that all meiotic recombination is associated with the formation of hybrid DNA.

Several models of genetic recombination have been proposed which involve the formation of hybrid DNA in their initial steps (see ref. 2). A crossed strand exchange between homologous DNA molecules (Fig. 1D) has been proposed by Holliday (4) as one possible intermediate in the formation of hybrid DNA. Model building by Sigal and Alberts (6) has shown that such a crossed strand connection can be formed between DNA duplexes without disruption of either base pairing or stacking and can migrate along the duplexes, perhaps by rotary diffusion (7), to produce extensive regions of hybrid DNA in both molecules. Strand equivalence in the connected structure allows the duplexes to undergo isomerization, i.e., the interchanging of crossing and noncrossing strands (Fig. 1D and D′), which can result in the formation of nearly equal numbers of crossover and noncrossover molecules (6, 8, 9).

Since crossed strand connections are a part of several models of genetic recombination and appear to be feasible from a physical standpoint, the manner in which they arise and the genetic consequences of their formation may be of considerable importance to the understanding of genetic recombination. We shall describe here a possible mechanism for the formation of crossed strand connections at specific sites on homologous DNA duplexes.

THE MECHANISM

This mechanism postulates that recombination initiates at and depends upon palindromic (i.e., inverted complementary repeat) sequences in the DNA (Fig. 1A) capable of forming the characteristic structures (palindromic loops) shown in Fig. 1B. Model building (10) shows that such structures must have at least two unpaired bases at the apex of the loop which could form base pairs with the complementary bases in an identical loop. This pairing, as well as formation of the palindromic loops themselves, may be facilitated or stabilized by a recombination protein. If nicks are introduced, possibly by the same protein, at sites identical with regard to structure, sequence, and polarity, cross-annealing will form a double-stranded bridge between the two molecules (Fig. 1C). Cross-annealing depends on the denaturation and renaturation of a short region of the palindromic loops. At present we are unable to imagine any means other than random denaturation and renaturation by which this exchange could be promoted as a consequence of the properties of the nucleic acid structure itself, but we recognize that such an exchange could be facilitated by proteins capable of lowering the energy barrier for the denaturation step (see ref. 11). It is important in this regard to note that the stability of the double-stranded bridge is greater than that of the individual palindromic loops due to its greater length of double helix and complete base pairing and stacking, so that once formed, it should not easily revert to individual loops. Once cross-annealing has formed the double-stranded bridge, limited rotation of the two stem DNA molecules about the axis of this bridge to unwind the annealed palindromic loops, accompanied by rotation of the stem duplexes about their helical axes to wind the outside loops and double-stranded bridge back into the stem, will produce a cross connected structure with no unpaired bases and a short region of hybrid DNA in each of the two molecules (Fig. 1D). This sugar-phosphate bridge between the two molecules can then migrate by rotary diffusion (7) and thus produce a variable length of hybrid DNA in both molecules. In addition, it is possible for the structure to undergo isomerization (refs. 6, 8, and 9; Fig. 1D′) to produce nearly equal numbers of crossover and noncrossover molecules following resolution. Resolution depends on the occurrence of additional nicks or breakage of the cross connection (see *Discussion*).

Because the recognition structure in this mechanism has a palindromic sequence, pairing and formation of the cross connected structure would be expected to occur with equal frequency between identical and complementary strands of the homologous DNA duplexes. A cross connected structure formed by the pairing of complementary strands would normally be resolved by falling apart at the nicks, since migration of the cross connection would be limited to the short region of the palindromic sequence by the lack of any additional homology. To insure that such resolution occurs and that improper recombinants are not formed, it is essential

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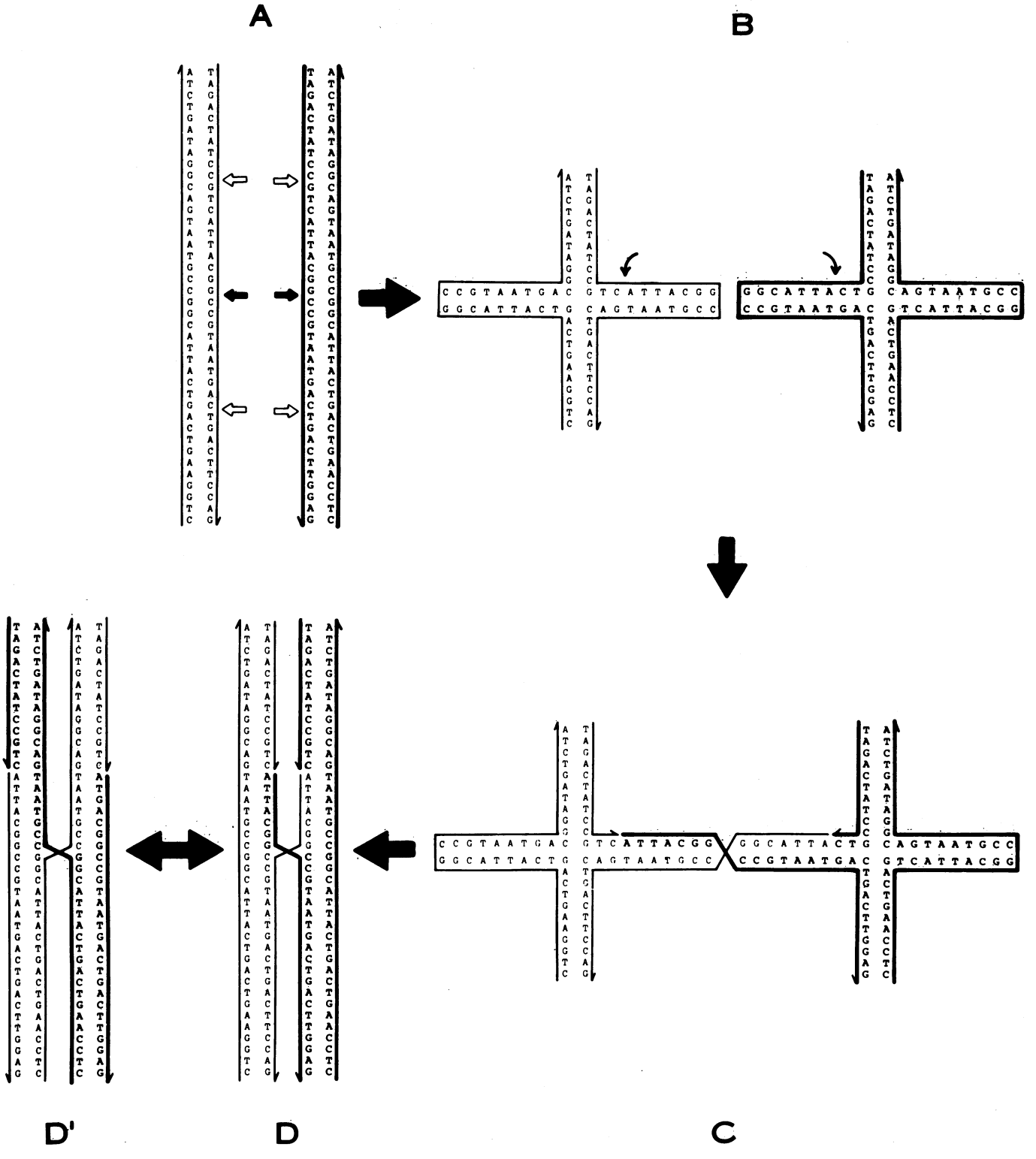

FIG. 1. (A) Homologous DNA duplexes with palindromic sequences. Solid arrows: apex of palindromic loop. Open arrows: base of palindromic loop. (B) Duplexes with palindromic loops. At least two bases at each apex are not paired and could facilitate pairing of the palindromic loops by pairing with the complementary bases at the apex of an identical loop. For clarity, this interpalindromic base pairing is not shown in the figure. Arrows indicate possible sites for specific endonuclease action. (C) Cross-annealed palindromic loops. Double-stranded bridge is formed by the introduction of nicks at identical sites on the palindromic loops as indicated in (B) and exchange of strands by denaturation and renaturation. All bases are paired. (D) Cross connected structure. Formed by limited rotation of the stem DNA molecules about the axis of the double-stranded bridge to unwind the annealed palindromic loops, accompanied by rotation of the stem duplexes about their helical axes to wind the outside loops and double-stranded bridge back into the stem. Cross strand connection may migrate by rotary diffusion to form extensive regions of hybrid DNA. (D') Isomerization. Strand equivalence in the cross connected structure allows crossing strands to become noncrossing strands (and vice versa) and consequently form crossover and noncrossover molecules with nearly equal frequency.

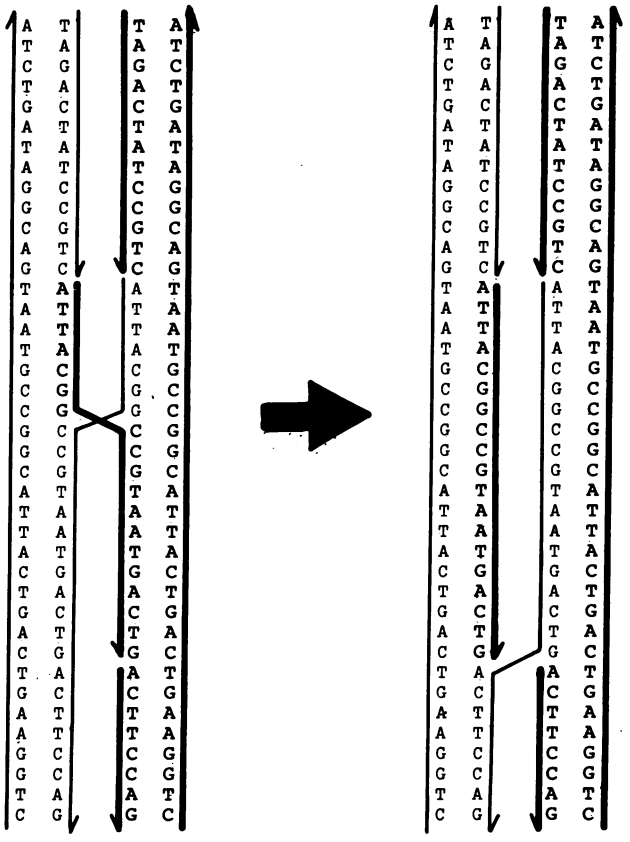

FIG. 2. Formation of a unitary strand connection. Migration of cross connection (left) by rotary diffusion produces a unitary strand connection (right) if a nick in only one strand is encountered.

that the nicks remain unsealed throughout the initial stages of the recombination event.

DISCUSSION

The mechanism for the initiation of genetic recombination proposed here provides for the formation of cross connections between homologous DNA duplexes in a simple, yet specific manner. The recognition sequence can be short and can be the same for all recombination sites within a genome, thus requiring only one sequence specific endonuclease for all nicking associated with the initiation of recombination. Although cross connections may be formed between improper sites, the lack of homology outside the recognition sequence will sharply limit migration of the cross connection and result in the molecules falling apart, as was described for the case of a cross connection formed between complementary strands.

A mechanism for genetic recombination proposed by Sobell (12) also makes use of looped structures for initiating synapsis. However, several features of this mechanism clearly distinguish it from the mechanism proposed here. The loops in Sobell's model are Gierer loops (13) with extensive nonpalindromic sequences at the apex, and his mechanism involves pairing of complementary strands, nicking at nonidentical sequences, and the generation and subsequent annealing of single-stranded ends leading to the formation of a structure with two cross connections.

A prediction of our mechanism is that recombination initiates at specific sites and consequently exhibits polarity. Polarity in the frequency of gene conversion has been observed in yeast (14) and other fungi (15–18). Palindromes, i.e., segments of single-stranded DNA resistant to single-strand deoxyribonucleases, have been observed in eukaryotic chromosomal DNA (19) and are found to average several hundred nucleotide pairs in length. Smaller palindromes, which may be preferable as initiation sites for genetic recombination, have been reported to occur in some prokaryotes (see ref. 19) and sequencing data indicate that in the case of restriction enzymes, small palindromic sequences can function as recognition sites for specific endonucleases (20–23).

Since the cross connection formed by the mechanism proposed here originates several base pairs away from the nicks (Fig. 1D) and since migration of the cross connection back to the nicks will result in the duplexes falling apart, hybrid DNA may be formed more often on the same side of the nicks as the original cross connection. Therefore, nicking at sequence specific sites may result in the formation of predominantly one hybrid overlap polarity for all recombination initiating at a given site. Such an asymmetry in the formation of hybrid overlaps has been reported for bacteriophage λ recombination by White and Fox (24).

The cross connected structure initially formed by this mechanism would produce hybrid DNA in both participating molecules and could be resolved by breaking both strands at the cross connection or by introducing nicks at identical sites in front of the migrating connection. If, however, a nick is encountered in only one crossing strand, a structure with a unitary strand connection results (Fig. 2) and migration by rotary diffusion may no longer be possible. Three outcomes of such an event can be envisioned: (*i*). The structure is resolved by breaking the unitary strand connection; (*ii*) isomerization occurs, generating a cross connection where rotary diffusion is again possible; and (*iii*) the unitary strand connection is driven by the concerted action of an exonuclease and polymerase (6, 8) and thus forms hybrid DNA in only one molecule. In those organisms where hybrid DNA usually forms in only one chromatid (25, 26), such a driven unitary strand connection may be the predominant means of producing hybrid DNA. Meselson and Radding (8) have proposed a model for genetic recombination which, unlike the mechanism proposed here, initiates with the formation of a driven unitary strand connection and in a later step involves isomerization to produce a crossed strand connection.

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