Transpositional recombination and site-specific recombination may be initiated by copy choice during DNA synthesis rather than break/join mechanism

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Abstract

Types of DNA recombination include homologous recombination and nonhomologous recombination. Homologous DNA recombination is a general term that includes exchange of information between chromatids: (reciprocal) crossing-over, gene conversion, and post-meiotic segregation. Gene conversion is now thought to be a type of non-Mendelian segregation of heterozygous markers near the recombination initiation site. Thus, it includes both gene conversion and post-meiotic segregation previously described. DNA non-HR including transpositional recombination and site-specific recombination. Our understanding of the molecular mechanism by which DNA recombination occurs has significantly increased in the past decades. Currently The synthesis-dependent strand annealing model is now thought to give rise to most or all noncrossovers, with the double-strand-break repair model forming mainly crossovers. The Shapiro model proposed by Dr. J. Shapiro explains the molecular mechanism of transpositional recombination. Site-specific recombination results from another distinct model. We previously proposed a novel theory which can provide a more reasonable and simpler explanation accounting for DNA HR including the 3 classes of recombinogenic events described above. In the new supplementedly molecular model, DNA meiotic recombination can be initiated by a copy choice mechanism, that is, copying part of 1 single-stranded DNA template, followed by DNA polymerase switching to another single-stranded DNA template, and then resuming the following DNA synthesis along the new template. The current review suggests that transpositional recombination and site-specific recombination should be initiated by copy choice during DNA synthesis rather than break/join mechanism. The work indicates that review of DNA nonhomologous recombination are very necessary. The novel theory would challenge earlier models accounting for transpositional recombination and site-specific recombination and would be critical to the understanding of the mechanisms. We hope copy choice initiating DNA nonhomologous recombination will be one of the concepts that are explored. Proper and specific experiments are required to reconstruct the detailed mechanism described here.

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Introduction

Types of DNA recombination include homologous recombination (HR) and nonhomologous recombination (non-HR). Considering that fully documented accounts for DNA recombination have previously been provided [1-4], the current introduction does not contain a thorough, point-by-point referral to the original literature.

HR is also known as general recombination. Initial evidence for the propensity of DNA to undergo HR arose from the early studies of chromosome behavior during meiosis. Meiosis is a cell division program in which a single round of DNA replication is followed by 2 consecutive rounds of chromosome segregation, thereby allowing the formation of haploid gametes from diploid germ cells [5][6]. To date, homologous DNA recombination is a general term that includes exchange of information between chromatids: (reciprocal) crossing-over, gene conversion, and post-meiotic segregation. Tetrad analysis of all of the products of individual meiotic recombination events revealed that recombinants between distant markers are produced in pairs, with all markers showing normal 4:4 segregation. Such recombination events are called crossovers (COs). Crossover reflects reciprocal exchange between homologous non-sister chromatids. In contrast to crossover, gene conversion reflects the non-reciprocal transfer of genetic information from 1 duplex to its homologue, with all markers showing 6:2 segregation. In another class of recombination, post-meiotic segregation, a spore is produced that divides to create 2 genetically different daughter cells. Post-meiotic segregation leads to aberrant 4:4 or 5:3 segregation. Therefore, any molecular model of homologous DNA recombination must reasonably explain the 3 classes of recombinogenic events described above. It is important to point out that gene conversion is now thought to be a type of non-Mendelian segregation of heterozygous markers near the recombination initiation site [7]. That is to say, it includes both gene conversion and post-meiotic segregation previously described.

With continuous expansion of experimental data, a more representative molecular model for DNA meiotic recombination usually requires repeated revisions and upgrades. For example, DNA meiotic recombination has undergone the Holliday model [8], the Meselson-Radding model [9],

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the double-strand-break repair (DSBR) model [1], and the synthesis-dependent strand annealing (SDSA) model [10]. The SDSA is now thought to give rise to most or all noncrossovers (NCOs), with the DSBR forming mainly COs. The SDSA arises from a common precursor, a DNA double-strand break, as the DSBR does. Additionally, we previously proposed a updated theory accounting for DNA HR including the 3 classes of recombinogenic events described above [11]. In the new supplementedly molecular model, DNA meiotic recombination can be initiated by a copy choice mechanism, that is, copying part of 1 single-stranded DNA template, followed by DNA polymerase switching to another single-stranded DNA template, and then resuming the following DNA synthesis along the new template (Figure 1). The model provides more reasonable and simpler explanations for DNA meiotic recombination via adding more patterns of Holliday junction (HJ) resolution than that recognized previously. The 3 recombinogenic events can arise from these different resolutions (Figure 2).

The application of the new model in transpositional recombination

In addition to HR, there exists DNA non-HR including transpositional recombination and site-specific recombination. Separate molecular models have been established to explain the two specific recombinant events respectively. A transposon can independently transpose from 1 DNA site to another in an event called transpositional recombination. Currently, the Shapiro model proposed by Dr. J. Shapiro explains the molecular mechanism underlying this event [3]. This model postulates a Shapiro intermediate and explains both a replicative transposition and nonreplicative transposition (Figure 3A). When located in the new theory, it is noted that the particular intermediate is not a unique structure. It can result more easily in the intertwinement model than in the Shapiro model (Figure 3B). When dissociating as Figure 2E, replicative transposition occurs. When dissociating as Figure 2F, non-replicative transposition results. Therefore, in addition to the function in interpreting HR, the new model indicates that there is a common pathway for transpositional recombination.

With respect to the reason why a gene can be translocated via recombination, the novel

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model provides the following interpretations: intertwinement can occur not only at colinear positions (i.e., homologous recombination), but also at non-colinear positions. Therefore, after copy choice based on an irregular double branched structures (BSs), an asymmetric dHJ would appear. The resolution of this dHJ would lead to transpositional recombination (Figures 3C and 3D). Then why can a transposable element be translocated as an independent part during the process? It is thought that the pair of inverted repeats flanking a transposon should to play a key role. Inverted repeats would readily induce the formation of a hairpin within a single strand during DNA replication. When the hairpin participates in intertwinement, the resulting structure containing a pair of BS shown in Figure 3E could appear. When similar procedures to Figure 2 occur, the whole hairpin can be moved as an independent component from the donor to the acceptor. It is important to point out that inverted repeats inducing a hairpin structure was once proposed by d'Alencon et al. in their research concerning nearly precise excision of a transposon to study illegitimate DNA recombination between short direct repeats [12].

The application of the new model in site-specific recombination

Another form of non-HR is site-specific recombination (summarized in [4]). Recombination occurring between specific DNA sequences is called site-specific recombination. It was first discovered during the genetic study of the λ phage. For sites located on the same chromosome, the outcome currently is thought to be determined by their relative orientation. Excision results from recombination between sites in a head-to-tail orientation, whereas inversion results from exchange between inverted (head-to-head) sites. According to the novel model, intertwinement can occur not only between single-stranded DNAs from 2 duplexes, but also between 2 strands of 1 duplex. In addition, such intertwinement is available between regions on 1 single-stranded DNA, resulting in intrastrand intertwinement. The latter 2 cases would naturally generate a gene excision (Figure 4A) or inversion (Figure 4B).

Senior studies were performed to concern the recombination events involving short direct repeated sequences. Such recombination events are one class of illegitimate recombination which

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occur between sequences of little or no homology [13], and have been reported in prokaryotes [14-16], as well as lower and higher eukaryotes, including man [17-21]. Several lines of genetic evidence suggest that short direct repeats might recombine by errors of DNA replication; a tip of a growing DNA chain could slip from one repeat to the other and be used as a primer for further DNA synthesis [14, 22]. Such a model was first suggested to explain frame-shift mutations [23] and is conceptually related to copy choice recombination [24]. Importantly, d'Alencon et al. used nearly precise excision of a transposon related to Tn10 from an *Escherichia coli* plasmid as a model to study illegitimate DNA recombination between short direct repeats [12]. They showed that nearly precise excision was stimulated greatly by rolling circle replication and it did not entail the transfer of DNA from the parental to the recombinant molecule. Both results clearly support a copy-choice mechanism of recombination between short direct repeats rather than break/join mechanism. And we are pleased to see that the new theory gets consensus with these experimental results.

Conclusions

The work suggests that review of DNA nonhomologous recombination aiming to explore mechanism details are very necessary. It clearly supports an easier copy-choice mechanism of transpositional recombination and site-specific recombination rather than previous break/join mechanism. The novel theory challenge the two earlier models accounting for transpositional recombination and site-specific recombination [3, 4]. We hope copy choice initiating DNA nonhomologous recombination will be one of the concepts that are explored. Proper and specific experiments are required to reconstruct the detailed mechanism described here.

Without being restricted to meiosis, the recombination events shown in Figure 4 might also occur during mitosis and result in mitotic recombination. In light of the current model, intertwinement between nuclear acid strands is an objective physical rule. Therefore, one can assume that such intertwinement as well as the resulting recombination events between DNAs of different species, or even between DNA and RNA, should not be an exception. Thus, some other

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unusual recombination events, such as DNA with RNA, could get a reasonable explanation.

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Figure Legends

Figure 1 HJs that arise from BS-based copy choice.

(A) During DNA synthesis, a BS forms between 2 single-stranded DNAs of the same polarity. When the BS is encountered consecutively by both polymerases, which engage maternal and paternal DNA, respectively, it mediates 2 rounds of copy choice, resulting in a single HJ.

(B) Another BS forms downstream the first HJ. When both maternal and paternal polymerases encounter a second BS, the same process of copy choice is performed as at the first BS, displaying a dHJ.

Figure 2 Various resolution patterns can lead to different recombination events.

(A) Meiosis I spindles begin to pull maternal and paternal chromosomes in opposite directions before both the HJs are resected. Thus, topologically, the dHJ is resolved and 2 symmetric heteroduplexes are generated. After subsequent mitosis, post-meiotic segregation occurs, displaying aberrant 4:4.

(B) Resolution of 2 junctions by cutting inner, crossed strands leads to crossover of genes between 2 HJs.

(C) Resolution of 2 junctions by cutting outer, noncrossed strands does not impact the products after repair synthesis.

(D) Compared to (B), opposite-sense cutting, e.g., in the left-hand HJ, the crossed strands are cut, and in the right-hand HJ, the noncrossed strands are cut, generates crossover containing upstream regions.

(E) Cuts are made on strand 1 and strand 3 in both HJs. After the pull of meiosis I spindle, 2 duplex form, each of which contains a single-strand gap. Both gaps are subsequently repaired by repair synthesis, leading to gene conversion (6:2 segregation).

(F) Cuts are made on strand 1, 2, and 3 in both HJs. After the pull and repair synthesis, the information on black chromatid is transferred to the homologous region of the red one. The original information on the black chromatid is deleted, leading to gene conversion of deletion.

(G) Cuts are made on strand 1 in both HJs. The resolution generates a duplex with a single-strand

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gap and another duplex with a 3-stranded helix region. Within the 3-stranded region, there first is a duplex composed of strand 1 and strand 3. Then, the duplex interacts with strand 4, forming a 3-stranded helical DNA. After mitosis, the 3-stranded helical DNA results in post-meiotic segregation, displaying normal 5:3 segregation.

(H) Contrary to (G), cuts are made on strand 2 in both HJs. Post-meiotic segregation results, but the products display aberrant 5:3 segregation.

(I) When cuts are made on strand 1 and 2 in both HJs, the information on black chromatid would be deleted and transferred to the red one. Then, a 4-strand helical DNA composed of 2 duplexes appears. After mitosis, the 4-strand helical DNA can result in post-meiotic segregation.

Figure 3 Comparison of the Shapiro model with the intertwinement model.

(A) The Shapiro model. The donor molecule is cleaved on either strand at the extremity of the transposable element. The target molecule is cleaved to yield 5- or 9-bp cohesive ends. The donor and target strands are ligated to generate a χ-shaped structure called a Shapiro intermediate. Subsequently, after breakage, and rejoining, non-replicative transposition occurs. After release, repair replication and homologous recombination, replicative transposition occurs. The purple region indicates the oligonucleotide target sequence. The blue region indicates the transposable element. The letters a, b, c, and d in the duplex arms flanking the transposable elements and target oligonucleotide serve to indicate the genetic structure of the various duplex products.

(B) Resolution of a dHJ would also result in a structure that is exactly the same to the Shapiro intermediate. The pattern of resolution here is the same as the one showed in Figure 2E.

(C) Intertwinement occurs at non-colinear positions. The length of 2 fragments of participants are equal, i.e., fragment a = fragment b. The resolution as shown in Figure 2E leads to transpositional gene conversion, similar to replicative transposition (Top). The resolution shown in Figure 2F leads to transpositional gene conversion of deletion, similar to non-replicative transposition (Bottom).

(D) The length of 2 participating fragments varies, where fragment a > fragment b. The resolution as shown in Figure 2E leads to transpositional gene conversion of insertion, i.e., replicative

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transposition (Top). While the resolution as shown in Figure 2F leads to transpositional gene conversion of insertion and deletion, i.e., non-replicative transposition (Bottom).

(E) During DNA replication, the duplex is released into single strands. Thereafter, inverted repeats within a single strand can readily induce a hairpin (Left). When the hairpin participates in the intertwinement with a single strand, the structure involving a pair of BSs could appear (Right). After recombination based on the pair of BSs, the whole hairpin would be moved as an independent element.

Figure 4 The interpretation for site-specific recombination by the intertwinement model.

(A) (Gene deletion) During DNA synthesis, an intrastrand BS forms via intrastrand intertwinement (within strand 2). After copy choice, DNA polymerase directly reaches the left part of the BS (Sequences homology flanking the BS site can induce the process). As a result of not being a template, the loop region is deleted.

(B) (Gene inversion) The loop could intertwine with strand 1, resulting in a pair of BSs. After copy choice, resolution, and repair synthesis as shown in Figure 2, the blue region is inverted in the progeny.

It should be pointed out that the illustration here only describes two of many possibilities.

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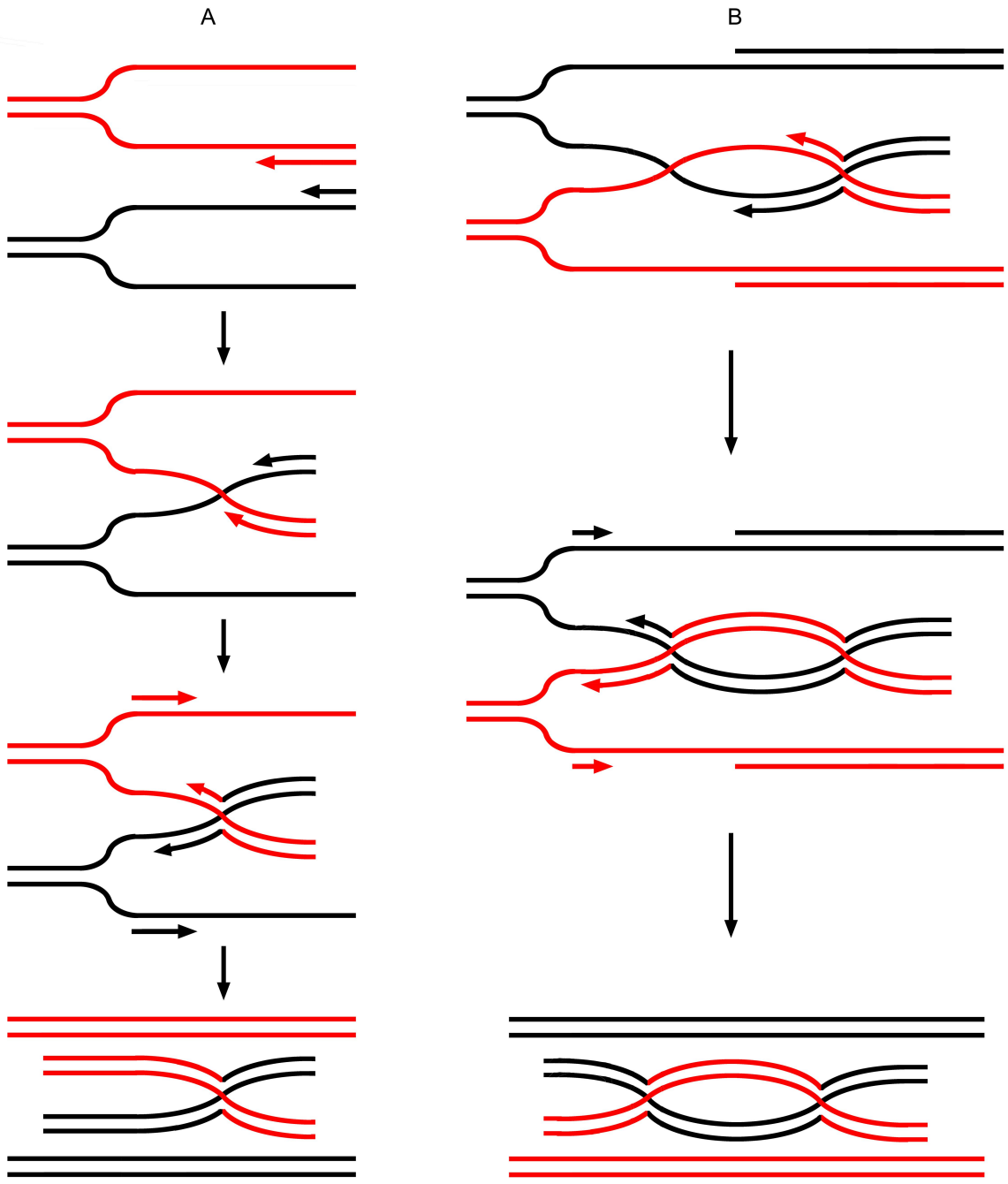

Figure 1

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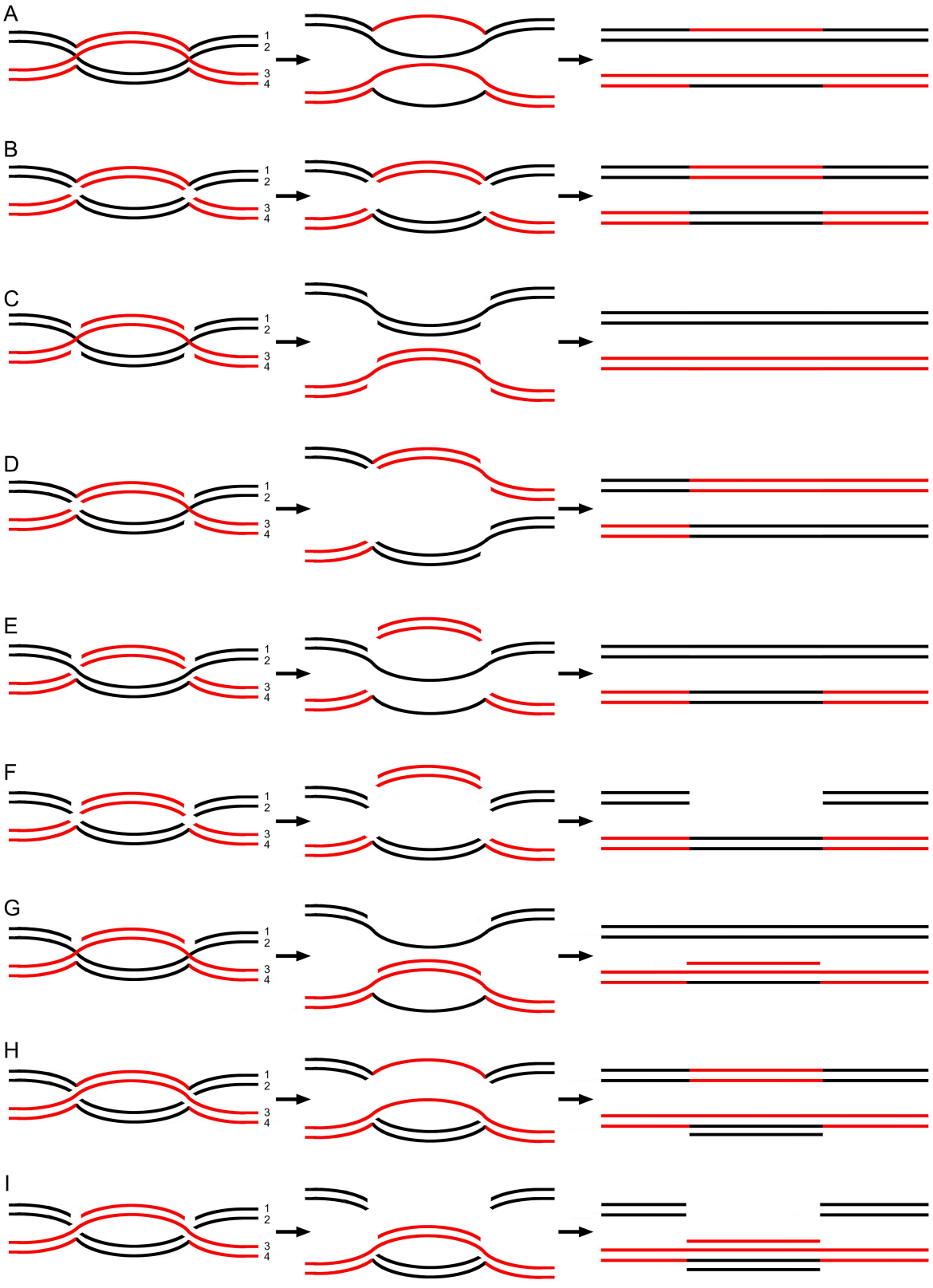

Figure 2

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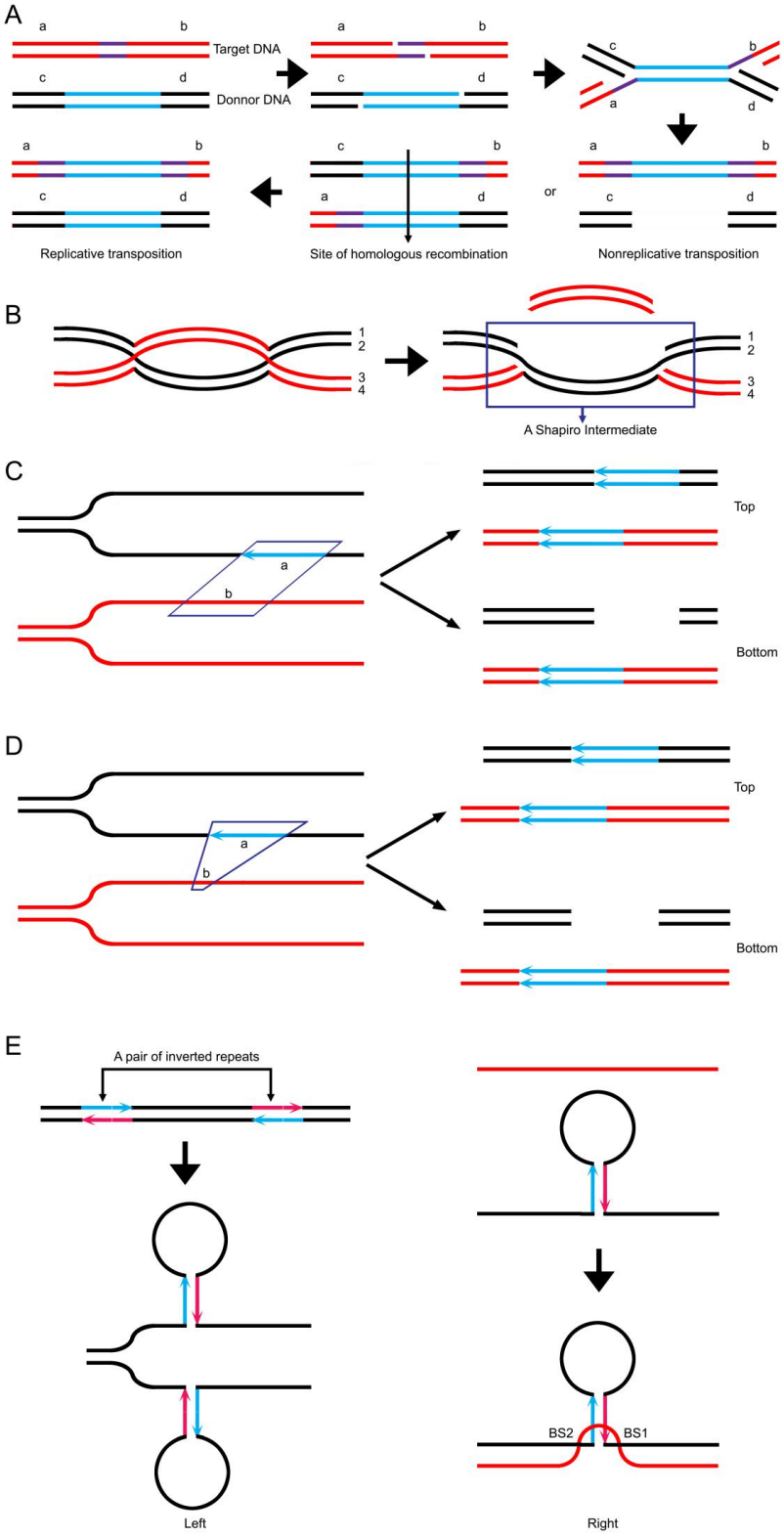

Figure 3

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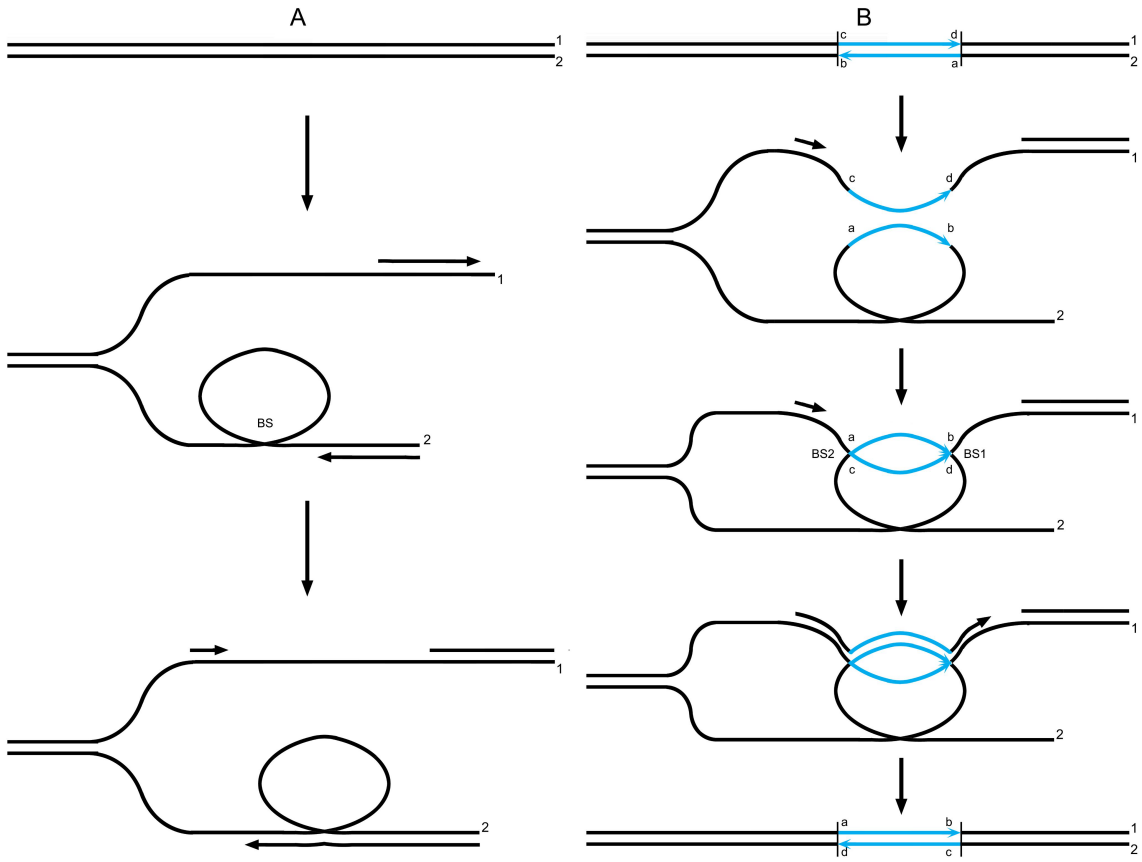

Figure 4