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Short Communication

A CRITICAL INSIGHT INTO THE DOUBLE STRAND BREAK REPAIR MODEL OF HOMOLOGOUS RECOMBINATION

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ABSTRACT
Double strand break repair (DSBR) model of homologous recombination proposed by Szostak *et al.* (1983) is widely accepted among the scientific community. Here we enlighten some of the unclear phases of recombination event occurring strictly by means of DSBR mechanism.

Key Words: *Homologous Recombination, Double Strand Break Repair, Holliday Junction (HJ)*

INTRODUCTION
Homologous recombination is found in all organisms from bacteria to higher eukaryotes including human. The DSBR model of homologous recombination in DNA proposed earlier (Szostak *et al*., 1983) is widely accepted. The detailed model is found in all modern molecular biology text books (Watson *et al*., 2008; Klein and Hoot, 2011 and Fig. 1).

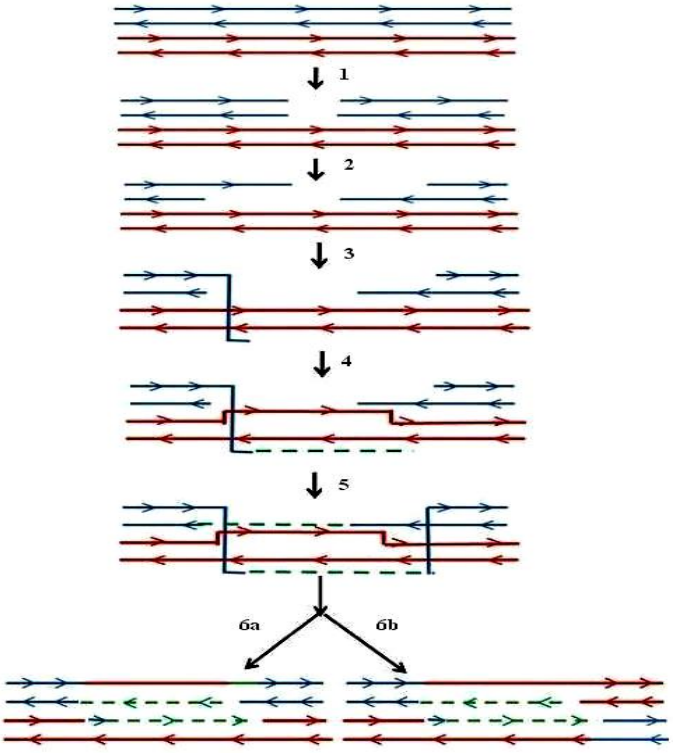

Figure 1: DSBR model of homologous recombination

According to the model, initially a double stranded break occurs in one of the homologous DNA molecules. By the action of RecBCD enzymes, 3' overhangs is generated in both the Watson and Crick strands of the damaged DNA molecule. Both single stranded DNA overhangs in Watson as well as in Crick strand is coated with RecA protein. The 3' overhangs either of the Watson or of the Crick strand

(suppose we consider here the Watson strand) invades the homologous DNA molecule and pairs with the non-sister complementary DNA (h-Crick strand; homologous DNA Crick strand). The displaced h-Watson strand (homologous DNA Watson strand) from the homologous DNA forms D-loop, which then pairs with the 3' overhangs of the Crick strand. DNA synthesis occurs from the 3' ends of both the Watson as well as the Crick strand of the cleaved DNA. After the synthesis the 3' ends join with their respective strands. The events of strand invasion and D-loop formation results in creation of two Holliday junctions (HJs) that are recognized by the RuvA and RuvB proteins. After branch migration, the strand resolution occurs with the aid of RuvC proteins. The resolution results either patch products or splice (crossover) products.

Though the above model satisfactorily explains several observations (Szostak *et al*., 1983), a critical insight into the model raised the following unclear events during the recombination process. Firstly, though 3' over hangs of both Watson and Crick strands of the damaged DNA molecule are coated with RecA protein independent of each other, it is not clear why only one of them invades into the homologous DNA molecule while both being capable of invading simultaneously. Secondly, how the D-loop, which is devoid of any RecA coating, is able to intrude the non-sister complementary DNA strand is not understood. Thirdly, it is not known whether synthesis of DNA at 3' flanking ends occur simultaneously or temporally.

To address the above ambiguities raised by the existing DSBR model, we propose here few modifications in the DSBR model, which we will refer to as m-DSBR (modified DSBR) model in the rest of the article. The m-DSBR model is depicted in Fig. 2. According to the model, both the Watson strand and the Crick strand coated with RecA will invade the homologous DNA and will pair with the respective non-sister complementary DNA strands. So, synthesis of DNA can occur simultaneously or in succession at both the 3' end of the invading strands. The formation of DNA branch points in our model is similar to given in DSBR model, which is in concordance with the electron micrograph pictures (Liu and West, 2004). The eventual outcomes of the DSBR as well as the m-DSBR are unaltered. The differences between the DSBR and the m-DSBR have been illustrated in the Table 1.

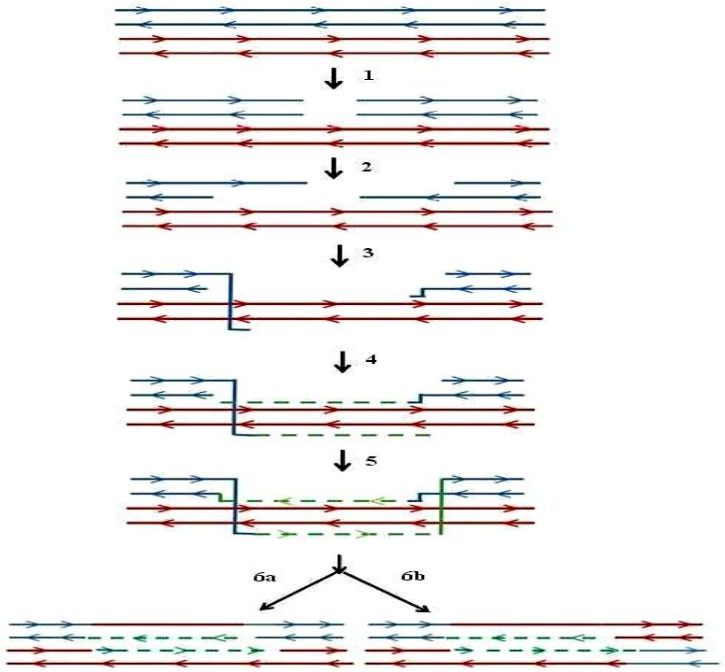

Figure 2: The proposed modification in the DSBR (m-DSBR)

Table 1: Features distinguishing m-DSBR from DSBR model

S. No.	DSBR model	m-DSBR model
1	One of the 3' over hangs invades the homologous DNA	Both the 3' over hangs invades the homologous DNA
2	D-loop formed in the homologous DNA invades the damaged DNA	D-loop do not invade
3	DNA synthesis starts at the 3' end of the invading strand. Only after the formation of D-loop and pairing, DNA synthesis occurs at the non-invasive 3' overhangs	DNA synthesis may occur simultaneously or temporally at the 3' ends of both the DNA strands
4	Single stranded DNA in the form of D-loop is retained for longer duration	Single stranded DNA in the form of D-loop is retained for short duration
5	So, the chances of cytosine deamination is higher	So, the chances of cytosine deamination is lesser

It is pertinent to note that the DSB model was proposed after the Holliday model (the first model for recombination event) and the Meselson and Radding model (Szostak *et al*., 1983). DSB model has features from Holliday model (Holliday junction) and the Meselson and Radding model (D-loop formation) with its own unique double strand break event. This might be the reason why the D-loop has been retained in the existing model. Further a critical look at D-loop formation during recombination will suggests that the displaced single stranded DNA is more prone to cytosine deamination (Francino and Ochman, 1987). Therefore DNA regions undergoing DSBR should gradually be enriched with A+T. It has been reported in *Escherichia coli* that regions surrounding *chi*-site is rich in G-nucleotides (Bell *et al*., 1998) but it has not been reported if the region is enriched with A+T.

DNA branch points known as Holliday Junctions (HJs) has been reported in electron micrograph and crystal structures of DNA recombinations (Ho and Eichman, 2001; Heyer, 2004 and Liu and West, 2004). The illustrations of structures as HJs (Schwacha and Kleckner, 1995; Hunter and Kleckner, 2001) are structures of DNA branch points during recombination. The DSBR is not an indispensable event during recombination. A recent experimental finding in yeast suggests that the DSBR model is not followed during the non-crossover product generation (Mitchel *et al*., 2010).

Finally we would like to say that our proposed m-DSBR is a simple theoretical argument over DSBR.

Two pairs of homologous dsDNA molecules drawn in blue and red respectively. Arrows depicts the strands in 5'-3' direction. In step (1), double strand break in one pair of homologous DNA strands occurs (here colored blue). In step (2), RecBCD complex recognizes the double strand breaks and generates 3' overhangs in both the Watson (upper strand) as well as the Crick (lower stand) strands. The single stranded 3' overhangs are coated with RecA proteins. In step (3), the 3' end of one of the DNA strands (here Watson strand) invades and pairs with the complementary non-sister DNA strand (h-Crick strand, Crick strand of homologous DNA) of the other pair of homologous DNA (red). In step (4), DNA synthesis occur on Watson's strand (the dashes painted in green illustrates replicating DNA). The partial displacement of the h-Watson strand (Watson strand of the homologous DNA in red) results in D-loop formation, which pairs with the 3' overhangs of Crick strand. Through step (5), replication is complete on the Crick strand. This follows joining of the newly synthesized DNA to their respective strands forming two HJs. Step (6), shows the resolution of the recombined DNA strands in two possible ways, giving rise to two products namely patch (6a) (resolution occurs either in the upper strands or in the lower strands of the two DNA molecules at the two HJs) and splice products (6b) (resolution occurs in the upper strands of the two DNA molecules at one HJ and in the other HJ resolution occurs in the lower strands of the two DNA molecules) respectively and this is in compliance with the existing DSB model being followed at present. Details of this model are given in text books.

Two pairs of homologous dsDNA molecules drawn in blue and red respectively. Arrows depicts the strands in 5'→3' direction. In step (1), double strand break in one pair of homologous DNA strands occurs (here colored blue). In step (2), RecBCD complex recognizes the double strand breaks and generates 3' overhangs in both the Watson (upper strand) as well as the Crick (lower stand) strands. The single stranded 3' overhangs are coated with RecA proteins. In step (3), 3' ends of both the cleaved DNA strands invade and pair with the complementary non-sister DNA strands of the other pair of homologous DNA strands (painted red). In step (4), 3'-ends of the intruding DNA strands starts DNA synthesis and extension using complementary non-sister strand as template, the dashes painted in green illustrates replicating DNA. Through step (5), replication is complete and joining of the newly synthesized region with the respective strands. Step (6) shows the resolution of the recombined DNA strands in two possible ways by which resolution can occur, giving rise to two products namely patch (6a) (resolution occurs either in the upper strand or in the lower strand of the two DNA molecules at the two branch points) and splice (6b) (resolution occurs in the upper strands of the two DNA molecules at one branch point and in the other branch point resolution occurs in the lower strands of the two DNA molecules) products respectively and this is in compliance with the existing DSBR model being followed at present.

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