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Holliday junctions, heteroduplex DNA and map expansion: a commentary on 'A mechanism for gene conversion in fungi' by Robin Holliday

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Perhaps there is truth in beauty. Robin Holliday's proposal for the mechanism of recombination (Holliday, 1964) was certainly beautifully elegant and has proved to be essentially correct. At the time of his proposal, all the elements of his model were circulating in the scientific ether: the observations on gene conversion and its association with crossing over (Perkins, 1962; Whitehouse, 1963), the association of postmeiotic segregation with recombination (Kitani *et al*., 1962; Lissouba *et al*., 1962; Stadler & Towe, 1963) and the association of DNA breakage with recombination (Kellenberger *et al*., 1961; Meselson & Weigle, 1961; Siddiqi, 1963), but it was the observation of map expansion that Holliday's paper grappled with in particular. Despite the prominence of the 1964 paper and its association with the 'Holliday junction', map expansion remains a poorly appreciated phenomenon even today.

I believe that the first depiction of the Holliday junction appeared in the model of break-induced replication proposed for bacteriophage lambda by Meselson & Weigle (1961), but it was Robin Holliday who explicitly recognized that this four-way junction could be formed and resolved in a way that would explain the patterns of recombination observed in fungal meioses. The old models of gene conversion by copying first one chromosome and then another (copy-choice) were no longer easy to understand, given the semi-conservative mechanism of DNA replication, and models of breakage and reunion that involved annealing of single-stranded DNA ends, such as those proposed by Meselson and Weigle and by Whitehouse, were either incompatible with meiotic recombination (Meselson & Weigle, 1961) or inelegant (Whitehouse, 1963). A detailed discussion of the history of recombination models had recently been published (Haber, 2007).

Robin Holliday's 1964 paper will be remembered rightly for proposing the centrality of the 'Holliday junction' (see Fig. 1A). Holliday junctions have been isolated from bacterial cells (Potter & Dressler, 1977) (Fig. 1B) and shown to be intermediates in the meiotic recombination of *Saccharomyces cerevisiae* (Schwacha & Kleckner, 1995) and *Shizosaccharomyces pombe* (Cromie *et al*., 2006). It was realized by model building that these junctions could form without any loss of base-pairing at the four-way junction (Sigal & Alberts, 1972) and physical studies have demonstrated the beauty of the molecular structures adopted by this form of DNA (Duckett *et al*., 1988; Ortiz-Lombardia *et al*., 1999) (Fig. 1C). Holliday's prediction that this junction could potentially be resolved by cleavage to produce crossover and non-crossover recombinants was confirmed by the isolation of nucleases, such as RuvC from bacteria that can cleave Holliday junctions in the predicted manner (Connolly *et al*., 1991; Dunderdale *et al*., 1991; Iwasaki *et al*., 1991). The identities of the eukaryotic nuclear Holliday junction resolvases have remained elusive though one complex able to carry out the reaction (Mus81/Eme1) has been identified (Boddy *et al*., 2001; Chen *et al*., 2001).

The strength of Holliday's paper lies not just in proposing a mechanism for the formation and resolution of 'Holliday junctions' but the association of these junctions with mismatches in heteroduplex DNA and the prediction that such mismatches could be corrected in such a way as to generate the patterns of recombination observed in tetrads and map expansion. Holliday's model predicts that 6:2 and 2:6 tetrads can be generated by correction of two symmetrically placed mismatches in heteroduplex DNA; 5:3 and 3:5 tetrads can be explained by one such mismatch remaining uncorrected, resulting in postmeiotic segregation; aberrant 4:4 tetrads can be generated in the absence of correction at both mismatches. Any of these patterns can be associated or not with crossing over according to the plane of cleavage of the junction. This is a remarkable set of divergent predictions to come from such a simple model.

Largely unknown to those who have not read it, the bulk of Holliday's paper is devoted to a discussion of

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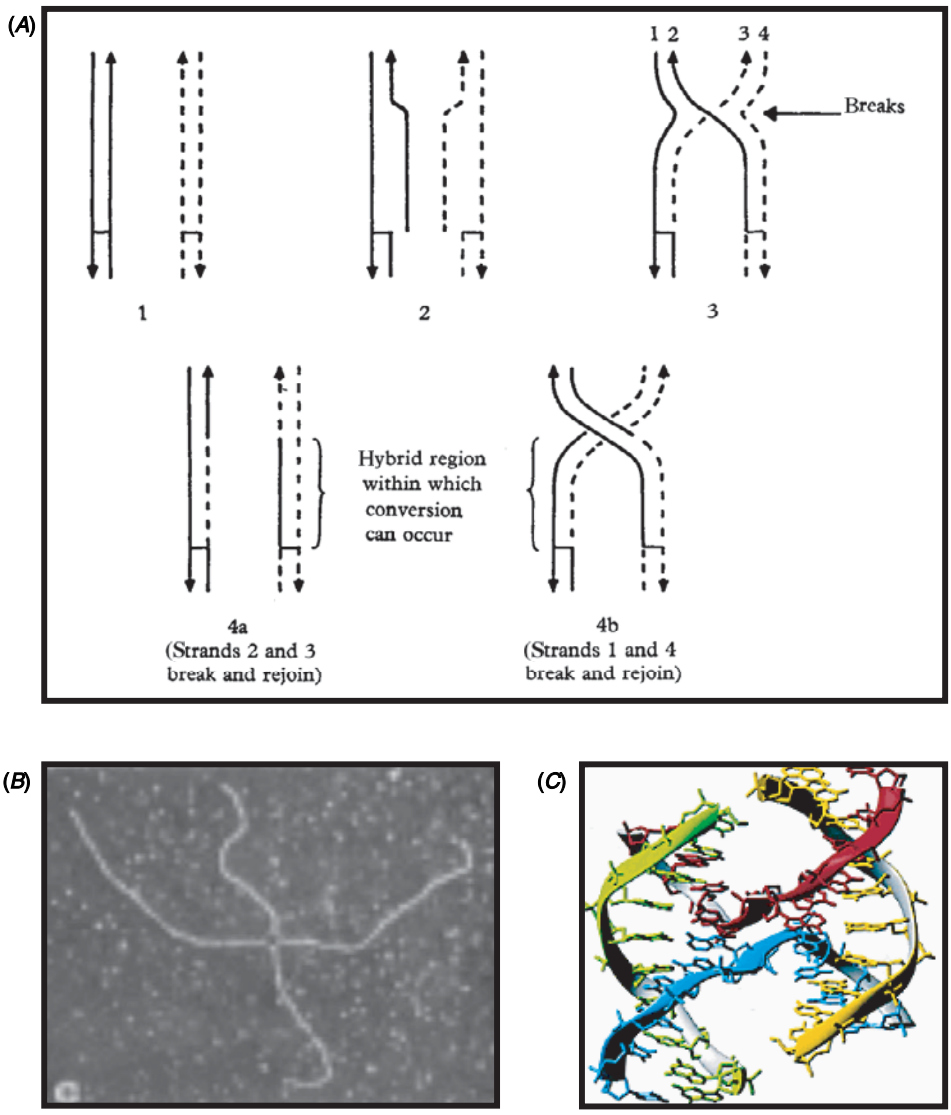

Fig. 1. (*A*) The model as drawn by Robin Holliday (Holliday, 1964). (*B*) Electron micrograph of a plasmid DNA molecule containing a Holliday junction (taken from Potter & Dressler, 1977, with permission from the *Proceedings of the National Academy of Sciences of the USA*). (*C*) Crystal structure of a Holliday junction (taken from Ortiz-Lombardia *et al.*, 1999, with permission from the Nature Publishing Group).

map expansion. So, what is map expansion? Map expansion is a phenomenon where, given three close (e.g. intragenic) markers A, B and C, the recombinant frequency between A and C (R_{AC}) is greater than the sum of the recombinant frequencies between A and B (R_{AB}), and between B and C (R_{BC}).

$$R_{AC} > R_{AB} + R_{BC}.$$

How can this come about? Holliday realized that this was likely to be an effect of the markers used in the cross and their behaviour in heteroduplex DNA. He proposed that the mutant sites were themselves interfering with intragenic recombination. In particular, he proposed an early version of heteroduplex rejection. He states: 'If there is an inhibiting effect by mutant sites on the opportunity for pairing, conversion or crossing-over, then the degree of inhibition might be inversely proportional to the distance apart of such sites.' In a later paper with John Fincham, he proposed a model based on the lengths of correction tracts (Fincham & Holliday, 1970). If two markers lie closer together than the length of correction tracts, they will tend to be co-corrected, thus reducing recombinant frequencies for very close markers. Fujitani and Kobayashi have returned to the idea of heteroduplex rejection (Fujitani & Kobayashi, 1997). In 1979, Stahl questioned the existence of map expansion (Stahl, 1979), but recent evidence using a sequenced region of the *S. pombe* genome has confirmed its existence (Baur *et al.*, 2005). Map expansion is in apparent contradiction with high negative interference also observed for close markers. However, Holliday argued that the two may be in harmony if the high negative interference involves markers in the

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heteroduplex DNA and markers flanking the site of initiation of recombination and/or resolution of the Holliday junction. Other influences on high negative interference may be system-specific (e.g. the nature of the mutations leading to independent correction or the effects of mating pools in the case of bacteriophage crosses).

Holliday's paper is remarkable for its pre-science. It anticipates the central importance of four-way junctions in recombination. It anticipates the importance of the behaviour of mismatches and of mismatch correction in recombination between close markers. Holliday was aware when he wrote his paper that the details of individual systems would vary from the exact format he drew in his figure (Fig. 1A) and he wrote: '… there are strong indications that whatever basic mechanism is operating, the details of this mechanism may not be the same in different organisms; therefore it does not seem profitable at the present time to attempt to make a model more specific by very detailed analysis of particular data from one organism or another.' The details of how recombination is initiated (e.g. at double-strand breaks, single-strand nicks or single-strand gaps), the extent of DNA degradation at the site of initiation, the extent and symmetry of heteroduplex DNA, the migration distance of Holliday junctions and the rejection and repair of mismatches will all contribute to the precise mechanism of recombination in any given system, but the general proposal put forward for the mechanism of recombination by Robin Holliday has stood the test of time.

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