

Holliday R. A mechanism for gene conversion in fungi.
Genet. Res. 5:282-304, 1964.
[John Innes Institute, Bayfordbury, Hertford, England]

A new model for genetic recombination based on the breakage and reunion of DNA chains, the formation of hybrid DNA, and the correction of mismatched bases was described. The model provides a molecular basis for the major features of crossing-over, gene conversion, and post-meiotic segregation that had been documented in several fungi. [The *SCI*® indicates that this paper has been cited in over 485 publications since 1964, making it the most highly cited paper in this journal.]

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Three discoveries stimulated new ideas in the field of recombination in the late 1950s. First came the recognition that genes could be split by recombination and mutant sites arranged in a linear order; second, that recombination could be nonreciprocal (gene conversion) as well as reciprocal; and third, that DNA has a double-helix structure. At the time it was widely believed that recombination must occur by copy choice, that is, the switching of a new DNA molecule between parental templates, although this conservative replication of DNA was totally contrary to the known facts of DNA structure and synthesis.

Alternative hybrid DNA (hDNA) models were formulated when I first went to work at the John Innes Institute in 1958, and I remember discussing some of these ideas with one of my PhD oral examiners, John Fincham, at lunch after the examination! The models depended on the unwinding of two parental DNA molecules and the reciprocal annealing of single strands with complementary partners. When the hDNA in the model spanned a heterozygous site in the parental molecules, two mismatched bases were produced, and it was further supposed that gene conversion or nonreciprocal recombination could occur by the repair of these mismatches. Failure of repair would lead to post-meiotic segregation, which had recently been discovered in *Sordaria*. The point where strands exchange partners was later known as the cruciform or Holliday structure or junction, and the resolu-

tion of this by breakage and reunion would produce 50 percent crossing-over of outside markers.

Although I discussed these features of the models with many in the field, only a summary was published.¹ I was concerned when Whitehouse independently proposed a cross-over model in 1963,² also based on hDNA and mismatch correction. This immediately stimulated me to write up my own ideas, and I chose what seems to me the very simplest molecular configuration capable of explaining most of the facts about recombination. Single-strand breaks occurred at homologous points and two reciprocal regions of hDNA were produced. Polarity in gene conversion was explained by the variable length of hDNA from the initiation point to the Holliday junction. My paper was rejected by *Nature* and *Genetics* and finally accepted by *Genetical Research*. (When it was in press, I received a kind letter from Max Delbruck, who had heard about the model, offering to publish it in the newly launched *Molecular and General Genetics*.)

The 1964 paper also discussed two apparent anomalies. First, if repair of mismatches occurred simply by the removal and replacement of bases, then even mutant sites close together would often yield recombinants, which was incompatible with linear fine-structure maps. Examination of existing data showed that, although maps were linear, they were not additive, since several demonstrated "map expansion." This was therefore invoked as a special marker effect—the closer the two sites were together, the greater the inhibition of hDNA formation and therefore the lower the recombination frequency. This problem of explaining fine-structure maps proved to be illusory when it was realised that repair involved degradation and re-synthesis of DNA.³ Second, data from *Ascomobolus* clearly indicated that nonreciprocal hDNA must sometimes be formed and Figure 5 in the paper suggested how this might occur. Later on, a much neater proposal for the formation of nonreciprocal hDNA was made by Meselson and Radding.⁴

Although the symmetry of the Holliday structure was demonstrated by a simple model in 1958, which still exists, accurate molecular models were not built until 1970 by Penny Jeggo in my laboratory at Mill Hill and by Sigal and Alberts.⁵ Evidence for the existence of the structure came from several studies of recombination in prokaryotes and especially electron microscopy.⁶ Looking back, it is striking that molecular biology and genetics make it possible to formalise the recombination mechanism without worrying about the enzymes that must be required. Nowadays, the properties of these enzymes are the focus of attention.

1. Holliday R. Mutation and replication in *Ustilago maydis*. *Genet. Res.* 3:472-86, 1962.
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3. Holliday R. Genetic recombination in fungi. (Peacock W J & Brook R D, eds.) *Replication and recombination of genetic material*. Canberra: Australian Academy of Science, 1968. p. 157-74.
4. Meselson M & Radding C M. A general model for genetic recombination. *Proc. Nat. Acad. Sci. US* 72:358-61, 1975. (Cited 285 times.)
5. Sigal N & Alberts B M. Genetic recombination: the nature of the crossed strand exchange between two homologous DNA molecules. *J. Mol. Biol.* 71:789-93, 1972. (Cited 105 times.)
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