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SHORT PAPER

A possible source of primary nucleotide chain breaks in recombinant structures in eukaryotes

By P. J. HASTINGS
Department of Genetics, University of Alberta, Edmonton, Alberta, Canada

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SUMMARY

It is argued that the primary break-points for meiotic recombination arise from the replication of regions in which DNA synthesis is delayed until meiotic prophase. The gaps would be in nucleotide chains of opposite polarity in sister chromatids. This would place a restriction on the recombination models of Holliday and of Whitehouse, so that neither is able to explain observed chromatid interference. It follows that if the primary breaks originate in the manner proposed here, the Whitehouse and Holliday models are either both wrong or both right.

It is commonly supposed that single nucleotide chain gaps are necessary for dissociation of DNA molecules so that a heteroduplex can form between two molecules (Whitehouse, 1963; Holliday, 1964; Paszewski, 1970; Kitani & Olive, 1969). It is postulated that these primary breaks are produced by an endonuclease (Whitehouse & Hastings, 1965; Holliday, 1968). This leads to some difficult assumptions about the ability of the endonuclease to recognize the correct positions on homologous chromatids (Whitehouse, 1966; Holliday, 1968). Further, since enzymes might function at any time in the cell cycle, it provides no explanation for the occurrence of both mitotic and meiotic crossing-over at a four-strand stage.

This paper offers an alternative origin for primary breaks in nucleotide chains – that they originate during replication. This explains why crossing-over occurs at the four-strand stage, and, based on the observations on meiotic DNA replication of Hotta and Stern (1971), it suggests an explanation for their localization between genes. It also suggests the mechanism of control of the distribution of recombination events. This idea is discussed by Chiu & Hastings (1972). It will be seen below that this origin of primary breaks leads to a restriction on crossing-over in the recombination models of Holliday (1964) and Whitehouse (1963).

It has been shown by Hotta & Stern (1971) that a part of the replication of the nuclear DNA does not occur in the premeiotic S-period, but is delayed until zygotene. By DNA–DNA hybridization, and by caesium chloride centrifugation, they have shown that the same sequences are delayed in their synthesis in every meiosis, and that these have a G + C content which differs from that of the bulk DNA.

Roth & Ito (1966) have shown synthesis of this zygotene DNA is required for chromosomes to synapse, since synaptonemal complex formation does not procede when DNA synthesis is inhibited.

The effect of DNA synthesis inhibitors, applied at the time of the pre-meiotic S-period in *Chlamydomonas reinhardi* is interpreted by Chiu & Hastings (1972) to mean that

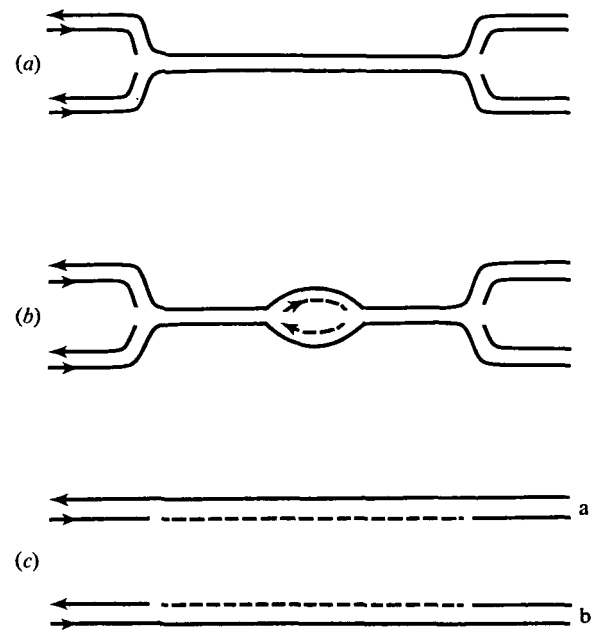

Fig. 1. The replication of a chromosome region in which replication is delayed (*a*) after premeiotic S-period (*b*) during replication and (*c*) after replication, but before ligation. Arrows show the polarity of nucleotide chains. Sister chromatids with gaps in chains of opposite polarity are designated 'a' and 'b'.

the amount of crossing over varies as the amount of DNA synthesis which is delayed until meiotic prophase. Also Rees & Evans (1966) reported a correlation between regions showing late mitotic DNA synthesis and chiasma frequency in the same regions. These results encourage one to look closely at the possibility that zygotene DNA synthesis is concerned in both synapsis and recombination, and that both can be explained in terms of variation of the same pattern of events associated with replication of the delayed regions.

Chiu & Hastings (1972) found that phenethyl alcohol, at a specific concentration, caused death of Chlamydomonas zygospores shortly before the pre-meiotic S-period, and again during early prophase, suggesting that prophase DNA synthesis needed to be initiated. This implies that it involves those parts of the chromosomes recognized as the beginning of the replication units. This, with the observation of Hotta & Stern (1971) that the delayed regions have atypically high G+C content, suggests that the delayed regions occur between structural genes. A large body of data on intragenic recombination in fungi implies that most recombination events originate between genes, extending lengths in which conversion occurs into the genes (Whitehouse & Hastings, 1965). More recently, intragenic reciprocal recombination has been found to occur commonly in yeast (Fogel & Hurst, 1967). Even in yeast, however, the majority of recombination events appear to originate outside the gene.

The model, then, is that between structural genes which replicated during the pre-meiotic S-period, regions occur in which replication occurs during zygotene, and that this provides the primary substrate for both synapsis and recombination.

Fig. 1 (*a*) shows a chromosome as a DNA molecule in which replication is delayed over a length. In Fig. 1 (*b*) the replication is occurring. It is shown to occur in both directions from an initiation point on the basis of the observations of Huberman & Riggs (1968).

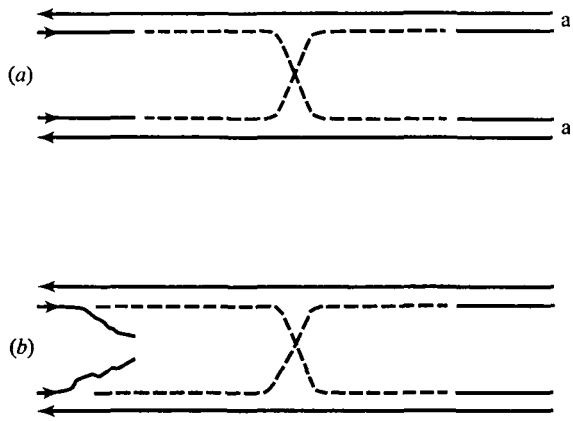

Fig. 2. (*a*) Recombining 'a' chromatids according to the model of Holliday (1964). (*b*) The same configuration after some nucleotide chain displacement during delayed replication.

With the delayed replication completed as in Fig. 1 (*c*) it is seen that both chromatids contain single strand gaps where the newly replicated lengths meet those lengths which replicated during S-period. It might be expected that the molecules would be preferentially broken at these points if DNA were extracted before they were closed. This might explain the observation of Hotta & Stern (1971) that DNA synthesized during zygotene is extracted at a lower molecular weight than is the bulk of the DNA. The single strand gaps can act as the primary breaks in a recombination event and remove the need to postulate the action of an endonuclease at this point. It will be seen that the single strand gaps occur in complementary nucleotide chains in the two sister chromatids, because of the manner in which they arose. So the two sister chromatids are different, and are designated 'a' and 'b' in Fig. 1 (*c*).

Since the annealing experiments of Hotta & Stern show that, at least in general, the same regions are always delayed, it is assumed here that the structures shown in Fig. 1 occur in homologous positions on both chromosomes.

On the Holliday model of recombination (Holliday, 1964) the primary breaks occur in chains of the same polarity. If the primary breaks originate by delayed replication, heteroduplex formation of Holliday's type would occur only between two 'a' chromatids or two 'b' chromatids. The resulting structure, shown in Fig. 2 (*a*) would not cause gene conversion, since the heteroduplex is confined to a delayed region between genes, unless either the delayed replication had extended into structural genes, displacing nucleotide chains formed during S-period (Fig. 2 (*b*)), or conversion was caused by migration of the half-chromatid chiasma, after ligation has closed the gaps. However, the results of Hotta & Stern (1971) do not preclude the possibility that structural genes may occasionally be delayed, in which case the Holliday configuration in Fig. 2 (*a*) could lead to gene conversion.

The Whitehouse model of recombination (Whitehouse, 1963; Hastings & Whitehouse, 1964) requires the primary breaks to occur in chains of unlike polarity, with replication displacing previously formed nucleotide chains. The resulting configuration, shown in Fig. 3, must concern one 'a' chromatid and one 'b' chromatid.

In both cases there are only two pairs of chromatids which can form cross-overs. On the assumption that the nucleotide chain giving rise to an 'a' chromatid or to a 'b' chromatid is continuous over long lengths of the chromosome, this would lead to two-

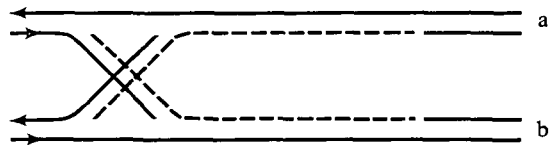

Fig. 3. Configuration of recombining chromatids with gaps in nucleotide chains of unlike polarity, according to Whitehouse (1963).

strand or four-strand double cross-overs on either model, but no three-strand double cross-overs. The general absence of chromatid interference implies that two of the four chromatids are chosen at random for any event.

One possibility is that sister chromatid crossing-over could occur to randomise the chromatids involved in detectable cross-overs. This would involve an 'a' and a 'b' chromatid, as in Fig. 3. However, the observed frequency of tetratype tetrads and of homozygosis in attached-X *Drosophila* makes this improbable (Perkins, 1955).

Alternatively, both types of recombinant configuration (Figs. 2, 3) could occur. If they were equally probable, this would lead to the observed ratios of two-strand to three-strand to four-strand double cross-overs.

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