The Relationship of Homologous Synapsis and Crossing Over in a Maize Inversion

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Manuscript received August 30, 1993
Accepted for publication January 18, 1994

ABSTRACT

Frequency of homologous synapsis at pachytene for a relatively short heterozygous inversion was compared to the frequency of crossover occurrence within the inversion and to the frequency of the presence of a recombination nodule within the homologously synapsed inverted region. Crossover frequencies were estimated from bridge-fragment frequencies at anaphase I and anaphase II. Recombination nodules (RN) were observed in electron micrographs. Results show very similar frequencies of homologous synapsis and the occurrence of reciprocal recombination within the inverted region, consistent with the interpretation that establishment of homologous synapsis in this case is related to at least commitment to the form of resolution of crossover intermediates which gives rise to reciprocal recombination, not conversion only, events. An RN was generally found at pachytene in homologously synapsed inverted regions.

For many years, conventional wisdom dictated that reciprocal crossing over followed the completion of synapsis at meiosis I, despite some early warning that this might not be the case. Early warning took the form of studies which indicated a strong correlation in heterozygotes between successful homologous synapsis of rearranged chromosome segments, and the occurrence of reciprocal exchange between them and the corresponding normal sequence segments (Maguire 1965, 1966, 1972, 1977). On the basis of these studies it was proposed that the stage of commitment to crossing over might indeed precede or accompany the full formation of the tripartite synaptonemal complex (SC) structure. Now it appears that, while there was insight in this interpretation, the understanding of the true complexity of the process now emerging should provide many answers.

Studies of meiosis in yeast have been rewarding. Recent reports suggest that genome-wide homology search, which leads to initial stages of homolog alignment, begins very early, immediately following bulk DNA replication or even premeiotically (Schürrth et al. 1992; Kleckner and Weiner 1993). From synchronized cultures (Kleckner et al. 1991; also reviewed by Hawley and Arbel 1993), the timing with respect to meiotic landmarks of appearance and disappearance of DNA configurations which are expected to be recombination intermediates has suggested the following sequence and insights. Homology is probably recognized in permissive chromosome structure at sites for prospective double strand breaks (DSBs) where an initial semi-stable physical connection is made. Then more intimate interaction is thought to occur where homology has been identified, and this probably requires 3' tails of DSBs with neither 3' nor 5' tails isolatable after this point. Appearance of DSBs with tightly coupled resection of 5' ends and first appearance of tripartite SC structure seem to be nearly contemporaneous, with heteroduplex formation relatively late in prophase. Resolution of heteroduplex structures seems to closely follow SC disintegration. It is guessed that bulk SC components may inhibit recombination "maturatio" until SCs have been disassembled. Researchers interpret the pattern to suggest that the SC serves some function other than provision for meiotic crossover events which it may in fact inhibit or stall. Additional support for this idea is provided by behavior of certain meiotic mutants in yeast, such as red1, mer1, hop1 and zip1, and dsy1 in maize which are defective for or totally lacking in important SC components but nevertheless show at least moderate levels of crossing over (Rockmill and Roeder 1990; Engbrecht et al. 1990; Hollingsworth et al. 1990; Sym et al. 1993; Maguire et al. 1993). On the basis of observations of the timing of appearance and distribution of recombinant nodules and synapsis in tomato, Stack and Anderson (1986) have also proposed that recombination may be initiated early and that the distribution of reciprocal recombination events is somehow dependent on some distance function of synaptic extension in accordance with interference. Egel (1978) and Maguire (1968, 1988) have inferred that the presence of tripartite SC may be inhibitory to reciprocal recombination on the basis of crossover and interference information.

The nature of the genetic map construct determines that crossovers shall be randomly distributed along it. However, wherever comparisons have been feasible, they seem to demonstrate that physical cytological maps of chromosomes do not correlate well with their genetic maps. In general, there appear to be hotspots and also coldspots for crossing over along the physical entities, where the genetic map is respectively expanded or...
contracted. Attention to localized recombination frequencies in the presence of translocations (Hawley 1980) and duplicating fragments (Rose et al. 1984) has led to the interpretation that centers specialized for homologous pairing indeed exist along chromosomes. Recently, various reports have suggested that hotspots for recombination may represent sites for pairing initiation [see Goldway et al. (1993) and references therein].

Late recombination nodules (RNs) which are found at mid to late pachytene with electron micrograph (EM) observation following appropriate fixation and staining have been generally shown to correlate for frequency and position with chiasmata or other crossover manifestations observed later (von Wettstein et al. 1984; Albin and Jones 1988; Carpenter 1988; Stack and Anderson 1989; Zicker et al. 1992; Stack et al. 1993; Herickhoff et al. 1993). They therefore provide a tool at pachytene for the observation of probable crossover locations. At zygotene in material with large chromosomes, initiation of meiotic pairing and synapsis are accompanied by the appearance of SC-associated nodules which are generally smaller and much more numerous but otherwise appear similar to pachytene RNs (Albin and Jones 1987; Anderson and Stack 1988). Some studies have suggested correlation between pairing initiation sites and positions of RNs, chiasmata and recombination (Zicker et al. 1992; Stack et al. 1993). Zicker et al. (1992) found correlations between frequency and location of reciprocal exchanges and frequency and location of late RNs in wild-type Sordaria but disparity between location of nodules and exchanges in those mutants which cause reduction in recombination but do not cause SC arrest or major abnormalities. They suggested that possible effects in the mutants, such as premature loss of nodules, or late synapsis might account for the disparities. Information for interrelationships of conversion, recombination and synapsis was more limited and less clear. Stack and Soulliere (1984) found a 1:1 relationship between synapsis of distal regions of chromosomes involved in complex translocation heterozygote configurations in Rhoea spathacea. But, in the absence of rearrangement, with long chromosomes the number of synaptic initiations may greatly exceed the number of chiasmata (Hasekampf 1984; Gillies 1985), and synaptic initiation in heterozygotes for structural change has been found in the absence of evidence for crossing over, e.g., in tomatoes heterozygous for translocations (Herickhoff et al. 1993). On the one hand it is possible that in cases where there is a 1:1 relationship between crossing over and synaptic initiation with change of pairing partner that the genetic map of the region involved is so large that this is the conventional expectation. On the other hand it is also possible that, where there is synaptic initiation in the absence of crossing over, this is due to late synapsis of unpaired ends, too late for crossing over. Other conceivable complicating factors may exist such as the existence of potential crossover sites with differing probabilities for initiations of crossovers and change in the use of these sites with chromosome structural change, as well as possible change in synaptic potential with chromosome rearrangement (Parker et al. 1982). McKim et al. (1993) have reported two types of sites required for meiotic chromosome pairing in Caenorhabditis elegans with one localized near one end of each chromosome.

The present study takes advantage of the fact that, for homologous pairing to occur in a heterozygously inverted region, pairing must be initiated within it rather than extended from an initiation elsewhere. It utilizes an inversion with an extent of about 30 map units so that conventional expectation would call for a maximum crossover frequency within it of about 60%. Maize chromosomes are sufficiently large to allow study of their synapsis at pachytene, and with current technology RNs can be reliably stained and identified in EM. The silver-staining procedure with EM viewing allows not only RN visualization but also much more accurate visualization of synaptic configurations than is possible with the light microscope observation of conventional acetocarmine smears used in the prior studies of maize rearrangements. In addition, a much smaller proportion of stained cells is unclassifiable. Bridge and fragment analysis at anaphase I and II provides information on the frequency of crossovers within the inverted region. Results suggest that homologous synapsis of the inverted region is associated with the presence of a crossover and an RN.

MATERIALS AND METHODS

Maize seeds heterozygous for Inv 5083 (provided by Gregory Doyle) were grown in a growth chamber with a controlled environment. Growth chamber culture has been found to reduce the sample to sample variations in synaptic and crossover frequencies such as those which were found in the field grown plants of the early study (Maguire 1966). The inversion is reported to have breakpoints at chromosome fl 0.70 and 0.87 of the length of the long arm from its centromere (Longley 1961). On the basis of the most recent tentative cytological map of maize chromosome I in the 1993 Maize Genetics Newsletter the inverted region constitutes about 30 map units. At meiotic stages samples were collected and prepared by two differing procedures. (1) Cells from fresh anthers at pachytene were spread on plastic coated slides following maceration of anthers and fixation by a technique described elsewhere (Maguire et al. 1998). Slides were then silver-stained by a procedure which allows reliable visualization of SC lateral elements and RNs (Sherman et al. 1992). EM grids were positioned on suitably spread and stained cells, and grids were floated on their plastic rafts on a water surface and picked up and dried on lens paper before examination and photography with a Siemens Elmiskop 1A electron microscope. (2) A second meiotic sample was collected and immediately fixed in ethanol-acetic 3:1 mixture and stored in a freezer until examination with systematic scanning of conventional acetocarmine squash preparations of anaphase I and also anaphase II slides. Anaphase I cells were scored as either no bridge or...
RESULTS

A total of 100 pachytene cells appropriately fixed and silver-stained were examined in EM for the presence of homologous synapsis of the heterozygously inverted segment. Of these, 29 showed at least a short homologously synapsed segment (with reverse pairing). The remainder (71) showed no such reverse synapsis and for the most part showed normal appearing, but necessarily nonhomologous, synapsis across the inverted region, a commonplace occurrence in maize inversion heterozygotes (Maguire 1972). Of the 29 cells with homologous synapsis of the inverted region, 24 contained a single identifiable RN within the region of reverse pairing (as illustrated in Figures 1-3), three contained no RN and two were unclassifiable for the presence of an RN. Thus it seems likely that usually homologous synapsis of the inverted region was accompanied by a crossover. In 15 of the cells in which reverse synapsis occurred and an RN was present in the homologously paired inverted region, stretching and distortion appeared to be absent, and the entire extent of chromosome I could be traced and measured. Results of measurements and positions of RNs for these cells are indicated graphically in Figure 4 for both the inverted and distal regions. These results are also tabulated in Table 1. In these configurations the distal region of the long arm of chromosome I was usually homologously synapsed, and in 9 of the 15 cells in which chromosome I was traceable, an RN was present in the distal region, three contained no RN and three were unclassifiable for the presence of an RN. Therefore, homologous synapsis of the distal region in the
probably also usually accompanied by a crossover event. The presence of inverted region homologous synapsis was probably also usually accompanied by a crossover event.

Findings from bridge and fragment counts in aceto-carmine squash preparations at anaphase I are presented in Table 2. In summary, 74% of 1000 cells at anaphase I showed no bridge or fragment, 20% showed a bridge and a fragment, as expected for cells with either a single crossover within the inversion (Figure 5) (by far the most common case) or for cells with a three-strand double within the inversion (Figure 6). 6% showed a fragment only (see Figure 7 and description below) and 0.40% showed a double bridge and two fragments as expected for cells with four-strand doubles within the inversion (Figure 8). Two-strand doubles within the inversion are totally invisible. With the assumption of no chromatid interference, three-strand doubles within should constitute 1/4 the doubles while two- and four-strand doubles should each be 1/2 of the total. Thus, use of the double bridge and fragment frequency to estimate total frequency of cells with double crossovers within the inversion calls for quadrupling of the 0.40% frequency to 1.60% frequency. An additional source of cells with at least one crossover within the inversion is represented by cells which show a fragment only at anaphase I and a bridge at anaphase II. These represent cells in which a single crossover occurred within the inversion, and a three-strand double type second crossover occurred in the proximal region (Figure 7).

TABLE 1

<table>
<thead>
<tr>
<th>Grid no.</th>
<th>Position of reverse pairing</th>
<th>Reverse pairing RN position</th>
<th>One homolog</th>
<th>Other homolog</th>
<th>Extent distal region pairing</th>
<th>Distal region RN position</th>
</tr>
</thead>
<tbody>
<tr>
<td>9D box 13</td>
<td>0.120</td>
<td>0.256</td>
<td>0.127</td>
<td>0.249</td>
<td>0.000</td>
<td>0.089</td>
</tr>
<tr>
<td>4D box 13</td>
<td>0.064</td>
<td>0.205</td>
<td>0.088</td>
<td>0.181</td>
<td>0.000</td>
<td>0.004</td>
</tr>
<tr>
<td>10A box 14</td>
<td>0.077</td>
<td>0.169</td>
<td>0.085</td>
<td>0.161</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>5B box 13</td>
<td>0.048</td>
<td>0.193</td>
<td>0.092</td>
<td>0.150</td>
<td>0.000</td>
<td>0.048</td>
</tr>
<tr>
<td>9E box 14</td>
<td>0.050</td>
<td>0.169</td>
<td>0.083</td>
<td>0.136</td>
<td>0.000</td>
<td>0.011</td>
</tr>
<tr>
<td>8A box 13</td>
<td>0.106</td>
<td>0.166</td>
<td>0.113</td>
<td>0.159</td>
<td>0.000</td>
<td>0.000</td>
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<tr>
<td>6C box 12</td>
<td>0.075</td>
<td>0.137</td>
<td>0.086</td>
<td>0.126</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>4A box 13</td>
<td>0.063</td>
<td>0.150</td>
<td>0.090</td>
<td>0.122</td>
<td>0.000</td>
<td>0.000</td>
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<tr>
<td>7D box 14</td>
<td>0.073</td>
<td>0.162</td>
<td>0.103</td>
<td>0.135</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>8D box 14</td>
<td>0.097</td>
<td>0.119</td>
<td>0.097</td>
<td>0.119</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>6C box 13</td>
<td>0.074</td>
<td>0.159</td>
<td>0.108</td>
<td>0.124</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>9E box 13</td>
<td>0.109</td>
<td>0.139</td>
<td>0.118</td>
<td>0.130</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>6A box 14</td>
<td>0.128</td>
<td>0.185</td>
<td>0.152</td>
<td>0.162</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>7C box 13</td>
<td>0.118</td>
<td>0.140</td>
<td>0.127</td>
<td>0.129</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>9A box 12</td>
<td>0.140</td>
<td>0.150</td>
<td>0.144</td>
<td>0.146</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Figure 4.—Graphical representation of the positions of homologous synapsis in the inverted and distal regions of the long arm of chromosome 1. Grid identities of these preparations are indicated at the left, and the horizontal axis is the scale representation of the fraction of total length which is involved of the entire chromosome from the distal end of the long arm. Lines at the left represent homologous synapsis extent of the distal region; lines to the right represent homologous synapsis of the inverted region. In each case, matching unsynapsed chromosome lengths of unsynapsed regions have been averaged for the two homologs. These tend to stretch more readily than synapsed regions, and the average value seems the most fair representation. RNs are represented by ×s for the two homologs so that each RN within the inversion appears here as two ×s in mirror image positions proximally and distally within the reverse pairing region. For the distal region each × represents an RN.
TABLE 2

Bridge and fragment frequencies at anaphase I

<table>
<thead>
<tr>
<th>No bridge or fragment</th>
<th>Bridge and fragment</th>
<th>Fragment only</th>
<th>Double bridge 2 fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
<td>Percent</td>
</tr>
<tr>
<td>744/1000</td>
<td>74</td>
<td>196/1000</td>
<td>20</td>
</tr>
</tbody>
</table>

FIGURE 5.—Diagrammatic representation of the inverted region at pachytene with a single crossover within the inversion. One homolog is represented by heavy lines, the other by light lines; boxes at the left represent centromeres; the × intersections represent a crossover. The second and fourth chromatids down from the top are involved in the crossover. Tracing from the left will indicate that these now represent one continuous dicentric chromatid which will form a bridge at anaphase I. Tracing from the right will indicate that the other ends of these chromatids now represent a single acentric fragment.

FIGURE 6.—Diagrammatic representation of the inverted region at pachytene with a three-strand double crossover within the inversion. One homolog is represented by heavy lines, the other by light lines; boxes at the left represent centromeres; the × intersections represent crossovers. Tracing the topmost chromatid from the left will indicate that it now represents a continuous dicentric chromatid with the fourth chromatid down from the top which will form a bridge at anaphase I. Tracing the second chromatid down from the right will indicate that it now represents a continuous acentric chromatid with the fourth chromatid down from the top which will form a fragment at anaphase I.

I cells of this class were found with a frequency of 6%. An additional estimate of the frequency of anaphase I cells of this last class is provided by the anaphase II observations where such cells are represented by a bridge. From scoring of 1074 anaphase II cells, 3% showed such a bridge, and since each meiosis I cell produces two anaphase II cells, only one of which will have the telltale bridge, the anaphase II cell information also suggests a frequency of 6% anaphase I cells with one crossover within the inversion and a second constituting a three-strand double in the proximal region. The total frequency of cells with at least one crossover within the inversion which is indicated by the anaphase data therefore represents 27.6%. This corresponds to the 29% frequency of cells found to have at least some homologous synopsis in the inverted region at pachytene and to the 24% which contained a single identifiable RN within that region, with 2% unclassifiable in this latter respect.

It must be emphasized that there is no test of conversion frequency in this material. Only reciprocal recombinants are scored, many of which may be accompanied by conversion, but the frequency of conversion only events is totally unknown.

Findings are therefore consistent with the suppositions that in this material, occurrence of homologous synopsis of the inversion at pachytene is closely corre-
lating with the occurrence of at least one crossover within it, and that the existence of an RN at this stage is also closely correlated with the occurrence of a crossover. Unfortunately, there is no test here of crossover frequency in the region distal to the inversion. If there are hotspots for crossing over within the inverted region, this study does not resolve them. However, with an estimate of 30 map units within the inverted region, if this region were homologously synapsed at pachytene throughout its extent in all cells, conventional expectation would call for crossing over to occur in this region in 60% of cells. But it is evident that even in those cells where there was homologous synapsis at pachytene of the inverted region, the extent of this synapsis was highly variable and often substantially less than the full inverted region. In addition there was no homologous synthesis of the inverted region in 71% of the cells. It is difficult to escape the conclusion that in this case there is an unconventional 1:1 relationship between occurrence of crossing over and an event of homologous synapsis at pachytene which is assumed to be stable in maize since synaptic adjustment apparently does not occur (Anderson et al. 1988).

Findings overall are similar to those of the first such study with this inversion (Maguire 1966), but frequencies differ both within the early study and between that study and the present results. In that experiment, three plants were studied with pachytene inversion homologous synopsis of 36.0, 30.1 and 34.9% compared to combined bridge-fragment and fragment only frequencies of 35.8, 29.6 and 34.2%, respectively. In addition, the frequency of the fragment only classes comprised a higher proportion of the total. Since the early study was done with field-grown plants in the presence of much environmental variation, perhaps differences among those plants and between those and the plants of the present study of growth chamber grown plants (with a constant climate) are not surprising. The coordinate variations of synaptic and crossover frequencies throughout may serve to demonstrate a strength of this relationship, however.

DISCUSSION

Findings of this report from studies of a eukaryote with large, complex chromosomes correspond well with expectations from research with yeast in that they are consistent with the interpretation that initiation of crossover events precedes or accompanies tripartite SC formation. Estimates of crossover frequencies within the inversion from bridge and fragment frequencies are expected to be accurate with only small margins of probable error, as a result of the fact that crossover frequencies may vary among samples from different parts of a tassel. Care was taken, however, to use samples for acetocarmine smears from the same general region of the tassel as those used for the pachytene spread preparations. A smaller number of cells at pachytene (100) were examined, however, than at anaphase I (1000) and anaphase II (1074), so that the frequencies observed may be somewhat less reliable at pachytene. The largest errors in this study are probably expected to exist in the measurements used to estimate positions of RNs within the inversion and distal regions. Breakpoints previously listed for Inv 5083 were estimated from empirical measurements of acetocarmine smear preparations where loop configurations were found and reported to be on the average at 0.70–0.87 of the long arm of chromosome I from the centromere (Longley 1961). From measurements in this study the average position of breaks, taking 1.32 as the ratio of long arm to short arm of chromosome I (Neuffer et al. 1968) differed somewhat from the previously reported values of 0.072–0.165 from the distal end of the long arm of the chromosome. Here they were 0.089–0.167. Within this study the length of the segment which was homologously synapsed was highly variable, but this is believed to be due not to variation with stage advancement, which (as stated above) seems not to occur in maize (Anderson et al. 1988) but to variation in stable synaptic extent, some of which may be nonhomologous.

It must be emphasized that, unlike noteworthy cases in yeast (Engebrecth et al. 1990) and Sordaria (Zickler et al. 1992) which have been studied, the close rela-
tionship of homologous synapsis here to the occurrence of crossing over is strictly a relationship to reciprocal recombination, not conversion, although many of the crossovers may also be associated with conversion. However, strong relationship of existence of overall homologous synapsis to conversion only events has been found in the mer1 mutant of yeast in cases where some of the usual defects are corrected by presence of the MER2 gene in high copy number. Synapsis and gene conversion are restored to normal by this condition, but reciprocal recombination frequency is still depressed, although some is found as was the case without the extra copies of MER2 (Engerbrecht et al. 1990). Exclusion of noncrossover or conversion only events from the relationship also exists for the many reports of close correspondence of pachytene RNs in frequency and position to chiasmata. Where this relationship is also related to synaptic initiation with change of pairing partner, important inferences can be drawn. Perhaps there is commitment at some kinds of synaptic initiation to resolution of crossover intermediates to the form which gives rise to reciprocal recombination. At each crossover event the DSB model requires opposite resolutions of two Holliday junctions ( Orr-Weaver and Szostak 1985).

On the basis of studies with synthetic Holliday junctions in vitro with purified bacterial enzymes, West (1990) has proposed that resolution may be unregulated to isomerization status (which would be difficult to envision when two DNA molecules are intertwined with a protein filament). Instead, the DNA helices may be held by RecA protein in such a way that resolution can occur in either of the two ways. West also suggests that these protein-DNA structures may represent a primitive form of the recombination nodules that occur within synaptonemal complexes of eukaryotes. If some RNs can play a role in commitment to resolution of crossover intermediates, perhaps it is some of those at points of synaptic initiation which serve in this capacity while others may more often relate to events of conversion only. Maybe SC extension at first takes the form of a series of button-up initiations in long chromosomes each followed by zipping up. SC metabolism could be more complex than commonly appreciated in that it might play a role in chiasma interference (Maguire 1968; Egel 1978; Maguire 1988). Foss et al. (1999) have noted that chiasma interference seems to depend upon genetic distance rather than physical distance and have proposed a model which is consistent with data from Drosophila and Neurospora. This model suggests that the alternative resolutions of randomly distributed recombination intermediates are somehow constrained so that neighboring reciprocal recombinations must have a certain number of conversion only or noncrossover events between them. The tripartite structural components of the SC may play roles currently not realized.

The lesson is that many factors contribute to synapsis and crossover relationships, and superficial appearances are determined by those which predominate. The task is to learn what these factors are and how they interact.

This work was supported by U.S. Department of Agriculture grant 92-01569. The authors are grateful to Gregory Doyle for supplying the seeds of the Inv 5083 stock.

LITERATURE CITED


Communicating editor: P. J. FukuRA