Synaptic Adjustment of Inversion Loops in *Neurospora crassa*

Maja Bojko

Department of Biological Sciences, Stanford University, Stanford, California 94305, and Department of Physiology, Carlsberg Laboratory, Gammel Carlsberg Vej 10, DK-2500 Copenhagen Valby, Denmark

Manuscript received June 19, 1989

Accepted for publication November 6, 1989

ABSTRACT

Heterozygotes for three long inversions on chromosome 1 were analyzed by serial reconstruction from electron micrographs. Measurements of loop lengths at different meiotic prophase substages revealed that the homologous synopsis of the inverted region was gradually replaced by nonhomologous synopsis as loops were eliminated during pachytene. This synaptic adjustment was apparently not affected by crossovers which occurred within the 150- and 160-cM long loops.

The synaptonemal complex (SC) is not just a framework holding the homologous chromosomes in alignment at pachytene, but is a dynamic structure, undergoing internal changes and rearrangements. These changes can be recognized as length reduction or increase (Maguire 1978; Gillies 1984). But even more dramatically, SC turnover is observed in structural heterozygotes or polyploid organisms, where it results in pairing correction (Rasmus sen 1977) or synaptic adjustment (Moses et al. 1978).

A pairing correction mechanism was observed in the achiasmatic female of silk moth, *Bombyx mori*. Reconstruction of two zygotene-early pachytene oocytes from triploid females revealed exchanges of pairing partner resulting in formation of 12 trivalents. In contrast, in mid-late pachytene nuclei only bivalents and univalents were present together with a number of nonhomologous associations (Rasmus sen 1977). A corresponding correction of quadrivalents leading to nearly exclusive bivalent formation at mid-pachytene was observed in autotetraploid Bombyx females, where seven early pachytene and 11 mid-late pachytene nuclei were investigated (Rasmus sen and Holm 1979). It was concluded that synopsis with SC formation is strictly homologous at zygotene, but is followed by a correction phase in which the pairing is optimized to yield a maximum number of bivalents and two-by-two associations. During the second phase, homology is no longer required for SC formation. Multivalents are much more stable in the autotetraploid Bombyx male, in contrast to in the female. A mean of 13.3 quadrivalents seen in 33 zygotene nuclei was reduced to 8.7 in 27 nuclei at late pachytene. The difference in the pairing pattern between the two sexes was ascribed to the occurrence of crossing over in the male. It was suggested that crossover events in relevant arms of the multivalents stabilize the SC and prevent multivalent resolution (Rasmus sen 1987).

Rearrangements of the SC are also reported from diploid organisms, where the initial zygotene pairing does not result in regular bivalents with continuous SCs. Para centric inversion heterozygotes of the mouse were investigated by surface spread SC (Moses 1977; Moses et al. 1982; Poorman et al. 1981). In early pachytene nuclei, a reversed loop was invariably observed. In the course of pachytene, desynapsis occurred at both ends of the loop followed by a nonhomologous pairing resulting in progressive reduction of the loop, until it was eliminated by late pachytene. No loops were observed in late pachytene nuclei, despite the fact that a significant number of crossovers occurred within the inversion, as indicated by 34% anaphase bridges. An analysis of bivalent formation in a mouse heterozygous for a tandem duplication showed an unpaired buckle in early pachytene bivalents but only straight pairing at late pachytene. This indicated that individual lateral components can change in length to compensate for differences in chromosome length (Moses and Poorman 1981). The process leading to formation of a straight, nonhomologous SC in structural heterozygotes is termed synaptic adjustment.

Observations consistent with the occurrence of synaptic adjustment are reported from such diverse animal species as the moth *Ephestia kuehniella* (Weith and Traut 1986), chicken (Kaelbling and Fechheimer 1985), sand rat (Ashley, Moses and Solari 1981), red kangaroo (Sharp 1986) and man (Guitingoua et al. 1985; Gabriel-Robez et al. 1986). Synaptic adjustment thus seems to be common in animals. However, in a population of Sitka deer mice polymorphic for a pericentric inversion, no loops...
could be detected in heterozygotes. All pachytene bivalents were fully paired with a nonhomologous SC (HALE 1986). Such heterosynapsis is of selective value for the population as it is believed to effectively suppress crossing over within the inverted segment and prevent formation of genetically unbalanced gametes.

The few studies of synaptic adjustment and pairing correction in plants point to different conclusions. A pairing correction mechanism is reported from euploid wheat (HOLM and WANG 1988), diploid hybrids of Lolium (JENKINS 1985), or tetraploid hybrids of Scilla (JENKINS, WHITE and PARKER 1988). In these plants, contrary to in Bombyx, nonhomologous pairing occurred at zygotene, as indicated by a presence of multivalents which were corrected to bivalents at pachytene. Inversion heterozygotes were investigated in maize and onion, and it was concluded that synaptic adjustment does not occur in these species (MAGUIRE 1981; LOIDL 1987; ANDERSON, STACK and SHERMAN 1988). However, RHODES' (1968) observations from maize microsporocytes indicate that the pairing behavior in maize could be more complex. He had studied pairing cytology and crossing over in a transposition, where a piece of chromosome 3 was inserted into chromosome 9, and in a chromosome 3 inversion. His camera lucida drawings of pairing configurations and the recombination data are consistent with the synaptic adjustment in chromosome 9 bivalents composed of a normal chromosome 9 and one with the transposition, but not in the normal/deficiency chromosome 3 bivalents. Similarly, the observations of bivalent 3 heterozygous for a paracentric inversion suggest occurrence of synaptic adjustment, but only when an abnormal chromosome 10 (K10) is present in the nucleus. Clearly, the genetic background is an essential factor in pairing behavior of maize chromosomes. Rhoades' observations of inversion loops and buckles of different sizes are of special interest, if the observed differences indeed result from the synaptic adjustment, as they indicate that synaptic adjustment operates at the chromatin level and not only at the level of the SC.

Less is known about the pairing behavior of rearranged chromosomes in fungi. No pairing correction mechanism was seen in a trilobid strain of the basidiomycete Coprinus where the trivalent configurations were maintained unaltered throughout pachytene (RAMMUSSEN et al. 1981). This lack of correction was not attributed to the stabilizing effect of crossovers, as 53% of analyzed trivalents had recombination nodules only between the same two homologues, or none at all.

The present study of Neurospora inversion heterozygotes provides the first evidence of synaptic adjustment in fungi. The length of the inversions chosen is such that at least one crossover event is expected within the loop. This gives an opportunity to inspect the notion that the occurrence of a crossover prevents SC rearrangement and loop elimination.

MATERIALS AND METHODS

The N. crassa strains and crosses have been described (Bojko 1988). Crosses used in the present investigation had one structurally normal parent while the other parent carried a long pericentric inversion, either In(IL;IR)H4250 [Fungal Genetics Stock Center (FGSC) numbers 13063 and 15641], In(IL;IR)OY323 (FGSC 3793) or In(IL;IR)OY348 (FGSC 3840).

Laboratory techniques were as described by PERKINS (1972). Crossing, harvesting, fixation of asci, processing for the electron microscope and techniques for serial section and reconstruction, were as described earlier (Bojko 1988).

RESULTS

Inversion In(IL;IR)H4250: Inversion In(IL;IR) H4250 was characterized by NEWMEYER and TAYLOR (1967) as a pericentric inversion, where one of the break points was between two proximal markers in linkage group I L, and the other was at or near the tip of IR. The latter break was distal to all known markers and there was no actual proof for its existence. However, evidence from higher organisms indicate that a break is necessary for other broken ends to attach, so the presence of a subterminal break in H4250 was postulated. The genetic length of the inverted segment was originally estimated as 50 map units. However, more recent estimation gives a value of 160 map units (PERKINS 1987).

Thirty-four nuclei from H4250 heterozygotes were reconstructed. Of these, six could not be used because the presence of abnormal SCs prevented the identification of chromosome 1 or prevented a meaningful analysis of the inversion loop. The SC anomalies, common in N. crassa, are described elsewhere (Bojko 1988). Analyzed nuclei are arranged according to stage in Table 1. Besides the nuclei listed in the table, four leptotene and three diplotene nuclei were reconstructed. The substage determination is based on ascus and nucleus morphology (Bojko 1988).

As shown in the table, full synopsis of chromosome 1 bivalents is seldom observed. Even at pachytene, most of the bivalents 1 had 20-30% of their length unpaired. This nonpairing is probably due to the turnover of the central region of the SC during the process of synaptic adjustment, as in six bivalents the unpaired region included a portion of the inverted region.

In bivalents with unpaired telomeric regions a precise determination of the extent of the loop was not possible. In these cases, the lengths of both lateral components were measured to calculate the maximum possible loop length. Such nuclei with maximum calculated loop length (and minimum arm length) are denoted in the table.
Loop and arm lengths expressed as percent of total length in chromosome 1 bivalents heterozygous for inversion In(II-IR)H4250 at different stages of meiotic prophase

<table>
<thead>
<tr>
<th>Stage</th>
<th>% Pairing</th>
<th>Loop</th>
<th>Long arm</th>
<th>Short arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early zygotene</td>
<td>20</td>
<td>69</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>&lt;10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid-zygotene</td>
<td>21</td>
<td>76</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>22</td>
<td>75</td>
<td>24</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>70</td>
<td>28</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>74</td>
<td>26</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>59*</td>
<td>56</td>
<td>44</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>78</td>
<td>63</td>
<td>37</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Late zygotene</td>
<td>72</td>
<td>74</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>Early pachytene</td>
<td>100</td>
<td>51</td>
<td>38</td>
<td>11</td>
</tr>
<tr>
<td>69</td>
<td></td>
<td>37</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Mid-pachytene</td>
<td>100</td>
<td>58</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>83</td>
<td>52</td>
<td>42</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>87</td>
<td>50</td>
<td>35</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>83</td>
<td>42</td>
<td>42</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Late pachytene</td>
<td>100</td>
<td>33</td>
<td>42</td>
<td>25</td>
</tr>
<tr>
<td>83*</td>
<td>17</td>
<td>44</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>76*</td>
<td>80</td>
<td>19</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* Unpaired segments prevented precise determination of the loop extent. A maximum loop length is calculated based on measurements of both lateral components.

Loop configurations and lengths found in nuclei at early, mid- and late zygotene are very similar despite great diversity in the extent of synopsis. The mean loop length is 70% (SE ± 2.5), and the long distal segment ("arm") is 30% (SE ± 2.5). There is no short arm, which indicates that the segment distal to the break (if it exists) does not contain a pairing initiation site. These observations are expected from the genetic data and are consistent with the notion that synopsis is strictly homologous at zygotene. A reconstruction of a mid-zygotene bivalent is shown in Figure 1a.

A short arm appears at early pachytene, and increases in length in the following stages (Table 1, Figure 1d). The increasing length of the short arm is compensated by a reduction of the loop. The long arm is relatively unchanged, at least until late pachytene. The mean loop length at mid-pachytene is 51% (SE ± 3.3). Reduction of the loop progresses in late pachytene resulting in complete elimination of the loop in some bivalents (Table 1, Figure 1, c–f). There is no indication of a loop in three early diplotene nuclei, where about 75% of the complement is still preserved and only linear fragments of SC are seen.

One late pachytene bivalent indicates that synaptic adjustment can also start within the loop and progress toward the telomeres. In this bivalent, the loop comprises 80% and the long arm 19% of the total length.

Inversions In(II;IR)OY323 and In(II;IR)OY348: Inversions In(II;IR)OY323 (BARRY and LESLIE 1982) and In(II;IR)OY348 (TURNER and PERKINS 1982) are partially overlapping, pericentric inversions with breakpoints in the central regions of each arm of chromosome 1. The genetic length of each has been estimated to be around 150 map units (PERKINS 1987).

Seven OY323 and 12 OY348 heterozygous nuclei were reconstructed. Only three OY323 and five OY348 inversion loops were analyzable. However, the results from these two inversions confirm those from H4250. All three OY323 observations were from mid-zygotene nuclei and the loop lengths and positions were as expected from homologous synopsis. The mean loop length was 69% (SE ± 0.6), with the long arm of 19% (SE ± 0.7), and the short arm 12% (SE ± 0.7).

Two nuclei from the OY348 inversion heterozygotes were at zygote. These had loops making up 64% and 67% of the length, short arms making up 13% and 9% of the length, and long arms making up 23% and 23% of the length, respectively. In one nucleus at early pachytene the loop was 53%, while only straight pairing was seen in a nucleus at mid-pachytene. One OY348 zygote bivalent lacked a loop and...
had the middle 47% portion unpaired. Both paired arms were of almost the same length, and it appeared as if heterosynapsis proceeded directly from the unpaired state without homosynapsis.

**Connection of a bivalent to the spindle pole body:**
In six mid- and late pachytene nuclei a connection was observed between a single telomere and the spindle pole body. The development of the spindle pole body and microtubule organizing centers was described earlier (Bojko 1988). In the present study, it is possible to identify with certainty the two longest bivalents: bivalent 1 by the inversion loop and bivalent 2 by the nucleolus. In three cases one telomere of chromosome 1 was attached to the nuclear envelope opposite the position where the spindle pole body was appressed to the outer nuclear membrane. In one nucleus, the non-nucleolus organizer telomere of chromosome 2 was present at this position, and in two other nuclei, telomeres of other, unidentified chromosomes were opposite the spindle pole body.

In a water mold *Allomyces macrogynus*, Borkhardt and Olson (1979) observed a bivalent attached to the nuclear envelope just opposite the centriole. The identity of the chromosome was not established, but it was hypothesized that the functioning centriole is associated with a particular bivalent. From the present observations it can be concluded that different chromosomes can be associated with the developing spindle pole body in different nuclei. Similar associations between spindle pole bodies and different bivalents have been reported in *Sordaria macrospora* (Zickler 1989).

**DISCUSSION**

This study describes the pairing pattern of pericentric inversions in three inversion heterozygotes during meiotic prophase.

**Crossing over does not hinder synaptic adjustment of inversion loops:** It is reasonable to assume that the constant dimensions of the zygotene loops, and the absence of one arm in the *H4250* inversion bivalents indicate a strict homologous pairing at zygotene. In the stages following zygotene, however, homology is no longer required. This is manifested by the appearance of a short arm in *H4250* and a progressive reduction of the loop. By late pachytene, some loops are completely replaced by straight pairing while others are much reduced.

These observations are similar to the observations from mice, where the turnover of the central region, leading to nonhomologous synopsis, is initiated in ars and proceeds toward the center of the loop (see Figure 5 in Moses 1982). One late pachytene bivalent appears different, however; the loop is growing at the expense of the long arm. Another exception is the *OY348* zygotene bivalent which has two equally long,

paired arms, no loop and 47% of the middle portion unpaired. This can be interpreted as a heterosynapsis proceeding directly from the unpaired state without homosynapsis.

Most observations, however, indicate that synaptic adjustment in *N. crassa* is similar to that in the mouse. Timing of the process may perhaps be different. In the mouse, synaptic adjustment does not commence before mid-pachytene and is completed by late pachytene (Moses et al. 1982). However, the staging criteria are not comparable between the two organisms and the different techniques used.

Since the inverted segment in *H4250* comprises a major part of the chromosome, one would expect that synaptic adjustment would be initiated within the loop with a high probability. Such initiation would minimize the length of SC to be rearranged and the length of the resulting, heterosynapsed region. However this is apparently not the case, as "arm reduction" is the exception rather than the rule. On the contrary, formation of the short arm indicates that the amount of heterosynapsis is not a factor in regulation of the adjustment process.

Inversion loops were eliminated in two *H4250* nuclei and in one *OY348* nucleus. It is not possible to determine from present data whether all loops would be completely resolved. Complete resolution seems likely, however, as no loop remnants are detected at diplotene. The genetic length of the inversion indicates a high frequency of recombination events. Therefore, the present observations do not seem to support the proposed role of crossing over as a stabilizer of synaptic configurations (Maguire 1972; Rasmusson 1977; Rasmusson and Holm 1979). However, it could be argued that the crossover frequency in the loop is reduced as compared with the wild type, and that the 150 and 160 map unit values do not apply when estimating the actual frequency of crossing over within the heterozygous inverted region. Evidence that there is really a high frequency of crossing over in the inversion loops is of three kinds.

In *N. crassa*, spores containing a significant chromosomal deficiency are inviable and can be detected by a lack of pigmentation. A single crossover within a pericentric loop, as well as some configurations of double and triple exchanges, result in complementary duplication/deficiency products with regard to the markers outside the inversion (Perkins 1974). With *OY348* and *OY323*, both of the complementary duplication/deficiency products are inviable. With *H4250*, one product is inviable, the other is not deficient for any essential genes and makes a viable duplication product with a characteristic morphology. It is possible to determine the minimum frequency of crossovers in the loop from the frequencies of inviable spores in unordered tetrads. From this type of data,
reported for H4250 heterozygotes by Newmeyer and Taylor (1967), one can calculate a probability of 95.4% for occurrence of at least one crossover within the loop. A similar calculation for OY348, from tetrad data of Turner and Perkins (1982) gives 63% of inversions having at least one crossover.

A more detailed analysis of H4250 was possible because the viability and unusual appearance of one duplication class permitted conventional genetic mapping. Among 25 ordered tetrads from a cross heterozygous for the inversion with three markers in the inversions having at least one crossover. Among 25 ordered tetrads from a cross heterozygous for the inversion with three markers in the numbers of recombinant nodules. The 11 pachytene bivalents, but a reciprocal exchange within the inversion loop would result in lateral components of unequal length. From the present observations, it can be concluded that such exchange of lateral components does not occur.

It is a pleasure to thank David D. Perkins for his constant support during the course of this work, for providing strains and laboratory facilities. David D. Perkins, Søren Rasmussen and Dennis Zickler are thanked for critical review of this manuscript. The length measurement program was written by Bryan Mason. The work was supported by grant 11/5416 from the Danish Natural Sciences Research Council, by U.S. Public Health Service International Research Fellowship F05 TW03851, and by U.S. Public Health Service Research Grant AI 01462.

LITERATURE CITED


PERKINS, D. D., 1979 Crossing over of flanking markers is less than 50% among meiotic convertants. Genetics 91: 94.


Communicating editor: R. L. METZENBERG