Normal Synaptonemal Complex and Abnormal Recombination Nodules in Two Alleles of the Drosophila Meiotic Mutant mei-W68

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ABSTRACT

The meiotic phenotypes of two mutant alleles of the mei-W68 gene, I and L1, were studied by genetics and by serial-section electron microscopy. Despite no or reduced exchange, both mutant alleles have normal synaptonemal complex. However, neither has any early recombination nodules; instead, both exhibit high numbers of very long (up to 2 μm) structures here named “noodles.” These are hypothesized to be formed by the unchecked extension of identical but much shorter structures ephemerally seen in wild type, which may be precursors of early recombination nodules. Although the mei-W68L1 allele is identical to the mei-W68 allele in both the absence of early recombination nodules and a high frequency of noodles (i.e., it is amorphic for the noodle phenotype), it is hypomorphic in its effects on exchange and late recombination nodules. The differential effects of this allele on early and late recombination nodules are consistent with the hypothesis that Drosophila females have two separate recombination pathways—one for simple gene conversion, the other for exchange.

RECOMBINATION nodules (RN; dense structures associated with synaptonemal complex and the bivalent around it during meiosis) were originally identified morphologically by conventional thin-section electron microscopy. Two types of RN have been described. The form that is present in mid-late pachytene is now called “late RN.” The close correlation between numbers and distributions of late RNs and exchanges first noted in Drosophila females (Carpenter 1975b) has since been widely confirmed across species (Roeder 1986; Zickler and Kleckner 1998) and the hypothesis that late RNs perform roles in the exchange process itself is now widely accepted. The other form, “early RN,” first described in human males (Rasmussen and Holm 1978) and Drosophila females (Carpenter 1979a) as a pachytene structure appearing earlier than late RNs, is present in many species as early as earliest zygotene. (Many workers consider exact time of presence to be less important than the fact that there are two types of RN and they continue to call these zygotene structures early RNs; others choose to give them a new name, “meiotic nodules.” I use early RN for both the zygotene and the pachytene structures.)

The conjecture that early RNs also have a role in recombination (Rasmussen and Holm 1978; Carpenter 1979a, 1981) has recently become more plausible since they have been found to be associated with enzymes thought to be involved in that pathway (Anderson et al. 1997; Moens et al. 2001). Moreover, the conjectures that early and late RNs perform distinct roles and are not necessarily precursor product (Powers and Smithies 1986; Smithies and Powers 1986; Carpenter 1987) are consistent with the results of Moens et al. (2001).

The functions of and relationships between RN types are addressed here from a different direction, the phenotypes of mei-W68 alleles. The hypothesis that early RNs perform a recombination function, specifically simple gene conversion (gene conversion without an accompanying exchange), is supported by the observation that alleles of the Drosophila homolog of the Saccharomyces cerevisiae SPO11 double-strand-break-producing enzyme (McKim et al. 1998) and early RNs (this work). The hypothesis that late RNs arise de novo rather than from early RNs is supported by the observation that mei-W68L1 homozygous females have late RNs but not early ones (this work). Furthermore, the unusual RN-like structures present in both mutant combinations, called here “noodles” in honor of the great lengths they can attain, allow sorting the amorphic (null) aspects from the hypomorphic (leaky) for the mei-W68L1 noodle-RN phenotypes, with the conclusion that the wild-type product of this gene performs two distinct roles in recombination processes—consistent with the hypothesis that Drosophila females, at least, use separate pathways to produce the two alternative outcomes (simple gene conversion vs. exchange; Carpenter 1987). mei-W68 is the Drosophila homolog of the Saccharomyces cerevisiae SPO11 topoisomerase II meiotic double-strand-break-producing enzyme (McKim and Hayashi-Hagihara 1998). The mouse SPO11 homolog has also been proposed to perform two distinct roles during meiosis (Romanienko and Camerini-Otero...
MATERIALS AND METHODS

Genetics: Crosses were done on standard cornmeal/molasses/agar medium and at 25°C unless otherwise specified; parents were transferred on day 5 (day 0 is day of setup), discarded on day 10, and progeny were counted through day 17 of their vial.

The first mutant allele of the mei-W68 gene (mei-W68\(^a\)) was identified during the Baker and Carpenter (1972) search for X-linked meiotic mutations (B. S. Baker, personal communication); since in that experiment the treated autosomes were specifically excluded by outcrossovers, and since mei-W68\(^a\) is on the second chromosome, this mutation must have occurred spontaneously either during those outcrossovers or in one of those outcross stocks—which is entirely consistent with its lesion being the insertion of \(\sim 5 \text{ kb}\) of DNA of unknown origin into the second exon (McKim and Hayashi-Hagihara 1998).

The second allele, mei-W68\(^b\), was identified by Dan L. Lindsley in the late 1970s (D. L. Lindsley, personal communication); although he no longer remembers its origin, it is likely to have been EMS mutagenesis. Three separate mei-W68\(^b\)-bearing second chromosomes were used in the genetic analyses of Tables 1 and 2 and Figure 1; they are designated \(a\), \(b\), and \(c\). Chromosome \(b\) was marked with \(ad f p b pr c v\); the other two were otherwise wild type. The mei-W68\(^c\) electron microscopy reported here was from females homozygous for the equivalent of chromosome \(a\).

Electron microscopy: Fixation and sectioning for electron microscopy was done as reported earlier (Carpenter 1975a) in 1972–1980 and the initial reconstructions of pachytene nuclei and measurement of synaptonemal complex (SC) lengths were mostly completed by 1982, also as in Carpenter (1975a). Initially I observed an electron-dense structure adjacent to the central region in a high proportion of SC images. Only after several failed attempts at analysis did I appreciate the 3-D structure they represent. I now think I understand what is happening, and I surely would not have had this work been published earlier, so the long delay at least has a happy ending!

Lengths of synapsed chromosomal arms and of noodles were calculated as usual, that is, using the thickness of sections transversed and linear projection as the two sides of a right-angle triangle to calculate the hypotenuse by the Pythagorean formula (see Figure 3), taking segments as required whenever slope or direction changed in the reconstructions. However, estimation of the proportion of SC where noodles would be detectable (see Results) and where they were detected was done using the linear projections of arms only; this means that segments where the SC is parallel to the plane of sectioning are overrepresented in this analysis and those where the SC is perpendicular are underrepresented in these estimates only.

Brief review of relevant Drosophila oogenesis: Drosophila females have two ovaries, each containing 10–20 blind tubes called ovarioles, with an anterior gerarium and posterior vitellarium. At the anterior end of each gerarium reside 1–3 (occasionally 4) germ-line stem cells; these divide asymmetrically to regenerate an anterior-residing stem-cell daughter and produce a free daughter called a cystoblast. The cystoblast then divides four times with incomplete cytokinesis to produce a cyst of 16 interconnected cells; these divisions occur after “capping” of preexisting connections (“ring canals”), so the pattern of interconnections of the 16-cell cyst is nearly invariant—there are two cells with four connections, two with three, four with two, and eight with one. One of the cells with four connections will eventually become the determined oocyte, but the nuclei of both cells with four connections initially enter meiosis, simultaneously and nearly indistinguishably (although one always seems to be a little ahead of the other), and both remain in meiosis until the latter part of pachytene, roughly at the germarial-vitellarial transition. Older cysts move down the gerarium both by being pushed by younger ones and by peristaltic movements of surrounding muscles, so the gerarium is basically a linear tube of cysts in developmental order. However, the gerarium is wide enough for two to four early 16-cell cysts to be at the same level, and when such a logjam breaks up the first down the tube may not be the oldest; cyst position within the gerarium is therefore only a guide to relative age. Moreover, 16-cell cysts do not appear to be produced at regular intervals, so the temporal interval between adjacent cysts is variable. Nevertheless, the Drosophila system does provide a lot of natural temporal information from each (lengthwise) serially sectioned ovariole.

RESULTS

Genetic studies: Extensive experiments performed by Bruce S. Baker in the late 1970s to study the genetic effects of the mei-W68\(^a\) and mei-W68\(^b\) alleles on meiotic chromosome behavior have never been published; with his kind permission summaries of these results are now presented in Tables 1 and 2. Since these experiments and their interpretation are standard (see Baker and Carpenter 1972; Carpenter and Sandler 1974) they will be only minimally discussed here.

Crossing over is reduced in females homozygous for either of the mutant alleles and also in mei-W68\(^c\)/mei-W68\(^a\) trans-heterozygotes (Table 1); the mutations are therefore alleles, although they differ dramatically in strength. mei-W68\(^a\) lacks meiotic crossing over (see below), yet mei-W68\(^c\) shows significant levels—\(\sim 50\%\) of wild type; that this is simply because the mei-W68\(^c\) lesion is hypomorphic (leaky) for this phene is shown by the greater reduction of crossing over in mei-W68\(^c\)/mei-W68\(^a\) females. That there is variation in strength of the mei-W68\(^c\) crossover defect depending on genetic background (Tables 1 and 2) is also consistent with its defect being hypomorphic.

Reduced crossing over means that the frequency of homologs with no exchanges at all (E\(_0\) tetrads) goes up; for low levels of E\(_0\) tetrads the backup distributive system ensures regular homologous segregation, but when two or more major chromosome E\(_0\) tetrads are in the same meiosis they may segregate, via the distributive system, irregularly. Frequencies of X, fourth, and third chromosome nondisjunction are presented in Table 2. For a detailed discussion of nondisjunction in Drosophila recombination-defective mutants, see Baker and Hall (1976); chromosome segregation in these mutant combinations is as expected from the levels of reduction in exchange—we need not postulate any additional meiotic defects in them to account for it.

Both mutant alleles increase mitotic recombination in somatic tissues (Baker et al. 1978) and, at least for mei-W68\(^a\) (mei-W68\(^c\) was not tested), in the male germ
TABLE 1
Crossing over along the left arm of chromosome 2

<table>
<thead>
<tr>
<th>Maternal meiotic genotype</th>
<th>Total progeny</th>
<th>No. female parents</th>
<th>Progeny per female</th>
<th>X exceptions</th>
<th>Map distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>13,472</td>
<td>40</td>
<td>253</td>
<td>0</td>
<td>13.8</td>
</tr>
<tr>
<td>+/H11001</td>
<td>10,117</td>
<td>95</td>
<td>104</td>
<td>2</td>
<td>26.8</td>
</tr>
<tr>
<td>mei-W68^L1 (a)</td>
<td>9,912</td>
<td>106</td>
<td>121</td>
<td>3</td>
<td>4.3</td>
</tr>
<tr>
<td>mei-W68^L1 (b)</td>
<td>12,882</td>
<td>184</td>
<td>89</td>
<td>5</td>
<td>1.1</td>
</tr>
<tr>
<td>mei-W68^L1 (c)</td>
<td>16,305</td>
<td>137</td>
<td>40</td>
<td>9</td>
<td>3.5</td>
</tr>
<tr>
<td>mei-W68^L1</td>
<td>5,058</td>
<td></td>
<td></td>
<td></td>
<td>1.7</td>
</tr>
<tr>
<td>mei-W68^L1</td>
<td>13,472</td>
<td>40</td>
<td>253</td>
<td>0</td>
<td>13.8</td>
</tr>
<tr>
<td>mei-W68^L1/mei-W68^L1</td>
<td>10,117</td>
<td>95</td>
<td>104</td>
<td>2</td>
<td>26.8</td>
</tr>
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<td>mei-W68^L1/mei-W68^L1</td>
<td>9,912</td>
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</tr>
<tr>
<td>mei-W68^L1/mei-W68^L1</td>
<td>5,058</td>
<td></td>
<td></td>
<td></td>
<td>1.7</td>
</tr>
</tbody>
</table>

Numbers are sums of the progeny of single y/y; al dp b pr c px sp females by +/Y; mei-W68^L1/mei-W68^L1 males except for mei-W68^L1/mei-W68^L1, where the females were y/y; al dp b pr c/+ + + + + + crossed to homozygous +/Y; al dp b pr c px sp males. a, b, and c designate three different mei-W68^L1-bearing second chromosomes. Data are from B. S. Baker (personal communication).

line (Lutkin and Baker 1979). In females, the mei-W68^L1 allele has too much residual meiotic exchange for any premeiotic exchanges to be detectable, but they obviously do occur in mei-W68^L1/mei-W68^L1; 16 of the progeny in Table 1 came from clusters of 2, 6, and 8 crossover progeny from each of three single females. Moreover, all three of these clusters included both reciprocal crossovers, so the crossovers must have occurred during stem-cell divisions (see Materials and Methods for a brief review of oogenesis in Drosophila). There was one additional “cluster” of unrelated events: One female gave one region 1 crossover progeny and one region 4. Five of the 16 cluster crossovers were X exceptions; none of the 8 unique crossovers was. Clustering of crossovers among the progeny of mei-W68^L1 females was also reported by McKim et al. (1998). Clusters are considered to represent one event in calculations.

An additional experiment with mei-W68^L1 involved the whole second chromosome (as y/y; al dp b pr c mei-W68^L1/px sp females crossed singly to +/Y; al dp b pr c px sp homozigous males) at 18°, 25°, and 29°; mei-W68^L1/+ at 29°; and controls at 18° and 29° (y/+; al dp b pr c px sp/+ + + + + + by +/Y; al dp b pr c px sp homoygotes).

As before, some of the crossovers from mei-W68^L1/mei-W68^L1 occurred premeiotically and present as identical clusters; again, each cluster is counted as only one event. There were no dramatic effects of temperature in mei-W68^L1/mei-W68^L1 so these results have been summed across temperatures for Figure 1, which plots the relative frequency of crossing over per unit physical for the various intervals (taken as the number of euchromatic salivary chromosome bands on the revised maps of Bridges between the markers used) with 95% Poisson confidence intervals (from tables of Crow and Gardner 1959). The few crossovers recovered from mei-W68^L1/mei-W68^L1 females do seem to be uniformly distributed along the euchromatin, as expected if they all derive from events during mitosis. However, unlike somatic crossovers induced with X rays, the data suggest that events one region 1 crossover progeny and one region 4. Five of the 16 cluster crossovers were X exceptions; none of the 8 unique crossovers was. Clustering of crossovers among the progeny of mei-W68^L1 females was also reported by McKim et al. (1998). Clusters are considered to represent one event in calculations.

Recombination data from the other genotypes have also been plotted as per unit physical in Figure 1. The control data from Table 1 (2L and centric region only) show the expected pattern of distal high, proximal low, minimum around the centromere; this is a graphic demonstration of the centromere effect. Both mei-W68^L1/mei-W68^L1 genotypes not only reduce the frequency of distal events but also flatten the curve relative to wild type and, although the frequency of events differs by a factor
of two between the two mei-W681 genotypes, the distributions of those events along the arm are similar: 2 × 4 contingency χ² of numbers of crossovers observed in the four regions in the two genotypes = 8.5 (3 d.f.): 0.05 > P > 0.01. In mei-W682/mei-W68 the curve is very nearly flat. This curve flattening is diagnostic of mutations defective in preconditions for exchange (events necessary before the actual initiation of the exchange process)—mutations defective in exchange itself have the same curve shape as wild type (Carpenter and Sandler 1974). The same broad trends are seen for both left and right arms in the whole-chromosome 18° and 29° control data; the effects of temperature on distribution of crossovers also present as effects on preconditions for exchange (Lifschytz 1975).

Both the mutant alleles mei-W681 and mei-W68 are recessive to wild type when nondisjunction frequencies are examined (Table 2), but both have dominant effects on crossing over. The effect for mei-W681 (Table 1 data) is slight but significant; 2 × 4 contingency χ² of control and mei-W681/+ crossovers by region (3 d.f.) gives 15.7, P < 0.01. The dominant effect of mei-W681 is striking. As can be seen in Figure 1, the map distances in proximal regions are considerably higher than those in the 29° control and less so or not at all in distal regions (2 × 6 contingency χ² = 12.1, 5 d.f.; 0.05 > P > 0.01). Again, the overall effect is a flattening of the curve, and this indicates that the dominant effect of mei-W68 on exchange is a precondition defect. Strong mutant alleles of other genes necessary for normal meiotic recombination also show dominant effects on crossing over (Hall 1972; Carpenter and Sandler 1974): The precondition-defective mei-218 gives an increase that is greatest around the centromere, exchange-defective mei-9 gives a decrease with the same distribution along the arm as wild type, and c(3)G1 also gives an increase greatest around the centromere (Hinton 1966; Hall 1972) and therefore was presumed also to be a precondition defect; the residual exchange in a partially functional construct is polar (Page and Hawley 2001) so this presumption is correct. mei-P26 heterozygotes present a polar decrease (Sekelsky et al. 1999). Both the recessive phenotype of the hypomorphic mei-W681 allele and the dominant effect of the amorphic mei-W68 allele strongly suggest that the crossover function of mei-W68 is a precondition, a function that is involved in establishing sites where exchange events can occur rather than a function involved exclusively in the production of those exchange events themselves.

**Cytological studies: Dynamics of meiotic progression:**
Four germaria from homozygous mei-W68 females, two from mei-W68, and one from mei-W681/mei-W68 were...
Genetics and Cytology of *mei-W68*

**Figure 1.**—Crossing over along chromosome 2 in various *mei-W68* genotypes. The abscissa is the genetic interval in number of salivary bands between markers; the ordinate is map distance observed divided by the number of bands for that interval. Top, controls, heterozygotes, and *mei-W68* homozygotes; curve A (crosses) is *mei-W68/1*/*H11001*, B is 18°/H11034 control, C is 29° control, D is *mei-W68/1* (a)/+ (solid circles), E is control from Table 1, F is *mei-W68/1* (b)/*mei-W68/1* (c), G is *mei-W68/1* (a)/*mei-W68/1* (b), and H is *mei-W68/1* (b)/*mei-W68/1*. Bottom, *mei-W68* homozygotes (curve I) with 95% confidence intervals. Numbers of progeny for crosses not in Table 1 are: /H11001/H11001 29/H11034, 284; /H11001/H11001 18/H11034, 576; *mei-W68*/H11001, 293; summed *mei-W68*/*mei-W68*, 5480, including four clusters of crossovers.

serially sectioned and low-magnification micrographs were taken every sixth to tenth section so that cysts and pro-oocytes could be identified by their ring-canal connections; the data for numbers of cysts of the various sizes are presented in Table 3 and for meiotic stages of 16-cell cysts in Table 4. Although there is a good deal of variability between germaria (as in wild type), the average numbers of cysts of the various sizes are well within the ranges seen in wild type; *mei-W68* mutations have no effect on the dynamics of germ-line mitotic divisions (Table 3).

They also have no effect on the dynamics of entry into meiosis or exit of the losing pro-oocyte (Table 4): In particular, neither the frequency of prezygotene nor of zygotene cysts is higher than that of controls—establishing complete SC formation is not delayed even in homozygous *mei-W68* females (*contra* HABER 1998). (Staging here is based solely on the low-magnification photographs, as were the controls; one cyst—A173 IV—was judged to be in pachytene from the low-magnification photographs but turned out to be in midzygotene once the high-magnification photographs were examined.) The one aspect in which these mutants may differ from wild type and other meiotic mutants is in the frequency of necrotic 16-cell cysts: 5/52 = 9.6% of cysts are dying in these seven germaria, whereas 9/189 = 4.8% were observed previously (Carpenter 1979a, b).

**Synaptonemal complex and recombination nodules:** All sections of 14 *mei-W68*/*mei-W68* nuclei were photographed at higher magnification (all of germarium A312 plus a few others) as well as all sections of 14 *mei-W68/1*/mei-W68/1* nuclei; synaptonemal complex (SC) was reconstructed by tracing its profiles from each photograph onto acetate sheets. Registration of the 40–50 sections per nucleus used all available information (SC, nuclear envelope, ring canals, and other cytoplasmic structures as needed; see Carpenter 1979a, Figure 13, for examples of how much information is available) but always sought the minimal SC length. Lengths and other measurements were then calculated as previously described (Carpenter 1975a). Euchromatic lengths of arms and other observations are in Tables 5 and 6. SC appears to be of normal morphology (Figure 2 for *mei-W68*; see Carpenter 1975a); and as in wild type, as *mei-W68* from wild type and other meiotic mutants is in the frequency of necrotic 16-cell cysts: 5/52 = 9.6% of cysts are dying in these seven germaria, whereas 9/189 = 4.8% were observed previously (Carpenter 1979a, b).

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TABLE 3
Germarium characteristics for *mei-W68* homozygous germaria

<table>
<thead>
<tr>
<th>Germarium</th>
<th>Stem</th>
<th>Stem-cb</th>
<th>cb</th>
<th>2 cells</th>
<th>4 cells</th>
<th>8 cells</th>
<th>16 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>mei-W68</em>/<em>mei-W68</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A312</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>10 + 1</td>
</tr>
<tr>
<td>A282</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>A108</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>6 + 1</td>
</tr>
<tr>
<td>A99</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>4 + 1 telo</td>
<td>0</td>
<td>2</td>
<td>1 + 1</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>4</td>
<td>8</td>
<td>10</td>
<td>2</td>
<td>5</td>
<td>29 + 3</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.7 ± 0.5</td>
<td>1.2 ± 1.0</td>
<td>2.0 ± 2.0</td>
<td>2.5 ± 2.1</td>
<td>0.5 ± 0.6</td>
<td>1.2 ± 1.0</td>
<td>7.2 ± 4.9</td>
</tr>
<tr>
<td><em>mei-W68</em>/<em>mei-W68</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A173</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3 + 1 ana</td>
<td>0</td>
<td>6 + 1</td>
</tr>
<tr>
<td>A170</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>6 + 1</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>18 + 2</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.0 ± 0</td>
<td>1.3 ± 1.2</td>
<td>1.0 ± 1.0</td>
<td>1.0 ± 1.0</td>
<td>2.3 ± 1.5</td>
<td>0</td>
<td>6.0 ± 0</td>
</tr>
</tbody>
</table>

| *mei-W68L1/mei-W68* | | | | | | | |
| A173      | 1    | 2       | 1   | 0       | 3 + 1 ana | 0       | 6 + 1    |
| A170      | 1    | 2       | 0   | 2       | 1       | 0       | 6 + 1    |
| Total     | 3    | 4       | 3   | 2       | 0       | 7       | 18 + 2   |
| Mean ± SD | 1.0 ± 0 | 1.3 ± 1.2 | 1.0 ± 1.0 | 1.0 ± 1.0 | 2.3 ± 1.5 | 0       | 6.0 ± 0  |

*Data are from Carpenter (1981).*

*Dead or dying.*

change-type recombination events are being attempted; there are no late RNs in *mei-W68*/*mei-W68* nuclei. At least 6 and possibly 10 nuclei are within the developmental range for late RN presence in wild type, to wit between the onset of cytoplasmic flow and oocyte determination (inclusive); 20–34 late RNs would have been predicted (Carpenter 1979a); none were observed. The role of early RNs is still unclear but, if they also function in recombination, then they must be at sites that resolve only as simple gene conversions (gene conversion without an accompanying exchange) or restorations without exchange (Carpenter 1987). Early RNs are smaller than late ones and consequently slightly harder to see, but in the 5 (4 from A312, 1 from A282) *mei-W68* nuclei at the right developmental stage (first cyst showing cytoplasmic flow plus next younger cyst) there was at most one thing that could possibly be an early RN (see Figure 6A; 14 expected, Carpenter 1979b). Like other precondition recombination-defective mutations that have been examined (Carpenter 1979b), *mei-W68* reduces meiotic exchange events and late RNs to the same extent (*i.e.*, to zero); unlike them, however, it also abolishes both simple gene conversion (McKim et al. 1998) and early RNs—providing the first clear link between the presence of early RNs and simple gene conversion.

Late RNs are present in *mei-W68*/mi:*mei-W68* nuclei (Figure 2) and are discussed below; only one possible early RN was found (see Figure 6B; again, 14 would have been expected in the five nuclei of the right developmental age). Unfortunately *mei-W68L1*’s effect on simple gene conversion is not yet known.

Noodle detection: However, both *mei-W68* alleles have a novel structure, which I call “noodle,” that resembles RNs in being adjacent to the central region of the SC (Figures 3 and 4). Noodles are smaller than RNs, being on average only 20 nm in diameter [*early RNs are 35 nm (Carpenter 1979a), where what are now called early RNs were called “ellipsoidal”*]. They are also less dense as measured by the degree to which the various stains react with their components: Even when the SC and the noodle are perpendicular to the plane of sectioning (cross section, Figure 4) so that 90 nm of noodle length is imaged onto the same part of the photograph, the structure still appears less dense than that of an equivalent early RN—in fact, it is closer to the density of chromatin (Figures 3 and 4). They also exhibit morphological variability (Figure 4).

The above three attributes hamper the analysis of noodles. First, like the lateral elements of the SC (Carpenter 1975a), they are not detectable in all planes of section, presumably because they do not make enough of an image to be seen against background unless they reinforce themselves through at least part of the section. This is obvious in slanting frontal series, where the SC is continuous through the series but the image of the
noodle is not—it becomes undetectable around the join between sections, in the region where it would be split between the two sections (Figure 3). Second, no noodles were ever observed when the SC was sectioned sagittally (sideways to Figure 2C, see Carpenter 1975a), either in the section with the SC or in the two flanking sections. This cannot be chance; although some noodle images might be rendered undetectable because they are in a section that also contains chromatin or even hidden under the image of the central element of the SC, most sagitally sectioned noodles must be undetectable from lack of reinforcement of the image. There are many examples where the noodle appears to end just at the point where the SC rotates into what is sagittal from the plane of sectioning. A similar problem occurs when the SC is caught in very accurate frontal section at the bottom/top of a loop; there are several examples of clear noodles before and after the (slanting frontal) loop that disappear right at its apex. The most convincing example of this (Figure 4D) is a case where there are noodles along both sides of a stretch of SC that is in cross section in the middle of this example, with both noodles visible; then as the SC bends into slanting frontal section in each direction both noodles disappear—at the same angle of SC bending even though this occurs in different sections on its two sides. One of the noodles then reappears in both directions as the SC bends back into cross section. Serial section electron microscopy (EM) alone is therefore insufficient to measure either the total number of noodles per nucleus or their maximum length (see below), although it is quite sufficient to establish minimum estimates of both. Third, noodles are sufficiently nondescript in appearance that they often cannot be unambiguously identified if they are short (less than two sections if in cross section, less than ~100 nm in slanting frontal); again, this means that their numbers are underestimated. Nevertheless, quite a lot of information about them can be extracted from the data.

Because noodles are hard to see, one needs a special tactic for finding and charting them. For early and late RNs, which are relatively conspicuous, short, dense bodies, examining all the SC profiles in each nucleus photograph by photograph detects them all; for these new structures, one must follow each arm individually as it meanders up, down, and around the nucleus (and the photographs), finding a possible noodle image in one photograph and then examining the continuation of that arm in the adjacent ones. In some cross-sectional series individual photographs have chromatin sufficiently around the top/bottom of the SC so that a noodle could not have been distinguished from it (see Figure 3F); as long as a noodle was present on both sides of this obfuscation it was counted as continuous. Many of the longer structures pass through a region of SC where they would be invisible from the plane of sectioning (see above); these too are considered to be continuous.
but carry the flag of being “gapped” and for most of these the gaps are relatively short. Figure 5 shows the tracings of the arms of a typical nucleus. The two gapped noodles of the bottom arm were each considered to be continuous, whereas the top right arm was scored as having three noodles. In addition, each noodle had recorded whether both ends were in regions where continuation could have been seen had it occurred (“unbounded” structures whose total length is certain) vs. one or both ends disappearing into regions where continuation could not be seen (“bounded” structures whose real total length may be greater than that measured). Finally, along each arm of the SC it was determined whether the plane of sectioning was such that a noodle could have been seen if one was present—over all nuclei, this averages to 50%. The data for lengths are presented in Figure 6 and Table 7 (lengths were measured and calculated on the three-dimensional reconstructions, not the two-dimensional projections). With but one exception, only structures that are clearly real noodles are included in Figure 6 and Table 7; additional images that for one reason or another are dubious have been excluded. However, since those are all short, even if some of them are real their absence has little effect on the following.

Noodle position and length: It is obvious from Figures 3–5 that a noodle can be along each of the two faces of a particular stretch of SC; when this occurs there is

---

**TABLE 5**

Lengths of arms of synaptonemal complex in *mei-W68/mei-W68* females in micrometers

<table>
<thead>
<tr>
<th>Cyst/cell</th>
<th>X</th>
<th>Bl</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>4</th>
<th>T</th>
<th>No. CC</th>
<th>cf?</th>
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<tr>
<td>A312 IIIc,d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Va</td>
<td>17.2</td>
<td>12.7</td>
<td>17.0</td>
<td>16.9</td>
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<td>1.2</td>
<td>78.9</td>
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<td>13.7</td>
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<td>1</td>
<td>−</td>
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<tr>
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<td>15.7</td>
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<td>61.7</td>
<td>2</td>
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<tr>
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<td>8.5</td>
<td>12.3</td>
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<td>10.6</td>
<td>0.3</td>
<td>54.5</td>
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<tr>
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<td>7.9</td>
<td>11.7</td>
<td>9.4</td>
<td>9.3</td>
<td>0.4</td>
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</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vi</td>
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<td>12.8</td>
<td>16.7</td>
<td>16.2</td>
<td>12.8</td>
<td>0.5</td>
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<td>1</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A99 Iia</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>i</td>
<td>7.3</td>
<td>6.2</td>
<td>8.5</td>
<td>8.0</td>
<td>7.1</td>
<td>0.3</td>
<td>37.1</td>
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<td>Ex</td>
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<tr>
<td>First vit</td>
<td>11.1</td>
<td>Unreconstructable fragments</td>
<td>2</td>
<td>+++</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

X is measured to NO, Blob arm to Blob, the rest to heterochromatin. No. CC, number of chromocenters; cf?, is cytoplasmic flow occurring, and if so how strong?

Small subtelomeric gaps in synaptonemal complex continuity of the X with an average of 0.8 ± 0.2 μm of SC followed by an average gap of 0.6 ± 0.2 μm; wild-type nuclei also show this subtelomeric gap, with an average of 0.7 μm of SC and a gap of 0.8 μm. This subtelomeric gap in *mei-W68* is no different in location or extent from wild type although it may be more frequent; in wild-type nuclei there were 7/36 X chromosomes with this gap = 0.2 ± 0.4; for *mei-W68* there were 5/12 = 0.4 ± 0.5. Wild type had 8 autosomal gaps out of 138 synapsed arms = 0.06; in *mei-W68* there were 4/48 = 0.08 and even regions where substructure may be less than perfect are rare (5 regions, 3.2 μm total, out of 682.1 μm). There were no instances of complete or nearly complete synaptic failure, whereas in wild type there were 5 failed autosomal arms/140 = 0.04 (predicts 2 in *mei-W68*, none observed).

If gap, SC thin and twisty, 0.3 μm at most.

SC branches just before the nucleolus; one branch clearly enters the nucleolus, the other may not.

Distal bulge of disorganized SC and proximal gap of at least 0.6 μm.

Subterminal gap or disorganized SC of 0.9 μm.

SC throughout arm unusually thin; this may be a case of partial synaptic failure.

The A282 sections proved to be too dirty for high-resolution work and photography was abandoned after

See footnote a, Table 7.

Includes gap of 0.4 μm.
no obvious relationship between either the ends of the two noodles or their apparent midpoints. Moreover, the “overlap” of such pairs of noodles is no more common than expected by chance, taking into account for each arm the length (in millimeters on the two-dimensional projections) where a noodle could have been seen if present (times two for the two faces of SC) and the total length (in millimeters on the two-dimensional projections) of noodles seen on that arm. For nuclei with only a few short noodles little overlap is expected and little or none is seen; for nuclei with lots of long noodles more overlap is expected and again that is what is seen. Summing over all 10 nuclei from the A312 germarium, 198.5 mm of overlapping noodles are expected; 169.5 mm were observed. Taken together, these two observations strongly suggest that there are no obligate or even preferred regions along the arms where noodle formation initiates.

From the data in Figure 6 and Table 7 it is clear that at least some noodles lengthen; both the mean and maximum lengths increase with increasing developmental age of the pachytene nuclei. Moreover, in both genotypes new noodles are initiated through at least part of the time course assayed; the number detected increases at least up to the time cytoplasmic flow begins (time of presence of late RNs in wild type) and, up to that point, noodle numbers and lengths in mei-68 and mei-68i are very similar. In mei-68, noodles persist post-cytoplasmic flow and probably continue to elongate but, as is discussed below, in mei-68i significant numbers disappear once cytoplasmic flow begins. Later pachytene (definitive oocyte determined) nuclei still have noodles, although their numbers may be decreasing. There is no information here on when (or even whether) noodles disappear in mei-68i; all of the older but pre-karyosome nuclei photographed still have them. No
occasional structure that looks a bit thicker and denser than that of a typical noodle (see Figure 4C, middle); almost all of these are continuous with bona fide noodles (either at one end or internally) and these possible intermediates are more frequent in mei-W68^2 (9/132) than in mei-W68 (1/154; Figure 6, a and b). However, they are found throughout the age range monitored rather than being restricted to the earlier nuclei as in wild type, which is consistent with them being either chance spurious images or the cell’s continuing futile attempt to “mature” a noodle into an early RN. A total of 28 early RNs would have been expected in the 5 mei-W68 and 5 mei-W68^2 nuclei of the right developmental age. No late RNs at all were observed in the 6–10 mei-W68 nuclei within the late RN period of wild type; 20–34 would have been expected.

mei-W68^2, on the other hand, does have late RNs, which first appear at the same time as late RNs in wild type (the youngest cyst with cytoplasmic flow, Table 6). Together, the three mei-W68^2 nuclei of the right age have a total of nine late-RN-type objects, but only three of these are “normal” late RNs (Figure 2, A and B); of the others, two have normal morphology but are in the wrong place—off the side of the SC, between sister chromatids rather than between homologs (Figure 7C); one is between homologs but of irregular shape, two are between homologs but are small, and the last one is of normal morphology but the SC is so twisty that its location is ambiguous. Late RNs of abnormal morphology have been observed in other meiotic mutants (Carpenter 1975b) but I have never observed a late RN between sister chromatids rather than between homologs (Figure 7C); one is between homologs but of irregular shape, two are between homologs but are small, and the last one is of normal morphology but the SC is so twisty that its location is ambiguous. Late RNs of abnormal morphology have been observed in other meiotic mutants (Carpenter 1975b) but I have never observed a late RN between sister chromatids rather than between homologs (Figure 7C).

Figure 2.—Synaptonemal complex and a normal late recombination nodule from mei-W68 homozygotes. (A and B) Two consecutive sections through a frontal segment of SC from A173 cyst VI cell d, showing normal substructure of synaptonemal complex and a normal late recombination nodule (arrows). (C) Diagram of the SC and RN as they were sectioned; A and B show these sections as viewed from the top. Magnification ×63,600; bar (in B), 100 nm.

karyosome nuclei were photographed but noodles would not be distinguishable from the condensed chromatin of that stage anyway.

Noodles in wild type: Long noodles have not been observed at any stage of pachytene in wild type. To look for short noodles, two wild-type germaria were chosen for careful analysis (A12 and A139, Carpenter 1979a) because they had pre-early RN pachytene cysts; all arms of all pachytene nuclei of both germaria were examined as discussed above for possible noodles. Although as expected most nuclei did have individual images that might be possible noodles, four nuclei really do have some short ones (Figure 7, A and B). They are A139 cyst II cell a (1) and IV i (4); and A12 III a (4) and III f (6). These are all pre-early RN cysts. See discussion for possible relationships between noodles and RNs, but noodle presence is not due to the mei-W68 defect; only noodle persistence and the phenomenal lengths that made them detectable in the first place are.

Early and late RNs: Neither mei-W68 nor mei-W68^2 has any unambiguous early RNs, although both have the

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Figure 3.—A long noodle from \textit{mei-W68/\textit{mei-W68} (A312 cyst VIII cell b).}

The first column presents the SC as it was reconstructed within the whole nucleus; this is a slanting frontal series. The indicated noodle first appears in B (arrow), continues through C–E (arrows), is obscured by chromatin in F (arrowhead), and is completed in G. The second column presents just the SC + noodle; the SC is emphasized with ink and the noodle is circled (dashed circle where the noodle cannot be distinguished in F). The third column is a diagram of this segment of SC with its noodle, as sectioned; each section is viewed from its top in the photographs of the first and second columns and the sections are rotated $90^\circ$. Section thickness is slightly exaggerated to fit the figure; in reality sections are slightly thinner than the width of SC. The indicated noodle is 1120 nm (1.1 $\mu$m) long; $6 \times 0.09$ $\mu$m (section thickness) = 0.54 $\mu$m, length of the figure is 22 mm/22,400 (magnification) = 0.98 $\mu$m. Square root of $[(0.54)^2 + (0.98)^2] = 1.12 \mu$m. A second noodle (not indicated) is along the other side of the central element in A–D. Magnification $\times22,400$; bar (in H), 120 nm.

**Noodle distribution:** Addressing noodle distributions along the arms is complicated by two things: the fact that the plane of sectioning is such that noodles are undetectable $\sim50\%$ of the time and the fact that noodles, especially in older nuclei, are not points. For the first, assume that “wrong plane of sectioning” is random along the length of each arm; this is not quite true (see below about tips of SC), but it nearly is, so the noodles detected can to a first approximation be considered to be a random sample of the total. For the second, the only solution is to plot the whole length of each noodle. Although it is clear that noodles get longer with time, we do not know whether they extend from both ends vs. unidirectionally, so taking either the apparent middle or one or the other end is making an unwarranted assumption; moreover, especially for the longer ones, noodles often disappear into a region of wrong plane of sectioning so it is unclear for those where its middle is. So the extent of each of the \textit{bona fide} noodles was marked on the three-dimensional reconstructions, and their extents and locations along the arms were calculated. Since the arms shorten with increasing developmental age, each arm was then normalized to 100\% of the distance between the telomere and entry into heterochromatin for the autosomal arms A$_2$, A$_3$, and A$_4$, which were sorted by relative length. This is reasonably reliable: One \textit{mei-W68} and six \textit{mei-W68L} nuclei had split chromocenters that separated the major autosomal arms and in all seven cases the other arm of the blob arm was A$_4$ (the shortest of the three arms that lack a landmark), which is reassuring in terms of reliability of arm identification by length and also permits tentative arm assignment. If the blob represents an elaboration of the clustered histone loci, which are close to the euchromatin/heterochromatin boundary on 2L, then A$_2$ is 2R and A$_3$ and A$_4$ are the arms of chromosome 3 with A$_3$ probably being 3R on the basis of relative euchromatic lengths.

The data for A$_2$, A$_3$, and A$_4$ are presented in Figure 8 in two ways: The outlines represent the sums of coverage, with each noodle being counted as present in each 1\% it extends over, and the symbols represent the apparent midpoints of individual noodles. Each arm is presented separately and the noodles from \textit{mei-W68} and \textit{mei-W68L} have different symbols. In addition, the nuclei...
Figure 4.—Three examples of noodle segments, illustrating the variety of presenting morphologies, all from mei-W68\(^{1}\) homozygotes (A173 cyst VI cell d; magnification, \(\times 44,600\); bar, 100 nm. (A) Four consecutive cross sections through SC and a long noodle (arrows); this series is from the top bend in D. (B) Three consecutive cross sections through SC with a long noodle along each side (arrows) and a diagram of the lowest section below it; this series is from the middle bend in D. (C) Three consecutive cross sections of SC and long noodle (arrows) and a diagram of the lowest section below it; the noodle image in the central section has an early-RN-like appearance. (D) Diagram of the two long noodles mentioned in text. Stippled bar, central element seen from its side; lines, noodles as detected. This series was sectioned perpendicularly to the diagram, in cross and slanting frontal section; the noodles could not be detected in the slanting frontal parts. Note that both noodles became undetectable at the same SC angles even though those photographs were of different sections.

are sorted into two age groups: those from cysts before the onset of cytoplasmic flow (pre-cf) and those after (post-cf). This time point corresponds to the initial appearance of late RNs in wild type. More pre-cf mei-W68\(^{1}\) nuclei than mei-W68\(^{4}\); in both cases, however, the average numbers of noodles per nucleus (or per arm, see below) are quite similar (Table 7).

If each arm has its own particular noodle pattern, then to a first approximation the distributions for each arm of mei-W68\(^{1}\) and mei-W68\(^{4}\) noodles would be expected to be the same and so would distributions pre-cf and post-cf. Departures from either of these expectations would be interesting (for example, noodles initiated after cf might be distributed differently from those initiated before), but such departures are expected to be arm independent. To facilitate comparisons the numbers of noodles were summed by midpoint per one-tenth arm (to reduce granularity) and plotted pre-cf vs. post-cf and mei-W68\(^{1}\) vs. mei-W68\(^{4}\). None of those distributions differed significantly—\(2 \times 10\) contingency \(\chi^2\) tests (9 d.f.) give \(P\) values of 0.1 and up for each, meaning that for all arms pre-cf and post-cf can be considered to have been drawn from the same population—likewise for mei-W68\(^{4}\) vs. mei-W68\(^{1}\). One can therefore use the summed data per arm to ask whether the numbers of noodles per tenth is random (uniform): \(\chi^2\) (9 d.f.) for the \(X = 7.4\) (\(P > 0.5\)), for \(A_2 = 11.4\) (\(P > 0.2\)), and for \(A_3 = 16.8\) (\(P > 0.05\)); the blob is marginally significantly nonrandom at 20.0 (0.05 \(> P > 0.01\)) and \(A_4\) is highly significant at 24.0 (\(P < 0.01\)). For both the blob arm and for \(A_4\) half the contribution to \(\chi^2\) comes from just one interval, 0.1–0.2.

The four autosomal arms can be considered to be different samples drawn from the same population (\(4 \times 10\) contingency \(\chi^2\) still using midpoints of noodles summed over tenths = 41.3, 27 d.f.; this gives a \(P\) value only very slightly <0.05); the total autosomal sample of 219 noodles is plotted in Figure 9a. This distribution is

Figure 5.—Tracings of SC (solid lines) and long noodles (side lines) from mei-W68\(^{1}\) A312 VIII b. Dashed lines indicate entry into heterochromatin; cross-hatching indicates SC whose plane of sectioning was such that noodles would not be detectable. NO, nucleolus; Bl, blob.
not uniform—\( \chi^2 \) (9 d.f.) = 34.2, \( P \ll 0.01 \), with the subterminal interval (0.1–0.2) and the three most proximal intervals (0.7–1.0) contributing 29 of the 34. However, all of the nonuniformity derives from the post-cf sample [Figure 9b; here, the numbers of pre-cf noodles per one-tenth arm have been adjusted down to the same number of arms (30) present in the post-cf sample to permit direct visual comparison (118 noodles across 52 arms reduced by a factor of 30/52) vs. post-cf (103 noodles across 30 arms)]. The pre-cf distribution is uniform; the post-cf distribution is highly significantly nonrandom, \( \chi^2 \) (9 d.f.) = 38.8, \( P \ll 0.01 \).

It was noted earlier that noodles appear to continue to be initiated and then elongate throughout at least the pre-cf segment of pachytene, so it is of interest to ask whether those initiated later have the same arm-length distribution as those initiated earlier. However, those initiating later will be doing so against a statistical

**Figure 6.**—Lengths of noodles in nanometers. x, unbounded (both ends of that noodle are known); o, bounded (one or both ends of that noodle disappear into a region where it would be undetectable from plane of sectioning); g, gapped (continuity through a short obfuscated region is assumed); E, might possibly be an early RN (see text); square, part of the noodle is early RN-like. Note breaks in abscissae. (a) mei-W68 A312; (b) mei-W68\( ^{\text{n}} \) A170 and A173.
### TABLE 7

Numbers of noodles per arm, per micrometer SC length, and lengths in nanometers

<table>
<thead>
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<th>Cyst</th>
<th>Noodles/arm</th>
<th>Avg/Mm SC</th>
<th>Length</th>
<th>Cyst</th>
<th>Noodles/arm</th>
<th>Avg/Mm SC</th>
<th>Length</th>
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<th>Noodles/arm</th>
<th>Avg/Mm SC</th>
<th>Length</th>
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<td>&lt;0.8</td>
<td>239</td>
<td>90–451</td>
<td></td>
<td></td>
<td>Vb</td>
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<td>1.6</td>
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<td>Vd</td>
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<td>9</td>
<td>1.8</td>
<td>230</td>
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<td>Vi</td>
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<tr>
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<td>13</td>
<td>2.6</td>
<td>208</td>
<td>76–461</td>
<td></td>
<td></td>
<td>IIIi</td>
<td>3, 2, 6, 0, 2, 0</td>
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<td>IVc</td>
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<td>3.2</td>
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<td>90–696</td>
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<td></td>
<td>IIIe</td>
<td>3, 3, 4, 1, 5, 0</td>
<td>16</td>
<td>3.2</td>
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<td>(VI dead)</td>
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<td>Vle</td>
<td>2, 3, 6, 1, 1</td>
<td>13+</td>
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**Onset of cytoplasmic flow**

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<th>Avg/Mm SC</th>
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<th>Noodles/arm</th>
<th>Avg/Mm SC</th>
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<td>516</td>
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<td>3.8</td>
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<td>15</td>
<td>3.0</td>
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<td>2.6+</td>
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<td>Xb</td>
<td>4, 5, 0, 5, 3</td>
<td>15+</td>
<td>3.0+</td>
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</table>

Other nuclei photographed: A312 IIIc and d, early to midzygotene, many short fragments of SC (c > d), no unambiguous noodles and no RNs; A282 Ii (zygotene), one possible noodle, no RNs; Vi (pachytene), lots of shortish noodles, no RNs; Xa, lots of long noodles, no RNs; A99 Ia and i, both have noodles but no RNs; first vit also still has noodles but probably fewer, no RNs; A173 Ii and c (zygotene), no unambiguous noodles, no RNs. g, gapped (see text).

* X, Bl, A5, A6, A8, 4 (if it had euchromatic SC).
* Plus a late RN between sister chromatids.
* Plus an irregular late RN between homologs.
* Plus a small late RN between homologs.
* Plus an apparently normal late RN between homologs.
* Plus a late RN whose location is ambiguous.
* All of the photographs of this nucleus are dirty, making noodle detection chancy. All noodles detected are long. They have not been included in other summaries.
all the “new” post-cf noodles initiated in the 10–40% interval, then the average length of noodles there is predicted to be significantly shorter than elsewhere (and this should be detectable, since 31/55 noodles are predicted to be new since the initiation of cf); however, as can be seen in Figure 9c, the size distribution of noodles in the 10–40% cf length interval is exactly the same as that on the other 70% of the arms, very strongly negating the hypothesis that later-initiating noodles are preferentially in either of these arm segments. An alternate hypothesis that accounts for this (nonsignificant) difference between pre-cf and post-cf noodle distributions is that their numbers are more representative of chromatin length than of SC length; SC shortening, which is continuing throughout the portion of pachytene surveyed here, is accompanied by thickening of the SC and its associated chromatin, but this thickening is much more pronounced in the more distal regions—consequently, noodles that have initiated earlier in the longer region that will become the 10–40% interval in the later nuclei will be accumulated there (see also Discussion).

Last, the apparent exception to uniform distribution of noodles, the very distalmost regions, may be real but two kinds of artifact may be affecting the data. One is that longer noodles are more likely to be detectable that longer region that will become the 10–40% interval in the later nuclei will be accumulated there (see also Discussion).

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**Figure 7.**—Other examples. (A and B) The two consecutive cross sections through a short noodle from wild type (from A12 cyst III; Carpenter 1975a), with tracings below; (C) a late RN between sister chromatids (from A173 mei-W68L1 cyst c) in cross-sectional view. The late RN is indicated by a single arrow; where it should be (to be between homologs) is indicated by arrowheads. Bar (in A), 117 nm.

**Figure 8.**—Patterns of noodle presence along autosomes A3, A4, and A6. Telomeres are at 0, entry into heterochromatin is at 100. For each arm, midpoints of noodles are given above and total coverage below. Solid triangles and solid bars, mei-W68 pre-cytoplasmic flow; open circles and open bars, mei-W68 post-cytoplasmic flow; solid circles and stippled bars, mei-W68 post-cf.
may just be chance, but regardless it means that even if noodles were present I could not detect them (see above).

DISCUSSION

Having two mutant alleles of the mei-W68 gene, of different origins and of different strengths, is very useful in unraveling the various aspects of the wild-type allele's function. The original allele, mei-W68, was spontaneous and is associated molecularly with the insertion of ~5 kb of DNA of unknown origin into its second exon (McKim and Hayashi-Hagihara 1998); this should kill the protein and indeed, at least in terms of its effect on exchange, the mei-W68 allele does appear to be amorphic (null). The second allele, mei-W681, was probably chemically induced and in most aspects of its phenotype (somatic exchange, Baker et al. 1978; meiotic exchange and presence of late recombination nodules, this study) it behaves as a hypomorph (leaky). However, in its effects on early recombination nodules and noodles it behaves as an amorph—at least in these phenes it is indistinguishable quantitatively and qualitatively from the amorphic allele mei-W68. This complexity at the genetic level implies that the wild-type protein performs several functions, some of which are more drastically affected than others by the lesion(s) in mei-W681.

The wild-type allele has been cloned and sequenced (McKim and Hayashi-Hagihara 1998); it has homology to the same archebacterial topoisomerase II that the S. cerevisiae SPO11 gene does. However, the phenotypes of amorphic alleles in the two species differ. In the first place, the yeast spo11 amorphic condition lacks SC (Roeder 1997); in mei-W68 mutant flies abundant SC not only is formed but also appears to be normal in all respects—time of initiation, between homologs, length, and length changes, etc. In the second place, the yeast spo11 protein is involved directly in the exchange process; in its absence neither recombinants nor double-strand breaks are produced. Although in flies meiotic (though not mitotic) recombination does depend absolutely on the mei-W68 protein, the genetic evidence indicates that its role is in a precondition for exchange (see RESULTS), that is, a function involved in determining the numbers and locations of exchange events rather than being exclusively part of the recombination enzymology. Although there is no a priori reason to deny that functions involved in permitting the precondition process to function normally might also be involved in DNA metabolism and therefore also function directly in exchange, or even that specific proteins might perform two or more sequential roles (in which case the phenotype of amorphic mutants would reflect the earliest role), it is possible that the wild-type mei-W68 topo-II-like protein actually functions as a normal topo II, relaxing DNA, rather than as a defective topo II like the yeast SPO11 wild-type gene, which can only make the nick, not repair it again.

Regardless of its molecular role, wild-type mei-W68 function is necessary for the occurrence of both early and late recombination nodules, and in fact the absence both of early RNs and of simple gene conversion (gene conversion without a concomitant crossover) in the amorphic mei-W68 allele is the first evidence that supports the hypothesis (Rasmussen and Holm 1978; Carpenter 1979a) that the structure, early RN, and the recombinational outcome, simple gene conversion, are related—at least in flies. In both mutant alleles, however, long novel structures here called noodles appear instead; like RNs, noodles are located directly over the central region of the SC (thus being between homologous chromatids) but they are even smaller in diameter than early RNs. However, there are more noodles per...
nucleus than the sum of early + late RNs (Table 7) and
noodle numbers are surely an underestimate by as much
as twofold because of the problems of detecting them.
Moreover, unlike RNs, which form, function, and go
away, in mei-W68L1 and mei-W68L1 females noodles form,
stay, and elongate.

So what are noodles? That short ones can be detected
in wild type, in pre-early RN nuclei, suggests that they
are normally a transient precursor of early RNs. [This
hypothesis predicts that noodle-bearing and early-RN
bearing cysts should be adjacent in age; unfortunately,
this early in pachytene several cysts are at the same level
of the germarium, forcing the use of SC length as the
only gauge of relative age. For germarium A12, this does
sort the noodle cyst (III) as next younger than the first
early RN cyst (V); for A139 it does not. A139 length
order is cyst II, late zygotene (1, 0)–cyst IV (4, 0)–cyst
III (0, 0)–cyst V (0, 0 but both early and late RNs); see
Carpenter (1979a). This could mean that wild-type
noodles are not early RN precursors but I think it is
more likely that SC length alone is not a sufficiently
accurate indicator of relative age.]

The long mei-W68-specific noodles probably represent
the unchecked development of such precursors, which
in wild type are transformed into early RNs very quickly
but whose transformation cannot occur in either mei-
W68 allele, leaving this putative early RN precursor to
carry on extending. Note that this hypothesis implies
that the pachytene nucleus has a way to monitor success-
ful early RN formation, since noodles continue to be
initiated throughout at least early pachytene in mei-W68
and mei-W68L1 females.

Consistent with the hypothesis that noodles are un-
transformed early RN precursors are the observations
of the occasional short noodle segment that does look
like a little like an early RN (Figure 4C, middle, is the best
example in the entire sample) and also the apparently
random distribution of noodles along the arms.

The fact that noodles—and very long ones as well—
remain over the central element during the SC’s mid-
pachytene substructural rearrangements probably tells
us that noodles (at least in mei-W68 alleles) are not
bound irreversibly to chromatin. The logic is as follows.
The very early pachytene bivalent—SC plus its associ-
ated chromatin—is very thin (top to bottom of Figure
2C; width of SC remains constant), but as pachytene
progresses the bivalents, including the SC itself, shorten
and thicken (Carpenter 1975a). For the SC, this proba-
ably involves simple rearrangement of SC substructural
components (possibly as a unit of piece of lateral ele-
ment + transverse fiber + core unit of central ele-
ment + other transverse fiber + other piece of lateral
element), but even if it involves local disassembly and
reassembly the important point is that the chromatin
goes too; chromatin is uniformly distributed across the
face of lateral elements regardless of whether they are
thick or thin (see Carpenter 1975a for pictures and
diagrams). In other words, the proportion of chromatin
that is near the top or bottom of the lateral elements
(and therefore near a noodle if one is over the central
element) decreases as pachytene progresses. One could
imagine that very short noodles could be holding on to
a segment of chromatin, keeping it near the top of the
SC during these rearrangements, but noodles can be very
long; moreover, there are several cases of very long
noodles on both sides of very thick SC. The rest of the
chromatin would have to undergo major contortions if
both those noodles maintained earlier chromatin con-
tacts along their lengths. This reasoning by no means
eliminates either transient noodle-chromatin associa-
tions or a few local, more permanent associations. It
should be noted that the same logic requires that the
association between a noodle and the central element
be either transient or local.

It is not unreasonable to expect that topoisomerase
function would be involved in a DNA-protein interac-
tion, so the hypothesis here is that, in wild type, the
mei-W68 protein mediates the initial DNA-short noodle
interaction; when this is “successful” (see below) the
short noodle matures into an early RN—and some sort
of signal goes out along the bivalent, halting or slowing
further short noodle initiations. When it is unsuccessful,
as it is in both mei-W68 mutant alleles, that short noodle
extends along the central element to become a recogniz-
able long noodle and additional short noodles initiate.
That there is no difference between mei-W68 and mei-
W68L1 in precytoplasmic flow noodle numbers or lengths
strongly implies that the lesion(s) in mei-W68L1 com-
pletely destroy this aspect of its protein’s function, how-
ever it may operate biochemically.

The effects on late RNs are different. Morphologically
normal late RNs are known to be present before the
completion of all enzymatic reactions at sites that, in wild
type, would become exchanges (Carpenter 1979b) and
quite possibly are present very early in, or before, the
data-metabolizing reactions. mei-W68 function is abso-
lutely required for late RN formation and exchange
because the mei-W68 allele lacks both; there is no alter-
native, redundant pathway in flies. However, although
the lesion(s) in the mei-W68 allele has an effect here,
it is really quite mild; late RNs are present and the
reduction in exchange is only to 50% of wild-type levels,
implying that the mei-W68L1 product is approximately
half as efficient as wild type in its role in the exchange
process (and in mei-W68L1/mei-W68, where there is only
one half-as much partly functional mei-W68L1 product as
in mei-W68L1/mei-W68L1, exchange is reduced by another
50%). This difference in the effect of mei-W68L1 on noo-
dles vs. late RNs/exchange implies that the wild-type
mei-W68 product performs two different roles during
meiosis and presumably during recombination; if it were
the same role at two different times then the mei-W68L1
lesion should be equivalently defective in both and it is
not. (It is formally possible that the two roles differ only
in level of efficiency required, but even if so the levels of efficiency are so different that they are functionally equivalent to different roles.) Two times of action of the mouse SPO11 homolog have also been suggested by Romanienko and Camerini-Otero (2000); see below.

It has been hypothesized above that, in wild type, a short noodle is normally converted with high efficiency into an early RN and that this process requires wild-type mei-W68 function. Can we deduce anything equivalent about late RNs? Only that, whatever pre-late RN structures might look like, they do not appear to be normal early RNs—because mei-W68\(^{-1}\), which does present late RNs, does not present normal early ones. Although other possibilities such as very ephemeral early RNs in mei-W68\(^{-1}\) cannot be excluded, this observation does strongly suggest, at least in flies, that early RNs and late RNs are independent structures and, therefore, that recombination events whose outcome will be simple gene conversion vs. those whose outcome will be exchange are separate events from the outset rather than alternative outcomes of one common set of events—as has been hypothesized previously (Carpenter 1987) and is also suggested by recent results of Moens et al. (2001).

On the other hand, the possibility that a noodle can be converted directly into a late RN cannot be excluded. The timing of the few short noodles detected in wild type suggests that this is not the normal progression, but mei-W68\(^{-1}\) is a mutant condition in which abnormal progressions might occur. What can be excluded is the possibility that the disappearance of noodles along mei-W68\(^{-1}\) arms that have a late RN between homologs is due solely to their material being converted into that late RN, because noodles disappear from both sides of the SC at that time. Those on the other side have to be disassociated/removed, so it is probable that those on the same side are, too—and that the late RN forms de novo. Even less can be said about precursors of those late RNs that are found between sisters: Noodles would not be detectable there because they look too much like chromatin. Early RNs should be detectable but none were seen.

The remarkable observation that arms in mei-W68\(^{-1}\) that have a late RN between homologs lack noodles, whereas arms in the same nuclei that lack homologous late RNs have, on average, as many noodles per arm as nuclei of the same age in mei-W68\(^{+}\), requires some sort of recognition and signal that reaches along the entire arm. On the one hand, the long-standing observation of positive interference between exchanges on the same arm in Drosophila implies such a long-distance signal; on the other hand, the disappearance of noodles is a rather dramatic illustration of the principle. Moreover, the fact that noodles did \textit{not} disappear on the two arms that have a late RN in the wrong place—between sister chromatids rather than between homologs—further emphasizes the precision required for that signal. Note that this signal, and its source, in mei-W68\(^{-1}\) is presumed to occur in wild type, too (rather than as some side effect of mei-W68\(^{-1}\)’s mutant state); and the fact that even the longest noodles, however much they deviate from anything yet observed in wild type, do respond to that signal in what is presumably the normal manner (i.e., by going away) strongly suggests that they are basically not that different from structures that are present in wild type, however hard to see/ephemeral those structures may be.

In wild type and other meiotic mutants, early RNs also disappear around the time that late ones appear, but this appears to be due to a nuclear rather than a brachial signal. On the one hand, nuclei that have both early and late RNs do have examples of both on the same arm, although this could reflect a brief developmental age that simply is not represented in the limited mei-W68\(^{-1}\) sample. On the other hand, later pachytene nuclei lack early RNs entirely even on arms that do not have a late RN, although if late RNs are asynchronous in their presence some of those arms in wild type might represent cases that had had a late RN earlier. This implies that early RN disappearance is regulated at the nuclear level. This difference in disappearance control suggests that long noodles are not just oddly shaped early RNs but rather a qualitatively different structure.

A speculative model: A hypothesis that ties everything together, which is surely wrong on some details but may provide testable predictions, builds on the observation that the gene conversion footprint in flies is longer in events without an exchange than in those associated with exchange (Curtis et al. 1989; Curtis and Bender 1991) and the previous hypothesis that there are two rounds of initiation of recombination events (Carpenter 1987). The first round, which in most organisms occurs early in zygotene but in flies occurs after full pachytene has been accomplished, involves early RNs and is hypothesized to resolve as simple gene conversion events only; moreover, it is hypothesized that these RNs and events are part of the homology-recognition system that is operating during synaptic initiation. The second round, which occurs during full pachytene in most organisms, involves late RNs and exchanges only.

Fly homologous chromosomes go into meiosis already aligned by the ubiquitous somatic pairing process (Bridges 1916; Grell 1972; Roeder 1997). The molecular basis of this somatic alignment is completely unknown, but it does mean that homologs do not have to search for their partners as they enter meiotic synopsis, and reasonably accurate alignment would result if, during zygotene in flies, there is no or only very cursory checking for homology at the DNA sequence level. I therefore propose that initial synopsis (with SC) in flies does depend on somatic alignment, but that the correctness of this alignment is then checked. First a short noodle forms and attempts to generate heteroduplex from adjacent DNA from the two homologs. In wild type extensive
homology is expected to be found fairly quickly; the 
noodle then matures into an early RN and the recombi-
nation intermediate resolves as either a simple gene 
conversion event or a restoration. What happens in wild 
type if homology is not found is unknown; only iso-
sequential genotypes have so far been examined at the 
EM level. The short noodle might disaggregate—or it 
might extend to become a longer noodle and widen 
the search. In both mei-W68 mutant alleles the short 
noodle is formed but, because of the mutant defect 
(whatever it may be molecularly), sufficient DNA cannot 
be unwound to make an extensive enough comparison 
of sequences; the test therefore neither succeeds nor 
fails—and the conversion of the short noodle to a con-
stantly extending noodle is either the normal response 
to this failure or mei-W68 mutant specific, depending 
what happens when the homology check fails in wild 
type. What does seem clear is that many more events 
are attempted in mei-W68 alleles than in wild type or in 
any other meiotic mutant examined: 23 noodles were 
detected in one nucleus (average 18.5 across the 6 
mei-W68 post-cf nuclei), whereas the maximum number of 
early RNs per nucleus observed in other genotypes is 8 
(wild type; Carpenter 1979a, Table 3) to 10 (mei-9; 
Carpenter 1979b, Table 4). Moreover, up to 7 noodles 
have been detected on a single arm; no more than 3 
early RNs have been seen on a single arm (Carpenter 
1979a, Table 4 and Carpenter 1979b, Table 5). Of 
course, the maximum numbers of early RNs per nucleus 
and per arm will be underestimates if early RN presence 
is asynchronous, and if late RNs also initiate from some-
thing that becomes a noodle in mei-W68 mutants then 
six events/nucleus should be added, but, on the other 
hand, the numbers of noodles observed is an underesti-
mate of up to twofold, owing to the problems of de-
tecting them. It seems likely that successful homology 
searches do signal that success in some manner that 
inhibits additional tests in wild type and the other mei-
otic mutants but not in mei-W68 mutant alleles, but even 
here there does seem to be a limit of either time (devel-
opal age) or numbers; numbers of noodles do not 
seem to increase further after the onset of cytoplasmic 
flow.

Very long Rad51p-containing structures are present 
at zygote/pachytene in the maize meiotic mutants 
as1 and dsy2 but not in wild type (A. Franklin, W. 
Pawlowski, I. Golubovskaya and W. Z. Cande, per-
sonal communication). It seems likely that these long 
structures are the maize analog of Drosophila long 
noo-
dles; if so, this suggests that longoodle formation is 
restricted neither to mei-W68 nor to flies. It will be very 
interesting to extend these observations in both organ-
isms.

Interspecific comparisons: There are so far two cate-
gories of organism with respect to cytological phenotype 
of SPO11: SPO11 nulls; those that continue to make normal 
SC (flies, this report; Caenorhabditis elegans, Dernburg 
et al. 1998) and those that do not (e.g., yeast, Roeder 
1997; mouse, Romanienko and Camerini-Otero 2000). 
Wild-type male mice have two distinct SPO11 protein 
expression times and patterns (Romanienko and Cam-
erini-Otero 2000): nonaxial foc in leptotene, hypothe-
sized to be the sites of recombination-initiating DNA 
double-strand breaks, and long tracts along the zy-
gotene-pachytene bivalents, hypothesized to reflect a 
second SPO11 function in synapsis. S. cerevisiae spo11 
amorphs fail to make double-strand breaks in leptotene; 
although the highest level of SPO11 RNA occurs during 
pachytene (Atcheson et al. 1987; Chu et al. 1998), no 
pachytene function has been proposed. Yeast also 
pre-
sents different lengths of homology requirements for 
the initiation of the simple gene conversion and ex-
change processes (Hayden and Byers 1992).

The present Drosophila data are in accord with two 
distinct bouts of SPO11 function, time shifted relative 
to yeast/mice: the first bout, early in pachytene, being 
necessary for the transformation of short noodles into 
early RNs and the second, later in pachytene, to promote 
exchange. Significantly, recent work in yeast (Allers 
and Lichter 2001) has demonstrated that the fundamen-
tal tenet of the double-strand-break repair (Szostak et al. 
1983) and other models that hypothesize that the simple 
gene conversion and exchange alternatives are formed 
at the same time from the same structure is false; in yeast, 
the noncrossover (simple gene conversion) outcome is 
produced significantly earlier than the crossover out-
come. Although all meiotic recombination events in 
yeast do seem to originate from a single round of initiat-
ing events, double-strand breaks in leptotene, with those 
predestined to become exchanges set aside for final 
metabolism in late pachytene, it is nevertheless possible 
that even in yeast there are two separate functions for 
the SPO11 protein, one early, one late. Similarly, it is 
formally possible that all recombination in Drosophila 
results from one bout of double-strand breaks produced 
(via mei-W68) very early in pachytene, with the very few 
such events produced in mei-W68 being sequestered 
to become exchanges via late RNs later, consistent with 
recombination measurements in spo11 hypomorphs in 
yeast (E. Martini and S. Keeney, personal communica-
tion). However, I personally think that two completely separate rounds of events—early going into simple gene conversion only, later going to exchanges (with simple gene conversion as a backout alternative if there are problems)—is a more parsimonious interpretation of mei-W687’s complex phenotype.

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LITERATURE CITED


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