Electron microscopy of meiosis in *Drosophila melanogaster* females: II: The recombination nodule—a recombination-associated structure at pachytene?

(crossing-over/serial reconstruction)

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ABSTRACT The recombination nodule is a transient structure present during pachytene in intimate association with the synaptonemal complex. The total numbers of these structures per nucleus and their locations along the bivalents correspond to the total numbers and distributions of genetic exchanges. It is suggested that this structure may be involved in the recombination process.

The mechanism of recombination in eukaryotes has been the subject of considerable speculation by both geneticists (in terms of crossing-over) and cytologists (in terms of chiasma formation) for over 70 years. Classical investigations of the mechanism of recombination have been hampered by the fact that only the end products of recombination can be studied (crossover chromosomes in the progeny or chiasma configurations at diplotene-diakinesis). The development of the technique of electron microscopy has permitted the fruitful observation of the fine structure of meiotic stages before chiasmata become visible, in particular of pachytene, the stage during which crossing-over is generally thought to occur (1). The most striking observation derived from electron microscopic studies of pachytene is that of the presence of the synaptonemal complex (SC), a structure which lies between the synapsed homologues (2).

In most organisms, the presence of the SC appears to be necessary, but not sufficient, to insure normal meiotic recombination (3–5). Moreover, observations of structural differences of the SC at pachytene between relatively long portions of the bivalent chromosomes have been correlated with the presence or absence of recombination in such portions (6–9). For example, the SC of the *Drosophila melanogaster* X chromosome shows longitudinal differentiation in that the distal portion associated with the euchromatic segment is morphologically different from the proximal portion associated with the heterochromatic segment. These differences correlate with the presence and absence, respectively, of meiotic crossing-over in those segments. However, although the SC of the euchromatic portion of the bivalent is quite long [up to 20 μm long (9) by 0.1 μm wide], there are only an average of 1.32 exchanges per meiosis (66 total map units for the X/50 map units per exchange). Thus, whereas such longitudinal differentiations may determine (or reflect) restrictions affecting the portion of a bivalent in which recombination may occur, additional structures, structural modifications, and/or processes would seem to be necessary both to

determine the precise site of recombination and to effect the recombinational event itself.

This report describes a structure, the recombination nodule, which is present at pachytene in *Drosophila melanogaster* females in intimate association with the SC. The agreement of the total numbers of nodules per nucleus and their locations along the bivalents with the total numbers and distributions of genetic exchanges suggests that this structure may, in some manner, be involved in the recombinational process.

MATERIALS AND METHODS

Details of fixation and embedding employed are described elsewhere (9); briefly, tissues were fixed in phosphate-buffered paraformaldehyde–glutaraldehyde, post-fixed in osmium tetroxide and stained with uranyl acetate, embedded in Epon–Araldite, sectioned, post-stained with uranyl magnesium acetate and with lead citrate, carbon-coated, and examined.

BACKGROUND: GENERAL MORPHOLOGY

This report presents further observations derived from serial section reconstruction analysis of 13 oocytes and pro-oocytes in pachytene from one wild-type *Drosophila melanogaster* germarium. The earlier observations (9) from this material will be briefly summarized. (1) The nearly linear arrangement of cysts in the germarium appears to reflect increasing developmental age of cysts, anterior to posterior. [Each cyst contains 16 interconnected cells which will become 15 nurse cells and one oocyte. However, in early cysts (anterior germarium), two cells per cyst enter pachytene as evidenced by completion of synaptonemal complex formation; these two cells, which can be recognized by their four ring canals (differentiated cytoplasmic connections to other cells in the cyst), are referred to as (sister) pro-oocytes. Later (posterior germarium), one cell loses its SC and reverts to nurse cell morphology; the other cell retains its SC and is now referred to as the oocyte. See ref. 10 for a detailed discussion of the morphology of the *Drosophila* germarium.] Thus the temporal progression of meiotic events can be followed by the examination of all pro-oocytes and oocytes in single germaria. (2) In this germarium the pro-oocytes of the first (youngest) two cysts (cysts 1 and 2) lack SC and are, therefore, in pre-pachytene stages; the pro-oocytes (cysts 3–8) and oocyte (cyst 9) of the next seven cysts contain fully formed SC and are therefore in various stages of pachytene. (3) The pachytene bivalents show a chromocentral arrangement. Each nu-

Abbreviation: SC, Synaptonemal Complex.

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FIG. 1. Three-dimensional aspects of the synaptonemal complex and the typical position of the recombination nodule (double arrow). ch = chromatin, le = lateral element, ce = central element; transverse filaments have been omitted here for clarity.

cleus contains five long portions of SC and associated chromatin representing the five major bivalent arms (XL, 2L, 2R, 3L, 3R); the proximal portions of the arms converge on a common region of the nucleus, the chromocenter. (4) The chromocentral portions of the bivalents contain SC; however, this SC differs in morphology from that present in more distal portions. Since the chromocentral region contains the proximal (pericentric) heterochromatin of the bivalents, the SC of chromocentral morphology is termed "heterochromatic SC" and that of the more distal portions is termed "euchromatic SC". The tiny fourth chromosome is found in the chromocentral region and has SC of heterochromatic morphology. (5) The length of euchromatic SC is maximal in the earliest pachytene cyst (cyst 3), steadily decreases in length until cyst 7, then increases slightly in cysts 8 and 9. Minimum length is approximately half of maximum. The SC increases in thickness (height as viewed in Fig. 1) as it decreases in length.

RESULTS

Twenty-five recombination nodules were detected among the 13 serially photographed and reconstructed pro-oocyte and oocyte nuclei. These nodules are roughly spherical and densely staining; they are found in a nearly constant relationship with the SC and chromatin of the bivalent. They lie adjacent to the central element and span the width of the SC; they are therefore located between and adjacent to the chromatin of the paired homologues (Fig. 1, Figs. 2-4). The nodules vary considerably in diameter (Fig. 5); the average diameter is 100 nm. There appears to be a slight correlation of diameter of the nodule with the developmental age of cyst in that the younger cysts (6 and 7) show greater variation in diameter of nodules than the older (8 and 9); however, the average diameter per cyst remains virtually constant (cyst 6, 101 nm; cyst 7, 98 nm; cyst 8, 100 nm; cyst 9, 129 nm).

Recombination nodules are internal structures (as opposed to surface contamination of the sections) since the ten largest nodules were observed to extend through two adjacent sections (Figs. 2 and 3). Moreover, in addition to the 25 recombination nodules from the extensively-analyzed germarium presented here, an additional 19 recombination nodules have been found in 11 nuclei from seven less-extensively analyzed germaria.

Three of the recombination nodules were atypical, both with respect to position and to diameter. The smallest (Fig. 5) appeared to be within, as opposed to adjacent to, the cen-

FIGS. 2-4. The relationship of the recombination nodule to the SC as seen in various planes of section. FIG. 2a and b. Two consecutive sections, cross section of SC. Large arrow in a indicates recombination nodule; small arrow indicates SC. ch = chromatin, N = nucleolus. cell a, cyst 9. FIG. 3a and b. Two consecutive sections, SC in slanting frontal section. Arrow in a indicates SC; arrow in b indicates small portion of recombination nodule included in this section. cell a, cyst 7. FIG. 4a and b. Two consecutive sections, SC in sagittal section. Arrows indicate central element in sagittal view. cell b, cyst 7. All $\times 36,000$; bar in FIG. 2a = 100 nm.

tral element; the two largest (Fig. 5) were not associated with the SC at all, but were instead embedded in the chromatin of one homologue to the side of the SC. These three atypical nodules were all found in the presumptive pro-

FIG. 5. Diameters of the various recombination nodules. Diameters were measured on the prints (final magnification $\times 36,000$) to the nearest 0.1 mm. The larger diameter was selected for those nodules extending through two sections. Cyst of origin is as indicated in the legend (see also Fig. 6).

FIG. 6. Numbers of recombination nodules per pachytene nucleus in the various 16-cell cysts previously reported (9). Cyst number indicates relative developmental age; pro-oocytes of cysts 1 and 2 lacked SC and were therefore in pre-pachytene stages. In cysts 3-8 both pro-oocytes (a and b) contained extensive continuous SC and were, therefore, in pachytene; in cyst 9, only one cell (a), the oocyte, contained continuous SC. For cysts 6-8 cell a is the presumptive oocyte (9).

nurse cell (cell b), as opposed to the presumptive pro-oocyte (cell a), of their respective cysts (9).

The numbers of recombination nodules found per nucleus are presented in Fig. 6. Since the SC of each of these nuclei has been subjected to reconstruction analysis (9), each nodule can be positioned along its bivalent arm (Fig. 7). All nodules were associated with euchromatic portions of bivalent arms; none were found associated with the heterochromatic (chromocentral) portions or with chromosome 4. Twenty-one bivalent arms had but one nodule each; two arms had two each. The position of each nodule with respect to the euchromatic length of its bivalent arm has been calculated by the method described previously (9); these positions are presented in Fig. 8. The position of the nodules along the length of the arms does not appear to be correlated with developmental age of cyst. Nodules do not appear to be associated with SC of unusual thickness.

DISCUSSION

The distribution of recombination nodules, both in terms of total numbers per cell and in terms of chromosomal location, shows strikingly good agreement with the distribution of exchange events as determined from conventional recombination analysis (11).

(1) The maximum number of five nodules per nucleus

FIG. 7. Two-dimensional tracing of the reconstructed synaptonemal complex of cell a, cyst 6. Solid lines = euchromatin, dashed lines = heterochromatin, N = nucleolus, B = blob (9). Large arrows indicate each of the 4 recombination nodules of this nucleus; the small arrow indicates chromosome 4. Bar = 1 μ m.

FIG. 8. Distribution of the 25 recombination nodules along the euchromatic length of the arms (each normalized to 1 for comparison). The two cases of two recombination nodules per arm are asterisked; (*, *) indicates one pair (on the X chromosome), (**, **) the other (on an autosome). Cyst of origin is indicated by shading; for legend, see Fig. 5.

observed in cyst 7 (Fig. 6) correlates well with the average expected number of exchanges per nucleus calculated from the total map [280 total map units/50 map units per exchange = 5.6 exchanges per meiosis (12)]. However, the number of nodules per nucleus appears to be correlated with developmental age of cyst (Fig. 6). Nuclei of the three youngest cysts (cysts 3, 4, and 5) contain no nodules, although these nuclei are in pachytene by the criterion of completion of SC formation (9); nuclei of the next two older cysts (cysts 6 and 7) contain increasing numbers, followed by decreasing numbers in the next two older cysts (cysts 8 and 9). Thus recombination nodules appear to be transient structures present only in mid-pachytene stages; meiotic crossing-over may be similarly restricted in time of occurrence (1, 13). The change in numbers of nodules during pachytene suggests that there is some asynchrony in the time of appearance and of disappearance; consequently, the maximum number of nodules observed per nucleus may be an underestimate of the total number that occur during a cell's passage through pachytene.

(2) All 25 recombination nodules were found in the distal portions of the bivalent arms, associated with SC of "euchromatic" morphology; none were found associated with the proximal, chromocentral portions of SC ("heterochromatic" morphology) or associated with chromosome 4. Meiotic crossing-over is restricted to the euchromatic portion of the major arms; the heterochromatic portions and chromosome 4 undergo virtually no meiotic crossing-over.

(3) Most (21/23) bivalent arms having an associated recombination nodule had but one; two bivalent arms were observed with two associated nodules. From genetic evidence, most exchanges occur as singles per bivalent arm (single-exchange tetrads); a much lower proportion of bivalent arms experience two exchanges (double-exchange tetrads). Typical proportions of no-, single-, and double-exchange tetrads, calculated following ref. 11, are: for the X; 0.09, 0.62, 0.28 (data from Table 5 of ref. 14); for the left arm of chromosome 2 (typical for the four major autosome arms); 0.15, 0.76, 0.09 (Table 6 of ref. 15). For the two cells of cyst 7, the numbers of arms with no, 1, and 2 nodules are 1, 8, and 1, respectively.

(4) In the two bivalent arms with two recombination nodules, the nodules are quite far apart (Fig. 8); exchanges tend to be far apart (= positive chiasma interference).

(5) Recombination nodules are distributed rather uniformly throughout the middle and distal portions of the bivalent arms (Fig. 8); crossing-over per unit physical length (DNA content) is quite uniform in the middle and distal

portions of the euchromatin (16). However, there is a paucity of crossovers per unit physical length (salivary chromosome bands, DNA content) in the proximal euchromatin, near the euchromatic-heterochromatic boundary (16); this nonuniformity is termed the centromere effect (17). There is a similar paucity of nodules in the proximal portion of the euchromatin, near the juncture between "euchromatic" and "heterochromatic" morphology. The centromere effect is least drastic for the X chromosome (that is, the probability of a very proximal crossover is highest for the X); thus, it is not surprising that the most proximal nodule found was associated with the X (Fig. 8).

(6) Arms with but one associated recombination nodule tend to have it in the middle of the arm; arms with two nodules have one proximal, one distal (Fig. 8). The exchange in single-exchange tetrads has a modal position in the middle of the arm; the two modal positions in two-exchange tetrads are proximal and distal (18).

On the basis of the strikingly good correlation between recombination nodules and exchange events, I suggest that this structure is in some manner involved in the recombination process; that it indicates the site where exchange is occurring or will occur. In the terminology of Sandler *et al.* (ref. 19, see also refs. 20 and 14-15), I suggest that the recombination nodule—or some component of it—is involved either in the performance of some of the functions necessary to prepare a chromosomal site for the possibility of exchange (precondition functions) or in the formation of physically recombinant DNA molecules (the exchange event itself) or both. This is a testable hypothesis; if recombination nodules bear a one-to-one correspondence with exchange events, then conditions that alter the distribution of exchanges along the bivalent arms [= affect precondition functions (14)] should show a parallel alteration in the distribution of nodules. [Whether conditions that alter the frequency of exchange events also alter the numbers of nodules depends upon the precise role(s) of the nodule in the recombination process.] However, if the recombination nodule does mediate precondition and/or exchange functions, then it is likely that the SC itself performs a general, rather than a specific, role in the process of recombination—presumably that of maintaining proximity of homologous chromosomal segments (3-4).

Since the recombinational functions with which the recombination nodule is associated (whatever they may be) are presumably not unique to *Drosophila melanogaster* females, one would expect to find similar structures in other organisms. In fact, bodies similar to recombination nodules both in morphology and in location relative to the SC have been observed in 12 species of fungi (21-23). Gillies (21) observed that these structures ("nodes") in *Neurospora* are present in low numbers (1-2) per bivalent arm and, if more than one is present per arm, are far apart (i.e., show interference); no hypothesis regarding function was suggested.

NOMENCLATURE

The similarities between the recombination nodules of *Drosophila* and the nodes of *Neurospora* should perhaps be reflected by similarity of name; "node" has precedence in this context. Unfortunately, the term "node" has already been employed in a formal sense in the genetic dissection of the recombination process in *Drosophila* to denote the establishment of precondition functions (see above; also refs. 24, 19-20, and 14-15). Consequently, in order to avoid confusion and premature functional implications, the term "recombination nodule" has been chosen.

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