SYNAPTONEMAL COMPLEXES DURING PREMEIOTIC DNA SYNTHESIS IN OOCYTES OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

Well-synchronized populations of oocytes obtained by means of the "pupal system" (GRELL, 1973a) have been examined to determine the time of appearance of the synaptonemal complex. The complex first appears in the most advanced oocytes between 132 and 138 hr of female development. Between 138 and 156 hr the complex apparently undergoes a fourfold increase in length. At 150 and 156 hr the complex system is extensive and present in virtually all oocytes. Previous studies using the pupal system have placed the period of premeiotic DNA synthesis between 132 and 162 hr. Thus, indirect evidence indicates that a significant portion of synaptonemal complex formation is coextensive with the main DNA replication in the oocyte. Direct evidence that DNA synthesis and complex formation occur simultaneously in oocytes has been obtained by electron microscope autoradiography. By definition, then, the stage of synaptonemal complex formation in Drosophila must include premeiotic interphase.

THE synaptonemal complex is a tripartite, ribbon-like structure found almost exclusively in meiotic cells along the axes of paired homologous chromosomes. Although the exact function of the complex is unclear, MOSES (1968) concluded that the complex plays "an essential role as synaptic center, providing the means for effective synapsis and structure within which genetic recombination in meiosis may occur." In a more recent review WESTERGAARD and VON WETTSTEIN (1972) maintain that "the synaptonemal complex in meiotic cells is the vector for chromosome pairing and crossing-over. . . ." According to the studies of MOENS (1968, 1969) with Lilium and Locusta, the complex is first visible as short segments during the zygotene stage of meiotic prophase. The dogma that the complex appears only during the zygotene-pachytene stages of meiosis appears to be so well accepted that its presence is assumed to be sufficient to identify these stages even in the absence of chromosome morphology at the light-microscope level. As an example, ZICKLER and OLSON (1975) have stated that the majority of yeast

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cells in "sporulation medium for 2–4 hr were in zygotene as evidenced by the presence of short pieces of completed synaptonemal complexes" and at 4–6 hr a large number of nuclei "were at pachytene as revealed by the presence of extensive synaptonemal complexes."

However, GRELL (1969, 1973a) has pointed out that KOCH, SMITH and KING (1967) observed complexes in Drosophila oocytes a few hours after their formation in the anterior region of the adult germarium, at which time it has been shown that the period of normal, premeiotic DNA replication is in progress (GRELL 1969, 1973a, b; GRELL and DAY 1974). In addition, CHAPRON and RELEXANS (1971) have demonstrated the presence of the complex at the leptotene stage in *Eisenia foetida*. The presence of the complex prior to the classical stage of pairing (i.e., zygotene) has obvious implications regarding not only the function of the complex, but also the mechanism of genetic exchange. Thus, in assigning a function to the complex it seems necessary to define clearly the duration of its existence with respect to other events in meiosis, especially those occurring prior to zygotene. This paper reports the time of appearance of the complex in carefully timed oocyte populations of *Drosophila melanogaster* obtained by means of the "pupal system" (GRELL 1967, 1973a). This time period is coextensive with the period of premeiotic DNA synthesis and the period of heat-induced recombination as determined in experiments using similarly timed populations of oocytes. Furthermore, studies utilizing combined electron microscopy and autoradiography which demonstrate the presence of synaptonemal complexes during DNA replication are reported.

MATERIALS AND METHODS

The purpose of this investigation was to determine the time and extent of formation of the synaptonemal complex in pre-zygotene oocytes. The proper timing was achieved by examining oocytes located in posterior-most 16-cell cysts in ovarioles of ovaries removed from immature females at 6-hr intervals from 132 to 156 hr of development. During this period of development each of the 12–20 ovarioles of an ovary consists of only the germarium portion, as the first egg chamber is not pinched off into the vitellarium until approximately 168 hr. For details of the structure of the Drosophila ovary the reader is referred to KOCH, SMITH and KING (1967).

The general shape of the germarium in longitudinal section is illustrated in Figure 1, part IV. Certain oogonia near the terminal filament (tf) begin a series of 4 divisions with incomplete cytokinesis, resulting in a cluster of 16 cells interconnected by ring canals (KOCH and KING 1966). The first 16-cell cluster in a germarium forms at about 126 hr (BUCHER 1957; GRELL 1967). From this time up until about 168 hr the 16 cells are indistinguishable from one another by our light microscope methods (but see GUYÉNOT and NAVILLE 1933). However, at the electron microscope level two cells, the pro-oocytes, can be recognized by virtue of their unique ring canal system and the presence of synaptonemal complexes in their nuclei. Complexes form in the nuclei of these two cells shortly after formation of the 16 cell cluster, disappear by 168 hr from one of them, which then becomes a nurse cell, and persist until much later in the other, which becomes the oocyte (KOCH, SMITH and KING 1967; BROWN and KING 1964; KING 1970).

Shortly after formation of the 16-cell cyst (at 132 hr) DNA synthesis begins in all 16 cells and continues for approximately 30 hr (GRELL 1973a, b; GRELL and DAY 1974; DAY 1973). Since the DNA synthetic period immediately prior to meiosis has been shown to occur in interphase, but in some cases may extend into early prophase (TAYLOR 1957) the 132- to 162-hr period in Drosophila oocytes is predominately, if not entirely, interphase.

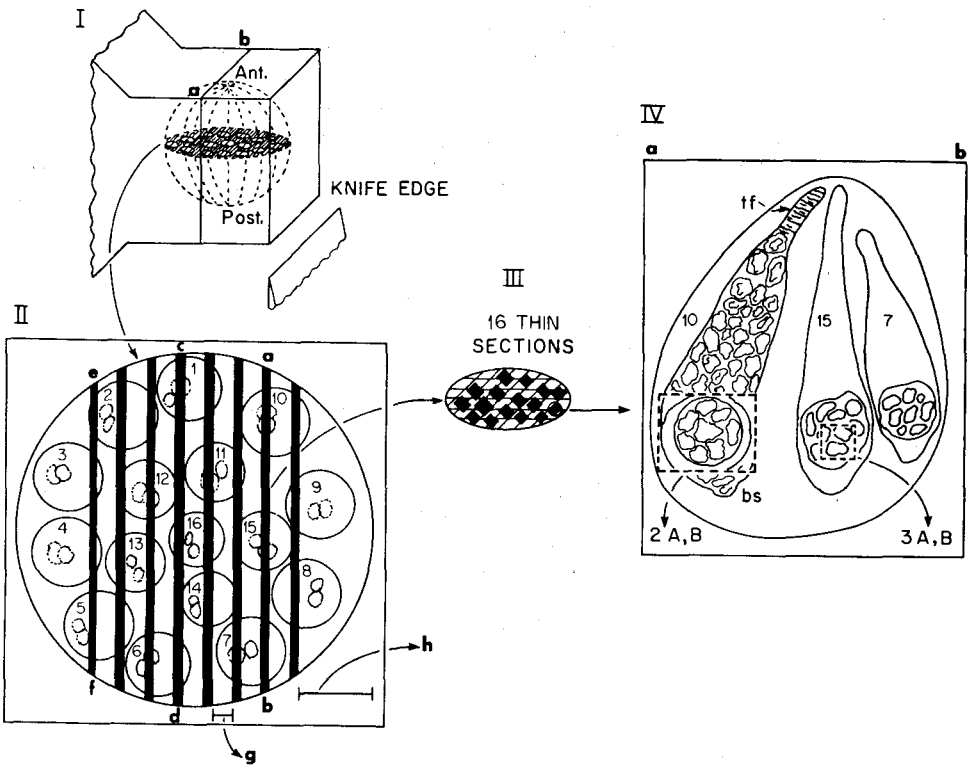

FIGURE 1.—Regimen followed in sectioning ovaries. (I) Orientation of an ovary embedded in a plastic block. Line *a-b* represents the area from which the section depicted in IV was cut. (II) Cross section of the ovary at the level indicated in I, showing the positions of ovarioles and pro-oocytes (dotted circles) in relation to areas (thick dark lines) from which thin sections were cut. Bar *g* indicates a distance of 6–15 μm separating adjacent areas from which thin sections were collected. Bar *h* indicates the first 1/4–1/3 of the ovary discarded before cutting thin sections. (III) Grid containing sections from area *a-b*, one of which is shown in IV. (IV) A section from area *a-b*, cut through ovarioles 10, 15 and 7, showing positions of their posterior-most 16-cell cyst. Electron micrographs of areas outlined are shown in Figures 2 and 3. *tf* = terminal filament, *bs* = basal stalk.

Synchronously developing pupae of the genotype y^2 *cv v f*/y^2 *sc car* · $y+$ were obtained by means of the "pupal system." For this purpose, eggs were collected over 1½- to 2-hr periods by serial transfer of parental flies to fresh culture bottles containing standard cornmeal-sugar-Brewer's yeast-agar medium. The surface of the medium was modified for optimum egg laying and larval growth by addition of moisture and a few granules of dry Baker's yeast. The cultures were maintained at 25 ± 1° throughout the experiments.

Pupae were removed from the cultures at 6-hr intervals from 132 to 156 hr after egg laying, inclusive. Ovaries were removed from pupae in ice-cold 0.1 M phosphate buffer, pH 7.2, and immediately transferred to ice-cold 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. Fixation time was 1 hr, followed by a minimum of three 15-min washes in ice-cold buffer, with occasional overnight storage in buffer at 0.4°. The ovaries were postfixed for 1 hr in ice-cold 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.2. The tissue was dehydrated in ascending alcohols and propylene oxide. After overnight infiltration in a 1:1 mixture of propylene oxide

and Epon, the ovaries were embedded in fresh plastic mixture in flat embedding molds. Polymerization was carried out at 60° for 2–3 days.

The simple technique of identifying oocytes in thick sections and then examining their ultrastructure in adjacent thin sections was not applicable, since oocytes and nurse cells at these stages of development were indistinguishable at the light-microscope level. At the electron-microscope level both identification of pro-oocytes by their 4-ring canals and determination of the presence or absence of synaptonemal complexes in their nuclei can be accomplished in serial section reconstructions of entire 16-cell cysts. To estimate the proportion of oocytes containing complexes at each of several developmental ages, a large number of reconstructions would be required. An alternative method was used which allowed reasonable estimates to be made of the proportion of oocytes containing complexes based on observations of larger numbers of oocytes than would be feasible by serial reconstructions. This method is described below and outlined schematically in Figures 1, 2, and 3.

The ovary is oriented in the block so that sections are cut in an anterior posterior direction, resulting in approximately longitudinal sections of ovarioles (Figure 1, part I). Groups of 10–20 thin sections (600–800A°) are cut from several areas of an ovary, each area separated by 6–15 μm, as indicated by the thick black lines in Figure 1, part II. The sections from each area are collected on Formvar-carbon-coated 200 mesh grids, double stained in uranyl acetate and lead citrate and scanned in Hitachi HU-11C electron microscope. The posterior-most 16-cell cyst were located at low magnifications and their nuclei were examined at higher magnifications to ascertain the presence of synaptonemal complexes. Figure 1, part IV, shows one typical section from a group of thin sections cut at one area of an ovary. Figures 2 and 3 show actual electron micrographs of posterior-most 16-cell cysts in which pro-oocytes containing complexes are present.

Using the sectioning regime illustrated in Figure 1, several posterior-most cysts are sectioned in each area, indicated by the thick dark lines. However, within a particular cyst, the plane of sectioning may completely miss the pro-oocytes (indicated by the dotted circles) or pass through one or both of them. For the ovary illustrated one or both pro-oocytes of only 7 of the 16 posterior-most cysts were actually sectioned. Since complexes develop in both pro-oocytes, observation of complexes in only one pro-oocyte of a cyst is interpreted to mean that the definitive oocyte of that cyst contains complexes. In this example if all the pro-oocytes sectioned were observed to contain complexes, a frequency would be recorded of seven oocytes with complexes

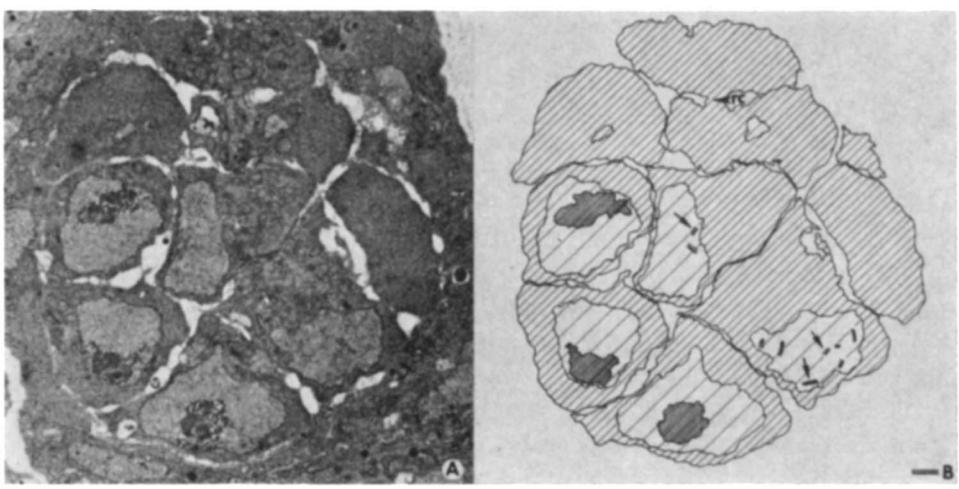

FIGURE 2.—An electron micrograph (A) and an accompanying drawing (B) of a section through a posterior-most 16-cell cyst comparable to that outlined in Figure 1, part IV (denoted by 2 *A,B*). Included are seven nurse cells and the two pro-oocytes, both containing synaptonemal complexes (*arrows*). The bar represents a distance of 1 μm. *rc* = ring canal.

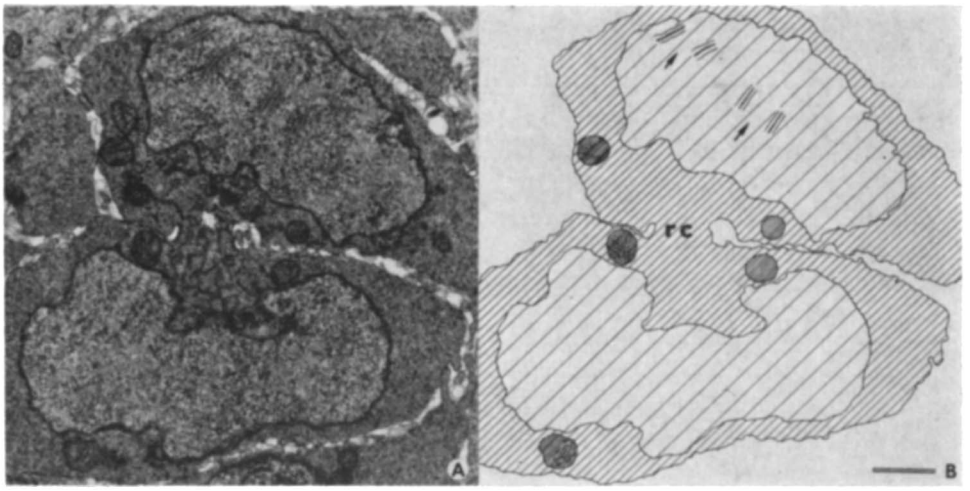

FIGURE 3.—An electron micrograph (A) and an accompanying drawing (B) of a section through a posterior-most 16-cell cyst comparable to that outlined in Figure 1, part IV (denoted by 3 *A,B*). Included are two cells interconnected by a ring canal (*rc*), one a pro-oocyte containing synaptonemal complexes (*arrows*), the other presumably a nurse cell since complexes are not evident in its nucleus. The bar represents a distance of 1 μm.

from eight levels examined, giving a frequency of 7/8 (0.88). On the other hand, if no complexes were observed, the frequency of 0/8 (0.00) would be recorded. Intermediate frequencies would be encountered depending on the actual proportion of pro-oocytes observed to contain complexes. With this method a maximum frequency would be expected when all pro-oocytes of the posterior-most cysts contain sufficiently extensive complex systems to ensure that complexes are virtually always observed when the plane of sectioning goes through their nuclei. The maximum frequency would then reflect the average number of posterior-most 16-cell cysts sectioned through at least one of their pro-oocytes per level examined.

Electron microscope autoradiograms of oocytes taken from females at 144 hr of development were obtained in the following manner. Ovaries were dissected in insect ringer solution, transferred to a solution of [^{3}H]thymidine in insect ringer (1 mc in 10 ml, specific activity 5 or 20 C/M), and incubated for 45–60 minutes at 25°. The ovaries were then rinsed three times in insect ringer and incubated for an additional 30 minutes. The ovaries were then fixed, embedded, sectioned and stained as described previously. The stained sections were coated with Ilford L4 emulsion according to the loop method of CARO and VAN TUBERGEN (1962) and exposed in sealed slide boxes at 4° for 7–20 days. The emulsion was developed in Microdol-X developer for 2 minutes, rinsed once in distilled water, fixed for 3 minutes in Kodak acid fixer, rinsed in running distilled water for 10 minutes and air-dried.

In a few cases alternating thick (1 μm) and thin sections were cut from labeled ovaries. The thick sections were affixed to subbed microscope slides by air-drying and coated with emulsion by the dipping method using a 1:1 mixture of Kodak NTB liquid emulsion and Photo-flo solution at 42°. After 4–12 days exposure at room temperature the emulsion was developed as described above and the sections were stained with toluidine blue. The adjacent thin sections were stained as described above for nonlabeled sections.

RESULTS

Observations on the occurrence of synaptonemal complexes in oocytes of 132–156 hr of development are summarized in Table 1. Examination of a total of 27

TABLE 1

Occurrence of synaptonemal complexes (SCs) in oocytes of ages 132–156 hr of female development

Age of ovary (hr)	Number of ovaries examined	Number of levels examined	Number of oocytes with SCs	Maximal length SCs (μm)	Frequency*
132	4	27	0	—	0
138	8	55	13	0.8	0.24
144	4	21	13	1.2	0.62
150	3	22	21	2.3	0.95
156	3	22	22	3.2	1.00
Total	22	147			

* Number of oocytes with SCs divided by number of levels examined.

levels in four ovaries at 132 hr fails to reveal synaptonemal complexes. That this is not due to failure to section through oocyte nuclei is apparent from the fact that at 144, 150, and 156 hr several oocyte nuclei (as indicated by the presence of complexes) were sectioned even though fewer levels were examined. It seems more likely that at this stage complexes either are not present or are present as a few very short segments per nucleus which escaped detection by our sampling method.

Complexes are first detected in oocytes at 138 hr of development. They are observed in 13 oocytes (one oocyte in each of 13 different posterior-most cysts) in total of 55 different levels examined in eight ovaries—a frequency of appearance of 0.24 (13/55). In other words, on the average, for every four levels examined, one posterior-most 16-cell cyst contains an oocyte in which synaptonemal complexes are observed. In the total of 147 levels examined in all age groups, the number of ovarioles sectioned through an appreciable portion of their posterior-most 16-cell cysts ranges from one to five for a single level, two to four being frequently observed. The number of these clusters observed to contain oocytes with complexes most frequently ranges from zero to two, although in this study three were observed in one case and four in another. Thus, if the frequencies had been based on the number of posterior-most cysts instead of levels examined, the values would have been lower by a factor of 2 to 4.

At the early stages of development examined in this study, the chromatin of nurse cells and oocyte nuclei is quite diffuse except in the area of the nucleolus. At 138 hr only one or two short segments of complex in approximately median longitudinal section are observed in any one nucleus. The lateral elements of these complexes are not associated with an substantial amount of condensed chromatin (Figure 3A). Due to this fact it is very difficult to find cross sections of complexes with any degree of certainty. As a result, in screening for the presence of complexes no attempt was made to find cross sections or to record their presence in any of the stages examined.

At 144 hr, 6 hr after the first detection of the complex, the frequency of complex occurrence more than doubles (0.62 *versus* 0.24). Another 6 hr later, at 150 hr, oocytes containing complexes are encountered approximately four times

as frequently (0.95 *versus* 0.24). During the next 6 hr, between 150 and 156 hr, the frequency does not change appreciably (1.00 *versus* 0.95). This is interpreted to mean that at 150 and 156 hr virtually all oocytes have developed sufficiently extensive complex systems that every oocyte sectioned through an appreciable portion of its nucleus is observed to contain complexes.

The total length of the complex system appears to increase considerably in the 18-hr period studied, based on a comparison of the maximum lengths of complex observed in single sections. Figure 4 shows electron micrographs of oocyte nuclei at 138, 144, 150, and 156 hr of female development. Each micrograph was selected to illustrate the maximum linear extent of the complex system seen in single sections for each of these age groups. Four such micrographs for each time period, representing the four nuclei with the longest single-section lengths of complexes for that period, were measured directly from prints with total magnifications of 17,000 to 20,000 ×. The average of the four values for each period (138, 144, 150, 156 hr) were 0.8, 1.2, 2.3, and 3.2 μm, respectively (Table 1). Although serial section reconstructions would be required for determinations of total lengths of the complex system, these data allow us to roughly estimate that a fourfold increase occurs between 138 and 156 hr.

The frequencies listed in Table 1 imply that the synaptonemal complex first appears between 132 and 138 hr and that the proportion of oocytes containing complexes increases gradually with age. As mentioned above, it was expected that the observed frequencies would reach a plateau value when all oocytes (pro-oocytes) contained extensive complex systems. At this time the actual proportion of oocytes containing complex is assumed to be 1.0, and the plateau frequency indicates the average number of oocytes with complexes sectioned per level. Thus, at 150 and 156 hr the actual proportion and observed frequency are essentially the same, i.e., ~1.0. However, at 138 and 144 hr the observed frequencies probably do not equal the actual proportions for the following two reasons:

First, the number of oocytes sampled per level examined is not the same as at 150 and 156 hr. Consideration of the data of KERKIS (1931) on ovary growth in pupal stages suggests that the volume of the ovaries in the present study increased approximately twofold between 138 and 156 hr. Thus, there were more oocytes per unit volume in an ovary at 138 hr than at 156 hr. It follows that more oocytes were sampled per level examined at the earlier stages. The frequencies listed for 138 and 144 hr, due to this factor alone, are overestimates of the proportion of oocytes containing complexes.

Second, fewer complexes will be detected when they are small and sparse within a nucleus. Since the amount of complex in nuclei of 138-hr oocytes is only 1/4 (0.8 μm/3.2 μm) that of 156-hr oocyte nuclei, it seems likely that a number of oocyte nuclei at the early stages could have been sectioned in areas devoid of complexes even though small segments of the complex were present in other areas. For instance, if 20 thin sections each 700 Å thick (a total segment 1.4 μm thick) were cut beginning at one end and proceeding along the axis of an ellipsoidal nucleus with long dimension 4 μm, only about 1/3 of the total nucleus (1.4 μm/4 μm) would be sampled. In addition, a few sections on a grid are

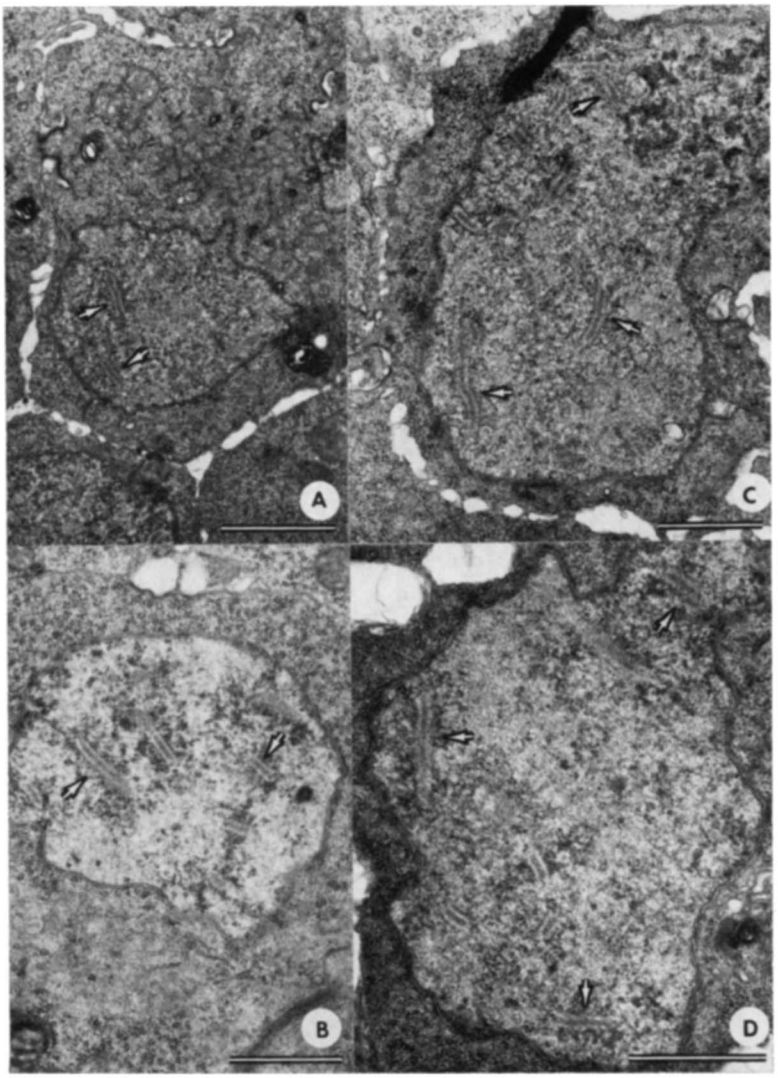

FIGURE 4.—Electron micrographs demonstrating the presence of synaptonemal complexes (*arrows*) in oocyte nuclei from pupae of (A) 138 hr, (B) 144 hr, (C) 150 hr, and (D) 156 hr of development after egg laying. The bars represent distances of 1 μm. [From: GRELL and DAY, 1974.]

normally obscured by grid bars, making the portion of the nucleus actually observed less than 1/3. This factor tends to make the frequencies at 138 hr and, to a lesser extent, at 144 hr underestimates of the proportion of oocytes containing complexes.

The tendency to underestimate due to the latter factor appears to be twice as great as the tendency to overestimate due to the volume factor (4 times less complex *versus* 2 times more oocytes sampled) at 138 hr. Consequently, at this

stage perhaps as many as 1/2 of the oocytes contain complexes. With these considerations in mind, it is also possible that a small proportion of oocytes at 132 hr may have contained complexes which were not detected by our sampling technique. Thus, it may be assumed that the complex system first forms in oocytes between 132 and 138 hr of female development and is extensive in all oocytes at 150 and 156 hr.

The conclusions reached with regard to the appearance and growth of the synaptonemal complex in oocytes are based on our observations that complexes are confined to only two nuclei of a cyst. Recent reports suggest that complexes may appear in a third cell of a 16-cell cyst (CARPENTER and BAKER 1974). These studies, which utilized adult material, gave no indication of the extent of the complexes nor the time of their appearance within the third cell. R. C. KING and his collaborators (see KING 1970) in their extensive investigations of the Drosophila oocyte, using serial reconstructions that should reveal all cells containing complexes within a cluster, reported that complexes were found only in the two pro-oocytes. If complexes do develop in a third cell of a cyst, this would seem to be a rare event, possibly confined to stages later than those studied in the present investigation.

Figure 5 illustrates the results of the autoradiographic studies of labeled pro-oocytes taken from 144 hr females. Electron microscope autoradiograms prepared on thin sections are shown in A and B; a light microscope autoradiogram of a thick section (bottom left) with an accompanying electron micrograph of an adjacent thin section (right) are shown in C. The black silver grains above the nuclei in A and B indicate foci of DNA synthesis while the arrows point to the simultaneous presence of synaptonemal complexes. In C, the arrow in the autoradiogram at the left indicates a labeled pro-oocyte while the arrows in the electron micrograph of this pro-oocyte at the right indicate synaptonemal complexes.

DISCUSSION

The adjective "synaptonemal," derived from the noun "synapsis," refers to the intimately paired state of homologs that is thought to be an attribute of the complex as well as a requirement for its existence. In terms of classical theory, the complex can come into being no earlier than the zygotene stage of prophase when homologous pairing is assumed to be initiated. In elaboration of these precepts, MOENS (1968) has described axial cores in unpaired leptotene chromosomes of *Lilium longiflorum* which he believes participate, two-by-two, in the formation of synaptonemal complexes at zygotene. In fact, MOENS redefines zygotene as the period when "axial cores of homologous chromosomes synapse to form synaptonemal complexes"; he further states that "the zygotene stage is completed when all axial cores have been incorporated into synaptonemal complexes." Beyond this, its presence at pachytene, a stage popularly identified as the time of crossing over, carries the implication that it plays a vital part in the exchange process (MOENS 1968; MEYER 1960, 1964). Thus, the nomenclature applied to

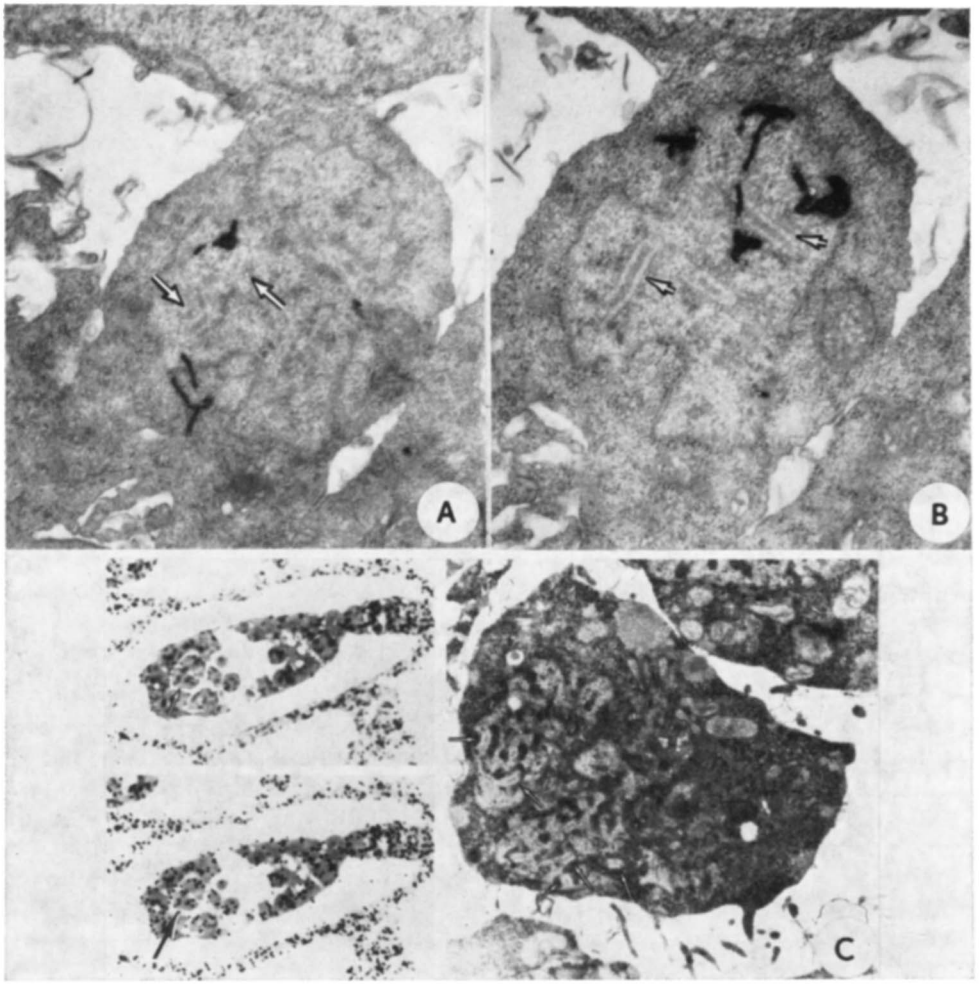

FIGURE 5.—Presence of synaptonemal complexes during DNA replication. A, B. Electron micrograph autoradiograms of pro-oocytes from 144 hr females showing label and synaptonemal complexes (*arrows*) within the same nuclei. C. Thick section of ovariole from a 144 hr female (left, above) and light microscope autoradiogram of same section (left, below). Black arrow indicates labeled pro-oocyte, whose adjacent thin section with synaptonemal complexes (*arrows*) is shown at right.

the structure confers upon it a narrowly circumscribed role, both functionally and temporally.

The present study is primarily concerned with the temporal role of the synaptonemal complex and, more specifically, with the time of its origin. Does its formation coincide with zygotene as current dogma requires' Unfortunately, the cytology of the Drosophila oocyte at the light-microscopic level continues to be intractable to analysis. Other lines of attack upon the problem are, however, available. For example, the main DNA synthesis may be used as a marker for premeiotic interphase and in this way serve as a reference point for complex

formation. On this basis, indirect evidence strongly suggesting that its appearance precedes meiotic prophase has been available for some time (GRELL 1969). KOCH, SMITH and KING (1967) observed complexes in oocytes residing in the anterior region of the adult germarium, and their calculations indicated that the complexes were present within 6 hr of oocyte formation. GRELL and CHANDLEY (1965) reported uptake of [^{3}H]thymidine by oocytes in identical regions of the adult germarium and at the same time showed that recombination could be enhanced by heat treatment during DNA synthesis. These studies suggested that DNA synthesis, genetic exchange, and synaptonemal complex formation were parallel events.

Subsequent studies by GRELL utilizing carefully timed pupal ovaries have more precisely defined the premeiotic S-phase and confirmed its coincidence with the sensitive period for crossover enhancement (GRELL 1967, 1973a, 1973b; GRELL and DAY 1974). Independent studies by DAY (1973) with a different autoradiographic technique confirm GRELL's observations on the coincidence of the S-phase and temperature sensitivity. GRELL and DAY (1974) have localized the period of DNA synthesis to between 132 and 162 hr and heat sensitivity to between 126 and 162 hr of female development for the most advanced oocyte in each germarium. The present result demonstrate that complexes are present in these oocytes at 138 hr; that they probably originate somewhat earlier, between 132 and 138 hr; and that they increase in length up to 156 hr, at least. Thus, a significant portion of their growth is coextensive with the S-phase as well as with the heat-sensitive period for enhancement of exchange. Direct evidence for the presence of synaptonemal complexes during DNA replication comes from the electron micrograph autoradiographs (Figure 5) showing both complexes and label within the same pro-oocyte nucleus. The pro-oocytes shown were taken from 144 hr-females, a time corresponding to the first half of DNA replication for those pro-oocytes in the posterior-most 16-cell cyst.

These results throw doubt on the traditional meiotic sequence, particularly the time of synapsis. Proof that pairing between homologs is initiated at zygotene has never been forthcoming. In fact, a variety of evidence suggests that homologous pairing may be a much earlier event (for discussion, see GRELL 1969). Our finding that the complex is present during the first quarter of the S-phase identifies the period of its formation as premeiotic interphase rather than zygotene. Serial reconstruction studies (GILLIES 1973; WETTSTEIN and SOTELO 1967; MOENS and PERKINS 1969) have made it clear that at pachytene the complex lies between paired homologs along their longitudinal axis. If the complex occupies the same position at premeiotic interphase, then we must conclude that homologs are paired and in a suitable position for exchange at that time.

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