THE CELLULAR PROGRAM FOR THE FORMATION AND DISSOLUTION OF THE SYNAPTONEMAL COMPLEX IN COPRINUS

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SUMMARY

Inhibition of protein synthesis by cycloheximide on processes in meiosis was used to probe the cellular program for the formation and dissolution of the synaptonemal complex (SC) in the synchronous meiotic system of Coprinus cinereus.

The pathway for the synthesis and assembly of the synaptonemal complex is proposed to be as follows: (1) synthesis and assembly of lateral components on the chromosomes; (2) synthesis and assembly of the central components in the nucleolus; (3) the lateral components of the homologous chromosomes are brought together to pair when the homologous pairing occurs at zygotene; (4) the transport of the central components from the nucleolus to join the paired lateral components and thus complete the synaptonemal complex.

Continued protein synthesis is required for all steps. Step (1) is nearly complete 2 h after the onset of karyogamy, because continued assembly is possible in the presence of cycloheximide. The transition point for step (2) is 4 h after the onset of karyogamy, as inhibition at this point results in accumulation of central components in the nucleolar dense body. The paired lateral components of step (3) are deprived of the central component. The transition point for step (4) is 5 h after the onset of karyogamy, for inhibition at this point no longer prevents transport.

Two steps are proposed for dissociation and dissolution of the SC at the end of pachytene. Protein synthesis is required for the dissolution of SC. Inhibition at this point causes accumulation of polycomplexes.

Mutations in various organisms from the literature relating to the SC support the validity of the proposed pathway.

INTRODUCTION

The synaptonemal complex (SC) was first discovered in crayfish by M. J. Moses (1958), who, by comparing thick and thin sections, defined it correctly as a structure involved in chromosome pairing. Since then, the complexes have been observed in the meiosis of all eukaryotes studied and have been the subject of several reviews (M. J. Moses, 1968; Westergaard & von Wettstein, 1972; von Wettstein, 1977; Gillies, 1975a; Moens, 1973). The sequence of events in the formation of the SC has been studied in some detail in a number of organisms (Lu, 1967; Moens, 1968; Zickler, 1977; Rasmussen & Holm, 1978; Gillies, 1975a; Westergaard & von Wettstein, 1970; von Wettstein, 1977; see also reviews by Westergaard & von Wettstein, 1972; Gillies, 1975b). It involves: (1) the production and assembly of lateral components associated with the chromosomes at leptotene; (2) the alignment of lateral components of the homologous
chromosomes side-by-side at zygotene; and finally (3) completion of the tripartite structure by the addition of the central components, which are thought to first assemble in the nucleolus prior to their transport to join the lateral components on the chromosomes. The most definitive studies of the sequence of events in the formation of the SC are those employing serial thin sectioning and three-dimensional reconstruction of the SC at leptotene, zygotene, pachytene and diplotene (Zickler, 1977; Rasmussen & Holm, 1978), which showed a continuous presence of lateral component from one end of the chromosome to the other at leptotene, followed by sequential additions of the central components at zygote (either zipping up or buttoning up) until completion of the SC at pachytene.

The SC is thought to be proteinaceous and to contain little or no DNA (Comings & Okada, 1970). This is based on the observations that the SC remains untouched after DNase digestion but is completely destroyed by trypsin digestion. The SC is also thought to contain RNA, based on observations using RNase and silver staining (Westergaard & von Wettstein, 1970).

The functions of the synaptonemal complex have also been extensively studied. Its involvement in chromosome pairing and recombination has come from the following observations: (1) achiasmate meiosis, with the exception of Bolbe (Gassner, 1969) and Panorpa (Welsh, 1973), appears to be correlated with the absence of the SC (Meyer, 1964; see also reviews by Westergaard & von Wettstein, 1972; Gillies, 1975b); (2) asynaptic mutants such as meI of Neurospora crassa (Lu & Galeazzi, 1978) or an oligochiasmatic man (Hulten, Solari & Skakkebæk, 1974) produce an incomplete SC; (3) recombination-deficient mutants such as c(3)G of Drosophila females produce no SC (Smith & King, 1968; Meyer, 1964; see also reviews by Baker et al. 1976; Gillies, 1975b); (4) in the synchronous meiotic system of Coprinus, the increase in genetic recombination induced by cold-temperature treatment is strictly correlated with the presence of the SC (Lu, 1970); the longer the SC is maintained, the higher the recombination frequency (Lu, 1974; Lu & Chiu, 1976); and finally, (5) the number and distribution of recombination nodules are such that suggest their involvement in recombination and chiasma formation (Carpenter, 1975, 1979a, b, 1981; Holm et al., 1981; Gillies, 1975a, 1979; Zickler, 1977; Rasmussen & Holm, 1978, 1980; Byers & Goetsch, 1975; von Wettstein, 1977; Carmi et al. 1978).

The dissolution of the SC normally occurs at diplotene (Moens, 1968; Zickler, 1977). Exceptions have been found in insects (Roth, 1966; Moens, 1969; Rasmussen, 1975), in yeast (Zickler & Olson, 1975), and in the domestic rooster (Schin, 1965) where dissolution is very much delayed. When the SC are sloughed off from the chromosomes, they form aggregated polycrplexes. In one case where SC are formed in achiasmate meiosis, e.g. Bolbe nigra, dissolution of the SC does not appear to occur and the SC may be found at meiotic metaphases and even in spermatids (Gassner, 1969).

More recently, SCs have been studied by a surface-spread technique (Counce & Meyer, 1972; Dresser & Moses, 1979; Tres, 1977), which allows a total view of SCs in many nuclei. This is especially valuable for assessing synaptic behaviour of rearranged chromosomes (Ashley, Moses & Solari, 1981; Ashley, Russel & Cacheiro,
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1982, 1983; K. J. Moses & Poorman, 1981; K. J. Moses, Poorman, Roderick & Davison, 1982; Gillies, 1981). From such observations, a new phenomenon of synaptic adjustment has emerged (Moses et al. 1982; Moses & Poorman, 1981). These authors suggest that pachytene be subdivided into five substages: at early pachytene (PI) chromosomes have strict homosynapsis; at PII to PIV, varying degrees of synaptic adjustment occur; and by PV, adjustment is complete and the chromosomes have heterosynapsis.

All of the above observations have raised the question of whether there are cellular programs for the synthesis, assembly and eventual dissolution of the SC. A cellular program is defined as a sequence of protein syntheses leading to the completion of a cellular event (e.g. formation of the SC).

In a previous paper my colleagues and I took advantage of the synchronous meiotic system of Coprinus (see Lu, 1982, for an overview) and used cycloheximide, a potent inhibitor of protein synthesis, as a probe to determine the transition point for the division program in meiosis (Lu & Chiu, 1978). The transition point was defined by Mitchison (1971) as the time at which protein or RNA synthesis for a given event is complete, and at which the cell is able to proceed to the subsequent stage. In the present paper, I have applied the same technique with the help of electron microscopy to determine the transition point(s) for the cellular programs for the formation and dissolution of the SC during the meiotic prophase I.

The results show that continued protein synthesis is required during zygotene for the formation and assembly of the SC, and that protein synthesis in the forming phase of the SC is complete 4—5 h after the onset of karyogamy; no dissolution of the SC can occur without protein synthesis, and the transition point for the dissolution was found to be 9 h after the beginning of karyogamy.

MATERIALS AND METHODS

Strains of Coprinus cinereus and the culturing techniques were the same as those used in previous studies (Lu, 1974; Lu & Jeng, 1975; Lu & Chiu, 1978). JR52 is a dendroid mutant and PR2301 is a methionine auxotroph. Dikaryotic cultures obtained from the cross JR52 × PR2301 were prepared on sterile horse dung in 90 mm × 50 mm crystallizing dishes and incubated in absolute darkness at 35 °C for 5 days. The dark-grown cultures were transferred on day 6 to an incubator at 25 °C fitted with a 6 W cool-white fluorescent lamp. The light regimen was set for a 16 h light/8 h dark cycle with the light cycle commencing at 1600 h. On day 10 at 2100 h, the cultures containing fruiting bodies that were judged to begin meiosis within 12 h were transferred to an incubator at 35 °C provided with a continuous light regimen to arrest the initiation of premeiotic S phase (Lu, 1974; Lu & Jeng, 1975). By so doing, all fruiting bodies were synchronized at the beginning of the premeiotic S phase. These arrested cultures were released on day 11 at 0900 h (this is referred to as arrest-release technique), and all fruiting bodies immediately started the premeiotic S phase, and after 5-5 h karyogamy began.

Treatment with cycloheximide

Soft nutrient agar (0·5 % agar) containing 50—100 µg/ml of cycloheximide was poured into a Petri dish to a depth of 2—3 mm, and allowed to cool and gel. Fruiting bodies to be treated first had their veil cells shaved off and then were covered with a layer of the drug-containing agar described by Raudaskoski & Lu (1980). The rate of entry of cycloheximide depends on the concentrations used. At 50—100 µg/ml, a positive effect can be observed in less than an hour. The minimal time required for an isotope to enter the meiotic tissue by cap feeding was determined to be 10 min.
Experimental

To study the effect of cycloheximide on the formation and dissolution of the SC, a synchronized population of fruiting bodies was achieved by the arrest-release technique. The timing of the formation and dissolution of the SC was carefully monitored previously (Lu & Chiu, 1978) and was found not to deviate from that of a normal culture.

Cycloheximide was applied to fruiting bodies at hourly intervals starting from 4 h after release (i.e., at late premeiotic S phase) until the first metaphase was reached. A gill sample was removed aseptically from each fruiting body before and at the end of treatment, when the control fruiting body has reached metaphase I, at midnight (or 15 h after the release) and fixed for electron microscopy. The pretreatment samples represented a set of control fruiting bodies in an hourly sequence of developmental stage for the formation and dissolution of the synaptonemal complexes. Another set of fruiting bodies was treated in the same way for the later stages, except that the post-treatment samples were collected and fixed 24 h after the treatment.

Collection of quantitative data

After thin sections were prepared and examined under the electron microscope, every basidium on a grid showing a nucleus was recorded for: (1) the presence or absence of the lateral component in the nucleus, regardless of prefusion or postfusion state; and (2) the presence or absence of central component in a tripartite structure. The purpose was not to determine whether the assembly of either the lateral components or the synaptonemal complexes was complete, but rather whether the production and assembly of lateral or central components had begun in each basidium. For the assembly of the central components in the nucleolus, every nucleolus in each sample was examined for the presence or absence of such components. The results are shown in Fig. 1.

RESULTS

Effect of cycloheximide on cell structure

Two ultrastructural changes were clearly evident as a result of treatment with cycloheximide. The first was the appearance of mitochondria whose matrix had become very electron-dense (Figs 5, 6, 7). This was in sharp contrast to the ones seen in samples taken before the treatment (Fig. 4). The second was the appearance of the nucleolus. In samples taken before the treatment, the nucleolus in most cases exhibited fibrillar components intermixed with granular components (Fig. 2), but in some cases a fibrillar dense body was seen (Fig. 3). In the samples taken some hours after the treatment, the fibrillar and granular components were segregated, with the granular component on the periphery and the fibrillar components aggregated in the centre (Figs 5–7). A donut-shaped nucleolar dense body was seen in some cases (Fig. 6). In samples taken after a longer period of treatment, no granular components were seen and the remains were fibrillar aggregates of the nucleolar dense body (Figs 9–12, 14–16).

The effect of cycloheximide treatment on the SC was unequivocal. If complexes had not been formed before the treatment, few complexes were formed after the treatment. If complexes had been formed before the treatment, the complexes remained even 24 h after the treatment and the morphology of the SC did not change appreciably (Figs 3, 4, pre-treatment; Figs 7, 8, post-treatment).

Effect of cycloheximide on the formation of synaptonemal complexes

The idea of dividing into stages the cellular program for the formation of the SC by treatment with cycloheximide was formed as follows: I assumed that protein
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Fig. 1. Effect of cycloheximide on the formation and dissolution of synaptonemal complex in C. cinereus. Fruiting bodies from arrest-release cultures were given 50 μg/ml cycloheximide (see Materials and Methods) at hourly intervals starting at the onset of karyogamy. Gill samples were removed before and after the treatment from the same fruiting body for electron microscopic observations for the presence of lateral components, central components and the synaptonemal complex (SC). K, karyogamy; P, pachytene; M, metaphase I. A. (■) Lateral component in pre-treatment samples; (□) lateral component in post-treatment samples; (●) SC in pre-treatment samples; (●) SC in post-treatment samples. B. (●) Assembled central components (CC) in the nucleolus in pre-treatment samples; (□) assembled central components in the nucleolus in post-treatment samples.
Figs 2–8
synthesis was required for the production of components and subsequent assembly into the SC. If synthesis of protein(s) for any of the components had not begun before the treatment with cycloheximide, the assembly of that component would not take place. But if the synthesis were 70% complete, the assembly might be 70% accomplished and could be observed in the electron microscope. Treatment with cycloheximide at different times during meiosis would allow one to delimit the 'transition point' between non-synthesis and synthesis of any component. By using this simple assumption, I was able to divide the formation and assembly of the synaptonemal complex into at least three distinct steps.

The first step was the production of the lateral components, which were assembled on the chromosomes at leptotene and later aligned at zygotene. Here abundant synthesis of such protein(s) was apparent in almost 70% of basidia within 1-2 h after the onset of karyogamy, and the assembly of the lateral components proceeded even in the presence of the inhibitor. This was particularly significant in the second bar in Fig. 1A, which shows a 30% increase in the axial component seen in the post-treatment sample over that in the pre-treatment sample (Fig. 1A). Continued protein synthesis was required to complete the assembly of the lateral components in the remaining basidia.

The second step was the production of the central components, which were believed to assemble first in the nucleolus and then to be transported to join the lateral components to complete the SC (Westergaard & von Wettstein, 1972). There was a 2 h lag time before the initial production of the central component was detected, as indicated by the increase in the appearance of tripartite synaptonemal complexes (Fig. 1A). At each time-point, the percentage of basidia with a SC was the same in pre-treatment and post-treatment samples. This suggested that before the synthesis of protein(s) for central components was complete, cycloheximide treatment completely prevented any further production and assembly of the SC. However, in the sample treated at 4 h post-karyogamy stage (i.e. completion of karyogamy), there was a striking anomaly, i.e. the presence of excessive accumulations of central components assembled in the nucleolus, as seen in Figs 10-12 and 14-16. The presence of central components in the nucleolus in an untreated cell has been observed (Fig. 9) but only

Abbreviations used in Figs 2-22: c, central component; ch, condensed chromosomes; f, fibrillar component; g, granular component; lc, lateral component; m, mitochondria; n, nucleus; ndb, nucleolar dense body; nu, nucleolus; rn, recombination nodule; SC, synaptonemal complex; pc, polycomplex.

Figs 2-8. Effect of cycloheximide treatment at early prophase I on ultrastructure of nucleolus, mitochondria and SC of C. cinereus. Bars, 0.5 μm.

Figs 2-4. Samples removed from a fruiting body before treatment (time of treatment is 4 h after the onset of karyogamy): Fig. 2 shows a normal nucleolus with fibrillar and granular components; Fig. 3 shows a nucleolus with a fibrillar dense body (ndb) and the pre-treatment SC; Fig. 4 shows SC and normal mitochondria.

Figs 5-8. Samples removed from the same fruiting body 6 h after the treatment: Fig. 5 shows abnormal mitochondria and the nucleolus where the nucleolar dense body is separated from the granular component; Fig. 6 shows a donut-shaped nucleolar dense body with a peripheral matrix of the granular component; Figs 7-8 show the post-treatment SC; note no difference between the pre-treatment and post-treatment SC.
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rarely, perhaps because the assembly and transport to the chromosomes were normally coordinated. Here the synthesis of proteins for central components was complete, and hence continued assembly of the central components in the nucleolus was possible in the presence of cycloheximide, but transport out to the chromosomes was impeded. This accumulation occurred only within one hour, as the subsequent sample showed no such accumulation. The data are shown in Fig. 1B. The above observation suggested that transport was the third step in the production and assembly of the synaptonemal complex. When transport was inhibited, central components became stranded in the nucleolus and so the paired lateral components were deprived of the central component (Figs 11, 13, 14, 15). However, some transport had occurred due to the presence of residual transport factor produced before treatment, as shown in Fig. 16, where the central component was still being transported out of the nucleolus and was continuous with the central component of a SC. This observation provided direct proof that the accumulated and assembled material in the nucleolus was the central component and that it was transported to the chromosomes to complete the SC. Fig. 12 also showed that a recombination nodule was associated with the central component before the transport was completed. The above observation also suggested that the transport was mediated by a protein transport factor, which was synthesized after a lag of an hour following the completion of the production of the central components. Thus the program for the production and assembly of the SC appears to include at least three overlapping but staggered steps.

Effect of cycloheximide on dissolution of synaptonemal complexes

In the normal course of events, synaptonemal complexes were removed from the chromosomes and were dissolved, except for the chiasmata, at the end of pachytene or at early diplotene. Treatment with cycloheximide prevented this removal and dissolution from taking place. This is shown in Fig. 1A, where the percentage of SCs from the same fruiting body was about the same before and after the treatment. However, at 9 h post-karyogamy (i.e. the end of pachytene), there was a 12% decrease in SCs (Fig. 1A) together with very condensed SCs and polycomplexes observed in the treated samples (Figs 18, 19).

Figs 9-16. Effect of cycloheximide on the assembly and transport of central components of *C. cinereus*. Fruiting bodies were treated at 4 h after the onset of karyogamy. Bars, 0.5 μm.

Fig. 9 shows a rarely observed assembly of central components in the nucleolus from a fruiting body before the treatment; a normal SC is seen attached to the nuclear envelope.

Figs 10–16, post-treatment samples from the same fruiting body, all showing massive accumulation of central components in the nucleolus; the same structure was seen in several consecutive sections: Figs 11, 13 and 14 show paired lateral components (l.c) deprived of the central component, suggesting that the central component is not transported to join the lateral components to complete the SC; Fig. 12 shows a recombination nodule associated with the assembled central component associated with the nucleolar dense body (the image may suggest that transport is imminent); Fig. 16 shows a continuation of the preassembled central component in the nucleolus with that in a SC.
It was apparent that protein synthesis was required for such an event. I considered this to be indicative of a cellular program for the removal and dissolution of the SC that was initiated at a precise time in meiosis. Before this program was complete, treatment with cycloheximide effectively stopped it and the SCs remained on the
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chromosomes. But after this program was complete, SCs were shed from the chromosomes and became condensed and aggregated to form polycomplexes (Fig. 19) as have been seen in many insects (Roth, 1966; Moens, 1969) and in yeast (Zickler & Olson, 1975). In time the chromosomes were expected to become condensed and the SC to disappear completely.

To confirm this expectation, another series of fruiting bodies was treated in the same manner, but treatment was left to continue overnight before the samples were taken, fixed and processed. It was found that samples treated before the transition point for dissolution of the SCs were seen to have the same number of SCs as the pretreatment samples (Fig. 1A). Fig. 17 shows a sample taken before treatment at the end of pachytene where the SC was normal, whereas Figs 20–22 show samples taken from the same fruiting body 24 h after treatment. No SC was observed; instead, the chromosomes were condensed. Here the fruiting body was treated after the transition point. The condensed chromosomes exhibit a dense core surrounded by chromatin material (Figs 20, 21). Fig. 22 shows very condensed chromosomes. In a few cases, formation of spindle tubules inside the nucleus was observed (not shown).

DISCUSSION

Treatment with cycloheximide, a potent inhibitor of protein synthesis, as used in the present study is a blunt instrument, which affects not only the synthesis of SC components, but also other protein-mediated events that might, in some way, influence the assembly of SC components that can be observed. Since such events are not known, the discussion in the following pages is deliberately presented in a simplistic way.

The effect of cycloheximide on structural changes in the nucleolus observed in the present studies deserves further attention. A similar effect on onion cells has also been reported (Medina, Risueno, Sanchez-Pina & Fernandez-Gomez, 1983). The transport of granular components (which are ribosomal precursors) to the peripheral regions may be taken to mean that synthesis of ribosomal RNA and assembly of ribosomal precursors have been indirectly inhibited by cycloheximide, and that the granular components already assembled are being moved from the nucleolus by the

Figs 17–22. Effect of cycloheximide on dissociation and dissolution of the SC. Bars, 0.5 μm.

Fig. 17, a sample taken before treatment at 9 h after the onset of karyogamy; normal SC with recombination nodule is still present.

Figs 18–19, sample taken after 1 h treatment from a fruiting body whose pre-treatment sample (not shown) is similar to that of Fig. 17, SC with thickened lateral components (Fig. 18) and polycomplexes (Fig. 19) are observed.

Figs 20–22, sample taken 24 h after treatment from a fruiting body whose pre-treatment sample is shown in Fig. 17; the SCs have disappeared and the chromosome condensation is apparent; Figs 21 and 22 show longitudinal and cross-sections, respectively, of initial stage of chromosome condensation, which shows condensing chromatin around a dense core.

Fig. 22 shows extremely condensed chromosomes.
existing transport mechanism. This leaves the fibrillar remains of the nucleolar matrix with the nucleolar dense body. Similar transformation has been observed in basidiocarps arrested at premeiotic S phase for 24 h by restrictive conditions (35°C, under continuous light) whereby all basidia have reverted to mitosis instead of proceeding to meiosis, and the nucleolus is reduced to 1/10 of the meiotic size (Lu, 1974). In this case, the nucleolus includes no granular components, and the fibrillar nucleolar dense body is donut-shaped, as a vacuole has formed in it (Lu, 1974). Thus the depletion of the granular components and the formation of the fibrillar nucleolar dense body appear to be associated with inactivity or regression of the meiotic nucleolus, either in the presence of cycloheximide or in the arrested basidia where the meiotic process has been interrupted. The nucleolar dense body has been seen rarely in control basidia. Cycloheximide treatment caused the depletion of the other components from the nucleolus, making the nucleolar dense body visible in every basidium. The nucleolar dense body has also been observed in yeast (Moens & Kundu, 1982; Horesh, Simchen & Friedmann, 1979; Zickler & Olson, 1975).

The steps involved in the formation of the synaptonemal complex have been well documented (Lu, 1967; Zickler, 1977; Moens, 1968; Rasmussen & Holm, 1978; von Wettstein, 1977; and reviews by Westergaard & von Wettstein, 1972; Gillies, 1975b). They may be summarized in the following sequence: (1) synthesis and assembly of lateral components on the chromosomes between two sister chromatids; (2) synthesis and assembly of the central components in the nucleolus; (3) when the homologous chromosomes pair-up, the lateral components are brought together and aligned in juxtaposition, what brings them together is not known; and (4) the transport of the central components from the nucleolus to join the paired lateral components to complete the synaptonemal complex on the chromosomes. The sequence of events is particularly clearly demonstrated by serial sectioning and three-dimensional reconstruction of different meiotic stages (Zickler, 1977; Rasmussen & Holm, 1978).

The present study has reconfirmed the presence of such a sequence of events in synchronized meiosis in Coprinus and also shown that each step is directly preceded by protein synthesis. As shown in Fig. 1A, the transition point for the synthesis of the lateral component is 3 h ahead of that of the central component, which in turn is 1 h ahead of that of transport protein. As I have suggested, cellular programs proceed in steps (Lu & Chiu, 1978; Lu, 1982), reminiscent of a pathway, such as: lateral component \(\rightarrow\) central component \(\rightarrow\) chromosome pairing \(\rightarrow\) transport of central component and assembly of synaptonemal complex. Any block, such as that caused by cycloheximide, not only prevents the forward movement but also causes accumulation of the components of the step before the block. For example, a block at 1 h post-karyogamy prevents completion of the SC, but allows continued accumulation of lateral components. This is true only when enough synthesis of proteins for that step has occurred (hence, the transition point).

The most significant observation is the massive accumulation of assembled central components in the nucleolar dense body when a block is effected at 4 h post-karyogamy (Fig. 1B). At this time, synthesis of central components is already complete, continued self-assembly is possible even in the presence of cycloheximide, but
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transport is blocked. An hour later, when the transport program is complete, a block is no longer possible and no accumulation of central components is seen. The accumulation of central components in the nucleolar dense body has been reported in yeast homozygous for a temperature-sensitive mutation spo10 (Moens & Kundu, 1982) and cdc4 (Horesh et al. 1979). Moens & Kundu (1982) called them polysynaptonemal complexes (pSC). They also attributed such an accumulation to excess synthesis of central component due to genetic imbalance in the regulation of central element production or to a deficiency of signal for the transition from zygotene–pachytene to diplotene–metaphase I. In the present study, the stage at which such accumulation occurs is clearly delimited at 4 h after the onset of karyogamy, which is still during late zygotene and early pachytene. A possible alternative interpretation, in the light of the present study, to the observations of Moens & Kundu is that the spo10 mutation really affects the transport of the central components. The same may be said about cdc4/cdcA of yeast. This interpretation is in keeping with the observations of Moens & Kundu (1982) and Horesh et al. (1979) that the longer the incubation under the restrictive conditions, the higher the percentage of cells with pSCs (or presynaptons) in the nucleolar dense body. Furthermore, in Coprinus, when the accumulation of central components is seen within the nucleolus, the paired lateral components are seen to be deprived of central components (Figs 13, 14). The photograph published by Moens & Kundu also showed a lack of central components, and they stated 'the tripartite structure of two lateral components with a central component is not well defined'. Thus, the accumulation of central components in the nucleolus and paired lateral components deprived of central components, strongly indicates that transport of the central components from the nucleolus to the chromosome sites is controlled by an independent genetic step.

The accumulation of central components in the nucleolus has been observed to occur before chromosome pairing in Neottiella rutilans (Westergaard & von Wettstein, 1970; von Wettstein, 1977). A similar accumulation has been seen in a variety of species (see review by Westergaard & von Wettstein, 1972). Westergaard & von Wettstein (1972) suggested that 'the central regions are first synthesized and assembled in the nucleolus, and then transported from the nucleolus to the pairing chromosomes and laid down between homologous chromosomes to join their lateral components'. They also noted that there is not sufficient evidence to support this suggestion. Fig. 16 shows a direct link between the central components accumulated in the nucleolus and that at the synaptonemal complex, which may be interpreted to mean that the accumulated central component is being transported to the homologous chromosomes to join the lateral component. Thus the present observation has provided the strongest visual evidence in support of the suggestion made by Westergaard & von Wettstein (1972).

The existence of a cellular program for the dissociation of synaptonemal complexes from the chromosomes and their final dissolution in the basidia is clear-cut. Before this program is complete (at the transition point), treatment with cycloheximide prevents the dissociation and dissolution from taking place. As shown in Fig. 1A, all SCs remain on the chromosomes. The transition point appears to be at 9 h after the
onset of karyogamy or the end of pachytene, at which time, the dissociated SCs is observed as polycomplexes in the presence of cycloheximide (Figs 18, 19). It should be noted that this sample was only treated for 1 h before electron microscopy. Within 1 h of treatment there is 12% less SC than in the post-treatment sample (Fig. 1A). Similar treatment, but continued overnight in the presence of cycloheximide before the samples were taken for electron microscopy, showed no sign of any remaining SC or polycomplexes. Instead, chromosomes condensation was observed (Figs 21, 22). Chromosome condensation in the presence of cycloheximide at this time has also been demonstrated by light microscopy (Lu & Chiu, 1978). Thus it is clear that the transition point for dissociation and dissolution occurs at the end of pachytene or thereabouts.

In summary, the formation of the SC appears to involve a staggered sequence of events, rather like a pathway, which includes at least four steps as outlined above. For the eventual dissolution, two steps may be involved. With the exception of alignment of lateral components (step (3)), the control of which is yet to be studied, each step is preceded by protein synthesis. At this juncture, one might speculate that each step involves a co-ordinated turning-on and turning-off of genes, and the pathway might look like the one proposed in Fig. 23.

Step (1) is the synthesis and assembly of the lateral component, which is probably controlled by L-gene(s). A mutation in this step would result in complete failure to make any component of the SC. An example has been found in c(3)G mutant of Drosophila (Meyer, 1964; Smith & King, 1968) in which no component of any kind is produced in the mutant homozygous for c(3)G. On the other hand, a precocious turning-on of the L-genes would lead to an accumulation of lateral components in the cytoplasm as was observed in Ascaris by Bogdanov (1977).

Step (2) is the synthesis and assembly of the central components in the nucleolus; it is suggested that their production is turned on by the completion of lateral components. Mutation at this step would be asynaptic in nature and would produce no central component. An example has been found in asynaptic wheat (LaCour & Wells, 1970) in which lateral components but no central component are produced.

Step (3) is the alignment of lateral components. This is brought about by pairing of homologous chromosomes. A mutation at this step would result in asynapsis of chromosomes such as mei1 or Neurospora (Lu & Galeazzi, 1978) in which lateral components are produced and the SC can be formed when the homologous chromosomes occasionally come together. What brings about pairing of homologous chromosomes is not understood.

Step (4) is the synthesis of transport factor whose production may be triggered by the central components. The assembled central components are transported with the help of the transport factor to join the paired lateral components to complete the assembly of the SC. Mutation at this step would result in the accumulation of the assembled central components in the nucleolus, leaving incomplete SCs. Examples have been found in an oligochiasmatic man (Hulten et al. 1974), in whom homologous chromosome alignments do occur but the SC is incomplete. Hulten et al. (1974) have documented the presence of well-formed lateral components and an occasional central
component in small segments. They also described the nucleolus as having 1000 Å channels surrounded by fibrillar material. It is possible that the channels they observed (though one picture is insufficient to assess the case), may be analogous to the accumulated central components described in this paper (Figs 9–16). If this were the case, the oligochiasmatic man should be considered to have a mutation in the transport factor. Another example is found in the yeast strain homozygous for a temperature-sensitive mutation cdc4 (Horesh et al. 1979), which accumulated central component in the nucleolar dense body when incubated at a restrictive temperature of 34°C. The longer the incubation at 34°C the higher the accumulation. I believe that yeast spo10 mutant described by Moens & Kundu (1982) also belongs to this step.
Table 1. Comparison of asynaptic mutants found in the literature, with respect to the assembly of the synaptonemal complex

<table>
<thead>
<tr>
<th>Mutants</th>
<th>Lateral component</th>
<th>Central component</th>
<th>Pairing of homologous chromosomes</th>
<th>Complete SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>cdc4/cdc4 yeast</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Oligochiasmatic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>man mei1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Neurospora</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Asynaptic wheat</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+, Events observed; −, events not observed.

In the case of the asynaptic mutants, asynaptic wheat, oligochiasmatic man, Neurospora homozygous for mei1 and yeast homozygous for cdc4, it should be of interest to compare their similarities and differences. These are listed in Table 1.

All these mutants produced lateral components and all except the asynaptic wheat produced central components, although central components were observed only in limited regions in homozygous mei1 of Neurospora (Lu & Galeazzi, 1978) and in the oligochiasmatic man (Hulten et al. 1974). The accumulation of central components in the nucleolus was not looked for in homozygous mei1 and could not be assessed, but was suggested to occur in the oligochiasmatic man. The pairing of homologous chromosomes was observed in the oligochiasmatic man and in yeast homozygous for cdc4, but not in homozygous mei1 of Neurospora nor in asynaptic wheat. All of these mutants failed to produce fully completed SCs. In the oligochiasmatic man, homologous chromosomes were paired, as seen by light microscopy, but the formation of the SC was incomplete. Thus chromosome pairing is necessary but not sufficient for the complete formation of the SC. Thus each of these mutants is unique on the basis of the available information and they appear to be consistent with the proposed pathway.

In a desynaptic mutant of Tradescantia paludosa, helicoidal polycomplexes are always found in dense bodies, which are nude in the wild type (Kehlhoffner & Dietrich, 1983). In such a mutant, complete pairing has never been found, regions of normal SC are interrupted by segments of structurally abnormal SC, and cytoplasmic polycomplexes are never found. It is possible that this mutant might also have some form of transport deficiency so that the central components are not transported out of or are returned to the dense body (or trapped). This mutant would fit in late step (4) but definitely before step (5), because cytoplasmic polycomplexes would have appeared after step (5).

Synaptic adjustment has been demonstrated in cases where chromosomal rearrangement occurs (Moses et al. 1982; Moses & Poorman, 1981). It appears that
such an adjustment may not be necessary when homologous pairing is perfect, as in normal meiosis. The synaptic adjustment at late pachytene may be a remedy, in case of chromosomal rearrangement, to safeguard proper disjunction. The synaptic adjustment may involve breakage and rejoicing of the SC.

Step (5) is the dissociation of the SC. Two possible misprogrammings could take place. First, the dissociating factor could be precociously produced. This mutation would result in desynapsis of chromosomes. An example has been found in a Drosophila female with a genotype of Df(3)sbd105/+, which results in premature dissolution of the SC and, as a consequence, recombination is drastically reduced (Smith & King, 1968). Second, no dissociating factor may be produced and, as a consequence, the SC would remain on the chromosomes at metaphase I and even in the spermatid. An example has been found in an achiasmatic male Bolbe (Gassner, 1969). It is interesting to note that achiasmatic meiosis normally produces no SC. However, a misprogramming can cause the SC to be produced while the dissociation and dissolution programs remain turned off, as expected in an achiasmatic meiosis. This interpretation may account for Gassner’s (1969) observations of SCs at metaphase I and later stages.

Step (6) is the production of enzymes responsible for the dissolution of the SC. A delay in this program would cause massive accumulation of polycomplexes, as is commonly seen in many insects (Roth, 1966; Moens, 1969; Rasmussen, 1975; Dudley, 1973) and in yeast (Zickler & Olsen, 1975). The dissolution appears to start from the central component, as observed in yeast homozygous for the temperature-sensitive cdc5 mutation (Horesh et al. 1979). Child & Byers (1980) suggested that the cdc5 mutation may have its effect at a later stage.

The above hypothesis, although speculative, appears to be scientifically reasonable. Each step is supported by one or more examples of genetic mutations. Further analysis of the program awaits the discovery of more mutations affecting SC formation.

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REFERENCES


The synaptonemal complex in Coprinus


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