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Meiosis in a Temperature-Sensitive DNA-Synthesis Mutant and in an Apomictic Yeast Strain (Saccharomyces cerevisiae) [and Discussion]

P. B. Moens, M. Mowat, M. S. Esposito, R. E. Esposito, G. Simchen and B. C. Lamb

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Meiosis in a temperature-sensitive DNA-synthesis mutant and in an apomictic yeast strain (*Saccharomyces cerevisiae*)

By P. B. Moens,† M. Mowat,‡ M. S. Esposito§ and R. E. Esposito§
† *Department of Biology, York University, Downsview, Ontario, Canada*
‡ *Department of Genetics, University of Alberta, Edmonton, Alberta, Canada*
§ *Department of Biology, University of Chicago, Chicago, Illinois* 60637, *U.S.A.*

[Plates 1 and 2]

It is shown that in the temperature-sensitive yeast mutant (*Saccharomyces cerevisiae*) *spo 11* at the restrictive temperature of 34 °C, (1) premeiotic DNA synthesis is nearly completely blocked; (2) the nucleus enters meiotic prophase indicated by the formation of axial cores and polysynaptonemal complexes; (3) the kinetic apparatus functions normally at meiosis I and II; (4) early spore formation occurs in nearly all cells but it is variable and all spores eventually degenerate. It is concluded that chromosome replication is not a prerequisite for the functions listed above.

The apomictic yeast strain 4117 produces 2 diploid spores. It is shown that a diploid which produces 2-spored asci, synthesized from 4117, no. 5, and an adenine requiring strain (1) has a normal meiotic prophase with abundant synaptonemal complexes; (2) has only one meiotic spindle; (3) has spores which form red clones more frequently than normal or u.v.-treated vegetative cells form *ade/ade* red sectors through mitotic recombination. It is concluded that this apomictic yeast has maintained meiotic prophase, but that one of the two meiotic divisions is suppressed.

1. Introduction

This report examines some details of the meiotic cell cycle through the description of two unusual meiotic mutants. Mutant *spo 11* does not undergo premeiotic DNA synthesis at 34 °C but continues through the cycle, presumably with unduplicated chromosomes. Information can be gained on the ability of such chromosomes to pair, form synaptonemal complexes and undergo recombination in the two-strand stage. The other unusual yeast produces only 2 spores per ascus by skipping one meiotic division. Here too the nature of chromosome pairing and exchange can be studied and conclusions reached about independence of meiotic recombination, disjunction, and chromosome segregation. In both cases the operation of the kinetic apparatus can be monitored under abnormal circumstances.

2. Materials and methods

When placed in sporulation medium (2% potassium acetate solution) control yeast (Z 190-8B) increases the ratio of ^{14}C in DNA, relative to the value at 0 h, from 1 at 0 h to 2.1 at 12 h. In *spo 11* the value increases from 1 to 1.25 (Esposito & Esposito 1974*a*). These values include increases in mitochondrial DNA. The changes in DNA content of *spo 11* cells used for electron microscopy in this study were determined fluorometrically (Simchen, Piñon & Salts 1972). Strain ATCC 4117 (2-spored asci) has 108 μg per 3×10^9 vegetative cells and 215 μg per

3×10^9 asci. The haploid value of strain X 901-35C is 51 μg per 3×10^9 cells (Grewal & Miller 1972).

For sporulation the cells were collected in log phase from presporulation medium and transferred, after two washes, to sporulation medium (Fast 1973). The *spo 11* and Z 190-8B strains were grown and sporulated at 25 or 34 °C, the other strains at 30 °C. The possible occurrence of meiotic levels of recombination in sporulating *spo 11* at 34 °C can be determined by returning samples to growth medium at different times during meiotic prophase and by scoring for intragenic recombination leading to prototrophy and exchange (Esposito & Esposito 1974*b*).

Strain 4117 with 2-spored asci was treated with EMS and then u.v. irradiated to induce recessive homozygosity for histidine auxotrophy. This 4117, no. 5, diploid was force-mated with a strain of *a* mating type and homozygous for histidine and adenine requirements. Homozygous *ade*/*ade* clones are red. Mated cells were selected through complementation on *his* plates. The resultant triploids were sporulated and 4-spored asci were dissected. Of the resulting clones, some were found to produce only 2-spored asci, and to be heterozygous for the adenine requirement as judged by red sectoring following u.v. irradiation, which changes mitotic recombination. In such clones, red sectoring was monitored in vegetative cells, u.v. irradiated cells and sporulated cells were scored for red sectoring.

The same 4117, no. 5, strain was also mated to the homozygous *a*/*a*, *his*1/*his*1, *ade*1/*ade*1 diploid 12C. The resulting tetraploid clones FM4 12X1, 2, 3, and 4 were selected by complementation for the histidine requirement. They produce 2, 3 and 4-spored asci. Diploid clones grown from dissected 4-spored asci were checked for 2-spored phenotype and heterozygosity of the adenine requirement. Three such strains, Fm4 12C2-18A, Fm4 12C1-10A, and Fm4 12C1-14C were grown vegetatively, u.v. irradiated, and sporulated. They are described here (table 1).

Electron micrography of samples were fixed and stained according to the method of Zickler & Olson (1975) using zymolase rather than glusulase to digest the cell walls.

3. Results

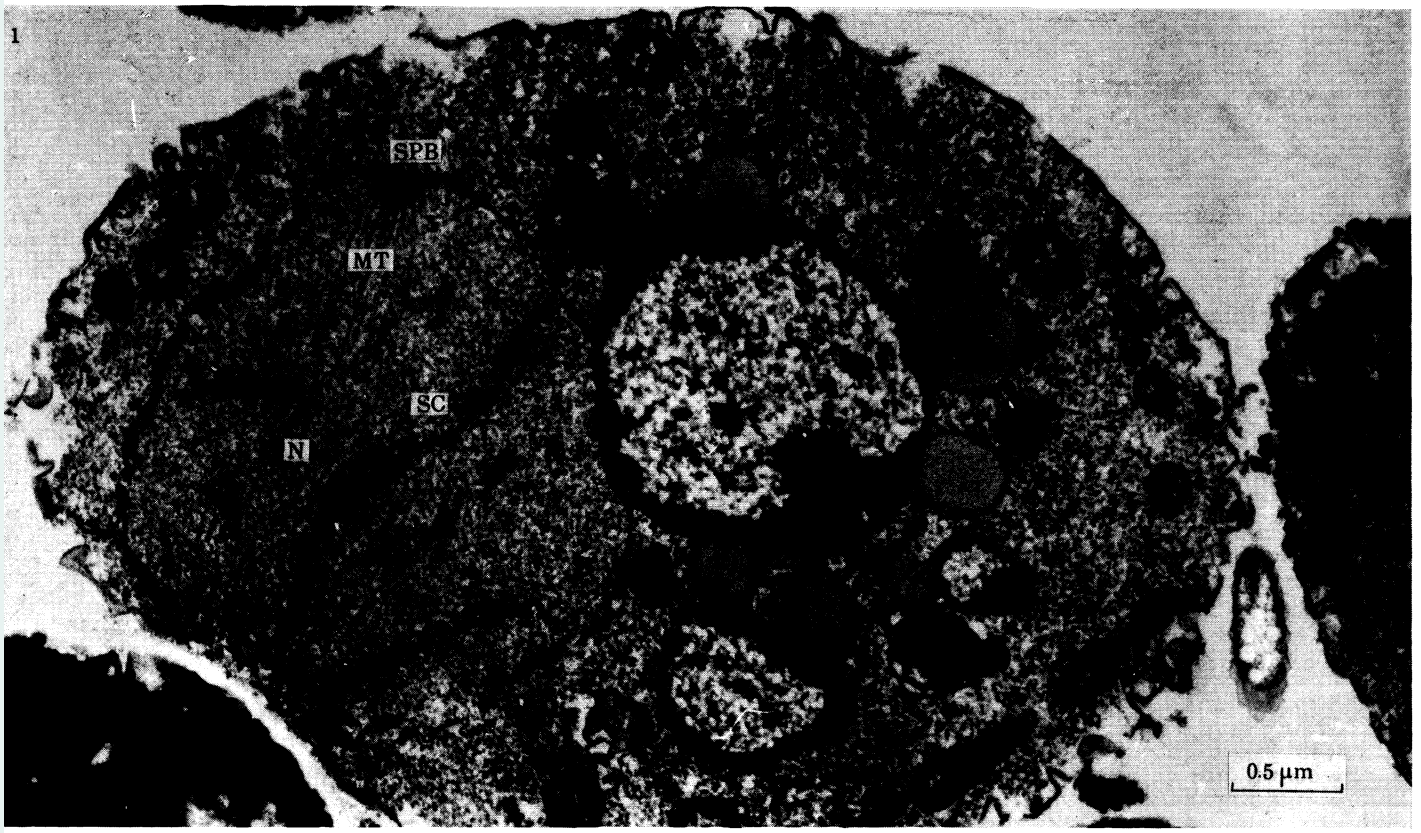
(*a*) spo 11

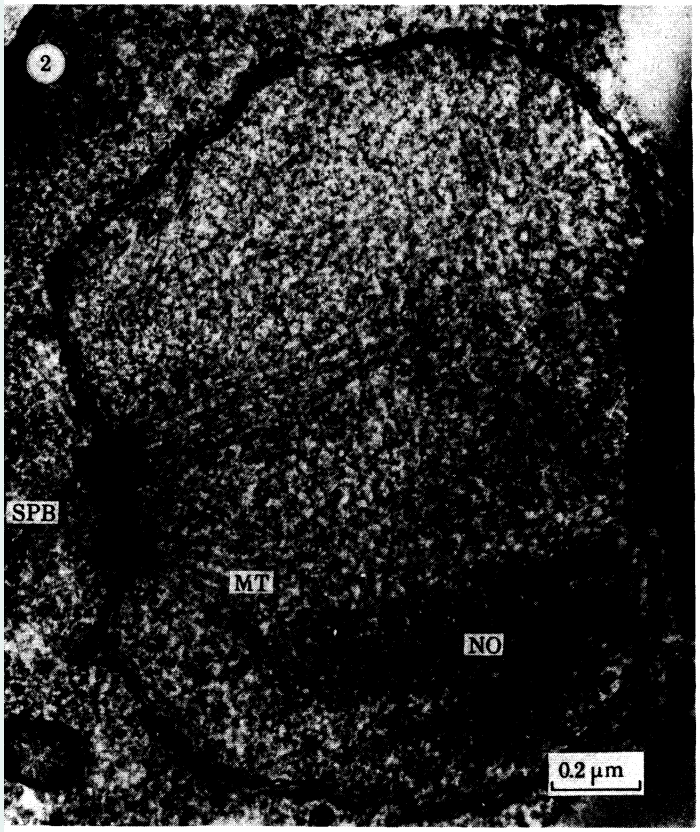
(i) *DNA metabolism at* 34 °C

The DNA content of cells used for electron microscopy analysis was checked fluorometrically. The values for *spo 11* remained at about 4 μg per 10^8 cells in the 3, 6, and 12 h samples while the values for the control Z 190-8B increased from 4 μg at 3 and 6 h to 6.7 μg at 12 h (averages of 3 measurements). At 24 and 48 h, *spo 11* had no spores visible with light- or interference microscopy. The control had about 60 % sporulated cells. At 25 °C *spo 11* and control have normal DNA metabolism and sporulation.

(ii) *Electron microscopy*

The control Z 190-8B at 34 °C for 12 h on sporulation medium has cells in meiotic prophase, cells with synaptonemal complexes (figure 1, plate 1), cells in meiotic division, and cells in early spore formation. Details of the normal sequence of events have been described previously (Moens & Rapport 1971; Zickler & Olson 1975). *spo 11* at 34 °C proceeds somewhat more rapidly through sporulation than Z 190-8B. Even at 6 h the nucleoli contain a dense staining body, the polycomplex body (which is assumed to produce axial core material), some of the





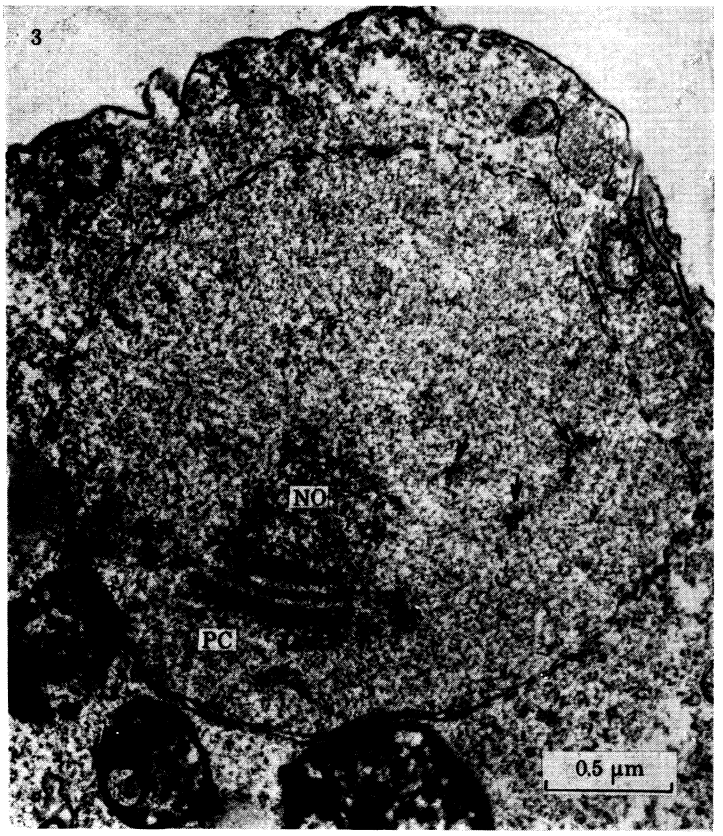


FIGURE 1. Control yeast Z 190-8B, 12 h on sporulation medium at 35 °C. The nucleus *N* contains synaptonemal complexes *Sc*, spindle pole body *SPB* with associated microtubules *MT*. (Magn. × 28 500.)

FIGURE 2. Mutant *spo 11* grown for 12 h on sporulation medium at 34 °C does not undergo DNA synthesis but the spindle pole body *SPB* undergoes normal duplication. Nucleolus *NO*. (Mag. × 57 000.)

FIGURE 3. Mutant *spo 11*, 12 h, 34 °C forms axial cores (arrows), polysynaptonemal complexes *PC*, usually in association with the nucleolus. Good complexes as in figure 1 were not found under these conditions nor in *spo 11* sporulated at 25 °C. (Magn. × 33 200.)

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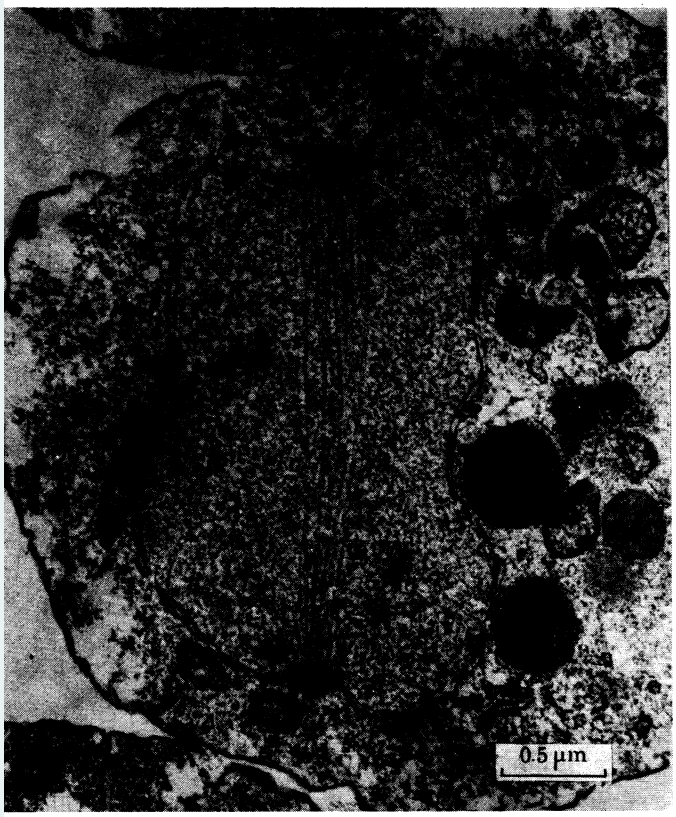

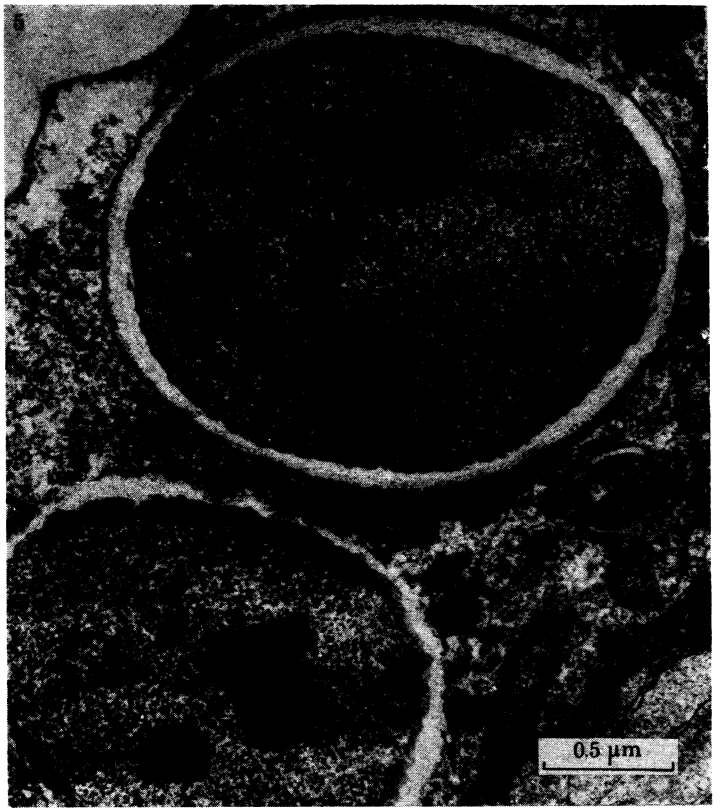

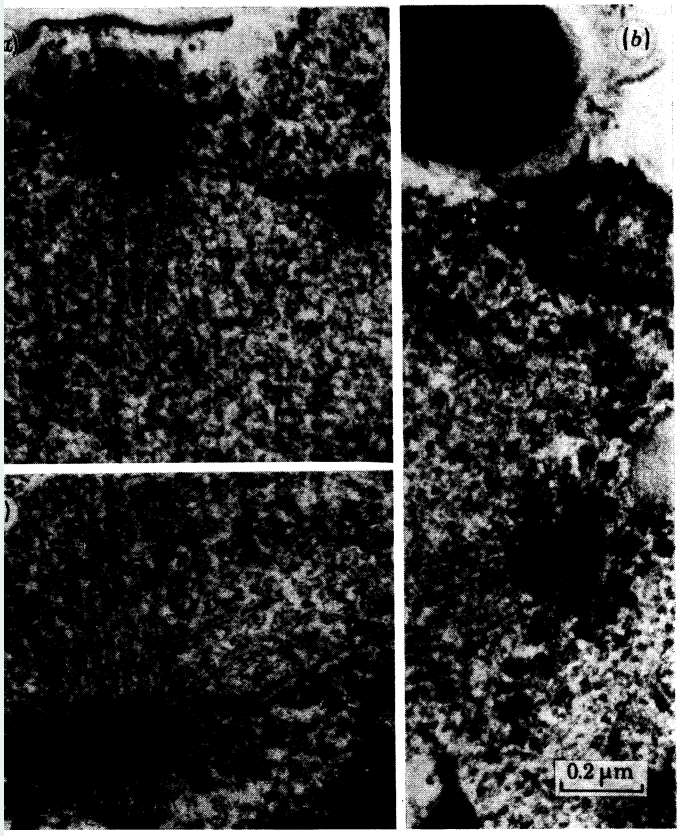

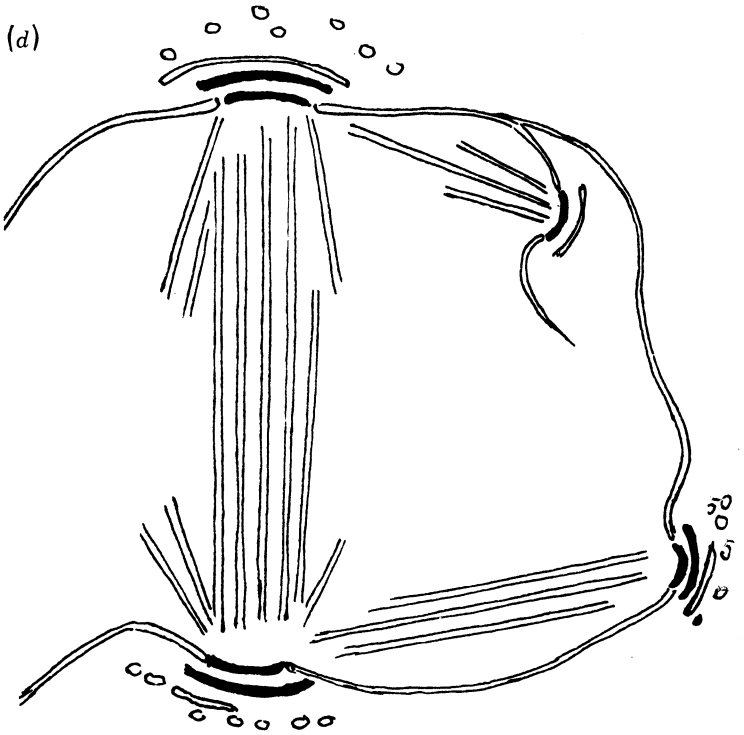

FIGURE 4. Mutant *spo 11*, at the restrictive temperature produces a normal anaphase I spindle indicating that the kinetic apparatus is not directly dependent on the presence of duplicated chromosomes. (Magn. × 26 600.)

FIGURE 5. Mutant *spo 11* at the restrictive temperature produces some aberrant and many normal looking early ascospores. All of these are aneuploid and degenerate shortly after this stage. (Magn. × 33 200.)

FIGURE 6. Strain Fm4 12C2-18A has the large spindle as in the single-division-meiosis parent 4117, no. 5, but the nucleus also contains poorly developed 3rd and 4th spindle pole bodies (*b*, *d*), presumably an incomplete expression of the 2-division meiosis parent 12C. (Magn. × 49 400.)

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cells already have numerous axial cores and some of the spindle pole bodies have divided (figure 2, plate 1). At 12 h the cells have axial cores, polycomplexes, but no obvious synaptonemal complexes (figure 3, plate 1), and all stages of meiotic division (figure 4, plate 2) and spore formation (figure 5, plate 2) are present. The spindle pole bodies and spindles are of normal appearance and behaviour (figures 2 and 4). The formation of the early spore wall is frequently irregular, failing to develop at one or more spindle poles or forming double walls

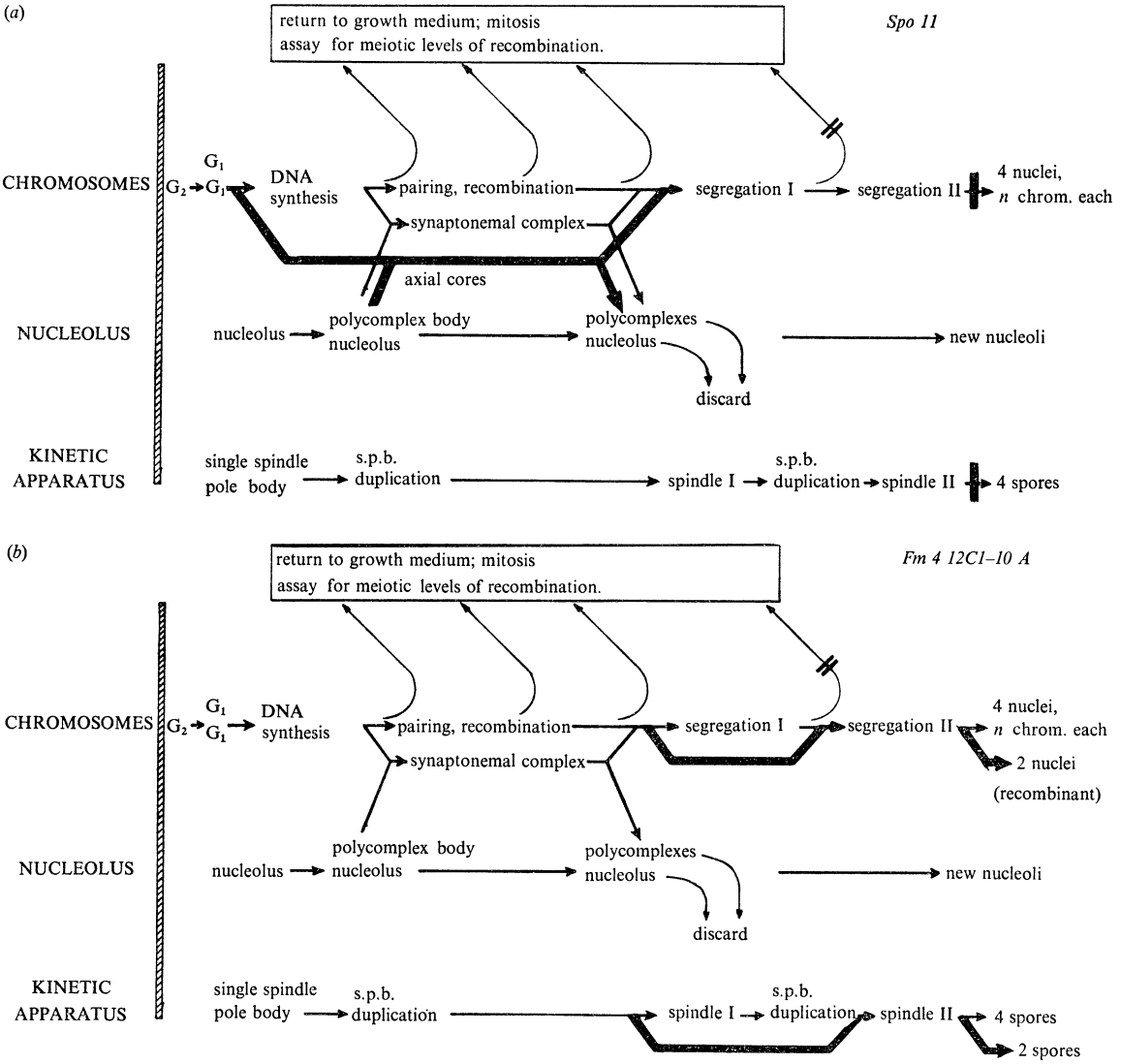

FIGURE 7. Diagrams of the development of chromosomes, nucleolus and kinetic apparatus in normal yeast. Superimposed in heavy bars are the characteristics of *spo 11* at 34 °C (*a*) and of Fm4 12C1-10A (*b*).

around a single spore. Although the early spores are obvious with the electron microscope in 60–80 % of the cells, they cannot be detected with the light microscope. They all degenerate, presumably each contains on the average only half a genome, and no refractile spores ever become visible in light microscopy at 24 or 48 h. *spo 11* at 25 °C develops normally but the samples examined so far show most cells in meiotic prophase with axial cores rather than synaptonemal complexes. The results are summarized in figure 7*a*.

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(iii) *Recombination*

The presence or absence of meiotic levels of recombination (Esposito & Esposito 1974*b*) among the unduplicated *spo 11* chromosomes in meiotic prophase at 34 °C is being examined at the time of preparation of this manuscript and will be reported separately.

(*b*) *Two-spored asci*

(i) *DNA metabolism*

No new determinations of DNA content were made and the values of Grewal & Miller (1972) are used here. These values indicate that strain 4117 is disploid, that it replicates DNA during premeiotic S phase, that each of the 2 spores is diploid and that prior to germination the spores replicate their DNA again so that the ascus now has an 8C content, and, through mitosis four diploid cells are produced. It is assumed that all cells have one or more copies of both mating factors *a* and α since no mating takes place and the cells are competent to sporulate.

(ii) *Electron microscopy*

The morphological aspects of meiosis in 4117 have been described by Moens (1974) and spore formation by Grewal & Miller (1972). Only two spores are formed because there is only one nuclear division in the ascus. There is no loss of nuclei or spores as is the case in strains which produce 2-spored asci but still have two meiotic divisions (James 1974). Using the new fixation and staining method according to Zickler & Olson (1975) it has become possible to recognize synaptonemal complexes with greater ease. Abundant axial cores and few synaptonemal complexes were observed in strain 4117. The tetraploid Fm4 12C (from 4117, no. 5, ×12C) has abundant synaptonemal complexes. Asci from tetraploid Fm4 12C contain 2, 3 or 4 spores. All spores of 4-spored asci produce clones which will sporulate again. The diploid clones selected from 4-spored asci had variable characteristics when sporulated again (Morrison *et al.* 1975).

Fm4 12C1-10A produces abundant synaptonemal complexes, only a single division as in the original 4117 strain, and only 2 spores. The spores, however, are different from the 2 spores in 4117 asci in that they do not fill the entire ascus and they do not have the connecting structure (Grewal & Miller 1972). The results are summarized in figure 7*b*.

Fm4 12C1-14C produces axial cores during meiotic prophase but no obvious synaptonemal complexes. They possibly occurred at earlier or later times but it is not likely that they are abundant at any time. The tendency to make 4 spores is moderately well suppressed but occasionally 3 spores are formed.

Fm4 12C2-18A has abundant synaptonemal complexes but the second spindle pole duplication is incompletely suppressed (figure 6, plate 29). In figure 6(*a*, *b*) there is a major spindle and each spindle pole body has produced a small satellite (figure 6*c*, *d*) with a minor spindle. Sporewall and spore formation are variable. Occasionally 2 good spindles and 4 normal looking spores are produced.

(iii) *Recombination*

One clone of each of the diploid strains heterozygous for adenine auxotrophy was grown in nutrient medium and then half were placed in sporulation medium and the other half was plated on nutrient agar and given 0–40 s u.v. irradiation. After 7 days, red sectors were scored.

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The sporulated cells were treated with glusulase and sodium thioglycolate and then put through a pressure cell to release spores randomly. These were plated on nutrient medium and scored for red clones (table 1).

A similar experiment with a triploid derivative of 4117, no. 5, gave 2–5 % red sectoring following u.v. irradiation and up to 15 % following sporulation. Strain 4117, no. 5, when forced, preferentially mates with *a* or *a/a* strains, rather than α or α/α strains (factor of 10).

TABLE 1. F_1 FROM 2-SPORED DIPLOID 4117, NO. 5, *a/α ADE/ADE* × 4-SPORED DIPLOID *a/a ade/ade*

	0 s u.v. (% red sectoring)	40 s u.v. (% red sectoring)	sporulated (% red clones)	complexes	spores
Fm4 12C2-18A (1)	0	0.3	3.6	yes	1–2–3–4
(2)	0	1.0	3.3		
Fm4 12C1-10A (1)	0	2.3	13.8	yes	1–2
(2)	0	3.0	15.3		
Fm4 12C1-14C (1)	0	0.6	0.3	no	2–3
(2)	0	0.8	1.0		

4. DISCUSSION

The development of chromosomes, nucleolus and kinetic apparatus during sporulation is shown in figure 7. Five *spo* mutants and 10 *cdc* mutants (Simchen 1973) block DNA synthesis at the restrictive temperature and also block meiosis completely or nearly so. This suggests that premeiotic DNA synthesis is a necessary prerequisite for meiosis to proceed. The mutant *spo 11* indicates, however, that the kinetic apparatus can function relatively independently from the normal DNA metabolism. In the case of the mitotic cell cycle the inhibitors and mutants which block DNA synthesis interfere with nuclear division (Hartwell *et al.* 1974). In analogy to meiosis, mutants may be produced which block DNA synthesis but do not affect the kinetic apparatus. At the restrictive temperature such cells will divide once and then die.

Mutant *spo 11*, furthermore, demonstrates that the formation of axial cores, and polycomplexes is not blocked by the inhibition of DNA synthesis. It indicates that the axial core can be formed in conjunction with a single, unduplicated chromosome. Thus, the axial core is not necessarily associated with a pair of sister chromatids. Possibly, in normal cells, the core is associated with only one of the two chromatids at any given point. The lateral elements then can break and rejoin at the site of a genetic crossover and so remain continuous along the newly constituted chromatid (Moens 1973).

The presence of axial cores and complexes in *spo 11* at 34 °C indicated that the material for making synaptonemal complexes is intact and that its production is independent of premeiotic DNA replication. Observations at 6 and 12 h samples did not show actual synaptonemal complexes which suggests that the mechanism of assembly may not be intact (Roth & Ito 1967). Yet *spo 11* at 25 °C for 12 h similarly had an abundance of cores but no complexes were observed. The control Z 190-8B at either temperature and at 6 or 12 h had cells with complexes. It is possible that *spo 11* has a generally low level of synaptonemal complexes or that they are present more abundantly after 8 and 10 h on sporulation medium.

Meiotic recombination always occurs at the 4-strand stage and *spo 11* offers the opportunity

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to examine recombination between unreplicated chromosomes in meiotic prophase. Samples are withdrawn from sporulation medium at various intervals and returned to growth medium (figure 7*a*) (Esposito & Esposito 1974*b*). This experiment is in progress at the time of preparation of the manuscript and the results will be reported separately.

Simchen (personal communication) similarly has found that *cdc4* mutant, blocked in the initiation of DNA synthesis, will also, when made into the diploid form, produce synaptonemal complexes at the permissive and restrictive temperatures. Withdrawal experiments as in figure 7*a* indicate meiotic levels of recombination, but in this case the cells must be returned to the permissive temperature.

Strain 4117 produces asci with 2 diploid spores. Its sexual reproductive cycle bypasses the formation of gametes as is common in apomictic plants. The single nuclear division superficially resembles a mitosis since each of the spores has the diploid chromosome number and is itself capable of producing a clone which can sporulate. Apparently the a/α parent produces 2 a/α offspring (Grewal & Miller 1972). The ultrastructure of the nucleus, however, has at first the characteristics of meiotic prophase including the presence of a polycomplex body in the nucleolus, frequent axial cores, rare synaptonemal complexes, and polycomplexes. When the meiosis I spindle develops the spindle pole bodies soon take on the characteristics of meiosis II spindle pole bodies, which have a large outer spindle plaque and the initiation of the spore wall (figure 6*a*). The observations suggest the possibility that the duplicated chromosomes synapse, recombine, completely desynapse in the absence of meiosis I, and then divide at meiosis II. These assumptions were tested through the analysis of red colony formation in a diploid strain heterozygous for an adenine requirement. The results indicate that 2 of the 3 strains have higher frequencies of red colonies from sporulated cells than red sectoring from u.v. treated and untreated vegetative cells (table 1). It can be argued that recombinational properties were brought in by 12C in the cross between diploids 4117, no. 5, $\times$ 12C. The presence of synaptonemal complexes in the original 4117 suggests, however, that that strain was least competent to undergo chromosome synapsis. Recombination in 4117 can also be disputed on the ground that it would lead to spores of a/a and α/α mating type. The data indicate that this is not so, but critical analysis has not been carried out. It is possible that the mating locus is duplicated so that 4117 is $a\alpha/a\alpha$. This would explain the continued ability of the diploid clones from Fm4 12C to sporulate (personal communication, P. Hastings, University of Alberta).

The alternative explanation for the appearance of red clones after sporulation is that reductional segregation occurs at meiosis I and that meiosis II is suppressed. This would lead to frequent homozygosis of centromere-linked markers such as *ade1*. In that case Fm 12C2-18A, Fm 12C1-10A, and -14C would be expected to all yield somewhat less than 50 % red clones following sporulation. The data do not agree with this expectation. In summary, it appears that the mechanism of chromosome segregation at meiosis I is suppressed and that following synapsis, recombination, and desynapsis meiosis II sets in with the division of centromeres and chromosomes.

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Discussion

G. Simchen (*Department of Genetics, The Hebrew University of Jerusalem, Israel*). The mitotic cell-cycle mutation *cdc4* has been reported by Hartwell (*J. molec. Biol.* **59** (1971), 183–194, to block the initiation of DNA replication at restrictive temperature. We found that meiosis in *cdc4/cdc4* diploids is normal at the permissive temperature (25 °C) and is arrested at the first division (one nucleus stage) at the restrictive temperature (34 °C). Arrested cells show a high degree of recombination commitment (50–95 % of controls), i.e. they form recombinant colonies when plated on vegetative medium, but are not committed to haploidization (the colonies formed are diploid). Arrested meiotic cells have undergone a limited synthesis of nuclear DNA (not more than 20 %) and small replication 'bubbles' appear upon electron microscopical examination of DNA molecules. Furthermore, these arrested cells contain synaptonemal complexes of normal or quasi-normal appearance. Thus commitment to meiotic recombination and the formation of synaptonemal complexes are associated with each other; both events are not dependent on the completion of the premeiotic DNA replication, and may only require its initiation.

B. C. Lamb (*Botany Department, Imperial College, London SW7 2BB*). Professor Moens showed a model of the synaptinemal complex with DNA double helices for one pair of sister chromatids shown as + + + + and + + + +, and for the other pair of sister chromatids as − − − − and − − − −. One + + + + and one − − − − helix were shown on the outside of the lateral and central regions, and one of each within those regions and able to pair intimately and form hybrid DNA.

Such a model, with only two out of the four chromatids of a bivalent able to pair at any one point, conflicts with the evidence from gene conversion studies in *Ascobolus immersus*. Conversion

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ratios such as 6+:2 *mutant*, 2:6, 5:3 and 3:5 from + × *mutant* crosses can be explained by pairing of two of the four chromatids of a bivalent to form hybrid DNA, with the possibility of enzymic correction of resulting mispairs in the DNA. In such crosses, Lamb & Wickramaratne (1973) also found 8+:0 *m* and 7+:1 *m* ratios, and control experiments ruled out reversion, aneuploidy or false clusters of spores as alternative explanations to conversion for their origin. This indicates that all four (or at least three) chromatids of a bivalent were involved in hybrid DNA formation at a single point of mutation, presumably with one + + + + chromatid paired with one − − − − chromatid, and the other + + + + paired with the other − − − −.

'Corresponding-site interference' was defined by Lamb & Wickramaratne as interference between the two pairs of non-sister chromatids in a bivalent in hybrid DNA formation at exactly corresponding sites. From the relative frequencies of different segregation ratios in a + × *mutant* cross, one can estimate the degree of corresponding-site interference. On the models of Moens and of von Wettstein (1971), with only two chromatids in a bivalent being able to pair at any one point, there should be complete, positive corresponding-site interference. In the *Ascobolus* experiments, corresponding-site interference was only weak, with slight positive interference in high conversion frequency crosses and slight negative interference in low conversion frequency crosses. The evidence is therefore that the two pairs of non-sister chromatids can independently pair with each other at the same point in a bivalent, without the extreme, positive corresponding-site interference that would follow from the Moens or von Wettstein models of pairing within the synaptonemal complex.

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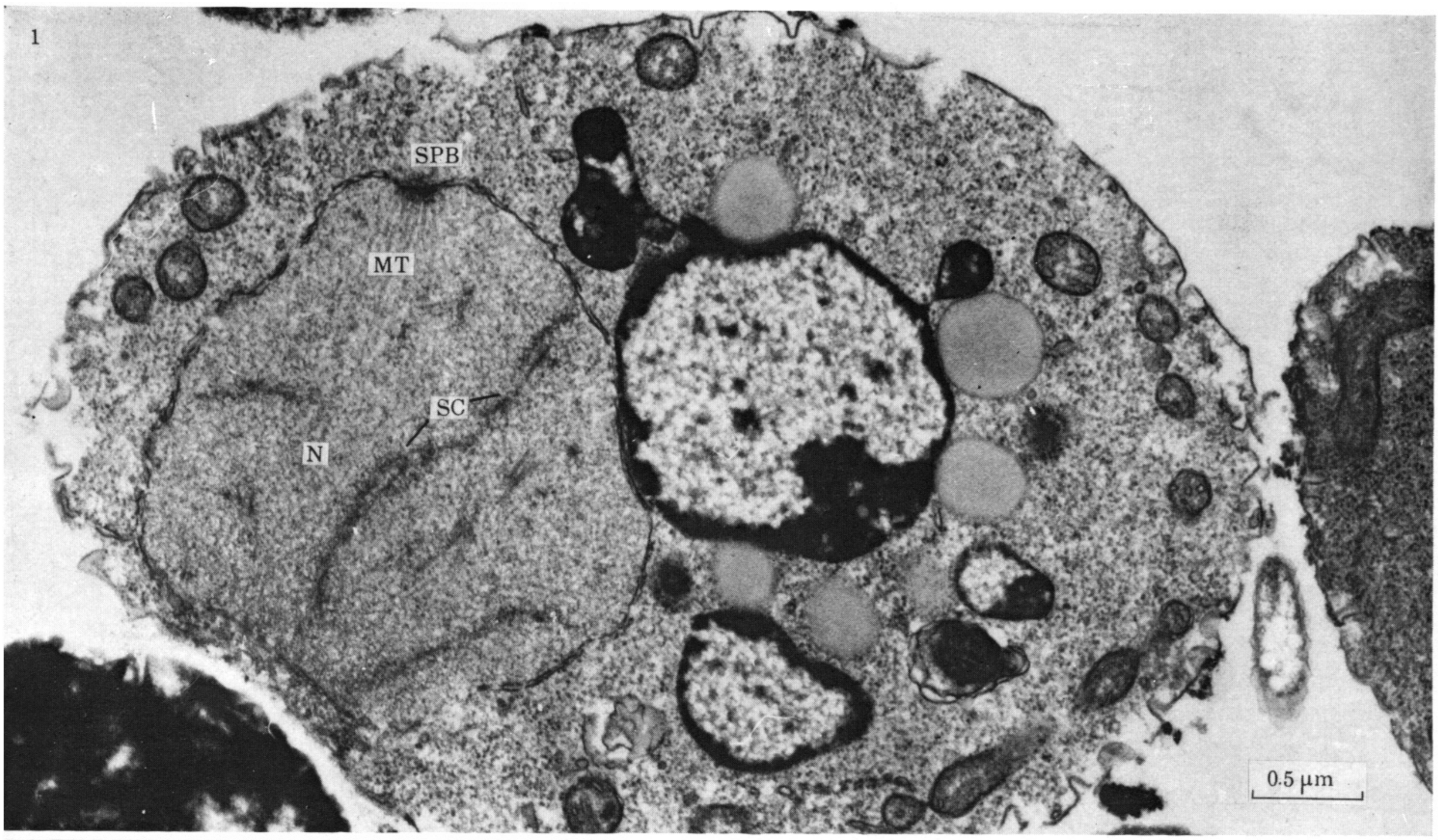
P. B. Moens. The participation of only two non-sister chromatids in a conversion or crossover event at a given site is so commonly reported that it needs strong evidence to postulate that both sets of non-sister chromatids can be involved simultaneously at any one site. For example, it should be possible to show that some 25 % of the 7:1 and 8:0 asci have a four-stranded double crossover for closely linked outside markers. Preferably one or more centromere-linked markers should show first division segregation to verify that meiosis has in fact taken place. Lamb & Wickramaratne have not reported on the behaviour of outside markers. Stadler, Towe & Rossignol (*Genetics* **66** (1970), 429), using outside markers, agree with Lamb that interference is weak, if present at all in *Ascobolus immersus*. This species, which has well developed synaptonemal complexes, and *Podospora anserina*, which has nearly complete interference but no detectable synaptonemal complexes (Zickler, *Chromosoma* **40** (1973), 401), are nails in the coffins of model-makers. It is comforting to remember, however, that the regulation of crossover frequencies is often a species-specific adaptation and as such a variety of regulatory mechanisms may exist.

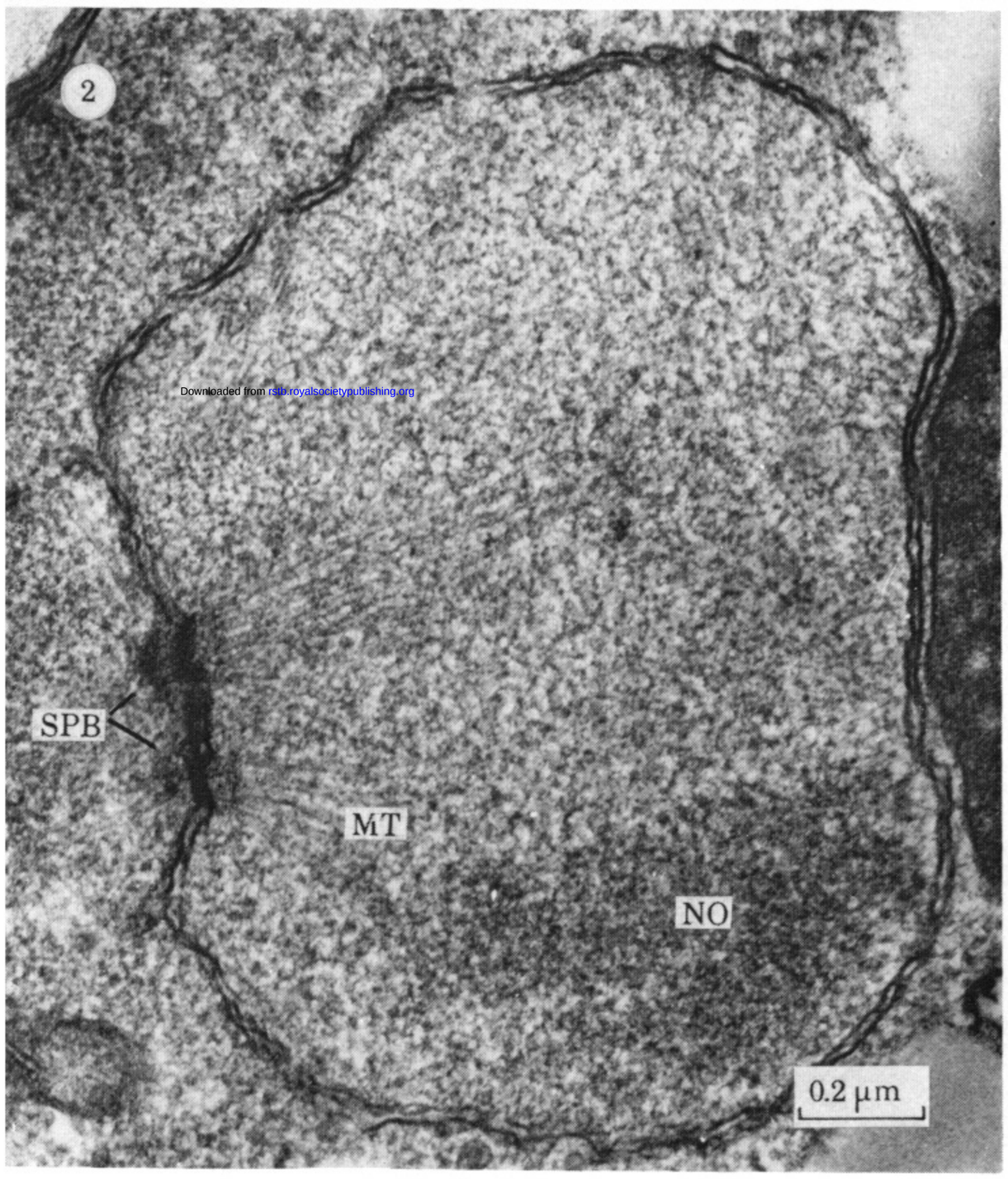
References

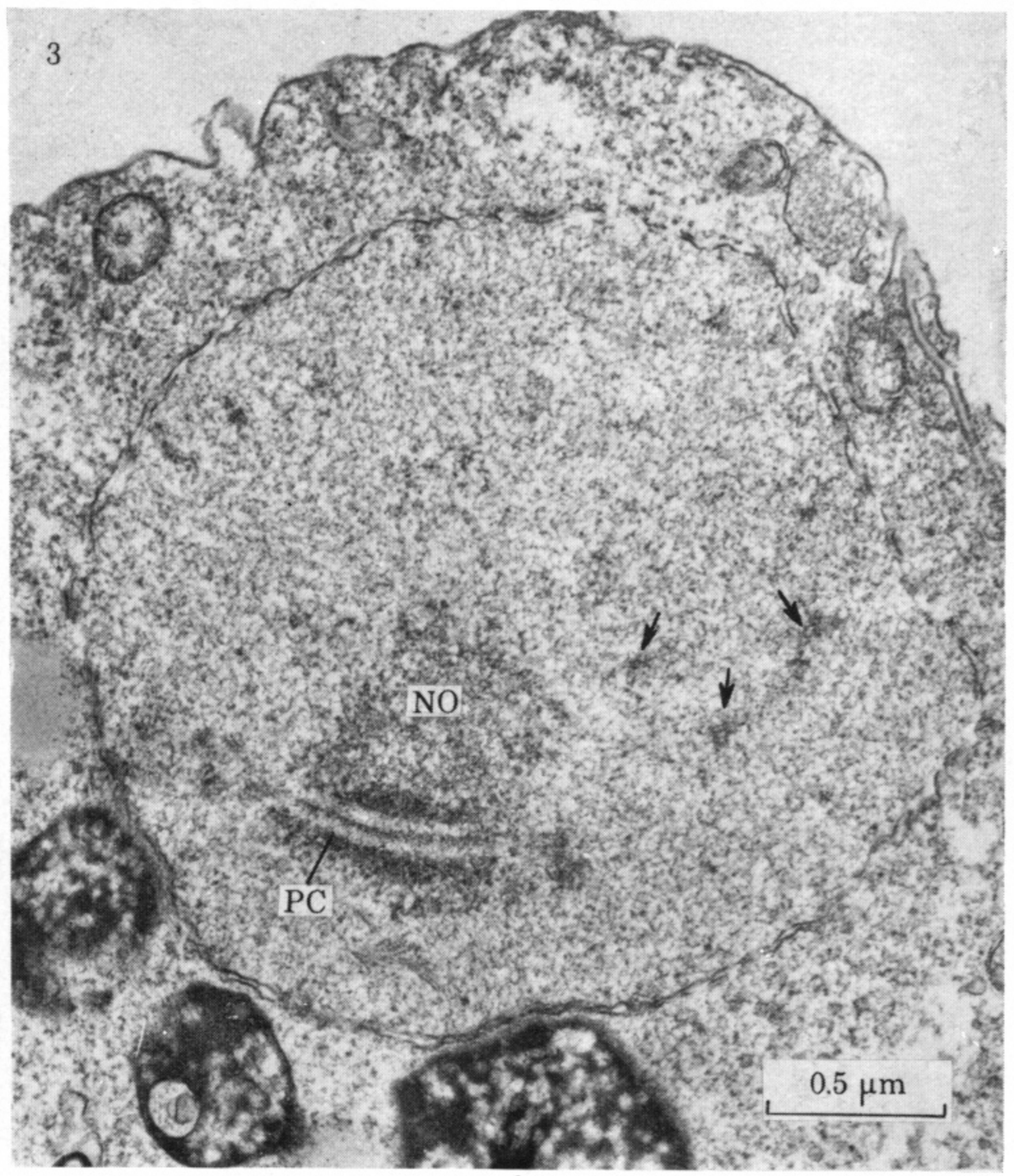
Stadler, D. R., A. M. Towe & J. L. Rossignol. 1970 Intragenetic recombination of ascospore color mutants in *Ascobolus* and its relationship to the segregation of outside markers. *Genetics* **66**, 429.

Zickler, D. 1973 Fine structure of chromosome pairing in ten Ascomycetes: meiotic and premeiotic synaptonemal complexes. *Chromosoma* **40**, 401.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES





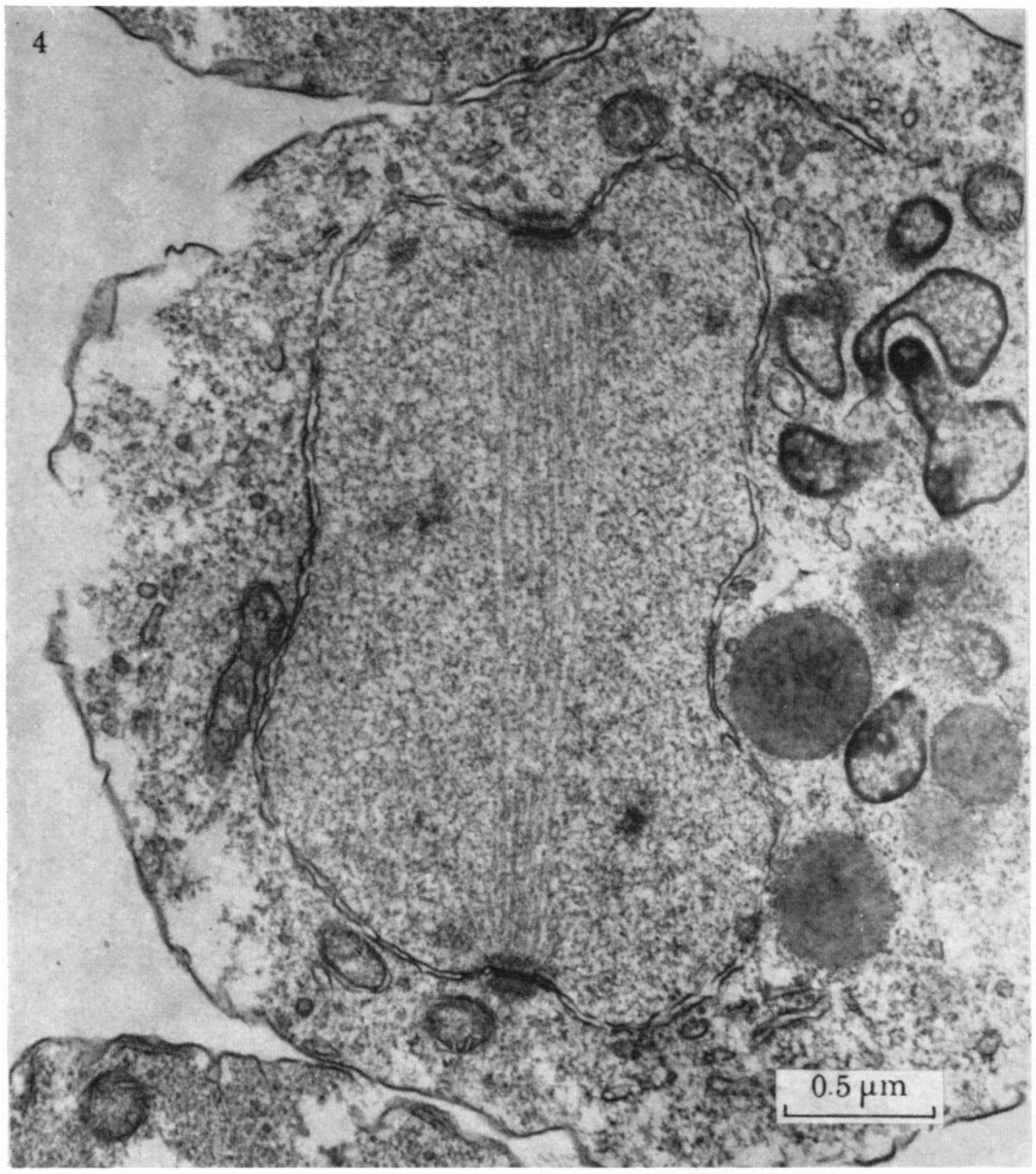


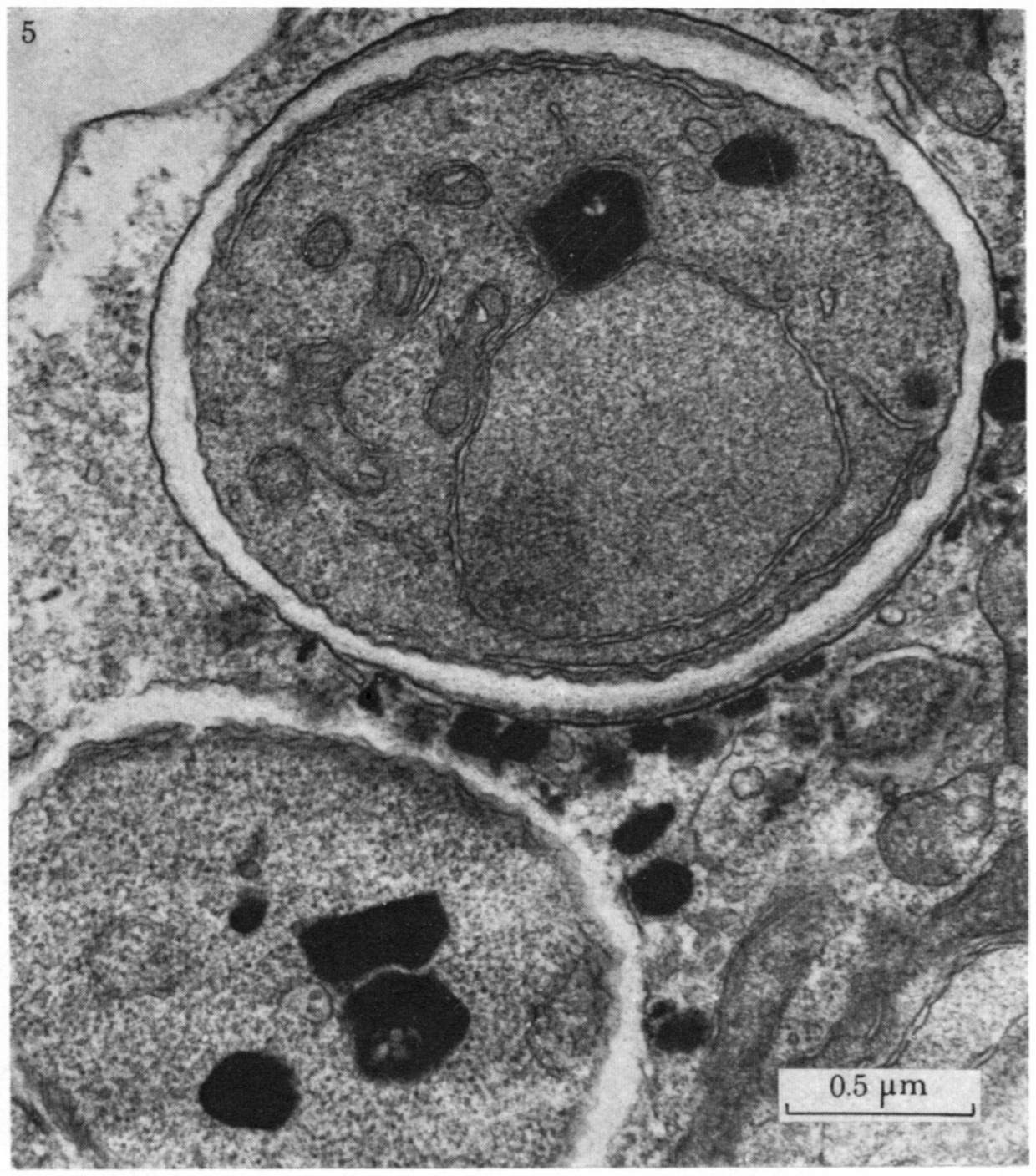
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

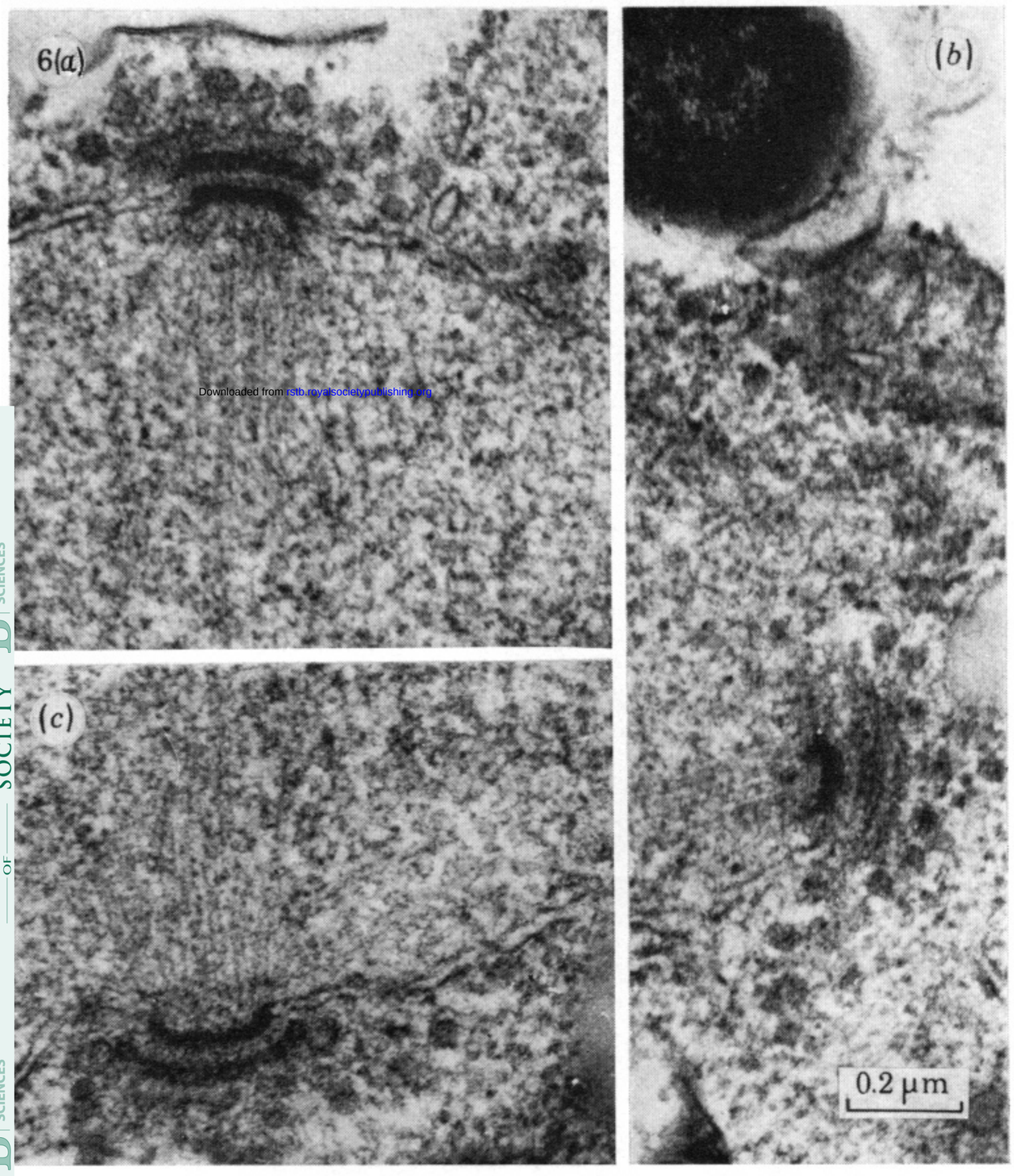
FIGURE 1. Control yeast Z 190-8B, 12 h on sporulation medium at 35 °C. The nucleus *N* contains synaptonemal complexes *Sc*, spindle pole body *SPB* with associated microtubules *MT*. (Magn. ×28 500.)

FIGURE 2. Mutant *spo 11* grown for 12 h on sporulation medium at 34 °C does not undergo DNA synthesis but the spindle pole body *SPB* undergoes normal duplication. Nucleolus *NO*. (Mag. ×57 000.)

FIGURE 3. Mutant *spo 11*, 12 h, 34 °C forms axial cores (arrows), polysynaptonemal complexes *PC*, usually in association with the nucleolus. Good complexes as in figure 1 were not found under these conditions nor in *spo 11* sporulated at 25 °C. (Magn. ×33 200.)







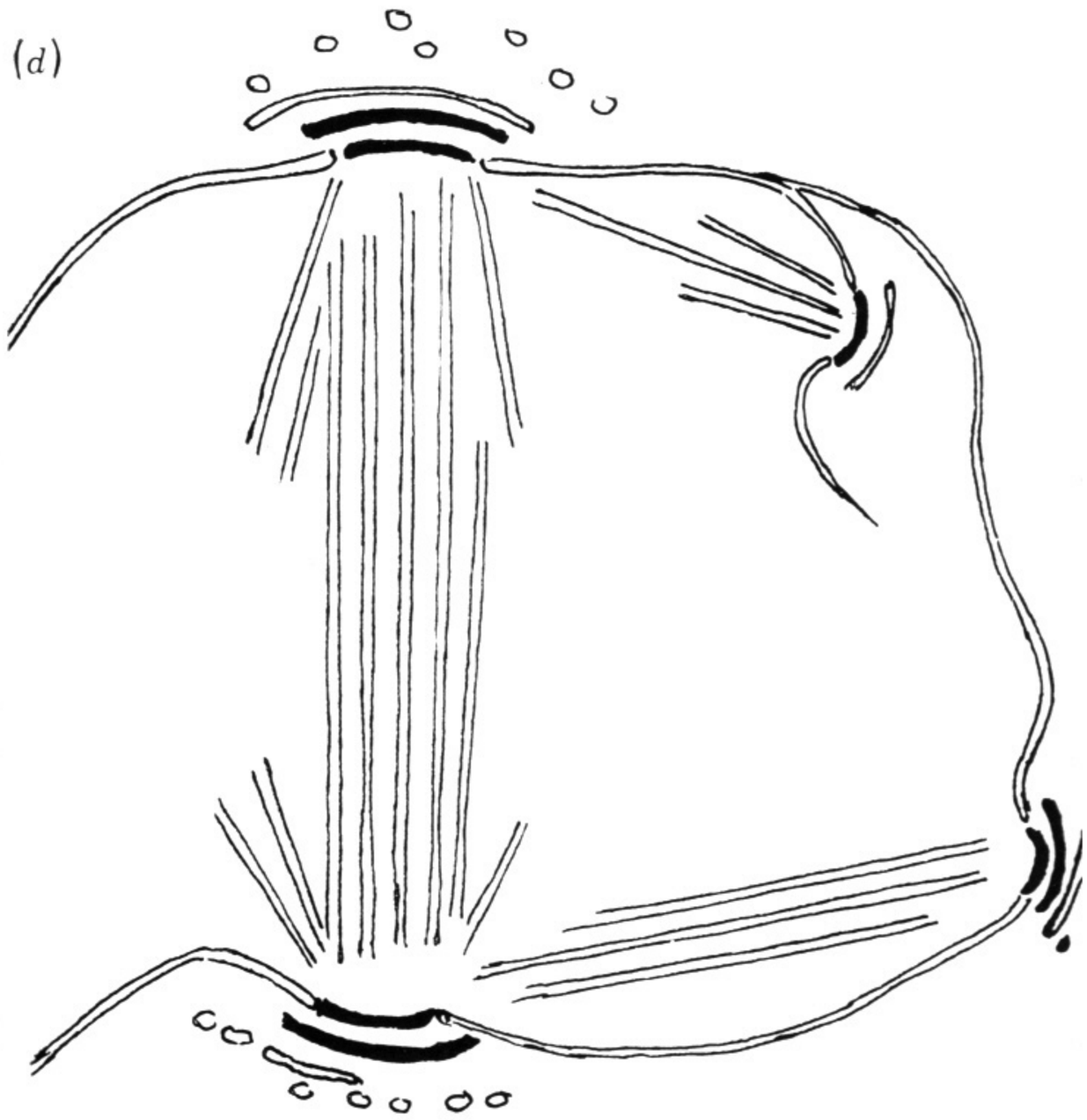


FIGURE 4. Mutant *spo 11*, at the restrictive temperature produces a normal anaphase I spindle indicating that the kinetic apparatus is not directly dependent on the presence of duplicated chromosomes. (Magn. × 26 600.)

FIGURE 5. Mutant *spo 11* at the restrictive temperature produces some aberrant and many normal looking early ascospores. All of these are aneuploid and degenerate shortly after this stage. (Magn. × 33 200.)

FIGURE 6. Strain Fm4 12C2-18A has the large spindle as in the single-division-meiosis parent 4117, no. 5, but the nucleus also contains poorly developed 3rd and 4th spindle pole bodies (*b*, *d*), presumably an incomplete expression of the 2-division meiosis parent 12C. (Magn. × 49 400.)