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Meiosis in Protists

Some Structural and Physiological Aspects of Meiosis in Algae, Fungi, and Protozoa

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INTRODUCTION

As the extant representatives of several evolutionary pathways, the protists (algae, fungi, and protozoa) exhibit characteristics which may be variously interpreted as being primitive or specialized. Consequently, a study of their biology frequently provides a valuable insight into the mechanics of cellular processes which could not have been obtained if the investigator had restricted himself to studying the relatively conservative behavior of "higher" organisms. This is particularly true in the case of meiosis, since not only is there frequently a range of meiotic behavior, but also protists are useful experimental systems which can often be cultured, synchronized, and manipulated in a manner which is rarely possible in higher organisms. It therefore seems appropriate to bring together for comparative purposes cytological, physiological, and biochemical observations of meiosis in algae, fungi, and protozoa. In so doing we have emphasized recent studies. To have done otherwise would have made the review unwieldy and, in any case, readers interested in earlier investigations should be able to locate these in the bibliographies of the papers we have referenced. We will also limit discussion of models of recombination (254, 313) and meiosis in *Lilium* (315) since these subjects have all been reviewed recently.

This review begins with a survey of protists in which meiosis is known to occur. Then, after a brief general account of meiotic cytology, the structural details of meiosis in protists are reviewed. Finally, the induction of meiosis, the physiological events during meiosis, and the mutants of meiosis are discussed.

OCCURRENCE OF MEIOSIS

The occurrence of meiosis in different groups of protists is summarized in Tables 1 to 3. In these tables the major groups of protists are printed in capital letters with their common names (if any) given in parentheses; the individual organisms are listed by their generic names. The most accurate indications of meiosis are based either on direct observations (i.e., reports of synaptonemal complexes or the

TABLE 1. *Occurrence of meiosis in algae*

Class	Meiotic stages observed	Synaptonemal complexes observed	Genetic evidence	Other indications of meiosis or sexuality[a]
RHODOPHYCEAE (Red algae)	*Furcellaria* (70) *Lemanea* (203)	*Gonimophyllum* (175) *Janczewskia* (175) *Levringiella* (175, 176) *Polycoryne* (175)		
CHLOROPHYCEAE (Green algae)	*Cladophora* (115, 306) *Cosmarium* (115) *Oedogonium* (115) *Spirogyra* (115) *Ulva* (24, 233) *Valonia* (253)	*Ulva* (24)	*Chlamydomonas* (40) *Cosmarium* (21) *Ulva* (99)	*Ulva* (23)
EUGLENOPHYCEAE (Euglenoids)	*Hyalophacus* (185) *Phacus* (170)			
CRYPTOPHYCEAE				
DINOPHYCEAE (Dinoflagellates)	*Gymnodinium* (340) *Noctiluca* (361) *Woloszynskia* (340)		*Crypthecodinium* (12, 333)	*Amphidinium* (334, 335) *Ceratium* (339) *Gymnodinium* (340) *Noctiluca* (36) *Woloszynskia* (340)
CHLOROMONADOPHYCEAE[b]				*Hornellia* (319)
CHRYSOPHYCEAE				*Kephyriopsis* (102)
HAPTOPHYCEAE				*Cricosphaera* (262) *Ochrosphaera* (292)
BACILLARIOPHYCEAE (Diatoms)	*Lithodesmium* (205-207)	*Lithodesmium* (205)		
PHAEOPHYCEAE (Brown algae)	*Fucus* (94) *Halidrys* (265) *Laminaria* (94) *Saccorhiza* (94) *Stictyosiphon* (265)	*Chorda* (330) *Pylaiella* (330)		
XANTHOPHYCEAE				*Vaucheria* (56)
EUSTIGMATOPHYCEAE				

[a] Papers presented at a recent colloquium on the sexual cycles and the alternation of generations in the algae contain much useful information; these have been published on pages 1-368 of the *Bulletin de la Société Botanique de France* (Mémoires, 1972).

[b] These algae are sometimes known by their alternative name, the *Raphidophyceae* (39).

appearance of meiotic stages in light and electron microscope preparations) or on the consequences of meiosis (genetic evidence). In some instances we have listed other indications of meiosis and sexuality, e.g., reports of gamete fusion, cytological evidence for an alternation of generations, and microspectrophotometric data of nuclear deoxyribonucleic acid (DNA) content. Additional information to that contained in Tables 1 to 3 can be found in recent surveys of fungal genetics (93, 97), protozoan genetics (251), and sexual reproduction in the protozoa (117, 118, 256). Useful recent information on sexuality and genetics of the algae is contained in various papers in *Selected Papers in Phycology* (272).

We have attempted to choose a widely accepted and relatively uncontroversial classificatory scheme. The classification system used for the algae is based on that proposed by Christensen (39). Our modifications to Christensen's scheme have been to include the Eustigmatophyceae (141); at the same time the Craspedophyceae, Loxophyceae, and Prasino-

TABLE 2. *Occurrence of meiosis in Fungi*

Class	Meiotic stages observed	Synaptonemal complexes observed	Genetic evidence
PHYCOMYCETES[a]	*Achlya* (81) *Pythium* (285) *Saprolegnia* (153)	*Achlya* (81) *Lagenidium* (6) *Saprolegnia* (153) *Thraustotheca* (132)	*Phytophthora* (18, 80)
ASCOMYCETES	*Ascobolus* (345, 358, 359) *Galactinia* (289) *Gelasinospora* (192) *Hansenula* (17) *Neottiella* (273, 351, 352) *Neurospora* (9) *Penicillium* (177) *Podospora* (305, 358, 359) *Pustularia* (289) *Saccharomyces* (122, 223, 228, 240, 361) *Schizosaccharomyces* (79, 357) *Sordaria* (108) *Wickerhamia* (270) *Xylaria* (289) *Xylosphaera* (14) *Candida* (110)	*Ascobolus* (345, 360) *Ascophanus* (360) *Galactinia* (289) *Neottiella* (350–353) *Neurospora* (114) *Pustularia* (289) *Podospora* (360) *Saccharomyces* (83, 227, 260, 361) *Sordaria* (360) *Xylaria* (289) *Xylosphaera* (14)	
BASIDIOMYCETES	*Boletus* (214) *Coprinus* (188, 193, 196, 257, 268, 326) *Poria* (294) *Schizophyllum* (255) *Ustilago* (264)	*Agaricus* (121) *Amanita* (121) *Boletus* (214) *Coprinus* (121, 193, 194, 268, 326) *Hypholoma* (121) *Panaeolus* (120, 121) *Poria* (294) *Russula* (121) *Schizophyllum* (255)	*Agaricus* (258) *Coprinus* (194)

[a] Microspectrophotometric analyses of nuclear DNA content provide evidence of gametic meiosis in two genera of *Phycomycetes, Apodachlya* (152) and *Saprolegnia* (28). Meiosis in the *Oomycetes* has recently been reviewed by Dick and Win-Tin (68).

phyceae are not treated as separate classes. Three groups of fungi are recognized: Phycomycetes, Ascomycetes, and Basidiomycetes (5, 30). The Fungi Imperfecti are omitted as they are thought to be a heterogeneous assemblage consisting of members from the other three classes whose principal feature in common is that sexual reproduction has not been described. The Myxomycetes are sometimes considered to be fungi (167), but we have chosen to treat these as a protozoan group of uncertain systematic affinities. The classification scheme used for protozoa is modified from Honigberg et al. (145) by grouping the Sporoza and the Cnidospora into a single subphylum, the Sporozoa. Thus, three subphyla are recognized: Sarcomastigophora, Sporozoa, and Ciliophora. For the purpose of this review, four natural groups of protozoa, the Acrasiales, Labyrinthulales, Myxomycetes, and Plasmodiophoromycetes, which were classified in the Sarcomastigophora (145), are listed separately in Table 3.

Meiosis has recently been demonstrated in two groups of organisms, the Acrasiales (Table 3) and the Dinophyceae (Table 1), which had been listed by Margulis (208) among "natural groups of protists in which fertilization and meiosis have been sought but never confirmed to the satisfaction of most investigators." It is therefore apparent that lack of evidence for meiosis does not necessarily indicate that this process is absent; it may merely reflect a generally poor understanding of a particular group of organisms. This is almost certainly the case in the algal classes Cryptophyceae, Chloromonadophyceae, and Eustigmatophyceae. In the Chloromonadophyceae there has been only

TABLE 3. *Occurrence of meiosis in Protozoa*

Class	Meiotic stages observed	Synaptonemal complexes observed	Genetic evidence
SARCOMASTIGOPHORA	*Barbulanympha* (49, 50) *Leptospironympha* (46) *Macrospironympha* (51) *Myxotheca* (291) *Notila* (45) *Oxymonas* (43) *Rhynchonympha* (48) *Saccinobaculus* (44) *Trichonympha* (42) *Urinympha* (47)	*Ceratiomyxa* (109)	
SPOROZOA	*Eimeria* (33)		*Plasmodium* (341, 342)
CILIOPHORA (Ciliates)	*Paramecium* (316) *Tetrahymena* (261, 322)	*Blepharisma* (161)	
ACRASIALES (Cellular slime molds)			*Dictyostelium* (199)
LABYRINTHULALES	*Labyrinthula* (239)	*Labyrinthula* (226, 239)	
MYXOMYCETES (Plasmodial slime molds	*Physarum* (2)	*Arcyria* (4) *Didymium* (3) *Echinostelium* (130) *Hemitrichia* (4) *Physarum* (2, 4) *Stemonitis* (4, 221, 222) *Tubifera* (4)	
PLASMODIOPHOROMYCETES	*Sorosphaera* (22)	*Sorosphaera* (22)	

a single indication of sexuality based on a report of fusion between two individuals (319). However, detailed observations over a number of years on two other genera of this class produced no evidence of gamete fusion in living cells or of meiotic stages in stained preparations (139). Knowledge of the Cryptophyceae is also limited, and several recent accounts (58, 102, 116) make no mention of either the presence or absence of sexuality. Similarly, as yet there have been no indications of meiosis in the Eustigmatophyceae (141).

In contrast, detailed cytological investigations of the Euglenophyceae (186) have failed to reveal any meiotic stages, with two possible exceptions (170, 185): Leedale (186) has concluded that "it seems probable that there is no sexuality in the majority of euglenoids, but it remains a possibility that the process does occur as a rare phenomenon." The absence of a genetic system in *Euglena* has limited its usefulness as a biochemical and physiological tool. One unusual observation about *Euglena* deserves mention: Epstein and Alloway (84) obtained a 50% reduction in the amount of DNA per cell when *Euglena gracilis* was grown in a medium containing low concentrations of phosphate. Chromosome preparations indicated that this corresponded to an approximate halving of the chromosome number. Davis and Epstein (65) speculated that this reduction was caused by nuclear division in the absence of chromosome replication. But it is also conceivable that a halving of the chromosome complement could have been the result of some type of meiotic process. It is interesting to note that meiosis has not been reported in the trypanosomes (251), which are a group of protozoa that may have evolved from the Euglenophyceae (20).

The apparent absence of meiosis can be interpreted in two ways: either sexuality is actually present in the organism but has not yet been observed, or sexuality is completely absent. If the latter is the case, then there are two possible explanations of this situation depending on whether absence of sexuality is an original or a derived condition. If meiosis

has arisen independently on a number of occasions (208), then extant forms which lack meiosis may be the descendants of organisms which never evolved this process. However, if meiosis arose at the beginning of eukaryote evolution, then all eukaryotes must have had sexuality at some stage in their evolutionary history. Absence of meiosis in extant forms would therefore be the result of a secondary loss.

There is good evidence that meiosis is present in those groups of extant protists considered to be present-day derivatives of primitive protists. The rhodophycean algae are widely assumed to be primitive (39, 166, 204). The body of evidence supporting this view includes the similarity of the photosynthetic apparatus in these organisms and the prokaryotic blue-green algae (113), their ancient fossil record (69, 288), the complete absence of flagella and centrioles [this condition is assumed to be primitive rather than derived (39, 204)], and one of the lowest molecular weight values of cytoplasmic ribosomal ribonucleic acids (RNAs) known for eukaryotes, i.e., closer to the molecular weights of the prokaryotic ribosomal RNAs (155). The value to be placed on each of these characteristics as an indicator of antiquity is still uncertain. Taken together, though, they probably indicate that these organisms are among the most ancient of extant eukaryotes. It is therefore of interest to note that meiosis is widespread in the Rhodophyceae (70) and that a synaptonemal complex has been recognized (175). Meiosis is known in the Chlorophyceae, which have an ancient fossil record (69, 288); in the Dinophyceae, whose nuclei are widely acknowledged to have some primitive features (172); and in the Labyrinthulales, where the molecular weights of ribosomal RNAs are similar to those in the Rhodophyceae and are much lower than those in other eukaryotes so far investigated (250).

It therefore is probable that meiosis may have had its origins early in eukaryotic evolution. The question of whether this process arose just once or evolved on a number of separate occasions remains open to speculation. The basic similarity of meiotic mechanisms and structures (e.g., the synaptonemal complex) might appear to argue for the former possibility. The alternative possibility was stated by Brown (27): "If meiosis has evolved independently in numerous evolutionary lines of organisms but has always converged to give essentially the same meiotic system, then there must be some property of cells or chromosomes that made such convergent evolution inevitable."

Regardless of the point in time at which meiosis was evolved, it appears that the most evolutionarily advanced organisms are diploid (259). This is clearly demonstrated by the increasing dominance of the diploid form in the evolutionary series leading from the Chlorophyceae (haploid and haplo-diploid life cycles) through the Bryophytes and Pteridophytes (haplo-diploid life cycles) to the seed plants (diploid with a brief gametophyte phase) (Table 4).

CYTOLOGICAL EVENTS DURING MEIOSIS

General Details of Meiosis

Meiosis consists of leptotene, zygotene, pachytene, diplotene, diakinesis, first meiotic division, and second meiotic division. Figures 14–23 are light micrographs of some of the earlier stages of meiosis. Each leptotene chromosome consists of two chromatids, but these cannot be distinguished in the light microscope, and the chromosome appears as a single fine thread with darkly stained granules (chromomeres) along its length. The ends (telomeres) of leptotene chromosomes are often attached to the nuclear envelope and may be grouped together: such clusters often occur close to the spindle pole bodies (which are unseparated at this stage). In some instances homologous chromosomes appear to be associated with each other even before the onset of meiosis (54, 55, 312); this premeiotic pairing may be a consequence of homologous chromosomes being paired as early as the last telophase of the preceding mitosis (312). However, premeiotic pairing does not appear to be the rule in all cases (343).

Precise alignment of pairs of homologous chromosomes occurs during zygotene. The onset of this phase is marked by the appearance of synaptonemal complexes which are initiated at the telomeres or at other points along the chromosome. The synaptonemal complexes extend and eventually the homologous chromosomes are specifically paired along their entire lengths. At this stage each pair of homologs is termed a bivalent. Contraction of the bivalents occurs during pachytene, a stage which may be long in duration. The end of pachytene is marked by the beginning of synaptonemal complex breakdown. This process is completed during diplotene (although traces of the synaptonemal complex may persist until diakinesis). Loss of the synaptonemal complexes allows separation of the homologs except at the chiasmata

TABLE 4. *Distribution of life cycles in eukaryotic organisms*

Organisms	Haploid	Haplo-diploid	Haplo-dikaryotic	Diploid
Protozoa	X	X	·	X
Higher animals	·	·	·	X
Myxomycetes	·	X	·	·
Algae				
Green	X	X	·	·
Red	X	X	·	·
Brown	·	X	·	X
Diatoms	·	·	·	X
Golden	X	?	·	·
Fungi				
Uniflagellate water moulds	X	X	·	X (rare)
Biflagellate water moulds	?	·	·	X
Zygomycetes	X	·	·	·
Hemiascomycetes	X	X	·	·
Euascomycetes	·	·	X	·
Basidiomycetes	·	·	X	·
Embryophyta				
Mosses and liverworts	·	X	·	·
Ferns, etc.	·	X	·	·
Seed plants	·	X	(–effectively → X)	

which may represent the points where recombination occurred during pachytene. Diplotene is the first point in meiosis when chromatids are readily distinguishable in the light microscope. In diakinesis the chiasmata move toward the ends of the bivalents (terminalization) and the nucleolus begins to disintegrate. Metaphase of the first meiotic division is characterized by dispersal of the nuclear envelope, formation of the spindle, migration of the poles to opposite ends of the nucleus, and movement of the chromosomes culminating in their alignment on the equator of the nucleus. At metaphase I, each of the chromatids has a kinetochore; the two kinetochores of each chromosome are closely associated with each other, and their spindle microtubules terminate at one pole, whereas the kinetochores of its homologue are attached to the opposite pole (8, 198). This arrangement ensures that the members of a homologous pair of chromosomes are separated at anaphase and become partitioned into different nuclei. During anaphase I, any remaining chiasmata are separated. This phase may lead into prophase II with little or no delay. Alternatively, these phases may be separated by telophase I and interkinesis. The latter is a modified form of interphase in which the nuclear envelope may be partially reformed and there may be some relaxation of the chromosomes. In prophase II and metaphase II any nuclear envelope that has reformed is once again broken down. A new spindle is formed and the chromosomes become aligned on the equator; the members of each pair of chromatids are attached to opposite poles. Chromatid separation occurs during anaphase II. The telophase II and interphase nuclei resulting from this separation contain the haploid number of chromosomes. Cytokinesis may take place after the second meiotic division; alternatively, it may take place at the end of each meiotic division.

Nuclear Cytology of the Protists

Before reviewing the structural features of meiosis in different protists, it is appropriate to discuss briefly the insights gained from ultrastructural studies of mitosis in higher organisms and protists (8, 73, 74, 132, 137, 187, 198, 242–246, 318). For example, there is a range in behavior of the nuclear envelope and nucleolus during mitosis. The nuclear envelope may break down completely during mitosis, it may remain intact throughout this process, or there may be partial breakdown (73, 104, 198, 245). Similarly, during mitosis the nucleolus may remain intact, may undergo partial dissolution, or may completely disperse (187, 243). Although chromosomes usually have a cycle of condensation, examples are known where chromosomes are condensed throughout the cell cycle (140, 172, 186), while in other organisms they remain uncondensed even during mitosis (241). Spindle microtubules may converge to a spindle pole body (131, 198, 244, 245) or they may not be associated with a morphologically recognizable structure. (The term spindle pole body [SPB] is employed as a useful general term [1, 137] to describe the variety of structures which occupy the polar areas of the division spindle.) Finally, although most spindles include both polar and chromosomal microtubules, examples are

known in which there is no attachment of spindle microtubules to the chromosomes (172, 186).

Although observations on meiotic nuclei are not as extensive as those on mitotic nuclei, the same range of nuclear behavior appears to be present. The following account will attempt to describe the morphological features encountered in meiotic nuclei and will also describe differences which may be present between meiosis and mitosis in the same organism. Most of the structural accounts reviewed here are based on electron microscopy, but we also include several light microscope studies such as Cleveland's painstaking investigations (41–52). Where observations were made with the light microscope, we refer to the spindle components as "fibers" and use the word "centromere" to denote the constricted region of the chromosome where they insert.

Synaptonemal Complexes

Synaptonemal complexes have been reported in a wide variety of protists (Tables 1–3). General details of the structure and possible pairing mechanisms of the synaptonemal complex will not be described here since they have been reviewed adequately elsewhere (55, 230, 353, 354). Westergaard and von Wettstein's account (353) is particularly valuable since it tabulates recent information on the structural details of synaptonemal complexes. The following generalizations can be made about the synaptonemal complexes of protists. They consist of a central region of width 90 to 120 nm, at the center of which is a central component of diameter 10 to 30 nm. On either side of the central region are lateral components of diameter 30 to 50 nm (Fig. 1–5). Some differences in dimensions may be the result of fixation (353). Inadequate fixation is also a possible explanation for the apparent absence of the central component of the synaptonemal complex in *Ulva* (24). However, it may also be that these synaptonemal complexes were at an early stage of formation at which time the central component is apparently absent in some organisms (193, 194).

Structural variations in the synaptonemal complex include dense nodes on the central component (144; Fig. 2a) and distinctive thickenings on the fibers connecting the central component to the lateral components (175). An interesting structural variation in the synaptonemal complex occurs in the Ascomycetes, where the lateral components consist of alternating thick and thin bands (353, 360; Fig. 1, 3–5). The spacing of the thick bands is constant, but there is a species-specific variation in the intervening thin bands. It may be too soon to generalize on these observations, but they tend to suggest that the Ascomycetes are a natural group of fungi in which banding in the lateral component of the synaptonemal complex occurred early in the evolutionary history. Further evolution within the Ascomycetes may have given rise to species specific differences in banding patterns.

The termination of synaptonemal complexes at the nuclear envelope has frequently been observed (4, 81, 114, 121, 132, 153, 176, 214, 226, 255, 294, 360) and its significance discussed (13, 96). In serially sectioned pachytene nuclei of *Neurospora*, both ends of six of the seven synaptonemal complexes terminated at the nuclear envelope; one end of the remaining synaptonemal complex also terminated at the nuclear envelope, but the other end, which was the satellite region of the nucleolar organizing chromosome, was apparently not attached (114). Serial sections through a meiotic prophase nucleus of *Labyrinthula* (Fig. 6) indicated the presence of nine synaptonemal complexes (226) and hence allowed an accurate determination of the chromosome number. This technique may be applicable to other protists having small chromosomes which are difficult to distinguish using the light microscope (175, 330). It would also seem that this technique could be used to determine other more fundamental features of the prophase nucleus. The possibility that chromatin has an ordered arrangement in the interphase nucleus has been discussed at length (54). It would be of interest to know whether the architecture of prophase nuclei were constant in a given species; this point might be resolved by comparing the positions of synaptonemal complexes in several pachytene nuclei using models reconstructured from serial sections.

The presence of synaptonemal complexes has been used as an indicator of meiosis and as a means of understanding life cycles (3, 4, 175). However, synaptonemal complexes sometimes occur in haploid nuclei. This has been documented in the case of a number of higher plants (e.g., 293), but it is also known to occur in the ascomycete *Ascophanus* (360), where some synaptonemal complexes were occasionally found in nonmeiotic nuclei. In some meiotic nuclei distinct synaptonemal complexes are rarely present in the nucleoplasm, but synaptonemal complex-like structures occur in the nucleoli (227, 360).

Synaptonemal complex-like structures are often grouped together to form polycomplexes (4, 6, 7), and their frequent association with nucleoli (4, 227) may indicate that the latter are involved in their synthesis. A nucleolar origin

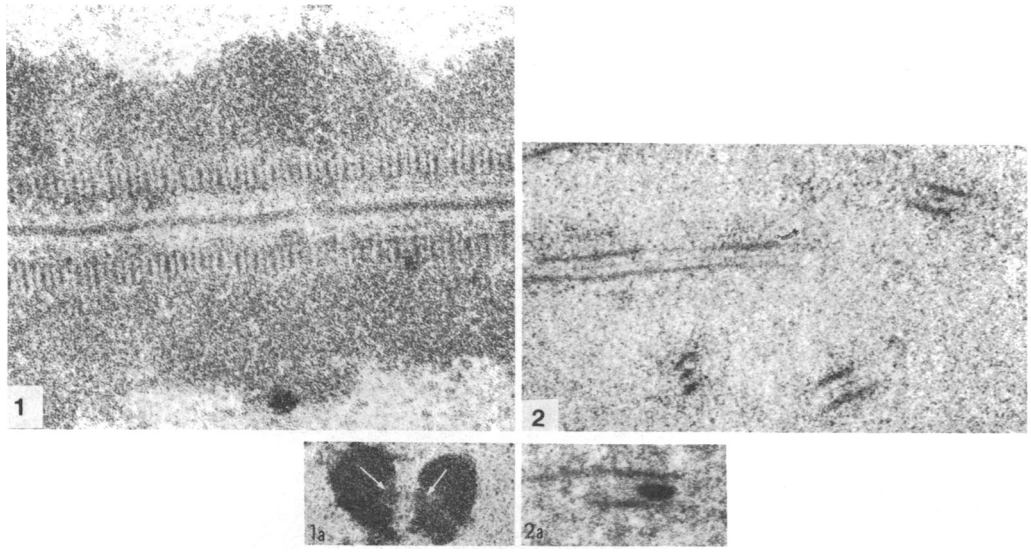


FIG. 1. *Synaptonemal complex in pachytene bivalent of Neottiella rutilans.* ×*77,000. Fig. 1a. Cross section of bivalent of Neottiella. The two lateral components of the synaptonemal complex are marked with arrows.* ×*27,500.*

FIG. 2. *Longitudinal and transverse sections of synaptonemal complexes of Neurospora crassa.* ×*16,000. Fig. 2a. Characteristic node in central region of synaptonemal complex in Neurospora. Such structures have been termed "recombination nodules" by Carpenter, 1975.* (Proc. Natl. Acad. Sci. U.S.A. 72:3186-3189.) ×*32,000. (Reproduced from reference 353 by kind permission of D. von Wettstein and the* Annual Review of Genetics.)

has also been proposed for the ribonucleoprotein material present in the central region of the *Neottiella* synaptonemal complex (352).

Some form of premeiotic pairing probably precedes the formation of synaptonemal complexes (55). In some organisms homologous chromosomes are paired even before premeiotic DNA synthesis (312), but this is not always the case since premeiotic DNA synthesis precedes karyogamy in *Neottiella* (273) and in *Coprinus* (194).

Synaptonemal complexes are formed during zygotene and begin to break down during diplotene (55). Complete synaptonemal complexes are present at pachytene and it is during this phase that most recombination occurs (194; Fig. 32). But the exact mode of action of the synaptonemal complex in chromosome exchange remains to be determined (353), and it is still not known whether crossing-over of DNA takes place within the synaptonemal complex or outside it. The fundamental morphological similarity between synaptonemal complexes of disparate organisms suggests a common mechanism. It would be of interest to know the reason for the minor morphological differences between the synaptonemal complexes of different organisms. In instances where interspecific crosses are possible, it might be worthwhile to examine the structure of the synaptonemal complex in the offspring and parents. Future investigations should deal not only with the descriptive cytology of the synaptonemal complex but should record structure and behavior from time of appearance to disappearance and should correlate these investigations with simultaneous observations on rates of recombination.

Nuclear Envelopes

The variety in behavior of the nuclear envelope during meiosis can be best illustrated by detailing the events in the fungi. Intranuclear meiosis, during which the nuclear envelope remains intact, appears to be widespread in the Phycomycetes (81, 153) and in the Ascomycetes (Fig. 7–9) (17, 122, 223, 228, 240, 270, 289, 357, 358). The nuclear envelope appears to disperse during meiosis in *Penicillium* (177), but this may be due to a fixation artifact; a similar explanation has been advanced (351) to account for the gaps in the nuclear envelopes of meiotic nuclei of another ascomycete, *Neottiella*. An unusual form of intranuclear meiosis has been reported in the ascomycetes *Saccharomyces* (122, 223, 228, 240) and *Wickerhamia* (270), and in the phycomycete *Saprolegnia* (153). This has been termed "uninuclear meiosis." As the name implies, both meiotic divisions occur within the original nuclear envelope (Fig. 10). This form of

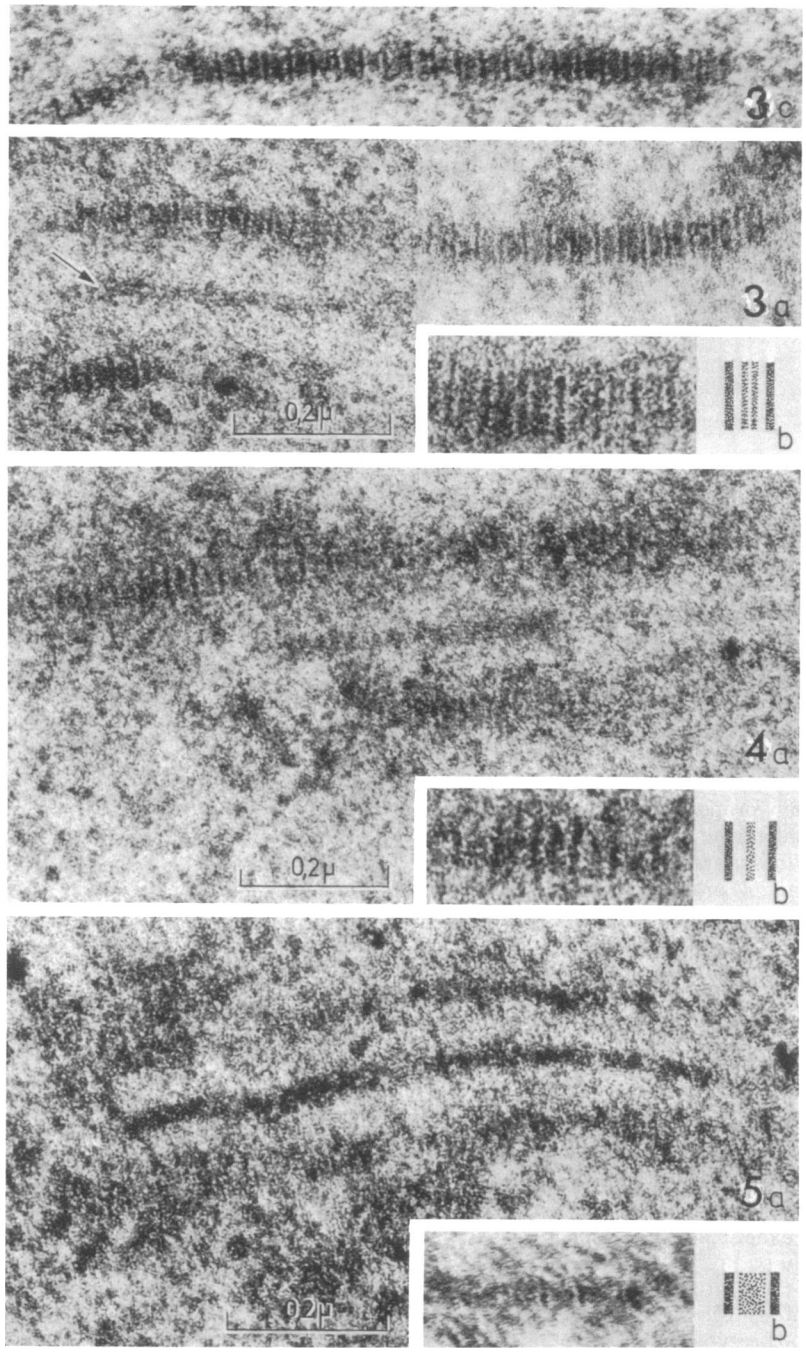


FIG. 3–5. *Synaptonemal complexes of three Ascobolus species. (Reproduced from reference 360 by kind permission of D. Zickler and* Chromosoma.)

FIG. 3. *A. immersus. (a) Some parts of the central component appear as a double structure (arrow); the lateral component consists of alternating thick and two thin bands. (b) Higher magnification of the banding. (c) The two thin bands are less clear in another race.*

FIG. 4. *A. stercorarius. The lateral component consists of alternating thick and thin bands.*

FIG. 5. *Ascobolus species. The "thin" band fills the space between two thick bands: note the thick and dense central component. In Fig. 3b, 4b, and 5b, magnification is ×150,000.*

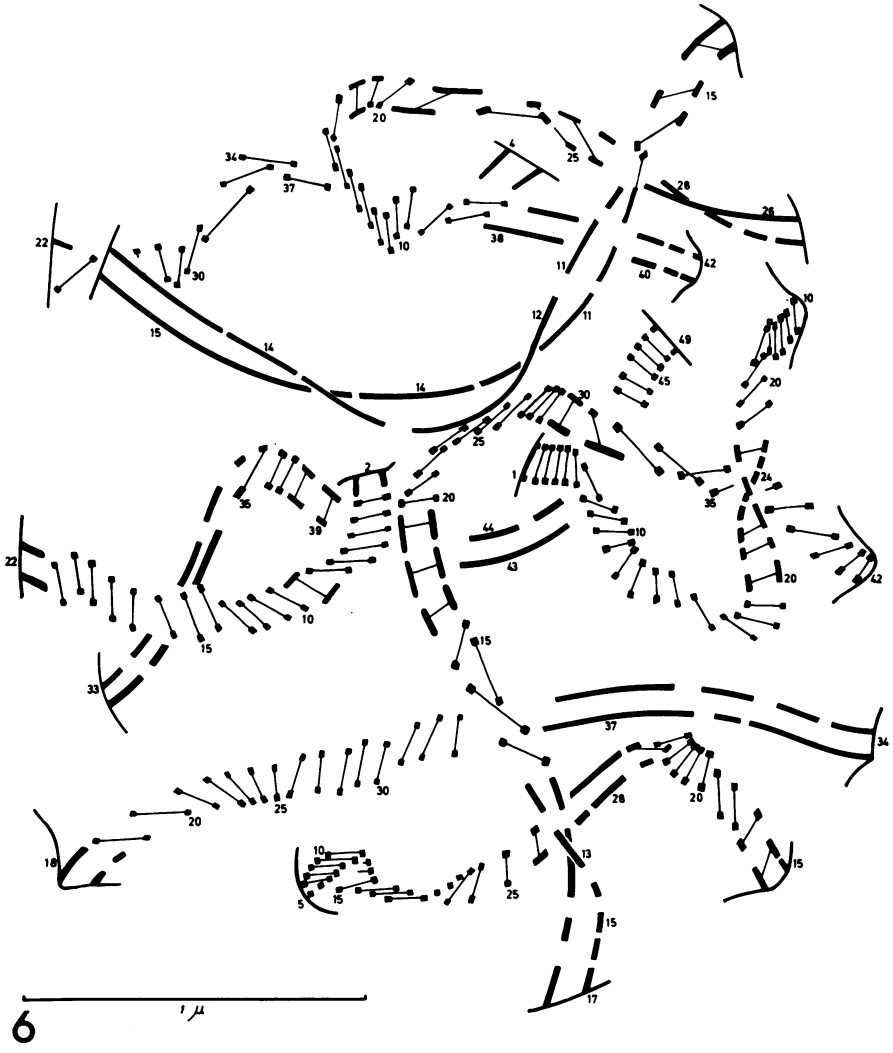


FIG. 6. *Diagram of the meiotic prophase nucleus of the mycetozoan Labyrinthula sp. The synaptonemal complexes that appeared in 49 consecutive sections of one nucleus are superimposed in this drawing. The lateral elements are represented by the fat black lines or squares. The numbers refer to the sections in which the segments of a complex were found. When the complex is attached to the nuclear membrane, the membrane is indicated by a solid line. (Reproduced from reference 226 by kind permission of P. Moens and* Science *magazine. This micrograph was originally published in* Science **166**:*1289-1291, 5 December 1969. Copyright 1969 by the American Association for the Advancement of Science.)*

meiosis is most clearly demonstrated by serial sections (228). In the absence of this three-dimensional perspective, the nuclear profiles observed in random sections through meiosis II nuclei might be misinterpreted as portions of two intranuclear meioses. For example, early reports of meiosis in yeast (129) concluded that two nuclei were formed at the end of meiosis I. Other examples of uninuclear meioses may have been misinterpreted and it may well be that this form of meiosis is more widespread than presently seems to be the case.

In contrast to the intranuclear meioses of the Phycomycetes and the Ascomycetes, the nuclear envelope appears to be partially or wholly dispersed during meiosis in most Basidiomycetes thus far investigated (188, 193, 214, 257). However, in *Poria,* breakdown of the nuclear envelope is restricted to the polar regions (294), whereas meiosis is intranuclear in *Coprinus*

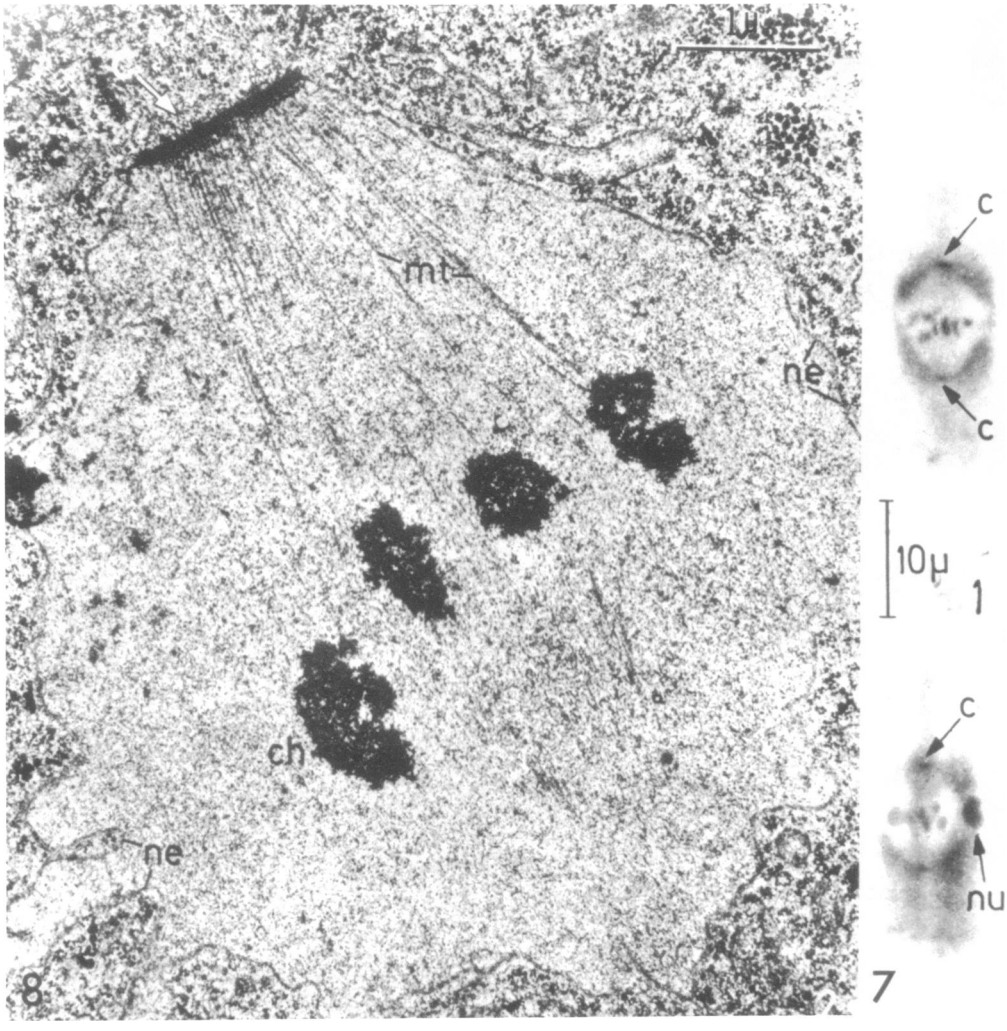


FIG. 7 and 8. *First meiotic metaphase in Ascobolus stercorarius.*

FIG. 7. *Light phase-contrast micrographs of the ascus after embedding in Epon. The arrows point to the centrosomal plaques (c) and the nucleolus (nu).*

FIG. 8. *Electron micrograph showing one centrosomal plaque (c), numerous spindle microtubules (mt), four bivalents (ch), and the intact nuclear envelope (ne). (Reproduced from reference 359 by kind permission of D. Zickler and* Chromosoma.)

radiatus (326). The latter report is especially intriguing, as a previous investigation (188) of the same species had shown that the nuclear envelope broke down during metaphase and reformed in telophase. This apparent dispersal of the nuclear envelope is probably the result of an artifact since, as Thielke (326) pointed out, it is more likely that faulty fixation could result in breakdown of an intact nuclear envelope rather than the converse. In view of this, the apparent dispersal of the nuclear envelope in other Basidiomycetes should be reexamined.

A recent review by Heath (132) indicated that mitosis is intranuclear in most Phycomycetes and Ascomycetes, while the nuclear envelope becomes dispersed during mitosis in most Basidiomycetes. Thus, in general, the behavior of the nuclear envelope at meiosis parallels that at mitosis in these three classes of fungi. It has been speculated that an intranuclear spindle is more primitive than an open spindle (187, 242, 245).

Considerably less is known about nuclear envelope ultrastructure during meiosis in the algae. In *Lithodesmium undulatum* (Bacillariophyceae), the nuclear envelope breaks down

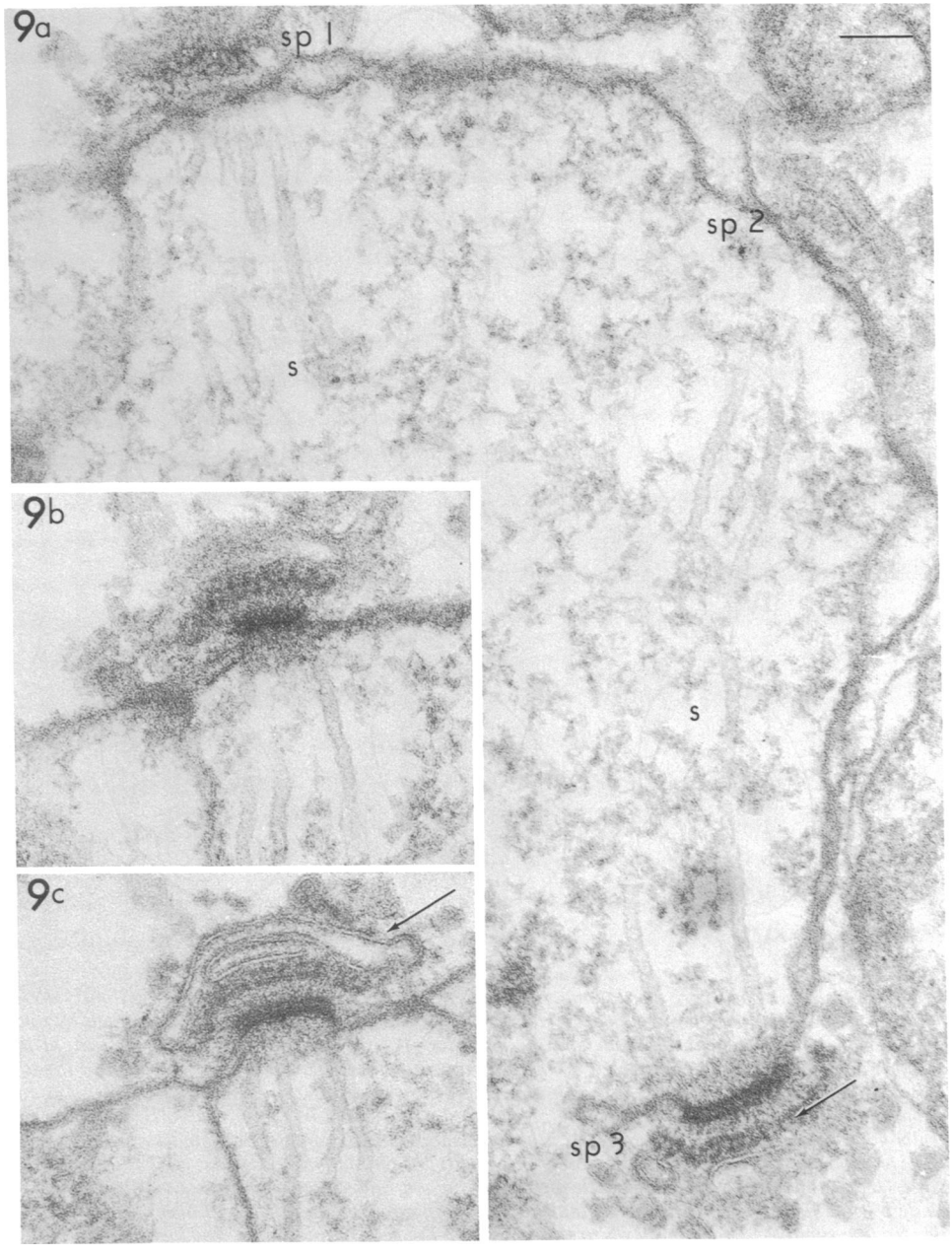


FIG. 9. *Meiosis II spindle plaques of Saccharomyces cerevisiae in profile. (a) Three of the four plaques present in a meiosis II nucleus are visible in this section. The components of spindle plaque 3 (sp 3) are most clearly seen. The outer zone of the plaque (arrow) is larger than in meiosis I and it is noticeably denser than the inner zone. The material comprising the region of low electron opacity between the outer and central zones of this plaque, as well as the outer zone itself, appears to be oriented in parallel arrays perpendicular to the surface of the nucleus. Only the edges of spindle plaques 1 (sp 1) and 2 (sp 2) are viewed in this section. Microtubules comprising both spindles (s) are present. ×84,000. (b and c) Spindle plaque 1 as observed in the next two sections. ×84,000. Flattened, membranous vesicles (arrow) appressed to the outer zone of the plaque can be observed in Fig. 9c. Calibration line represents 0.1 μm. (Reproduced from reference 240 by kind permission of J. B. Peterson and the* Journal of Cell Biology.)

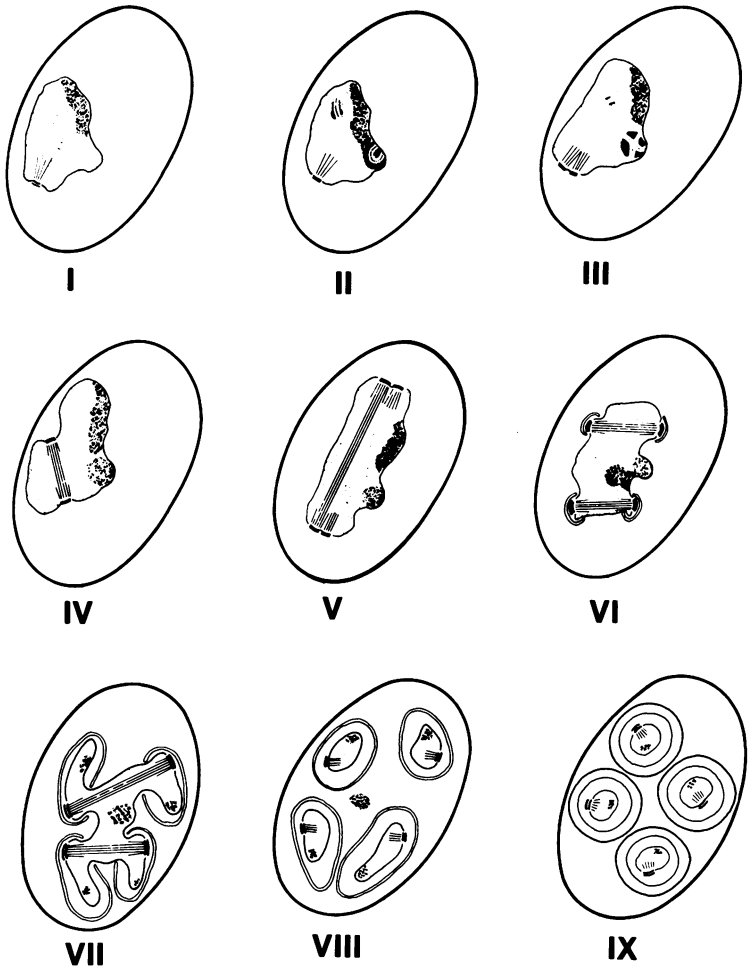


FIG. 10. *Meiosis and ascospore development in Saccharomyces cerevisiae. The nucleus is lightly dotted, the nucleolus is hatched, the polycomplex body is densely dotted, the spindle plaques are indicated by two short parallel lines at the nuclear envelope, the spindle microtubules are indicated by groups of straight parallel lines, the synaptonemal complex indicated by the dotted, tripartite structure in the nucleus, and the developing spore wall is indicated by the thick line on the cytoplasmic side of the spindle plaque. (I) During the first 4 h on sporulation medium, there is a single plaque and a nucleolus in the nucleus. (II) At about 6 h, the large cells have synaptonemal complexes and multiple synaptonemal complex-like structures aggregated in a single polycomplex body. In analogy with other fungi, higher plants, and animals, recombination occurs at this stage. (III) At about 6 to 8 h, the spindle plaques of the most advanced cells replicate. The two plaques remain side by side for some time. (IV) During the 9 h, the plaques move apart and bracket a short (0.8 μm) spindle. No free synaptonemal complexes are present, and the polycomplex body also loses the synaptic structures at this stage. The remaining fine granular round body persists until stage VI. (V) The spindle elongates and each of the plaques duplicates. Presumably this stage represents the first meiotic division up to telophase I. (VI) The two plaques of each pair separate and bracket a spindle. The second meiotic division is probably initiated at this time. The future ascospore walls have begun to develop next to each of the four plaques. (VII) Both spindles elongate and simultaneously cytoplasm, mitochondria, and nuclear material flow into the developing ascospores. This stage represents the end of the second meiotic division. Apparently, in this yeast both meiotic divisions occur within a single nuclear mass, and genome segregation is accomplished through the budding of the nucleus into the four developing ascospores. The parent nucleus decreases in size and the nucleolus remains behind. (VIII and IX) After 10 h on sporulation medium, the first asci with immature ascospores are present. (Reproduced with modifications from reference 228 by kind permission of P. B. Moens and the* Journal of Cell Biology.)

during prophase of both meiotic divisions (Fig. 11). It is reformed in telophase beginning at the polar side of each progeny group of chromosomes (205, 207). In the chlorophycean alga *Ulva*, the nuclear envelope is intact apart from polar fenestrae (24). A curious feature of this meiosis is that at prophase I the nucleus has an irregular outline due to invaginations of the nuclear envelope; these disappear by metaphase I. No explanation was offered for this observation, but it is conceivable that the invaginations at prophase represent newly synthesized membrane whose intercalation into the existing nuclear envelope allows expansion of the nucleus from an approximately spherical structure to the elongated cylindrical shape which it assumes later in meiosis.

Meiosis is intranuclear in those ciliates (316; Fig. 12) and sarcomastigophoran protozoa (42–51, 144, 291) so far investigated. Cleveland's detailed light microscope studies (50) of meiosis in *Barbulanympha* uncovered an intriguing sequence of events. In meiosis I the progeny chromosomes are pulled apart within the intact nuclear envelope. Later in meiosis I, the central portion of the nucleus is constricted on both sides. This leaves each of the progeny nuclei complete with its original nuclear envelope, while the central region of the nuclear envelope together with its contents (including the nucleolus) is left in the cytoplasm where it eventually disintegrates. Cytokinesis follows the first meiotic division. Meiosis II is also intranuclear; in this case cytokinesis occurs in telophase so that each progeny cell contains a nucleus whose chromosomes have not condensed. During telophase, a new nuclear envelope is formed within the confines of the old one which is breaking down. Formation of new nuclear envelope in this manner is unusual but not unique; similar events occur during mitosis in the micronucleus of the ciliate *Blepharisma* (160).

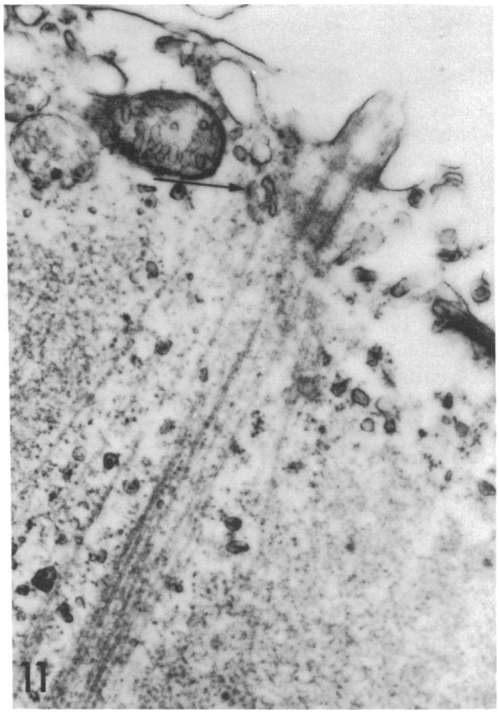


FIG. 11. *Meiosis II in the marine centric diatom Lithodesmium undulatum. View of one end of the spindle showing traces of the curved polar plate (arrow) beside the flagellar base. ca. ×30,000. (Reproduced from reference 207 by kind permission of I. Manton, K. Kowallik, and H. A. von Stosch and the* Journal of Cell Science.)

In *Echinostelium minutum*, meiosis I is intranuclear (130), whereas in *Physarum*, another genus of Myxomycetes, the nuclear envelope remains intact until anaphase (2) and is reformed around the progeny nuclei at telophase. In the plasmodiophoromycete *Sorosphaera*, the meiotic nuclear envelope is intact apart from polar fenestrae (22; Fig. 13). In *Labyrinthula* (239) there is partial breakdown of the nuclear envelope at the end of prophase I. Some fragments of membrane material move to the poles and are reformed around the progeny nuclei. A similar sequence of events occurs in the second meiotic division and in the subsequent mitosis. The events in *Labyrinthula* indicate that material from the original nuclear envelope contributes to the new nuclear envelopes. This is probably true in other instances where there is limited dissolution of the original nuclear envelope. And it is probably safe to assume that in many intranuclear meioses the new nuclear envelope is formed directly from the old nuclear envelope, although this is apparently not the case in all intranuclear meioses as indicated by the above description of events in *Barbulanympha*.

The significance of the range of behavior exhibited by the nuclear envelope remains obscure: why should it disperse in some organisms and not in others? In coenocytes, nuclear divisions are usually synchronous and hence the persistence of the nuclear envelope during meiosis could prevent intermingling of the meiotic spindles (which might result in chromosomal imbalance). It could also be argued that

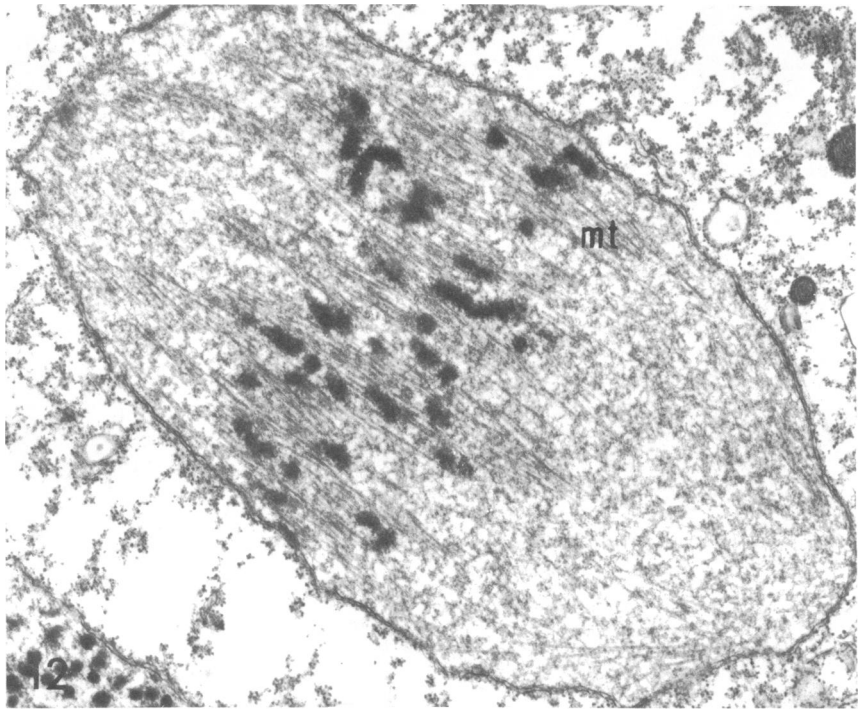


FIG. 12. *Meiosis in the micronucleus of the ciliate Paramecium aurelia. The chromosomes congregate to form a typical metaphase. A variety of shapes and sizes are evident. Microtubules (mt) are not so well organized away from the metaphase plate. There are no well-defined poles at this stage, and no centrioles are associated with the nucleus. ×15,200. (Reproduced from reference 316 by kind permission of I. Stevenson and the* Australian Journal of Biological Sciences.)

intranuclear meiosis serves to maintain the internal environment of the nucleus by preventing direct contact between the nucleoplasm and the cytoplasm. But this immediately raises the question of what occurs in those organisms where the nuclear envelope disperses during meiosis. The fact that the latter organisms include the most advanced forms in at least three separate evolutionary pathways (higher fungi, higher plants, higher animals) indicates that dispersal of the nuclear envelope during meiosis might be an advantageous characteristic.

Nucleoli

Nucleoli frequently disperse in early meiosis, but in a number of instances they persist to varying degrees. For example, in the flagellate *Barbulanympha* most of the nucleoli fuse in prophase; this mass of nucleolar material remains at the equator during meiosis I and is not incorporated into the nuclei at telophase (50). In some species of *Ascobolus* and *Podospora*, the nucleolus does not disperse during meiosis but instead occupies a protrusion of the nuclear envelope (359). In contrast, the nuclear envelope of *Neottiella* invaginates at the end of prophase causing the nucleolus (which was formed by fusion of two nucleoli following karyogamy) to leave the nucleus (351); it remains attached to the nuclear envelope and is recognizable until telophase. In this organism pronucleoli are formed in telophase and fuse to give the new nucleolus (351). The formation of a single nucleolus in *Neottiella* following karyogamy probably reflects the commencement of some form of pairing between the nucleolar-organizing chromosomes. In another ascomycete, *Gelasinospora*, the nucleolus enlarges at zygotene but decreases in size again at diffuse diplotene (192). Lu correlated the increased size of the nucleolus to the need to provide ribosomes for synthesis of material to be used in the enlargement of the ascus. Following this increase, the nucleolus returns to its normal size and persists through the meiotic divisions.

Recombination appears to be unaffected by the presence of nucleoli, but the dispersals of these structures later in meiosis might allow

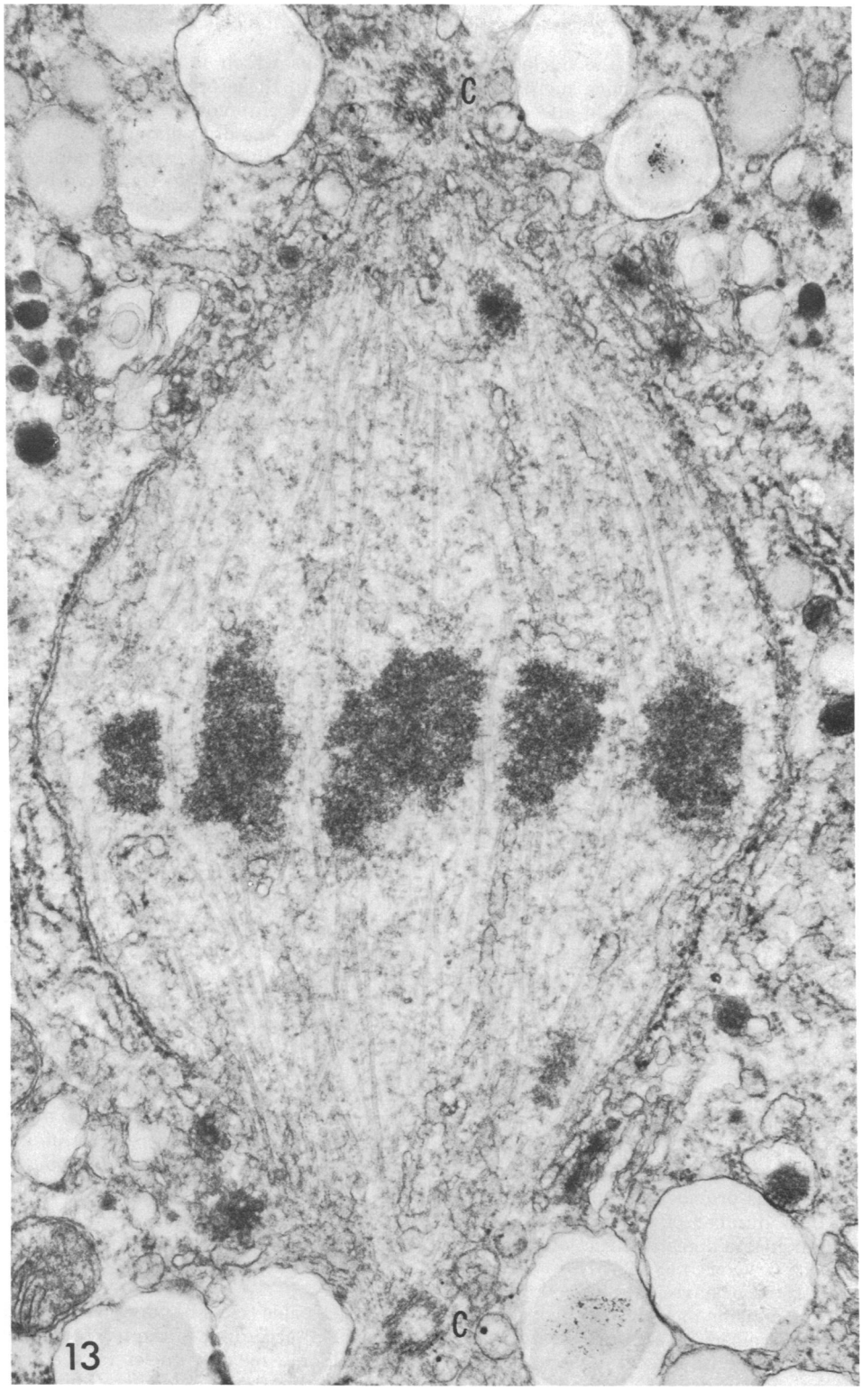


FIG. 13. *Sorosphaera veronicae. Survey electron micrograph of a metaphase nucleus (presumably meiosis I) with a centriole (C) at each pole. Note that the nuclear envelope is still present, although it is disrupted at the poles, and microtubules penetrate the nuclear envelope in those regions. (Reproduced from reference 22 by kind permission of J. P. Braselton and* Mycologia.)

the chromosomes (especially the nucleolar-organizing chromosomes) to move more easily. Dispersal of the nucleolus would also create a supply of ribosome precursors which would be needed in the meiotic products. This would be especially necessary in those organisms where ribosomes are broken down prior to or during meiosis (see p. 223).

Chromosomes and Kinetochores

The degree of chromosome condensation prior to and during meiosis varies considerably. For example, yeast chromosomes are difficult to recognize during interphase and meiosis (223, 228, 240). But in *Neottiella*, another ascomycete, chromosomes are contracted both during interphase (350) and for most of meiosis (273). However, between pachytene and diplotene there is a "disintegration" stage (273) during which chromosomes appear thin and less distinct. A comparable stage during which the appearance of the chromosomes has been described as "fuzzy" (360) or "diffuse" (9, 192) has been observed in other ascomycetes (9, 192, 360; Fig. 20–23), in the basidiomycete *Coprinus* (196), and in some rhodophycean algae (70, 71). It has been variously suggested that these changes may be associated with increased gene activity (192, 196) or with genetic crossing-over (9, 273). Diplotene chromosomes also become diffuse in *Paramecium* (316). The micronucleus at this time is considerably elongated and is referred to as the "crescent stage"; later the micronucleus diminishes in size and enters metaphase I (316). A similar crescent stage has been observed during meiotic prophase in *Tetrahymena* (261, 322) and has been shown to correspond to a period of active RNA synthesis. During this stage in *Tetrahymena* the chromosomes become arranged into two chromatin threads each apparently consisting of five chromosomes in end-to-end association. The two chromatin threads are paired along their lengths. The chromatin threads later contract to form a thick rod-like structure; this subsequently breaks up into individual chromosomes which then enter diakinesis and metaphase I.

Cleveland has provided detailed accounts of meiosis in a number of symbiotic flagellates which occur in the digestive tract of the wood-eating roach *Cryptocercus* (42–51). Five genera of these protozoa have an unusual form of meiosis, one-division meiosis, in which chromosomes enter meiosis in an unreplicated state (43–47). At metaphase a certain degree of chromosome pairing occurs (45, 46) and the homologs separate to opposite poles (46). Apparently it is the random segregation of homologous chromosomes which is the source of genetic variability in these organisms, since crossing over between chromosomes does not occur in this particular one-division meiosis (43, 45, 46). Consequently, this process is evidently at an evolutionary disadvantage compared with two-division meiosis (43). Nonetheless, organisms with both types of meiosis are able to coexist in the same environment, and hence those with one-division meiosis do not appear to be greatly disadvantaged. These protozoa may be very ancient symbionts of the hindgut that are by now well adapted to that relatively constant environment and so do not have such a great need for genetic diversity.

Cleveland investigated two other genera, *Trichonympha* (42) and *Barbulanympha* (49, 50), from the gut of *Cryptocercus* and determined that these had two-division meiosis. The centromeres of *Trichonympha* and *Barbulanympha* are attached to the nuclear envelope during meiosis (42, 50). Probably growth of the nuclear envelope (172, 187, 242) is at least partially instrumental in effecting the initial separation of the meiotic chromosomes. Electron micrographs of mitotic nuclei of *Trichonympha* (171) indicate that kinetochores occur in close association with the nuclear envelope. In telophase II of *Barbulanympha*, when the nuclear envelope is formed inside the original nuclear envelope, the centromeres leave the old envelope and become attached to the new one (50). The ultrastructural details of this event would doubtless be interesting; possibly the kinetochore is transferred as part of a fragment of nuclear envelope.

The kinetochore of *Paramecium* is indistinct at both mitosis (317) and meiosis (316), but light micrographs of another ciliate, *Tetrahymena*, clearly show that the chromosomes have median and submedian centromeres (261). Similarly, structures recognizable as kinetochores were not observed in the alga *Lithodesmium*, even though contacts exist between chromosomes and spindle microtubules (206) and light microscopy indicates the presence of centromeres (207). The genetics of *Lithodesmium* have not been investigated, and consequently the presence of a centromere cannot be verified by genetic means.

Spindle Pole Bodies

Spindle poles can be divided into three classes (198) according to the type of structure present. Some meiotic nuclei lack a distinct polar structure: the spindle microtubules terminate in an area of cytoplasm or, in the case of intranuclear meioses, close to the nuclear enve-

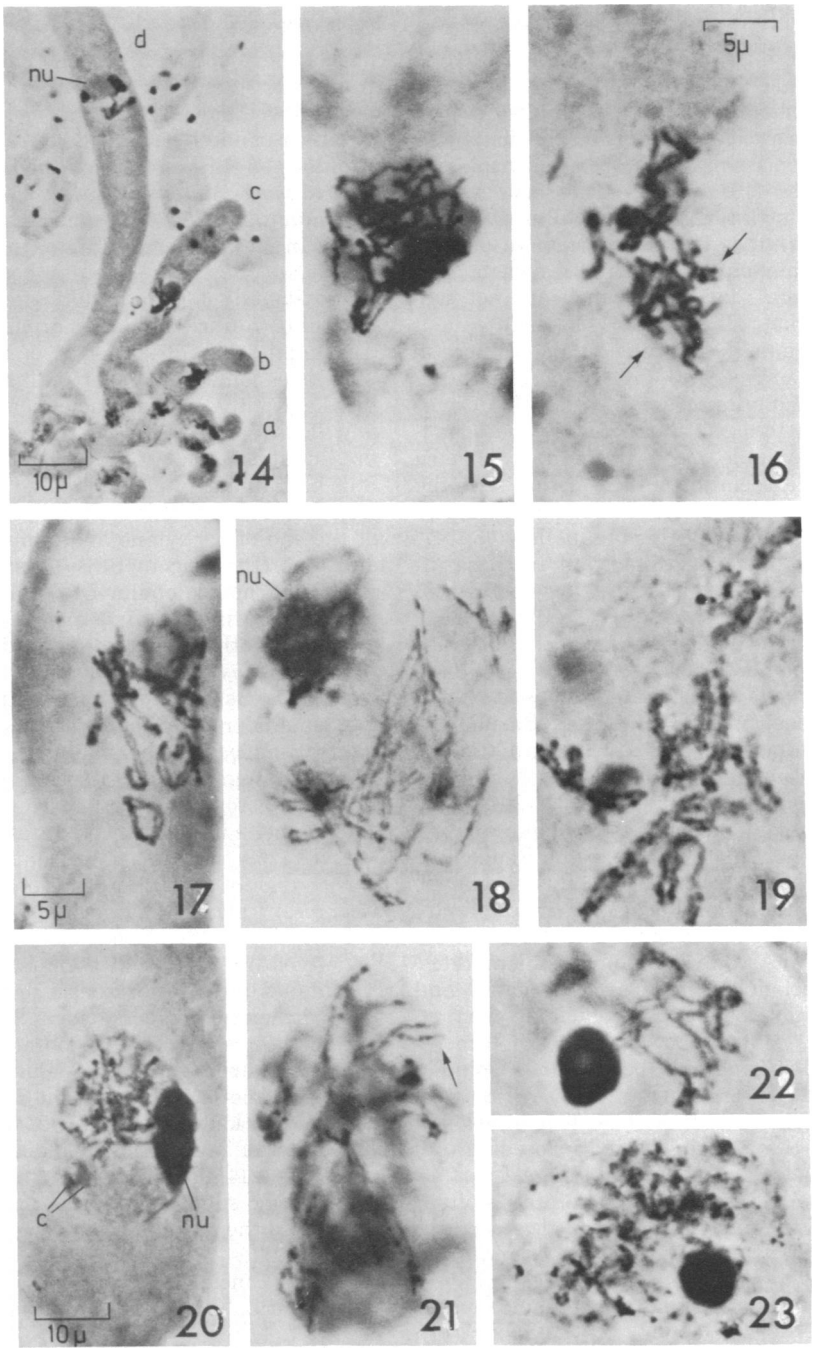


FIG. 14–23. *Light micrographs of the first meiotic division in Ascomycetes. (Reproduced from reference 360 by kind permission of D. Zickler and* Chromosoma.*) Fig. 15, 16, 18, 19, 21, 22, and 23 have the magnification shown in Fig. 16.*

FIG. 14. *Asci of advancing development in Podospora anserina; note the important increase of the nucleolus size from a to d. (a) Crozier; (b) early pachytene; (c) full pachytene; (d) late pachytene; nu, nucleolus.* FIG. 15. *Karyogamy in Ascobolus immersus.* FIG. 16. *Zygotene of A. immersus, some chromosomes are paired (arrows).* FIG. 17. *Early pachytene of A. immersus; the chromosome pairing is complete.* FIG. 18. *Full pachytene of A. immersus; note the straight bivalents.* FIG. 19. *Late pachytene of A. immersus; the chromosomes are fluffy and less straight.* FIG. 20–22. *Early diffuse stage; the two homologues of each bivalent are widely separated (arrow).* FIG. 20. *A. immersus. Note that the centrosomal plaque (c) divides at this stage.* FIG. 21. *A. immersus.* FIG. 22. *Podospora anserina.* FIG. 23. *Ascophanus carneus diffuse stage; the chromosomes are most indistinct.*

lope. The remaining two categories, involve recognizable SPBs; these may either be a centriole or some other structure. The word "centriole" is reserved exclusively for the distinctive organelle consisting of nine skewed triplets of microtubules (107) which may serve as the basal body for a flagellum or cilium. It is now recognized that it is inappropriate to use the word centriole (or derivatives of it such as "centriolar plaque") for SPBs which are not centrioles. Pickett-Heaps (244) has pointed out that this organelle is but one type of microtubule-organizing-center (MTOC). The other types of SPBs are presumably also MTOCs, as they appear to direct the assembly and behavior of the spindle. Aside from its role as a MTOC, the association of the centriole with the meiotic apparatus ensures that each progeny cell receives a copy of this organelle (105, 242, 244); this is especially important in those instances where centrioles cannot be synthesized de novo and where there are flagellated stages in the life-cycle. It appears that centrioles are absent in members of those natural groups of protists which lack flagellated stages, e.g., the Rhodophyceae, Ascomycetes, and Basidiomycetes. The Rhodophyceae are thought to be a primitive group of protists which arose prior to the evolution of the centriole (39, 204, 246). Light microscopy indicates that a polar body is present in some meiotic cells of the Rhodophyceae (70). The polar body is probably similar in structure to the polar ring seen in electron micrographs of rhodophycean algae (211).

The SPB in Ascomycetes and Basidiomycetes has been variously termed: archontosome, centriole, centriole-like body, centriolar plaque, centrosome, centrosomal plaque, kinetochore equivalent, peripheral body, and spindle plaque (214, 257). Many of these SPBs are closely associated with the nuclear envelope. Frequently the SPB is closely apposed to the nuclear envelope or is even an integral part of its structure, while spindle microtubules often arise inside the nucleus opposite this structure (228, 240, 359; Fig. 8, 9). In *Coprinus radiatus* the SPBs are present outside the nuclear envelope early in prophase I but then penetrate the nuclear envelope and form the poles of the intranuclear spindle (326).

Detailed knowledge of the morphology and behavior of one type of SPB results from investigations on three species on the basidiomycete *Coprinus: C. lagopus* (193, 196, 257; Fig. 24–27); *C. radiatus* (188, 326); and *C. stercorarius* (268). In its unreplicated state, the SPB of *Coprinus lagopus* is a globular body (Fig. 26), approximately 0.3 μm in diameter, which consists of both fibrillar and amorphous material. The duplicated SPB consists of two of these structures joined by a short isthmus (Fig. 27); breakage of the isthmus region leaves the progeny SPBs free to migrate to the poles. SPBs are present at mitosis, but they are absent from the cells both immediately before and immediately after karyogamy. This organelle arises de novo at the end of pachytene and duplicates in late diplotene. The progeny SPBs separate in diakinesis, and monoglobular SPBs are present at the poles during the first meiotic division. In prophase II, the SPBs are again double structures, but they divide to give rise to the monoglobular SPBs which occur at the poles during the remainder of meiosis. The morphology of the SPB appears to be similar in *C. radiatus* (188), although in this account there are no electron micrographs to show the structure of this organelle in its duplicated state. In *C. stercorarius* (268) there is a significant difference in the behavior of the SPB. In this organism SPBs are present prior to karyogamy, and the two SPBs in the postfusion nucleus are thought to be derived from different parents. Duplication of SPBs occurs in interkinesis so that each of the four meiotic products has an SPB. In other basidiomycetes the SPB was observed to be diglobular in structure not only immediately before each meiotic division (as in *Coprinus*) but also prior to karyogamy (121, 214) and at the completion of meiosis (121). McLaughlin's observations (214) have been queried by Raju and Lu (257) who suggested that he may have misinterpreted the stage of the life cycle; but more recent studies (121) appear to support McLaughlin's conclusions.

Raju and Lu (257) draw attention to the simi-

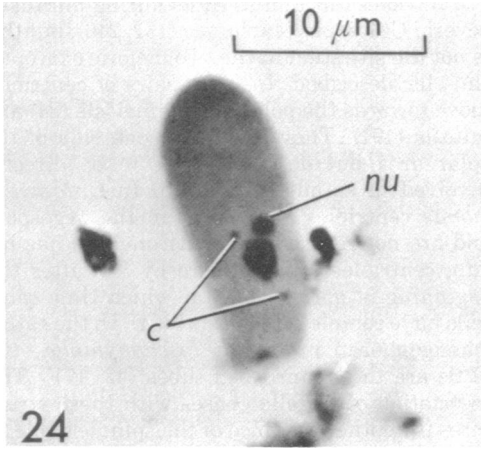
FIG. 24–27. *Spindle pole body behavior during meiosis in Coprinus lagopus. Abbreviations: c, spindle; n, nucleus; ne, nuclear envelope; nu, nucleolus. (Reproduced from reference 257 by kind permission of B. C. Lu and* Chromosoma.)

FIG. 24. *Metaphase of premeiotic mitosis. Spindle pole bodies are spherical (monoglobular).* ×2,500.

FIG. 25. *Young basidia 15 h before karyogamy. One spindle pole body is visible in each basidium.* ×2,500.

FIG. 26. *Electron micrograph of a basidium at early diplotene. Spindle pole body is attached to an indented portion of the nuclear envelope.* OsO_4 *fixation.* ×60,000.

FIG. 27. *Electron micrograph of late diplotene. Spindle pole body is diglobular. Glutaraldehyde-*OsO_4 *fixation.* ×46,000. *The inset is a photomicrograph of a basidium at late diplotene.* ×2,500.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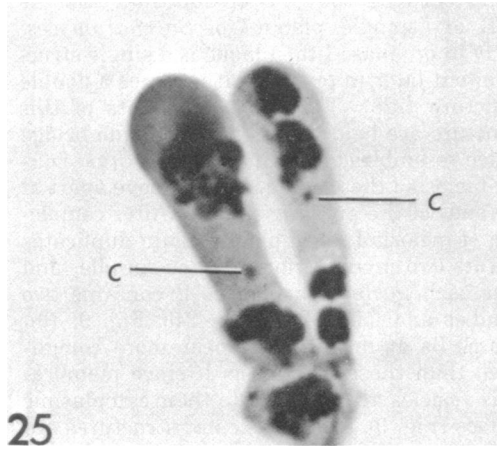
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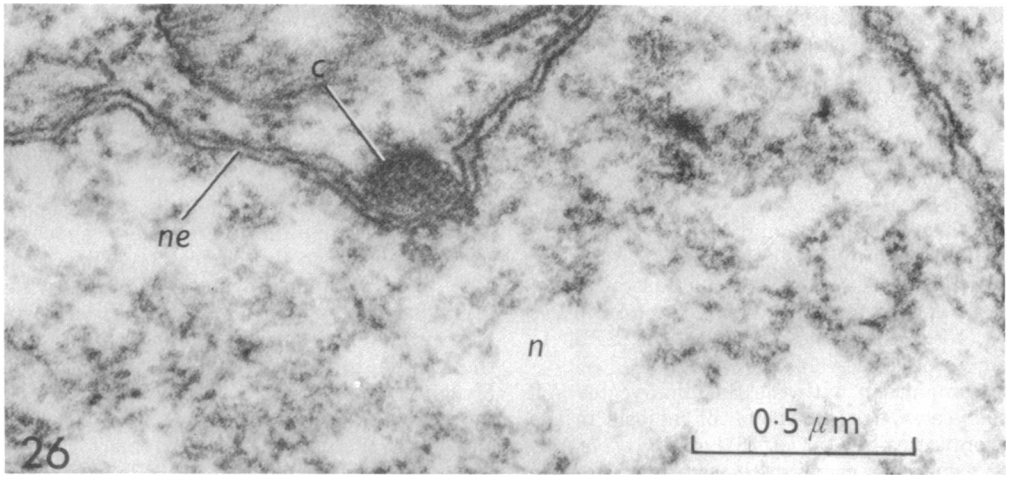
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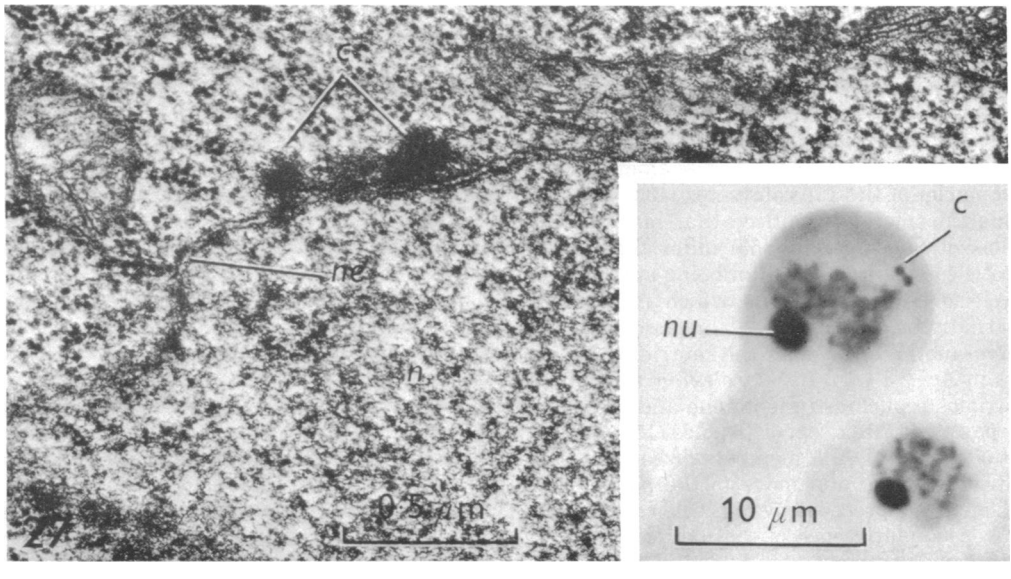
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larity between the SPB of *Coprinus* and the SPB or "spindle plaque" of *Saccharomyces:* early in prophase I the plaque is a single structure, but later in prophase it becomes a double structure (228). The two components of this structure are held together by a plaque bridge which resembles the isthmus of *Coprinus*. During meiosis I the spindle plaques move apart at the ends of the growing spindle. After completion of meiosis I, each plaque again duplicates and its two products form a new spindle, and thus each nucleus in meiosis II contains two spindles and four SPBs (228, 240; Fig. 9, 10). The SPBs at meiosis II appear more complicated than those at meiosis I, since membranous vesicles are attached to their cytoplasmic surface (Fig. 9). These vesicular structures are probably involved in ascospore formation, a process begun before the completion of meiosis (228; Fig. 10). It is of interest to note that the SPBs of *Saccharomyces* are also involved in karyogamy (31).

The SPBs in some other species of Ascomycetes have features in common with those of *Saccharomyces,* for example, close association with the nuclear envelope (14, 345, 358, 359) and differences in size and/or structure at different stages of meiosis or between meiotic and mitotic nuclei (14, 192). Although astral microtubules appear to be absent in *Saccharomyces* (240), they are associated with the SPBs in other Ascomycetes (14, 345, 359).

In comparison with the Basidiomycetes and Ascomycetes, less is known of meiosis in the Phycomycetes. In *Achlya* (81) and *Saprolegnia* (153), centrioles occur in the cytoplasm at the poles of meiotic nuclei. Since meiosis is intranuclear there is no direct contact between centrioles and spindle microtubules, but there appears to be a close relationship between them as spindle microtubules terminate at the nuclear envelope directly across from the centrioles. This resembles the arrangement in mitotic nuclei of the Phycomycetes (133, 156), although it should be noticed that meiotic centrioles of *Saprolegnia* (153) differ from their mitotic counterparts (133) in being surrounded by electron-dense material which is bounded externally by a membrane. This association of membrane material with the centriole also occurs in *Achlya* (81). In *Saprolegnia* the pair of centrioles replicates in leptotene and the progeny pairs migrate to opposite poles (153). At the end of meiosis I, the members of each pair of centrioles divide and move to the poles. This division is not preceded by centriole replication, and consequently each of the meiotic products possess one centriole.

Centrioles function as SPBs during mitosis in several Chlorophycean algae (131, 246), but this is not the situation in the two meiotic examples thus far described. In *Ulva* pairs of centrioles move towards the poles during meiosis (24) and mitosis (191). They remain at one side of the polar area but do not appear to be directly involved in spindle formation. In *Chlamydomonas* centrioles are absent in the zygospore and are not reassembled until meiosis has begun; centrioles are still rare by 8 h after the beginning of germination at which time some cells have completed meiosis I (34). In the sarcomastigophoran protozoan *Trichonympha,* the SPBs are the hemirostral tubes (42, 171). The association of flagellar bases with these structures is another instance of the spindle's role in effecting the distribution of centrioles to progeny cells where they can function as the basal bodies of flagella. The situation is different in the ciliates since the presence of hundreds of cilia over the surface of the cell ensures that each of the progeny cells automatically inherits some cilia. Consequently it is not surprising that centrioles are absent from the poles of mitotic (161, 317) and meiotic (316; Fig. 12) ciliate nuclei; spindle microtubules appear to terminate close to the nuclear envelope. Similarly, it would appear that in organisms where centrioles can be formed de novo there should be no need for them to be located at the poles of the spindle. In fact, centrioles are absent in the Myxomycetes during meiosis but are present in the cytoplasm of the resulting spore (2, 4, 220). Some other organisms which can form centrioles de novo have different SPBs at different stages in the life-cycle. An example of this phenomenon is provided by the sarcomastigophoran protozoan *Myxotheca,* in which "centrosomal bodies" function as the SPBs during meiosis, while centrioles are present at the poles during the mitotic divisions preceding formation of flagellated cells (291). A somewhat similar situation exists in the bacillariophycean alga *Lithodesmium*. For most of its life-cycle *Lithodesmium* is non-flagellated, centrioles are absent, and the poles of the spindles at mitosis and meiosis I are occupied by polar plates (Fig. 28–31). Centrioles form at the poles during interkinesis and flagella begin to grow; both centrioles and polar plates occur in close association at the poles during meiosis II (Fig. 11). The meiotic products are flagellated gametes (207). The de novo formation of centrioles during interkinesis in *Lithodesmium* may indicate that these structures have some active involvement in meiosis II, since otherwise they might be formed after meiosis as is the case in

the Myxomycetes. Similarly the de novo synthesis of centrioles during prophase I in *Labyrinthula* (238, 239) and their location at the poles during both meiotic divisions may indicate that these structures are active in meiosis and that in this case their presence at the poles is not merely a means of ensuring their distribution to the meiotic products.

The significance of the SPB varies according to the organism. In those instances where this structure is a centriole, its location at the poles may ensure its distribution to the progeny cells (242), i.e., the centriole as such has little or no involvement in the production of the spindle. However, in the Ascomycetes and the Basidiomycetes, the fact that the SPB often arises de novo before nuclear division and can exist in the mono- or diglobular forms according to the stage of the nuclear cycle suggests that here the SPB is involved only with nuclear function (and possibly also with ascospore wall formation).

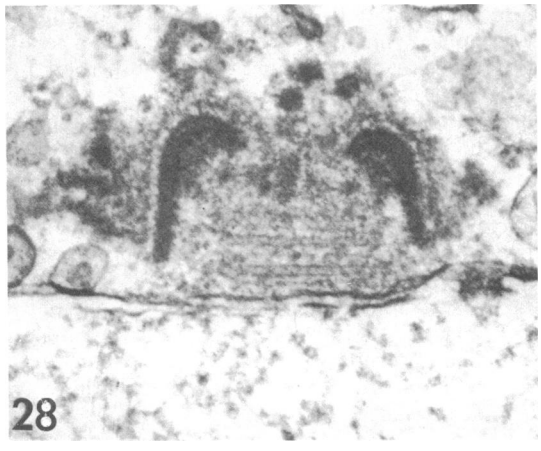
Spindle Microtubules

There are three main types of spindle microtubules: chromosomal microtubules, polar microtubules, and astral microtubules. Chromosomal microtubules extend from the poles to the chromosomes where they usually terminate at kinetochores. Polar microtubules do not make contact with the chromosomes; they either extend from one pole to the other (in which case they are often termed continuous microtubules) or they overlap at the equator of the nucleus with polar microtubules from the opposite pole of the spindle. Astral microtubules radiate from the spindle poles into the surrounding cytoplasm; they are often absent or inconspicuous. Interaction between microtubules is thought to be responsible for alignment of chromosomes across the equator of the nucleus at metaphase (7, 136, 212). Anaphase chromosome movement in mitotic cells usually involves two components (26, 210, 213): an increase in the distance between the poles, and a shortening of the pole-to-chromosome distance. Both these movements may be involved in anaphase, e.g., in *Ascobolus* (345), or one may predominate over the other, e.g., in *Paramecium* (316). In the latter case it can be calculated from Fig. 14 and 17 (Stevenson, 316) that the length of the nucleus extends from about 8.7 μm at metaphase II to approximately 25 μm at telophase II. In spite of this, there is no significant decrease in the distance of the chromosomes from the poles. Thus, although chromosomal microtubules are apparently present [Stevenson notes that "Each chromosome is small (up to 1 μm long, usually less), and many appear round, attached to a bundle of microtubules at an indistinct site" (316)], their major function appears to be one of attaching the chromosomes to the poles, and there appears to be little, if any, chromosome to pole movement. During mitosis in the micronucleus of *Paramecium* there is also pronounced elongation of the nucleus and eventually a separation spindle over 30 μm extends between the progeny nuclei (317).

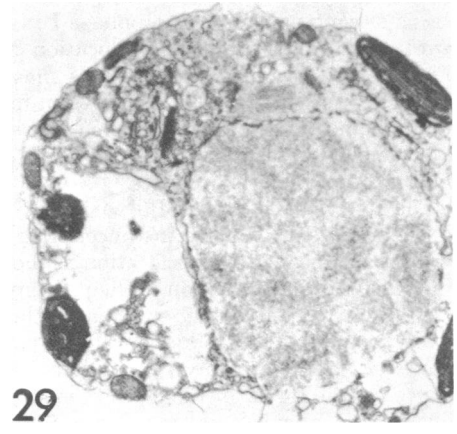
Extranuclear spindle fibers are present during meiosis in several genera of sarcomastigophoran protozoa (42-51). The nuclear envelope remains intact and consequently there is no direct contact between chromosomes and spindle fibers. Chromosomal fibers extend from the SPBs to the centromeres which are associated with the nuclear envelope. Continuous spindle fibers extend from one SPB to the other. Elongation of the continuous spindle is the major cause of nuclear extension and separation in these organisms; shortening of the chromosomal fibers appears to be of minor importance. In mitotic cells of *Trichonympha*, the nuclear envelope may be involved in the initial separation of progeny chromosomes (171), and a similar mechanism in meiosis is quite possible.

Manton and her colleagues have provided a detailed description of spindle structure at different times during meiosis in *Lithodesmium* (205-207). At prophase I an extranuclear spindle forms close to the nuclear envelope between the SPBs (205; Fig. 28-31). The spindle elongates and eventually sinks into the nucleus when the nuclear envelope breaks down. Longitudinal sections of metaphase nuclei show a central spindle extending from pole to pole across the nucleus (205, 207). Transverse sections across the spindle at prophase indicate that approximately 10% of the spindle microtubules are continuous from one pole to the other. The remaining microtubules extend from one pole to just beyond the equator (205, 206). Bundles of microtubules which are regularly arranged with respect to each other were observed in the equatorial region at metaphase. The numbers of bundles are similar in both divisions and approach the haploid chromosome number, but the bundles at metaphase I contain approximately twice as many microtubules as their counterparts in the second meiotic division (207). Evidently bundles attached to chromosomes contain double the numbers of microtubules present in bundles attached to chromatids.

These observations on *Lithodesmium* suggest that there is no net synthesis of microtu-



28



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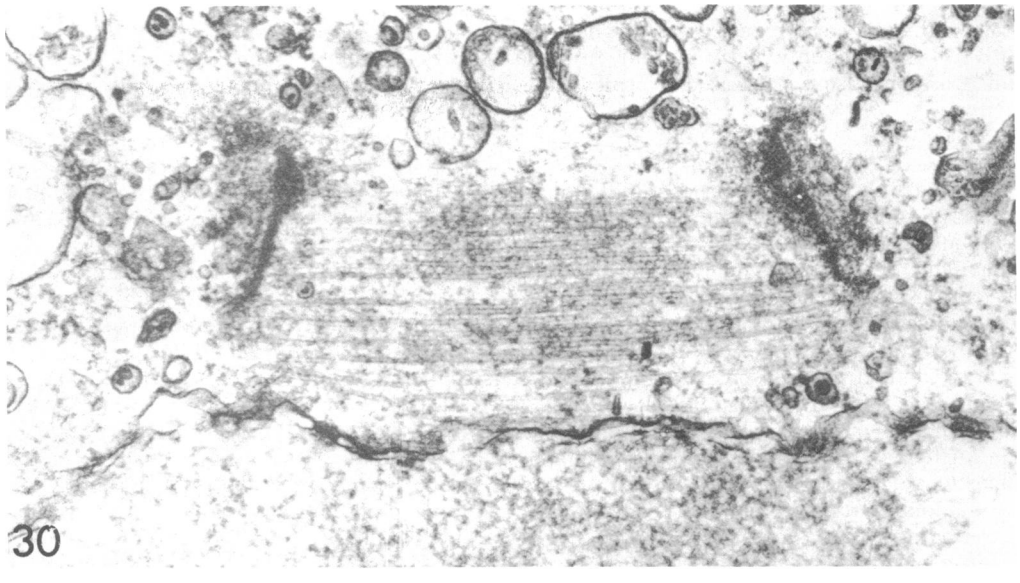

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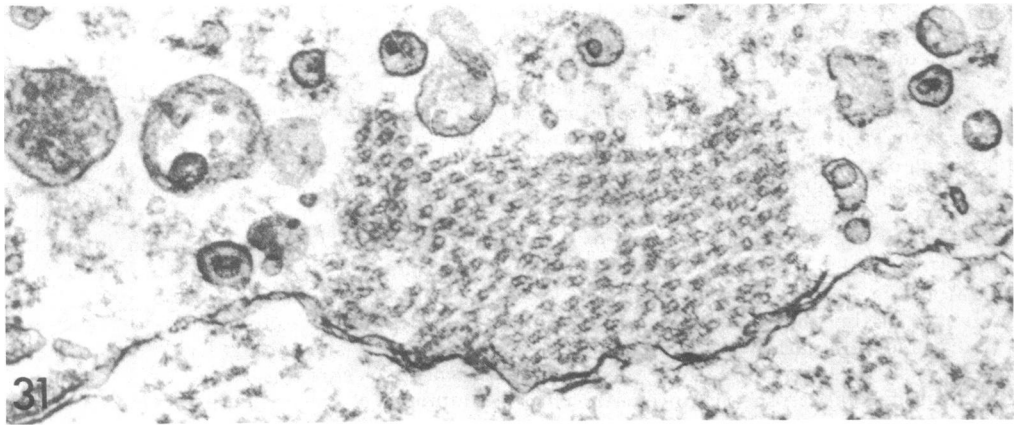

31

bules between the two meiotic divisions and that microtubule subunits from meiosis I are used to form the spindle at meiosis II. This is hardly surprising in view of the ease with which microtubules are broken down and reassembled (296, 327). Similarly, in the basidiomycete *Boletus* cytoplasmic microtubules disappear at the time of meiotic spindle formation (214). Presumably they depolymerize to provide the microtubule subunits which are used in the spindle. *Paramecium* provides another example of microtubule versatility since in this organism microtubules are abundant in the nucleus during the crescent stage of prophase, in each of the meiotic divisions, in the division of the surviving meiotic product, and in the male pronucleus (316). They are absent or inconspicuous between each of these stages. The presence of microtubules in the male pronucleus is of interest since they could be involved in its migration (usually over a distance of several microns) before it fuses with the female pronucleus (316). It is possible that microtubules and/or microfilaments might also be involved in nuclear rotation at prophase I in *Gymnodinium* (340) and at the end of meiosis in *Ulva* (24). Ultrastructural aspects of meiotic spindle organization are now known for a number of protists. What is urgently needed are detailed investigations of meiotic spindles similar to those undertaken in mitotic systems, e.g., time-lapse phase-contrast photography of meiotic divisions in untreated cells and those exposed to different spindle inhibitors, biochemical analyses of isolated and purified meiotic apparatuses, and micromanipulation of meiotic chromosomes.

Duration of Meiosis

There are relatively few estimates of the duration of meiosis. Approximately 4.5 h elapse between the start of prophase I and the end of telophase II in *Lithodesmium* (207), about 2 h in *Saccharomyces* (148, 328), and less than 100 min in Allomyces (82). In *Coprinus radiatus* the two meiotic divisions occur in a time period of 30 to 40 min (188). Cells of the myxomycete *Stemonitis herbatica* progress from metaphase I to telophase II in 10 to 15 min (4). These observations indicate that not only are the meiotic divisions themselves speedily completed, but that interkinesis must be brief, and it is sometimes noted that meiosis II occurs immediately after meiosis I, e.g., in *Paramecium* (316). In contrast, there is a prolonged interkinesis in some dinoflagellates and in one of these, *Wolosyznskia*, the two meiotic divisions frequently (but not always) occur in different cells (340). There is also an interphase period between the two meiotic divisions in the Rhodophyceae (70, 71); the duration of this interphase is not known, but it has to be long enough to allow for chromosomal DNA to be replicated. This is necessary as meiosis I evidently involves pairing and separation of unreplicated homologous chromosomes (70, 71). This type of meiotic behavior may well be primitive as suggested by DuPraw (74), and may, in fact, be a form of one-division meiosis.

Fate of the Meiotic Products

In the majority of algae and fungi all the products of meiosis can be recovered, often directly as the tetrad. However, some organisms have one or more mitotic divisions following shortly after meiosis. In this manner, 8 and 16 flagellated zoospores are released after meiosis in *Labyrinthula* (239) and *Ulva* (24), respectively. In contrast, instances are known where some of the meiotic products (usually three out of four) degenerate (130, 270). Observations on ciliates have provided an insight into the factors which determine the fate of the meiotic products. In *Tetrahymena* (261) the nucleus closest to the point of attachment between the two conjugants survives while the other three nuclei disintegrate. Instances where all the meiotic products degenerated were thought to have occurred because none of the nuclei was

FIG. 28–31. *Meiosis I in the marine centric diatom, Lithodesmium undulatum. (Reproduced from reference 205 by kind permission of I. Manton, K. Kowallik, and H. A. von Stosch and the* Journal of Cell Science.)

FIG. 28. *Spindle precursor and young spindle cut in a plane perpendicular to the nuclear envelope. ×50,000.*

FIG. 29. *A spermatocyte towards the end of prophase of the first meiotic division reduced to its normal size after completion of the swelling phase and with the spindle beginning to elongate after breakdown of the center of the spindle precursor. ×5,000.*

FIG. 30. *The spindle from the cell of Fig. 29, showing the centrosomal plates enlarged and moving apart. The differentiation characteristic of the equator versus the spindle ends is already clearly visible; the nuclear envelope is still intact. ×30,000.*

FIG. 31. *Transverse section through the equator of an exceptionally large specimen at approximately the stage of Fig. 30, showing the spindle tubules in slightly curved rows with a few gaps; apart from these the arrangement approximates to "square" close-packing. ×60,000.*

sufficiently close enough to this point. The situation is similar in *Paramecium* (316) except that in this organism there are two micronuclei which give rise to eight meiotic products. The one of these that is in the paroral cone region of the cell survives, and degeneration of the remaining seven occurs in less than 30 min. In both *Tetrahymena* and *Paramecium*, the surviving haploid nucleus divides to give two pronuclei, one of which remains stationary while the other migrates into the conjugant and fuses with the stationary nucleus of that individual (261, 316). It therefore appears that survival of the nucleus closest to the attachment point between the two individuals ensures that its division products are conveniently located to allow reciprocal cross-fertilization between the conjugants.

INDUCTION OF MEIOSIS

In many organisms the occurrence of meiosis in natural isolates has been reported but no attempt has been made to determine the conditions necessary for the process. In the part of the review, we confine ourselves to the organisms where such conditions are partially or completely defined and emphasize those that have the most promise in yielding information about the process of meiosis itself.

The point at which commitment to meiosis (or to karyogamy in those organisms where zygotic reduction is the rule) occurs has not been carefully defined for most organisms, but where it has been investigated it is found to occur surprisingly late. For example, the gametes of *Chlamydomonas* can revert to vegetative growth at any time up to fusion (165), whereas yeast will remain diploid if returned to vegetative medium any time up to the first meiotic division (111, 301).

Organisms which undergo zygotic reduction usually require some sort of environmental stimulus in order to achieve sexual maturity. Culture of the haploid forms of *Chlamydomonas reinhardi* in nitrogen-free medium under strong illumination and aeration leads to 100% gamete formation (37). Meiosis takes place in the zygospores, which are formed after fusion of the gametes (36).

In *Neurospora*, sexual differentiation (protoperithecium formation) occurs on a solid medium with nitrogen being supplied in the form of KNO_3 (349). An exception is *N. tetrasperma*, which forms protoperithecia under all growth conditions (348). Fusion of the trichogyne of the protoperithecium with a cell of the opposite mating type leads to formation of a heterokaryon, which gives rise to numerous croziers in which karyogamy and meiosis take place. Each crozier forms an ascus containing the haploid ascospore progeny. The ascus spores may be binucleate heterokaryons (*N. tetrasperma*), or binucleate homokaryons with the nuclei being derived from a mitosis after the spore wall is delineated (*N. crassa* and *N. sitophila*). Other Pyrenomycetes, such as *Podospora anserina*, have a very similar life cycle (92). A recent report has described the isolation of two compounds called "erogens," which seem to act like hormones in stimulating the sexual development cycle in *N. crassa* (337). Addition of an extract from a mating culture has been reported to cause the formation of mature perithecia and subsequently mature asci (338).

When two haploids of opposite mating type in *Saccharomyces cerevisiae* are mixed, fusion occurs, followed almost immediately by karyogamy. The resulting diploid can grow mitotically. Meiosis and ascus formation can be introduced by transfer of the diploids to medium containing potassium acetate (or another respirable substrate) but no nitrogen source. The ascus contains four haploid spores. Largely through the pioneering work of Miller (216, 249), a great deal is known about the physiological requirements for this process. Since the subject has been reviewed several times in the last few years (103, 126, 328), only a brief outline will be given here. Cells will sporulate if transferred to the proper medium at a time when they are respiratory sufficient (103, 278), with extent and synchrony of meiosis and sporulation being better in cells grown on a carbon source which does not repress the tricarboxylic acid cycle enzymes (95, 278). Cyclic adenosine monophosphate appears to stimulate sporulation if it is added to cultures growing in glucose medium (331, 332). The exposure to the carbon source after transfer can be as short as 4 h; at the end of that time, half the sample will complete meiosis and sporulate even if removed to distilled water. After 7 h, the cells will complete sporulation if transferred back to vegetative medium. Simchen et al. (301) call this state commitment to sporulation; it occurs just before meiosis I. For a high percentage of sporulation, aeration is necessary. However, newly made *genotypically* petite (respiratory deficient) cells which have not yet lost their mitochondrial function will sporulate (174); the spores they produce are all petites (328). Cells at the end of the cell cycle, near scission, seem to sporulate more readily than cells at other stages (125), although a conflicting report has appeared (219). Diploids which are homozygous for the mating type can be constructed. Such diploids

do not normally undergo meiosis. Other factors which have been reported to influence sporulation in *S. cerevisiae* are pH (217) and temperature (103).

In *Schizosaccharomyces pombe,* the normal life cycle is one of zygotic reduction, and diploids do not divide mitotically. However, Leupold discovered that some diploids do not immediately form asci and that these can be cultured as stable diploids (190). Meiosis and ascus formation (termed *azygotic meiosis,* since it is not immediately preceded by zygote formation) occur in cultures of stable diploids in nitrogen-free medium. There are three mating type alleles in *S. pombe:* h^+, h^-, and h^{90}. The first two are heterothallic, mate with the opposite sign but not with self, and either will mate with h^{90}. The third, h^{90}, is homothallic and omnifertile; it will mate with itself or with either of the other two alleles. Diploids which are h^{90}/h^{90} will undergo meiosis and sporulate if shifted to medium containing no nitrogen source; diploids which are h^+/h^+ or h^-/h^- will not undergo meiosis. From two such diploids of opposite mating type heterokaryons can be made; these are able to undergo karyogamy and tetraploid meiosis, giving rise to four diploid spores (75). In a small number of cells, however, the nuclei do not fuse; each diploid nucleus undergoes meiosis by itself, giving rise to an eight-spored ascus of which four spores are descended from one diploid parent and four from the other, as shown by genetic analysis (123). Thus there is a sort of complementation in the heterokaryon between the two mating type alleles.

In most other systems, the conditions leading to meiosis are much less well studied. The phycomycete *Allomyces* forms "resistant sporangia" at the end of apparently normal growth. These sporangia require two to three weeks of ripening; at the end of that period they will undergo meiosis and spore formation within 100 to 130 min after being placed in water at 20 to 25 C (82). The chlorophycean alga *Ulva mutabilis* Føyn undergoes meiosis in a very synchronous manner when the diploid is fragmented and suspended in fresh growth medium (234). In Myxomycetes the plasmodium differentiates into a sporangium when the medium is exhausted (5). Meiosis as judged by the appearance of synaptonemal complexes occurs shortly after spore formation (221). In diatoms, meiosis is necessary to reverse the trend towards increasingly smaller cells which occurs in vegetative cultures (151, 346, 347). Cells are at a maximum size immediately after meiosis, and then successive mitoses decrease the size of progeny cells until a point is reached at which further mitosis is impossible (346). Immediately after meiosis cells are not sexually inducible, but after reduction in size a stage is reached at which the potential for sexual induction is maximal (346, 347). At this point spermatogenesis is inducible by raising the temperature and increasing the illumination or by changing the growth conditions from unaerated to aerated with the simultaneous addition of 40 mg of methanol per liter of culture (347). Changes in temperature and illumination have also been used to synchronize *Coprinus* (195). At 25 C this Basidiomycete can undergo meiosis when illuminated continuously or cyclically (16 h of light alternating with 8 h of dark), but at 35 C cells exposed to continuous illumination will not carry out meiosis. This sensitivity begins 7 h before karyogamy and is reversible for up to 20 h; karyogamy occurs synchronously 6 h after the fungus is returned to 25 C.

In a number of protozoa which are intestinal symbionts of the wood-feeding roach *Cryptocercus,* meiosis or gametogenesis occurs under the influence of the host's moulting hormones (41, 53). Meiosis occurred in the first 3 days after ecdysis (moulting) in most of the 10 genera studied but occurred at 36 days, 29 days, and 9 days before ecdysis in *Rhynchonympha, Leptospironympha,* and *Urinympha,* respectively (53). *Trichonympha* forms gametes beginning 6 to 8 days before the moulting period; meiosis takes place in the resulting zygote 1 or 2 days after ecdysis. Specimens of *Trichonympha* removed from an adult *Cryptocercus* survive transfaunation into nymphs that are 10 or more days from ecdysis; but transfer into nymphs within 9 days of ecdysis is fatal to the protozoa (53).

In some ciliates, preconjugant cells are morphologically distinguishable from vegetative cells (256). Preconjugation mitosis of the micronucleus may differ from other mitoses, e.g., in the size of the mitotic spindle. Furthermore, there may be incomplete decondensation of the micronucleus following preconjugant mitosis; in such cases micronuclei of conjugating individuals may be in a stage of meiotic prophase. In other ciliates, e.g. *Paramecium,* there is no special preconjugation mitosis (256). In *Paramecium* the micronucleus of each conjugant enters meiotic prophase shortly after the individuals have mated. It would be tempting to assume that conjugation triggers meiosis in *Paramecium,* and this may indeed be the case. But it is also possible that some earlier event prepared the cell both for conjugation and meiosis. It has been shown that potassium is necessary for the agglutination that precedes conjugation

in *Paramecium* (323). This explains the early observation that baked lettuce medium stimulates conjugation (311), since lettuce is rich in potassium.

Induction of meiosis in protists is generally a response to unfavorable culture conditions. Since a necessary result of recombination and segregation of genetic characters is highly variant progeny, it is tempting to speculate that meiosis evolved in part as a means of providing variants to cope with changing conditions.

PHYSIOLOGICAL EVENTS DURING MEIOSIS

A number of major questions about the physiological events in meiosis remain unanswered; indeed, until rather recently there were no investigations of, e.g., macromolecular metabolism during meiosis in any organism. Yet meiosis is a developmental process and as such is amenable to study from a physiological point of view. We do not yet know, for example, what, if any, is the first event which is common to almost all meioses. Other problems which are not yet solved are the relationship of DNA replication to recombination, the number of functions specific to meiosis (including which of these are universal), and the role of the progeny genome in the meiotic process. In microorganisms we may add other questions specific to the particular differentiation process which accompanies meiosis, ranging from ascus formation to zoospore liberation. It seems likely that a comparative approach may at least help to bring these questions into focus; by comparing knowledge of the physiology of various groups, the common events can be distinguished from those specific to a given organism.

The study of the physiological events which accompany meiosis is a relatively recent endeavor, and investigations in microorganisms presently lag behind those in explanted microsporocytes in *Lillium* (315). One problem in such studies is to distinguish which of the observed events are meiosis-specific and which are continuations of vegetative functions or are related to a generalized response to the conditions which induce meiosis. Suitable controls are often difficult to arrange.

An ideal microorganism for such studies ought to have several specific properties. First it must be cultivatable in a defined medium, preferably a rather simple one so that precursors may be added to label specific classes of macromolecules. Second, meiosis should be inducible by a simple stimulus. Third, meiosis should be synchronous, and if all members of the population do not undergo meiosis, the meiotic cells should be easily separable from the vegetative ones. Fourth, the organism should have chromosomes large enough so that the cytology of the various stages of meiosis can be followed. Fifth, the course of meiosis should be neither too rapid nor too slow, so that distinct events can be measured. Sixth, the genetics of the organism should be well enough studied so that mutants blocked at various stages in the process can be isolated, for without such mutants, physiological studies cannot progress beyond the descriptive phase.

No organism seems to conform to all these criteria. *Chlamydomonas reinhardi, Saccharomyces cerevisiae,* and *Ulva mutabilis* all fit the first two rather well, but *Ulva,* while its meiosis is quite synchronous, with 82% of the cells simultaneously in meiotic prophase, lacks a good genetic system. *Saccharomyces* has a very well studied genetic system, but its meiosis is not altogether synchronous and is completed by only 70% of the cells. Furthermore, only telophase I and II are distinguishable in this organism. *Chlamydomonas* appears to satisfy all the above criteria, except that the chromosomes are small. Surprisingly, while DNA synthesis and recombination have been studied in this alga, there are very few data on other macromolecular syntheses. Meiosis in *Allomyces* is extremely rapid and individual events might be very difficult to detect; again, no physiological or genetic studies have been made of meiosis in this phycomycete. In filamentous fungi, the difficulty of separating the meiocytes from the vegetative cells becomes overwhelming, and meiotic synchrony is not usually present.

Meiosis in protozoa is either totally asynchronous, as in conjugation in *Paramecium,* or takes place under extremely complex conditions, as in the intestinal symbionts of *Cryptocercus.* In addition there is very little genetic information on most of these organisms.

Since no organism has been exhaustively studied but many have been investigated from one aspect or another, we will compare the results from different organisms in an attempt to determine which physiological processes are general features of meiosis and which are special to the particular organism studied.

DNA Replication and Its Relationship to Recombination

Meiosis is accompanied in almost all organisms by a heightened recombination rate; recombination seems to be a fundamental part of

the process. Whether recombination is a concomitant of DNA synthesis is still not clear. In at least one case, *Neottiella* (273), replication has been shown to occur before karyogamy and thus necessarily before recombination. Premeiotic DNA synthesis may be required for recombination, since the latter usually takes place at the *4n* level, and there are several reports of experiments in which inhibition of DNA synthesis blocks recombination (38, 64, 298). However, the drastic effect that blocking DNA synthesis has on meiosis may lead to nonspecific effects. In this context it should be mentioned that there is no hard evidence that recombination occurs at the time of chiasma formation (134). It seems very likely that premeiotic DNA synthesis does not differ qualitatively from meiotic DNA synthesis, since the products of premeiotic replication can be used for mitotic growth in a number of organisms.

Timing and extent. Since all two-division meioses involve a tetrad stage, the DNA content of the meiocyte is normally 4n at some point. However, there are cases where a cell in the 2n stage can begin meiosis (147). Such cells lose viability at a chaotic rate, possibly due to the formation of aneuploid progeny. This implies that there must be a round of DNA replication between the last cell division and meiosis. The time of this replication varies, but it always takes place before meiotic prophase.

In *S. cerevisiae*, DNA synthesis takes place several hours after transfer to a nitrogenous acetate-containing medium. In cells grown to early stationary phase on glucose, DNA synthesis begins at 4 h and ends at 12 h with a 65% increase in DNA content (60, 85). When cells are pregrown in acetate, nuclear DNA replication extends for 6 h, beginning at 2 to 4 h (148, 279, 301). Mitochondrial DNA synthesis begins immediately and continues throughout the sporulation period (173, 248a). In a/a and α/α cells, mitochondrial DNA synthesis is suppressed (248a). In this case the net increase is close to 80%. Hopper et al. (148) showed that DNA synthesis preceded telophase I by about 1 h. This implies that the early division stages must both take place within this hour.

In *S. pombe*, premeiotic DNA synthesis occurs from 2.5 to 5.5 h after the shift to sporulation medium during azygotic meiosis; in zygotic meiosis it appears to occur just after fusion of the haploid cells (78).

In *Chlamydomonas reinhardi* there is one round of DNA replication which takes place in the zygospore. This replication is semiconservative (320, 321) and takes place at about 6.5 to 8 h after germination begins (38). The timing and extent are the same whether the strain yields four or eight meiotic products. Sueoka et al. (320) presented data that each of the two gametes which fuse to form the zygote contains two haploid complements of DNA; this is borne out by the results of Kates et al. on gametic differentiation (165). Thus the original zygote is 4n. The replication must raise the DNA content to 8n. In an eight-spore-producing strain, the zoospores are said to form a tetrad of genetically identical pairs, a result which is compatible with meiosis at the 4n but not the 8n level (40). Therefore, Sueoka et al. call the replication in the zygospore "post-synaptic" and argue that recombination takes place before DNA replication, at the 4n level.

The evidence for genetic identity of the progeny rests upon the observation that linked markers in the zoospores fall into four groups; that is, that each chromosome configuration (parental or recombinant) occurs in two of the eight progeny. This result could be explained either by synapsis and recombination at the 4n level followed by meiosis at the 8n level, or by a normal 4n meiosis followed by a mitotic replication. In the former case, however, each pair of spores of a tetrad ought to differ with respect to unlinked markers, since any two chromosomes should segregate independently. In the latter case, the pairs should be wholly identical. There are no data available on unlinked markers (Chiang, personal communication); so the issue cannot be resolved at the present time. Cytological studies reveal that nuclear division occurs at 8 to 9 h, that is, after replication (62). However, the small size of the chromosomes of *Chlamydomonas* might result in meiosis I and II being unobservable if the products remain intranuclear. If that were the case, the replication in the zygospore might be mitotic and the nuclear divisions after replication be mitotic ones.

In *Ulva mutabilis*, another green alga, DNA synthesis occurs sometime during the 46 h prior to the appearance of meiotic prophase (234). The net DNA increase is 2.4-fold. The authors interpret this to mean that there are two rounds of DNA replication after the last mitotic division and before meiotic prophase, so that here too meiosis takes place at the octad rather than the tetrad state. One further round of replication after meiosis provides enough DNA for the 16-zoospore progeny. The 2.4-fold increase in DNA would be explainable by completion of any nascent rounds by the fraction of the cell population synthesizing DNA, entry into G2 (the period between DNA synthesis and cell division) by all cells not there at transfer, and a

premeiotic round of DNA replication in all the sporangia. After this round the sporangia would be 8n and meiosis would take place at this stage. However, no attempt was made to distinguish between replication of nuclear DNA and replication of chloroplast DNA. If there is a large replication of organellar DNA, one can draw no conclusions about the ploidy of the cells at meiosis. In *Ulva* the cytological evidence is highly convincing that meiosis occurs after the 2.4-fold increase in DNA content (and thus possibly at the 8n level), but there are no data on the genotypes of the zoospore progeny. If the 16 zoospores should turn out to be composed of four genetically identical quartets, the interpretation of Sueoka et al. for *Chlamydomonas* would be greatly strengthened. In the absence of these data, both interpretations of the *Chlamydomonas* data are plausible, although we prefer the mitotic one. For *Ulva*, it is also possible that an 8n meiosis occurs. There is, of course, ample precedent for this in tetraploid meioses, e.g. in *Saccharomyces*, but these lead to four genetically heterogeneous diploid progeny (269).

The relationship of premeiotic to mitotic DNA synthesis. Premeiotic DNA synthesis does not seem to differ qualitatively from mitotic replication, and the product can serve for a mitotic division. *Saccharomyces* will return to mitotic growth if removed from sporulation medium before the "binucleate" stage (111, 301). Intragenic and intergenic recombination not only occur without reduction division (295) but can approach meiotic frequencies in cells which are not yet committed to meiosis (89, 91). Commitment, defined as the continuation of the sporulation process even when the cells are returned to nitrogen-containing medium, occurs about 1 h after DNA replication. A yeast strain disomic for chromosome III and containing the mating type alleles a and α can be transferred to sporulation medium and made to undergo intragenic recombination in the disomic chromosome (277). Since the cells, being haploid for all but one chromosome, cannot undergo meiosis, this is further evidence that the recombination events associated with premeiotic DNA synthesis do not prevent this replication from functioning in normal cell division. There do appear to be functions necessary for premeiotic DNA synthesis which are not required for mitotic growth (275).

The relationship of DNA replication to recombination. The question of whether recombination takes place during premeiotic DNA synthesis or at some later stage in meiosis is important in evaluating models of recombination. Copy-choice models can be ruled out if recombination takes place at a time different from replication, although breakage and reunion models can accommodate either simultaneous or sequential replication and recombination. While it seems most attractive to envisage a universal mechanism for recombination, there is no evidence that such is the case. Nevertheless, the best evidence points to recombination occurring at or after DNA replication.

The most straightforward and unequivocal evidence that replication precedes recombination was provided in *Neottiella rutilans*, where it was shown with Feulgen microspectrophotometry that the haploid nuclei doubled their DNA content *before* karyogamy (nuclear fusion), so that there was no possibility of recombination during the process of replication (273). Unfortunately, this fungus, which is useful for such studies because of the large size of its nuclei, cannot be grown in the laboratory, so it must be inferred rather than demonstrated that recombination occurs.

The argument that recombination in *Chlamydomonas* occurs *before* DNA synthesis has been discussed above, but Chiu and Hastings suggest that recombination follows the major DNA replication in *Chlamydomonas* (38). They treated a four-spore producing strain of *C. reinhardi* with various inhibitors and noted that treatments around the time of DNA synthesis affected the appearance of recombinants. Radioactive phosphorous incorporation into DNA occurred at 6.5 to 7 h after the zygospore began to germinate, and most treatments, including phenethyl alcohol, ribonuclease, and deoxyadenosine, were effective between 5.5 and 8.5 h.

Chiu and Hastings propose that recombination is the result of delayed replication, and that those agents which delay replication enhance recombination. This conclusion seems unjustified, due to the lack of specificity of the phenethyl-alcohol effect, the fact that the biochemical part of the inhibition studies was performed on vegetative cells, not zygospores, and the lack of any supporting data that any inhibitor does affect initiation of DNA synthesis. Whatever the reason, the sensitive period for recombination in *C. reinhardi* is 5.5 to 7.0 h after the beginning of germination of the zygospores. Agents known to break DNA strands, such as mitomycin C and gamma rays, stimulate recombination if applied during some limited period, possibly as a result of stimulation of DNA repair. In *Lillium*, a small amount of late DNA synthesis occurs during pachytene (315); this replication, if it occurs in *Chlamydomonas*, might be the specific target of the inhibitors.

One problem with the above explanation is that all inhibitor studies were carried out on a four-zoospore-producing strain; there is no mitotic *division* after meiosis in such a strain. There may, however, be a mitotic *replication*, so that the zoospores are 2D, like the gametes in the octospore-producing strain.

In *Saccharomyces*, the chronological relationship of DNA synthesis and recombination can be determined by removal of the cells from meiotic conditions and returning them to vegetative growth. If the strain is heteroallelic for an auxotrophic marker, prototrophic recombination can easily be measured. Sherman and Roman (295) first showed that intragenic recombination preceded haploidization, but they were unable to determine the time at which DNA synthesis occurred. Roth and Fogel (277) and later Hopper et al. (148) measured DNA synthesis and recombination in yeast; both groups found that 50% of the final yield of recombinants was detectable at 4 h, which was approximately the same time that 50% of DNA synthesis had been completed. Hydroxyurea, which blocks replication, blocks recombination even if the inhibitor is administered as late as 20 h, well after the bulk of DNA synthesis is completed (298). This argues that recombination is dependent upon completion of chromosomal replication and that there may be a late period of replication essential for recombination. Inhibitor studies are open to the criticism that the process of recombination may take place in several steps and any or all of these may be sensitive to the inhibitor. Recombination may begin during replication but be reversible at later stages. Recently evidence has been reported for single-strand breaks in nuclear DNA during the period of recombination in yeast (158). It is very difficult to evaluate these results since the size of the DNA was determined using sedimentation velocity on sucrose gradients, and comparison of the gradients of meiotic DNA with those of mitotic DNA indicates a lack of resolution in the former. Perhaps the recent observation of sporulation in protoplasts (169) will lead to experiments where the DNA is extracted from the cells in a more intact form.

In summary, only in *Neottiella* has it been clearly shown that recombination follows replication. Inhibitor studies are at best a blunt tool with which to separate the two processes; careful studies of timing are impossible in many organisms and are often difficult to interpret where they have been performed. A biochemical as distinct from a genetic assay for recombination would greatly aid the resolution of this question, since then the timing of molecular events could be investigated.

Miscellaneous effects on recombination. A number of agents have been shown to affect recombination more drastically than they affect other aspects of meiosis. One of the most common treatments is temperature change. All that can be said about this sort of experiment is that the results vary according to the organism, the regimen, the loci examined, and the kind of recombination measured. Temperature shifts can affect the frequency of recombination in *Neurospora*, but the direction (increase or decrease) of the effect is variable (89). Cold shock increases recombination in *Chlamydomonas* (183) as well as in *Neurospora* (180). In *Sordaria fimicola* two loci affecting spore color were studied for the effect of temperature on second-division segregation and gene conversion frequency. The results were locus specific (179). In *Coprinus* shifts to higher temperatures during synapsis or to either higher or lower temperatures during pachytene stimulates recombination (Fig. 32; 194). Lamb has proposed a plausible but strictly hypothetical two-factor model to account generally for temperature shift results (178).

Since some of the ultraviolet repair mechanisms have been implicated in recombination (see *Meiotic mutants*), it is interesting that in *Chlamydomonas* (49, 183) and in yeast (302)

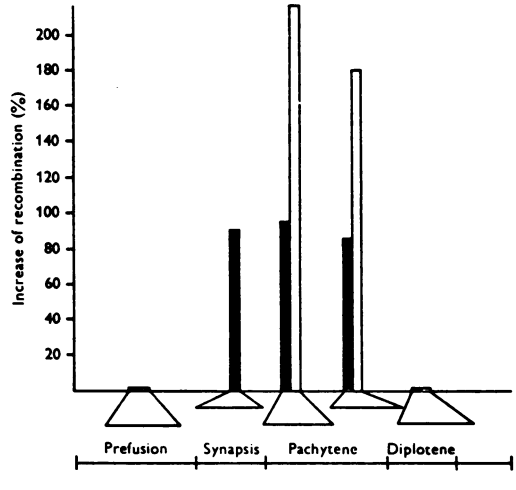

FIG. 32. *Effect of high temperature (solid histogram) and low temperature (empty histogram) on genetic recombination between den and me-I in Coprinus lagopus. At the root of each histogram the approximate times of treatment with respect to meiotic stages are indicated. (Reproduced from reference 194 by kind permission of B. C. Lu and the* Journal of Cell Science.)

meiotic cells have been shown to undergo a period of ultraviolet sensitivity. There has been one report that iron deficiency, induced by treatment with ethylenediaminetetraacetic acid or $CoCl_2$, inhibits meiotic recombination in *Neurospora* (181). In *Chlamydomonas,* inhibition of protein synthesis reduces intergenic recombination (184).

Several investigations have been made of the genetic regulation of recombination. In general, recombination frequencies are not independent of the total genetic backgrounds, and the frequencies of recombination between various loci vary independently (300, 303, 307). In one case a very high homoallelic reversion frequency was found (10). Whether such differences are due to a gene(s) whose primary function is to control recombination frequencies or to some nonspecific genetic difference is not clear at this time.

Transmission of cytoplasmically inherited determinants during meiosis. Cytoplasmic hereditary elements are defined by their non-Mendelian inheritance patterns in that they do not distribute evenly to meiotic progeny. Although *poky* in *Neurospora* and ρ in yeast were discovered more than 20 years ago, the details of cytoplasmic inheritance and particularly recombination are just beginning to be worked out. Classically, non-Mendelian inheritance has been the criterion and uniparental inheritance the rule with cytoplasmic elements. The alleles of one parent predominate in the progeny, although usually the exclusion of the other set of alleles is not complete (40, 154). In the haplodiplontic life cycle of *S. cerevisiae,* a cross of cytoplasmically heteroallelic parents leads to segregation during the mitotic growth of the diploid formed. After about 20 generations, the daughter cells have become homozygous (197). In general, cells carrying any combination of alleles occur in the population, but the fraction of cells of any one genotype is determined by many factors. Among those implicated have been the mating type (29, 32), a mitochondrial sex factor (19), and a nuclear function (289). When a cytoplasmically homozygous diploid undergoes meiosis, it gives rise to progeny identical to the mother cell.

In organisms such as *Chlamydomonas* and *Neurospora,* which undergo zygotic reduction, there is usually evidence for uniparental inheritance, meaning that the alleles of one parent are in great excess in the meiotic progeny, although the exclusion of the other set of parental alleles is not total. In *N. crassa, poky* is the classical example of a cytoplasmic gene of this type, although many other similar mutations have been isolated (16). In all of these the allele of the protoperithecial parent is transmitted to the offspring. Mixed heterokaryons have shown that the uniparental transmission does not occur in simple hyphal fusion, since conidia from such heterokaryons may show either the wild type or the mutant allele.

In *Chlamydomonas* uniparental inheritance is the case, with the cytoplasmic alleles of the mt^+ parent mating type predominating. However, ultraviolet irradiation of the mt^+ parent blocks material inheritance, and alleles from both parents are transmitted to each of the zoospores, with segregation occuring during the subsequent mitotic growth (283). One explanation for uniparental inheritance in the alga is destruction in the zygote of the DNA of the mt^- parent (282). Although the data of Sager and Lane clearly indicate a great loss of mt^- chloroplast DNA, the redundancy of the chloroplast genome and the difficulty of explaining the ultraviolet irradiation effect still leaves the question somewhat open as suggested by Chu-Der et al. (40); nevertheless, Sager's explanation is a very attractive one.

In *Paramecium,* where the exchange of cytoplasm is very limited during conjugation, the cytoplasmic character of each parent appears to remain, although induction of cytoplasmic bridges can lead to some exchange of cytoplasmic genetic markers (11).

Synthesis of Macromolecules Other than DNA

Although premeiotic DNA synthesis and recombination have been the focus of most investigations on meiosis, the other biochemical and physiological events which take place during the process have lately come under considerable scrutiny. If meiosis is regarded as a developmental process, i.e., a series of programmed events leading from one differentiated state (zygote or diploid) to another (gamete or haploid), then the only way to understand what happens is to identify the various events and determine their interdependence. In microorganisms, where the product of meiosis is often morphologically distant from the zygote (as in *Chlamydomonas),* the developmental nature of the process is most clear, but the principle is the same for all organisms.

Biochemical and physiological studies can help to answer a number of questions about meiosis. First, a knowledge of the periods of protein and RNA synthesis and of their extent can give a rough idea of when and what amount of new gene products are required, although the dependence of events like recombination

upon specific gene products can best be answered by studying mutants. Second, until we know in detail the sequence of biochemical events, we cannot determine which of them, if any, are universally associated with meiosis. One idea which has emerged from recent studies on macromolecular breakdown is that a great deal of the protein-synthesizing machinery of the cell is turned over. At least ribosomes and transfer RNA appear to be largely degraded beginning in the early stages of meiosis, or gametogenesis, with synthesis accelerating at late stages. Third, by studying the physiology of heterozygotic diploids one can determine when the progeny (haploid) genome begins to be expressed, since recessive characters should appear at that time. In this way the question of the role of the progeny genome – when it begins to function, what parts of the process are dependent upon its function, and when the parental gene products stop being necessary – may be explored.

A complication in such studies is the problem of separating the events specific to the meiotic process from the adaptive response of the organism to the conditions required for the induction of meiosis. The best attempts at controls have been in yeast, where diploids unable to sporulate due to homozogosity at the mating type locus have been monitored (148, 164, 279). The validity of these controls depends on how far along the developmental pathway the control cells progress before they are blocked. Such diploids do not undergo premeiotic DNA synthesis or recombination (106, 148, 279); however, they have served as a starting point for the isolation of mutants whose capacity to complete sporulation is no longer completely blocked by homozygosity at the mating type locus. It is certainly possible that a number of other events specific to ascospore formation occur in the control strains under sporulation conditions. A possible control which has not been exploited is suggested by the discovery in *Schizophyllum commune* of a strain which does not undergo karyogamy (168). Such mutants would be very useful as controls in organisms undergoing zygotic reduction. Another possible control in those organisms that exhibit heterophasic alternation of generations is the haploid cell under meiosis-inducing conditions. This control has been used seldom if at all.

In systems where the meiotic cells cannot easily be separated from the majority of mitotic cells, autoradiographic techniques must be used to decide when RNA and protein synthesis is occurring. This technique has been used in the ciliate *Stylonychia mytilus* (286). In this organism conjugation lasts for 24 h; at the end of this period the conjugates separate, but macromolecular development continues for about 15 h after separation. A high rate of [^{3}H]uridine incorporation was seen at 5 to 6 h. This synthesis terminated at about 6 h, and no more incorporation occurred until 15 h after separation. Protein synthesis continues throughout the process, but at a level lower than in the vegetative cells. Actinomycin D causes no effect up to 5 h after conjugate formation; at 5 to 6 h, the drug seems to block the process; the pairs do not separate even after 48 h. After 6 h, the organisms seem immune to the effects of the drug and the developmental process continues whether actinomycin D is present or not. Ribonuclease, which penetrates the cell membrane of these ciliates, blocks development at any time up to 15 h after separation. Vegetative cells can recover from ribonuclease treatment, whereas conjugants and exconjugants apparently cannot. These data are interpreted to indicate the presence of a stable messenger RNA, between 5 and 6 h, which functions throughout the nuclear developmental cycle. Such an interpretation is open to considerable question until the ability of [^{3}H]uridine and actinomycin D to penetrate conjugating cells has been demonstrated. It is quite conceivable that conjugation is accompanied by membrane changes which prevent uptake of both the labeled precursor and the drug. In *Stylonichia,* inhibition of premeiotic DNA synthesis does not block associated developmental events such as the allocation of the structure of the feeding apparatus (15, 287).

In certain organisms where the uptake of labeled precursors does not occur efficiently but meiosis is relatively synchronous, the amount of RNA and protein synthesis can be estimated by biochemical assays. Such measurements may drastically underestimate the amount of synthesis, since, under the sort of nutrient-poor conditions which induce meiosis in a variety of organisms, there may be significant turnover of preexisting macromolecules. Such turnover has been documented extensively in *S. cerevisiae* (see below). However, determination of net macromolecular changes can at least provide a minimum estimate of the amount of synthesis (or breakdown).

In *Ulva mutabilis* there is a 2-fold increase in RNA content and a 1.6-fold increase in protein during the development of the sporangium, although the number of cells stays constant (234). Presumably photosynthesis is continuing throughout the period of germination of the zygospore in this organism, for the lipid-free

mass of the sporangium more than doubles during development while chlorophyll content stays practically constant. No measurements have been made of RNA and protein turnover or carbohydrate synthesis, although the latter might account for a great deal of the mass increase, as it does in yeast (274).

During ascosporogenesis in *Saccharomyces cerevisiae*, the accumulation and degradation of lipid and glycogen was observed using stains specific for these macromolecules (249). A large part of the increased mass of sporulating cells is due to accumulation of trehalose, glucan, and glycogen. Kane and Roth (164) were able to document quantitatively the accumulation and subsequent loss of glucan and glycogen. The latter event occurs at the time of ascus formation (148, 164). Strains deficient in glycogen and trehalose accumulation sporulate very badly, and few of the asci contain four spores (252).

Determination of the timing of RNA and protein synthetic events during ascus formation in yeast is complicated by the use of two different regimens for inducing sporulation. In the classical system (system I), the cells are grown on glucose-containing medium to early stationary phase and are then switched to acetate-containing sporulation medium. They require 1 to 2 h to adapt to acetate metabolism (60). Sporulation reaches a maximum (70%) at the end of 24 h. On the regime most utilized in the past few years (system II), the cells are grown to mid-logarithmic phase in a medium containing buffered potassium acetate and a nitrogen source (278). Upon transfer to potassium acetate alone, 70% of the cells sporulate by 15 h. The synchrony in the latter system is better than in the former; the time from first appearance of an ascus to the maximum sporulation is 10 h in the older medium and 7 h in the newer. This difference makes it difficult to compare the relative timing in various events, but results using the two systems have been in quite good agreement about the actual course of events. Protein and RNA synthesis in system I have been monitored both by means of net increase of macromolecular content (59) and by 1- or 3-h incorporation periods using radioactive precursors (86, 87). Protein content increases 10% at the start of the sporulation period, remains largely unchanged for 12 h, and then declines to about 60% of the initial value. [Claims for a significant decrease in protein content at 4 h are not supported by the data (59).] The incorporation data show a different pattern; a single large peak of protein synthesis occurs from 2 to 20 h and a possible second peak is found at 23 h (86). Since the pulses were 1 h long and since there is evidence for very large pools of precursors in such cells (see below), it is conceivable that some fine structure of these peaks might have been overlooked. However, other studies by the same authors using 3-h incorporation periods and a different amino acid gave very similar results (87). The fraction of ribosomes engaged in protein synthesis appears to remain high throughout the first 24 h of sporulation (218).

The uptake of labeled RNA and protein precursors decreases during sporulation as the pH rises; to be valid, a test of incorporation must be carried out at a pH no greater than 6.4 (217). Taking these precautions, Mills showed that in system I cells the rate of adenine incorporation during a 10-min pulse increased from 0 to 6 h and then declined until 12 h. These results are somewhat different from those of Esposito et al. (87), who used 1-h pulses and did not adjust the pH. They found a high initial rate, a period of decline at 5 h, a peak at 10 h, and a second peak at 15 h. However, they were incubating their sporulating cells at 34 C not 30 C. Mills' results are perhaps the more reliable, since he measured intracellular nucleoside triphosphate pools in order to be certain that changes in rate were not due to changes in the specific activity of the precursors (217). It is interesting that protein and RNA synthesis rates are high *during* DNA synthesis at 30 C, while at 34 C DNA synthesis is delayed more than protein and RNA synthesis, and the bulk of the latter is complete when DNA synthesis begins (87).

In system II a series of measurements used 10-min pulse labels of [^{35}S]methionine and [^{3}H]adenine together, so that the temporal relationship of the changes in rate could be monitored (148). The pulses were carried out in medium adjusted to pH 6.0, to maximize uptake. A significant peak of protein synthesis occurred at 2 h, i.e., before RNA synthesis began to increase. The maximum in RNA synthesis rate was around 4 to 6 h after inoculation into sporulation medium. The specific activity of the pools of radioactive precursors did not change significantly; thus the rates reported are real and do not reflect differences in intracellular concentrations (148).

Although a series of new proteins is synthesized by a/α cells placed in sporulation medium (and a number of vegetative proteins are no longer synthesized), the pattern on sporulation medium is not unique to cells able to sporulate but is found in nonsporulating diploids and in haploids (148). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of extracts of cells pulse-labeled for 10 min at times corre-

sponding to the beginning of premeiotic DNA synthesis, the beginning of meiosis I, and the beginning of sporulation showed the same patterns in a/α, a/a, α/α, and haploid cells. Cycloheximide inhibition experiments indicate that 15% of the cells reach meiosis I if protein synthesis is inhibited at 6 h and that 30% form asci if inhibitor is added at 10 h (201). One would therefore expect to see some specific proteins from the spindle apparatus and some from the ascospores being made at these times. The failure to observe these indicates that they must constitute a small fraction of the total protein synthesis in the culture.

RNA synthesis and processing during ascosporogenesis in *Saccharomyces* differs from vegetative synthesis in a number of ways. Kadowaki and Halvorson showed that a 20*S* RNA species, specific for sporulation and nuclear in origin, accumulated beginning at about 3 h and continued until 36 h in cells sporulating under system I conditions (162, 163). This species does not seem to appear in at least one well-sporulating strain (J. Harper, unpublished results). Sogin et al. (310) later showed via competition hybridization that the new species was 70 to 80% homologous to the 18*S* ribosomal RNA, although it was not methylated. An auxotroph deprived of methionine made 18*S* but not 5.8*S* or 26*S* ribosomal RNA during sporulation (344). There is, in addition, a slowdown in the processing of ribosomal RNA during meiosis (310). Arginyl transfer RNA seems to be altered during sporulation (202). There is a change in the chromatographic profiles of the RNA polymerases from yeast during sporulation (200); a new peak of RNA polymerase appears at about 9 h in system II cells. RNA synthesis seems less stringently controlled during sporulation (201) than during vegetative growth (276). All these reports make the study of RNA synthesis and its control a promising point of attack on the problem of the regulation of ascosporogenesis.

Very few measurements have been made of lipid biosynthesis during meiosis. In yeast there are two periods of extensive lipid synthesis in cells pregrown in glucose and then switched to sporulation medium (135, 157), but neither seems to be related to meiosis. The first period probably is related to the adjustment to sporulation medium, the second to ascospore formation.

Turnover of RNA and Protein During Meiosis

In organisms in which meiosis is induced by a shift to culture conditions where an essential nutrient is lacking, there must be breakdown of preexisting cellular material in order that the meiosis-specific gene products can be synthesized. Such organisms include the myxomycetes, some of the ascomycetes including *S. cerevisiae*, and *Allomyces*. This breakdown has been documented in *Saccharomyces* by Chen and Miller (35), who reported a sporulation-specific increase in protease activity. Net protein and RNA content decrease during meiosis and ascospore formation (59), and protein turnover amounts to 35% of the acid-insoluble [^{3}H]lysine incorporated during a 26-h period before the shift to sporulation medium (86). Surprisingly, acid-insoluble [^{3}H]lysine continues to increase for 5 h after the shift to sporulation medium, which lacks the label. This implies a very large pool of lysine at the time of transfer and makes interpretation of the pulse-label data in the same paper difficult. Hopper et al. (148) showed that protein degradation amounted to 30 to 40% of preexisting label ([^{35}S]methionine) in a/α cells, while in a/a or α/α cells protein turnover was non-existent during the first 24 h. RNA breakdown amounted to 70%. In nonsporulating diploids, 20% of preexisting RNA is broken down. These estimates are minimal since the measure of breakdown was loss of label to the medium, and active protein and RNA synthesis was shown to take place at the same time. Thus, re-utilization of intracellular breakdown products must have occurred. A very large fraction of the ribosomes must therefore be broken down. This breakdown could have the function of getting rid of (possibly) senescent protein-synthesizing machinery in which coding errors have accumulated,an event predicted by Orgel (236). Evidence for a large-scale resynthesis of ribosomes corresponding to the daughter genome has been obtained by A. Hopper and P. T. Magee (unpublished observations). Half of the progeny of a diploid heterozygous for the recessive cycloheximide resistance locus *cyh2* will germinate on cycloheximide-containing medium. This implies that the progeny contain a large fraction of their own resistant ribosomes, rather than a complement totally derived from the diploid parent. In *Chlamydomonas* a similar extensive breakdown of ribosomes occurs during gametic differentiation. Both chloroplast and cytoplasmic ribosomes are broken down, with the release of prelabel approximating 90%. The mature gametes contain only 15% of the number of ribosomes found in vegetative cells (297).

A number of inhibitors have been used to study the role of macromolecular synthesis in meiosis and sporulation in yeast. The amino acid analogue ethionine affects DNA synthesis

(but not protein synthesis) when added at any time up to 4 h after shift to sporulation medium. If continually present it prevents DNA synthesis; if it is added and removed, DNA synthesis is delayed. The later ethionine is added, the shorter is the delay (60).

In system II cells, all physiological events leading to ascospore formation, except protein degradation, are sensitive to cycloheximide. DNA replication appears to have no insensitive period at all, a result which implies either that elongation is blocked by the drug, in contrast to mitotic replication (138, 356), or that the time for replication is less than 45 min and the 6-h spread over which DNA synthesis occurs is the result of asynchrony. Meiosis II is greatly inhibited by addition of cycloheximide at 6 h, when 75% of the DNA replication is complete. The final fraction of tetranucleate cells (15%) in such cultures corresponds reasonably well with the number of binucleate cells at the time of inhibition. RNA degradation is drastically arrested at any time up to 12 h by the inhibitor, as is glycogen synthesis and breakdown (201). Interestingly, recombination seems to be reversed by cycloheximide, an effect analogous to that of hydroxyurea (298).

Ascus formation in system I cells becomes insensitive to inhibition about 1 h before asci are observable in the microscope. In system II, the time was estimated at 2 to 3 h (201). One would expect ascus formation to be far enough removed in time and development from preculture conditions so that the two systems would not be different; the difference may be attributable to the fact that the strain used in the latter experiments was heterozygous for cycloheximide resistance.

Despite the rather fragmentary nature of the data, a few generalizations can be made about the biochemistry of meiosis in microorganisms. First, the period up to and including meiotic prophase is a very active one metabolically, but not all (nor even a majority of) these metabolic processes seem to be meiosis-specific. Second, there appears to be a great deal of turnover of preexisting macromolecules. Whether this is a universal feature of meiosis or is specific to those microorganisms in which meiosis is triggered by starvation conditions is moot at the present time. Third, RNA metabolism appears to be an area where a number of fairly specific effects (e.g., long-lived messenger RNA, RNA polymerase changes, new RNA species appearing) have been noted. Thus, it may be the area of choice in which to try to isolate meiosis-specific functions. Other possibilities are to focus on such phenomena as the synthesis or aggregation of proteins specifically involved in observable processes (such as chromosome segregation or synaptonemal complex formation) or on events which seem to be specifically absent in mutants.

Meiosis may involve drastic changes in the metabolism of the cell, as implied by the degradation of ribosomes, the halt in processing of RNA, and the possible existence of a new RNA polymerase peak (all effects noted in yeast). On the other hand, the lack of specificity in gross macromolecular synthetic patterns in meiocytes as compared to vegetative cells, the failure to find meiosis-specific proteins, and the evidence that commitment to meiosis occurs at about the time of nuclear division all argue that meiosis involves at most relatively minor perturbations of the cell at least in the early stages, and that any gross changes may be related to the differentiation processes which accompany formation of the meiotic products, rather than meiosis itself. More extensive studies in other organisms, such as *Chlamydomonas*, may help clarify the issue.

MEIOTIC MUTANTS

One very promising approach to understanding meiosis is the use of mutants conditionally blocked in the process. Such mutants can be used to answer questions about the presence of coupled developmental sequences, about which steps are common to meiosis and mitosis, and about the way in which physiological conditions are translated into the signal for meiosis. For example, one important question which has arisen is whether recombination, which ordinarily accompanies meiosis, is necessary for its completion. Two recently isolated mutants, one in *Podospora* and one in *Saccharomyces*, argue that it is. The *Podospora* mutation blocks meiosis in most cells and affects recombination in the few which complete the process (304). The yeast mutation allows premeiotic DNA synthesis to proceed normally but only a fraction of the cells undergo recombination; those cells sporulate normally (137). This implies that recombination and meiosis are tightly coupled. As more of the events common to meiosis become known and more mutants are isolated, we may expect to discover other such relationships in the developmental pathway.

Microorganisms offer uniquely advantageous systems for isolating meiotic mutants, since in several fungi and in *Chlamydomonas* the genetics is quite far advanced, the requirements for induction of meiosis are well known, and the meiotic cells are separable from mitotic cells.

An important factor in selecting ameiotic

mutants is the method by which the mutant cells are identified. The method of identification determines to some extent the stage at which the mutants will be blocked. Recombination-deficient mutants, for example, will most likely have a lesion early in meiosis, while mutants lacking the differentiated structure containing the meiotic products, e.g., zoospores in *Chlamydomonas*, may be expected to be blocked anywhere during the developmental process. Recently a method using ethyl ether has been described for selective killing of nonsporulating cells in *Saccharomyces* (66). The generality of this method for other organisms whose meiotic products are spores warrants exploration.

In general, the types of mutations isolated fall into three classes: morphological mutants, whose development during or after meiosis is aberrant; recombination-deficient mutants (which may complete meiosis); and ameiotic mutants, blocked in the process itself. Of course, the last two classes overlap considerably.

Srb's group (235, 247, 248, 280) has worked a great deal on mutants of *Neurospora* with altered ascus morphology. These mutants have been called *scruffy, scumbo, peak,* and *ascus* mutants. The mutations seem to affect the orientation of nuclear spindles during ascus development (248). In *N. crassa,* a strain carrying *pk-2* in the homozygous state has faulty alignment of the spindle at the mitotic division; thus the spores are arranged nonlinearly. *Scruffy* acts to cause the spores in the center of the ascus to be arranged nonlinearly; it also affects the apical region of the ascus, leading in extreme cases to dichotomization. *Scumbo* is similar to *scruffy* in its effects. These three recessive genes are not allelic, *scruffy* lying on linkage group II, *pk-2* on V, and *scumbo* on III. Other such mutations have been isolated in *N. tetrasperma* (235). In total there are at least three and possibly four genes in *Neurospora* which affect spindle formation at one or another of the steps in ascus development.

Grewal and Miller (119) have reported that about 2% (3 out of 140) of diploid strains of *Saccharomyces* produce two-spored asci which are diploid. The diploid spores are able to sporulate without further conjugation and they do not mate. They are therefore presumably a/α. The single division that takes place in these yeasts during sporulation appears to resemble mitosis more than meiosis I (224). Whether this behavior is due to a single gene has not been resolved.

In studying recombination, the success in bacteria with radiation-sensitive strains has led to an extensive search for analogous mutants in other protists, and a number of papers have described their isolation. In only a few cases, however, have the effects of the mutation on recombination and on meiosis itself been studied. Among the ultraviolet-light-sensitive mutants of *Neurospora,* none seem to affect meiotic crossing-over, but at least two of them are infertile in homozygous crosses, being blocked at or before nuclear fusion (290, 314). The mutants *uvs-3* and *uvs-5* are both recessive and they complement. The cross, however, is sterile. Two other mutants, *uvs-1* and *uvs-4*, produce a large fraction of inviable ascospores and grow slowly when homozygous. Another ultraviolet-sensitive mutant, *uvs-2*, which appears to be cytoplasmic in nature, fails to form protoperithecia, although it functions well as the "male" parent (290). A protoperithecia-negative mutant, *ff-1,* has been isolated; it seems to result from a nuclear mutation (325). The ultraviolet sensitivity of the strain has not been tested. Several other mutants have been reported but not carefully studied (72, 98, 149, 150, 215, 348). An ultraviolet-sensitive mutation isolated in *Ustilago maydis* blocks meiosis in the homozygous state (144); other *uvs* mutations in *U. maydis* and *U. violacea* (67) have no effect on meiosis or on meiotic recombination.

Of four *uvs* mutants of *A. nidulans* isolated by Jansen (159) and by Fortuin (101), all are affected in some aspect of meiosis. All are recessive, so that the effects are seen only in the homozygous diploids. *uvsB* seems to affect disjunction; it gives rise to spores that are odd-shaped, about half of which are diploid or aneuploid (159). In *uvsC*, cleistothecia are formed and ascus primordia are visible, but no ascospores are formed, so one or another of the stages of meiosis itself may be blocked (182). *UvsE* is sterile in homozygous crosses, while *uvsD* is similar to *uvsB* in that a large fraction of the ascospores are diploid. Analysis of the haploid progeny of *uvsD/uvsD* diploids showed a 10-fold reduction in intragenic recombination rate at the *paba* locus compared to homozygous wild type or heterozygous *uvsD/+* crosses. The recombinants seemed largely the result of reciprocal cross-over events, rather than gene conversions, as judged by outside marker segregation (101).

One ultraviolet-sensitive strain of *Chlamydomonas* has been found to be reduced 25% in recombination in some groups compared to wild-type frequency (271). The mutation did not seem to block any other processes related to meiosis. Four other *uvs* mutations of *Chlamydomonas* had no effect on meiosis (63).

A number of radiation-sensitive mutants

have been isolated in *S. cerevisiae* (57, 232, 263, 266, 309). Of these, at least two have been found to be blocked in meiosis; a number of others show a reduced level of ascospore formation (57, 267). One set seems to affect gene conversion but not reciprocal recombination (267).

Many of the cell-cycle mutants characterized by Hartwell (128) fail to sporulate at restrictive temperatures when they are homozygous, but whether the block is in meiosis or some function required for adjustment to the medium is not clear (299). Simchen et al. have isolated a dominant mutant in *Saccharomyces cerevisiae* which appears to be blocked after the initiation of DNA synthesis (248a, 301). The mutant does not complete DNA synthesis (248a).

A technique for detecting meiotic mutants in *Neurospora* has recently been described by Smith (308). Hypohaploid spores are white, while viable aneuploid ones are black. This technique allowed Smith to detect a recessive mutation leading to a lesion in synapsis or termination of synapsis. The locus has been called *mei-1*. Ninety percent of the ascospores from a homozygous cross are nonviable; the viable ones are disomic for more linkage groups. The chromosomes are always parental in their marker assays; that is, there is no crossing over. Another mutation, *mei-2*, is dominant and causes nondisjunction along with a decrease in crossing over (308). Both mutations lead to cytological abnormalities during meiosis (308).

A direct approach to the selection of recombination-deficient mutants has been devised by Roth and Fogel (277). They used yeast strains which were disomic for chromosome III. In addition, these strains were heteroallelic for the mating type locus and contained noncomplementing alleles for the *leu2* gene. Such strains, when shifted to sporulation medium, attempt to undergo meiosis but, presumably due to the fact that they are 2n + 2 rather than 4n, give rise to nonviable spores. In the initial stages of meiosis they undergo intragenic recombination at the *leu2* locus, and the recombinants can be detected if they are transferred to selective glucose-containing medium. Mutants blocked in recombination can be detected by the failure of leucine prototrophs to appear. Five have been carefully studied; three are blocked between premeiotic DNA synthesis and intragenic recombination. Of these, one sporulates at a very low frequency when homozygous, one reaches 5% asci, and one sporulates to 24%. All are recessive; they have been named *con1*, *con2*, and *con3* (100). Two others are blocked before premeiotic DNA synthesis and they fail to undergo recombination. Protein, RNA, and carbohydrate synthesis seem normal. The genes involved have been termed *mei1*, *mei2*, and *mei3* (275).

One well-characterized group of mutants blocked during meiosis is the temperature-sensitive set isolated by the Espositos (85, 85a, 87, 88, 89, 90, 91, 225). These mutations were induced in ascospores of homothallic strains of *S. cerevisiae*. When the spores germinate, they mate with themselves, and the resulting strains are wholly homozygous diploids. Such diploids will express any recessive mutations (85). Altogether 11 different complementation groups have been identified by recessive mutations, and in addition, three dominant mutants have been found (90). The mutants sporulate at 23 C but not at 34 C, an unfortunate choice of temperature since sporulation is not very good at 34 C even in the parent strain. At 30 C, the optimum temperature for sporulation in the parent, the mutants tend to show intermediate sporulation. Three of the mutants have been examined biochemically and physiologically. None of the three appears to be tightly blocked at or before premeiotic DNA synthesis, although the amount of DNA synthesized in *spo2* and *spo3* is slightly less than in the wild type (87). RNA and protein synthesis also do not differ from the parent in these strains when they are put in sporulation medium at the restrictive temperature, consistent with the results showing that a/a and α/α diploids do not differ from a/α diploids in their macromolecular biosynthetic patterns (87). *spo1* seems to be blocked between recombination and spindle pole body duplication (225). Its temperature-sensitive period begins about halfway through the sporulation cycle and ends three-fourths of the way through (87). (The temperature-sensitive periods are given in fractions of the sporulation cycle because the cycle is much longer and less synchronous at 34 C than at 30 C.) *spo2* seems to lead to abnormal meiotic divisions; the nucleus divides at meiosis instead of remaining intact. Ascospore wall formation excludes the daughter nuclei, and the anucleate ascospores formed do not mature (225). The lesion leading to this phenotype seems to be an early event; the temperature-sensitive period for *spo2* is from 0.22 to 0.56 of the sporulation cycle (87). *spo3*, temperature-sensitive for a very brief time around 0.64 of the sporulation cycle, fails to coordinate wall formation and nuclear division; it forms spores containing greater or lesser amounts of nuclear material (225; Fig. 33). At 30 C, largely two- and one-spored asci are found (225). The spores are nor-

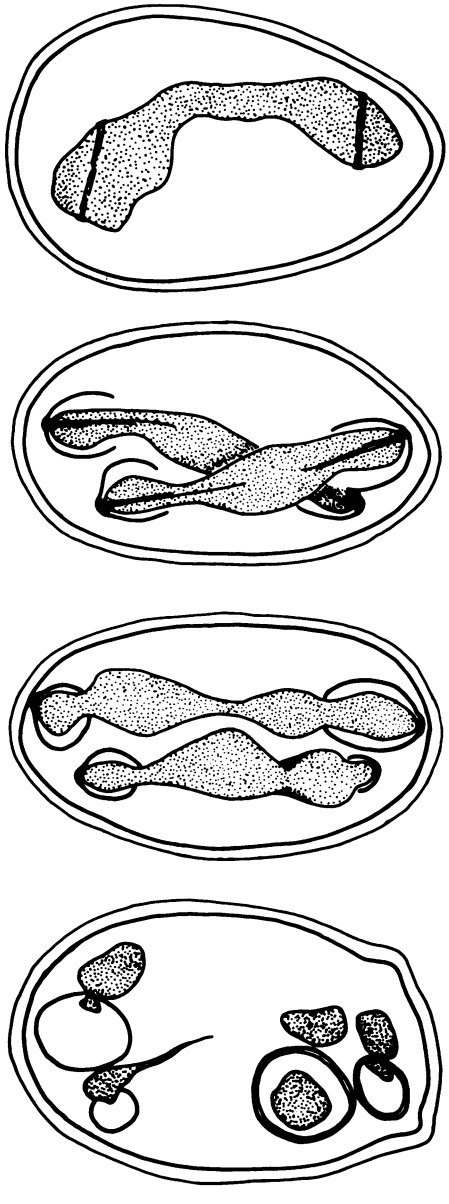

FIG. 33. *Ascus development in the mutant spo3 of Saccharomyces cerevisiae. Meiosis is normal through part of the second meiotic division. However, the nuclear material (dotted) seems slow in moving into the developing ascospore walls. These later close while the bulk of the nuclear material remains outside, free in the cytoplasm. (Reproduced with modifications from reference 225 by kind permission of P. B. Moens and* Experimental Cell Research.)

mal with respect to recombination and appear to be haploid (88). Thus these mutants are all blocked at stages relating to nuclear division or ascospore wall formation. Among the other mutants isolated, some seem to be blocked in metabolic events necessary for the adjustment to sporulation medium and completion of the cell cycle as judged by their terminal phenotype (85). None of these progress to premeiotic DNA synthesis. Others which do seem able to adjust also fail to synthesize DNA in sporulation medium (85a). It would be interesting to know whether the first class lose viability when shifted to sporulation medium, as do cells blocked in adjustment by inhibition of protein synthesis (201). Doubtless further analysis of these mutants will be very helpful in elucidating the developmental pathway. However, the nonpermissive temperature of 34 C is an unfortunate choice, since, as noted above, sporulation is reduced even in the wild type at this temperature. From the analysis of these mutants an estimate of the number of genes essential to meiosis and sporulation has been derived: 47 ± 27 (90). This is clearly a minimum estimate, since not all genes would be expected to mutate to yield a product that was temperature sensitive.

Bresch et al. (25) isolated asporogenic mutants in *S. pombe* by using the homothallic h^{90} mating type allele (77). Of the first 230 spontaneous mutants isolated, 228 were dominant in that they failed to sporulate when crossed with h^+ or h^-, or both, and two were recessive. With the aid of mutagens, more recessive mutants were isolated. They fell into 26 complementation groups. Three were linked, so the authors postulate 24 genes. Mutation in *fus 1* blocked cell fusion; specifically, the walls did not dissolve in the agglutinated cells. Four mutations, called *meI-1* to *-4* (later called *mei 1–4*), led to cells blocked at the mononucleate stage. One, meII-1 (*mes-1*), caused arrest at the binucleate stage, and 18 genes, *spo-1* to *18*, when mutated, caused arrest at some stage after meiosis but prior to ascus formation. There were no mutants isolated that seemed uncoupled in the sense that spore formation proceeded in the absence of meiosis, but this may be due to the method of isolation, which depends upon distinguishing asporogenic colonies by their lack of staining with iodine vapor. It is also possible that observations at the ultrastructural level might show formation of rudimentary or incomplete spores indistinguishable in the light microscope.

A more complete analysis of the *mei* genes showed that *mei-1, -2;* and *-3* all block at some stage before commitment to meiosis (76). Evidence for this is a 10- to 500-fold increase in the number of stable diploids which are formed in *mei* h^{90} cultures and the fact that *mei-1* and

mei-3 block premeiotic DNA synthesis (79). The mutant *mei-4*, by contrast, seems to block after commitment, since it gave the normal level of stable diploids and did synthesize DNA in sporulation medium. Evidence that the gene affected in *mei-4* acts in the developmental program after the gene affected in *mei-1* is that the double mutant *mei-1 mei-4* h^{90} gives the number of diploids characteristic of *mei-1*. The results with these mutants are in accord with the finding that commitment in *S. cerevisiae* is at or very near meiosis I (301).

Hopper and Hall (146, 147) have isolated, in *S. cerevisiae*, a series of mutants which undergo meiosis even though they are homozygous at the mating type locus. The most interesting of these seem to be the result of a single dominant mutation; they resemble the parent in that they mate like normal a/a or α/α strains but sporulate at 5 to 30% efficiency. In haploid strains the mutation allows the synthesis of a round of DNA replication analogous to premeiotic DNA synthesis. The mutants appear to act like a/α diploids through DNA synthesis; the net increase in DNA content is 70 to 80%. After replication, the efficiency of sporulation falls off and only a fraction of the cells form complete asci. Some step between DNA synthesis and recombination seems to be completed in only a fraction of the population, since the low sporulation in the one mutant tested is accompanied by normal recombination in the spores but very little in the nonsporulating cells. The population as a whole resembles a/a or α/α rather than a/α diploids in mitotic recombination frequency and X-ray sensitivity. The mutation(s) called CSP (control of sporulation) is effective in a/a or α/α cells. No data are yet available on the number of alleles, but none tested are linked to the mating type locus. Since the mutations are effective in the haploid at least in allowing DNA synthesis, it is interesting that CSP strains disomic at chromosome III but a/a or α/α at the mating type locus undergo recombination at a level equivalent to the CSP a/a or α/α diploids when placed in sporulation medium. A preliminary report of the independent isolation of similar mutants has appeared (112).

An elaborate cytological and genetic study by Simonet and Zickler (305) has provided a very detailed analysis of 40 ameiotic mutants in *Podospora anserina*. This ascomycete has chromosomes large enough to be observed with the light microscope, so that abnormalities in meiosis can be easily detected. Since the four ascospores of *P. anserina* contain two nuclei that differ in genes which show second-division segregation, recessive ameiotic mutants can be identified by screening several spores from the cross of a mutagenized stock to a wild-type strain. The less tightly linked the mutation is to the centromere, the greater the likelihood that some of the spores will be homozygous for it and thus unable to proceed through meiosis.

Among the 40 mutants isolated, 10 complementation groups were found affecting caryogamy, the first nuclear division, control of plaque formation, chromosomal alignment, and the spindles in the post-meiotic mitosis (see Table 5). In *mei-2* homozygotes, the first meiotic division does occur rarely; in the tetrad thus formed, recombination is abnormal. Recombination in centromere-proximal regions is increased; in centromere-distal regions it is diminished. Thus mating type, which normally shows a 97 to 99% second division segregation frequency, shows only 66% in the mutant. The latter effect was shown to be the result of a decrease in chiasma interference; the 66% figure is the theoretical one expected for the absence of interference. In regions close to the centromere, interallelic recombination is increased and the ratio of reciprocal events to conversions is higher than in the wild type. Simonet proposes that the primary defect is in some gene which controls the repair of DNA (304).

The elegant cytological and genetic studies which are possible with the *Podospora* mutants are very illuminating. However, the fact that these mutants are absolute, not conditional, makes them useless for a kinetic analysis of the process of meiosis. For example, we know where *mei-3* is blocked—at diplotene—but we

TABLE 5. *Ameiotic mutants of Podospora anserina*

Complementation group	Percentage post-reduction	Phenotype	No. of isolates
car-1	60	No asci	2
mei-1	<2	Asci but no spores	4
mei-2	0.1	Asci but no spores	3[a]
mei-3	70	Rare asci	2
mei-4	83	1-3 Spores in each fruiting body	1
mei-5	74	1-3 Spores in each fruiting body	1
kin-1	16.5	No spores	1
kin-2	96	Rare spores	1
kin-3	50	No spores	1
spo-1	40	Abnormalities of the nuclear distribution of the spores	1
spo-2	2	Abnormalities of the nuclear distribution of the spores	1

[a] One allele of *mei-2* gives spores after 20 days.

do not know when the altered gene product is made. Neither do we know whether the block is the result of altered structural components or a failure in some meiosis-specific metabolic process. It is interesting that *mei-2*, which has such general effects on recombination, also completely blocks meiosis in two of three alleles.

It is evident that elucidation of the developmental program leading to meiosis and haploid progeny formation will require analysis of large numbers of mutants. A good start has been made in amassing such mutants, almost exclusively in the fungi. None of the mutants so far reported are ideal. Only one group was isolated as conditional mutants, and there the restrictive conditions were inhibitory for the wild type. Some of the most interesting have been found in organisms in which the biochemistry and physiology of the process are almost impossible to study. Furthermore, all the isolation techniques so far employed have in theory excluded large numbers of potential mutants. In *Podospora,* lesions in these genes linked to the centromere will not be represented among the mutants. In the disomic selection system, lesions in functions after recombination will not be found. In the yeast temperature selection system, those genes whose products cannot be altered so as to undergo inactivation between 23 and 34 C will not be represented. Selection for radiation sensitivity, of course, leads to a very specific class of mutants.

In any future mutant hunts, the following guidelines should be very helpful. (i) The organisms chosen should have, as well as good genetic system, a meiotic cycle which allows studies of physiology and biochemistry. (ii) The identification or selection of the mutants should be if possible via some late event, so that as much of the cycle as possible is covered. (Of course, this assumes that the events are ordered and that late events are completely dependent upon early events; so far this assumption appears to be borne out.) (iii) The mutants should be conditional, so that strains blocked under one set of conditions can be released under others. This allows determination of what Hartwell, studying the mitotic cycle in yeast, has called the execution point or point where the cell acquires the capacity to complete the process, as distinct from the point of arrest, where the cycle is blocked (128). (iv) Finally, the conditions should be chosen so that as many kinds of mutants as possible may be expected to be found. One possible way to assure this is to use temperature-sensitive suppressors of the short described in *S. cerevisiae* (281). In theory, at least, lesions in every essential gene coding for a protein can be found with this method.

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ADDENDUM IN PROOF

Byers and Goetsch (Proc. Natl. Acad. Sci. U.S.A. 72:5056–5060, 1975) have demonstrated by serial sections the presence of 17 synaptonemal complexes of conventional appearance in one yeast strain. This strain is homozygous for the cell cycle mutation *cdc4* and must be sporulated under permissive conditions. The relationship of these findings to the polycomplex body described by Moens is not clear at this time.

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