Studies on the Fine Structure of Microorganisms

V. Morphogenesis of Nuclear and Membrane Structures during Ascospore Formation in Yeast

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ABSTRACT

The fine structure of cells of *Saccharomyces cerevisiae* engaged in the formation of ascospores was studied in electron micrographs of ultrathin sections. Although the mode of the first reduction division could not be clearly determined, the second nuclear division appeared to proceed in a manner similar to that observed previously during vegetative division. That is, division by constriction of the existing nucleus occurs without dissolution of the nuclear membrane and without involvement of discrete chromosomes. Variously shaped areas of low electron density were discerned within the nucleoplasm; these had not been previously seen in the vegetative nucleus. The significance of this nuclear differentiation and its possible similarity to nuclear structures reported in bacteria and an imperfect fungus are discussed.

The cytoplasmic membrane appears first in the developing ascospore. The formation of an outer coat and an inner coat then follows. The cytoplasmic vacuole was observed not to be incorporated into the spore. An unusual intracytoplasmic membrane was observed in the spore and appeared to be at least temporarily continuous with the nuclear membrane.

INTRODUCTION

Disagreement has existed and still persists on the pattern of nuclear division in both vegetative and sporulating yeast cells. In our previous studies (1–3) of vegetatively dividing cells of *Saccharomyces cerevisiae*, we sought to exploit the superior resolution obtainable with electron microscopy of ultrathin sections. The results were difficult to reconcile with earlier reports (4, 5), based on light microscopy, which had pictured classical mitotic figures and distinct chromosome structures. Rather, we concluded that vegetative nuclear division is characterized by (*a*) the absence of distinct chromosomes of the type seen in higher organisms, (*b*) the persistence of the nuclear membrane throughout the division process, and (*c*) the division of the nucleus by apparently autonomous constriction and subsequent migration of the two parts into the newly formed daughter cells. It seemed unwise, however, to deny the existence of chromosomes in yeast nuclei altogether in view of the experience of Moses (6) and of Gibbons and Bradfield (7) that chromosomes visible in light images of Feulgen preparations of thick sections may be unrecognizable in electron micrographs of adjacent thin ones.

Because of the unusual appearance of the sectioned vegetative nucleus in electron micrographs, similar techniques were employed to examine yeast during the first and second nuclear divisions which precede the formation of four ascospores, when classical meiosis allegedly occurs (8–11). In addition to characterizing the nucleus, we were also concerned with the origin and order of forma-

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tion of the spore coats, and the behavior of the vacuole and other cellular components during the sporulation process.

Methods

A readily sporulating strain of distillers yeast, *Saccharomyces cerevisiae* strain M-1, was used in the present investigation and previously (1–3). Young cells growing on a molasses agar medium were collected, washed twice with distilled water, and inoculated heavily onto acetate-glucose sporulation agar similar in composition to that described by Adams (12). The incubation temperature was 30°C. for both vegetative growth and sporulation. Indications of spore formation could not be detected by either light or electron microscopy during the first 10 to 11 hours of incubation on the sporulation medium. From that time on, however, the number of sporulating cells increased gradually, and a majority of the cell population completed sporulation within 24 hours.

The method of preparing thin sections for electron microscopy was the same as previously reported (1, 13, 14); after fixation with 1.5 per cent potassium permanganate, the cells were dehydrated in alcohol and embedded in butyl methacrylate.

OBSERVATIONS

In the sequence of events leading to the formation of four ascospores, the nucleus of the yeast cell undergoes two successive divisions. The mechanism of both these processes was examined, the second successfully. Although considerable effort was also directed to studying the first such division, the results were considered to be inconclusive owing to the following difficulties in interpreting electron micrographs: (*a*) There was no way of determining with certainty whether a given cell containing a large dividing nucleus represented a stage of vegetative division or an atypical first meiotic division. (*b*) The interpretation of cells that appeared to be binucleate was similarly difficult, although the frequent occurrence of vegative division under the experimental conditions seemed quite improbable since 95 per cent of the total population would become sporulated within 40 hours. Thus, we are inclined to share the view of Ganesan *et al.* (15) that most of the binucleate cells represent stages of meiotic division. (*c*) A cell without an observable nucleus was likewise difficult to distinguish from a cell in which the nuclear membrane may have been dissolved during the first division, as expected in typical meiosis. The absence of a nucleus, of course, could also be due simply to the plane of sectioning. The solution of these difficulties in interpretation must await future experimentation with more refined techniques, but it appears that careful serial sectioning will be most useful.

Perhaps the most informative observation relative to the first nuclear division during sporulation was that there is some differentiation within the nucleus early in the process, as shown in Fig. 1. Small but distinct structures of low electron density were seen to be distributed within the nuclei. The nature of these structures is not completely understood, but the occurrence of such structures in the nucleus during sporulation is intriguing in view of the apparent absence of such structures in vegetatively dividing nuclei. The behavior of these structures was followed throughout the process of spore formation and is described and discussed below.

The mechanism of the second division of sporulating yeast is much clearer. A series of electron micrographs suggests that this process closely resembles that which occurs during vegetative division; that is, constriction of the existing nuclei occurs without dissolution of the nuclear membrane and without involvement of visible chromosomes. The upper nucleus of the cell shown in Fig. 2 is believed to represent an early stage of the second division, because its size is approximately twice that of the nucleus of a mature spore and because its shape suggests that it is about to divide. The two nuclei of extremely irregular shape are seen to be separated from the large vacuole on the left. Areas of low electron density can be discerned within the nucleus in most of the figures presented and perhaps represent the structures referred to as chromosomes by some workers.

As sporulation progresses, the second nuclear division appears to be completed in a manner similar to that of vegetative division (3). A nucleus, presumably typical of this stage, can be found in the center of Fig. 3; what is considered to be the point of constriction is indicated. Two partially developed ascospores can be observed above and below the large dividing nucleus. Close examination of the figure also reveals that the two smaller ascospore nuclei are already encased within a thin membrane, which is considered to be the cytoplasmic membrane of the ascospore.

The uppermost nucleus in Fig. 4 appears to possess a long tail-like extension of the nuclear structure, which suggests that the nuclear mem-

brane persists throughout this stage of the division process. Similar figures had also been observed during the latter stages of vegetative nuclear division (3).

Following or sometimes even prior to completion of the second division, a membrane begins to form around the nucleus. Fig. 5 illustrates an early stage of this membrane formation. The membrane appears to be laid down *in situ* and is destined to become the cytoplasmic membrane of the ascospore.

Fig. 6 represents a stage when the spore nuclei appear to be completely encased within a distinct cytoplasmic membrane. The shapes shown by the spore limits suggest that they are still flexible. Within the nuclei one can again discern structures characterized by an electron density lower than the rest of the nucleoplasm. It was interesting to find such differentiation in the sporulating yeast nucleus since it had not been observed either in germinating or proliferating yeast nuclei (1, 3). No portion of the vacuole was observed to be incorporated into the cytoplasm of the spore, in contrast to other reports (16).

The next structural development during yeast sporogenesis is the synthesis of the outer spore coat, which remains during dormancy and is shed upon germination. An early stage in the formation of the outer coat is illustrated in Fig. 7. It is interesting to note that at this stage the spore cytoplasmic membrane and outer coat are in close proximity.

A later stage of development is represented by the cells shown in Fig. 8. The most conspicious change is in the appearance of the transparent intranuclear structures, which now tend to be dispersed or fragmented. A definite layering in the structure of the outer coat is disclosed. Lamellae have been reported in previous studies (1) as being one of the characteristics of the outer coat of the ascospore. It is at this terminal stage of development that the inner coat, which becomes the cell wall of the future germ, starts to develop between the cytoplasmic membrane and the outer coat and apparently is synthesized *in situ*. Eddy and Williamson (17) have reported formation of ascospores in yeast "protoplasts" prepared by digestion with snail enzyme, which also suggests that the cell wall of the ascospores actually is synthesized within the cytoplasm of the ascus during sporulation. As reported earlier (1), the inner coat of germinating yeast cells usually exhibits remarkably low density to electrons but can be stained by treatment of the section with a 1 per cent solution of lanthanum nitrate. Gradual widening of the low density zone in cells from a maturing population can be considered to reflect development of this inner coat, which finally attains the stage represented by Figs. 9 and 10. Prominent fragmentation of the low density structures may be seen in the ascospore nuclei shown in Fig. 9. Some nuclei show an internal structure which can be best described as an aggregation of the electron-transparent material, as represented in Fig. 10. It is thus evident that, at the same stage of development, these intranuclear structures may or may not appear fragmented. From this time on, these intranuclear structures gradually become undetectable and the spore nucleus becomes almost uniformly less electron-dense than the cytoplasm, as reported earlier (1).

A few remaining observations of structures other than the nucleus and membranes can be made from this series of micrographs. The small, internal, vacuole-like bodies observed in the dormant spores were considered, in accordance with our previous studies (1), to be lipoidal in nature. However, we have been unable to identify the dark inclusion bodies often seen in the spore cytoplasm (Fig. 7); there appear to be no distinct cristae, which characterize the mitochondria of yeast. Not infrequently a dense, membrane-like structure was observed lying either immediately under the spore cytoplasmic membrane or within the spore cytoplasm. An interesting feature of this structure is that it appears to be continuous with the nuclear membrane of the ascospore nucleus, as seen most readily in Fig. 8. A number of other observations indicate that this membrane system has a definite connection with the nuclear membrane. The significance of this observation is not completely understood at this time.

DISCUSSION

Investigators in the past have described the mode of reduction division in sporulating yeast as being a "convential meiotic division as close as could be expected in anything as small and unique as a yeast cell" (8), "echte Meiosis" (9), "classic meiosis" (10) and "conventional meiosis" (11). However, a few of these investigators (18) have recently reviewed the field of yeast cytology and stated, with reference to their own work, that "The chromosomes in this material (*Saccharomyces*) were not . . . so clearly evident as in the

material investigated by Lietz, in spite of the fact that these authors had employed the best optical equipment available to them." The point seems well taken. The virtual impossibility of observing by light microscopy the complicated and fine structural differentiation of nuclei during meiosis and mitosis in so small a cell led us to attempt to exploit the superior resolving power of the electron microscope.

With this advantage employed apparently for the first time in sporulating yeast, it was observed that the nuclear membrane remained intact and, after the first reduction division, the nucleus appeared to divide simply by constriction without the participation of distinct chromosomes. Thus, this situation resembles closely that observed previously in vegetatively dividing yeast nuclei (2, 3).

Intranuclear detail which may be of considerable significance was noted at all stages of sporulation: the nucleus contained distinct areas of lower electron density than the rest of the nucleoplasm. These structures assumed various forms, sometimes aggregated and sometimes fragmented, but apparently not in any order which could be correlated with the stage of sporulation. Instead, these structures seemed to be influenced more by unknown factors. It is tempting to speculate that these structures correspond to the mass of electron-transparent chromatin found in bacteria (19–21) and even more closely to that recently found in a deuteromycete by McAlear and Edwards (22). There are no means of determining whether the aggregated form represents the normal state of the structure or whether it is merely the result of the assembling of normally dispersed or fragmented structures. In any event, it is worth noting that there is a definite differentiation within the yeast nucleus during sporulation. Future experiments employing the electron microscope in combination with specific cytochemical staining of sections cut from the same cells may give conclusive data. At the present time, however, one can only speculate that the electron-transparent intranuclear areas may correspond to the structural units which, when Feulgen-stained, have been interpreted to be "chromosomes."

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Explanation of Plates

Explanation of Plates

Plate 146

Fig. 1. The nucleus (*N*) of a yeast cell in a very early stage of sporulation, approximately 10 hours after transfer onto the acetate-glucose medium. Note the transparent areas (*TA*) within the nucleus and its membrane (*NM*). Also discernible are the cell wall (*CW*), cytoplasmic membrane (*CM*), lipoidal inclusions (*L*), and mitochondrion-like bodies (*M*). × 54,000.

Fig. 2. An early stage of the second nuclear division. Note that the upper nucleus (*N*) is approximately twice as large as the normal mature ascospore nucleus, which is about 1 micron. Also note the structures of low electron density (*TA*) within the dividing nucleus and the fairly distinct nuclear membrane (*NM*). The large vacuole (*V*) appears to contain some solid material of an unknown nature. The vacuole remains undivided while the nuclei already show distinct division. × 28,500.

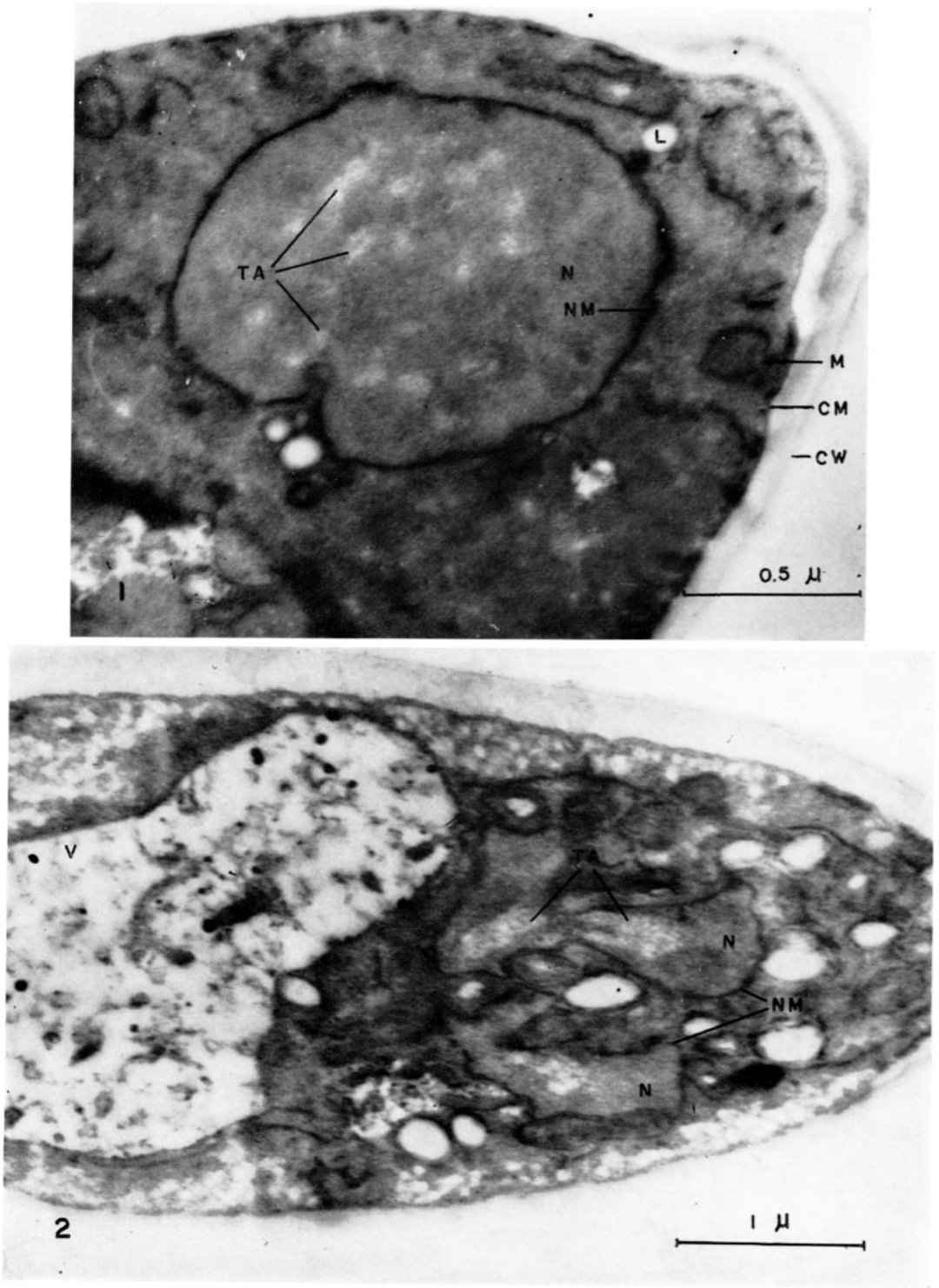

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PLATE 147

FIG. 3. A typical second division. The centrally located nucleus (*N*) appears to be constricted at the point (*CP*) indicated. The two other nuclei, which apparently have undergone division, can be observed above and below the dividing nucleus. Compare the size of the nuclei and also note that a membrane (*SCM*), which is destined to become the cytoplasmic membrane of the spore, can be faintly observed around the divided and dividing nuclei. × 27,500.

FIG. 4. The uppermost nucleus (*N*) is considered to have just completed separation during the second division. It carries a long tail-like structure stretching out into the cytoplasm. The central nucleus also displays a distinct extrusion. A structure of low electron density (*TA*) is apparent within this nucleus. Also note the membrane (*SCM*) forming around the central and lower nuclei. × 34,200.

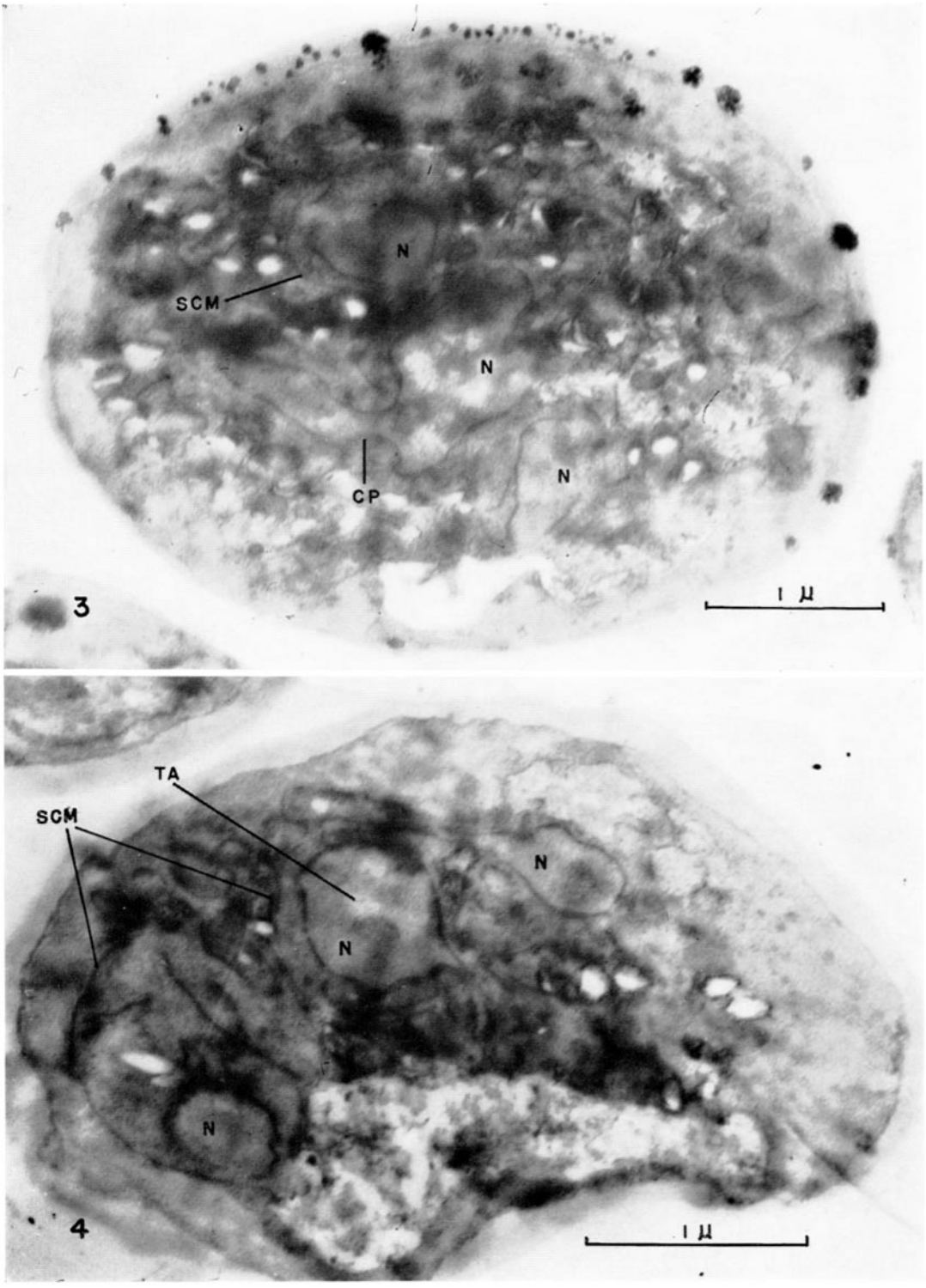

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PLATE 148

FIG. 5. This electronmicrograph more clearly illustrates the formation of the structural precursor of the spore cytoplasmic membrane (*SCM*) around the nucleus (*N*). Mitochondrion-like bodies (*M*) and lipoidal inclusions (*L*) are also evident. × 43,000.

FIG. 6. The cytoplasmic membrane (*SCM*) is already formed around the two nuclei (*N*) seen in this cell and a part of the outer spore coat (*SOC*) is starting to appear. Note that the structures of low electron density (*TA*) appear aggregated in both nuclei, in contrast to their fragmented appearance in the preceding figures. Four membrane layers are apparent in the ascus coat (*1*, *2*, *3*, *4*). × 35,000.

FIG. 7. A distinct spore coat (*SOC*) is now formed outside the cytoplasmic membrane (*SCM*), with a space between. Note that the electron transparent areas (*TA*) within the upper nucleus are homogeneous rather than fragmented. Unidentified dark inclusion bodies (*I*), in addition to the lipoidal granules (*L*), are located within the spores. × 35,000.

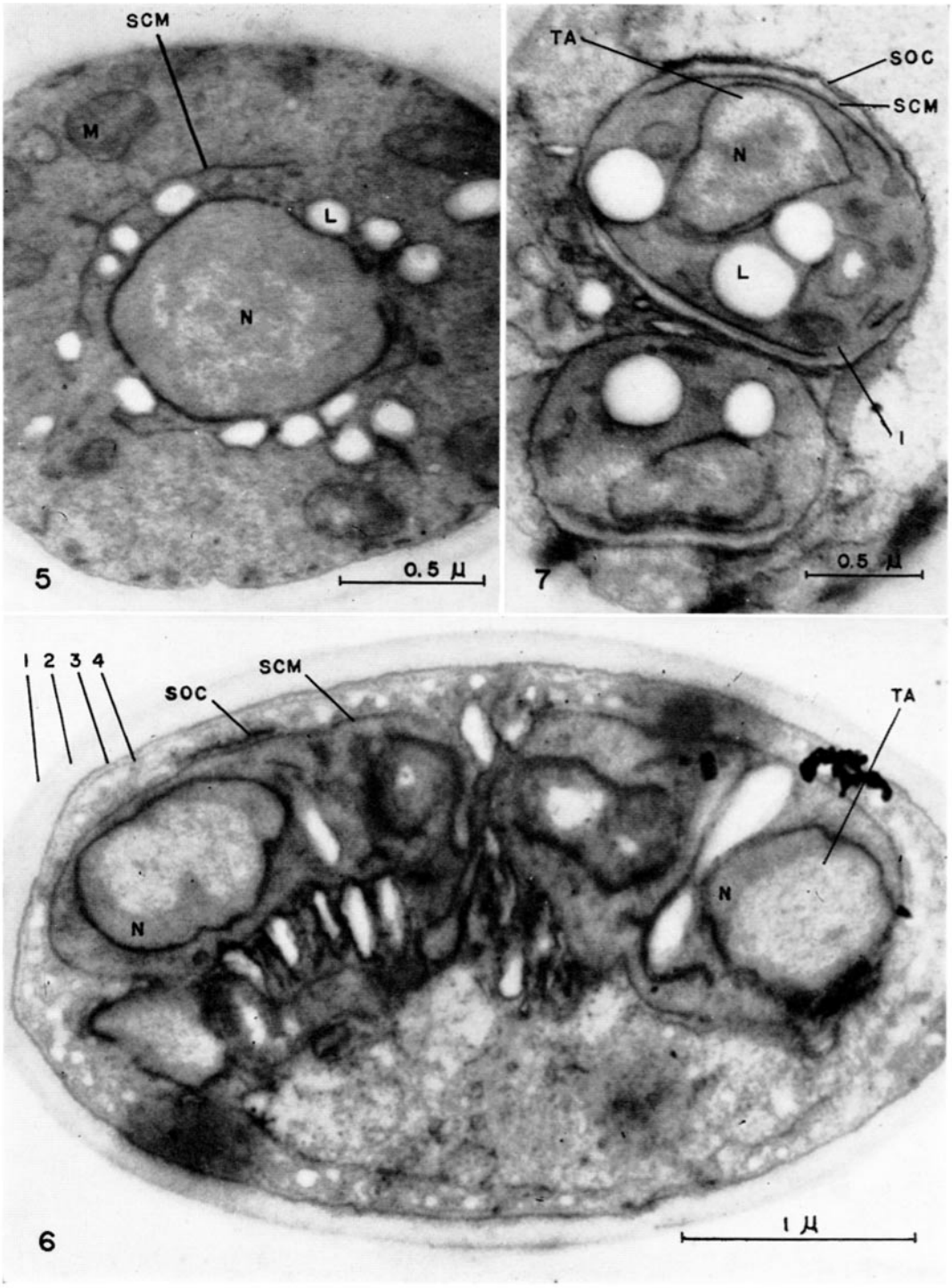

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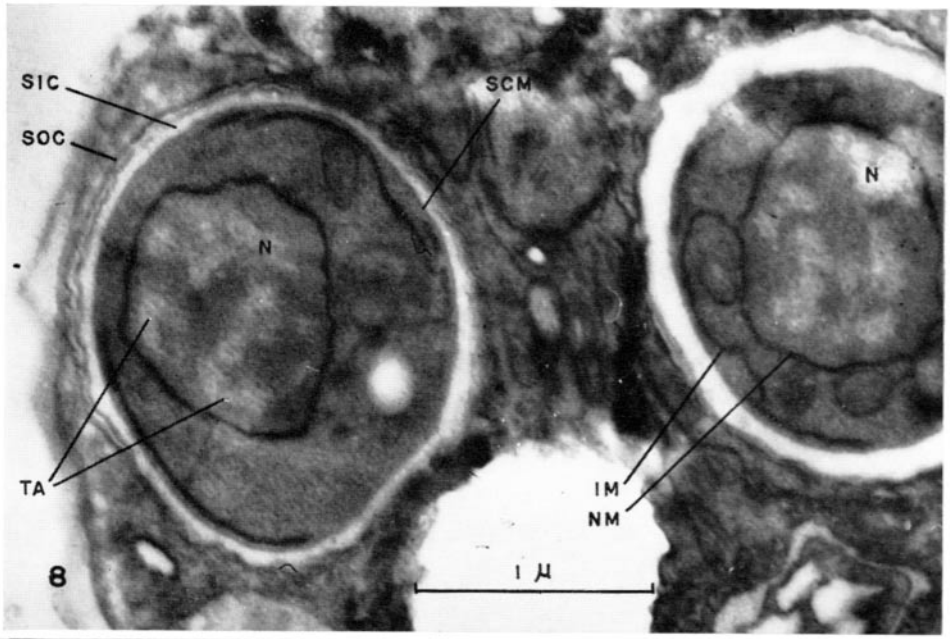
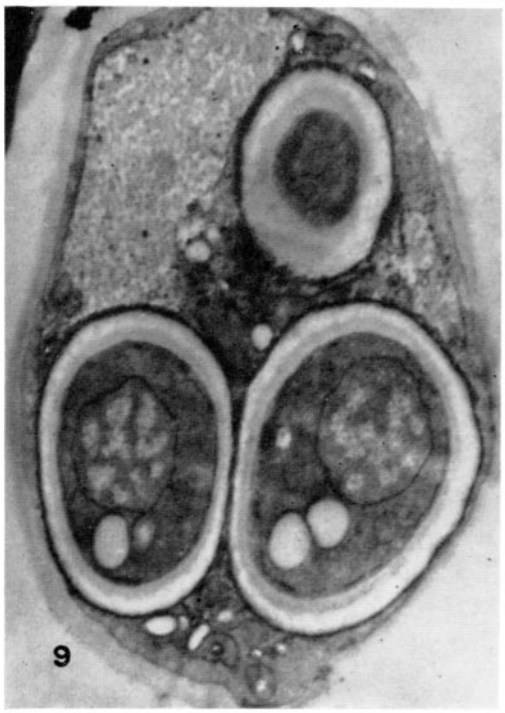
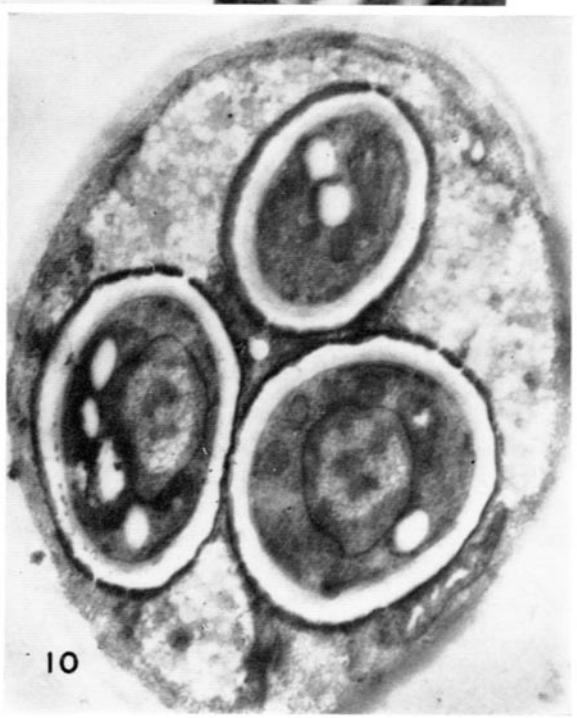
PLATE 149

FIG. 8. A more advanced stage of sporulation. The lamellar structure of the outer coat (*SOC*) is apparent. The inner coat (*SIC*) is beginning to form between the cytoplasmic membrane (*SCM*) and the outer coat and appears as a zone which is less dense to electrons. Note that the transparent areas (*TA*) within the nucleus (*N*) now appear fragmented. Continuation of the nuclear membrane (*NM*) with an unidentified intracytoplasmic membrane (*IM*) of the ascospore on the right can also be observed. × 31,500.

FIGS. 9 and 10. The final stages of sporulation are represented in these two figures. These ascospores possess all of the structural components of the mature ascospore. The electron transparent structures within the nuclei appear aggregated in Fig. 10 but fragmented in Fig. 9. × 21,000.







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