THE OCCURRENCE OF SYNAPTONEMAL COMPLEXES IN THE SLIME MOLD *ECHINOSTELIUM MINUTUM* DE BARY

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INTRODUCTION

Elongate tripartite structures termed synaptonemal complexes have been observed during meiotic prophase in a number of sexually reproducing animals and plants (5, 10, 11, 12, 13, 17). A complex seen in longitudinal section ("frontal view," 14) appears as three parallel strands. Apparently this triple-stranded structure forms along the entire length of synapsed homologous chromosomes and is a concomitant of chiasma formation and crossing over (4, 13, 14, 15). On the basis of cytochemical observations it is generally agreed that the synaptonemal complex is composed of protein and nucleic acid (4, 14). Both DNA (4) and RNA (21) have been reported as components; however, conclusions regarding their presence and localization are inconsistent.

The synaptonemal complex is a valuable indicator of meiosis in studies of the life histories of organisms with minute chromosomes such as myxomycetes. That the postcleavage spore is the site of meiosis in the life histories of four of the five orders of the subclass Myxogastromycetidae (9) is indicated by synaptonemal complexes demonstrated by ultrastructural studies (1, 3).

In this report we present the first description of normal and anomalous synaptonemal complexes (14) in a species of the Echinosteliales. The arrangement of the anomalous synaptonemal complexes with the biphasic nucleolus of *Echinostelium minutum* de Bary has to the best of our knowledge not been previously observed in any member of the Protista.

MATERIALS AND METHODS

E. minutum, isolate D-3, was used in this investigation (7). Abundant spore material was obtained 48 hr after transfer of protoplasmodia to Millipore filters (HAWP 047 00) (Millipore Corp., Bedford, Mass.) which were placed in Petri dishes on the surface of 1.5% Ionagar # 2 (Oxoid) (Colab Laboratories, Inc., Chicago Heights, Ill.) and incubated at 22°C.

The sporangia were fixed for electron microscopy with 3% glutaraldehyde in 0.025 M phosphate buffer at pH 6.8 for 1 hr, then postfixed in 1% osmium tetroxide for 1 hr. The material was dehydrated in ethanol, transferred into xylene, and embedded in Epon 812 according to the method of Luft (8). Silver sections, cut on a Reichert Om U2 ultramicrotome, were picked up on uncoated 200-mesh copper grids. The sections were stained with uranyl acetate and lead citrate and then examined with a Philips EM 300 or a Zeiss EM 9S-2 electron microscope.

The sporangia were fixed for light microscopy with Schaudinn's fluid for 1 hr, stained with Harris' hematoxylin, dehydrated in ethanol, transferred into xylene, and mounted in Permount (Fisher Scientific Company, Pittsburgh, Pa.) Photomicrographs were made with a Zeiss WL-phase-contrast microscope equipped with a Leitz Mikroblitz electronic flash attachment and were recorded on 35 mm Kodak Panatomic X film.

RESULTS AND DISCUSSION

A protoplasmodium of *E. minutum* gives rise in 2–4 hr to a single sporophore consisting of a globose mass of 75–100 pellucid spores 8–14 μ in diameter mounted atop a tapering, acellular stalk 150–450 μ in height. During differentiation a wave of nuclear division occurs in the sporangium. Concomitantly the sporogenous protoplasm undergoes centripetal cleavage, forming uninucleate spores. The spore wall has prominent circular patches of blunt spines which represent contact points between adjacent spores before separation.

Approximately 24–36 hr after spore formation two nuclear divisions, probably meiosis I and II, occur within each spore. One to several eccentrically placed refractile bodies are present during the early stages of the first division (Figs. 1, 2, 4, 5, and 6). We believe these bodies are nucleoli because they disappear during division and reform in reconstructing daughter nuclei, because they stain intensely with basic dyes such as toluidine blue and methylene blue and because they are Feulgen negative and do not stain with gallocyanin chrome alum after ribonuclease treatment (16).

During the early stages of the first division a

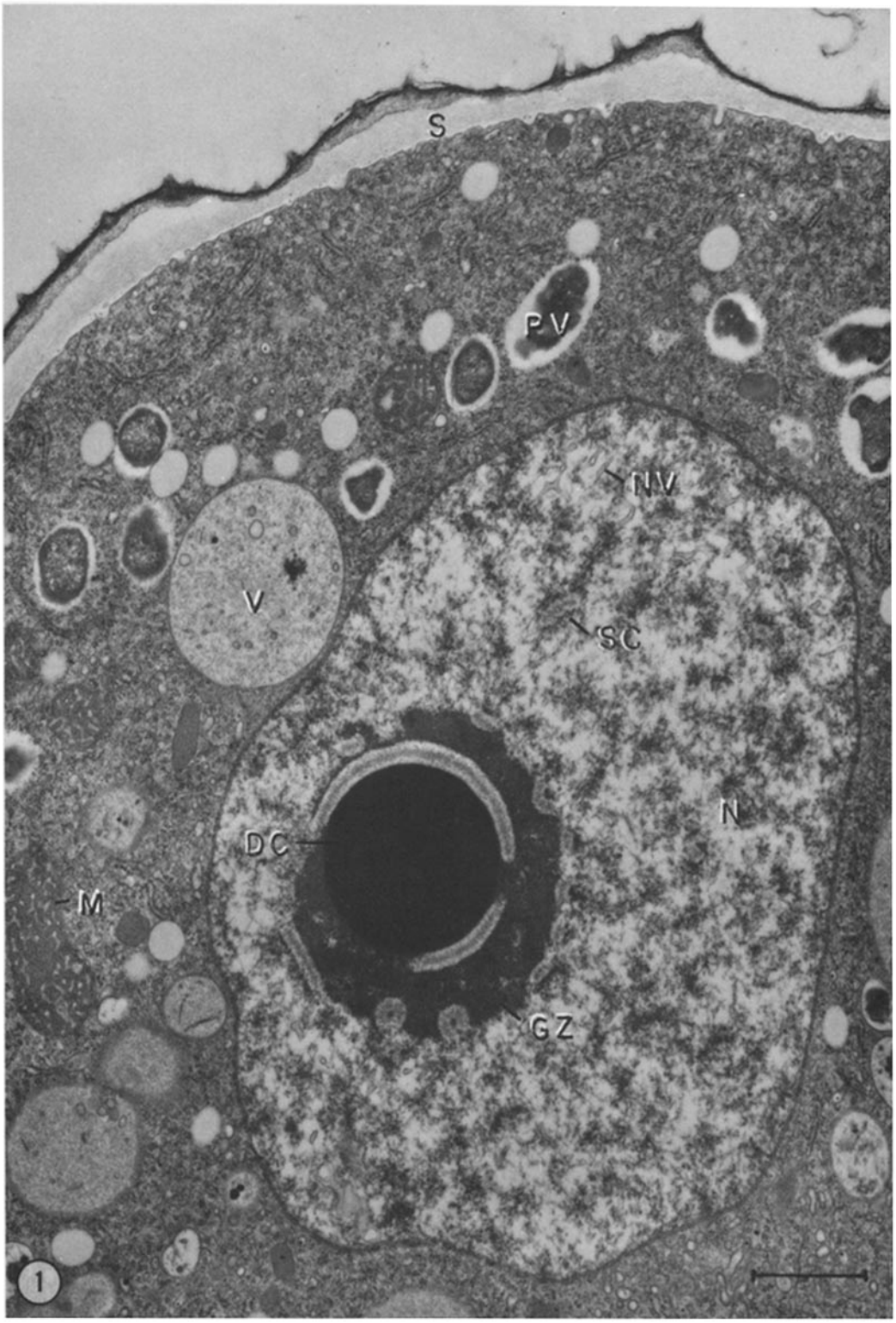
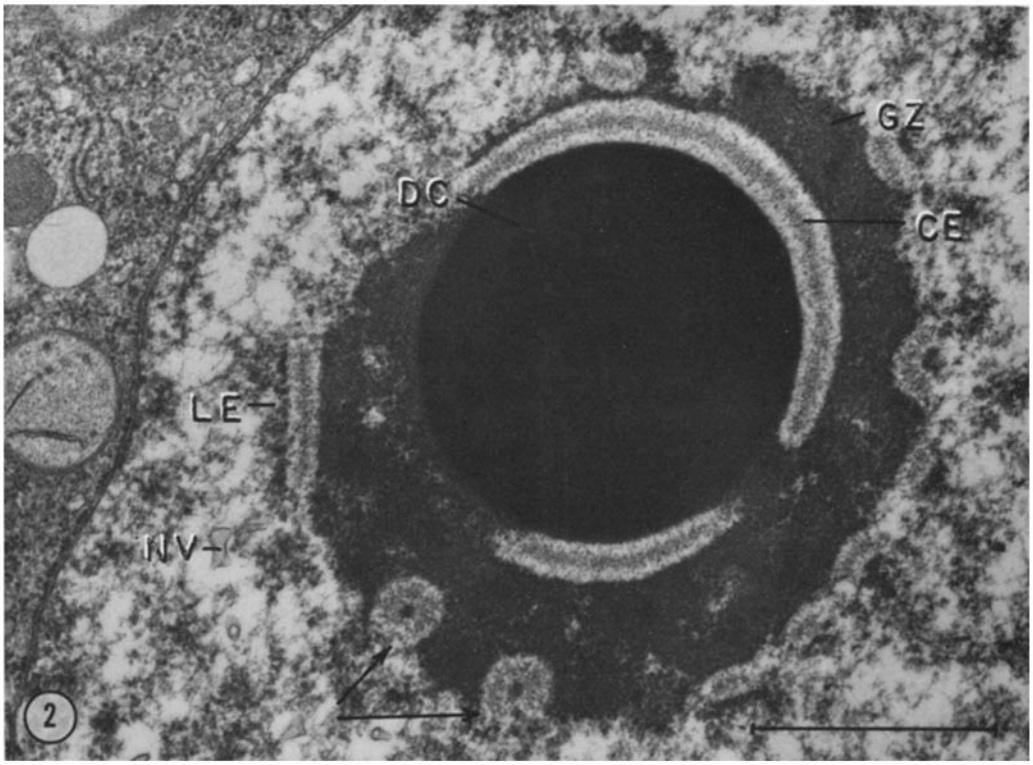


FIGURE 1 Thin section of spore. Multiple synaptonemal complexes associated with a nucleolus. *N*, nucleus; *GZ*, granular zone of nucleolus; *DC*, dense core of nucleolus; *SC*, synaptonemal complex; *NV*, nuclear vesicle; *PV*, phagocytic vacuole containing a bacterium; *V*, vacuole; *M*, mitochondrion; *S*, sporoderm. Scale in Figs. 1–3 is 1 μ. × 17,875.


GZ
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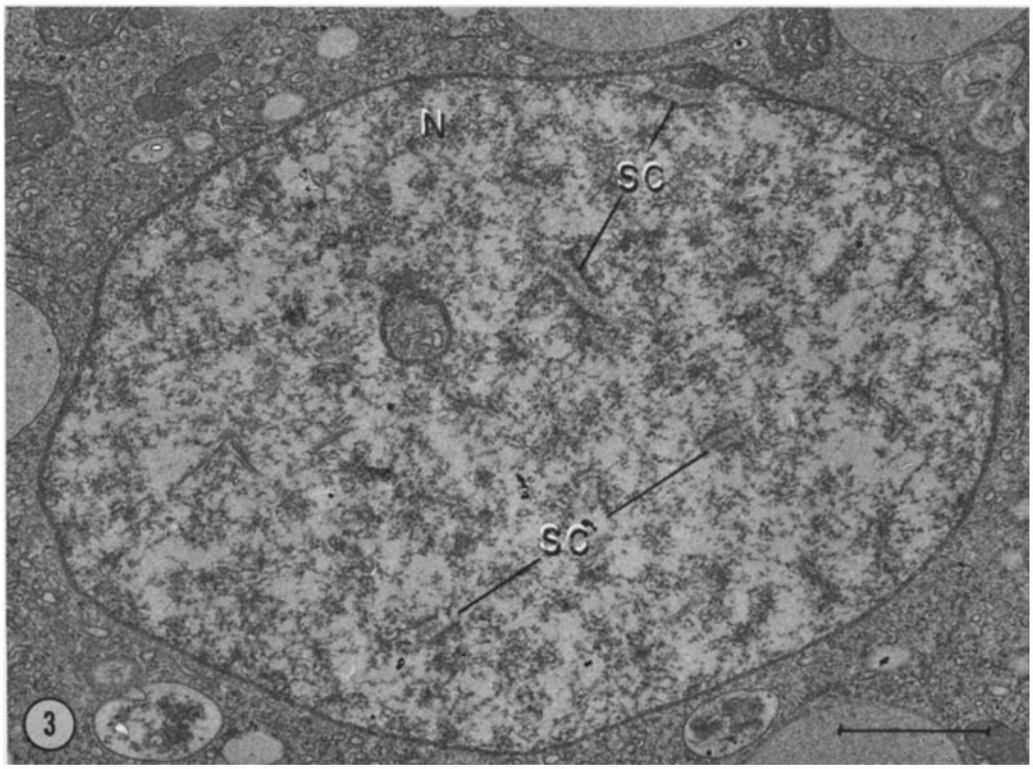
2


N
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SC
3

nucleolus consists of a granular zone and a dense core. Rectilinear and curvilinear synaptonemal complexes are embedded in the outer margin of the granular zone and curvilinear complexes border the dense core (Figs. 1 and 2). We have never observed these complexes in presporogenous material. Two examples of what may be "cross-sectional views" (14) of synaptonemal complexes are present in the granular zone of the nucleolus (Fig. 2, arrows). Alternatively, these profiles may be interpreted as looped or folded complexes in "frontal view." Because these profiles occur rarely in our material, we have not been able to complete successfully an analysis of serial thin sections to distinguish between these two hypotheses.

Synaptonemal complexes in the surrounding nucleoplasm (Figs. 1 and 3) consist of two "lateral components" (14) 500–600 A in diameter and a "central region" (14) 1500–1800 A thick which contains a "central element" (14) 300–600 A in diameter. Nucleolar complexes correspond in size with those complexes found in the surrounding nucleoplasm; however, it is difficult to identify "lateral elements."

Although the significance of the multiple complexes embedded in the nucleolus (Figs. 1 and 2) remains unclear, they may represent a stage in the synthesis of synaptonemal complexes (6, 21, 22) or perhaps a manifestation of pairing and/or crossing-over of homologous chromosomes.

It will be of considerable interest to discover the function of the numerous nuclear vesicles (Figs. 1 and 2). Do they play a role in chromosome alignment and pairing during meiosis?

Light- and electron microscope studies indicate that the first division in the spore is intranuclear. Evidence from light microscope studies suggest that the nuclear envelope may break down during the second division (Figs. 5). This type of division occurs in haploid slime mold amoebae (2). Light microscope studies indicate that one daughter nucleus become pycnotic after each division (Figs. 5 and 6). The two pycnotic nuclei ultimately degenerate, leaving a mature spore with one nucleus which presumably is haploid. Nuclear degeneration after meiosis in the spore has been reported previously for a number of slime molds (1, 18, 19).

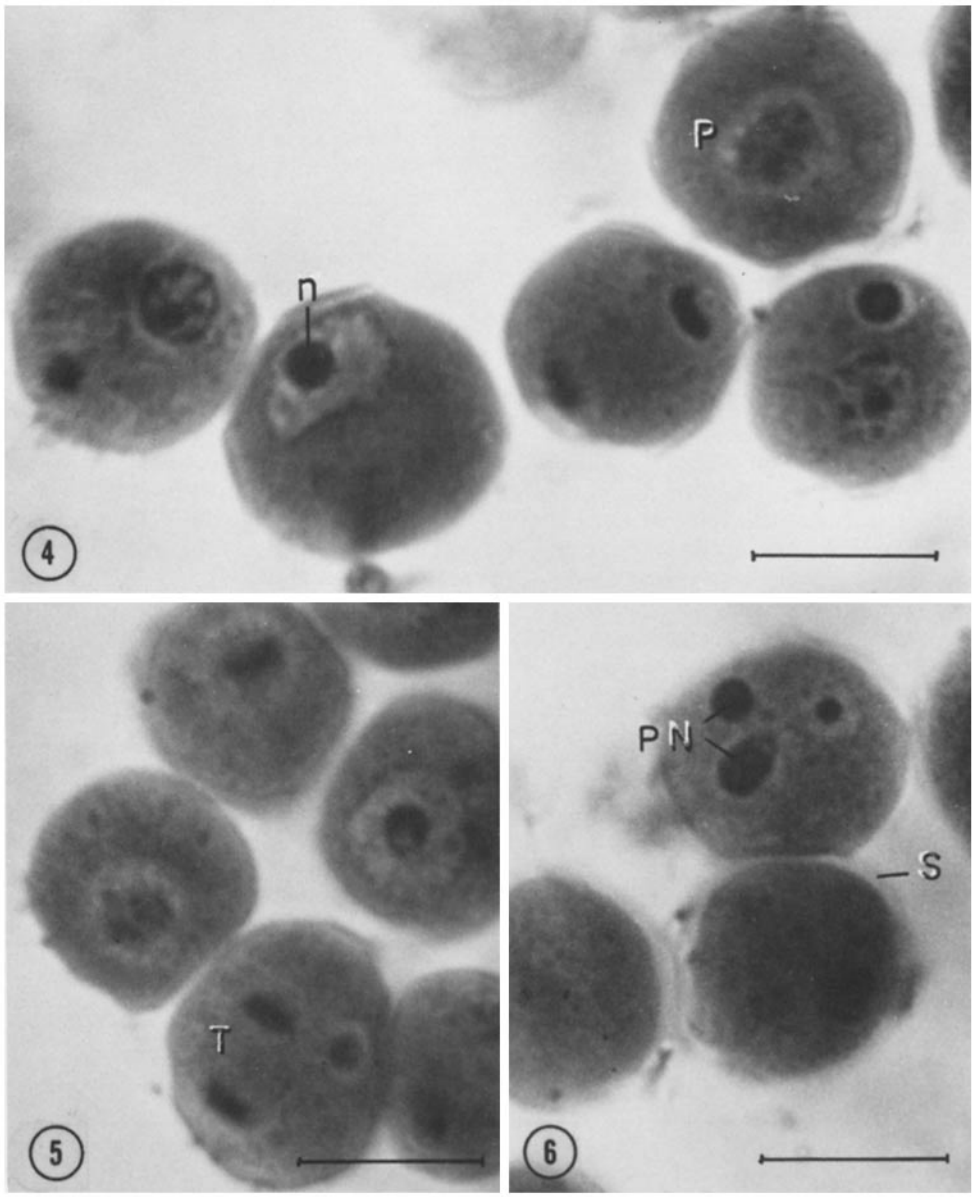
That detection of synaptonemal complexes is a reliable criterion of meiosis in a life history has been questioned by the recent discovery of these structures in haploid tomato and maize plants (10, 20). In the former investigation an average of one complex per haploid nucleus was found, a number much lower than observed during meiosis in diploid tomato plants (10). It was postulated that the complexes noted in the haploid nuclei represented pairing of nonhomologous chromosomes. We feel that in *E. minutum* the number of synaptonemal complexes observed per nucleus is higher than would be found if these represented pairing of nonhomologous chromosomes in a haploid nucleus. The possibility that the complexes observed in the spore are persistent structures from meiotic divisions occurring earlier in the life history must be considered in view of the persistence of such complexes in insects (23). We have found no evidence of synaptonemal complexes in precleavage sporogenous material and therefore conclude that these structures arise initially in the spores.

SUMMARY

It is postulated that the two nuclear divisions observed in the spore of *E. minutum* are meiotic. This contention is supported by the observation of normal and anomalous synaptonemal complexes in the early stages of the first nuclear division. The anomalous complexes are associated with a nucleolus consisting of an electron-opaque core and an outer granular cortex in which complexes are embedded. We suggest that the nucleolus may play a role in the synthesis of synaptonemal complexes or may function in the collocation and effective synapsis and crossing over of homologous chromosomes.

FIGURE 2 Electron micrograph of a portion of a spore nucleus showing multiple synaptonemal complexes associated with a nucleolus. *GZ*, granular zone of nucleolus; *DC*, dense core of nucleolus; *LE*, lateral element of synaptonemal complex; *CE*, central element of synaptonemal complex; *NV*, nuclear vesicle. Arrows indicate putative cross-sectional views of synaptonemal complexes. × 32,600.

FIGURE 3 Thin section of a spore nucleus showing synaptonemal complexes. *N*, nucleus; *SC*, synaptonemal complexes. × 20,000.



FIGURES 4–6 Photomicrographs of intact spores stained with Harris' hematoxylin. *P*, prophase; *n*, nucleolus; *T*, telophase; *PN*, pycnotic nucleus; *S*, sporoderm. Scale in Figs. 4–6 is 10 μ. × 2500.

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