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Abstract. Nuclei of the vegetative body of *Stemonitis fusca* have a nuclear membrane that is double and has pores and annuli. The nucleoplasm contains numerous chromocenters and a nucleolus that is without a limiting membrane and has the appearance of a nucleolonema. What appeared to be extensions of the nuclear membrane were often seen within the nucleoplasm, and structures resembling annulate lamellae were found near the outside of nuclei and elsewhere in the cytoplasm. Large blebs were sometimes seen extending outward from the nuclear membrane, and vesicles in the neighboring cytoplasm contained material identical in appearance with the contents of the blebs.

Modern cytological techniques such as phase contrast and electron microscope studies have produced much more information on cytoplasmic organelles than on nuclear structure. This discrepancy, according to Hans Ris (1), is not due to unsatisfactory methods of fixation and preparation, but rather to difficulties of interpretation. The three dimensional orientation of complicated structures is very hard to get at through ultrathin sections.

Myxomycetes are usually grouped with the fungi, and their nuclei might be expected to have much in common with the nuclei of fungi. Although the number and range of fungal organisms that have been studied by electron microscopy is still small, much attention has centered on their nuclei. Many of the recorded observations have been made on stages of mitosis. Olive (2) concluded in 1953 that vegetative nuclei divided by mitosis, but some later evidence challenges this view. Robinow (3) thinks that although meiosis in fungi appears to be typical, "division of vegetative nuclei might well be of a less highly evolved type, resembling the division of the nuclear bodies of the bacteria."

There are many other investigations of fungi in all the major groups in which the typical structures of mitosis have not been found. Rather, they have shown an amitotic process consisting of a condensation of chromatin material, degeneration of the nucleolus, and the pulling apart of the chomatin as two teardrop daughters. There are only a few exceptions, notably *Neurospora crassa, Puccinia graminis,* and two species of *Hypomyces.*

The vegetative stage of myxomycetes, the plasmodium, has

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been used extensively for physiological studies of protoplasm. The nuclei of the most widely used species, *Physarum polycephalum,* have been reported to divide synchronously throughout the plasmodium, by mitosis (4). The only detailed study of the fine structure of the nuclei is by Terada (5).

The plasmodium of *Stemonitis fusca* differs from that of *Physarum polycephalum* in a number of features of its gross morphology (6), and has been designated the "aphanoplasmodium" type (7).

Materials and Methods

The aphanoplasmodium of *Stemonitis fusca* was examined in both the living and the fixed state by phase contrast microscopy. The plasmodium spread itself out on the glass bottom of a Petri dish. Most of the fluid surrounding it was removed, the bottom of the Petri dish was placed on the stage of the microscope, and the living plasmodium was examined and photographed.

Small plasmodia growing in Stender dishes were flooded with 1% OsO_4 buffered with veronal acetate at pH 7.6 and fixed for 40 minutes. They were then dehydrated in a graded series of ethanols, embedded in 3:2 butyl-ethyl methacrylate and sectioned at 40 mμ. They were examined with a model RCA EMU-3F electron microscope and photographed at 100 Kv. Staining was with $KMnO_4$.

Observations

Nuclei were found randomly distributed in the protoplasm excepting that they were not immediately adjacent to the plasma membrane. They were plastic, deformed where they were pressed against other structures. The karyoplasm was uniformly granular and more dense than the surrounding cytoplasm. Numerous areas of increased density scattered in the karyoplasm corresponded to the structures usually interpreted as chromocenters. The nucleolus is evident; it has no limiting membrane and in many sections had the appearance of a loosely coiled thread.

The nuclear envelope is double, about 15 mμ thick. Nuclear pores and annuli could be identified, but it was not possible to determine whether the pores are closed by a diaphragm. What appeared to be extensions of the complete (double) nuclear membrane passed from the nuclear envelope into the karyoplasm, sometimes at three or four different places in the same nucleus. This gave the appearance of a nucleus which might fragment into more than two parts.

The nuclear envelope appeared to have short extensions out-

ward into the cytoplasm, but these could never be followed for more than a very short distance. Lying beside some of the nuclei, structures having the appearance of annulate lamellae (8) were sometimes seen. Similar lamellar structures farther away in the cytoplasm were also noted.

In some of the micrographs, the two layers of the nuclear envelope appeared to be pushed apart by the accumulation of a fairly electron-transparent material between them. This occurred in small localized areas only, but there were often several such areas in one nucleus. Beside these nuclei, vesicles containing electron-transparent material similar to that seen in the swelling were sometimes seen.

Discussion

Although it was constantly sought, no evidence of typical mitosis was ever found in the present investigation. It has been reported several times that the nuclei of various plasmodia divide synchronously in a diurnal cycle, so it is possible that all our examinations missed the division phase of the cycle.

Protozoa often have nuclei that divide by fragmentation, and it is possible that the nuclei of myxomycete plasmodia might also undergo this type of division. The inward extensions of the nuclear envelope seen here suggest this.

Both the origin and the function of the annulate lamellae originally described by Swift (8) have been somewhat controversial. Afzelius (9) at first thought that they were fragments of the nuclear membrane remaining after nuclear breakdown at mitosis. Rebhun (10) suggested that they arise by delamination from the nuclear membrane, and Swift (8) suggested that they are synthesized on the surface of the nuclear envelope and then move out into the cytoplasm. Merriam (11) thinks that they form inside the nuclear membrane and are later sloughed off into the cytoplasm. Hsu (12) has observed them on both sides of the nuclear envelope.

In the oocytes of *Necturus*, Kessel (13) has recently shown that they arise by fusion of vesicles derived by blebbing from the outer membrane of the nuclear envelope. Their function or significance has not yet been determined.

There are many reports of passage of nucleolar substance into cytoplasm in protozoa, and it is possible that the nuclear pores of all organisms in which pores occur function in passage of materials between nucleus and cytoplasm. Chevremont (14) has evidence that large DNA precursors are manufactured in mitochondria and pass *into* the nucleus.

The most direct type of evidence for passage of nuclear material into cytoplasm is the pinching off of blebs from the nuclear

surface. This has been seen in the ameba *Pelomyxa carolinensis* (15) and has been described in higher animals, as well.

In the egg cells of the fern *Pteridium aequilinum*, Bell (16) has found evaginations of the nuclear membrane that are more elaborate than simple blebs. He interprets his sections as showing the formation of a hooded protrusion of the double membrane, containing cytoplasm within the hood. A section across such a structure would show nuclear membrane surrounding cytoplasm within nuclear membrane surrounding karyoplasm, or what might appear as a nucleus within a nucleus. In a few of our micrographs we found conformations that conform to Bell's description.

Bell believes that these hoods detach themselves from the nucleus and are transformed into mitochondria, in the fern which he investigated, and that less elaborate evaginations detach themselves and become proplastids. In our material, we found no evidence on which we could base an opinion as to the fate of these structures.

Summary

We have described here a number of features found in nuclei of myxomycetes that are reminiscent of features described in other animals and plants. As additional information of this kind accumulates, more light may be shed on the affinities of the myxomycetes and on the dynamic activities and functions of nuclei in general.

Literature Cited

1. Ris, Hans. 1962. Interpretation of the ultrastructure of the cell nucleus. In *The Interpretation of Ultrastructure*, Vol. I (R. J. C. Harris, ed.), Academic Press, New York. p. 69.
2. Olive, L. S. 1953. Botan. Rev. *19:* 439.
3. Robinow, C. F. 1956. Bacteriol. Rev. *20:* 207.
4. Daniel, J. W. and H. P. Husch. 1956. (Abs.) Fed. Am. Soc. Exp. Biol. Fed. Proc. *15:* 513.
5. Terada, T. 1962. Fac. of Sci., Osaka Univ., Osaka, Japan. *1962:* 47.
6. McManus, S. M. A. 1961. Amer. Jour. Bot. *48:* 582.
7. Alexopoulos, C. J. 1960 (1961). Mycologia *52:* 1.
8. Swift, H. 1956. J. Biophys. Biochem. Cytol. *2:* 415.
9. Afzelius, B. A. 1955. Exptl. Cell Res. *8:* 147.
10. Rebhun, L. I. 1956. J. Biophys. Biochem. Cytol. *2:* 93.
11. Merriam, R. W. 1959. J. Biophys. Biochem. Cytol. *5:* 117.
12. Hsu, W. S. 1962. Mikroscop. Anat. *58:* 660.
13. Kessel, R. G. 1963. Anat. Record *145:* 363.
14. Chevremont, M., R. Bassler, and E. Baekeland. 1961. Arch. Biol. *72:* 501.
15. Brandt, P. W., and G. D. Pappas. 1959. J. Biophys. Biochem. Cytol. *6:* 91.
16. Bell, P. R. 1964. The membranes of the fern egg. In *Cellular Membranes in Development* (M. Locke, ed.) Academic Press, New York. p. 221.