Electron Microscopic Observations of Dividing Somatic Nuclei in *Saprolegnia*

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SUMMARY

An intranuclear spindle of about 40 microtubular fibrils, 200 Å in diameter, convergent to poles close to the inner membrane, with attendant centrioles external to the nuclear envelope, has been identified in sections of synchronously dividing nuclei in vegetative hyphae of *Saprolegnia ferax*.

INTRODUCTION

The mode of division of the somatic nuclei of *Saprolegnia* species has proved difficult to elucidate with the light microscope and has become the source of considerable argument since Hartog's investigations of 1895, which included *Saprolegnia ferax*, when he reported the duplication and partition of the chromosomes by an essentially mitotic process. Diverse and often inconclusive results have been obtained by subsequent investigators. Recently *S. ferax*, among other species, was examined again by Bakerspiegel (1960) and *S. delica* by Slifkin (1967), the latter including colchicine treatments. Both of these authors suggested that no nuclear spindle was formed and supported the view that the nuclear division is generally amitotic. Centrioles were reported in *S. ferax* by Gay & Greenwood (1966). Further investigations with the electron microscope now show that an intranuclear spindle accompanied by centrioles does exist in the dividing nuclei of *S. ferax*.

METHODS

The strain of *Saprolegnia ferax* (Gruithuisen) Thuret was obtained from vegetative subcultures originating from a single zoospore (Manton, Clarke & Greenwood, 1951). *S. furcata* Maurizia was obtained from Dr M. Dick (Reading University).

Petri dish cultures were made at 25° on a glucose, yeast-extract, peptone and salts agar (1 %) medium on which they remained indefinitely vegetative. Actively growing subcultures were fixed *in situ* with 5 % glutaraldehyde buffered at pH 7.0 with M/15 phosphate followed by similarly buffered 1 % osmic acid, dehydrated in ethanol and embedded in Epon 812. Ultrathin sections were stained with 2 % aqueous uranyl acetate followed by Reynolds lead citrate.

RESULTS

In eight randomly selected young hyphae from the margin of a colony of about 2.5 cm. diam. all the nuclei were found to be in phases of division. This enabled a large number of nuclei to be examined in given states of division.

The spindle was small and at early metaphase, as seen in *Saprolegnia ferax* (Pl. 1, fig. 1), was situated eccentrically within the nuclear envelope which also enclosed a relatively large nucleolus, not included in the spindle but accounting for the greater part of the nuclear diameter at the equator of the mitotic figure.

The spindle consisted of about 40 microtubules of about 200 Å in diameter, spaced separately and fairly widely apart (Pl. 2, fig. 3) though convergent towards the poles. Some of the tubules ran from pole to pole whilst others terminated in the region of the equator on what appeared to be chromosomes (Pl. 1, fig. 1; Pl. 2, fig. 4). The terminations were less well defined than the kinetochores shown by Brinkley & Stubblefield (1966) in hamsters. The spindle tubules terminated abruptly at the poles where they approached close to the inner nuclear membrane but did not penetrate it. At each pole there was at this stage a centriole situated outside the membranes of the nucleus in a well-defined pocket of its envelope. The centrioles (Pl. 2, fig. 5) resembled in structure those described by Berlin & Bowen (1964) in *Albugo candida*; their mode of division is the subject of further work. From the centriolar region microtubules radiated into the cytoplasm and along the outside of the nuclear membranes. Pole to pole microtubules persisted during chromosomal separation and towards the completion of division (Pl. 2, fig. 2), the nucleus including the spindle became elongated. The nuclear envelope appeared to remain intact at all stages of division. Preliminary observations on similarly prepared material of *S. furcata* (not illustrated) showed centrioles and polar intranuclear microtubules comparable to those of *S. ferax* but the metaphase spindle was not observed.

DISCUSSION

The structural organization of the centrioles, microtubules and chromosome-like bodies in different stages of nuclear division of the *Saprolegnia* species here studied is consistent with the activity of a functional mitotic apparatus. The persistent nuclear envelope and small size of the spindle combined with the low number of widely separated microtubules in the latter and its eccentric position relative to the large nucleolus do not favour its detection with the light microscope. For these reasons Bakerspiegel (1960) and others may have found the critical feature of a spindle impossible to detect in *S. ferax* and other species. However, if a similar situation exists in *S. delica* the results of Slifkin (1967) with colchicine remain unexplained. In view of the present observations reports of amitotic divisions based on the absence of a spindle in *Saprolegnia* species and allied fungi, where information about their fine structure is lacking, should now be regarded as uncertain.

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EXPLANATION OF PLATES

Key to symbols: N = nucleus, Nu = nucleolus, C = centriole, G = Golgi body, M = mitochondrion, Cp = centriolar position, F = spindle fibre, T = microtubular termination, Mt = cytoplasmic microtubule.

PLATE I

Fig. 1. *Saprolegnia ferax*. Median longitudinal section through an early metaphase nucleus with eccentrically placed spindle and large nucleolus showing two centrioles (C) and spindle fibres some of which appear to terminate at chromosome like bodies in the equator of the figure. Note cytoplasmic microtubules (Mt). Neg. 8518.*

PLATE 2

Fig. 2. *S. ferax*. Longitudinal section of a telophase nucleus with 2 nucleoli (Nu) showing centriolar positions (Cp) and spindle fibres (F). Neg. 8772.

Fig. 3. (a) *S. ferax*. A transverse section of spindle in the equatorial region showing the microtubular nature of the spindle fibres. Arrowed tubule represents a probable chromosomal termination in T.S. Neg. 8588. (b) The next section in the series showing continuity of the majority of tubules. Note absence of the tubule arrowed in (a). Neg. 8587.

Fig. 4. *S. ferax*. Longitudinal section of equatorial portion of spindle showing microtubular termination (T) resembling a kinetochore. Cf. fig. 3(a). Neg. 8516.

Fig. 5. *S. ferax*. Transverse section of a centriole, showing the nine triple fibres and central 'cartwheel', situated in a pocket of the nuclear envelope with polar terminations of the spindle fibres adjacent to its inner surface. Neg. 8592.

* Number of negative in collection of Botany Department, Imperial Collection.