SUMMARY

The electron-dense material which regularly occurs at the poles of the nuclei of certain fungi during division has been studied in hyphal interphase nuclei of 4 basidiomycetes and 3 ascomycetes. The shape of this material varies with the species and the nuclear age and may be that of a sphere or a dumbbell. In some instances paired diskoidal structures are found in the place of the globular elements of the dumbbell configuration. The organelle in question lies in an invagination of the nuclear envelope preferentially at the forward pointing pole of the nucleus. Its size (0.1-0.5 μm) is correlated with the size of the nucleus. In *Polystictus versicolor* the diameter of both globular elements of the polar organelle increases during initiation of the division process.

The structure is identical with the fungal 'centriole' of light-microscopical studies but lacks the characteristic organization of a true centriole. It is suggested that the polar organelle is the equivalent of a kinetochore because it maintains continuous and direct contact with the nuclear envelope (on its cytoplasmic aspect), seems to be connected with the karyoplasm, exhibits oscillating movements, is continuous with microtubules during division and resembles in its fine structure the kinetochores of the chromosomes of higher plants and animals. In addition to its function as an organelle of nuclear motility it also seems to play a part in the initiation of DNA synthesis and nuclear division. If this is correct one would expect to find the genome of the fungal nucleus having the form of a unitary, coherent compound structure (chain) extending from or incorporating a single, solitary kinetochore.

INTRODUCTION

In recent years much evidence has been presented that concentrations of ill-defined electron-dense material are regularly present at the poles of dividing nuclei of certain kinds of fungi. Microtubules may extend from these centres into the interior of the nucleus, as well as into the cytoplasm. Disk-like aggregates are seen in ascomycetes (Robinow & Marak, 1966; Zickler, 1969, 1970; Schrantz, 1969; Aist (postscript), 1969), spherical ones in basidiomycetes (Lu, 1967; Girbardt, 1968; Lerbs & Thielke, 1969; Motta, 1969). They lack the characteristic fine structure of the true centrioles which have been found at the poles of dividing nuclei of phycomycetes capable of forming motile zoospores (Heath & Greenwood, 1968; Lessie & Lovett, 1968; Ichida & Fuller, 1968), but nevertheless give the impression of having an important

* Dedicated to my teacher Prof. Dr H. Drawert, Hamburg, on the occasion of the 60th anniversary of his birthday.
part to play in nuclear division. Almost nothing is known about their fate during interphase at the ultrastructural level.

An earlier paper (Girbardt, 1970) has emphasized similarities in fine structure between fungal nuclei and the nuclei of cells of higher eucaryotes. The present paper and future communications from this laboratory will be concerned with special features and modes of division of fungal nuclei. They will bear out the prediction (Girbardt, 1962a) that as regards fine structure the nuclei of fungi will be found to occupy a position intermediate between the genophore of procaryotes and the nucleus of higher eucaryotes.

MATERIALS AND METHODS

All species were grown on malt peptone agar (4% malt extract, 0.5% peptone, 2% agar) at 24 °C. The fixative was plain glutaraldehyde or mixtures of it with formaldehyde. The 'kinetochore equivalent' (KCE, Girbardt, 1968), which is the subject of this paper, is not equally well preserved in all species by one and the same fixative of unvarying composition. Numerous trials have convinced us that the optimal preservation of the KCE of different species requires different strengths of glutaraldehyde (GA), different amounts of formaldehyde (FA) mixed with it and buffers of different molality. The following 5 procedures have been found satisfactory:

1. 30 min GA (2%) in 0.005 M phosphate buffer;
2. 30 min GA (5%) in 0.005 M phosphate buffer;
3. 30 min GA (1%) in 0.05 M phosphate buffer;
4. 20 min GA (2%) + FA (0.75%) in 0.05 M phosphate buffer;
5. 20 min GA (1.25%) + FA (1.5%) in 0.05 M phosphate buffer.

The pH of all fixatives was adjusted to 7.0 and they were used at room temperature. The osmolality was determined by freezing-point depression (last column of Table 1, p. 457). After washing for 1 h with several changes of the corresponding buffer specimens were post-fixed with 1% OsO₄ (dissolved in buffer) for 2 or 3 h at room temperature. Fixed specimens were again washed in distilled water for 30 min and soaked in 0.5% uranyl acetate for 2 or 3 h. After dehydration in ascending series of acetone they were embedded in 'Mikropal' (= Vestopal W).

A nucleus-containing area of a hyphal tip cell was selected by light microscopy in flat embeddings without prior examination of the living cell (Girbardt, 1965). This preselected hyphal region was serially sectioned with a Porter-Blum or Ultratome I ultramicrotome. Sections were stained with lead citrate according to Reynolds (1963). Preparations were examined with either a SEM 3 or SEM 3/1 (VEB Werk für Fernsehelektronik, Berlin) or Elmiskop I (Siemens) electron microscope at 80 kV, using 50- or 70-μm objective apertures. Both SEM 3/1 and Elmiskop were equipped with anticontamination cold fingers.

OBSERVATIONS

Shape of the Polystictus versicolor KCE

The above-mentioned electron-dense material observable at the poles of dividing fungal nuclei is well developed in the basidiomycete Polystictus versicolor (Girbardt, 1968). During most of interphase the KCE of this species has the shape of two globular elements (GE) connected by a flat plate-like middle piece (MP). Depending on 'nuclear age' (determined by distance of a nucleus (Girbardt, 1955) from the site of its last division, namely cross-wall or clamp connexion), a nucleus may possess only one GE (Figs. 3, 4, monoglobular stage) or a complete KCE (diglobular stage).
Ultrastructure of fungal KCE

The latter configuration is assumed by the KCE as early as 30–40 min after the previous nuclear division.

Single sections of a KCE cut at right angles to its longitudinal axis mislead the viewer into assuming the presence of but a single GE (Fig. 2, KCE 2).

The KCE maintains intimate contact with the cytoplasmic face of the nuclear envelope. Single sections may give the impression that a KCE is lying free in the cytoplasm (Fig. 4, sections 30–32), but serial sections invariably reveal points of contact with the nuclear envelope at other levels (Fig. 4, section 34). Occasionally, especially at times of high nuclear motility, the area of contact between the KCE and the nuclear envelope may be very narrow. More frequently, however, particularly in the interval of 40–120 min after division, the globular extremities of the KCE are in contact with the nuclear envelope on a much broader base.

At no stage of their growth cycle are the GEs or the MP separated from the cytoplasm by a membrane. In some instances GEs of several species are surrounded by a region of low electron density (Figs. 4–7, 11). It cannot at present be decided whether these regions are really occupied by special material of low density or whether they represent an artificial space created by shrinkage of the KCE.

Concentrations of dense intranuclear particles opposite the KCE

The nucleus of *Polystictus* contains in all phases of its cycle of rest and division groups of small particles of higher density than those forming the rest of the nucleoplasm. Such intranuclear accumulations of dense particles are always found opposite the KCE during interphase when these granules are the only nuclear components of this degree of density (chr in Figs. 2, 3, 5 and 6).

Many of our electron micrographs suggest that the GEs are in direct contact with the local concentrations of dense particles on the inner face of the nuclear envelope (arrows, Figs. 4–6). No evidence has been obtained of similar connexions between the flat middle part of the KCE and the interior of the nucleus. This is in agreement with observations (Girbardt, in preparation) on the behaviour of the KCE during the phases of strong motility which preceded nuclear division.

Spatial relationships of the KCE to the nuclear envelope in *Polystictus*

The KCE lies in a shallow groove of the nuclear envelope which always runs parallel to the long axis of the KCE but may be inclined at any angle to the long axis of the nucleus. The infolding of the nuclear envelope is best seen in electron micrographs where the KCE has been sectioned at right angles to its long axis (Fig. 6). As might be expected, in series of sections in the plane of the long axis of the KCE it is only the most peripheral, glancing ones (Fig. 5, section 27) which convey the impression that the KCE lies in an invagination of the nuclear envelope. Reconstructions based on series of sections taken through KCEs at different angles to their long axis reveal that KCEs lie in a long fold of the nuclear envelope, which extends for a considerable distance beyond the limits of the GEs at either end of the KCE.

The KCE usually lies close to the forward pole of the nucleus (Fig. 2, KCE 1,
Only during special stages of the nuclear cycle are KCEs seen near the equator or the rear of the nucleus. A possible significance of the position of KCEs in relation to the direction of nuclear movements will be discussed elsewhere.

The KCE of other fungi

We have looked for a KCE in phycomycetes, ascomycetes and basidiomycetes. Table 1 lists the species that have been examined.

All fixatives were based on aldehydes. In several instances the fixative adopted for optimal preservation was arrived at only after a lengthy series of systematic experiments. In favourable cases KCEs were consistently obvious after the first few exploratory observations. However, different species differ greatly in the ease with which they permit the presence of KCEs to be detected. Small size and ill-defined shape account for this difficulty in most instances.

Table 1 shows that we have been unable to find a KCE in phycomycetes. This is surprising because a ‘band of electron dense material’ some 50 nm wide has been demonstrated in the close vicinity of the centrioles of water moulds (Blastocladiales, see Ichida & Fuller, 1968) and structures which might conceivably represent a KCE have been demonstrated by Renaud & Swift (1964, fig. 5). It remains to be seen whether in Mucorales in particular a true KCE is lacking. The number of species examined in our laboratory is not large enough to allow us to decide this question, though it might be mentioned that we have entirely failed to find KCEs in many series of sections of hyphae of specimens of Absidia glauca prepared in a variety of different ways. Naked globular structures with diameters of 0.6 μm have sometimes been seen in the vicinity of nuclei of Phycomyces and Mucor. It cannot be decided at present whether solitary electron-dense bodies of this kind represent KCEs, since none of the nuclei seen were in a state of division. The bodies in question are always single and are never connected to a second body by a flat middle portion.

The size of the globular elements (GEs) in typical KCEs is directly correlated with the size of the nuclei. Large nuclei have large GEs and vice versa, regardless of whether they are nuclei of ascomycetes or basidiomycetes. An exception is the KCE of Aspergillus niger with its disk-like substructure. Particularly large GEs accompany the large nuclei of basidiomycetes (Fomes, Polystictus, Schizophyllum).

Behaviour of the KCE during the cell cycle of Polystictus versicolor

The volume of the globular elements changes in a regular manner in the course of the cell cycle. All measurements of the largest diameter of GEs are based on the examination of serial sections. The ‘nuclear age’ (i.e. time elapsed since the last division) was determined by measuring the distance of the nucleus from the clamp connexion. Fig. 1 shows graphically how the size of the GE changes little for the first 125 min after nuclear division but is doubled precipitously at about 130 min (anaphase) after division (compare Figs. 5–7). Increase in size of the GE is not accompanied by any noticeable loosening of the close packing of its constituent electron-dense granules. The observed enlargement therefore probably represents true growth and neither a mere swelling nor dilution of pre-existing materials.
Table 1. List of investigated species

<table>
<thead>
<tr>
<th>Species</th>
<th>Origin</th>
<th>No. of investigated variants of fixation</th>
<th>Average size of nuclei, μm</th>
<th>Diameter of GE or DE, nm</th>
<th>Fixation (see Materials and Methods)</th>
<th>Osmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phycomycetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>Absidia glauca</em></td>
<td>B.I.</td>
<td>13</td>
<td>- (?), 2.5 x 1.0</td>
<td>- ( ? )</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Mucor racemosus</em></td>
<td>B.I.</td>
<td>1</td>
<td>2.5 x 1.5</td>
<td>- ( ? )</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Phycomyces blakesleeanus</em></td>
<td>B.H.</td>
<td>1</td>
<td>3.2 x 1.5, 400–600?</td>
<td>- ( ? )</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Rhizopus nigricans</em></td>
<td>B.I.</td>
<td>1</td>
<td>3.3 x 1.6</td>
<td>2</td>
<td>0.48</td>
<td>-</td>
</tr>
<tr>
<td>Ascomycetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>B.I.</td>
<td>2</td>
<td>2.3 x 1.3, 140</td>
<td>3</td>
<td>0.36</td>
<td>-</td>
</tr>
<tr>
<td><em>Chaeomium globosum</em></td>
<td>B.I.</td>
<td>14</td>
<td>4.2 x 1.0, 90</td>
<td>4</td>
<td>0.64</td>
<td>-</td>
</tr>
<tr>
<td><em>Neurospora sitophila</em></td>
<td>B.H.</td>
<td>11</td>
<td>2.6 x 1.6, 70</td>
<td>5</td>
<td>1.07</td>
<td>-</td>
</tr>
<tr>
<td>Basidiomycetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Coprinus micaceus</em></td>
<td>M.W.</td>
<td>1</td>
<td>3.0 x 1.5, 70</td>
<td>4</td>
<td>0.64</td>
<td>-</td>
</tr>
<tr>
<td><em>Fomes fomentarius</em></td>
<td>M.W.</td>
<td>1</td>
<td>1.3 x 1.6, 200</td>
<td>8</td>
<td>4.64</td>
<td>-</td>
</tr>
<tr>
<td><em>Polystictus versicolor</em></td>
<td>pers.</td>
<td>&gt; 100</td>
<td>12.0 x 2.2, 200–400</td>
<td>3</td>
<td>0.36</td>
<td>-</td>
</tr>
<tr>
<td><em>Schizophyllum commune</em></td>
<td>H.U.</td>
<td>1</td>
<td>1.0 x 2.4, 150</td>
<td>2</td>
<td>0.48</td>
<td>-</td>
</tr>
</tbody>
</table>

The negative results in showing a KCE in phycomycetes probably means for *Absidia* that none is present. Size of GE is dependent on size of nuclei. The relatively high value for *Aspergillus* represents the diameter of the discoidal entity (DE).

Enlargement of the GEs starts before condensation of the chromatin first becomes apparent and appears to be an early step on the road to division. This may be concluded from the fact that in one case the enlarged GEs belonged to the nuclei of a cell in an early stage of clamp formation. In other cases the distance of nuclei with enlarged GEs from the last-formed cross-wall indicated that the nuclei had been near the end of one growth cycle and were approaching division. The enlarged GEs undergo no further increase in size during the ensuing nuclear division and retain their size during the first 20 min in the life of young daughter nuclei. We are currently investigating how the diameter of the GE is later on reduced to about one half of its premitosis value. The evidence of our time-sequence places the moment of change from the mono- to the diglobular stage of the GE in the interval between the 15th and 35th minute after nuclear division.

![Graph showing diameters and distances of GEs during cell cycle of Polystictus versicolor.](image)

Fig. 1. Diameters and distances of GEs during cell cycle of Polystictus versicolor. Arithmetic mean of diameters of GEs from 20 to 125 min after division (n = 11) is 0.23 µm (0.21–0.24 at a 95% confidence interval). Arithmetic mean of diameters of GEs during nuclear division up to 20 min after division (n = 62) is 0.41 (0.39–0.42 at a 95% confidence interval). The difference of the two means is significant. (Level of significance α = 0.002.) The distance of both GEs from each other is measured from GE-centre to GE-centre in equatorial sections.

The separation between GE (distance of GEs), which is indicative of the length of the middle part, remains nearly constant at 0.3–0.4 µm during interphase. It does not noticeably increase either during the phase of enlargement of the GEs or prior to the start of nuclear division. The shape of the connecting middle part, however, does undergo a visible change during the enlargement of the GE. Ribbon-like at first, it is gradually narrowed down to a cord (Figs. 5, 7, 8) of especially high electron density.
Ultrastructure of fungal KCE

DISCUSSION

Why 'kinetochore equivalent'?

Our understanding of the peculiarities of nuclear division in fungi (Girbardt, 1962a; Robinow & Caten, 1969) would be considerably advanced if it were possible to assign a definite role and clear-cut morphological affinities to the organelle that has been variously called 'centriolar plaque' (Robinow & Marak, 1966), 'plaque atractophorienne' (Schrantz, 1969), KCE (Girbardt, 1968), or MTOC ('Microtubule Organizing Centre') (Pickett-Heaps, 1969). Do these designations refer to homologous variants of essentially the same kind of structure or to organelles that cannot be compared with each other? This is a matter that cannot yet be decided. More fine-structure studies of the nuclei of protists other than fungi than have, so far, been carried out are required before the dictum can be either proved or refuted that 'the intranuclear position of the centrosome is the only truly primitive feature discovered in a wide survey of protozoal nuclei' (Belar, 1926). An example of the oddly convergent observations that will eventually, one hopes, become amenable to description within the framework of a general scheme is provided by the strange similarity between nuclear division in the flagellate protozoon*Joenia* (Cleveland, 1963) and in *Saccharomyces.*

It may be profitably discussed however, even now, whether the dumbbell-shaped body at the periphery of the nuclei of basidiomycetes is the equivalent of a centrosome, a centriole, or perhaps a kinetochore (Girbardt, 1968) or whether it represents an altogether novel organelle peculiar to the fungal nucleus. Such a discussion might consider the following points.

(a) The KCE as described and illustrated in the present paper is obviously identical with the 'centriole' (Moreau, 1958) and the 'lateral granule' of light microscopists (Matile, Moor & Robinow, 1969, and earlier authors). It is also identical with the 'centrosomelike polar vesicle' demonstrable in the living fungal cell by phase-contrast microscopy (Girbardt, 1960). Doubts about the reality of this structure (Heim, 1952, 1954) can no longer be reasonably held. The light microscope can however do no more than establish the existence of the organelle in question. The decision whether the KCE is a true centriole or something else (Lepper, 1956) cannot be made on the basis of light-microscopical evidence.

(b) Kinetochores and centrioles have in common an involvement with motion, are of similar dimensions and share the ability to organize microtubular proteins. One might be tempted therefore to assume that we are dealing here with 2 morphologically dissimilar structures that perform identical or similar functions. A comparison of animal and plant mitoses and centrosome-free spindle-poles in animal spermatoocytes (Dietz, 1959) suggests, however, that the orderly and equitable separation of chromatids depends only on the presence of kinetochores and not on centrioles. The latter may act as additional organizing centres but may also be absent. (Even the mode of division of bacterial nuclei can be accommodated in this scheme if one is prepared to regard the putative 'attachment point' of the chromosome as the equivalent of a kinetochore.) Similar considerations apply also to the 'dinomitosis' as described
by Kubai & Ris (1969) and the division of the macronucleus in ciliates (Tamura, Tsuruhara & Watanabe, 1969). Kinetochores and centrioles have certain capabilities in common but differ from each other in that the former are chromosomal, i.e. nuclear organelles, whereas the latter belong to the cytoplasm even though they are frequently closely associated with the nucleus. Centrioles are, in fact, capable of considerable activity in enucleated cells where they not only form asters but also act as kinetosomes (Cleveland, 1956).

(c) The identity of a centriole is established by its characteristic fine structure (de Harven, 1968). Nothing resembling this unique organization has been detected in the multilayered disks or KCE at the periphery of fungal nuclei. The term 'centrosome' has been proposed for these structures by Westergaard & von Wettstein (1970). It would seem, however, that in proper usage that term refers to a region of the cytoplasm clearly marked off from the rest of the cell and enclosing a specific central structure, usually a typical centriole. There is no evidence that the KCEs or 'lateral granules' of fungi are organized in this way.

Perhaps it is unwise to look for parallels between KCEs and conventional mature centrioles. It has several times been observed that new centrioles arise from vaguely delimited accumulations of granular, electron-dense material, so called 'procentrioles' (Stockinger & Cireli, 1965; Dirksen & Crocker, 1966; Kalnins & Porter, 1969). Ought one then to regard KCEs and similar bodies as organelles that have remained at the evolutionary level of procentrioles? This would seem unsound for two reasons: first, aquatic fungi and terrestrial ones with motile zoospores possess perfectly normal centrioles; secondly, as Outka & Kluss (1967) and Sorokin (1968) have shown, procentriolar aggregations of granular matter arise only in the course of development of kinetosomes, whereas true centrioles arise in most instances by orthogonal budding (Robbins, Jentzsch & Micali, 1968).

(d) At first sight the extranuclear position of the 'kinetochores equivalent' (KCE) would seem to argue against the functional interpretation implied in its name. Serial sections do, however, show that at least the globular elements of the KCEs are in intimate contact with the outer component of the nuclear envelope during interphase and up to the early stages (anaphase) of mitosis (see Fig. 4). GEs which appear to be lying free in the cytoplasm are invariably seen to be in contact with the nucleus, provided their position is traced in a sufficiently large number of serial sections.

(e) Changes in shape of the nucleolus are directly correlated with movements of the KCE (the 'activity centre' or 'nucleolus attachment point' (NA) of Girbardt, 1960). The behaviour of the nucleolus could be accounted for by assuming the existence of a chromosomal nucleolar organizer somehow attached at the same time to the nucleolus and to the nuclear envelope in the vicinity of the GE. That is, in fact, the very site where, following mitosis, the development of a new nucleolus can be seen to be taking place in the living cell. The known behaviour of nucleolar organizers in the nuclei of cells of higher organisms suggests that the connexion, be it a direct or indirect one, between the nucleolus and the KCE, may be quite stable over the greater part of the cell cycle (see, for example, Hsu, Brinkley & Arrighi, 1967).
(f) The to-and-fro movements of the KCE during some phases of nuclear division (Girbardt, 1968, and in preparation) recall similar movements of true kinetochores during prometaphase and metaphase (Bajer & Molè-Bajer, 1956). Slight movements of the kinetochores accompanying changes of shape of nuclei entering prophase and their greatly increased motility after the breakdown of the nuclear envelope, observed in higher organisms (Bajer & Molè-Bajer, 1969) and protozoa (Cleveland, 1954; Hollande & Valentin, 1968), suggest that in these forms, too, the kinetochores may be attached to the nuclear envelope during interphase. Rotation of nuclei, observable in many tissues (Girbardt, 1962b; Puza & Gayer, 1967) could similarly be viewed as an expression of the motility of envelope-attached kinetochores. Arguments unfavourable to the assumption discussed in this section are provided by the reported disappearance of kinetochores of animal chromosomes at the end of telophase (Brinkley, Stubblefield & Hsu, 1967) and their reappearance during breakdown of the nuclear envelope (Brinkley & Stubblefield, 1966).

(g) During nuclear division the globular elements of the KCE are in direct contact with microtubules (Girbardt, in preparation). This is also true of kinetochores and centriolar satellites (de Haven, 1968). By contrast, there is apparently no continuity between true centrioles and microtubules (Stey, 1969).

(h) Light-microscopical studies (Lima-de-Faria, 1958) have revealed differences of shape and size between kinetochores of different organisms. Surprisingly, the KCEs of basidiomycetes and ascomycetes seem less far removed from the types of kinetochore commonly encountered in higher organisms than might be expected. Those of basidiomycetes and ascomycetes resemble, in fact, the kinetochores of higher plants (Wilson, 1968), whereas the diskoidal forms seen in some ascomycetes recall the kinetochores of animal chromosomes (Brinkley & Stubblefield, 1966; Jokelainen, 1967).

(i) Structures which resemble the KCE of fungal nuclei have been described attached to the nuclei of diatoms (Drum & Pankratz, 1963) and archimycetes (Temmink & Campbell, 1968, fig. 7). Like the KCEs these structures, too, are in direct contact with microtubules during nuclear division (see, however, Manton, Kowallik & von Stosch, 1970). Thus it seems unlikely that the organelle described in the present paper is present only in the cells of higher fungi. From the point of view of phylogeny, too, it seems unlikely that in one particular group of protists (namely the fungi) there should have arisen an organelle, involved in the equitable distribution of genetic material, which could not be homologized with corresponding organelles in eucaryotes.

Function of the KCE

The principal function of the KCE is to act as an organ of nuclear motility, not only during division but also during interphase (Girbardt, 1968). Furthermore, from the onset of nuclear division, the GEs serve as organizing centres for microtubules and thus play a decisive role in the segregation of the genetic material. It seems conceivable that the changes in morphology of the GEs during interphase are correlated with the phases of the nuclear growth cycle and that the KCE in addition
to its involvement in the segregation of the genetic material may have a role to play in the initiation of DNA synthesis. It is with this thought in mind that the designation ‘kinetochore equivalent’ has been tentatively employed in the present paper.

The monoglobular stage, lasting about 20 min, might correspond to the $G_1$ phase (Guttes & Guttes, 1969). The diglobular configuration might signalize the beginning (or a time-point shortly after starting), and the onset of the enlargement of the GEs, 100 min later, the end of the $S$ phase. The ‘ante-phase’ of 15 min duration, during which enlargement of the GEs continues, might in this scheme be compared with the $G_2$ phase.

Attempts to obtain evidence supporting this hypothesis have, so far, failed. The chromatin of the Polystictus nucleus responds positively to the Feulgen test but the colour produced is too faint to permit cytophotometry (Girbardt, 1963). Attempts to label the DNA with tritiated thymidine have likewise been unsuccessful, as radioactivity was confined to components of the cytoplasm and was not diminished after treatment with protease-free DNase.

The demonstration that the bacterial chromosome is attached to a particular point on the cytoplasmic membrane (Lark, 1966) has been followed in recent years by a number of observations on the attachment of the chromosomes to the envelope of the nuclei of higher organisms (Comings, 1968; Comings & Okada, 1970; Moens, 1969; Beams & Mueller, 1969). The autoradiographic studies of Comings & Kakefuda (1968) strongly suggest that initiation of the synthesis as well as the replication of DNA take place at the nuclear envelope. Both these activities are, perhaps, as in bacteria (Bleecken, 1969) carried on at the same site. Regarding the organization of the fungal nucleus in the light of the hypothesis here proposed, one is led to postulate that the KCE with its globular elements occupies a special site on the nuclear envelope which is also the point of attachment during interphase of the genome, and that the latter has, perhaps, the form of a single collective chromosome or chain of chromosomes.

It may further be supposed that the rapid enlargement of the GEs at the start of nuclear division coincides with putative ‘trigger’ events that initiate the sequence of mitosis. To judge from its staining properties (Girbardt, 1960, 1962a; Matile et al. 1969) the KCE probably consists of or contains protein. Precise cytochemical evidence bearing on this point is not yet available. If there is indeed protein in the KCE then it might correspond to the ‘division protein’ whose existence has been proposed by Mazia (1961, 1966). The results of numerous experiments performed on a variety of organisms support the assumption that nuclear division starts as soon as the synthesis of essential protein reaches a certain level (Fautrez-Firlefyn & Fautrez, 1967). It is not difficult to think of the KCE as performing such a function since its GEs become organizing centres for the formation of microtubules before and during nuclear division (Girbardt, in preparation).

If there is only one kinetochoore as suggested in these pages (at least for a haploid nucleus) then it follows that during division the whole of the chromosomal material must move as a unit. The chromatin granules generally interpreted as chromosomes would in reality be parts of a compound chain-like chromosome (Dowding & Weijer,
Ultrastructure of fungal KCE

1962; Dowding, 1966; Namboodiri & Lowry, 1967; Rosenberger & Kessel, 1968). Chromosome associations of this kind have, in fact, been described in higher plants (Wagenaar, 1969), where they have been called 'interphase associations'. The lack of a metaphase plate characteristic of nuclear division in many species of fungi (Robinow & Caten, 1969) can be explained by invoking mechanisms involving compound chromosomes. In the interphase nucleus one GE presumably belongs to one chromatid. This suggestion recalls Harper's insistence (1905) that the chromosomes (of ascomycetes) are in continuous contact with the 'central body' (used in the sense of 'centriolar body'). One solitary compound chromatid derived from the previous division would be present in the nucleus during the monoglobular stage of the KCE. The change to the diglobular condition of the KCE would be an indication that synthesis of DNA has started. At this stage the flat middle part of the KCE still prevents the separation of the 2 GEs from each other and therefore probably also the separation of the newly formed sister chromatids, at least until sufficient 'division protein' has been synthesized and formation of microtubules has begun. More work is required to enable us to see whether the hypothesis that has been put forward (in its present or later, suitably modified versions) will improve our understanding of the peculiarities of the mode of division of fungal nuclei and help us to define the place of fungal nuclear behaviour in a tentative evolutionary scheme of mitotic processes.

The author gratefully acknowledges the help of Dr C. Robinow, London (Canada) in correcting the English text and the skilful technical assistance of Mrs Bähring, Miss Fritsche and Mr Wachsmuth.

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Ultrastructure of fungal KCE


M. Girbardt


(Received 23 December 1970)

Fig. 2. *Polystictus versicolor*. Interphase nucleus about 120 min after nuclear division. To show the KCEs of both nuclei parts of different sections are used (sections 32 and 38 of the series). These parts are separated by a black line. Numbers in triangles refer to section numbers, the black angles of the triangles indicate direction of sectioning. The longitudinal axis of KCE 1 lies parallel to longitudinal axis of the nucleus (n1); because of this, both globular elements (ge), the middle part (mp) and KCE-associated chromatin (chr) become visible simultaneously. Nucleoli (nu) are very large at this stage of nuclear cycle. gd, growing direction of the hypha; mi, mitochondria; mt, microtubules; ne, nuclear envelope. Fixation 2; × 15 500.

Fig. 3. *Polystictus versicolor*. The KCE of a young nucleus (n) about 25 min old is composed of only one globular element (ge). The small nucleolus (nu) and KCE-associated chromatin (chr) are visible. The GE is surrounded by a cap of ER (er) and lies at the forward pole of the nucleus. mi, mitochondria. Fixation 2; × 15 500.
Ultrastructure of fungal KCE
Fig. 4. *Polystictus versicolor*. Serial sections through a young nucleus (n) with already well developed nucleolus (nu) in the subterminal cell. Section number and direction of sectioning in marked triangles. The nuclear envelope (ne) reveals direct contact with globular elements (ge) at a small area (section 34). Microtubules (mt) are during this stage still radiating from the ge. Arrows point to possible connexions between ge and karyoplasm. cy, cytosome; gd, growing direction of the hypha; h, uncontrasted area around ge; mvtb, multivesicular body; np, pores of nuclear envelope. Fixation 3; x 40000.
Ultrastructure of fungal KCE
Fig. 5. *Polystictus versicolor*. Serial sections through a KCE parallel to its long axis. Nucleus (n) about 130 min after division. The KCE is located in an infolding of the nuclear envelope (ne) which is cut peripherally in section 27. Around the globular elements (ge) sometimes the hardly contrasted area (h) may be seen. Possible connexions between chromatin (chr) and ge are designated by arrows. Cytoplasmic microtubules (mt) are not connected with the KCE. Fixation 2; × 50000.

Fig. 6. *Polystictus versicolor*. Serial sections through a KCE transverse to its longitudinal axis. Nucleus (n) about 120 min after division. The infolding of the nuclear envelope (ne) is well shown in all sections. The middle part (mp) is still plate-like. Abbreviations as in Fig. 5. Fixation 2; × 50000.
Fig. 7. *Polystictus versicolor*. KCE just before nuclear division begins. GEs are enlarged and the cord-like middle part (mp) shows enhanced contrast. After rotation of the nucleus (n) the KCE is located at the pole of the nucleus which is turned away from the tip. gd, growing direction of the hypha. Possible connections between chromatin (chr) and GE (ge) through pores (np) of the nuclear envelope (ne) are marked by arrows. h, poorly contrasted area. Fixation 1; ×100 000.

Fig. 8. *Fomes fomentarius*. Plate-like middle part (mp) of the KCE sectioned obliquely and lying in an infolding of the nuclear envelope (ne). chr, KCE-associated chromatin. Fixation 4; ×100 000.

Fig. 9. *Neurospora sitophila*. Both GEs (ge) are lying in grooves of the nuclear envelope (ne). This fungus also shows association between chromatin (chr) and KCE. Fixation 5; ×100 000.

Fig. 10. *Chaetomium globosum*. Similar to Fig. 9. Fixation 3; ×100 000.

Fig. 11. *Aspergillus niger*. KCE with diskoidal entities (de). A poorly contrasted area (h) is present. Fixation 3; ×100 000.