MITOSIS IN ASPERGILLUS NIDULANS

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SUMMARY

Mitosis in growing hyphae of haploid and diploid Aspergillus nidulans has been studied with the light microscope in Helly-fixed preparations. At the periphery of the nucleus a sharply defined granule is invariably found which has strong affinity for acid fuchsin. Mitosis begins with the duplication of this granule and the development inside the nucleus of a gradually lengthening fibre between the daughter granules. This fibre is also visible in dividing nuclei of living hyphae examined by phase-contrast microscopy, when it appears as a grey bar traversing the non-nucleolar region. Division of the chromatin has been studied in preparations stained with aceto orcein, directly or after hydrolysis. It proceeds through four phases, beginning with the condensation of distinct chromosomes from the chromatin network of the resting nuclei. Later the chromosomes become arranged in two parallel rows of chromatinic masses in which individual chromosomes can no longer be distinguished. Division is completed by migration of the chromatin to the ends of each row, giving the appearance of a transverse break in the double bar. The chromatin accumulating at the ends of the double bar condenses to form the two daughter nuclei. The number of chromatinic elements appears to be the same in both haploid and diploid nuclei, although the individual elements are larger in the latter.

Successive staining of the same dividing nucleus, first for the fibre (acid fuchsin) and then for the chromosomes (HCl-aceto orcein or HCl-Giemsa) has established that the fibre lies among the chromosomes, and that its elongation is closely related to the anaphase-telophase movements of the chromosomes. This suggests that the fibre is the equivalent of a mitotic spindle. That the fibre is connected with the chromosomes is further suggested by the observation that, in stained preparations, the fibre is considerably thicker in dividing diploid than in haploid nuclei.

Electron microscopy of dividing nuclei has revealed that mitosis is carried out within the intact nuclear envelope. The mitotic spindle is composed of a bundle of fibrils which traverses the nucleus between two plaques of dense material associated with the envelope.

In order to explain the configurations seen during the division of the chromatin it is proposed that the chromosomes become attached to the central spindle at various points along its length. With the elongation of the spindle the sister chromatids separate and pass, asynchronously, to opposite ends of the spindle along two preferred lines, thereby producing the characteristic double bar figure. The absence of a metaphase plate is thus accounted for by the scattered points of attachment of the chromosomes to the spindle and by their asynchronous division.

INTRODUCTION

Despite the now considerable number of accounts of mitosis in hyphae of fungi, no generally accepted picture has emerged. Some accounts reveal nothing out of the ordinary (Somers, Wagner & Hsu, 1960; Robinow, 1963; Knox-Davies, 1966; Ichida

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but many others give the impression that division occurs by a process which differs in several respects from the mode of division of the nuclei of higher organisms (Robinow, 1957a, b, 1961; Bakerspigel, 1959; Duncan & Macdonald, 1965; Weijer & Weisberg, 1966; Brushaber, Wilson & Aist, 1967; Namboodiri & Lowry, 1967; Laane, 1967). The literature leaves the reader in doubt whether this impression is due to the small size of fungal nuclei, the techniques employed in studying them or to some genuine peculiarity of behaviour.

We have tried to reduce the area of uncertainty by examining *Aspergillus nidulans*. This mould seemed a suitable organism in which to study mitosis of hyphal nuclei, for a number of reasons. (i) It is a well-known member of a widely distributed genus which has but recently been the subject of a monograph (Raper & Fennell, 1965). (ii) The haploid chromosome number has been established as 8 by cytological examination of meiosis (Elliott, 1960). This figure is in complete agreement with the number of linkage groups (Käfer, 1958; Dorn, 1967). (iii) A single hypha contains a limited number of well-separated nuclei, thus allowing the development of individual nuclei to be followed by phase-contrast microscopy and minimizing the possibility of confusion between adjacent nuclei in stained preparations. (iv) Nuclei of relatively large size are available in diploid strains. (v) Under our conditions of cultivation the division of the nuclei within a single cell is not perfectly synchronized, and the complete sequence can occasionally be followed within a single hypha (Fig. 19). (vi) Studies with heterozygous diploid strains of *A. nidulans* have demonstrated that somatic recombinants may arise by both crossing over and non-disjunction during mitosis (Pontecorvo et al. 1953; Käfer, 1961). The occurrence of these processes is inferred purely from genetic analysis and their cytological basis has remained obscure (Pontecorvo, 1958). It was hoped that comparison of mitosis in haploid and diploid strains might help fill this gap in our knowledge.

**MATERIALS AND METHODS**

**Origin and maintenance of strains used**

The haploid strain was a Glasgow wild type obtained from the Fungal Genetics Stock Centre (FGSC no. 4). The diploid was synthesized from two haploids using Roper's method (1952). The haploid parents of the diploid were also obtained from the Stock Centre and were of genotypes *Acri w3; mthi and proi pan y bi; pyro4*, respectively (FGSC nos. 100 and 101 respectively). The former of these two haploid strains carries a translocation involving chromosomes VI and VII, while the latter has the normal chromosome complement (Käfer, 1965; Barratt, Johnson & Ogata, 1965). The diploid would therefore be heterozygous for this translocation. Cultures of both the haploid and the diploid were maintained on *Aspergillus minimal medium* (Pontecorvo et al. 1953).

**Phase-contrast microscopy of resting and dividing nuclei in living, growing germ tubes and young branching hyphae**

For phase-contrast microscopy, germinating conidia were examined in a medium consisting of 2 % glucose, 0.5 % Difco yeast extract and 48 % gelatine. Thin films of this medium on 0.8-mm thick slides were inoculated with a fine flexible glass fibre. The inoculum was prepared by dispersing conidia in a sterile 'Tween' solution (one drop 'Tween 80' in 10 ml distilled water). The suspension was centrifuged, resuspended in plain water and used after
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a further centrifugation and resuspension in water. Inoculated slides were left overnight at room temperature resting on triangles of glass rod or a layer of glass beads, over a film of water in a Petri dish. The next morning all but a narrow rectangle of gelatine measuring about 3 x 5 mm was scraped off the slide. A no. 0 coverslip was placed over the growing hyphae and the assembly sealed with wax from a small candle. Zeiss phase-contrast optics were used, condenser VZ of n.a. 1.4, a fluorite immersion objective x 100, n.a. 1.3 in conjunction with a x 10 or x 16 compensating eyepiece. The microscope was illuminated with light from a Bausch and Lomb tungsten ribbon lamp passed through a green no. 11 Wratten filter. Photographs were taken on Kodak Royal Pan or Super Panchrom Press cut film which under these conditions required an exposure of 4 s. Prints were made at twice the original enlargements of x 1100 or x 1800.

Preparation of hyphae with dividing nuclei for electron microscopy

The method we have used is based on the work of Girbardt (1956, 1965). Conidia were allowed to germinate on the surface of thin films of 1-5 % agar containing 2 % glucose and 0.5 % yeast extract (yeast extract glucose agar). The position on the slide of living hyphae with nuclei in the earliest stages of division detectable by phase-contrast microscopy was recorded and the culture immediately fixed by the procedure of Ryter & Kellenberger (1957). The dehydrated specimen was embedded in Vestopal. Sections were cut on a Porter-Blum microtome, stained with uranyl acetate and lead citrate according to Reynolds (1963) and examined in a Philips 200 electron microscope. So far we have only examined diploid nuclei. The electron-microscopical work continues and we hope to report its results at a later date.

Fixation and staining for light microscopy

Fixation. Germ tubes and young mycelia were obtained from conidia that had been spread on sheets of opened-out dialysis tubing, sterilized by boiling in water, lying on the surface of yeast extract glucose agar. This is essentially the method of Fleming & Smith (1944). Fixation throughout was in modified Helly solution: 5 % mercuric chloride, 3 % potassium dichromate (w/v) in water with 6 % (v/v) of a 3 % solution of formaldehyde added immediately before use (Robinow, 1961). Fixation was carried out by transferring mycelia-bearing sheets face down, to a dish with freshly prepared fixative. After 10—15 min the sheets were rinsed several times with 70 % alcohol and either stained immediately or stored in the cold in Newcomer's (1953) preservative.

Acid fuchsin. Fixed specimens in alcohol were rinsed with 1 % acetic acid, immersed for 1-5—2 min in 1:60 000 acid fuchsin in 1 % acetic acid and examined in 1 % acetic acid under a no. 0 coverslip (Robinow & Marak, 1966). Preparations were sealed with wax from a small candle or with ' Glyceel' (G. T. Gurr, London, England.).

Direct aceto orcein. Fixed material was transferred from alcohol to 60 % acetic acid and after 5 min to 1 % synthetic orcein in 60 % acetic acid ('aceto orcein': Elliott, 1960), which was allowed to act for 30 min. Stained specimens were mounted in the stain under a no. 0 coverslip, flattened by pressure through several layers of absorbent paper and sealed with wax ('Glyceel' causes a precipitate along the edges of acetic-acid mounts).

Aceto orcein after hydrolysis. Fixed specimens were hydrolysed for 9 min in NHCl at 60 °C, rinsed with water, left in 60 % acetic acid for 5 min and stained for 30 min with aceto orcein. Stained specimens were examined in the stain under a coverslip.

Giemsa after hydrolysis. This widely used procedure has the disadvantage that in many fungi it colours mitotic constellations so deeply that they are hard to resolve into their constituent chromosomes, a defect which is enhanced by photomicrography. This is in keeping with the independent experience of Knox-Davies (1967), who noted that more structural detail was apparent in orcein-stained nuclei of Macrophomina than in Giemsa-stained ones. We have used HCl-Giemsa in some instances as a second procedure after primary staining with acid fuchsin.

Fixed specimens were carried directly from 70 % alcohol into NHCl at 60 °C and hydrolysed for 12 min. They were rinsed with tap water and distilled water and stained for 1 h or more with Gurr's 'Improved Giemsa stain R 66' diluted 1 in 10 with Gurr's Giemsa (phosphate) buffer at pH 6.8. The quality of the staining was checked with a x 50 Zeiss Jena water-immersion
lens, and overstained specimens were differentiated in a Petri dish with 40 ml of distilled water
to which a loopful of acetic acid had been added, giving it a pH of about 4.5.

Feulgen procedure. Specimens fixed in Helly were hydrolysed in N HCl at 60 °C for about
10 min, rinsed with tap water and distilled water and left for 3.5 h in the Feulgen reagent.
After 7 or more quick rinses with standard SO4 water they were washed for 20 min in running
tap water and mounted over a drop of aceto carmine (McIntosh, 1954). Some preparations
were examined without having been counterstained.

Preparation of specimens for the successive staining of spindles and chromosomes in the
same nucleus

It proved difficult, using the procedure described above, to make preparations in which the
same hypha could first be stained with acid fuchsin and then be hydrolysed and stained with
Giemsa solution or aceto orcein, without causing it to detach itself from the dialysis tubing.
Alternative methods, e.g. one using hyphae growing on a coverslip at the edge of a hanging drop
of nutrient medium, proved equally unreliable. Adaptation to our purposes of a method
published by Clutterbuck & Roper (1966) provided a way out of this difficulty. We proceed as
follows: The centrifuged deposit of a suspension of conidia is dispersed in about 1 ml of
filtered fresh 5 % egg white (v/v) in distilled water which also contains 1 % glucose (w/v).
A drop provided by a wire loop 2 mm in diameter is spread over a 22 x 22 mm coverslip and
allowed to dry on the bench or in a 35 °C incubator for 10—15 min. Dialysis tubing (cellulose)
is boiled for 10 min in distilled water and cut into strips wide enough to cover the centre third
of the coverslip and long enough to protrude a bit beyond the sides. The wet strip is lightly
blotted between sheets of absorbent paper and placed over the dried film of conidia. A narrow
slab of yeast extract glucose agar is placed over the cellulose strip and the assembled culture
resting, agar up, on an object slide, to which it had been attached with a few dabs of wax for
ease of handling, is incubated at 35 °C in a Petri dish in which it rests on a layer of glass beads
half covered by water. At the time of fixation the coverslip is detached from the carrier slide,
and the protruding bit of cellulose is seized with forceps and peeled off. The coverslip is
instantly plunged into a Columbia staining dish with Helly's fixative. The preparation is first
stained with acid fuchsin, stainable hyphae photographed, and then hydrolysed and re-stained
with aceto orcein.

RESULTS

Phase-contrast microscopy (Figs. 1, 2)

The nuclei in living hyphae of A. nidulans look much like other fungal nuclei
that have been described in the living state. They have oval or pear-shaped, smooth,
continuously changing outlines, are not bounded by a visible membrane, are of evenly
low density and contain a relatively large, dense nucleolus that lies usually closer to
one end of the nucleus than to the other (Fig. 1: 0). As they approach division the
nuclei get larger and brighter. A short cigar-shaped grey bar may now be seen travers-
ing the clear portion of the nucleus on one side of the nucleolus (Fig. 1: 8, 9; Fig. 2: 3).
The bar becomes longer and thinner, the nucleolus becomes increasingly transparent
and assumes irregular, 'moth-eaten' outlines, finally it fades from sight (Fig. 2: 7).
The clear portion of the nucleus can at this stage of rapid changes also no longer be
readily distinguished from the cytoplasm (Fig. 1: 12). Minutes later a pair of minute
daughter nuclei can be distinguished (Fig. 1: 14; Fig. 2: 18). These increase rapidly
in size and visibility, each containing a new nucleolus (Fig. 1: 18). One of the daughter
nuclei stays close to the site of mitosis, the other one moves rapidly away, proximally
or distally, over distances in the order of 10-15 μm. The time occupied by the visible
changes accompanying the division process is about 6–8 min from the first fading of
the nucleolus to the reappearance of daughter nuclei. All the nuclei in a germ tube or
hyphal branch divide within a few minutes of each other, indicating a high degree of
synchrony within single cells. It is obvious during life, however, that synchrony is not
perfect and some nuclei start to divide earlier than others.

Observations on preparations stained with acid fuchsin (Figs. 3–14)

Used as described above, the stain is taken up mainly by the nucleolus and, in the
nuclei of one in every 20 or 30 germ tubes or cells of young mycelia, by a structure
resembling in size, shape and orientation in the nucleus the grey bar seen in living
nuclei early in division. In diploid nuclei this element is distinctly spindle shaped and
it will henceforth be referred to as the spindle. If one nucleus in a germ tube or cell
contains a spindle then all the others in the same hypha contain spindles. This
synchrony of spindle development has been noted in cells containing up to six nuclei.
Under our conditions a gradient of increasing spindle length along the hypha could
frequently be detected, indicating that synchrony was not perfect. In a sample of
58 germ tubes with spindles, 35 had this gradient running from the conidium to the
tip, 12 had a similar gradient running in the opposite direction, while no gradient
could be detected in the remaining 11. The existence of this gradient has allowed us
to trace the development of spindles in acid-fuchsin preparations from a small com-
pact, deeply stained body, the ‘lateral granule’, regularly to be found at the periphery
of resting nuclei (Fig. 7). Formation of the spindle begins with the appearance of a new
granule alongside the existing one (Figs. 3, 8). Subsequently the two granules elongate
into short rodlets which are initially inclined towards each other, forming a charac-
teristic V shape (Figs. 9, 35A), before fusing and straightening into a single cigar-
shaped unit (Figs. 3, 4, 10). A plausible, if unproved, interpretation of this mode of
spindle formation in terms of fine structure will be advanced in the discussion.

There are striking differences between the spindles in dividing haploid and diploid
nuclei. The former (Figs. 3–6) are thin and occasionally bent, the latter (Figs. 7–14)
are thicker, often spindle-shaped and usually straight. At telophase the spindles
expand into a thin straight fibre which is similar in both kinds of nuclei but longer in
diploids. In diploids the fully extended spindle is 6–8 μm long, representing a 15–
to 20-fold increase over the diameter of the ‘lateral granule’ in resting nuclei.

During the early stages of its development from the lateral granule, the spindle may
be inclined at various angles to the long axis of the hypha and frequently lies across
the hypha (Figs. 3, 4, 9–11). With increasing length, however, they become aligned
parallel to that axis (Figs. 5, 6, 13, 14).

In a series of experiments to be reported in detail elsewhere we have found that the
spindle of Aspergillus is very sensitive to a sudden lowering of the temperature of its
environment. To judge from fixed and stained preparations spindles become dis-
organized within a few minutes at 4 °C. Only spindles of normal appearance are found
in acid-fuchsin stained preparations fixed soon after the return of chilled cultures to a
favourable temperature; for example, 35 °C.
Distribution of chromatin (Figs. 15-33)

Direct staining of fixed hyphae with aceto orcein revealed only the most contracted stages of the chromosomes of dividing nuclei (Figs. 15-18). Rees & Jinks (1952), using an iron-acetocarmine stain, have had the same experience in work with *Penicillium*. As will be shown below, better contrast between chromosomes and cytoplasm at all stages of the nuclear cycle is obtained by hydrolysis before staining. Directly stained aceto orcein preparations have, however, one advantage over hydrolysed ones in that they show not only mitotic chromosomes but also the nucleolus, which tends to be invisible in hydrolysed aceto orcein (though not in HCl-Giemsa) preparations. It can be seen in Figs. 15 and 16 that well-defined nucleoli may still be present at a time when the chromosomes are already in advanced stages of mitosis. This finding indicates that the breakdown of the nucleolus, which is the first detectable sign of mitosis in living nuclei, does not really mark the start of nuclear division.

The complete division sequence was revealed by both HCl-Giemsa (Fig. 19) and HCl-aceto orcein (Figs. 20-33). We have relied mainly on the latter method. The distribution of Feulgen-positive material corresponded closely with that of materials having marked affinity for aceto orcein both before and after hydrolysis. However, the Feulgen reaction, though unambiguous, was faint and unsuitable for regular use.

In interphase nuclei (Figs. 20, 32) the chromatin appears finely granular. The bulk of it lies to one side of the nucleolus. Where it overlies this organelle the chromatin is reduced to a thin shell. The peripheral granule does not appear to be chromatinic and cannot be recognized in our HCl-Giemsa and HCl-aceto orcein preparations. The spindle is also not revealed by these procedures.

Four phases of mitosis can readily be distinguished. In stage I (prophase-metaphase) chromatinic filaments and granules having the appearance of chromosomes are clustered together to one side of the nucleolus (Figs. 20, 21, 26, 27). The number of chromosomes in the dividing haploid nuclei of Fig. 20 is close to the expected number of 8. Successive staining reveals that a short spindle is inside stage I clusters (Figs. 34, 35). It is often seen that a thin chromatinic thread extends from the cluster into the nucleolus (Fig. 26). In stage II, separation of the chromosomes has begun and the tight cluster of the previous stage has been transformed into 2 or more chains of chromatinic bodies parallel to each other and to the long axis of the hypha (Figs. 23, 24, 28–30). A change of focus frequently reveals the looping over of chromatinic elements from one chain to another (Fig. 29). The gap between the parallel chains is occupied by a long spindle (Fig. 36). This 'track' stage, which has no equivalent in the usual forms of mitosis, is seen with great regularity and has also been described in other fungi, e.g. by Duncan & Macdonald (1965). Limitations of optical resolution do not allow us to decide whether the chromatinic units aligned lengthwise in the track pattern are connected end to end. Configurations intermediate between stages I and II are common but the details of the process by which the track stage is arrived at have remained obscure. In stage III there is a movement of chromatin towards both ends in each chain, giving the appearance of a transverse break in the paired array (Figs. 25, 31, 32). At this stage the spindle is long and straight (Fig. 37). Shortly afterwards it
can no longer be seen in stained preparations under the light microscope. Stage IV sees the reconstruction of a pair of relatively small daughter nuclei (Figs. 33, 38) from densely contracted, often paired (‘bilobed’) chromatinic masses which gradually become more granular and less stainable with aceto orcein. Stages III and IV appear to be the equivalent of anaphase and telophase respectively of standard forms of mitosis.

The aceto orcein and Giemsa preparations reveal that all the nuclei in a single germ tube are either in the resting stage or in division; that is, division is synchronous within a single cell (Figs. 20, 23). As with the development of the spindle, however, synchrony is not perfect and a sequence of division stages is frequently found within a single hypha (Fig. 19). The direction of this sequence has not been examined, as was done for the gradient of spindle development. However, because of the association between the development of the spindle and the division of the chromatin, the observations on the former should apply equally to the latter.

The nucleolus is not divided. Early in karyokinesis its shape becomes irregular, its density, both in living and in fixed and stained preparations, is seen to wane, and by the time the daughter nuclei have been formed, the old nucleolus, if it has not already dissolved, is small. However, the timing of these events is variable and a sizeable nucleolus may still be encountered at telophase (Fig. 38). It usually lies closer to one daughter nucleus than to the other. Dr A. Bakerspigel informs us that he has made the same observation in a study of dividing nuclei of *Trichophyton mentagrophytes* and of several other species.

The morphology of mitosis as described in the preceding paragraphs is the same in haploid and diploid nuclei. Reliable counts of chromatinic elements are difficult to make. Comparisons of the two strains at comparable stages of division, however, give no indication that diploid nuclei contain twice the number of chromosomes of haploid (compare Figs. 20, 21 with 26, 27). Such comparisons, in fact, give no reason to believe that the diploid contains any more chromatinic elements than the haploid, the difference appearing to be one of size and stainability of individual elements and not of number. This failure to discern the expected increase in numbers is particularly striking at the track stage, where both haploid and diploid appear to have similar numbers of concentrations of chromatin in each chain (compare Figs. 23, 24 with 28, 30).

**Electron microscopy**

Owing to unsolved problems of fixation we have, so far, obtained few useful electron micrographs of dividing *Aspergillus* nuclei. Those available confirm and add to the observations made with the light microscope. It seems that the nuclear envelope remains intact throughout the course of division. The manner of extrusion of the nucleolus during mitosis (Fig. 2:9; Figs. 15-18), is still obscure. There are indications in our sections that the nucleolus is so to speak budded off, enclosed within a portion of the 'self-sealing' nuclear envelope. The spindle is revealed as a set of microtubules varying in length and lying parallel to each other along a straight line connecting two dense bars or plaques at opposite points of the nuclear envelope. In some of our
sections are profiles of complete spindles, in most of the others the spindle lies only partly on the plane of the section. So far we have not seen anything resembling kinetochores. These findings are illustrated in Figs. 39–42.

**DISCUSSION**

Since much that follows deals with appearances in stained preparations it seems worth while to point out that our view of mitosis in *Aspergillus* is partly derived from observations on living dividing nuclei and partly from the study of fixed and stained ones. Direct observation shows that nuclei reach their largest size just before division, that the spindle gets longer during the course of mitosis and that recently reconstituted nuclei are small. Beginning, middle and end of mitosis are thus securely established. In our preparations chromosomes are not visible in the nuclei of living cells. However, since we know that the spindle gets longer during mitosis, it is only necessary to find out which chromosome constellations are regularly associated with spindles of different length in order to arrive at a reliable sequence of chromosome movements.

Our views on the course of mitosis are in good agreement with recent independent observations on the behaviour of the chromosomes and the spindle in living dividing nuclei of *Fusarium oxysporum* (Aist & Wilson, 1968). The beautifully distinct phase-contrast images of mitosis in living *Fusarium* nuclei demonstrated to us by Mr J. R. Aist and published (1969) since this paper was first submitted should soon end current controversies.

The intranuclear fibre

The peripheral acidophilic granule of the *Aspergillus* nucleus that expands into a fibre during mitosis closely resembles corresponding structures in bakers’ yeast (Robinow & Marak, 1966; Moor 1966, 1967), *Polystictus versicolor* (Girbardt, 1968) and *Verticillium albo-atrum* (Heale, Gafoor & Rajasingham, 1968). Electron microscopy reaffirms these similarities. In *Aspergillus*, yeast and *Polystictus* the intranuclear fibre (Girbardt’s ‘Zentralstrang’) has been shown to be composed of a bundle of microtubules, and to be anchored at or near a dense, plate-like region of the nuclear envelope (the ‘centriolar plaque’ and ‘kinetochore equivalent’ of the yeast and *Polystictus* nucleus respectively).

The peripheral granule of *Saccharomyces* and *Aspergillus* nuclei recalls the ‘central body’ or ‘lateral granule’ of *Phyllactinia* nuclei described by Harper (1905) and Colson (1938). Both authors concluded that the division products of the lateral granule become the centrosomes or centrioles of the asters of meiotic and postmeiotic divisions of the nuclei of developing ascospores. Harper (1905) points out that a lateral body is also present in the mycelial nuclei of *Phyllactinia*. However, it seems that neither he nor Colson (1938) have examined the behaviour of that body during mitosis of hyphal nuclei. Our observations show that in hyphal nuclei too the ‘lateral body’ gives rise to a mitotic apparatus, though to a relatively less voluminous one than in ascus nuclei. Harper’s fig. 52 (fig. 22 in Olive, 1965) suggests that the V-shaped developmental stage of the intranuclear spindle in our material (Figs. 9, 34A) might
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be interpreted as two cones of divergent submicroscopic fibrils about to fuse in the region where they overlap. Growth in length of the fused fibres would lead to a straightening of the V-shape and change it into that of a cigar or spindle. Recent electron micrographs of replicas of frozen-etched nuclei of Saccharomyces and of Wickerhamia fluorescens have revealed that in yeasts new intranuclear fibres may indeed be formed in this manner (Moor, 1967; Heinz Bauer, personal communication) but we still lack electron-microscopical evidence that this also happens in Aspergillus.

In Saccharomyces (Robinow & Marak, 1966) and in Polystictus (Girbardt, 1968) there is, so far, no evidence that the intranuclear fibre is connected with the chromosomes and directly involved in their distribution to daughter nuclei. In Aspergillus direct evidence of this kind is also lacking, but there is good correlation between the elongation of the intranuclear fibre and the separation of daughter sets of chromosomes to opposite poles. We have therefore felt justified in calling the fibre a mitotic spindle. Its construction from submicroscopic fibrils fits this interpretation as does its sensitivity to cold, which recalls the experience of Inoué (1964) with the spindles of chilled dividing plant cell nuclei. It is not easy to compare our observations with those made by Wakayama (1931) on very differently prepared Aspergillus material. His figs. 5 and 15 portray typical intranuclear spindles, and his figs. 16, 20, 22 and 23 closely resemble anaphases common in our preparations. However, we have found no evidence for the author's view that the caryosome (nucleolus) becomes differentiated into chromosomes.

Behaviour of the chromosomes

The stage I patterns in haploid and diploid Aspergillus nuclei are not very different from the prophase configurations of standard forms of mitosis. Difficulties of interpretation arise from the direct transition from this prophase stage to the parallel alignment of chains of chromatinic elements (stage II), and the transverse separation of the chains without the intervention of a metaphase stage when sister chromatids are separated and oriented towards opposite poles. The absence of obvious metaphase plates gives a special character to nuclear-division figures of many fungi. Examples (not always commented upon) may be found among illustrations of mitosis in Pyrolena confluent (Gwynne-Vaughan & Williamson, 1931), Coleosporium vernoniae (at meiosis) (Olive, 1949), Schizopyllum commune (Bakerspigel, 1959), Polystictus versicolor (Girbardt, 1962) and Marasmius androsaceus (at mitosis and meiosis) (Duncan & Macdonald, 1965). The cord-like anaphase stage in Lipomyces (Robinow, 1961) and the half-way phase of nuclear division in Saccharomyces (Ramirez & Miller, 1962; Robinow & Marak, 1966) are presumably other instances of the same behaviour.

Of particular interest is a recent detailed study of the division of hyphal and basidial nuclei in Marasmius androsaceus by Duncan & Macdonald (1965). The problem that emerges from our own observations and those of Duncan & Macdonald is to explain how an anaphase constellation in which parallel rows of chromatinic units are broken transversely can achieve the equitable separation of two genomes. Before discussing this further two points should be made. First, as Duncan & Macdonald (1965) have already remarked, the number of chromosomes involved must be larger than the number of chromatinic units separately distinguishable along the chains. Thus,
although the diploid *Aspergillus* which has provided most of our illustrations has 16 chromosomes and presumably 32 at anaphase, nothing approaching this number of separately distinguishable chromatinic units was ever seen at anaphase. Secondly, careful focusing frequently reveals looping over of chromatinic strands from one anaphase chain to the other, suggesting that more than two chains may be involved in these constellations. Explanations starting from the assumption that the problematic figures are composed of only two chains may therefore not be correct.

Duncan & Macdonald (1965) solved the problem of genome separation by postulating that one of the paired chains (they believed only two were involved) is somehow turned through 180° relative to the other one before the pair is broken across to form the daughter nuclei. The essence of their scheme is summarized below:

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\begin{align*}
A & B & C & D \\
A' & B' & C' & D'
\end{align*}
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We have no evidence of inversion of one of the chains and, as indicated above, the limited resolving power of the light microscope does not permit one to be sure of a physical association of the elements within a chain. These considerations together with the fact, not known to Duncan & Macdonald, that a spindle apparatus is involved in somatic nuclear division, render more common schemes of chromosome behaviour more plausible. Our own proposal takes into account that in *Aspergillus* the size of the spindle reflects the degree of ploidy of the nucleus. This relationship indicates that the spindle is not organized solely by the centriolar plaques, unless diploid nuclei also contain diploid plaques. It is likely that at least part of the spindle, as in the mitotic apparatus of higher organisms, is generated by the chromosomes. This is plausible and probable. At present we have evidence only of the development of the spindle in connexion with the lateral granule.

Pooling assumptions and observations one arrives at the view that, at the onset of mitosis, the chromosomes become attached by their centromeres to the short spindle at randomly distributed sites on its surface. Hence nothing akin to a metaphase plate is ever formed. Coincident with the onset of spindle elongation during the transition from stage I to stage II the centromeres would divide and guide sister chromatids (or aggregates of them, in our case) in opposite directions along the surface of the spindle. The chromosomes may not go through this procedure all at the same time. This would account for their being strung out along the spindle at stage II of division (e.g. in Fig. 30); sister chromatids of early dividers farthest apart at opposite poles, those of late performers closer together in the middle. The movement of the chromosomes along two preferred lines—that is, the two chains of stage II—can be accounted for by the assumption that the spindle is asymmetrical with regard to chromosome attachment. Olive (1949) arrived at a similar interpretation of chromosome movements at anaphase II of meiosis in *Coleosporium*. The early phase of the events here speculated upon is not without precedent. In a *Meridinium* species parasitic on the radiolarian *Collozum inerme* and erroneously (but splendidly) described by Belar (1926) under the name of its host, the spindle of mitosis is an inconspicuous wire-like fibre at the periphery of the nucleus. Chromosomes are attached to points along the spindle at a
time when they are still long and loosely coiled. A metaphase stage is lacking. The chromosomes do not markedly contract until telophase, when the points of attachment of sister chromatids have become widely separated through the elongation of the spindle and the migration of chromatids to the poles. The spindle and chromosomes of the mitotic divisions that provide nuclei for microgametes of the sporozoan *Aggregation eberthi* (Belar, 1926) behave in a similar manner.

Our account of nuclear division in germinating conidia of *A. nidulans* differs markedly from that given by Weijer & Weisberg (1966). This discrepancy is undoubtedly due in part to the different methods of growing and fixing the material for study. However, since a number of the figures given by the authors resemble configurations frequently encountered by us, it is apparent that questions of interpretation are also involved.

**Synchrony of division**

Both the observations on living hyphae and those on fixed and stained preparations indicate that division is synchronous within a single hypha. Rosenberger & Kessel (1967) arrived at the same conclusion from a consideration of the number of nuclei within single hyphae. Small differences in the time at which individual nuclei enter division are frequently apparent in living hyphae, however. These differences are revealed in fixed and stained preparations as a gradient of division stages along the hypha. The presence of this gradient indicates that the nucleus at one end of the conidium/germ-tube unit begins to divide and is quickly followed by successive nuclei along the hypha. The excess of hyphae with the gradient running from conidium to tip indicates a preference for the nucleus at the hyphal tip to enter division first.

**The number of chromatinic elements**

The observation of a number of stage I haploid nuclei with close to 8 chromatinic elements indicates that at the early stages of division these elements are individual chromosomes. As division proceeds, however, the number of separately discernible elements decreases as a result of condensation of the chromatin on the spindle. We therefore agree with the conclusion of Duncan & Macdonald (1965) from their study of *Marasmius* that at the track and subsequent stages, chromatinic elements cannot be equated with chromosomes.

Because of the small size of the mitotic figure, consistent counts of the number of chromatinic elements could not be made. Nevertheless it is the authors' opinion that a doubling in number would have been readily apparent. This expected difference between haploid and diploid was not observed. Instead the number of chromatinic elements appeared to be the same at both levels of ploidy. This similarity persisted throughout the division cycle. However, because of the condensation of the chromatin, only stage I figures provide information about the number of chromosomes.

This question of the number of chromatinic units present in dividing diploid nuclei is relevant to the mechanism of mitotic crossing over in diploids of *A. nidulans* (Pontecorvo *et al.* 1953; Pontecorvo, 1958). The reciprocal nature of the process
(Roper & Pritchard, 1955) indicates that intimate association of homologous chromosomes is essential for crossing over. From the genetic evidence it is impossible to determine whether somatic pairing is a regular feature of diploids of this fungus, or whether it occurs sporadically and in only a small proportion of the nuclei. Westergaard (1964) argues from evolutionary considerations that homologous pairing may be the normal condition in transient diploid cells of habitually haploid species. In indicating that all the dividing nuclei in a diploid strain of *A. nidulans* contain the haploid number of chromatinic elements and that the individual elements are larger than in a haploid strain, our observations clearly support Westergaard’s hypothesis.

Dr E. Käfer-Boothroyd has pointed out to us that somatic homologous pairing in the diploid examined should give rise to fewer chromatinic elements than are found in the haploid (7 as opposed to 8), as the result of the formation of a translocation multiple. Unfortunately the demonstration of so small a difference requires accurate counts of the number of elements, and this was not possible with our preparations. The examination of diploids heterozygous for a number of translocations would appear, however, to offer a possible test for the hypothesis of regular somatic pairing.

Since our paper was first submitted for publication we have learned that the number of distinct chromosomes is also approximately the same in both haploid and diploid sporidia of the basidiomycete *Ustilago violacea* (A. W. Day & J. K. Jones, in preparation). This provides further support for the idea that homologous chromosomes may be associated in somatic cells of diploid strains of fungi.

In summary, in our view, mitosis of hyphal nuclei of *A. nidulans* differs from the usual form of mitosis in being intranuclear, in the attachment of chromosomes at various points along a slender central cord-like spindle and in the staggered separation and movement of sister chromatids. As Duncan & Macdonald (1965), writing of another fungus (*Marasmius*) have put it: ‘The end products of all the hyphal divisions are the same as in organisms having a conventional mitosis but some of the steps by which this is achieved are unusual….’

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REFERENCES


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Figs. 1, 2. Time-lapse phase-contrast micrographs of nuclear division in living hyphae of diploid A. nidulans. The first picture in each row shows a resting nucleus immediately prior to division. A clear chromatin-containing area (ch) encloses a dense nucleus (nu). Numbers indicate minutes elapsed since the first picture in each row was taken. The intranuclear spindle (sp) is visible in Fig. 1, 8 and 9 min and in Fig. 2, 3 and 7 min. Remains of the nucleolus may be seen in Fig. 1, 14 min, characteristically nearer to the proximal than to the distal daughter nucleus. Note difference in size between the nucleus in Fig. 1, 0 min, which is ready to divide, and the newly reconstituted daughter nucleus at the top of Fig. 1, 18 min. A delicate strand of nuclear substance still persists between the telophase nuclei in the upper half of Fig. 2, 18 min. Fig. 1, × 2700; Fig. 2, × 2200.
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Figs. 3–6. Acid-fuchsins-stained hyphae showing the development of the intranuclear spindle (sp) in dividing nuclei of haploid *A. nidulans*. The spindles are seen as rods of increasing length adjacent to the deeply stained nucleolus. The chromatin is not apparent in these preparations. All figures × 3600.
Figs. 7–14. Development of the intranuclear spindle in dividing nuclei of diploid *A. nidulans*. The larger size of the spindles in the diploid is apparent from a comparison of these figures with Figs. 3–6. All figures × 3600.

Fig. 7. Above the bean-shaped nucleolus is the sharply defined 'lateral granule' (/g).

Fig. 8. Duplication of the lateral granule. Compare with Fig. 3.

Fig. 9. Early stage in the formation of the spindle (sp) from the two granules. The young spindle is characteristically V-shaped.

Figs. 10–12. Thick, cigar-shaped spindles by the side of deeply stained nucleoli.

Fig. 13. Thin, straight spindle running along the long axis of the hypha.

Fig. 14. Daughter nucleoli (dnv) are forming at either end of the fully elongated spindle (sp). The old nucleolus (nu) is still very conspicuous, however. Compare with Fig. 38.
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Figs. 15-18. Dividing nuclei of diploid *A. nidulans* stained directly with aceto orcein. All × 2700.

Fig. 15. An elongated constellation of chromosomes which at its lower left is in contact with the nucleolus (*nu*).

Figs. 16-18. Anaphase-telophase constellations. In Figs. 16 and 17 the lower of the two sister clusters of chromosomes is in close contact with the nucleolus (*nu*). In Fig. 18 the remains of the nucleolus (*nu*) can be discerned as a light patch midway between the developing daughter nuclei.

Fig. 19. A sequence of stages of nuclear division in a hydrolysed Giemsa-stained hypha of the diploid strain of *A. nidulans*. Division is least advanced close to the conidium (top) and completed at the bottom of the figure. × 2700.
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Figs. 20–25. Stages of the division of haploid nuclei. Aceto orcein after hydrolysis. x 3600.

Figs. 20A, B. Different levels of focus of the same group of hyphae. Resting nuclei on the left, showing finely granular chromatin (ch) and clear nucleolus (nu). Nuclei in stage I of division on the right. The white patch beneath the division figure at the top of Fig. 20B represents the nucleolus.

Figs. 21, 22. Dividing nuclei in transition from stage I to stage II.

Figs. 23, 24. Nuclei in stage II. Chromatin arranged in two more or less parallel chains. Compare with the corresponding stage of diploid nuclei in Figs. 28–30.

Fig. 25. Anaphase. The chromatin forms 'bilobed' masses at either end of the division figure.
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Figs. 26–33. Stages of the division of diploid nuclei. Stained with aceto orcein after hydrolysis. × 3600.

Fig. 26. At left, out of focus, a resting nucleus. Other resting nuclei are shown in Fig. 32. At the right a nucleus in stage I of division. Note threads (th) which connect a nucleolus-associated piece of chromatin with the main cluster of chromosomes.

Fig. 27. Similar to Fig. 26, chromosomes more contracted. Compare with the corresponding stage of haploid nuclei in Fig. 21.

Figs. 28–30. Typical examples of stage II of division. See also Figs. 19 and 36B. Figs. 29A, B are of the same nucleus photographed at two different levels of focus. In Fig. 29A a strand of chromatin is seen crossing the gap between the two parallel chains of chromatinic elements. In Fig. 30 note V-shaped elements at either end of the upper one of the two tracts. Their similarity suggests that they may be homologous. See text for further explanation.

Figs. 31–33. Anaphase, telophase. Figs. 31 and 32 illustrate the movement of chromatin to the ends of the parallel tracts. Fig. 33 shows an early stage in the formation of daughter nuclei. Compare Figs. 28–32 with corresponding stages of directly stained nuclei in Figs. 15–18.
Figs. 34–38. Five pairs of photographs in which the A picture shows a hypha stained with acid fuchsin for spindle and nucleolus and the B picture the same hypha stained with aceto orcein or Giemsa for chromatin. × 3600.

Fig. 34. The earliest stage of spindle formation in nuclei whose chromatin, seen in B, has barely begun to condense. Aceto orcein.

Fig. 35. A short spindle is seen to occupy a site within a cluster of chromosomes at stage I of division. The nucleolus, below the spindle, appears solid and deeply stained. Giemsa.

Fig. 36. The spindle has become elongated into a straight fibre which B shows to be lying between the chromatinic elements of stage II of nuclear division. The nucleolus at the lower end of the spindle appears less solid than in Fig. 35A. Aceto orcein.

Fig. 37. The spindle is long, thin and straight. The nucleolus is nearly dissolved. The anaphase movement of chromatin to the ends of the division figure has begun. Comparison of the two figures shows that the spindle runs between the thinning strands of chromatin. Giemsa.

Fig. 38. Advanced telophase. New nuclei have formed at the end of the thin, maximally extended spindle. The nucleolus, characteristically nearer to one new nucleus than to the other, is still remarkably solid. Compare this stage with Fig. 1, 14 min, and Fig. 18. Giemsa.
Figs. 39–42. Electron micrographs of sections of hyphae of diploid *A. nidulans* fixed at the moment when their nuclei were first seen to enter division.

Fig. 39. Component fibrils (which better preservation would probably reveal to be microtubules) are diverging from a recessed dense portion of the nuclear envelope. In sections of other specimens fibrils have been seen running straight across the nucleus from one dense plaque to another one on the opposite side of the envelope. In the present section the spindle has been cut obliquely. ×30,000.

Fig. 40. The middle of the hypha is filled by an elongated nucleus. The nuclear envelope is intact. Fibrils can be discerned in the interior of the nucleus. They run parallel to its long axis. The nucleus of this section was probably in stage II of division, in which parallel chains of chromatinic masses are stretched out on either side of the rapidly elongating spindle. Compare with Figs. 28–30. ×30,000.

Figs. 41, 42. Section of a dividing nucleus probably in early anaphase corresponding to Figs. 31, 32. The nuclear envelope appears intact. Its corrugated outline is partly an artifact of fixation. Note the broad membrane-bound corridor between developing daughter nuclei. Fibrils are seen where the corridor joins the right nucleus. This region is shown in greater detail in Fig. 42. Dense matter in the right nucleus is part of the developing nucleolus. Fig. 41, ×16,000; Fig. 42, ×63,000.
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