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# Mitotic mutants of *Aspergillus nidulans*

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SUMMARY

Forty-five temperature-sensitive mutants of *Aspergillus nidulans* which are defective in nuclear division, septation or distribution of nuclei along the mycelium have been isolated, and most have been subjected to complementation analysis and mapped to chromosome. Thirty-five of the mutants were unable to complete nuclear division at the restrictive temperature. Twenty-six of these mutants exhibited a co-ordinate drop in both spindle and chromosome mitotic indices at 42 °C, indicating that they fail to enter mitosis. These mutants have been assigned to the gene symbol *nim*. Nine mutants exhibited a co-ordinate rise in spindle and chromosome mitotic indices at 42 °C, indicating that they are arrested in mitosis. These mutants were assigned the gene symbol *bim*. Five mutants failed to form septa and were given the gene symbol *sep*; and five mutants had an abnormal nuclear distribution and were given the gene symbol *nud*. All of the mutations were recessive. Most of the mutants were in different complementation groups. Mutants in the same complementation groups were phenotypically similar, but phenotypically similar mutants were not necessarily or usually in the same complementation group. There was no evidence for genetic clustering of phenotypically similar mutants. The mutants were located on all eight chromosomes.

## 1. INTRODUCTION

The fungus *Aspergillus nidulans* may be a useful model system for analysing the biochemical events of mitosis. Although the morphological events of mitosis are well known, the biochemical processes which underlie these events are not well understood. For example, the chemical events which lead to condensation of the chromosomes, formation of the mitotic spindle, separation of the chromosomes at anaphase and depolymerization of the spindle and relaxation of the chromosomes at interphase are mostly unknown. Equally mysterious are the mechanisms responsible for synchronizing chromosome condensation with assembly of the mitotic spindle, and chromosome relaxation with disassembly of the spindle, for aligning nuclear division with cytoplasmic division, and for coupling nuclear division to DNA doubling. There are disadvantages to studying the biochemistry of mitosis in higher organisms, notably the difficulty of obtaining large amounts of material for biochemical analysis and the fact that rapid and convenient systems for production and genetic analysis of mutations affecting the mitotic process

are not available. The problem of obtaining sufficient material for biochemical analysis has partly been solved through the use of lower organisms, e.g. the sea urchin, as sources for isolation of the mitotic apparatus (Mazia & Dan, 1952); however, these organisms are not amenable to genetic analysis. Systems for studying the genetics of mitosis have also recently become available. Mutants defective in mitosis and cell division have been isolated from *Saccharomyces cerevisiae* (Hartwell, Culotti & Reid, 1970; Hartwell *et al.* 1973) and from *Chlamydomonas reinhardi* (Howell & Naliboff, 1973). The yeast mutants comprise a large series and have been extensively studied. However, *S. cerevisiae* is not entirely suitable for studies of mitosis, since the chromosomes of this organism do not visibly condense at metaphase (Robinow & Marak, 1966). Mitosis in *Chlamydomonas reinhardi* is also unusual in that four daughter cells are produced rather than the usual two. For these reasons, in order to develop a system for genetic and biochemical analysis of mitosis in which most of the events of mitosis conform more closely to the normal eukariotic picture, I have isolated a set of mitotic mutants from *Aspergillus nidulans*.

The primary features of eukariotic mitosis are evident in *A. nidulans* (Robinow & Caten, 1969). The chromosomes are condensed at metaphase and are easily demonstrated after staining with aceto-orcein or giemsa. A mitotic spindle can also be demonstrated by light microscopy after staining with acid-fuchsin, and the spindle is seen to be microtubular in structure under the electron microscope. Some features of mitosis in *A. nidulans*, however, are not similar to those of higher eukariotes. The chromosomes orient along the spindle rather than on a metaphase plate; the nuclear membrane does not disappear during mitosis; and there are no centrioles. Instead of centrioles structures known as spindle plaques are seen to be embedded in the nuclear membrane at the ends of the spindle (Robinow & Caten, 1969; Moens and Rapport, 1971). Despite the morphological differences, many of the chemical events of mitosis in *A. nidulans* especially those related to chromosome condensation and the mitotic spindle, may be expected to resemble the events occurring in higher eukariotes.

*A. nidulans* grows as a branching mycelium which is divided into multinucleated hyphal segments by incomplete septa. Its nuclear division and nuclear DNA synthesis are regulated as a function of growth rate (Kessel & Rosenberger, 1968). Nuclear division is synchronous within a hyphal segment (Rosenberger & Kessel, 1967) and septation is coupled to nuclear division (Clutterbuck, 1970). The genetic system of *A. nidulans* is also well understood, and both sexual and parasexual cycles have been used for genetic analysis (Pontecorvo *et al.* 1953; Pontecorvo & Kafer, 1958). *A. nidulans* has eight chromosomes (Elliot, 1960) represented by eight well-marked linkage groups (Kafer, 1958). It is normally haploid; however, heterokaryons and diploids are easily produced, and it is also homothallic; therefore, independently isolated mutants can be tested directly for genetic complementation. There are reported to be $1{\cdot}3 \times 10^{10}$ daltons of DNA per haploid conidial nucleus (Bainbridge, 1971); therefore the size of the average chromosome should be approximately one-half that of the *E. coli* chromosome. *A. nidulans*

grows rapidly, either submerged, or on the surface of simple or defined culture media. In rich liquid medium it grows to a final yield of approximately 30 g/l. DNA, RNA and proteins can easily be labelled with radioisotopes by using suitable auxotrophs. An additional potential advantage of *A. nidulans* for this work is that the nuclear membrane does not disappear during mitosis (Robinow & Caten, 1969). Thus it may be possible to study mitosis in intact nuclei. In this paper a set of temperature-sensitive conditional lethal mutants of *Aspergillus nidulans* is described which are defective in nuclear division, nuclear distribution or septum formation.

## 2. METHODS

### (i) *Strains and nomenclature*

FGSC 154, the parental strain used for isolation of mitotic mutants, and also FGSC 73 and FGSC 99, were obtained from the Fungal Genetics Stock Center at Humboldt State College in Arcata, California. FGSC 154 carries the following markers in each of the following linkage groups: *adE20* (IR), *biA1* (IR), *wA2* (IIL), *cnxE* (IIR), *sC12* (III) *methG1* (IVL), *nicA2* (V), *lacA1* (VI), *choA1* (VII) *chaA1* (VIIR). FGSC 73 and FGSC 99 were used in mapping studies to assign new mutations to linkage groups. FGSC 73 has the following markers: *pabaA1* (IR), *yA2* (IR), *adE20* (IR), *AcrA1* (IIL), *phenA2* (III), *pyroA4* (IV), *lysB5* (V), *sB3* (VI), *riboB2* (VIIIR) and FGSC 99 the following: *sulA1 adE20* (IL), *proA1* (IR), *pabaA1* (IR), *adE20* (IR), *AcrA1* (IIL). The mutants described in this paper were produced by ultraviolet irradiation and are temperature-sensitive at 42 °C, therefore they are designated UVts 1, 2, 3, .... Gene symbols have been assigned in accordance with the new rules proposed by Clutterbuck (1973).

### (ii) *Culture techniques*

The growth media used were YG (0·5 % yeast extract, 2 % glucose), YAG (0·5 % yeast extract, 1·9 % agar, 2 % glucose). YAGD (YAG plus 0·08 % deoxycholate) and Czapek-Dox broth (Difco) with 3 % saccharose, 1·9 % agar and supplementary nutrients appropriate for the nutritional markers in use (see above).

### (iii) *Procedure for isolation of mitotic mutants*

Conidia of FGSC 154 were mutagenized with ultraviolet light. Conidia were harvested from 3- to 7-day-old mycelia and were diluted with $H_2O$ to a concentration of $1{\cdot}5 \times 10^5$/ml. Twenty ml of conidial suspension were irradiated for 9 min with stirring in a glass Petri dish at a distance of 24 cm from a General Electric G8T5 ultraviolet source prewarmed for 30 min. 0·1–0·2 ml samples of the irradiated conidia were plated on YAGD and incubated at 32 °C in a Thelco Model 4 air incubator for 4 days. On the fourth day, surviving colonies (10–100/plate) were inoculated on duplicate YAGD plates in a grid pattern and one plate incubated at 32 °C, the other at 42 °C for 3 days. Presumptive temperature-sensitive (ts) mutants, identified by their failure to grow at 42 °C were streaked to give single colonies from the 32 °C master grid and incubated at 32 °C for 4 days. Temperature

regulation in the Thelco incubator was not perfect; therefore, when an incubation temperature is given as 42 °C it implies some variation between 40 and 42 °C. When more precise temperature control was required for determination of mitotic indices and germination characteristics (see below), a water bath was used and the temperature of the agar monitored by a thermistor probe embedded in the agar. The temperature sensitivity of the putative ts mutants was reverified by punch-transferring single colonies on to YAGD plates and incubating them at 42 and 32 °C respectively for 2 days. The yield of temperature-sensitive mutants was 3–5 %. The nuclear morphology of the ts mutants was studied by light microscopy in order to identify mitotic mutants.

(iv) *Microscopic examination of ts mutants*

For microscopic examination ts mutants were inoculated on a single layer of sterile dialysis tubing spread on the surface of YAG agar and incubated overnight at 32 °C to allow the conidia to germinate and produce germ-tubes of a size convenient for microscopic examination. The optimal time for this was found to be 11 h. The dialysis tubing was then transferred to YAG at 42 °C in a water bath for 2 or 4 h after which the dialysis tubing with adhering mycelia was removed, fixed with modified Helly solution (5 % merchloric chloride, 3 % potassium dichromate (w/v) in water with 6 % (v/v) of a 3 % solution of formaldehyde added immediately before use) and stained with either aceto-orcein or acid-fuchsin according to the method described by Robinow & Caten (1969). Only aceto-orcein obtained from Fisher Scientific Co. produced well-stained nuclei. Alternatively mutants were germinated directly at 42 °C. The stained mycelia were examined by light microscopy for abnormalities associated with defective mitosis or cell division at 790–1260 × magnification using a Zeiss microscope equipped with a 63 × Planapo objective. Approximately 5–10 % of the ts mutants were putative mitotic mutants; however, about half of the mutants isolated were leaky or grew at a reduced rate and were discarded. Putative mitotic mutants were stored at 4 °C on silica gel (Ogata, 1962).

(v) *Determination of mitotic indices*

*A. nidulans* is coenocytic. Under the conditions of these experiments, nuclear division is synchronous for all the nuclei in a hyphal segment (Rosenberger & Kessel, 1967). Thus, the mitotic index as it is usually described, i.e. the percentage of nuclei observed to be in mitosis, is not useful as a measure of nuclear division in *A. nidulans* because the data are affected by the varying numbers of nuclei in the particular segments scored. A better measure of mitotic index in *A. nidulans* is the percentage of hyphal segments in mitosis. At early times after germination most mycelia consist of only one or two hyphal segments. As the hyphal tips at the periphery of the culture presumably divide more rapidly than those nearer the centre of the culture, I have used the percentage of peripheral apical hyphal segments observed to be in mitosis in fixed and stained material as my measure of the mitotic index. Two different measures of mitotic index were used, one based on the percentage of hyphal tips with condensed chromatin and the other on the

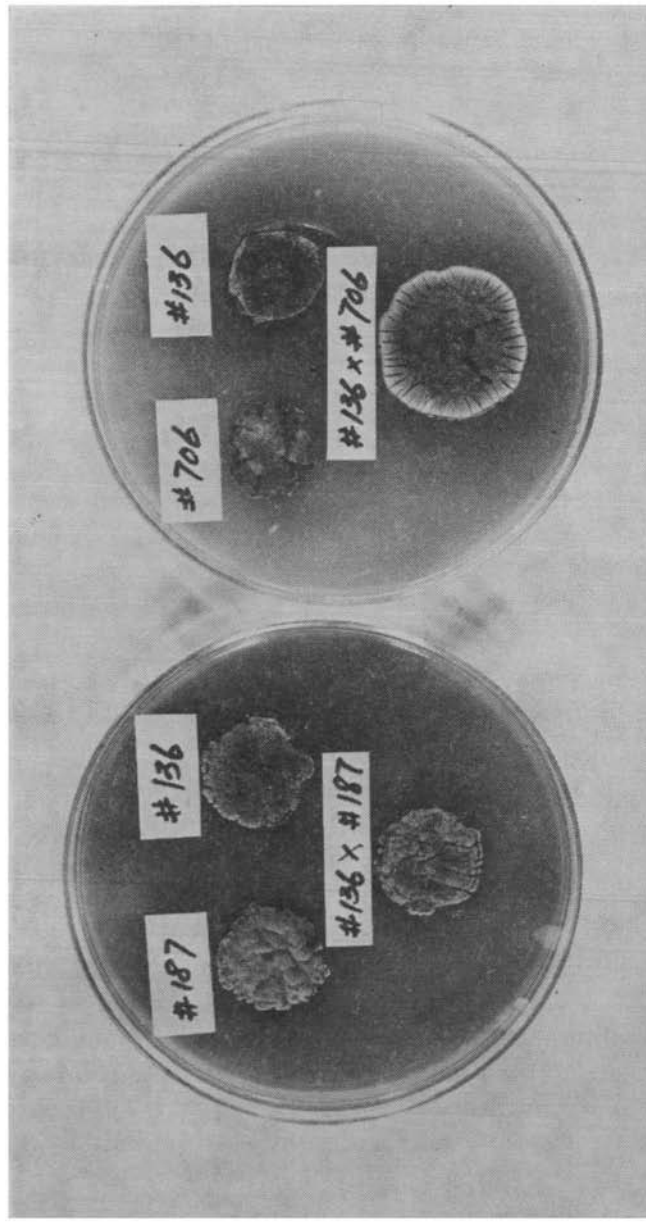


Fig. 1. Complementation analysis of ts mutants. Mycelial mats from het tubes incubated on YAGD for 4 days. On left, UVts 136 × 187 fail to complement, as shown by the very limited growth of the mycelial mat after transfer. On right, UVts 136 × 706 complement, as shown by unlimited outgrowth of the mycelium.

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percentage of hyphal tips in which mitotic spindles were seen. The former, termed the chromosome index (CMI), was obtained from cultures in which the nuclear material was stained with aceto-orcein. The latter, the spindle mitotic index (SMI), was determined from cultures in which mitotic spindles were stained with acid-fuchsin. Five hundred hyphal tips were scored for each data point.

### (vi) *Complementation analysis*

Independently isolated ts mutants were tested for genetic complementation by measuring their ability to form heterokaryons which could grow at restrictive temperature (Fig. 1). Since *A. nidulans* is homothallic this could be done directly by forming heterokaryons between mutants. 'Heterokaryon tubes' (100 mm screw-cap test-tubes containing 1–2 ml of YG agar freshly overlaid with 1–2 ml of $H_2O$ were inoculated with conidia of the two strains to be tested for complementation and incubated at 32 °C until a mycelial mat, 1–2 mm thick, formed (3–4 days). This mat was then transferred to the surface of YAGD in a Petri dish and incubated at 42 °C. The radial outgrowth from the original mat was measured after 4 and 6 days growth at 42 °C. Since some of the mutants were leaky, if outgrowth clearly exceeded that of the leakier parent the mutants were considered able to complement each other. If a pair of mutants failed to complement, the test was repeated five times or until complementation occurred. All mutants which complemented each other were tested for syntrophism by placing slices of mycelial mats of two mutants to be tested about 1 mm apart on YAG and incubating at 42 °C. All pairs tested for complementation were also tested for syntrophism. No syntrophic outgrowth was seen between any two pairs.

### (vii) *Assignment of mutations to linkage groups*

Mutations were assigned to linkage groups by traditional methods of parasexual analysis (Forbes, 1959; McCully & Forbes, 1965). Heterokaryons were formed by nutritional selection between the mutants being analysed which all carry the recessive gene *w* and are white and either FGSC 73 or FGSC 99, both of which carry the recessive gene *y* and are yellow. A diploid strain, which was heterozygous for both markers and consequently green, was isolated on selective medium from each heterokaryon and plated on YAG containing 0·013 % *p*-fluorophenylalanine (Morpurgo, 1961). Under these conditions random chromosome loss occurs, and unknown markers can be assigned to chromosomes by virtue of their association with markers whose chromosomal locations are already known.

## 3. RESULTS

Forty-five mutants which by morphological criteria appeared to be defective with respect to nuclear division, cell division or nuclear distribution were isolated from among approximately 1000 temperature-sensitive, conditionally lethal mutants of *A. nidulans*. Three classes of mutant were recognized: (1) mutants

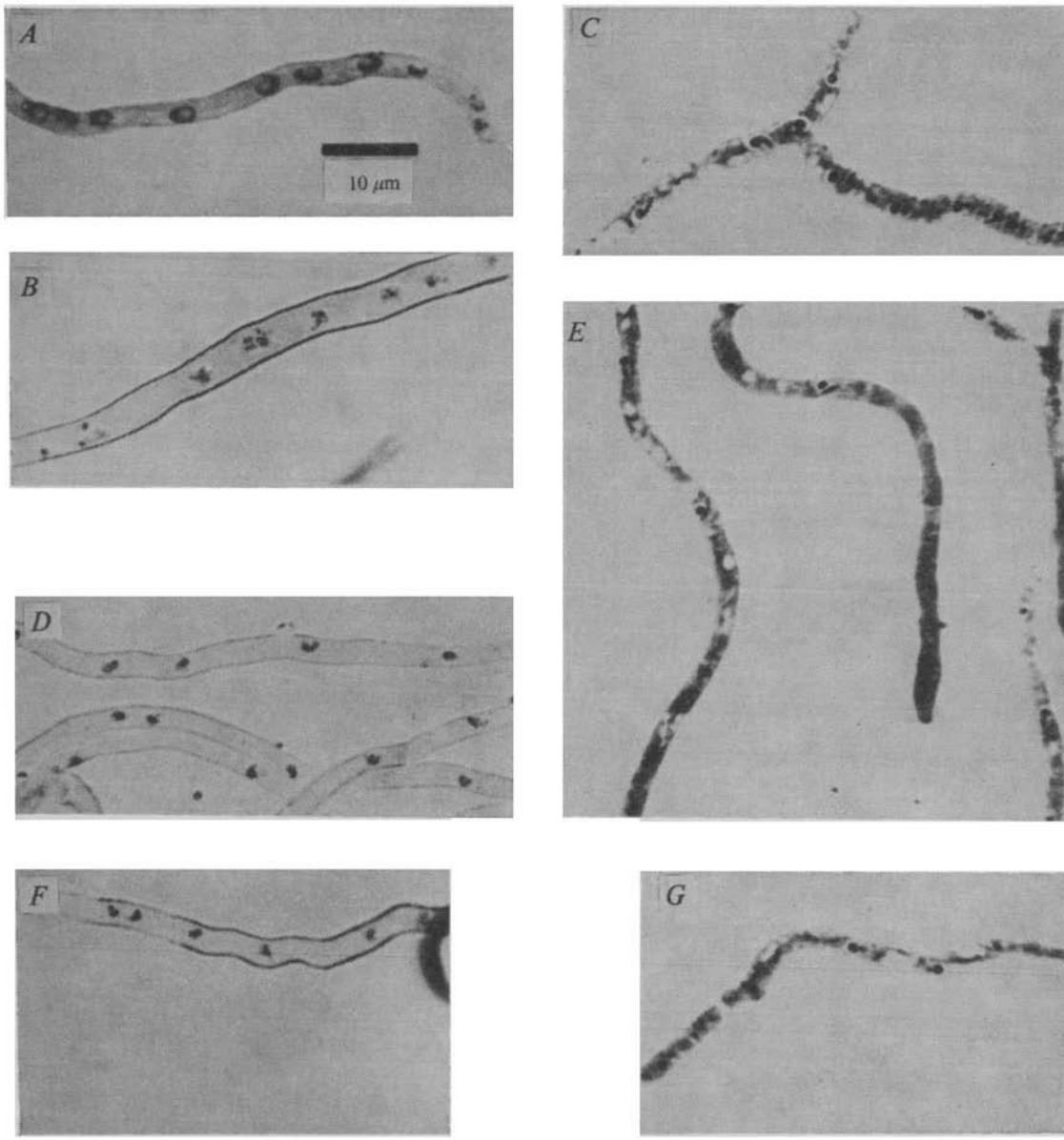


Fig. 2. Nuclear and spindle morphology of wild-type and *bim* mutants. (*A*) Wild-type mycelium (FGSC 154) grown at 32 °C for 12 h and stained with aceto-orcein to demonstrate interphase nuclei. (*B*) Wild-type mycelium grown as above and stained with aceto-orcein to demonstrate metaphase nuclei. (*C*) Wild-type mycelium grown as above and stained with acid-fuchsin to demonstrate mitotic spindles. (*D*) *bimE7* mycelia grown at 32 °C for 12 h, switched to 42 °C for 2 h and stained with aceto-orcein to demonstrate metaphase nuclei. (*E*) *bimE7* mycelia grown as in (*D*) and stained with acid-fuchsin to demonstrate mitotic spindles. (*F*) *bimC4* mycelia grown as in (*D*) and stained with aceto-orcein to demonstrate metaphase chromosomes. (*G*) *bimC4* grown as in (*D*) and stained with acid-fuchsin to demonstrate 'minute' spindles.

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defective in mitosis, (2) mutants which failed to form septa and (3) mutants which were abnormal with respect to nuclear distribution.

### (i) *Mutants defective in mitosis*

By far the largest group of mutants were those which appeared to be defective in mitosis. There were 35 mutants in this group (UVts 69, 92, 136, 165, 175, 187, 228, 244, 333, 462, 469, 537, 546, 555, 597, 622, 706, 711, 758, 759, 764, 765, 783, 826, 840, 843, 862, 895, 903, 911, 935, 945, 966, 967 and 990). They were recognized initially by their distinctive appearance when stained with aceto-orcein and examined under the microscope after overnight growth at 32 °C followed by 4 h at restrictive temperature (42 °C). On initial screening, the features of these mutants which first drew attention were either an increased number of nuclei in mitosis at restrictive temperature, as in the case of strains UVts 69, 706, 967 and 990 (Fig. 2), or nuclei which were more or less abnormally spaced, large and elongated, as in the case of strains UVts 92, 136, 165, 175, 187, 228, 244, 333, 462,

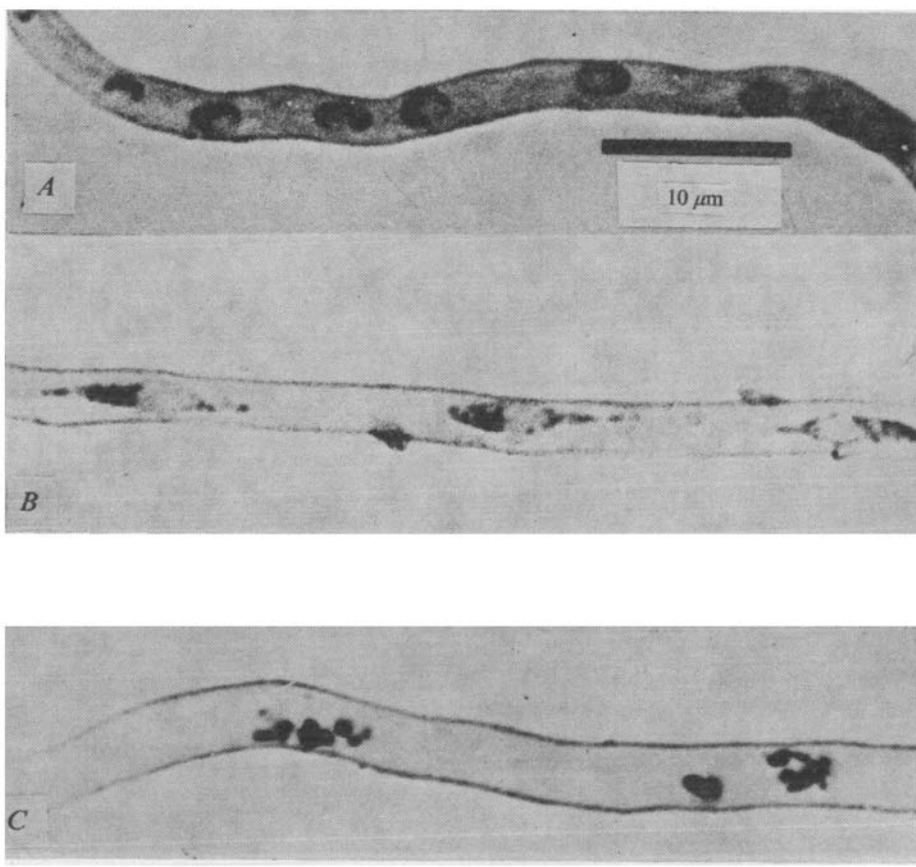


Fig. 3. Nuclear morphology of *nim* mutants. (*A*) Wild type, FGSC 154, mycelium grown at 32 °C for 12 h and stained with aceto-orcein. (*B*) UVts 136 grown at 32 °C for 12 h, at 42 °C for 4 h and stained with aceto-orcein. (*C*) Same as (*B*), metaphase figures.

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469, 537, 546, 555, 597, 622, 711, 758, 764, 783, 826, 840, 843, 862, 895, 903, 911, 935, 945 and 966 (Fig. 3). Some mutants, UVts 136, 187, 244, 537 and 555, exhibited both increased numbers of nuclei in mitosis and increased internuclear distances.

Internuclear distances vary during the cell cycle in *A. nidulans* (Robinow & Caten, 1969). The internuclear distance is least just after nuclear division, and becomes largest just before nuclear division. Thus it seemed likely that mutants with increased internuclear distances might be arrested at about the time of nuclear division. These mutants could either be blocked during mitosis, in which case they would exhibit increased numbers of nuclei in mitosis at 42 °C, or be unable to enter mitosis, in which case they would exhibit decreased numbers of nuclei in mitosis. Determination of mitotic indices should therefore discriminate between these two classes of mutant.

### (ii) *Mutants with elevated mitotic indices*

Mutants with increased numbers of nuclei in mitosis were obviously arrested in mitosis irrespective of whether or not intranuclear distances were increased. In order to quantitate the degree of mitotic block in these mutants chromosome

Table 1. *Mutants blocked in mitosis*

| | | 2 h at 42 °C | | 4 h at 42 °C | | 18 h at 42 °C | | |
|---|---|---|---|---|---|---|---|---|
| Gene symbol | Strain no. | CMI* | SMI* | CMI | SMI | Divs | *L*/*N*§ (*μ*) | Linkage group |
| WT* | FGSC 154 | 4 | 3 | 3·4 | 2 | 8 | 4–5 | — |
| bimA1 | 69 | 12 | 10 | 27 | 26 | —† | — | I |
| bimB2 | 136 | 50 | 27 | 0 | 2 | 0·63 | 112·7 | III‡ |
| bimB3 | 187 | 12 | 15 | 0 | 4 | 0·63 | 122·3 | III |
| bimC4 | 244 | 43 | 38‡ | 13 | 9‡ | 1·7 | 28 | VI |
| bimD5 | 537 | 8·2 | 10 | 3 | 0 | 1·9 | 17·5 | IV |
| bimD6 | 555 | 7·4 | 15 | 4 | 1 | 2·6 | 13·5 | IV |
| bimE7 | 706 | 90 | 60 | 90 | 10 | 0·1 | 14·8 | VI |
| bimF8 | 967 | 25 | 16 | 51 | 21 | —† | — | — |
| bimA9 | 990 | 16 | 9 | 32 | 17 | —† | — | I |

* CMI and SMI stand for 'chromosome mitotic index' and 'spindle mitotic index'. WT stands for 'wild type'.

† Failed to germinate.

‡ Spindles were 'minute'.

§ Average mycelial length for nucleus.

mitotic indices were determined after 2 and 4 h at restrictive temperature (Table 1). Spindle mitotic indices (SMI) were also determined on these strains, since in theory the SMI might change in a direction opposite to the CMI (such as mitotic arrest by colchicine). Nine mutants – UVts 69, 136, 187, 244, 537, 555, 706, 967 and 990 – had CMI's significantly higher than the wild type at restrictive temperature. All of these mutants also had increased SMI's at 42 °C (Table 1, Fig. 2). In most of the strains the morphology of the mitotic spindle by light microscopy

was indistinguishable from that of the wild type; however, UVts 244 had spindles which appeared to be abnormally small – approximately one-third the length of the normal haploid spindle (Fig. 2). The precise nature of the spindle abnormality in this strain could not be determined by light microscopy, as the minute spindles in this strain are very close to the limit of resolution of the light microscope. Electron microscopic studies of the UVts 244 spindle are in progress. The nine mutants with increased CMI's and SMI's at restrictive temperature have been assigned the gene symbol *bim* standing for *b*locked *i*n *m*itosis.

Table 2. *Amitotic mutants*

| | | 2 h at 42 °C | | 4 h at 42 °C | | 18 h at 42 °C | | |
|---|---|---|---|---|---|---|---|---|
| Gene symbol | Strain no. | CMI | SMI | CMI | SMI | Divs | *L/N* (*μ*) | Linkage group |
| WT | FGSC 154 | 4 | 3 | 3·4 | 2 | 8 | 4·5 | — |
| nimA1 | UVts 92 | 0 | 0·8 | — | — | 1 | 28 | III |
| nimB2 | 165 | 0 | 0 | — | — | 1·2 | 62 | V |
| nimC3 | 175 | 0 | 1·6 | 0 | 0 | 0·3 | 14 | V |
| nimD4 | 228 | 3·5 | 3·6 | 0 | 0 | 0·5 | 61 | VII |
| nimA5 | 333 | 0 | 0·8 | — | — | 0·2 | 60 | III |
| nimE6 | 462 | 0·3 | 0 | — | — | 1·2 | 55 | II |
| nimA7 | 469 | 0·5 | 0·5 | — | — | 0·1 | 23 | III |
| nimF8 | 546 | 0·6 | 0 | 0 | 0 | 0 | 9 | — |
| nimA9 | 597 | 0 | 0 | — | — | 0·2 | 62 | III |
| nimG10 | 622 | 0 | 0 | 0 | 0·2 | 0·3 | 62 | II |
| nimH11 | 711 | 2·4 | 2·2 | 0·8 | 1·6 | 2·3 | 18 | VII |
| nimI12 | 758 | 0 | 1 | 0 | 0·6 | 1·8 | 34 | I |
| nimJ13 | 759 | 4 | 4 | 0·2 | 0·6 | 1·9 | 9 | V |
| nimK14 | 764 | 0·2 | 0 | 0·3 | 0 | 1·5 | 38 | I |
| nimL15 | 765 | 0 | 0 | 0 | 0 | 0 | 9 | IV |
| nimM16 | 783 | 0 | 0 | 0 | 0·6 | — | — | I |
| nimN17 | 826 | 0·6 | 0 | 1 | 0 | 2·4 | 15 | VIII |
| nimO18 | 840 | 0 | 0 | 0 | 0 | 0·2 | 24·1 | VII |
| nimP19 | 843 | 1 | 1 | 1 | 0 | 0·1 | 35 | VI |
| nimQ20 | 862 | 5 | 4 | 0 | 0 | 0·1 | 26 | VII |
| nimR21 | 895 | 1·6 | 0·8 | 0·2 | 0·6 | 0·1 | 26 | II |
| nimS22 | 903 | 0 | 0·2 | 0 | 0 | 0·4 | 33 | VI |
| nimT23 | 911 | 0 | 0·2 | — | — | 0·7 | 53 | II |
| nimU24 | 935 | 0 | 0·2 | — | — | 2·5 | 17 | VII |
| nimV25 | 945 | 2 | 1 | 0 | 0 | 1·8 | 17 | I |
| nimW26 | 966 | 1 | 0 | 1·6 | 0 | 0·4 | 19 | — |

### (iii) *Mutants with decreased mitotic indices*

Twenty-six mutant strains which had been originally isolated on the basis of increased internuclear distances exhibited a decrease in both CMI and SMI at 42 °C. In order to determine whether nuclear division was blocked in these strains a method was developed to measure nuclear division directly. This was based on the fact that conidia of *A. nidulans* are uninucleate and that nuclear division is synchronous in young, rapidly growing germlings until the first septum is formed (Rosenberger & Kessel, 1967). Thus the number of nuclear doublings (generations) that occur during any germination time, *t*, starting with a uninucleate spore, can

be determined by counting the number of nuclei per germling at $t$, since the number of nuclear doublings that occur during any germination time, $t$, equals the log of the number of nuclei per germling at time $t$ divided by the log of 2. In this way the number of nuclear divisions occurring under restrictive conditions of germination was determined for each of the mutants defective in mitosis (Tables 1, 2). Mutant conidia were inoculated on dialysis tubing on YAG and incubated at 42 °C for 18 h. The germlings were then fixed, stained with acid-fuchsin and scored under the microscope for the number of nuclei per germling. Although the wild-type parental strain underwent approximately eight nuclear doublings under these experimental conditions, many of the mutants failed to undergo any significant nuclear division ($D \leqslant 0{\cdot}4$). Some mutants underwent limited nuclear division during 18 h at restrictive temperature. These mutants were either leaky or were delayed with respect to the onset of the block. It was important to demonstrate that in these strains nuclear division was inhibited before inhibition of growth. In order to do this the length of the germlings was measured and divided by the number of nuclei per germling to give a measurement of average mycelial length per nucleus ($L/N$) for each of the mutants (Tables 1, 2). As controls, germlings of the wild type were grown to 10–40 $\mu$m (about 11 h), a length comparable with that produced by the mutants in 18 h at 42 °C. The wild-type germlings had a $L/N$ of 4–5 $\mu$m, but the mutants which germinated at 42 °C had $L/N$'s at least twice and often 5–10 times greater than those of the wild type. UVts 136 and 187 had $L/N$'s approximately 30 times greater than the wild type. The hyphal diameter of the mutants were similar to those of the wild type. The increased $L/N$'s of these mutants demonstrates that nuclear division stopped earlier than hyphal growth (and therefore before protein synthesis, etc.) even in those strains in which the block of nuclear division was leaky or delayed. The mutants in which nuclear division stopped before hyphal growth and in which the SMI and CMI dropped at restrictive temperature were clearly unable to enter mitosis and were therefore assigned the gene symbol *nim*, standing for *n*ever *i*n *m*itosis. Most of the *bim* mutants germinated poorly at 42 °C, but those conidia that germinated were usually able to undergo some hyphal elongation in the absence of nuclear division. *Bim*B2 and B3 underwent considerable hyphal elongation. Only one *nim* mutant *nim*M16 failed to germinate at 42 °C. This mutant was assigned the *nim* classification on the basis of its decreased mitotic indices at 42 °C.

### (iv) *Mutants defective in septation*

Five mutants (UVts 401, 402, 802, 864 and 964) which were defective in septation at restrictive temperature were given the gene symbol *sep* (Table 3). Two of these, UVts 802 and 864, were otherwise morphologically normal. The other three mutants, UVts 401, 402 and 964, had fat hyphae more than twice the diameter of the wild type. The ability of the aseptate mutants to grow and to undergo nuclear division was studied by allowing the mutants to germinate and grow at 42 °C for 18 h. All five mutants grew and underwent numerous divisions under these conditions (Fig. 4). It can be concluded that growth and nuclear division are not

Table 3. *Aseptate mutants*

| Gene symbol | Strain no. | Linkage group |
|---|---|---|
| sepA1 | UVts 401 | I |
| sepA2 | 402 | I |
| sepB3 | 802 | V |
| sepC4 | 864 | I |
| sepD5 | 964 | — |

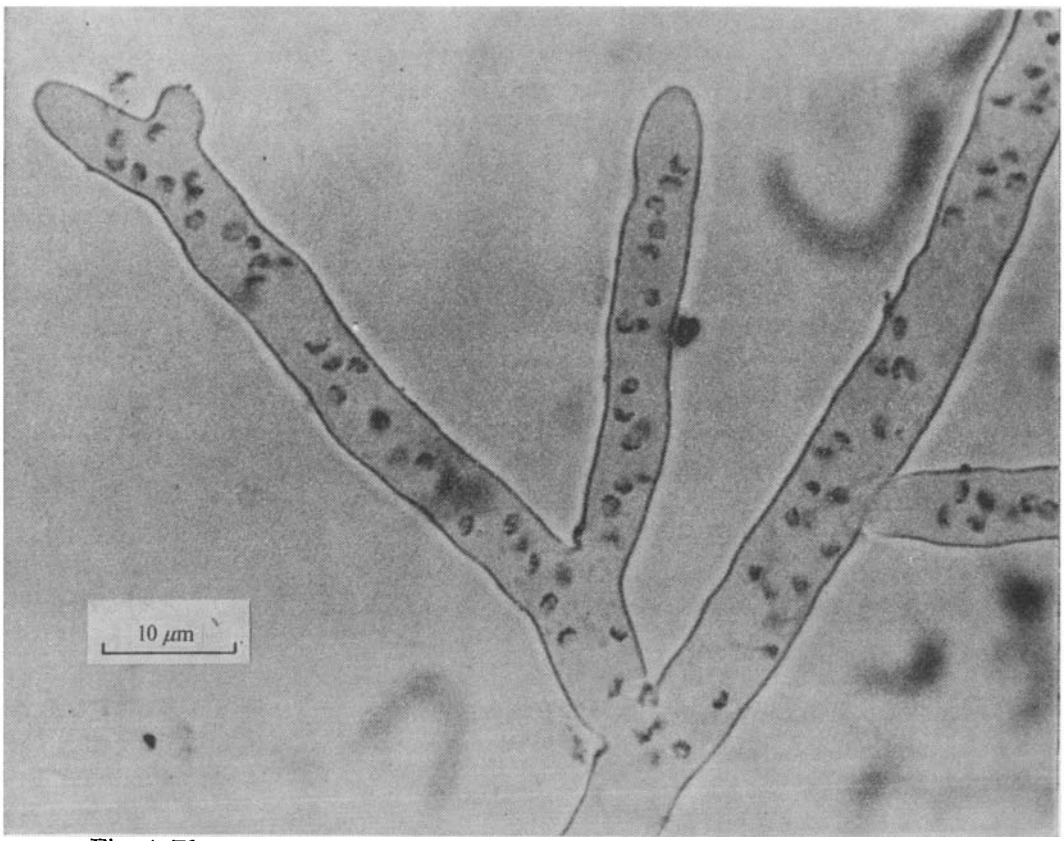


Fig. 4. Phenotype of *SepA2* grown at 32 °C overnight, switched to 42 °C for 4 h and stained with aceto-orcein.

necessarily dependent upon septation in *A. nidulans*. These mutants were originally selected for their inability to form colonies at 42 °C. Thus although growth and nuclear division are not limited at early times, failure to septate must impose some eventual restriction to unlimited increase in mass, such that colony formation is inhibited.

Normally, in *A. nidulans*, nuclei which occupy to same cytoplasm, i.e. are in the same hyphal segment, are synchronized in mitosis (Rosenberger & Kessel, 1967). In the *sep* mutants the absence of septa caused large numbers of nuclei to occupy the same cytoplasm. It seemed possible that much larger numbers of nuclei might be in mitotic synchrony in the *sep* mutants than in wild-type strains. This was observed. After 2 h at 42 °C individual hyphae in mitosis were observed to have

as many as 90 nuclei simultaneously in mitosis. However, not all the nuclei in the aseptate mycelium were in synchronous mitosis. But rather mitosis appeared to progress as a wave with nuclei in different stages of division in different parts of the mycelium, with some nuclei having completed and others not yet having entered mitosis. Mitosis also progresses as a wave in the wild type (Clutterbuck, 1970), but the size and extent of the wave is much greater in the mutant. The extended duration of mitosis was reflected in a CMI for UVts 401 of 14% after 2 h at 42 °C.

### (v) *Mutants with abnormal nuclear distribution*

Nuclei are normally distributed more or less uniformly along the mycelium in the parental strain FGSC 154 (Fig. 2). Five mutants (UVts 235, 320, 768, 970 and 976) have been found which after 4 h at restrictive temperature have an abnormal nuclear distribution such that nuclei were clustered near the hyphal septa and were few in number or absent along considerable lengths of mycelium (Fig. 5). The nuclei clustered near the septa tended to be small and to stain darkly with aceto-orcein. Although septal clusters of nuclei were prominent, nuclear clusters were also observed which were not associated with septa (Fig. 5). A few isolated nuclei were always present which were distant from clusters. These nuclei were widely separated, were normal in size, i.e. significantly larger than the clustered nuclei, and stained less intensely with aceto-orcein. In clustered nuclei mitotic spindles were observed to appear synchronously. Spindles were also occasionally observed in distant single nuclei of hyphal segments in which the clustered nuclei were in mitosis. The gene symbol *nud*, standing for *nu*clear *d*istribution, has been assigned to these mutants (Table 4).

Table 4. *Nuclear distribution mutants*

| Gene symbol | Strain no. | Linkage group |
|---|---|---|
| nudA1 | UVts 235 | — |
| nudB2 | 320 | VIII |
| nudC3 | 768 | I |
| nudD4 | 970 | — |
| nudE5 | 976 | — |

### (vi) *Complementation analysis*

Mutants were tested for complementation by determining the ability of heterokaryons formed between mutant pairs to grow at restrictive temperature (Fig. 1). All of the mutants formed heterokaryons easily except for UVts 244, which formed heterokaryons with a greatly reduced frequency. Five non-complementing groups of mutants were found: UVts 69 and 990 (*bim A1* and *A9*), UVts 136 and 187 (*bim B2* and *B3*), UVts 537 and 555 (*bim D5* and *D6*), UVts 92, 333, 469 and 597 (*nim A1*, *A5*, *A7* and *A8*), and UVts 401 and 402 (*Sep A1* and *A2*). The remaining mutants complemented each other. Mutants in the same complementation group were phenotypically similar, but phenotypically similar mutants were not necessarily or even usually in the same complementation group. All of the complementing strains were tested for syntrophism with negative results.

(vii) *Assignment to linkage groups*

Most of the mutants were mapped to linkage groups. Heterokaryons and diploids were formed between each of the mutants and FGSC 73 or FGSC 99 or both. Interestingly, UVts 244, although a poor heterokaryon former gave a high incidence of spontaneous diploid formation. All of the temperature-sensitive mutations tested were found to be recessive in both heterokaryons and heterozygous diploids. The diploids were haploidized on *p*-fluorophenylalanine and/or benlate, and the linkage between temperature sensitivity and known markers in the haploid segregants was used to determine the chromosomal assignments of the mutants. The chromosomal assignments and complementation groups of the mutants are given in Tables 1 and 2. Most of the diploids produced uncomplicated

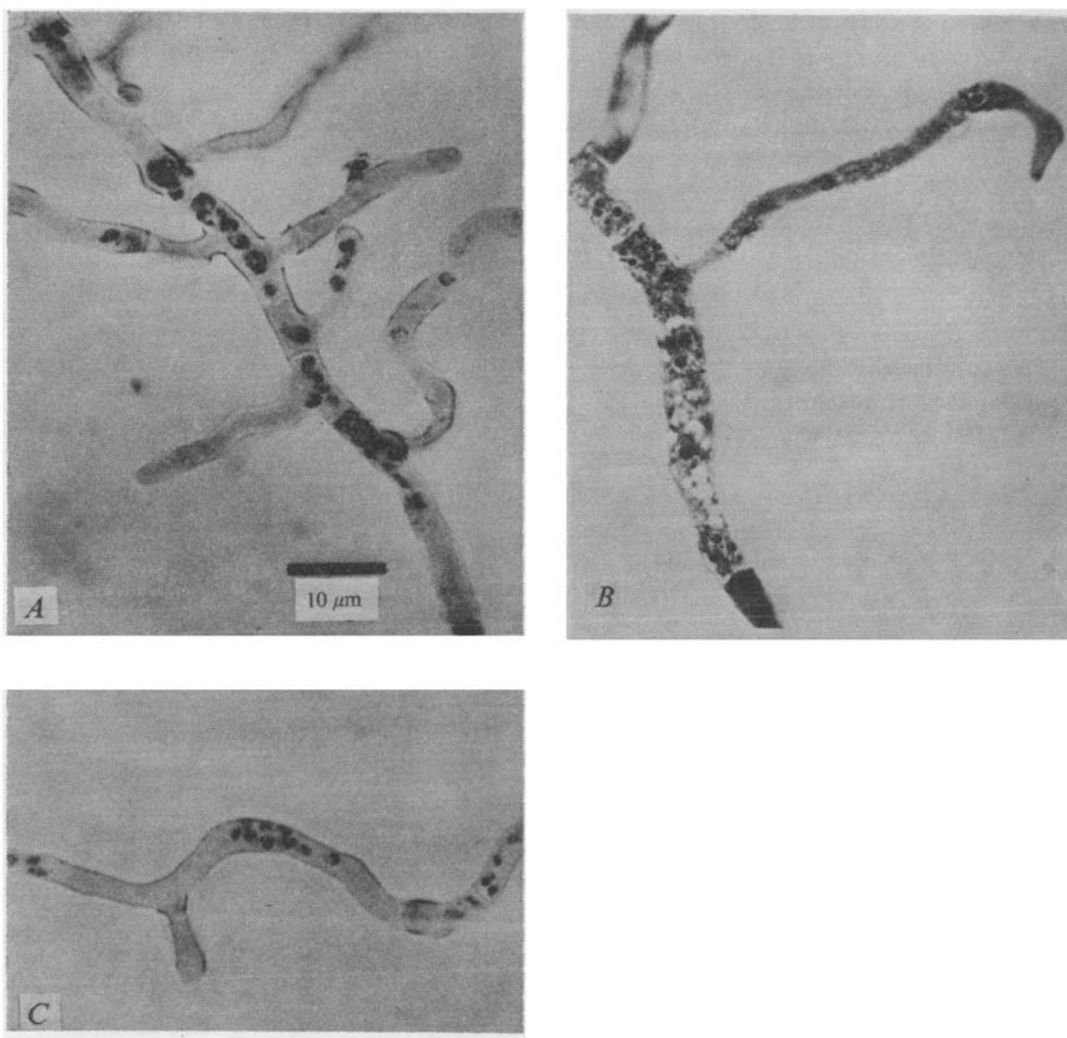


Fig. 5. Phenotype of *nud* mutants. *nudC3* grown for 12 h at 32 °C, switched to 42 °C for 4 h and stained (*A*) with aceto-orcein, (*B*) with acid-fuchsin to demonstrate mitotic spindles, (*C*) with aceto-orcein to demonstrate a non-septal cluster.

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results except that abnormal segregation ratios were found for genes located on chromosome III, because the marker used to identify this chromosome, *phen2*, caused segregants carrying this gene to be more sensitive to growth inhibition by *p*-fluorophenylalanine. A similar situation obtained in the case of the *bimE7* mutation in strain UVts 706: the number of temperature-sensitive haploid segregants recovered following haploidization on *p*-fluorophenylalanine was much lower than the expected 50 %. Only 21 of 658 segregants tested (3·2 %) were temperature-sensitive. Recovery of the *lac* marker on chromosome VI was equally low in this cross; however, *lac* and temperature sensitivity were linked and UVts 706 was therefore assigned to chromosome VI. This assignment to linkage group was confirmed in a separate experiment in which benlate was used to induce haploidization (Hastie, 1970). The number of ts and *lac* segregates recovered from benlate was also reduced but not so much as with *p*-fluorophenylalanine. In crosses of UVts 706 which were haploidized on benlate only 10 % of the segregants were temperature-sensitive.

The poor recovery of the *bimE7* and *lac* genes was eventually explained by the finding that strains carrying *bimE7* were more sensitive to growth inhibition by *p*-fluorophenylalanine than the wild-type parental strain (data not shown). Thus in the presence of *p*-fluorophenylalanine (and also to a lesser extent with benlate) there was a co-ordinate loss of both 6th chromosome markers. It is possible that *bimE7* also confers increased sensitivity to benlate. It was necessary to collect larger numbers of haploid segregants in order to map *bimE7* than to map the other mutations. No clustering of *bim* or *nim* genes with respect to linkage groups was noted. The *bim* mutations were located on chromosome I, III, IV and VI, and the *nim* mutations were located on all chromosomes except IV. The *sep* mutants mapped to chromosomes I and V, and the *nud* mutants to chromosomes I and VII. Thus there was no evidence of genetic clustering of phenotypically similar mutants.

## 4. DISCUSSION

Forty-five temperature-sensitive mutants of *Aspergillus nidulans* blocked in nuclear division, septation or nuclear distribution have been isolated, tested for genetic complementation and mapped to chromosome as a first step in the development of a new system for studying the biochemical genetics of mitosis. All of the mutations tested were recessive and all mapped to known chromosomal linkage groups. Most of the mutants were unique with respect to complementation analysis, therefore there must still be other mitotic mutants to be found, and for this reason we will continue to look for new mutants. The few mutants that were found to belong to the same complementation groups had similar phenotypes; however, mutants with similar phenotypes were not necessarily found to be in the same complementation group, as would be expected if many genes were involved in the regulation of mitosis. No obvious clustering of mutants on any one chromosome was apparent and mutations mapped to all eight linkage groups.

The most numerous mutants were those which were defective in mitosis. On the basis of mitotic index determinations these mutants were divided into two

classes: (1) mutants blocked in mitosis in which the mitotic indices rose at restrictive temperature, and (2) mutants unable to enter mitosis in which the mitotic indices dropped at restrictive temperature. Nine mutants exhibited increased chromosome and spindle mitotic indices at 42 °C. These mutants appeared either to be blocked in mitosis or to suffer a prolongation of mitosis and were assigned the gene symbol *bim* standing for *b*locked *i*n *m*itosis. The *bim* mutants comprise at least five complementation groups and map on at least five different chromosomes. The effect of the *bim* mutations was pleiotropic, i.e. both chromosome relaxation and disassembly of the mitotic spindle were blocked at restrictive temperature. Thus these processes would appear to be tightly coupled to each other and to be under common genetic control. It is possible that these strains are defective in a biochemical reaction which is either involved in or necessary for both chromosome relaxation and spindle disassembly. Two of the *bim* mutations were unusual. *Bim-7* exhibited a very rapid rise in both SMI and CMI and promises to be most useful as a tool for generating large numbers of nuclei in mitosis for biochemical and morphological study. *Bim-4* (UVts 244) was unique in that by light microscopy the spindle was minute. The details of spindle structure in this strain await elucidation by electron microscopy, but it is possible that the spindle is unipolar or resembles the structure found in Hartwell's cell cycle mutant *cdc28* of *Saccharomyces cerevisiae* in which the block results in an aberrant spindle structure apparently required for nuclear fusion (Byers & Goetsch, 1973). UVts 244 also has a genetic peculiarity. Although it was a poor heterokaryon former, it formed diploids at a high frequency: roughly 50% of the fusion events with FGSC 73 or FGSC 99 resulted in diploid fans. This may be of some practical use in *A. nidulans* genetics since it allows rapid one-step recovery of diploids from heterokaryon mats.

Twenty-six mutants exhibited decreased spindle and chromosome mitotic indices. These mutants appeared to be unable to enter mitosis and were assigned the gene symbol *nim*, standing for *n*ever *i*n *m*itosis. In theory two different types of mutation would be expected to yield the *nim* phenotype: (1) mutations affecting events which are obligatory prerequisites to mitosis, e.g. DNA synthesis (see Mitchison, 1971) and (2) mutations causing defects in the processes of chromosome condensation and spindle assembly *per se*. The existence of obvious morphological differences (e.g. differences in $L/N$ ratios (see Table 2)) and of at least 23 different complementation groups among the *nim* mutants implies that defects in a number of genes can cause the *nim* phenotype. Although some of the *nim* mutants may have their primary lesion in DNA synthesis, it is unlikely that all of these mutants are so deficient, and many may be defective in as yet unknown functions. The ability of these strains to synthesize DNA at restrictive temperature has not yet been tested. It is interesting that in all of the mutants both chromosome condensation and spindle assembly are blocked. This suggests that these processes are coupled. It is possible that among the *nim* mutants we may find mutants defective in some biochemical reaction(s) necessary for both chromosome condensation and spindle assembly.

Five mutants in at least three complementation groups were isolated which were unable to form septa at restrictive temperature. These mutants, which were assigned the gene symbol *sep*, grow and are able to undergo several rounds of nuclear division at 42 °C, thus septation does not appear to be required for growth or mitosis in *A. nidulans*. This is consistent with observations on other eukariotes that growth and DNA synthesis can occur in the absence of cell division (Mitchison, 1971). Three of these strains not only lack septa but have fat mycelia, implying that some sort of generalized defect in regulation of wall synthesis occurs in these mutants.

Five strains in at least two different complementation groups have been isolated which are defective in nuclear distribution. These were assigned the gene symbol *nud*. The existence of these strains indicates that in the wild type there is a special mechanism which is responsible for the regular distribution of nuclei along the mycelium and that this mechanism is defective in the mutants. Such a mechanism is also suggested by the elongated nuclear shape of some of the *nim* mutants at 42 °C which look as though the nuclei are stretched between two attachment sites on the hyphal wall which have moved apart. If nuclei in *A. nidulans* are normally attached to the cell wall, then the *nud* mutants may be defective with respect to this attachment.

One of the unique advantages of *A. nidulans* for studying mitosis is that the chromosomes condense visibly at mitosis, in comparison, for example, to the chromosomes of *Saccharomyces cerevisiae*, 'which appear to be in much of the same condition at rest and during mitosis' (Robinow & Marak, 1966). It is not yet known whether yeast chromosomes fail to condense or are so small that they cannot be seen in the condensed state. Nevertheless, because the *A. nidulans* chromosomes condense visibly, this organism has an advantage over *S. cerevisiae* for studies of the inter-relationship between chromosome condensation and spindle formation and for studies of the biochemistry of chromosome condensation.

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