JOURNAL OF BACTERIOLOGY, Oct. 1978, 341-351
0021-9193/78/0136-0341$02.00/0

Vol. 136, No. 1
Printed in U.S.A.

Nuclear Division Cycle in Germinating Conidia of *Neurospora crassa*

LETICIA SERNA† AND DAVID STADLER*
Department of Genetics, University of Washington, Seattle, Washington 98195

Received for publication 26 May 1978

A temperature-sensitive mutant has been shown to be blocked at a specific point in the nuclear division cycle: just before the initiation of DNA synthesis at the time when the spindle pole bodies have duplicated but not separated. The metabolic activities of conidia of this mutant strain at the nonpermissive temperature have led us to conclude that the nuclei in a population of dormant conidia are arrested at various points in the nuclear division cycle. This conclusion is substantiated by the activities of conidia in the presence of the inhibitory drugs cycloheximide and hydroxyurea. In each inhibitory situation we observed that some, but not all, of the conidia were able to accomplish DNA synthesis and/or nuclear division.

The macroconidium of *Neurospora* is a vegetative spore which can remain dormant for weeks or months and then germinate and grow when placed in a suitable medium. We might expect this specialized cell to be arrested at a specific point in the mitotic division cycle, and thus a population of these conidia placed in growth medium would provide a synchronized population of growing cells. This situation has been reported for the germinating spores of some fungi and bacteria (15). However, the germinating conidia of *Neurospora* show no evidence of synchrony. Whether one monitors germination or nuclear division or DNA synthesis, one finds that different cells in the population begin these activities at different times over a range of several hours.

Two hypotheses to account for the asynchrony are diagrammed in Fig. 1. The first states that all conidia are arrested at the same point in the nuclear division cycle, but they experience differing lag periods before commencing growth. The second hypothesis states that all the conidia resume progress through the mitotic cycle at the same time, but they start from different arrest points in that cycle.

This paper describes studies of a temperature-sensitive mutant which is blocked at a specific point in the nuclear division cycle. The activities of germinating conidia of this strain at permissive and nonpermissive temperatures and in the presence and absence of inhibitory drugs lead us to favor the second hypothesis: that the nuclei of dormant conidia are arrested at various points in the nuclear division cycle.

† Present address: Facultad de Education, Universidad de Caldas, Manizales, Caldas, Colombia.

MATERIALS AND METHODS

Strains. An inositol-requiring *Neurospora crassa* strain (*inl* **a**, allele number 89601) was used as the wild-type control for all of the experiments reported here, because the temperature-sensitive mutant strain *inl 3B* **a** was isolated from it as a vegetative descendent after UV treatment. These two strains will be referred to as *inl* and *3B*. All growth studies in both strains were performed in medium supplemented with inositol.

Isolation of temperature-sensitive mutants. Condia of *inl* were treated with UV (to yield about 20% survival) and then subjected to inositol-less death (incubation in minimal medium at the nonpermissive temperature) to enrich the surviving population for temperature-sensitive mutants (8). Surviving conidia were then permitted to form colonies on inositol-supplemented medium at 20°C. These colonies were isolated and grown, and conidial suspensions were tested for growth. Those which failed to grow at 37°C but grew well at 20°C were deemed to be temperature-sensitive mutants.

Microscopic analysis of germination and nuclear number. Germination was scored in stained or unstained material as the visible protuberance of a germ tube from the roughly spherical conidium. Nuclei were stained by a modification of the Giemsa technique of Loo (10). Conidia were fixed in 3.7% formaldehyde in 0.9% NaCl for at least 30 min and then pelleted and rinsed once with water. Hydrolysis was done at 60°C for 15 min by resuspending the pellet in 1 M HCl. After hydrolysis the suspension (still in HCl) was cooled in ice and spread on microscope slides. Air-dried slides were rinsed in buffer (0.067 M potassium phosphate, pH 6.9) and immersed in stain (20 parts buffer to 1 part stock Giemsa stain from Fisher Sci-

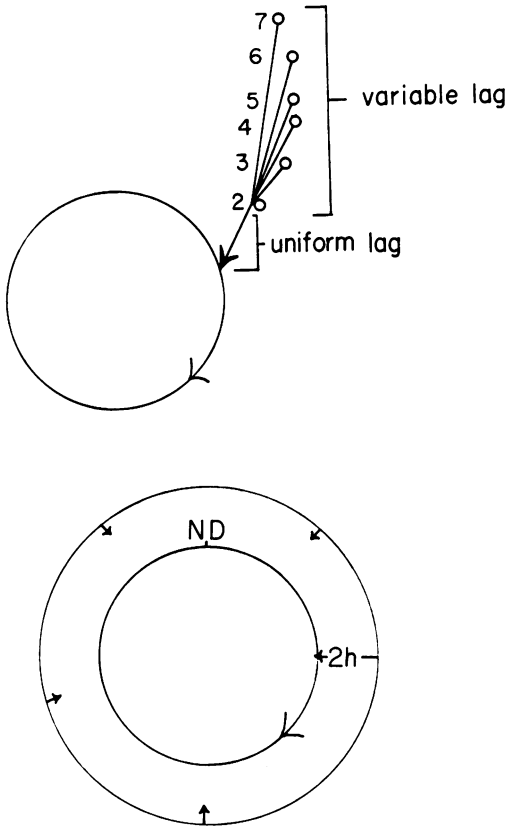

FIG. 1. *Two hypotheses to account for the asynchrony of germinating conidia. The circles represent the mitotic cycle. ND, Nuclear division.*

entific Co.) for 25 to 30 min. Slides were rinsed with water until no more leaching was visible, air dried, and examined at ×1,000 under immersion oil. Such preparations could be kept for more than 3 months in good condition.

Assays of nucleic acids and proteins. The fluorometric assay of DNA with 3,5-diaminobenzoic acid was done by a modification of the method of Kissane and Robins (7) as described by Hopper et al. (6). Triplicate samples of 2×10^6 cells were taken for each measurement.

Nucleic acid and protein synthesis were monitored by the incorporation of [^{14}C]uracil and [^{3}H]leucine into trichloroacetic acid-precipitable material. DNA was measured by ^{14}C incorporation into material precipitated in trichloroacetic acid after overnight hydrolysis in 1 M NaOH (9).

EM examination. A total of 10^8 cells was fixed in gluteraldehyde solution (3% gluteraldehyde–0.1 M cacodylate, pH 6.8) and rinsed twice with water. Cell wall was digested with 0.2% glusulase in 0.03 M phosphate buffer, pH 6, containing 0.6 M sucrose, for 1 h at 37°C. The cells were prepared for thin-section electron microscopy (EM) as described by Byers and Goetsch (2).

Measurement of DNA content of conidia by flow-microfluorimetry. A total of 10^8 cells was fixed in 70% ethanol, pelleted, and stained with 1 ml of mithramycin solution (0.1 mg of mithramycin per ml and 0.015 M $MgCl_2$ in 0.9% saline) at 22°C for 20 min in the dark. The suspension was then diluted with $MgCl_2$-saline and assayed in a flow-microfluorimeter. This instrument measures the DNA content of a large number of individual cells. The cells flow in single file past a laser beam. As each cell passes the beam, the individual fluorescence is detected, amplified, and recorded by a computer terminal which then provides a plot of the distribution of the DNA content within the population of cells.

RESULTS

Germination of wild-type conidia. The time courses of germination, DNA synthesis and nuclear division in freshly incubated conidia of *inl* are shown in Fig. 2, 3, and 4. A steady rise in the percentage of germinated cells, amount of DNA, and nuclear number is seen to continue over a period of several hours. Evidence reported later in this paper indicates that S, G2, and nuclear division all occupy rather small proportions of the nuclear division cycle. For a population of cells with synchronized nuclei, we should expect the plot of DNA or nuclear number to show an abrupt rise with a plateau on either side. No such indication of synchrony is observed.

One possibility is that the asynchrony of germinating conidia results from different cells experiencing varying lag periods. What feature of the physiological state of the conidium might determine this lag period?

One known variable within a population of conidia is age. A fresh slant culture may start forming conidia on the second day and continue

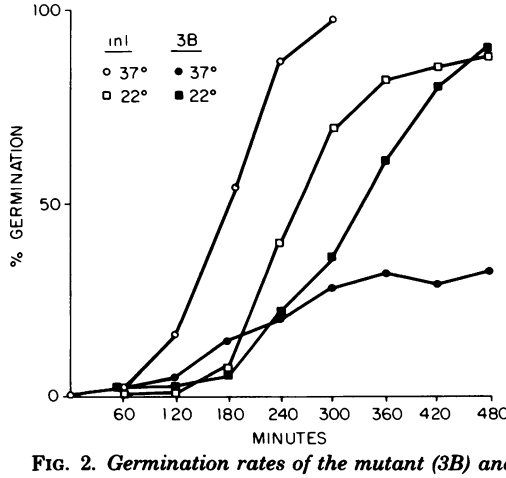

FIG. 2. *Germination rates of the mutant (3B) and the control (inl) strains at 22 and at 37°C.*

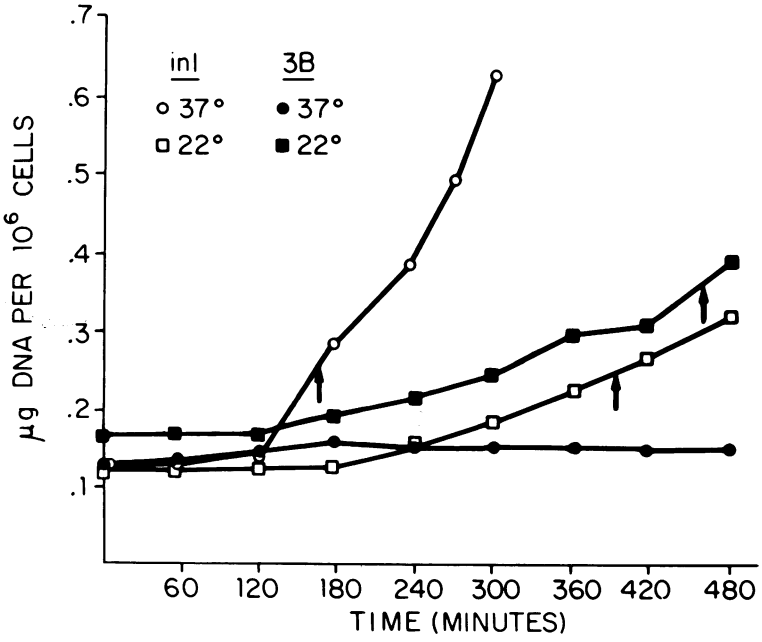

FIG. 3. *DNA synthesis by germinating conidia of the mutant (3B) and the control (inl) strains at 22 and at 37°C.*

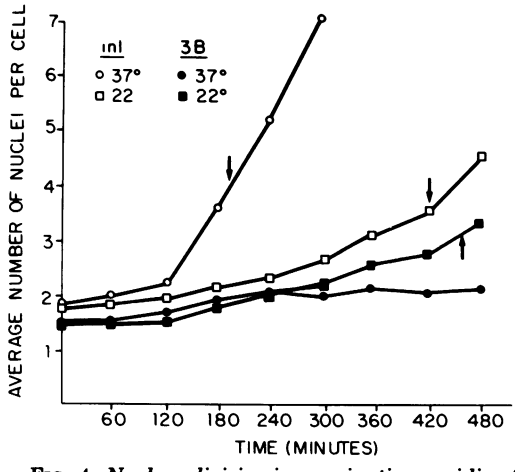

FIG. 4. *Nuclear division in germinating conidia of the mutant (3B) and the control (inl) strains at 22 and at 37°C.*

for several days; thus, there may be a considerable spectrum of ages for different conidia in the same tube. We tested the effect of conidial age on germination rate by measuring the time course of germination for conidia from four identical slant cultures of *inl* which were 5, 10, 15, and 20 days old. All four cultures showed identical germination kinetics, indicating no effect of conidial age.

Another variable within a population of conidia is nuclear number. The macroconidia of *Neurospora* may have one to four haploid nuclei (and a few have even more). The relationship of nuclear number to germination has been studied by Loo (10) by counting nuclei in cells which were stained after various periods in growth medium. There was no evidence that rate of germination was related to nuclear number in the conidium. As germination proceeded, the average nuclear number of ungerminated cells remained relatively constant, suggesting that cells were being withdrawn from the ungerminated population without regard to nuclear number.

Temperature-sensitive mutant *3B*. Forty-three mutant strains were isolated from *inl* conidia after UV treatment and inositol starvation at 37°C and were screened for macromolecular synthesis during conidial germination by the incorporation of radioactive substrates (9; D. Stadler, unpublished data). In about half the mutant strains, the level of incorporation was significantly reduced at 37°C compared with that of the wild-type conidia; in nearly all cases the reduced incorporation was seen at least as early in protein as in RNA or DNA. However, conidia of *3B* stopped incorporating label into DNA about 20 min before the arrest in protein and RNA. This focused our attention on *3B* as a mutant which might have a lesion in DNA synthesis or nuclear division.

The activities of freshly incubated conidia of strain *3B* are shown in Fig. 2, 3 and 4. Germination, DNA synthesis, and nuclear division proceed in normal fashion at 22°C, although the lag period is somewhat longer than in the wild-type cells. At 37°C, these activities all begin in the *3B* cells and then arrest abruptly. There is about 20% of a doubling of the DNA before arrest, and about one-third of the nuclei divide and a similar fraction of the cells germinate.

The temperature sensitivity of strain *3B* is not limited to the conidial stage. Conidia were permitted to germinate and form young mycelia at 22°C and then transferred to 37°C. Growth ceased within 2 h after the shift. Other observations indicate that this strain is temperature sensitive at all stages of the vegetative life cycle.

Conidia of *3B* were incubated for 4 h at 37°C and then prepared for thin-section EM. All 20 nuclei which were examined showed the same phenotype: the spindle pole body had divided but failed to separate (Fig. 5). Conidia grown for 6 h at 22°C followed by 6 h at 37°C were prepared in the same manner. The same stage (double-spindle pole body) was seen in all nuclei. The EM observation suggested that strain *3B* was blocked at a specific point in the nuclear division cycle when incubated at 37°C, and temperature shift experiments (described below) have confirmed this interpretation. Therefore, we have designated the mutant *ndc-1*.

(The control experiments for the EM study suggest that this stage is not as precise and restricted in *Neurospora* as it is in the mitotic cycle of other organisms. EM preparations of *3B* conidia after 6 h of incubation at the permissive temperature or of *inl* conidia after incubation at either temperature all showed a high frequency of nuclei in the double-spindle pole body stage. At least half the nuclei in each preparation were clearly in this stage, and some of the remaining ones were doubtful. In yeast [2], microtubules are seen to be attached to the spindle pole body, and they aid in the identification of the stages of its development. In our *Neurospora* preparations, microtubules were seen only rarely in association with the spindle pole bodies. An earlier EM study of *Neurospora* [16] reported the reg-

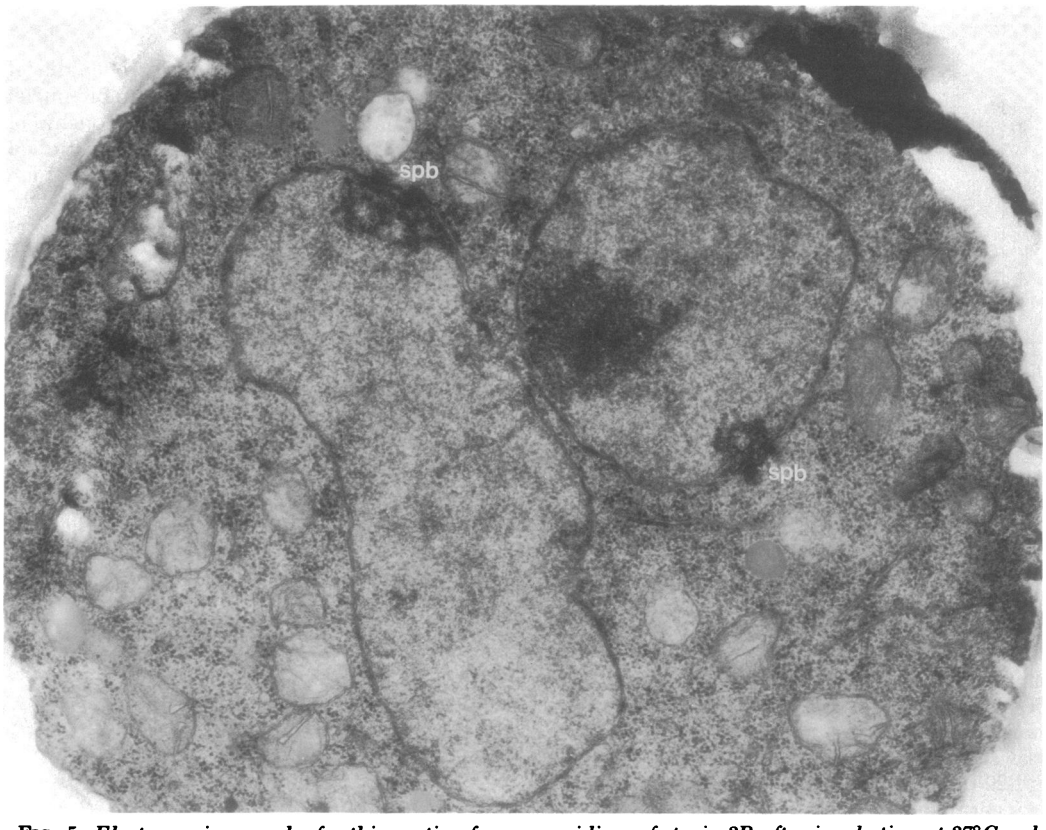

FIG. 5. *Electron micrograph of a thin section from a conidium of strain 3B after incubation at 37°C. spb, Spindle pole body.*

ular presence of a conspicuous dense granule which the authors suggested was the initiator of spindle pole body formation. In our material this dense granule was seen only sporadically.)

The dominance of the *ndc-1* mutant gene was studied by measuring the growth rate at 37°C of a heterokaryon that was *ndc-1*/+. The + allele at the *ndc-1* locus was provided by a strain carrying the mutant gene *pan-2*. Thus, the heterokaryon could be "forced" on minimal medium by the inositol requirement of one component and the pantothenate requirement of the other. The growth rate of this heterokaryon at 37°C was compared with that of a control heterokaryon: *inl* + *pan-2*(+/+ at the *ndc-1* locus). The growth rates of the two heterokaryons were found to be identical, whether measured as weight of conidia growing in shaking liquid culture (doubling time, 2 h) or as linear growth rate along an agar surface in a "race tube" (4.8 mm/h). Thus, the *ndc-1* mutant gene appears to be completely recessive.

The EM phenotype of nuclei at the *ndc-1* block suggests some direct control of the mitotic apparatus, so we were led to enquire whether *ndc-1* might be nucleus limited in its action. If so it would impede the division of the *ndc-1* nucleus at 37°C, even in a heterokaryon with a nucleus carrying the wild-type allele. Duplicate cultures of the *ndc-1*/+ heterokaryon used in the dominance test were grown at various temperatures, and the nuclear ratio was monitored by measuring the frequencies of the three genetic types of conidia (Table 1). The relative frequencies of the *ndc-1* nuclei were the same in heterokaryons grown at permissive and nonpermissive temperatures; thus, there was no evidence of a nucleus-limited effect on division. This experiment was repeated with a second *ndc-1*/+ heterokaryon in which *met-5* (methionine requiring) replaced *pan-2* as the forcing marker, and it was compared with a control heterokaryon which had the same markers without the *ts* mutant (Table 1). Again the results indicate no nucleus-limited effect of *ndc-1* on mitotic rate at 37°C.

TABLE 1. *Genetic ratios of conidia produced by heterokaryons grown at different temperatures*

Heterokaryon	Heterokaryotic culture growth temp (°C)	No. of conidia			Percent *inl* homokaryons
		Heterokaryons (prototrophs)	Homokaryons		
			pan met	*inl*	
ndc inl + / + + *pan*	20	65	74	13	8.6
	30	38	55	9	8.8
	37	42	46	9	9.3
ndc inl + / + + *met*	20	36	27	33	34
	37	61	23	37	31
+ *inl* + / + + *met*	20	40	30	28	29
	37	100	17	40	25

Crosses of *ndc-1* to the wild type yielded only about 20% temperature-sensitive cultures among the viable ascospore progeny. However, dissected tetrads showed that this departure from 1:1 segregation resulted from reduced viability of the mutant ascospores; in the few tetrads in which all four spore pairs grew there was regular 2:2 segregation, showing that *ndc-1* is determined by a single gene. Furthermore, these crosses showed that *ndc-1* was linked to *inl* and was thus on linkage group V. A cross to another group V marker, *arg-4*, gave the results shown in Table 2 and led to the conclusion that the *ndc-1* gene is about two map units from *arg-4* and proximal to it (order is *ndc-arg-inl*).

Effects of drugs on conidia. Cycloheximide, an inhibitor of protein synthesis, has been shown to inhibit DNA synthesis at the beginning of the S phase in yeast (5, 14, 17). In *Physarum*, cycloheximide continues to inhibit synthesis later in S (3). It has been suggested that protein synthesis is needed only for the initiation of replication forks, and these start all at once at the beginning of S in yeast, but in a sequential fashion in *Physarum*.

Conidia of *ndc-1* were incubated at 22°C in the presence of 100 μg of cycloheximide per ml. Protein synthesis was completely arrested, and DNA synthesis stopped after 7 ± 3% of a doubling (Fig. 6). There was no nuclear division and no germination; this is not surprising, because both these processes are known to require protein synthesis (10, 11).

Hydroxyurea (HU) has been shown to specifically block DNA synthesis, probably stopping elongation by inhibiting nucleoside diphosphate reductase (13). Conidia of mutant *ndc-1* were incubated for 8 h at 22°C in the presence of 0.1 M HU. Viability platings showed that virtually 100% of the conidia survived this treatment. There was no DNA synthesis (Fig. 6), and meas-

TABLE 2. *Analysis of the cross* $\frac{ndc\text{-}1 \; + \; inl}{+ \; arg\text{-}4 \; +}$

Genotype	Random-germinated ascospore progeny	
	No. of ascospores	Crossover region
+ *arg* +	92	None
ndc + *inl*	23	None
+ *arg inl*	3	*arg-inl*
ndc + +	2	*arg-inl*
+ + *inl*	2	*ndc-arg*

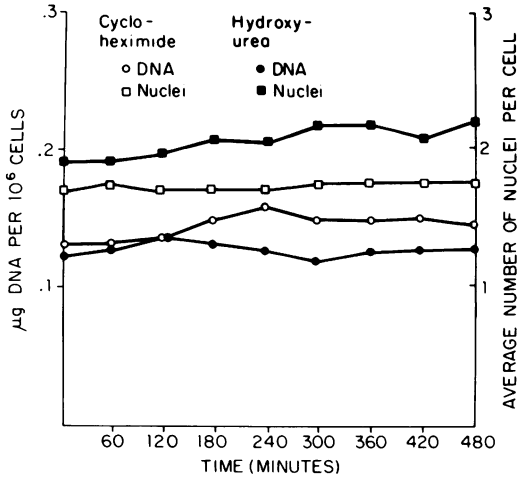

FIG. 6. *DNA synthesis and nuclear division by conidia of strain ndc-1 in the presence of cycloheximide or hydroxyurea.*

urements of incorporation of radioactive substrates indicated that protein and RNA synthesis were much reduced and stopped at about 8 h. However, about 15% of the nuclei divided (Fig. 6), and about 20% of the cells germinated.

We have attempted to determine the sequential relationship of the *ndc-1* gene function to the HU block of DNA synthesis. The rationale of this kind of experiment has been clearly explained by Hartwell (4). If *ndc-1* and HU block two different steps which are necessary to complete a developmental sequence, the *ndc-1* step can be related to the HU step in one of four ways (Table 3). In relationship 1, they may be related in a dependent sequence such that *ndc-1* function cannot occur until the HU-sensitive step is completed. In relationship 2, they may be related in a dependent sequence such that the HU-sensitive step cannot occur without completion of the *ndc-1* function. In relationship 3, the two steps may be independent such that either the HU or *ndc-1* step can occur in the absence of the other. In relationship 4, they may be interdependent such that neither can occur in the absence of the other. To test for these possibilities it is important that: (i) the two restrictive conditions be independent of one another, and (ii) the blocks are reversible.

These four possibilities can be distinguished by following DNA synthesis as 3,5-diaminobenzoic acid-reactive material and counting nuclei in Giemsa-stained cells in the two following experiments.

Experiment 1. Experiment 1 consisted of two successive incubations, the first one at 37°C for 5 h in the absence of HU. These conditions are restrictive for the temperature-sensitive step and permissive for the HU step. Then cells were

TABLE 3. *Possible relationships between 3B and HU steps and expected results*

Relationship	Expt 1[a]	Expt 2[b]
1. Dependent HU → *3B* →	+	−
2. Dependent *3B* → HU →	−	+
3. Independent HU → ; *3B* →	+	+
4. Interdependent (HU, *3B*) →	−	−

[a] For experiment 1, incubation 1 was at 37°C without HU, and incubation 2 was at 22°C with HU. + indicates that DNA synthesis and nuclear division would occur; − indicates that they would not.

[b] For experiment 2, incubation 1 was at 22°C with HU, and incubation 2 was at 37°C without HU.

transferred to the second incubation in which 0.3 M HU was added for 7 h at 22°C. These conditions are restrictive for HU and permissive for the temperature-sensitive step. If a doubling of DNA and nuclear number occurred, the cells had completed the HU-sensitive step when the temperature-sensitive step was blocked; in this case the relationships 2 and 4 of Table 3 would be ruled out. If no DNA synthesis and nuclear division occurred, the HU-sensitive step had not been completed and relationships 1 and 3 of Table 3 would be ruled out.

Experiment 2. Experiment 2 was done to distinguish the remaining relationships. Conidia of *ndc-1* were incubated at 22°C in the presence of 0.1 M HU for 5 h. These conditions are restrictive for the HU-sensitive step and permissive for the temperature-sensitive step. Then the HU was removed and the incubation was continued for 7 h at 37°C (a lower HU concentration is used in this experiment to keep the cells blocked but allow good recovery; the controls show that this was valid). If DNA synthesis and nuclear division occurred, the temperature-sensitive step was completed while the HU-sensitive step was blocked, ruling out relationships 1 and 4 of Table 3. If no DNA synthesis and nuclear division occurred, the temperature-sensitive step was not completed, and relationships 2 and 3 of Table 3 could be ruled out.

In experiment 1 (Fig. 7) neither DNA synthesis nor nuclear division occurred after the shift, ruling out relationships 1 and 3 from Table 3. The other two possibilities are distinguished by experiment 2 (Fig. 8), in which a doubling of DNA and about 70% increase in number of nuclei occurred after the shift, ruling out relationship 4. Therefore, it can be concluded that relationship 2 holds and that the product of gene *ndc-1* is needed before the HU block and presumably before initiation of DNA synthesis. (The fact that nuclear division did not complete

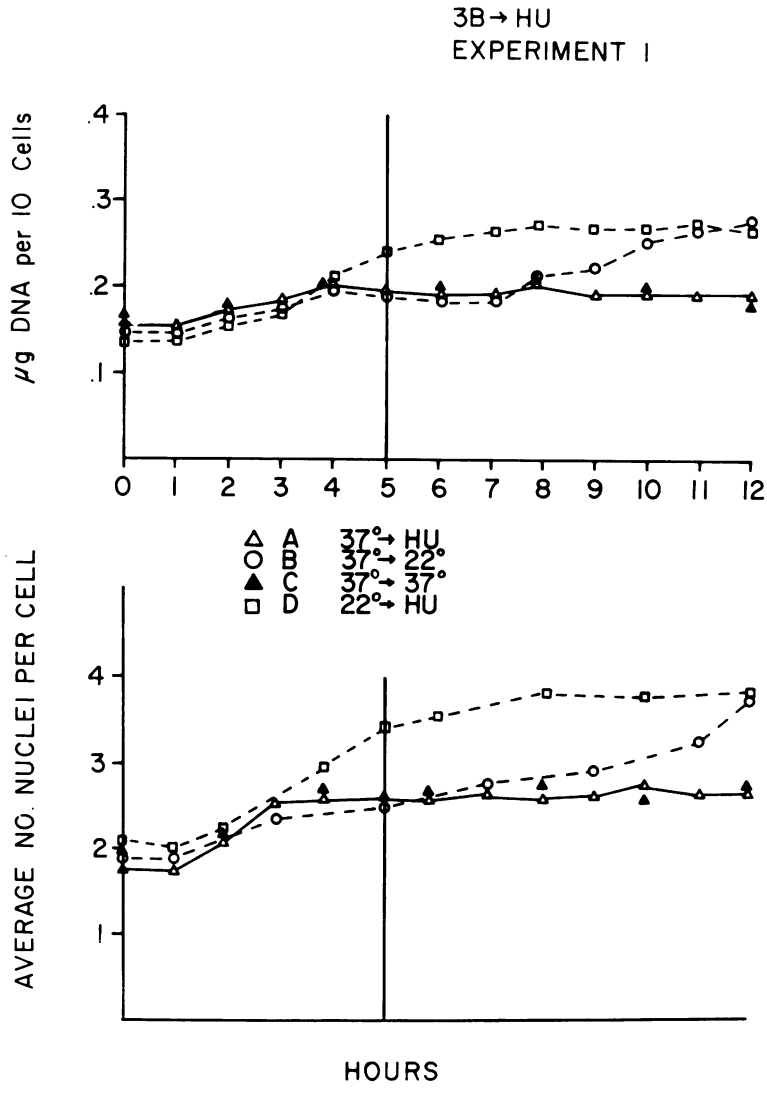

FIG. 7. *Experiment 1.* △, *Temperature restriction followed by HU restriction; top: DNA measured as 3,5-diaminobenzoic acid-reactive material; bottom: number of nuclei counted in Giemsa-stained cells. Controls (dashed lines):* ○, *temperature restriction followed by permissive conditions;* ▲, *temperature restriction throughout;* □, *permissive conditions followed by HU restriction.*

a doubling could be due to side effects of the *ndc-1* mutation.)

Observations by flow-microfluorimetry. Conidia of *ndc-1* were stained with mithromycin solution at 0, 3, and 6 h of incubation at 22°C and analyzed by flow-microfluorimetry (Fig. 9). There are well-defined peaks representing cells with DNA contents of 1c, 2c, and 3c. The valleys between successive peaks fall nearly to the base line, indicating that there are few cells with amounts of DNA between 1c and 2c or between 2c and 3c. Other experiments (Fig. 3) have demonstrated that this is an unsynchronized population in which DNA is being synthesized continuously. Therefore, we conclude that S must be brief, so that few cells are in S at any given time.

DISCUSSION

The EM analysis (Fig. 5) shows that when conidia of mutant strain *ndc-1* are incubated at 37°C, all of the nuclei arrest at the same point in the mitotic cycle. But one-third of these nuclei divide before reaching the arrest point. Similarly, HU is known to block a particular stage of

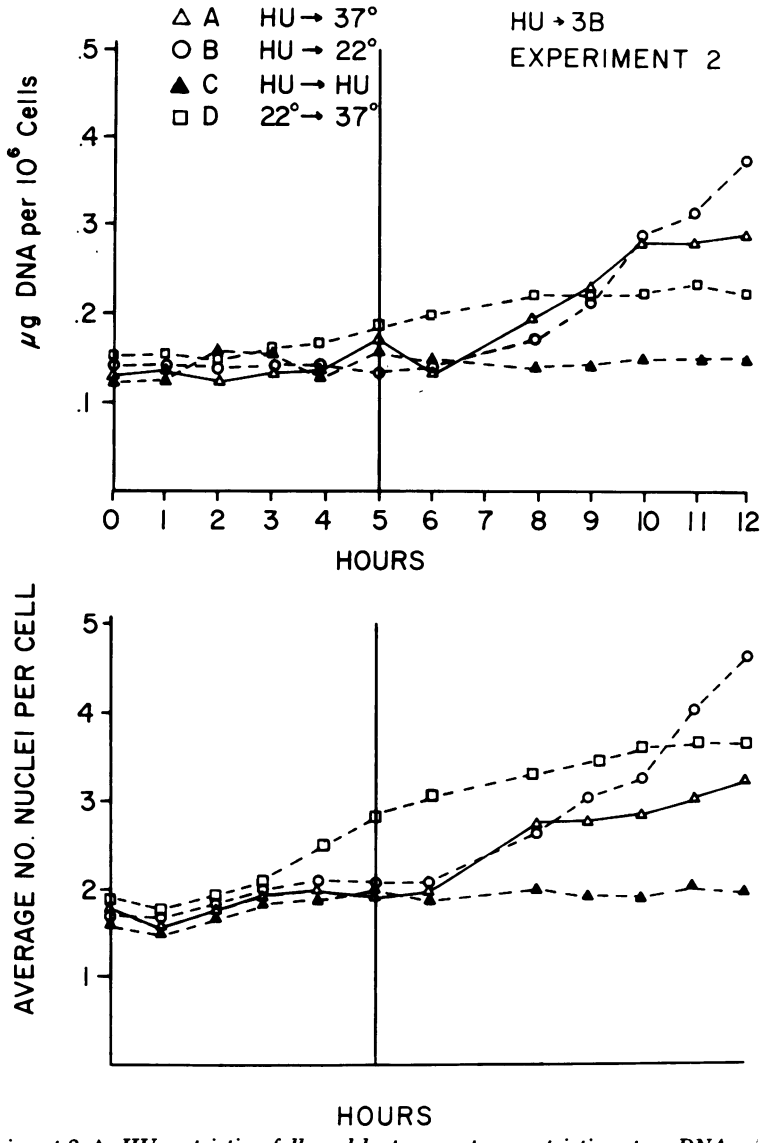

FIG. 8. *Experiment 2. △, HU restriction followed by temperature restriction; top: DNA measured as 3,5-diaminobenzoic acid-reactive material; bottom: number of nuclei counted in Giemsa-stained cells. Controls (dashed lines): ○, HU restriction followed by permissive conditions; ▲, HU restriction throughout; □, permissive conditions followed by temperature restriction.*

the mitotic cycle (DNA synthesis); yet a proportion of conidial nuclei (15%) divide before reaching this block. We believe that these observations can best be explained by the hypothesis that the nuclei of a population of dormant conidia are arrested at different points in the mitotic cycle.

Loo (10) studied another temperature-sensitive mutant of *Neurospora* which blocked protein synthesis. When incubating conidia were shifted from 37 to 20°C, she observed that some of the events which normally precede germination remained arrested until after nuclear division and germination began. One such event was DNA synthesis. She also observed, as we have here, that some nuclear division and germination occur in conidia in which DNA synthesis is prevented by HU. She concluded that some of the nuclei in conidia are arrested in G2.

The contention that some of the nuclei in dormant conidia are in G2 is substantiated by calculating the fraction of these cells which have

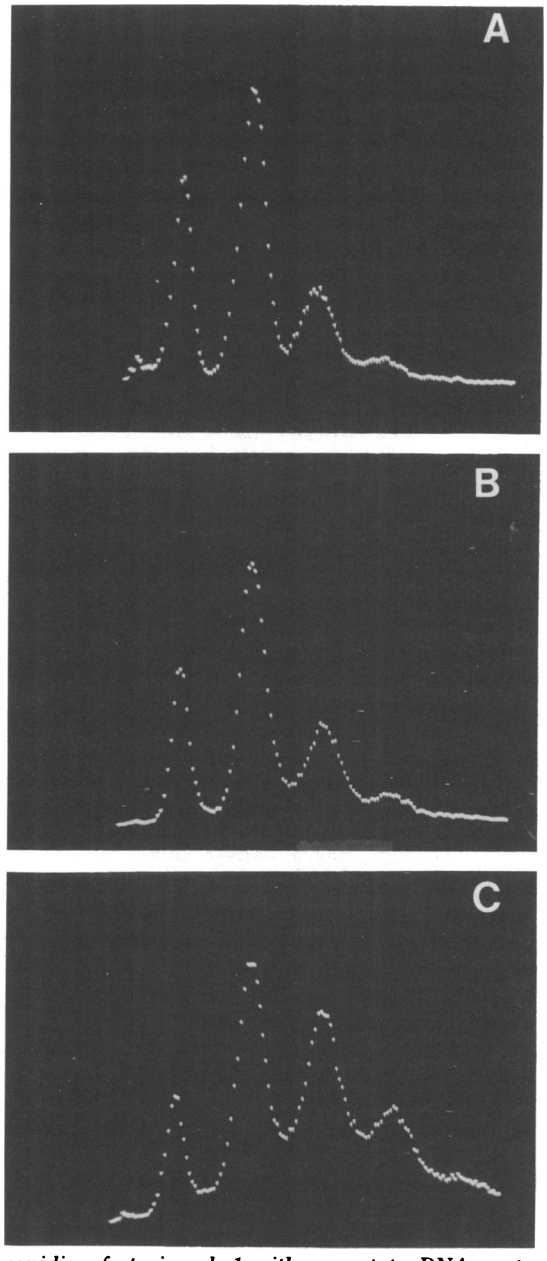

FIG. 9. *Distribution of conidia of strain ndc-1 with respect to DNA content as determined by flow-microfluorimeter analysis. The vertical axis is a linear plot of number of cells, and the horizontal axis is a linear plot of DNA per cell. (A) Freshly suspended conidia; (B) cells which had incubated for 3 h at 22°C; (C) represents cells after 6 h at 22°C.*

a single haploid complement (1c) of DNA. This is done by measuring the fraction of the total area which is under the left-hand peak of the flow-microfluorimetric analysis (Fig. 9, top), and the result is 25%. However, nuclear staining shows that 43% of *ndc-1* conidia are uninucleate. Thus, a significant fraction of the uninucleate cells must be in the 2c peak.

Let us assume that these nuclei may be arrested at any point in the cycle and that the fraction of the nuclei in any given stage is a measure of the comparative length of that stage.

This permits us to estimate the lengths of some of these stages. The 15% of the nuclei which divide in the presence of HU represent the fraction of the cycle taken up by G2 and mitosis. The 7% of a doubling of DNA which takes place in cycloheximide represents 14% of the nuclei caught at various points in S beyond initiation (assuming all the replication forks initiate at the beginning of S, as in yeast). The 33% of the nuclei of conidia of *ndc-1* which divide at 37°C place the *ndc-1* block a short time (about 4% of the cycle) before the beginning of S. These conclusions are represented in Fig. 10.

The macroconidia of *Neurospora* are specialized cells which have distinct differences in levels of various metabolites from those shown by germinating conidia or mycelia (13). The experiments reported here indicate that these metabolic changes are not dependent on any specific stage of the nuclear division cycle.

It is not clear whether germination is directly dependent on the nuclear division cycle. Loo (10) has observed that the average nuclear number in freshly germinated conidia is one (or slightly more) greater than that of the ungerminated conidia, suggesting that a single mitosis might be a prerequisite to the formation of a germination tube. In yeast Byers and Goetsch (1) have observed that bud emergence invariably requires the double-plaque stage of mitotic spindle formation. The bud arises very near the double plaque and is connected to it by microtubules. It is possible that germination has a similar dependence on a particular stage of mitotic spindle formation.

The nonsynchrony of different nuclei in a population of germinating conidia may even be manifest for the different nuclei within the same multinucleate cell. If all nuclei in such a conidium were arrested at the same point in the mitotic cycle, we might expect them to divide simultaneously. Loo (10) pointed out that in such a population we would expect the number of cells with odd numbers of nuclei (especially three) to decrease rapidly. Her results show that this does not happen and suggest that the separate nuclei in a multinucleate conidium are autonomous in the control of mitosis. Asynchrony of different nuclei in the same conidium was also observed in *Aspergillus,* when germination took place in suboptimal growth conditions (12). This is in contrast to the situation which has been demonstrated in *Physarum* and other ceonocytes and even in "heterokaryons" between chick erythrocytes and HeLa cells (11).

Nothing is known of the nature of the product of gene *ndc-1* except that its function is required for the continuation of the mitotic cycle at about the time of initiation of DNA synthesis. Our heterokaryon experiments show that the normal allele is completely dominant to the mutant, and the normal gene product moves freely through the cytoplasm. The yeast mutant *cdc-4* corresponds to *ndc-1* in its EM phenotype, being blocked at the double-spindle pole body stage (1). *cdc-4* and two other mutants at other loci (*cdc-7* and *cdc-28*) are all blocked at the initiation of DNA synthesis, and their block points precede the HU block (4). However, each of these three mutants is blocked at a different point in the formation of the pole bodies and the spindle (1). Thus, they must control separate steps which are all prerequisites for DNA synthesis.

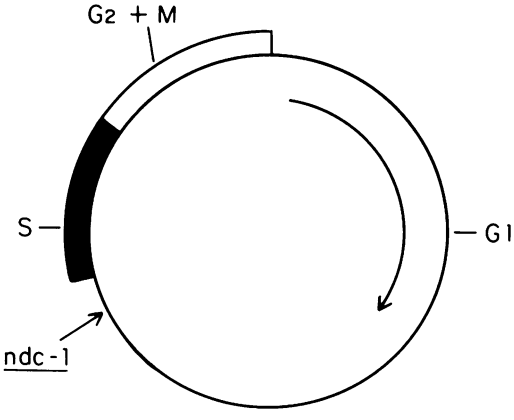

FIG. 10. *Relative lengths of the stages of the mitotic nuclear division cycle in conidia of Neurospora as indicated by the experiments reported here. The arrest point of mutant ndc-1 is indicated.*

ACKNOWLEDGMENTS

We are indebted to Loretta Goetsch and Breck Byers for the EM studies and to James Callus for the flow-microfluorimeter analysis. We are grateful to Melanie Loo for discussion and advice during the preparation of the manuscript.

This research was supported by a grant from the National Science Foundation. L. S. was supported by a scholarship from the Latin American Scholarship Program of American Universities.

LITERATURE CITED

1. **Byers, B., and L. Goetsch.** 1974. Duplication of spindle plaques and integration of the yeast cell cycle. Cold Spring Harbor Symp. Quant. Biol. **38**:123-131.
2. **Byers, B., and L. Goetsch.** 1975. Behavior of spindles and spindle plaques in the cell cycle and conjugation of *Saccharomyces cerevisiae.* J. Bacteriol. **124**:511-523.
3. **Cummins, J. E., and H. P. Rusch.** 1966. Limited DNA synthesis in the absence of protein synthesis in *Physarum polycephalum.* J. Cell. Biol. **31**:577.
4. **Hartwell, L. H.** 1976. Sequential function of gene products relative to DNA synthesis in yeast cell cycle. J. Mol. Biol. **104**:803-817.
5. **Hereford, L. M., and L. H. Hartwell.** 1973. Role of protein synthesis in the replication of yeast DNA. Na-

ture (London) New Biol. **244**:129-131.
6. **Hopper, A. K., P. T. Magee, S. K. Welch, M. Friedman, and B. D. Hall.** 1974. Macromolecule synthesis and breakdown in relation to sporulation and meiosis in yeast. J. Bacteriol. **119**:619-628.
7. **Kissane, J. M., and E. Robins.** 1958. The fluorometric measurement of deoxyribonucleic acid in animal tissues with special reference to the central nervous system. J. Biol. Chem. **233**:184-188.
8. **Lester, H. E., and S. R. Gross.** 1959. Efficient method for selection of auxotrophic mutants of *Neurospora*. Science **129**:572.
9. **Loo, M.** 1975. *Neurospora crassa* temperature-sensitive mutant apparently defective in the initiation of protein synthesis. J. Bacteriol. **121**:286-295.
10. **Loo, M.** 1976. Some required events in conidial germination of *Neurospora crassa*. Dev. Biol. **54**:201-213.
11. **Prescott, D. M.** 1976. Reproduction of eukaryotic cells. Academic Press Inc., New York.
12. **Rosenberger, R. F., and M. Kessel.** 1967. Synchrony of nuclear replication in individual hyphae of *Aspergillus nidulans*. J. Bacteriol. **94**:1464-1469.
13. **Schmit, J. C., and S. Brody.** 1976. Biochemical genetics of *Neurospora crassa* conidial germination. Bacteriol. Rev. **40**:1-41.
14. **Slater, M. L.** 1973. Effect of reversible inhibition of deoxyribonucleic acid synthesis on the yeast cell cycle. J. Bacteriol. **113**:263-270.
15. **Sussman, A. S., and H. O. Halvorson.** 1966. Spores, their dormancy and germination. Harper & Row, Publishers, New York.
16. **Van Winkle, W. B., J. J. Biesele, and R. P. Wagner.** 1971. The mitotic spindle apparatus of *Neurospora crassa*. Can. J. Genet. Cytol. **13**:873-887.
17. **Williamson, D. H.** 1973. Replication of the nuclear genome in yeast does not require concomitant protein synthesis. Biochem. Biophys. Res. Commun. **52**: 731-740.