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Initial Characterization of *Aspergillus nidulans* Mutants Blocked in the Nuclear Replication Cycle

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Several hundred temperature-sensitive mutants of *Aspergillus nidulans* were screened for ability of their conidia to produce germ tubes at the nonpermissive temperature while still remaining with the original single conidial nucleus. Eleven such strains representing 11 different complementation groups were found. One of the strains accumulated condensed chromosomes at 42 C, the nonpermissive temperature, and thus had a temperature-sensitive step in the mitotic sequence. Five mutants made protein and ribonucleic acid at 42 C, but synthesized deoxyribonucleic acid at a greatly reduced rate. These were presumed to have mutations in steps related to chromosome replication. Other strains continued to make deoxyribonucleic acid as well as ribonucleic acid and proteins at the nonpermissive temperature and accumulated abnormally large nuclei and nucleoli. The deoxyribonucleic acid made at 42 C banded together with the parent deoxyribonucleic acid in CsCl gradients. These strains have mutations in steps required solely for nuclear division.

The events involved in the replication of eukaryotic chromosomes and in the division of the eukaryotic nucleus are as yet poorly characterized. One approach which has provided useful information has been the study of temperature-sensitive fungal mutants blocked in specific steps of nuclear replication. Strains unable to synthesize deoxyribonucleic acid have been obtained from *Ustilago maydis* (26) and from *Saccharomyces cerevisiae* (8), and in one *U. maydis* mutant the inability to make DNA has been related to the presence of a temperature-sensitive DNA polymerase (11). Mutations in a number of loci in *S. cerevisiae* allowed cells to synthesize DNA and to replicate their chromosomes but prevented nuclear division (9). Significantly, some of these mutants were affected in very early steps of the cell cycle, such as bud initiation. Thus, the inability to complete steps in a different sequence, related to cell enlargement, also blocked nuclear division.

Mutants have so far been sought mainly among mononucleate organisms which multiply by budding or fission. However, coenocytic hyphal species may offer advantages for studying specific aspects. Mutants could be detected by the relatively simple technique of looking for cells with less than the normal number of nuclei. Because mitosis is not followed by septation and cell division, there will be no mechanisms which interlock the nuclear and division cycles. This may facilitate characterization of the steps involved solely in nuclear replication. These considerations have led us to look for mutants in the hyphal ascomycete, *Aspergillus nidulans*, which has the additional property, not possessed by yeasts, of forming recognizably condensed chromosomes at mitosis (23). Some of the mutants we isolated were blocked in DNA synthesis or mitosis. Others continued to make DNA and finally contained large nuclei with several times the normal amount of DNA.

MATERIALS AND METHODS

Organisms and media. Mutants were isolated from *A. nidulans* strain 46NXW, having the genotype ad15 bil w cnx and an unmapped mutation for ultraviolet sensitivity (for gene symbols see ref. 2). For mapping experiments, master strain F (19) was used.

Medium A contained 2% (wt/vol) malt extract (Difco Laboratories, Detroit, Mich.), 2% (wt/vol) glucose, and, when added, 2% (wt/vol) agar. Defined media used were as described previously (21). Growth factors required by the strains were routinely added to all media. Conidial suspensions were prepared as described previously (13).

Isolation of temperature-sensitive mutants. Conidia of 46NXW were germinated in medium A for 2 h at 30 C, collected on a membrane filter, washed with 0.01 M Tris-maleate buffer (pH 6), and resuspended in the same buffer containing 100 μg of nitrosoguanidine per ml. After 30 min the conidia were washed, resuspended in water containing 0.5% (wt/vol) Tween 80, and sown as a thick suspen-

sion on medium A solidified with agar. The fraction of conidia surviving nitrosoguanidine treatment was 10 to 20%. After growth and conidiation, the fresh crop of conidia were collected and contained, in our experience, a higher proportion of mutants than conidia plated directly after nitrosoguanidine treatment.

Mutants able to grow on medium A at 30 C but not at 42 C were detected by replica plating (18) and purified.

Cytology. Conidia were spread on sterile cellophane sheets placed on the surface of medium A solidified with agar. After incubation at 30 or 42 C, the cellophane with the germinated conidia was fixed in Helly solution (23). Staining for nuclei, nucleoli, and mitotic spindles was then carried out as described by Robinow and Caten (23). Samples for electron microscopy were prepared as previously described (25).

Measurements of the rates of macromolecular synthesis. Identical numbers of conidia were sown in a series of flasks containing medium A. Duplicate samples each consisting of the contents of a whole flask were taken at intervals, and the germinated conidia were harvested on membrane filters. Pools were removed with cold 5% trichloroacetic acid, and the ribonucleic acid (RNA) and DNA were extracted separately as described by Kennell (14). Protein was estimated on the residue after extracting the nucleic acids by the method of Lowry et al. (17). Labeling with [1-^{14}C]leucine during germination showed that the extraction for nucleic acids did not remove any protein components.

RNA and DNA in the above extracts were estimated either chemically (3, 14, 24) or by following radioactivity due to the incorporation of [8-^{3}H]adenine (The Radiochemical Centre, Amersham, specific activity, 20 Ci/mM). [8-^{3}H]adenine could be used for this purpose because 46NXW is a purine-requiring strain, carries a cnx mutation which blocks catabolic breakdown of purines (5) and because ^{3}H attached to carbon 8 of the purine molecule does not label histidine. Extraction of labeled hyphae with hot 5% (wt/vol) trichloroacetic acid showed that essentially all the radioactivity was soluble in trichloroacetic acid. When RNA and DNA synthesis was followed radiochemically, radioactive conidia were prepared from hyphae growing on medium A with 0.1 μCi of [8-^{3}H]adenine per ml, and these were used as the inoculum. The growth medium also contained 0.1 μCi of [8-^{3}H]adenine per ml, and the rate of polymer synthesis could be obtained directly from the increase in radioactivity. The chemical and isotopic estimations gave closely corresponding results.

Distribution of DNA in CsCl gradients. Conidia were germinated in medium A containing [8-^{3}H]adenine (1 μCi/ml) or [^{32}P]O_4^{3-} (1 μC/ml), collected on membrane filters, and washed with 0.1 M EDTA, pH 8. The cells were shaken in chloroform-methanol (1:1) for 60 min at 4 C and lyophilized. The dry material was ground to a powder and resuspended in 0.1 M EDTA and 0.15 M NaCl, pH 8. In most of the experiments RNA was degraded before the CsCl run by incubating the suspension with 200 μg of pancreatic ribonuclease per ml and 50 units of T1 ribonuclease per ml at 37 C for 2 h. Pronase (100 μg/ml) was then added, and the suspension was incubated for a further hour at 37 C. In some experiments the above RNA degradation was omitted, and the RNA was hydrolyzed with KOH (see below).

Sodium lauryl sulfate was then added to the suspension to give a final concentration of 2% (wt/vol), and the mixture was incubated for 15 min at 60 C. CsCl was added to give a final density of 1.700 g/ml, and the mixture was centrifuged for 70 h at 22 C at 110,000 $\times$ g. Fractions of 0.1 ml were collected directly on glass fiber filters (GF/C, Whatman Ltd., England) when ribonuclease-treated cells had been loaded onto the gradient. When the ribonuclease treatment had been omitted, fractions were collected in tubes. KOH was added to give a final concentration of 0.3 N and, after 2 h at 37 C, the precipitate was collected on GF/C filters. In all cases, the filters were washed with cold 10% (wt/vol) trichloroacetic acid, 95% (wt/vol) ethanol, and acetone in that order, and radioactivity was determined by liquid scintillation counting. The scintillant contained 3 g of 2,5-diphenyloxazole (PPO) and 0.3 g of 1,4-bis-(5-phenyloxazolyl)-benzene (POPOP) per liter of toluene.

Genetical techniques. Conventional methods were used to produce heterokaryons and heterozygous diploids and for the haploidization of diploids (19, 21).

Distribution of conidial sizes. The distribution of conidial volumes was determined with a Coulter Counter Model B (Coulter Electronics, Inc., Hialeah, Fla.), fitted with a 100-μm orifice and a recorder to plot the relative frequency of pulses of different heights.

RESULTS

Screening of mutants. Over 300 mutants which grew on the malt extract medium at 30 C but not at 42 C were isolated. Eleven of these mutants formed germ tubes at 42 C but remained with one nucleus or nucleolus (Fig. 1, A-F). The maximal lengths which the germ tubes reached before growth ceased was constant for a particular strain but varied from one mutant to another. In all cases, however, an equivalent length of germ tube in the parent contained more than the single nucleus of the mutants (Fig. 1). Thus, these 11 strains behaved as if they were blocked in nuclear replication, and their cytology and physiology were further investigated.

Cytology. Staining with aceto-orcein showed that, after incubation at the nonpermissive temperature, almost all the nuclei of strain 48 contained condensed chromosomes and appeared blocked in mitosis (Fig. 2B). None of the other 10 mutants accumulated condensed chromosomes or mitotic figures at 42 C. Strains which synthesized appreciable amounts of DNA at the nonpermissive temperature (see

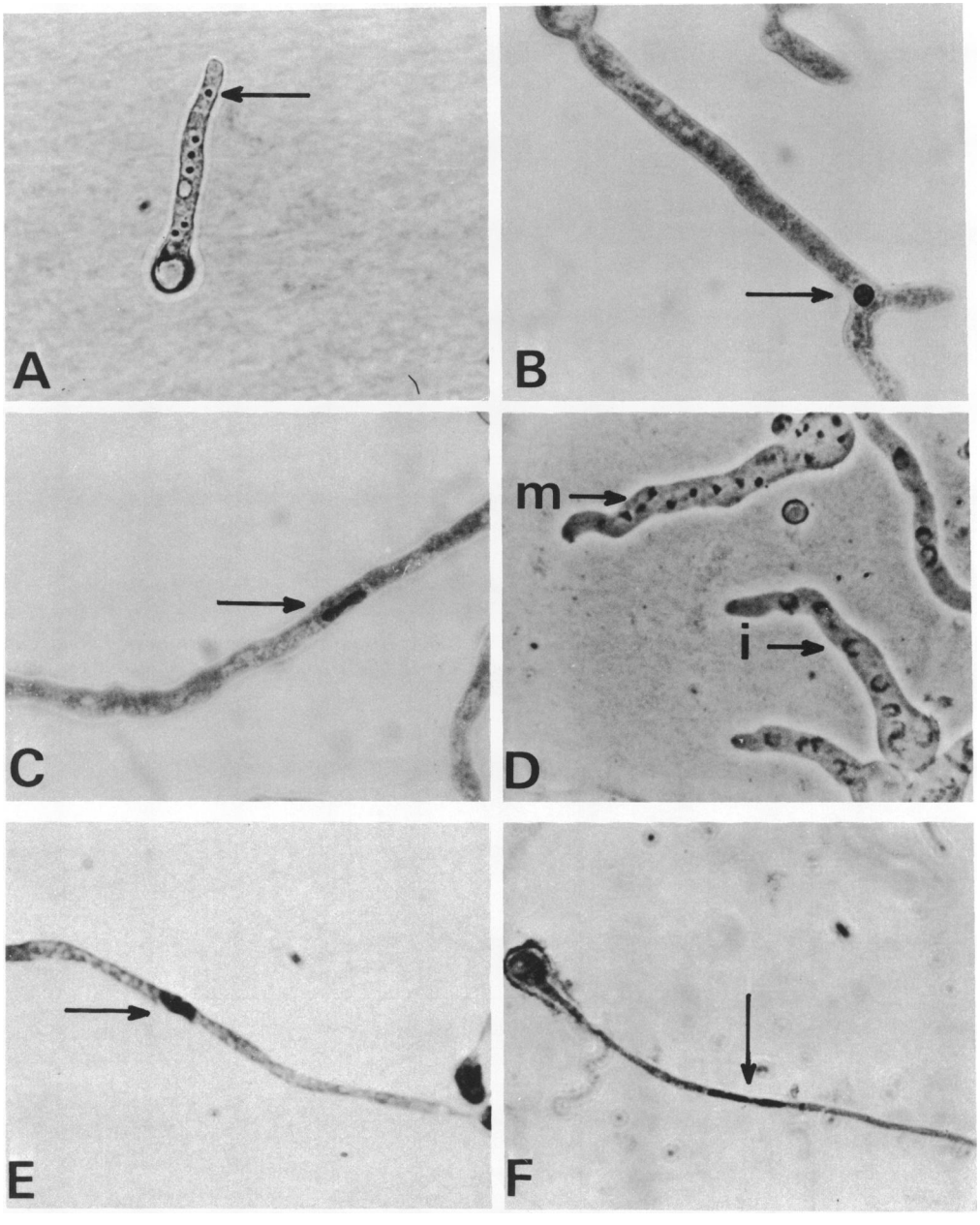

FIG. 1. *Nuclei and nucleoli in parent and mutant strains of A. nidulans grown at 42 C. A, B, and C were stained with acid fuchsin for nucleoli (arrow); D was stained with aceto-orcein for chromatin; E and F were stained with hematoxylin for nuclei (arrow). (A) Parent strain (× 800), (B) and (C) strain 59 (× 1,000), (D) parent strain (× 980), abbreviations: i, interphase nucleus; m, nucleus in mitosis, (E) strain 59 (× 600), (F) strain 50 (× 500).*

below) had markedly larger nuclei and nucleoli at 42 C than those of the parent (Fig. 1). Ultrathin sections examined under the electron microscope confirmed the presence of large nuclei and nucleoli in these mutants but did not indicate any other abnormalities (Fig. 2A).

Macromolecular synthesis at the nonpermissive temperature. To determine the effect of the nonpermissive temperature on the synthesis of DNA, RNA, and protein, conidia were germinated at 30 C for 15 h and then part of the cultures were switched to 42 C and compared

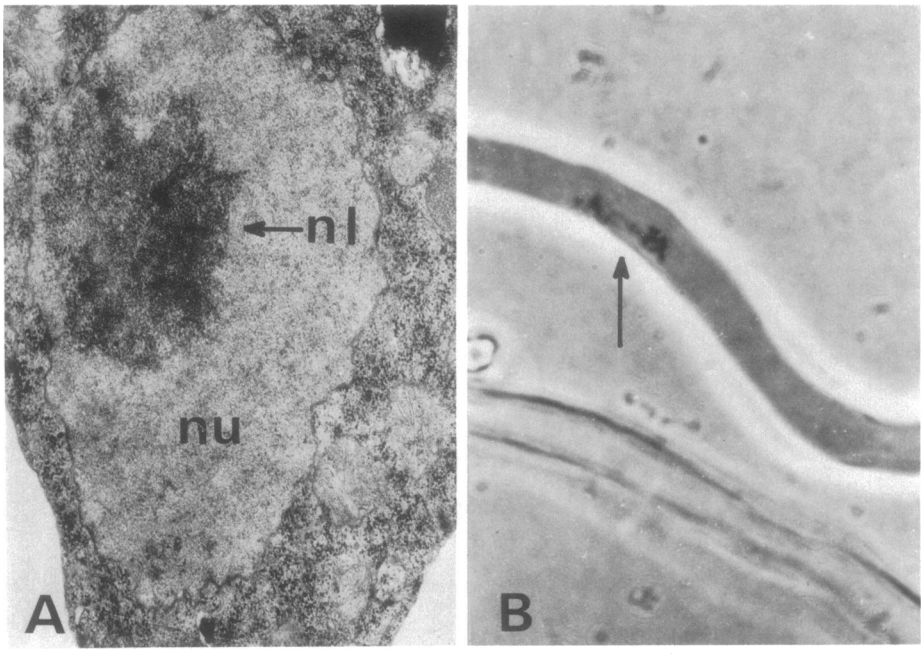

FIG. 2. *Nuclei in mutants of A. nidulans grown at 42 C. (A) Strain 59 stained with uranyl acetate and lead citrate. Abbreviations: nu, nucleus; nl, nucleolus (× 19,000). (B) Strain 48 showing condensed chromosomes (arrow). Stained with aceto-orcein (× 1,560).*

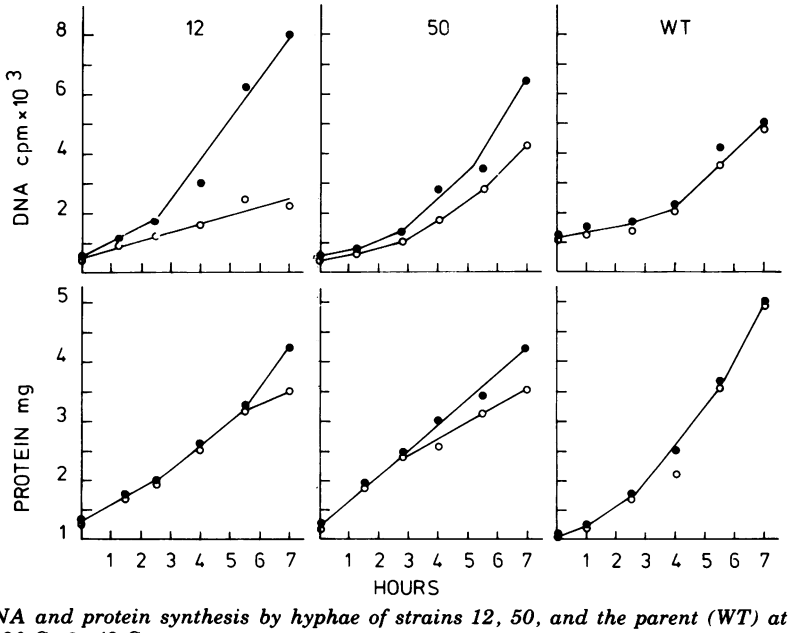

FIG. 3. *DNA and protein synthesis by hyphae of strains 12, 50, and the parent (WT) at 30 and 42 C. Symbols: ●, 30 C; ○, 42 C.*

with those remaining at 30 C. The parent strain synthesized polymers at an almost identical rate at these two temperatures (Fig. 3).

All the 11 mutants continued to make proteins at 42 C, and the rates equaled those at 30 C for several hours (Fig. 3, 4; Table 1). After 7 h, the ratio of protein made at 42 C per protein made at 30 C (micrograms per culture) was between 0.7 and 0.88.

Some DNA continued to be made at the non-

permissive temperature by all the strains. However, the rates of synthesis and the total increment after 7 h varied markedly between the different mutants (Fig. 3, 4; Table 1). Three mutants (35, 50, and 181) made DNA at rates close to those at 30 C for several hours, and the DNA increment in strain 50 after 7 h at 42 C regularly reached 700%. In seven other mutants DNA synthesis was markedly slowed by the high temperature, and the increase in DNA after 7 h lay between 60 and 130%. Strain 59 was intermediate between these two groups.

Strain 48, shown cytologically to be blocked in a step acting on mitosis, synthesized little RNA at 42 C (Fig. 4). At the nonpermissive temperature, the rates of polymer synthesis by this mutant were, in fact, very similar to those of the parent grown in the presence of benomyl (20), an inhibitor of mitosis in fungi (6, 12, 20). Two other mutants (strains 296 and 35) made RNA at reduced rates at 42 C. The remaining mutants synthesized RNA at roughly the same rate as at 30 C (Fig. 5).

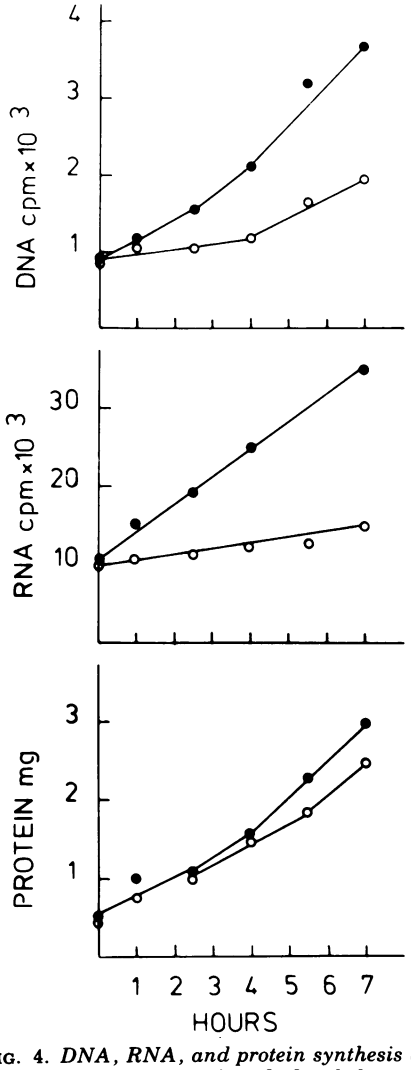

FIG. 4. *DNA, RNA, and protein synthesis by hyphae of strain 48 at 30 and 42 C. Symbols: ●, 30 C; ○, 42 C.*

TABLE 1. *DNA and protein synthesis by mutants transferred from the permissive to the nonpermissive temperature*

Strain	DNA synthesis[a]		Protein synthesis[a]
	ΔDNA 7 h, 42 C/ΔDNA 7 h, 30 C	DNA 7 h, 42 C/DNA time 0	ΔProtein 7 h, 42 C/ ΔProtein 7 h, 30 C
46NXW	1.0	5	1.0
13	0.14	1.6	0.84
12	0.18	1.6	0.88
48	0.2	2.2	0.86
52	0.26	2.0	0.78
316	0.26	1.9	0.74
59	0.315	3.3	0.7
161	0.67	5.0	0.84
35	0.77	3.4	0.85
50	0.65	8.6	0.77

[a] A part of replicate cultures in medium A were harvested at the time of the shift and the remainder after 7 h further incubation at the two temperatures.

Polymer synthesis was also followed under slightly different conditions by incubating conidial suspensions of the various strains at 42 C without prior growth at 30 C. Results from such experiments agreed with those from temperature shifts.

When germinated from conidia at 42 C, all the mutants made protein, with strain 296 synthesizing the lowest amounts (Table 2). DNA again was made by all the strains, but five mutants synthesized significantly more than the others (Table 2). These were the same strains (Table 1) that continued to make DNA in the temperature-shift experiments (strain 182 has not been thoroughly tested in temperature shifts). In spite of the fourfold or higher increase in DNA in these mutants, almost all of the germinated conidia still contained a single nucleus.

When germinated from conidia at 42 C strain 48, like the parent strain in the presence of benomyl (20), synthesized large amounts of RNA (Table 2). This indicates that strain 48 accumulates RNA at 42 C and that interference with mitosis affects RNA synthesis much more drastically in growing hyphae than in germinating conidia.

Buoyant density of DNA synthesized at the nonpermissive temperature. To check for ma-

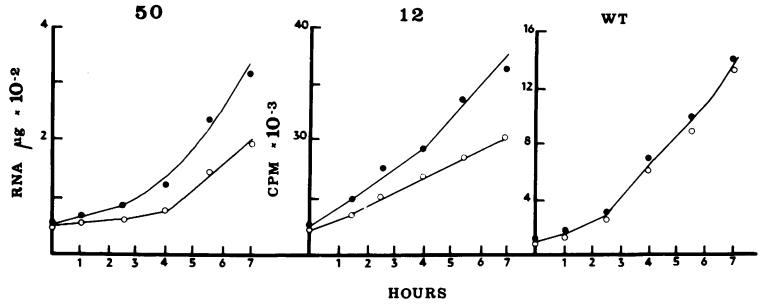

FIG. 5. *RNA synthesis by hyphae of strains 12, 50, and WT at 30 and 42 C. Symbols: ●, 30 C; ○, 42 C. Strain 50, chemical estimation; strains 59 and WT, isotopic.*

TABLE 2. *DNA, RNA, and protein synthesis by conidia germinated at 42 C*[a]

Strain	DNA 12 h 42 C/DNA time 0	RNA 12 h 42 C/RNA time 0	Protein 12 h 42 C/Protein time 0
46NXW	7.1	42	46
296	1.5	2.5	5.2
13	1.8	14	13.5
12	1.9	10	12
52	1.9	11	11
316	2.2	9	6.5
48	2.8	20	23
35	3.4	6	14.4
161	3.6	16	24
182	3.8	15	23
59	4.2	25	30
50	5.6	8.4	22

[a] A part of replicate cultures in medium A were harvested at the start of incubation and the remainder after 12 h at 42 C.

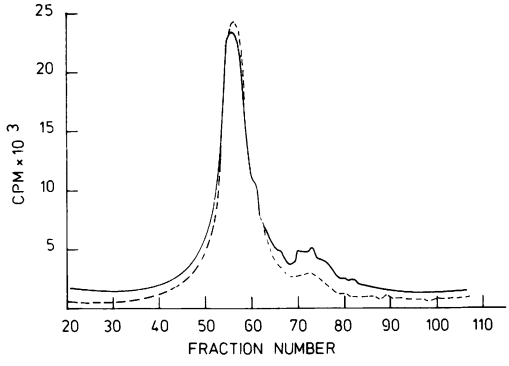

FIG. 6. *Buoyant density of DNA made at 42 C by strain 59. Symbols: (- - -) DNA from parent labeled with [³H]adenine; (——) DNA from 59 labeled with ³²P.*

jor differences between DNA made at the nonpermissive temperature by mutants and that of the parent strain, the DNAs were banded in CsCl gradients. In all the mutants, the DNA made at 42 C was indistinguishable from that of the parent (Fig. 6), and all the mutants made some mitochondrial DNA at the restrictive temperature. No differences were detected between gradients prepared from conidia germinated and grown at 42 C and from those germinated at 30 C and then transferred to 42 C.

Phenotypes of heterokaryons and heterozygous diploids. Heterokaryons and heterozygous diploids between the mutants and master strain F were prepared by standard techniques (19). Master strain F carries known nutritional and color markers but is wild type for nuclear replication (19). Both heterokaryons and diploids grew well at 42 C, showing that all the mutations were recessive. When combined pairwise in forced heterokaryons, all the mutants complemented each other. They thus represent distinct complementation groups or, according to the original definition of the term, distinct cistrons.

Volumes of conidia formed after exposure to the nonpermissive temperature. Exposing mutants, which synthesize DNA without nuclear division, to 42 C should lead to an increased number of chromosomes per nucleus. If this increase persists during subsequent growth at permissive temperatures, the conidia formed will also contain more than the normal amount of DNA. In wild-type strains of *A. nidulans*, the conidial volume is related to the DNA content of the conidial nucleus (4, 10). Assuming that this is also true for mutants, a haploid strain carrying the 59 mutation was grown at 30 C and at 37 C, an intermediate temperature supporting poor growth and some conidiation. The conidial volume distribution was markedly different at the two temperatures (Fig. 7A, B), with 37 C producing more large conidia. In the case of a diploid with genotype y pyro4 nic8 ts59/ad15 w ribo2 ts59, the nonpermissive temperature led to a breakdown of the diploid (Fig. 7C, D). Breakdown of the diploid was also indicated by growing conidia, after exposure to 42 C, at 30 C when almost every colony contained yellow and white sectors.

DISCUSSION

We screened for mutations in the nuclear replication cycle by looking for conidia which germinated at the nonpermissive temperature but remained with one or at most two nuclei. The assumptions underlying the screening were that such mutations would not inhibit conidial germination and would allow at least some cell elongation. These assumptions appear to have been justified, since the 11 mutants synthesized RNA and protein at quite similar rates when conidia were germinated at the permissive or the nonpermissive temperature. It may well be, however, that not all classes of mutants can be detected in this way.

At least a proportion of mutants blocked in nuclear replication would be expected to be unable to continue synthesis of DNA while still making RNA and protein (9, 26). Five and possibly six (strain 296) of the mutants we isolated have these characters. The mutation in one of the strains of this group could be defined more closely. This is strain 48 which by cytological techniques has been shown to have a temperature-sensitive step required for mitosis. With bacteria, it has been usual to classify mutants affected in DNA synthesis into elongation and initiation mutants according to the increment in DNA made at the nonpermissive temperature (7, 16). In *A. nidulans*, however, the S period is only 20 min out of a dry weight-doubling time of 120 min (15), and in asynchronous cultures most nuclei will not be making DNA at any particular instant. Measurements of the DNA increments will therefore not differentiate between these two types, particularly since mitochondrial DNA synthesis gives a high background. Synchronous cultures, which could solve this problem, have so far proved difficult to obtain. However, enzyme assays indicate that strain 52 may have a temperature-sensitive DNA polymerase (E. Orr, manuscript in preparation) and could thus be blocked in DNA chain elongation.

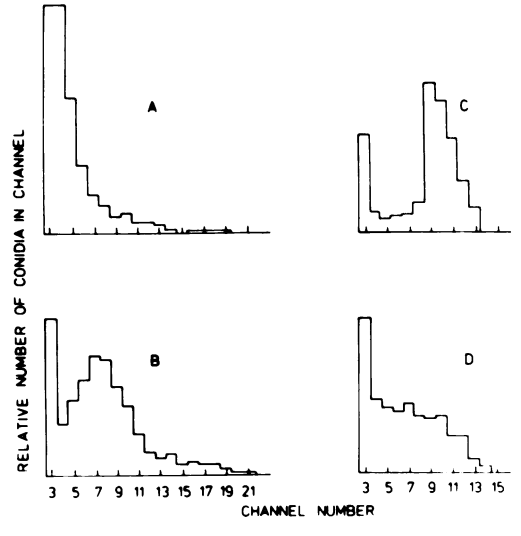

FIG. 7. *Volume distribution of conidia in haploid and diploid strains of mutant 59. Haploid strains: (A) grown at 30 C; (B) grown at 37 C. Diploid strains: (C) grown at 30 C; (D) 7 h at 42 C, then grown at 30 C. Increasing channel number shows increasing volume.*

The nonpermissive temperature markedly depressed RNA synthesis in hyphae of strain 48. This, however, was not a direct effect of the high temperature on the formation of stable RNA, since germinating conidia accumulated RNA at 42 C. The behavior of the parent strain in the presence of benomyl (20) supported the view that a block in mitosis inhibits RNA synthesis and does so much more drastically in growing hyphae than in germinating conidia. Presumably the changes undergone by the nucleolus during mitosis (22) suppress the synthesis of ribosomal RNA when the nucleus is frozen in this state. In germinating conidia, RNA synthesis starts some hours before DNA replication (1), and the greater amount of RNA made by conidia at the nonpermissive temperature may simply reflect the longer interval before the nucleus enters mitosis.

Four, and possibly five, mutants continued to synthesize DNA as well as RNA and protein at the nonpermissive temperature. Under these conditions they formed abnormally large nuclei with a single nucleolus. The buoyant density of the DNA made at 42 C appeared identical to that at 30 C, suggesting that the whole genome can be duplicated. The increase in the conidial volumes of the haploid strain 59 after exposure to restrictive temperatures is at least in keeping with this suggestion. Such mutants, blocked in steps which inhibit nuclear division but not DNA replication, appear to be a class not described so far in other organisms (9) and cannot readily be explained by attributing leakiness to the mutations. They could be particularly frequent in coenocytic species, where the nuclear cycle is not linked to a parallel cycle of cell growth, septation, and division.

Determining the point in the nuclear cycle where the mutations act would help to clarify the reactions they affect. We have attempted to carry out such determinations, and these are described in a subsequent paper (20).

LITERATURE CITED

1. **Bainbridge, B. W.** 1971. Macromolecular composition and nuclear division during spore germination in *Aspergillus nidulans*. J. Gen. Microbiol. 66:319-325.

2. **Barrat, R. W., G. B. Johnson, and W. N. Ogata.** 1965. Wild type and mutant stocks of *Aspergillus nidulans*. Genetics **52**:233-246.
3. **Bonting, S. L., and M. Jones.** 1957. Determination of microgram quantities of DNA and protein in tissue grown *in vitro*. Arch. Biochem. Biophys. **66**:340-353.
4. **Clutterbuck, A. J.** 1969. Cell volume per nucleus in haploid and diploid strains of *Aspergillus nidulans*. J. Gen. Microbiol. **55**:291-299.
5. **Darlington, A. J., C. Scazzocchio, and J. A. Pateman.** 1965. Biochemical and genetical studies of purine breakdown in *Aspergillus*. Nature (London) **206**:599-600.
6. **Georgopolous, S. G.** 1975. Mechanisms of fungicide tolerance. Proc. Soc. Gen. Microbiol. **2**:59-60.
7. **Gross, J. D., D. Karamata, and P. G. Hempstead.** 1968. Temperature sensitive mutants of *B. subtilis* defective in DNA synthesis. Cold Spring Harbor Symp. Quant. Biol. **33**:307-312.
8. **Hartwell, L. H.** 1967. Macromolecular synthesis in temperature-sensitive mutants of yeast. J. Bacteriol. **93**:1662-1670.
9. **Hartwell, L. H.** 1974. *Saccharomyces cerevisiae* cell cycle. Bacteriol. Rev. **38**:164-198.
10. **Heagy, F. C., and J. A. Roper.** 1952. Deoxyribonucleic acid content of haploid and diploid *Aspergillus* conidia. Nature (London) **170**:713-714.
11. **Jeggo, P. A. P. Unrau, G. R. Banks, and R. Holliday.** 1973. A temperature sensitive DNA polymerase mutant of *Ustilago maydis*. Nature (London) **242**:14-16.
12. **Kappas, A., S. G. Georgopolous, and A. C. Hastie.** 1974. On the genetic activity of benzimidazole and thiophenate fungicides on diploid *Aspergillus nidulans*. Mutat. Res. **26**:12-27.
13. **Katz, D., D. Goldstein, and R. F. Rosenberger.** 1972. Model for branch initiation in *Aspergillus nidulans* based on measurements of growth parameters. J. Bacteriol. **109**:1097-1100.
14. **Kennell, D.** 1967. Use of filters to separate radioactivity in RNA, DNA and protein, p. 686-693. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology. vol. 12A. Academic Press Inc., New York.
15. **Kessel, M., and R. F. Rosenberger.** 1968. Regulation and timing of deoxyribonucleic acid synthesis in hyphae of *Aspergillus nidulans*. J. Bacteriol. **95**:2275-2281.
16. **Klein, A., and F. Bonhoeffer.** 1972. DNA replication. Ann. Rev. Biochem. **41**:301-332.
17. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
18. **Mackintosh, M. E., and R. H. Pritchard.** 1963. The production and replica plating of microcolonies of *Aspergillus nidulans*. Genet. Res. **4**:217-231.
19. **McCully, K. S., and E. Forbes.** 1955. The use of p-fluorophenylalanine with "master strains" of *Aspergillus nidulans* for assigning genes to linkage groups. Genet. Res. **6**:352-359.
20. **Orr, E., and R. F. Rosenberger.** 1975. Determination of the execution points of mutations in the nuclear replication cycle of *Aspergillus nidulans*. J. Bacteriol. **126**:903-906.
21. **Pontecorvo, G., J. A. Roper, L. M. Hemmons, K. D. Macdonald, and A. W. J. Bufton.** 1953. The genetics of *Aspergillus nidulans*. Advan. Genet. **5**:141-238.
22. **Prescott, D. M.** 1964. The normal cell cycle, p. 71-97. *In* E. Zeuthen (ed.), Synchrony in cell division and growth. Interscience Publishers, Inc., New York.
23. **Robinow, C. P., and C. E. Caten.** 1969. Mitosis in *Aspergillus nidulans*. J. Cell Sci. **5**:403-431.
24. **Schneider, W. C.** 1957. Determination of nucleic acids in tissues by pentose analysis, p. 680-684. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 3. Academic Press Inc., New York.
25. **Sternlicht, E., D. Katz, and R. F. Rosenberger.** 1973. Subapical wall synthesis and wall thickening induced by cycloheximide in hyphae of *Aspergillus nidulans*. J. Bacteriol. **114**:819-823.
26. **Unrau, P., and R. Holliday.** 1970. A search for temperature sensitive mutants of *Ustilago maydis* blocked in DNA synthesis. Genet. Res. **15**:157-169.