PHASE-SPECIFIC GENES FOR MACROCONIDIATION IN *NEUROSPORA CRASSA*[1]

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ABSTRACT

Two new mutant genes in *Neurospora crassa* prevent the formation of free macroconidia from proconidial chains. These genes, called conidial separation-1 and conidial separation-2, are phase-specific, playing no role in either the sexual life cycle or other aspects of the asexual life cycle. A cell-wall-associated autolytic activity was found to increase in wild-type cultures at the time of active formation of free conidia from proconidial chains; no such increase was detected in mutant cultures. It appears that the products of these genes are both essential for and unique to macroconidiation.

SPORULATION exemplifies the development of terminally differentiated resting cells from a population of actively growing and multiplying stem cells. In multicellular organisms, the identification of gene-controlled events unique to this process presents difficulties which may be overcome in part by the isolation and characterization of phase-specific genes, that is, genes whose expressions are *limited* to a single developmental process or cell change. While the identification of such mutants is difficult in most eukaryotes, they can be profitably sought in a simple organism such as *Neurospora crassa* in which viability and genetic analyses do not depend upon the development of a full complement of cell types. Asexual sporulation in Neurospora involves an ordered series of relatively simple and well described events (SIEGEL, SELITRENNIKOFF and NELSON 1974). Vegetative hyphal cells can be called upon to form an intermediary cell type, called aerial hyphae, and these will yield asexual spores known as macroconidia. It has been shown that the development of macroconidia can be blocked by single-gene mutations (SHENG and RYAN 1948; S. S. MATSUYAMA, manuscript in preparation). However, most conidiation mutants have demonstrable effects on other phases of the life cycle and are not phase-specific (S. S. MATSUYAMA, manuscript in preparation).

We report two genes, called conidial separation-1 and conidial separation-2, whose expressions are phase-specific and required for the conversion of proconidial chains into free spores.

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MATERIALS AND METHODS

Strains were maintained as slant cultures grown at 25° on Vogel's N (VOGEL 1956) plus 1.5% (w/v) sucrose and 1.5% agar supplemented when appropriate with 500 μg/ml L-arginine (Cal Biochem) or 50 μg/ml nicotinamide (Sigma) and stored on silica gel (DAVIS and DE SERRES 1970). Crosses were performed by coinoculating strains of opposite mating type onto WESTERGAARD and MITCHELL's (1947) crossing medium. Resulting ascospores were washed once with double distilled water and placed on a 4% agar block. Individual ascospores were transferred to appropriate agar slants and germination was induced by heat shock at 60° for 45–60 min.

74-OR8–1a and 74-OR8–23A were used as wild type strains and were assumed to be isogenic. Strains used for mapping and the construction of heterokaryons were *nic-3* (Y31881), *nic-2* (43002), *arg-3* (30300), the triple mutant *nic-3 wc arg-10* (Y31881, P829, B317) and the "alcoy" linkage tester strain, T (I;II) 4637 *al-1*; T (IV;V) R2355, *cot-1* (C102t); T (III;VI) 1, *ylo-1* (Y30539y), and were obtained from the Fungal Genetics Stock Center, California State University, Humboldt, Arcata, CA.

Ethyl methane sulfonate (EMS) treatment of macroconidia (modified from MALLING and DE SERRES 1968) was used to obtain strain UCLA 37. A suspension of macroconidia from 74-OR8–1a in 0.01 M sodium phosphate buffer pH 7.0 (buffer A) was obtained from a 6–8 day agar slant culture. The suspension was filtered through 4 layers of sterile cheesecloth and washed twice with buffer A by centrifugation (5,000 × g, 5 min). The conidia were resuspended in buffer A and adjusted to a concentration of 2×10^7 conidia/ml. EMS (Eastman Chemical) was added to a final concentration of 25 μl/ml. The reaction was allowed to proceed for 120 min at 35° (with vigorous shaking) and then quenched by the addition of 4 volumes of 0.04 M sodium thiosulfate (made in buffer A). The suspension was diluted and conidia distributed to 9-cm petri plates (30–40 survivors/plate) containing solid sorbose-sucrose medium (NEWMEYER 1954). The plates were incubated in continuous light for 4–5 days at 35° and colonies macroscopically distinguishable from untreated wild-type colonies were transferred to 10 mm × 75 mm tubes containing 1 ml solid Vogel's minimal medium. After incubation for 3 days in continuous light at 35°, those cultures which had produced macroconidia were inverted and each tube was tapped sharply in front of a strong fluorescent light. Among 3500 cultures so tested, only one which failed to release free conidia was discovered (SELITRENNIKOFF and NELSON 1973).

Subsequently, three additional strains which failed to liberate conidia when slant cultures were "tapped" were found. Strain UCLA 101 was isolated as a third mutation in the *cr, sn* strain obtained from the Fungal Genetics Stock Center and is otherwise of unknown origin (NELSON, CHANDLER and SELITRENNIKOFF 1973). Strains FS 590 and FS 591 were obtained from DR. A. G. DEBUSK and were ultraviolet light-induced (DEBUSK, personal communication). Genetic analyses and full phenotypic characterizations were carried out on progeny derived from the third or fourth backcross of each original mutant to wild type. Assignment of the new mutations to linkage group position was accomplished by crosses to the *alcoy* linkage tester strain (PERKINS 1964) followed by 3-point crosses utilizing *arg-3* and the mating type locus for UCLA 37 and *wc, arg-10* for UCLA 101, FS 591 and FS 590.

The growth rates of vegetative hyphae were determined from dry weight measurements of stationary cultures grown in 125-ml flasks containing 25 mls of Vogel's N (supplemented with 1.5% sucrose, 0.23% sodium acetate w/v and 0.01% v/v Tween 80) in darkness at 25° and also from measurements of hyphal elongation in "race" tubes (DAVIS and DE SERRES 1970). Flask cultures were started from 0.2-ml conidial suspensions obtained by adding 3 ml of Vogel's N (supplemented as above) and 1–2 g sterile glass beads (5 mm) to 5–8-day-old slant cultures; slants were then vigorously agitated on a Vortex Genie mixer for 30 sec in order to mechanically disrupt chains of conjoined proconidia. At predetermined times the resulting mycelium was poured onto preweighed filter paper, washed with distilled water and dried to constant weight. Race tubes (500 mm × 15 mm) containing minimal medium were inoculated and incubated at 25° in continuous light. At selected times, the position of the leading edge of the growing mycelium was recorded.

Vegetative hyphae were induced to form aerial hyphae using a method previously described

(UREY 1971; SIEGEL, MATSUYAMA and UREY 1968). In brief, vegetative hyphae were grown in darkness in Vogel's N liquid medium (supplemented as above) in stationary flasks at 25° for 90 hours. These conditions are known to prohibit the production of aerial hyphae (SIEGEL, MATSUYAMA and UREY 1968). The vegetative hyphal cells were harvested, washed (so as to abruptly exclude an external source of nutrients), then transferred to glass finger bowls, sealed with a glass plate and exposed to continuous light at 25°. Under these conditions the reproduction of vegetative hyphae was promptly replaced by the formation and growth of aerial hyphae. Aerial hyphae begin to form macroconidia at approximately the sixth hour following induction; macroconidiation continues until the eleventh hour. At predetermined times after induction, aerial hyphae weer removed from underlying vegetative hyphae with a rubber policeman, dispersed in a known volume of water, and their lengths were determined by light microscopy. To determine the dry weight of an aerial hyphal mass (aerial hyphae and associated conidiogenic structures), this material was gently removed from the underlying mycelia with a scalpel, transferred to a preweighed millipore filter and dried to constant weight. During this procedure some conidia were mechanically displaced from the aerial mass to the underlying vegetative mycelia and hence were not directly weighed. The number of these displaced conidia was counted in a hemocytometer after the mycelia had been disrupted. Their dry weight (the average dry weight of a conidium is 31 $\mu\mu$ grams) was added to the dry weight of the aerial hyphal mass to give the corrected dry weight.

To determine the number of single free macroconidia formed by mutant and wild type following induction, cultures were transferred to SS-34 centrifuge tubes containing 5 ml water, agitated with the aid of a Vortex Genie mixer for 30 sec, and several aliquots were counted in a hemocytometer (SIEGEL, MATSUYAMA and UREY 1968).

The four mutants were crossed to heterokaryon-compatible strains, *nic-2* and *nic-3*, and double mutant progeny (conidiation-defective; auxotroph) were selected. From among these double mutants, those which were heterokaryon-compatible *inter se* were then selected. Nutritionally balanced heterokaryons were then used for tests of dominance, recessivity and complementation. Vegetative mycelia of each heterokaryon were induced (as described above) and at selected times the numbers of single free conidia were determined.

In order to obtain mutant and wild-type aerial material for scanning electron microscopy, clean paired 22-mm No. 1 circular coverslips were placed on a radius perpendicular to and touching the vegetative mycelia. Cultures were induced and at 10 and 26 hours of development the coverslips were removed and the adhering aerial material fixed in OsO_4 vapor (1% solution) for 16–20 hours at room temperature. The fixed material was dried by immersing the coverslips in 25%, 50%, 75%, 90%, 100% (twice) ethanol for one hour. The coverslips were transferred to a dessicator containing KOH pellets for 24–48 hours, glued to 24-mm brass SEM mounts and coated with 150–200 nm of gold-pallidium in a Jelco vacuum evaporator. The samples were viewed with a Jelco JSM-2 Scanning Electron Microscope at 15 KEV. Photographs were taken using Pan-X Kodak film.

The endogenous autolytic activity of cell wall preparations was determined by a method modified from MAHADEVAN and MAHADKAR (1970). Stationary flask cultures were grown for 90 hours at 25° in the liquid medium described above supplemented with 1 μC/ml ^{14}C-D-glucose (general label-specific activity 9.6 mC/mM; Schwarz-Mann). The resulting vegetative mycelia were induced to form aerial masses in the presence of 25 μC ^{14}C-D-glucose (9.6 mC/mM). The aerial hyphal mass was removed from the underlying vegetative hyphae, washed with cold distilled water (centrifugation, 10,000 $\times$ g, 5 min), resuspended in cold water, and the cells disrupted by two passages through a Hughes press. The cell wall fraction was separated from soluble cellular components by centrifugation (4000 $\times$ g, 10 min) and washed twice with cold water, then with cold 0.1-M NaCl and twice with cold 0.1-M sodium phosphate buffer (pH 7.0) containing 400 units/ml Penicillin G (Squibb) and 10 μg/ml Streptomycin sulfate (Pfizer) (buffer B). Aliquots of the cell wall fraction resuspended in buffer B were incubated for 0 and 20 hours at 25° in a rotary water bath. After incubation, the samples were quantitatively poured onto millipore filters (0.45 μ pore size) and washed twice with 5 ml of cold water. The filters were placed in glass scintillation vials, dried, and the radioactivity (cpm) present was determined

by a Beckman Liquid Scintillation Counter using a toluene, Triton X-100, 2,5 Diphenyloxazole (PPO) Scintillation fluid (2 liters toluene, 1 liter Triton, 12 grams PPO). Autolytic activity was computed by dividing the initial radioactivity present into the difference between the initial and the final (20 hours' incubation) radioactivity.

RESULTS

Preliminary characterization and genetics of conidial separation defective strains: When strains UCLA 37, UCLA 101, FS 590 and FS 591 were grown as slant cultures each formed an aerial hyphal mass (aerial hyphae and macroconidia) macroscopically identical to that of the wild type. But when such cultures were inverted and tapped they failed to liberate "clouds" of conidia; light microscopic examination revealed that the majority of conidia produced by mutant cultures remain conjoined and in this respect are in sharp contrast to wild type (Figure 1). The few free conidia which are produced by the mutants were found to be as viable as those formed by wild type. The mutant phenotype was irreparable by growth on complete medium (DAVIS and DE SERRES 1970), Vogel's N + 0.5% casamino acids medium, and was independent of temperature. Each strain was crossed to wild type and in each cross the mutant phenotype, conjoined conidia, segregated 1:1 with the wild-type phenotype (Table 1), demonstrating single nuclear gene differences. Analyses of unordered asci revealed 4:4 segregations as expected.

Isolates from the four mutants were crossed in all pairwise combinations and the progeny examined for the presence of wild-type recombinants. The results shown in Table 2 indicate that FS 591, FS 590 and UCLA 101 are allelic and are unlinked to UCLA 37. This conclusion was confirmed by completmentation results presented below.

Preliminary crosses to the *alcoy* linkage tester strain (PERKINS 1964) and

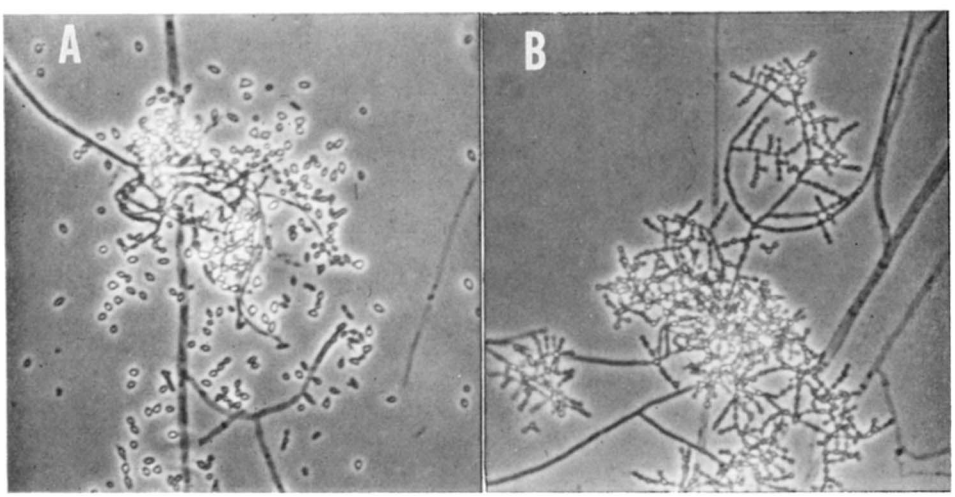

FIGURE 1.—Conidiating material from wild-type (A) and UCLA 37 (B) cultures (phase-contrast—150 ×).

TABLE 1

Crosses of mutant strains to wild type

Cross	Wild-type progeny	Mutant progeny	Percent germination
UCLA 37 × 74-OR8-1a	48	42	89
FS 591 × 74-OR8-1a	51	46	93
FS 590 × 74-OR8-1a	42	50	90
UCLA 101 × 74-OR8-1a	32	36	86

TABLE 2

Wild-type progeny recovered from crosses among mutants

	UCLA 37	FS 591	FS 590	UCLA 101
UCLA 37	0/400*	34/121	26/102	22/69
FS 591		0/165	0/195	0/218
FS 590			0/116	0/48
UCLA 101				0/122

* The number of wild-type progeny among the total progeny examined.

subsequent crosses to appropriately marked strains demonstrated that UCLA 37 was located in linkage group I and that FS 591, FS 590, and UCLA 101 were located in linkage group VII. Three-point crosses indicated that the position of UCLA 37 is 0.64 centimorgans proximal to *arg-3* in linkage group I, left arm, and FS 591, FS 590 and UCLA 101 are located 4.6 centimorgans to the left of *wc* in linkage group VII (see Table 3). We have assigned UCLA 37 to the new locus, conidial separation-1 (*csp-1*) (SELITRENNIKOFF and NELSON 1973), and FS 590, FS 591 and UCLA 101 as alleles of a second new locus, conidial separation-2 (*csp-2*) (SIEGEL, SELITRENNIKOFF and NELSON 1974).

Phase-specific effects of the csp *mutants:* Each mutant was found to produce morphologically normal and functional protoperithecia, perithecia and ascospores; all mutants could act as male parents. The double mutant recovered from the cross *csp-1* × *csp-2* (FS 591), and recognized by its failure to yield wild-type recombinants when backcrossed to each parental mutant, was also found to have

TABLE 3

Three-point crosses establishing gene order

Parental strains	Parental progeny	Single crossovers		Doubles
		Region I	Region II	
$\frac{+\ \text{I}\ arg\text{-}3^-\ \text{II}\ a}{37^-\ +\ A}$	280	2	31	0
$\frac{+\ \text{I}\ wc^-\ \text{II}\ arg\text{-}10^-}{591^-\ +\ +}$	102	6	22	1

TABLE 4

The growth of vegetative hyphae of mutant and wild-type strains

Strain	Stationary flask cultures 48 hours	72 hours	96 hours	Race tubes
74-OR8-1a	30*	70	92	2.08†
csp-1 (UCLA 37)	33	68	85	1.95
csp-2 (FS 590)	26	67	81	1.95
csp-2 (FS 591)	29	65	79	1.91
csp-2 (UCLA 101)	28	65	85	1.91
csp-1, *csp-2* (FS 591)	30	70	93	1.90

* mg dry weight—average of two determinations.
† Rate of mycelial extension in mm/hour at 25°—average of two determinations.

a fully normal sexual cycle. These results indicate that *csp-1*+ and *csp-2*+ play no essential roles in the sexual phase of the life cycle.

Growth rates of the vegetative mycelia of single mutants and double mutant strains show neither consistent nor significant differences from that of wild type (see Table 4). Results indicate that the mutants produce aerial hyphae comparable to wild type with respect to elongation in time (Table 5), and are indistinguishable from wild type in total aerial mass (Table 6). The number of single free conidia produced by wild-type and mutant cultures is shown in Figure 2. The mutants form no detectable single free conidia prior to the ninth hour following induction, although proconidial chains, comparable in numbers to those observed in wild-type cultures, are present. Later the *csp-1* mutant forms free conidia, though in fewer numbers than wild type. It may be noted that identical conidial crops are eventually produced by the three *csp-2* mutants and are two orders of magnitude lower than wild type. Also the rates at which

TABLE 5

Aerial hyphae from induced wild-type and mutant cultures

Strains	Hours of development	Average length (μ)
74-OR8-1a	4	325 (170–425)*
	5	425 (340–595)
	6	680 (510–1190)
csp-1 (UCLA 37)	4	380 (170–510)
	5	470 (340–765)
	6	800 (680–1300)
csp-2 (FS 591)	4	340 (170–425)
	5	470 (340–765)
	6	680 (605–850)
csp-1; *csp-2* (FS 591)	4	425 (170–510)
	5	550 (340–850)
	6	635 (510–940)

* Range of lengths.

TABLE 6

Dry weight of aerial hyphal masses from induced mutant and wild-type cultures

Strain	Hours of development 6	8	10	24
74-OR8–1a	2.4 ± 0.8*	7.4 ± 3.6	11.1 ± 1.6	13.2 ± 0.7
csp-1	4.1 ± 1.6	6.5 ± 1.3	12.6 ± 1.8	15.2 ± 2.5
csp-2 (FS 591)	1.8 ± 0.3	5.8 ± 0.2	9.6 ± 4.2	11.2 ± 1.8
csp-1, csp-2 (FS 591)	1.9 ± 0.6	6.0 ± 2.0	10.1 ± 1.9	10.4 ± 1.8

* Corrected dry weight in mg; standard deviation; N=8.

csp-1 and *csp-2* form free conidia differ from each other and from wild type. Double mutant cultures produce no detectable free conidia, although they are otherwise phenotypically indistinguishable from single-mutant and wild-type cultures. These observations lead to the conclusion that the roles of these genes in the vegetative phase of the life cycle in Neurospora is restricted to the formation of single free conidia from proconidial chains.

The arrest of conidial morphogenesis due to mutation at the csp *loci:* Scanning electron microscopy reveals the details of conidial morphogenesis and suggests the

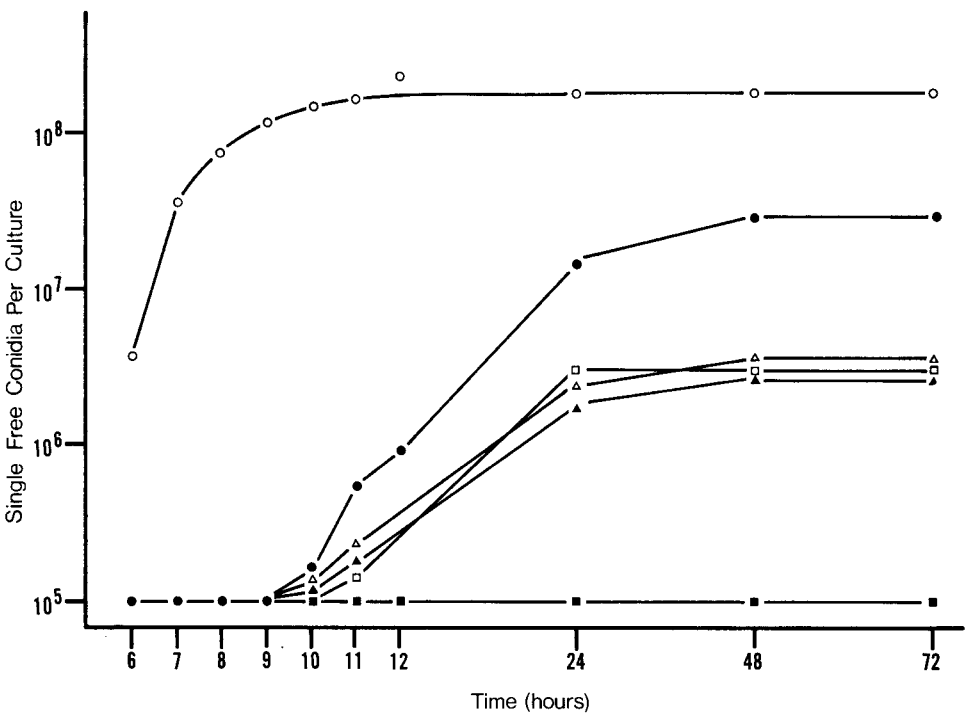

FIGURE 2.—The production of free conidia by wild-type (○—○), UCLA 37 (●—●), FS 591 (△—△), FS 590 (▲—▲), UCLA 101 (□—□) and UCLA 37; FS 591 (■—■) cultures following induction. Conidial yields of 10^5 or less canont be discriminated by the experimental procedure used.

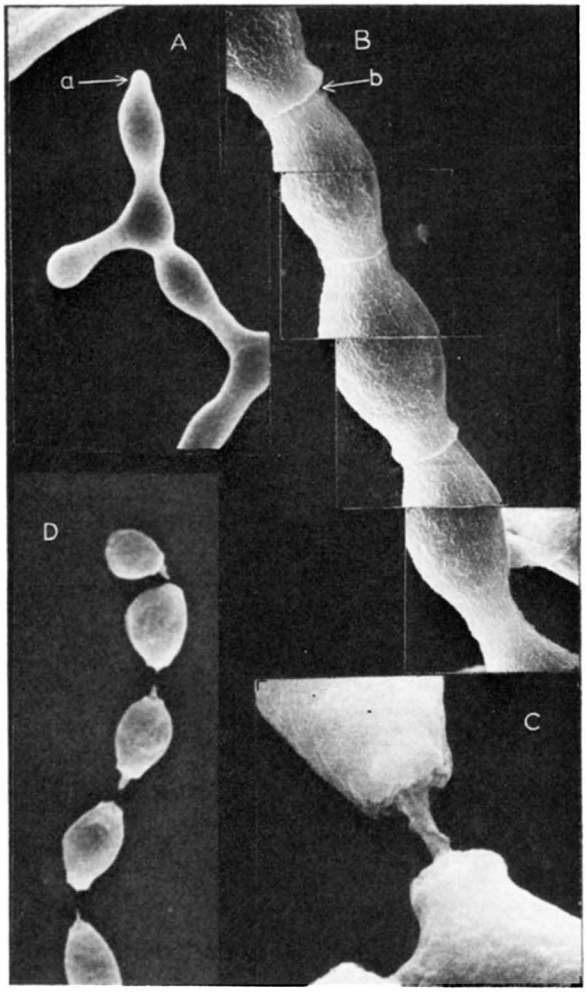

FIGURE 3.—Scanning electron photomicrographs of conidiating material from induced wild-type cultures. A—chain of proconidia formed by terminal and lateral budding; a—terminal bud (2000 ×); B—chain of proconidia with interconidial septal furrows (b) (6000 ×); C—interconidial connective between adjacent conidia (9000 ×); D—conidia with connectives attached (2800 ×). (Photographs B, C, and D have been previously published in SIEGEL, SELITRENNIKOFF and NELSON 1974).

stage at which mutant development is arrested. Chains of proconidia (Figure 3A) as well as single free conidia (Figure 3D) were present in wild-type cultures fixed after 10 and 26 hours of development. Septal furrows between adjacent proconidia (Figure 3B) and interconidial connectives (Figure 3C) were evident. On the other hand, *csp-1* cultures at 10 hours contained only chains of proconidia; no septal furrows, interconidial connectives or free conidia were found. But after 26 hours of development a small number of septal furrows, interconidial connectives, and free conidia were observed. In contrast, material from *csp-2* (FS

591) and from the double mutant contained *only* proconidial chains at *both* 10 and 26 hours of development; furrows, connectives and free conidia were not seen. The aerial hyphae and conidiogenic structures produced by the mutant cultures appeared indistinguishable from those of wild type; no aberrant structures were detected. These data indicate that the formation of septal furrows, a process essential for the formation of conidia, is blocked by mutation at the *csp-1* and *csp-2* loci.

Complementation analyses: The provisional conclusion that mutants FS 591, FS 590 and UCLA 101 are allelic was tested by complementation analysis. Heterokaryotic vegetative hyphae were induced to form aerial hyphae and conidia and after 8, 11 and 24 hours of development the number of single free conidia was determined (Table 7). Heterokaryon and "selfed" homokaryons of strains FS 591, FS 590 and UCLA 101 yield low and similar numbers of free conidia. Hence these mutants do not complement. As expected, UCLA 37 (*csp-1*) complements with all of the *csp-2* mutants. Since all heterokaryons containing mutant and wild-type nuclei with respect to the conidiation trait produce conidial crops similar to those formed by the wild-type "selfed" homokaryon, we conclude that the mutant genes are recessive.

Autolytic activity of cell-wall preparations from wild-type and mutant strains: TURIAN and BIANCHI (1972) have speculated that the separation proconidia into free conidia involves the autolysis of the cell walls between presumptive conidia. Since *csp-1* and *csp-2* mutants are unable to convert proconidial chains

TABLE 7

Conidial yield of induced heterokaryons

	Time of development		
	8 hours	11 hours	24 hours
"Selfed" homokaryons:			
nic-2/nic-3	5.1×10^7*	9.3×10^7	1.9×10^8
UCLA 37/UCLA 37	$< 10^5$	1.8×10^6	1.6×10^7
FS 591/FS 591	1.3×10^5	7.0×10^5	6.4×10^6
FS 590/FS 590	$< 10^5$	1.2×10^6	1.6×10^6
UCLA 101/UCLA 101	$< 10^5$	1.0×10^6	2.0×10^6
Intra-allelic heterokaryons:			
FS 590/FS 591	2.1×10^5	1.3×10^6	4.3×10^6
UCLA 101/FS 590	$< 10^5$	1.2×10^6	2.9×10^6
UCLA 101/FS 591	$< 10^5$	1.4×10^6	1.2×10^6
FS 591/+	5.3×10^7	8.8×10^7	1.1×10^8
FS 590/+	5.5×10^7	1.1×10^8	1.2×10^8
UCLA 101/+	7.7×10^6	7.7×10^7	1.0×10^8
UCLA 37/+	1.5×10^7	1.6×10^8	1.6×10^8
Interallelic heterokaryons:			
UCLA 37/FS 591	5.2×10^6	1.4×10^8	1.9×10^8
UCLA 37/FS 590	4.5×10^6	1.4×10^8	2.2×10^8
UCLA 37/UCLA 101	9.4×10^6	1.1×10^8	1.3×10^8

* Average of two determinations.

TABLE 8

Autolytic activity of mutant and wild-type preparations

Strain	0 hours (Vegetative hyphae)	5 hours (Aerial hyphae)	9.5 hours (Aerial hyphal mass)
74-OR8–1a	9.8*(6.4–13.2)†	4.6 (2.0–11.1)	27.9 (26.4–29.5)
csp-1 (UCLA 37)	8.5 (7.5–9.3)	0 (—0.8–0.3)	5.5 (1.7–9.5)
csp-2 (FS 591)	5.0 (1.1–8.6)	2.7 (0.4–5.2)	10.8 (8.5–13.0)

* Autolytic activity × 100; average of 3 determinations.
† Range.

to free spores, the capacity to autodigest cell wall material might be impaired. To test this possibility, radioactively labelled vegetative hyphae from wild type, *csp-1* and *csp-2* (FS 591) were induced to form aerial hyphae and conidia in the presence of additional label; at various times during development the autolytic activity of the cell wall fraction was determined. Vegetative and aerial hyphal material isolated from both wild-type and mutant cultures prior to the onset of conidiogenesis (0 and 5 hours of development) possessed low levels of autolytic activity. In contrast, only the cell wall fraction prepared from wild-type aerial hyphae after 9.5 hours of development showed a dramatic increase in autolytic activity (see Table 8). It should be emphasized that at 9.5 hours of development wild-type cultures are actively forming free conidia from proconidial chains; each culture produces about 10^8 new conidia between the ninth and tenth hour of development (see Figure 2). But during this period the mutants form very few free conidia from proconidial chains. Thus the level of cell-wall-associated autolytic activity is directly correlated with the conversion of proconidial chains into free conidiospores.

DISCUSSION

The familiar finding that different cell types synthesize unique proteins and messages means that fractions of the total genome are selectively expressed in phenotypically different cell lines. This has led to a general notion that differential gene expression is in fact the necessary basis for cell changes. In theory, this predicts the existence of a class of mutants which fail to form a particular cell type but which are otherwise entirely normal. Such mutants, referred to as phase-specific, should permit the discovery of gene products unique to and necessary for a given cell change. Our data strongly indicate that the expression of the *csp* loci is phase-specific and necessary for the completion of macroconidiation.

Mutations at the *csp* loci prevent the conversion of proconidial chains into free spores but do not interfere with other phases of the vegetative and sexual cycles. We have also discovered that autolytic activity associated with wild-type cell walls increases sharply at the time of separation of proconidial chains. Hence autolytic activity appears to be phase-specific. The fact that *csp* mutants show no comparable increase in such cell-wall-associated autolytic activity is notable.

Granting that the products of the *csp* genes are functionally phase-specific, the

question remains as to whether the loci are transcribed and translated only during that period of the life cycle. Whatever the specific functions of these genes may be, the fact that *csp-1* and *csp-2* complement indicates that their products are cytoplasmically diffusible. Taken together the data encourage future investigations aimed at the eventual isolation and characterization of the *csp* gene products.

Our scanning electron micrographs suggest a sequence of morphogenetic events for the final stages of conidiation that is in full agreement with and extends that previously described by TURIAN and BIANCHI (1972) and others (SEALE 1972; LOWRY, DURKEE and SUSSMAN 1967). However, the intracellular events remain unknown. It is possible that an arrangement of microfilaments (SCHROEDER 1973) could constrict the autolysis-weakened cell wall; or intraconidial cell walls may be constructed and then conidia freed by subsequent autolytic action. These and other mechanisms could be explored with the aid of transmission electron microscopy.

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