Polyamine-Deficient *Neurospora crassa* Mutants and Synthesis of Cadaverine

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The polyamine path of *Neurospora crassa* originates with the decarboxylation of ornithine to form putrescine (1,4-diaminobutane). Putrescine acquires one or two aminopropyl groups to form spermidine or spermine, respectively. We isolated an ornithine decarboxylase-deficient mutant and showed the mutation to be allelic with two previously isolated polyamine-requiring mutants. We here name the locus *spe-1*. The three *spe-1* mutants form little or no polyamines and grow well on medium supplemented with putrescine, spermidine, or spermine. Cadaverine (1,5-diaminopentane), a putrescine analog, supports very slow growth of *spe-1* mutants. An arginase-deficient mutant (*aga*) can be deprived of ornithine by growth in the presence of arginine, because arginine feedback inhibits ornithine synthesis. Like *spe-1* cultures, the ornithine-deprived *aga* culture failed to make the normal polyamines. However, unlike *spe-1* cultures, it had highly derepressed ornithine decarboxylase activity and contained cadaverine and aminopropylcadaverine (a spermidine analog), especially when lysine was added to cells. Moreover, the ornithine-deprived *aga* culture was capable of indefinite growth. It is likely that the continued growth is due to the presence of cadaverine and its derivatives and that ornithine decarboxylase is responsible for cadaverine synthesis from lysine. In keeping with this, an inefficient lysine decarboxylase activity ($K_m > 20$ mM) was detectable in *N. crassa*. It varied in constant ratio with ornithine decarboxylase activity and was wholly absent in the *spe-1* mutants.

Polyamine synthesis in *Neurospora crassa* (Fig. 1) has been studied by several groups over the last decade. The sole origin of putrescine (1,4-diaminobutane) was identified as ornithine (8), and the enzyme responsible for putrescine formation, ornithine decarboxylase, was shown to be cytosolic (3, 13, 21). Two putrescine-requiring mutants of *N. crassa* were isolated by Deters et al. (Genetics 77:s16–s17, 1974), although there has been some doubt about their ornithine decarboxylase deficiency (14). The existence of spermine in *N. crassa* and other filamentous fungi has been debated recently (11, 15), with general agreement presently that spermine does occur in low amounts in *N. crassa* (T. J. Paulus and R. H. Davis, Methods Enzymol., in press). A study of polyamine pool dynamics in *N. crassa* (16) suggests that polyamines are normally maintained in a narrow range of values (on a dry-weight basis) by a control mechanism for ornithine decarboxylase which responds with high efficiency to spermidine. It was also shown that little or no polyamine degradation takes place during exponential growth in *N. crassa* and that a substantial fraction of the polyamine pools may be bound (nondiffusible) within the cell (16, 17).

In previous work and the work reported here, use has been made of the arginaseless (*aga*) mutation. Because strains carrying an *aga* mutation cannot form ornithine from arginine (Fig. 1) and because arginine feedback inhibits ornithine biosynthesis, *aga* mutants grown with arginine develop severe ornithine and polyamine depletion. The resulting nutritional needs are met by addition of ornithine or polyamines (8).

In this report, we wish to extend and clarify our knowledge of polyamine metabolism in *N. crassa*. We confirm the previous work on genetic control of ornithine decarboxylase and show that ornithine decarboxylase is highly derepressed in the *aga* strain when depleted of ornithine. Finally, we explore the origin of cadaverine (1,5-diaminopentane), an analog of putrescine found in the ornithine-starved *aga* mutant, and its role in supporting the slow and indefinite growth of this strain despite the lack of the normal polyamines.

MATERIALS AND METHODS

Strains, media, and growth. The strains of *N. crassa* used were IC-1 and IC-2 (wild types) and strains carrying the *aga*, various *spe-1*, and *inl* (inositol) mutations (Table 1). In this paper, we rename the *put-1*
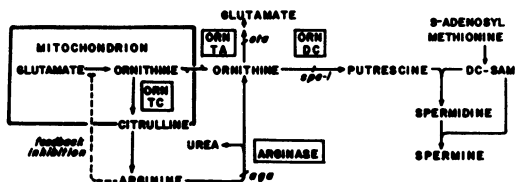
FIG. 1. Scheme of putrescine, spermidine, and spermine synthesis and its relation to ornithine. Mutations (in italics): *aga*, arginase-deficient; *ota*, ornithine transaminase-deficient; *spe-1*, ornithine decarboxylase-deficient. Abbreviations: DC-SAM, decarboxylated S-adenosylmethionine; ORN DC, ornithine decarboxylase (EC 4.1.1.17); ORN TA, ornithine transaminase (EC 2.6.1.13); ORN TC, ornithine transcarbamylase (EC 2.1.3.3).

mutations (462JM and 521KW) of McDougall *spe-1* to accommodate mutants of other steps of polyamine synthesis under the same locus symbol.

The media used were Vogel medium N for growth, corn meal agar without dextrose and supplemented as appropriate for crosses, and sorbose plating and spot-testing media for genetic analysis (7).

Growth was done in 10-ml Vogel medium in 50-ml flasks for stationary cultures, in 1,000-ml aerated exponential cultures for enzyme and polyamine pool determination, and in 25-ml solidified medium in 125-ml Erlenmeyer flasks to grow conidia used for inocula and for mutational work (7). Media were supplemented as noted in the text.

Genetic methods. Mutagenesis of *N. crassa* conidia of both wild-type and polyamine-depleted *aga* strains was done. Polyamine-depleted *aga* conidia were prepared by growing cultures in 1 mM arginine agar medium and limiting (0.2 mM) putrescine. Mutagenesis of conidia with UV light to 20 to 80% survival, followed by the filtration-enrichment technique for spermidine-requiring mutants, was done by standard methods (7). Crosses were made by standard methods (7); most media in which ascospores were plated were supplemented with 500 μg of spermidine per ml to counteract the very poor germination of Spe⁻ ascospores.

Complementation tests were performed by combining drops of conidia in 1 ml of liquid minimal medium in culture tubes (13 by 100 mm) and incubating them for up to 10 days at room temperature.

Ornithine decarboxylase extraction and assay. Assays were performed on extracts of cells grown in exponential cultures. Mycelia collected by filtration were ground with sand in a cold mortar in 0.05 M K^+-phosphate buffer (pH 7.1) containing 1 mM EDTA. Slurries were centrifuged at 18,500 × *g*, and the supernatants were desalted on Sephadex G-25 columns equilibrated with the extraction buffer. Extracts were frozen (−70°C) with little loss of activity. Enzyme assays were performed as described previously (21; R. H. Davis and T. Paulus, Methods Enzymol., in press) in reaction mixtures (0.3 ml) containing 100 mM K^+ phosphate (pH 7.1), 1 mM EDTA, 1.7 mM β-mercaptoethanol, 50 μM pyridoxal phosphate, 2 mM L-ornithine, and sufficient L-[1-¹⁴C]ornithine to bring the specific radioactivity to 200 to 700 cpm/nmol. Incubations were carried out at 37°C for 10 to 90 min; specific activity is expressed as nanomoles per hour per milligram of protein. Lysine decarboxylase activity was measured in the above reaction mixture, in which L-[U-¹⁴C]lysine (5,800 cpm/nmole) was used in place of ornithine. Due account was taken of the fact that only one of the six lysine C atoms is released upon decarboxylation.

Polyamine determinations and identification. Polyamine pools were determined in perchloric acid extracts of mycelia by the double-isotope derivative assay described previously (16; Paulus and Davis, in press). The method depends upon dilution of added ¹⁴C-labeled polyamine by polyamines in the extract. The quantitation of polyamines recovered from silica gel, thin-layer chromatograms is done by use of [³H]dansyl-chloride of known specific activity. Dansyl-polyamines dissolved in benzene were spotted on Sil G plates (20 by 20 cm). For one-dimensional separations, the solvent used was ethyl acetate-cyclohexane (2:3, vol/vol) (16). For two-dimensional separations, the first dimension was run with the solvent above and the second dimension was run with chloroform–n-butyl-alcohol–dioxane (48:1:1) (1). Two-dimensional separations are essential for dependable resolution of the dansyl derivatives of putrescine,

TABLE 1. *N. crassa* mutants used in this study

Strain (locus)	Mutant allele and mating type	Deficiency	Source or origin
Single mutants			
IC-1 (74A)	A	None	R. H. Davis
IC-2 (73a)	a	None	R. H. Davis
IC-3 (*aga*)	UM-906A	Arginase	R. H. Davis (8)
IC-4 (*spe-1*)	462JM A	ODCase[a]	K. McDougall (14)
IC-5 (*spe-1*)	521KW a	ODCase	K. McDougall (14)
IC-6 (*spe-1*)	TP-138 A	ODCase	This paper
IC-7 (*spe-1*)	TP-138 a	ODCase	This paper
IC-8 (*inl*)	89601 a	Inositol	FGSC[b]
Multiple mutant IC-9 (*spe-1, aga*)	TP-138, UM-906		This paper

[a] ODCase, Ornithine decarboxylase.
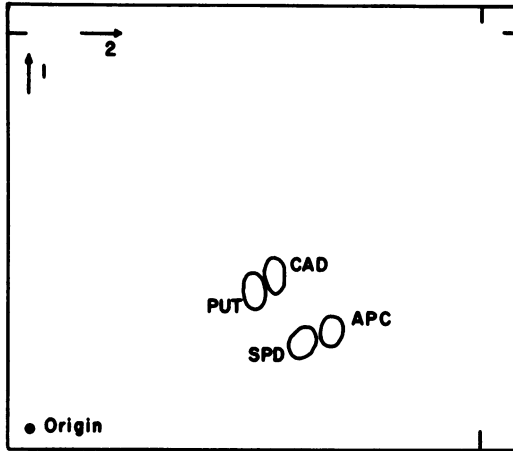[b] FGSC, Fungal Genetics Stock Center, Humboldt State University Foundation, Arcata, Calif.

FIG. 2. Two-dimensional thin-layer chromatographic separation of the dansyl derivatives of putrescine (PUT), cadaverine (CAD), spermidine (SPD), and aminopropylcadaverine (APC). First dimension, ethyl acetate-cyclohexane (2:3); second dimension, chloroform–*n*-butyl alcohol–dioxane (48:1:1).

cadaverine, spermidine, and aminopropylcadaverine (Fig. 2). No attempt was made to measure the amount of aminopropylcadaverine, but both this compound and cadaverine were further identified by rechromatographing their dansyl derivatives eluted from two-dimensional plates in additional one-dimensional plates with standards.

Materials. All chemicals were of reagent grade. Most biochemicals were obtained from Sigma Chemical Co. Triton X-100 was purchased from Research Products International, and Polygram Sil G thin-layer chromatograms were obtained from Brinkman Instru-

ments, Inc. Isotopes were purchased from Amersham-Searle (L-[1-^{14}C]ornithine) and New England Nuclear Corp. (polyamines and dansyl-chloride). Aminopropylcadaverine was a gift from David R. Morris, University of Washington, Seattle.

RESULTS

Mutants requiring polyamines. In the course of numerous attempts, only one polyamine-requiring strain was isolated from conidia of the *aga* strain 1C-3, as described in Materials and Methods. It grew very slightly in minimal medium but grew well in medium supplemented with putrescine, spermidine, or spermine. By a cross to the wild type, strains 1C-6 and 1C-7, carrying the *spe* mutation TP-138 in otherwise wild-type backgrounds, were isolated for study.

The TP-138 mutation, like other polyamine-requiring mutations (14), caused poor ascospore germination even in supplemented media, particularly in allelic crosses. Despite this, crosses of strain IC-6 with wild type gave no indication that the putrescine-requiring phenotype was other than monogenic (Table 2). A cross of strains IC-6 or IC-7 with strains carrying previously isolated putrescine auxotrophic mutations (IC-4 or IC-5) yielded few spores, but none were prototrophic (Table 2). This is significant in view of the high degree of selection for prototrophs in germination. A cross of strain IC-6 to the *inl*-bearing IC-8 strain demonstrated linkage of TP-138 and *inl* (ca. 5 centimorgans [cM], based on Spe$^+$ progeny). This was confirmed in a second cross, *spe, aga × inl* (Table 2), in which the map distance was approximately 4 cM. These map distances are somewhat shorter than those de-

TABLE 2. Crosses involving *spe-1* alleles[a]

Cross	Progeny phenotypes[b]		Remarks
	Spe$^+$	Spe$^-$	
IC-6 × IC-2 (TP-138 × wild type)	34	13	
IC-7 × IC-4 (TP-138 × 462JM)	0	63	450 spores plated
IC-6 × IC-5 (TP-138 × 521KW)	0	23	
IC-4 × IC-5 (462JM × 521KW)	0	41	
IC-6 × IC-2 (TP-138 × wild type)	30	20	All Spe$^-$ sparse at 35°C
IC-4 × IC-2 (462JM × wild type)	24	26	All Spe$^-$ vigorous at 35°C
IC-6 × IC-8 (TP-138 × *inl*)	3 Inl$^+$	24 Inl$^+$	Linkage: 3/62 × 100 = 5 cM between
	59 Inl$^-$	0 Inl$^-$	*inl* and *spe-1*
IC-4 × IC-8 (462JM × *inl*)	6 Inl$^+$	35 Inl$^+$	Linkage: 10/85 = 12 cM
	40 Inl$^-$	4 Inl$^-$	between *inl* and *spe-1*
IC-9 × IC-2 (TP-138, *aga* × wild type)	13 Aga$^+$	13 Aga$^+$	No linkage between *aga* and *spe-1*
	20 Aga$^-$	6 Aga$^-$	
IC-9 × IC-8 (TP-138, *aga* × *inl*)	38 Aga$^-$ Inl$^-$	1 Aga$^-$ Inl$^-$	Linkage: 3/77 × 100 = 4 cM between
	36 Aga$^+$ Inl$^-$	2 Aga$^+$ Inl$^-$	*inl* and *spe-1*
	0 Aga$^-$ Inl$^+$	8 Aga$^-$ Inl$^+$	
	3 Aga$^+$ Inl$^+$	5 Aga$^+$ Inl$^+$	

[a] Alleles of *spe-1* are identified in each cross.

[b] Spe and Inl, Requirements for polyamine and inositol, respectively; Aga, presence or absence of arginase, detected as ability or inability to use arginine as nitrogen source (8).
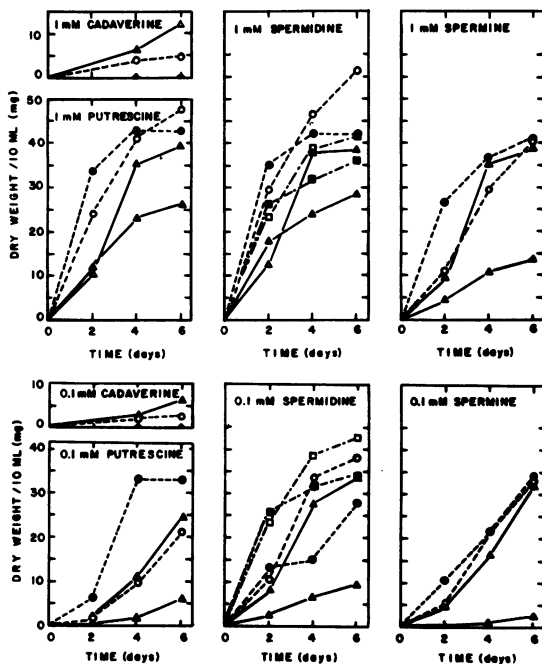
FIG. 3. Growth of *spe-1* mutants in stationary cultures supplemented with 0.1 and 1.0 mM polyamines at 25°C (open symbols) and 35°C (closed symbols). △ and ▲, strain IC-6 (TP-138 allele); ○ and ●, strain IC-4 (462JM allele); □ and ■, strain IC-1 (wild-type). Growth of wild type was not influenced by the presence or absence of polyamines; only the data for spermidine are given. Strain IC-6 grown in minimal medium yielded a trace of growth at 25°C and none at 35°C. Strain IC-4 did not grow at all in minimal medium.

termined by McDougall et al. (14) (ca. 10 cM) and by us (Table 2) with *spe-1* allele 462JM. A test of complementation among three *spe-1* strains of the same mating type and carrying different alleles (TP-138, 462JM, and 521KW) was negative. We conclude on the basis of intercrosses (Table 2), complementation, and linkage data that the three mutations are allelic. The locus is hereby named *spe-1* with the consent of K. J. McDougall. The symbol, derived from the end product(s) of the pathway, supplants *put-1*, which is based on the intermediate.

All three *spe-1* mutants grew well with 1.0 mM putrescine, spermidine, or spermine at 25°C (Fig. 3). The same was true at 35°C for strains carrying the earlier mutations, 462JM (IC-4) and 521KW (IC-5). Strains carrying the TP-138 mutation (e.g., IC-6), however, grew much more poorly at 35°C even on 1 mM (Fig. 3) or 5 mM polyamines. This character cosegregated in small progenies with TP-138 (Table 2), but its genetic and physiological basis cannot be inferred until more *spe-1* mutants are isolated from the parent strain.

Ornithine decarboxylase activity. Ornithine decarboxylase activity was measured in extracts of wild-type, an *aga*-bearing strain, and strains carrying the three *spe-1* mutations grown exponentially under various nutritional conditions (Table 3). Wild-type *N. crassa* had an activity of about 20 U/mg of protein, a value that did not change significantly when the strain was grown in putrescine or arginine. This is in contrast to the finding of Sikora and McDougall (19) that ornithine decarboxylase activity of wild type was augmented fourfold in arginine-grown cultures.

The arginaseless strain grown in minimal medium had normal ornithine decarboxylase activity. When grown in the presence of arginine, it became ornithine- and polyamine-starved (see above) and grew more slowly. As expected on the basis of previous data (16, 19), the strain had about 60-fold normal enzyme activity (Table 3),

TABLE 3. Ornithine and lysine decarboxylase activities of wild-type and mutant strains

Strain	Supplement to medium (concn [mM])	Doubling time (h)	Activity (U/mg) of [a]:	
			Ornithine decarboxylase	Lysine decarboxylase
1C-1 (wild type)	None	2.8	20	0.065
	Arginine (1.0)	2.8	25	
	Putrescine (0.2)	2.8	24	
	Putrescine (1.0)	2.8	24	
1C-3 (*aga*)	None	3.0	26	
	Arginine (1.0)	5.0	1,459	3.1
1C-4 (*spe-1*)	Putrescine (0.2)	4.8[b]	<0.1	
	Putrescine (1.0)	2.7	<0.1	
1C-5 (*spe-1*)	Putrescine (0.2)	4.0[b]	<0.1	
	Putrescine (1.0)	2.5	<0.1	
1C-6 (*spe-1*)	Putrescine (0.2)	4.0[b]	<0.1	<0.01
	Putrescine (1.0)	2.8	<0.1	

[a] Ornithine decarboxylase activity was measured with 2 mM ornithine; lysine decarboxylase activity was measured with 1 mM lysine in reaction mixtures. One unit equals 1 nmol of CO_2 per h at 37°C.

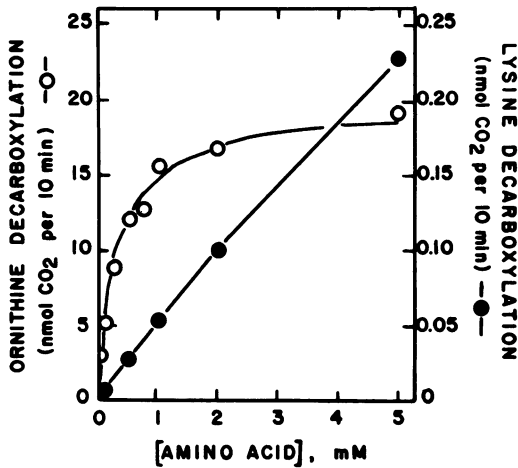[b] These doubling times lengthen as growth continues.

FIG. 4. Decarboxylation of ornithine (left ordinate) and lysine (right ordinate) by a desalted extract of IC-3 (*aga*) grown in arginine, as a function of amino acid concentration.

in keeping with the postulated negative control of the enzyme by polyamines (16).

The three putrescine-requiring strains, whether grown on limiting or unlimiting putrescine, displayed no detectable ornithine decarboxylase activity in assays that would have measured less than 1% wild-type activity (Table 3). None of the *spe-1* extracts altered the ornithine decarboxylase activity of wild-type or *aga* extracts when mixed with them.

A test of the extracts for lysine decarboxylase showed easily detectable activity in the arginine-grown *aga* strain, IC-3. Wild-type extracts had little activity, and *spe-1* extracts had none (Table 3). The proportional variation of lysine and ornithine decarboxylase activities suggests that the activities are properties of the same enzyme, particularly in view of their simultaneous loss by

mutation. When the extract of the arginine-grown *aga* strain was used, the K_m value for ornithine was estimated to be 0.3 mM. The K_m for lysine was too high to be measured (Fig. 4). At 5 mM substrate (saturating with respect to ornithine), lysine was decarboxylated at 1.2% the rate of ornithine (Fig. 4). Lysine (5 mM) did not inhibit ornithine decarboxylase activity in reactions with 0.5 mM ornithine. Ornithine (5 mM), however, inhibited lysine decarboxylation by 95% in reactions with 5 mM lysine.

Polyamine pools and growth. In exponential cultures of wild type (strain IC-1) or *aga* (IC-3) growing in minimal medium, mycelia contained 1.1 nmol of putrescine, 16.2 nmol of spermidine, and 0.5 nmol of spermine per mg (dry weight). The *spe-1* mutants grew somewhat initially in minimal medium, but the amount of polyamines found in such cultures can be accounted for by carry-over from the inoculum. Growth of *spe-1* mutants on 1 mM putrescine restored their pools to the normal range. Growth on spermidine did not lead to the appearance of putrescine (Davis and Paulus, in press). Strains given exogenous spermidine did not contain much more spermidine than normal, as though polyamine uptake was inefficient or polyamine capacity was fixed.

Of primary interest here are the polyamine pools of the *aga* strain during nutritional manipulations (see above). The polyamine pools of strain IC-3 (*aga*) were compared after growth in minimal medium or in arginine-supplemented medium. The data (Table 4) show that arginine caused a complete loss of the ornithine and putrescine pools, and the small amount of spermidine present could be accounted for by the spermidine of the inoculum. (Pools of wild-type cultures on arginine-supplemented medium are quite normal [Davis and Paulus, in press].) However, in contrast to the *spe-1* cultures, cadaverine (0.6 nmol/mg [dry weight]) and a detectable

TABLE 4. Polyamine pools of the *aga* and *spe* strains during polyamine deprivation

Strain	Supplement[a]	Doubling time (h)	Presence of[b]:					
			ODCase	ORN	PUT	SPD	CAD	APC
1C-1 (wild type)[c]	None	2.8	+	+	+	+	−	NT
1C-3 (*aga*)	None	2.9	+	+	+	+	−	−
	Arginine	5.5	+++	−	−	±[d]	+	+
1C-6 (*spe-1*)	None	14.0	−	+	−	±[d]	−	−
	Putrescine	2.7	−	+	+	+	−	NT
	Cadaverine	7.0	−	+	−	±[d]	+	NT

[a] The concentration of supplement, when present, was 1 mM.

[b] ODCase, Ornithine decarboxylase; ORN, ornithine; PUT, putrescine; SPD, spermidine; CAD, cadaverine; APC, aminopropylcadaverine; NT, not tested; +, present or normal; +++, greatly augmented; ±, trace; −, absent.

[c] Pools of wild type (in nanomoles per milligram) are, generally: ornithine, 30; putrescine, 1.1; and spermidine, 16.2 (Davis and Paulus, in press).

[d] Carry-over from the inoculum accounts for spermidine in polyamine-starved cultures.

amount of aminopropylcadaverine appeared in strains carrying *aga*. The amounts of these compounds were greater after lysine was added to such cultures. These observations suggest that the lack of ornithine and the extreme derepression of ornithine decarboxylase led to decarboxylation of lysine (endogenous or exogenous). The fact that starving *spe-1* cultures did not show any trace of cadaverine or its aminopropyl derivative suggests that cadaverine synthesis requires ornithine decarboxylase.

It now might be asked whether cadaverine serves as a polyamine substitute in the growth of *N. crassa*. Indeed, cadaverine did stimulate growth of *spe-1* mutants (Fig. 3). This was confirmed (Table 4) by the reduction of doubling time for exponential cultures. It is very likely that the indefinite growth of arginine-grown *aga* cultures (8) can be attributed to the endogenous synthesis of cadaverine, because they grew at a rate similar to that of *spe-1* cultures supplemented with added cadaverine (Table 4). It is not known whether the analog(s) is less effective than its normal counterpart(s) on a molar basis, because no cultures are available with intracellular levels of analogs equal to those of normal polyamines.

DISCUSSION

Our major findings can be summarized as follows. (i) There is a gene controlling ornithine decarboxylase in *N. crassa* on the right arm of linkage group V, confirming the original work of McDougall. It is not known whether the gene is the structural gene for the enzyme. (ii) Polyamine-deprived mycelia have greatly enhanced (60- to 100-fold) ornithine decarboxylase activity, confirming expectations of earlier work (16). (iii) Cadaverine and its aminopropyl derivative appear only in cells having high ornithine decarboxylase activity and a lack of ornithine. Ornithine decarboxylase appears to be the sole enzyme of lysine decarboxylation in the preparations we have used.

There are several discrepancies between our results and those of McDougall (14, 19). First, as noted above, the map distance (4 to 5 cM) between our new mutation, TP-138, and *inl* was less than the distance between 462JM and *inl* found by McDougall. We have confirmed the higher recombination rate between mutation 462JM and *inl* and consider that this may reflect pecularities of the chromosome segments carrying the 462JM and TP-138 alleles. Precedents for such differences are frequent in *N. crassa* (4). The fact that the TP-138 and 462JM neither complement nor recombine defines them as alleles of the same locus, *spe-1*.

We have been unable to confirm the fourfold augmentation of ornithine decarboxylase in argi-

nine-supplemented wild-type cultures. More important, we have been unable to confirm the rather high ornithine decarboxylase activities reported by McDougall (14) for his mutants (carrying alleles 462JM and 521KW). We find that they conform to his original report (Genetics 77:s16–s17, 1974) in having less than 0.5% wild-type activity. The discrepancy may be related to our finding that strains carrying allele 462JM quite often revert to a Spe$^+$ phenotype; mass transfers in nonselective media may allow revertants to contaminate an initially Spe$^-$ culture.

Yeast mutants lacking enzymes of polyamine synthesis have been isolated by others (5, 6, 22). The *spe-10* locus (originally designated *spe-1*) controls ornithine decarboxylase, but its action does not appear to be straightforward (6). Yeast polyamine mutants share with those of *N. crassa* the ability to grow considerably in unsupplemented media until the exhaustion of internal polyamines (22). Yeast mutants, moreover, are exquisitely sensitive to the addition of polyamines to the medium. As little as 10^{-10} M of any polyamine elicits a growth response, and 10^{-6} M spermidine suffices for optimal growth (6). In contrast, *N. crassa* mutants grow optimally only with 5×10^{-4} M or more polyamine. The difference between species probably reflects differences in polyamine uptake capability rather than differences in demand or catabolism (16).

The synthesis of cadaverine and aminopropylcadaverine upon starvation of mutant cells for the normal polyamines was first described by Dion and Cohen (9) working with *Escherichia coli*. Using cells having only the ornithine decarboxylase route of polyamine synthesis, they imposed ornithine deprivation by feedback inhibiting ornithine formation with addition of arginine to the medium. (*E. coli* cannot make ornithine from arginine.) The derivation of cadaverine and aminopropylcadaverine from lysine was proven, and both lysine and cadaverine in the medium stimulated the growth of ornithine-deprived cells (9). Studies of spermidine analogs on growth and DNA replication of polyamine-requiring *E. coli* showed that aminopropylcadaverine and aminopropyl-1,6-diaminohexane were stimulatory (10, 12). The synthesis of cadaverine in *E. coli* is due largely to activity of a lysine decarboxylase (20). Strains lacking this enzyme make only traces of the compound. (It is not excluded that the ornithine decarboxylases of *E. coli* can weakly catalyze lysine decarboxylation.) Whereas *E. coli* strains unable to make any polyamine grow slowly, the fungal mutants eventually do not grow at all. In both *N. crassa* and *E. coli* (20), however, cadaverine stimulates growth slightly when added to the

medium. It is probable that the indefinite growth of the arginaseless strain of *N. crassa* when grown on arginine is due in large measure to its ability to synthesize cadaverine endogenously.

In animal cells, cadaverine occasionally appears in certain tissues. In a study of lysine decarboxylase activity in vitro, Pegg and McGill (18) could attribute all activity to a nonspecific action of ornithine decarboxylase with which the lysine decarboxylase activity copurified. (Results from cultured mammalian cells which suggested the presence of a specific lysine decarboxylase [1] were later shown to be attributable to mycoplasmas in the culture [2].) Thus, *N. crassa* fits the animal pattern for cadaverine synthesis, inasmuch as ornithine decarboxylase-deficient mutants cannot decarboxylate lysine.

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