Developmental Defects Resulting from Arginine Auxotrophy in Aspergillus nidulans

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A mutant of Aspergillus nidulans, isolated for inability to form asexual spores (conidia) on complete medium, was found to regain the ability to conidiate if the medium was supplemented with arginine. On minimal medium the mutant required arginine for growth but at a much lower concentration than that required for conidiation. This mutant, designated argB12, thus defines a phase-critical gene, i.e. a gene whose function is in greater demand for development than for growth. In addition to its aconidial phenotype, the mutant also exhibited (depending on the medium) aberrant sexual development and a low efficiency of conidial germination. In crosses, each of these developmental phenotypes segregated with arginine auxotrophy. Genetic and biochemical analyses showed the argB12 mutation to be an allele of the previously described argB locus, mutants of which lack ornithine transcarbamylase. Arginine-requiring mutants at at least two other loci were also found to be defective in asexual sporulation, but the germination defect appears to be specific to argB mutants.

INTRODUCTION

Spore-originated surface colonies of the homothallic ascomycete Aspergillus nidulans sporulate in two morphologically and temporally distinct phases. In the first phase, conidia (asexual spores) are generated by mitosis on the surface of specialized aerial hyphae (conidiophores), while in the second phase, sexual spores are formed by meiosis within closed spherical shells (cleistothecia). Each of the two phases is accompanied by the synthesis of a specific phenol oxidase (laccase I for the asexual phase and laccase II for the sexual phase) which can serve as convenient biochemical event markers (Clutterbuck, 1972; Hermann et al., 1983). Growth in submerged liquid culture completely suppresses the development of spores and spore-bearing structures, but differentiation can be readily induced by transferring the mycelium from liquid medium to a solid nutrient surface (Axelrod et al., 1973).

In a mutational analysis of the conidiation genes of A. nidulans, Martinelli & Clutterbuck (1971) estimated that several hundred genes are required specifically for conidiation. Surprisingly, very few mutants were found that have defects in the terminal stages of sporulation after conidiophores are formed, suggesting that most genes function at early stages of the conidiation process (Clutterbuck, 1969). By using thermosensitive aconidial mutants in temperature-shift experiments we have obtained results that support this view: the majority of such mutants begin to lose their thermosensitivity in a relatively short period just before the appearance of conidiophores (Yager et al., 1982).

Mutants which are blocked in sporulation but which are apparently otherwise normal are of interest for the identification of phase-specific genes, i.e. genes that are expressed only during...
one phase of development. However, a serious limitation to the value of mutants isolated solely for a developmental aberration is that they usually give no clue to the nature of their biochemical defect. Another conceivable class of mutants is one which requires a nutritional factor at low concentration for normal growth but at high concentration for normal development. Such mutants would identify what Schmit & Brody (1976) have termed phase-specific genes, i.e. genes whose functions are in greater demand for development than for growth. Such genes may not be as directly relevant to development as phase-specific genes, but the mutants can at least reveal which of the many metabolic processes are in high demand during development and the time at which this demand is imposed. In this report we describe an arginine-requiring mutant of A. nidulans that is abnormal in both asexual and sexual development and, under certain conditions, in conidial germination.

METHODS

Strains of Aspergillus nidulans. Strains R-21 (yA2 pabaA1) and R-153 (wA; pyrA4) were obtained from Dr C. F. Roberts, University of Leicester, U.K., and have been described by Armit et al. (1976). An arginine-requiring derivative of R-21 (WIM-180) was obtained by UV mutagenesis as previously described (Kurtz & Champe, 1981). Strain WIM-181 is a yA2+ pabaA1+ pyrA4+ recombinant from a cross of WIM-180 with R-153 and carries the induced arg mutation of WIM-180. The strains carrying the standard arg markers were FGSC-230 (yA2; wA2; argA1; veA+), FGSC-256 (pabaA1; wA3; argC3 facB101 riboB2) and G034 (bA1; argB2). The former two strains were obtained from the Fungal Genetics Stock Center and the latter from Dr A. J. Clutterbuck, University of Glasgow, U.K. The aconidal fluffy variant flu-11 is in a strain WIM-46 of genetic background yA2; palA1 galA1 phenA2 as described by Dorn (1970). For purposes of genetic analysis various derivatives of the above arginine-auxotrophic strains were constructed by crosses with R-21 or R-153. Genetic symbols are as listed by Clutterbuck (1974).

Media. The complete medium (YGT) was composed of 0.5% (w/v) yeast extract (Difco), 2% (w/v) glucose and trace elements at concentrations previously described (Kurtz & Champe, 1981). The arginine concentration of this medium was determined to be 70 μg ml⁻¹ by the method of Atfield & Morris (1961). Minimal medium (MM), containing 0.01 M-sodium nitrate as the nitrogen source, was as described by Cove (1966). The carbon source of MM was 1% (w/v) glucose unless stated otherwise. These media supplemented with arginine (200 μg ml⁻¹) are designated YGT(Arg) and MM(Arg). For plates these media were solidified with 1.5% (w/v) agar (Difco).

Genetic analysis. The methods employed were those described by Pontecorvo et al. (1953).

Assay of conidial yield. The viable conidia formed by isolated colonies were assayed as the number of colony-forming units in a filtered homogenate of pooled colonies. As previously described in detail (Yager et al., 1982) the filtration passes 100% of the conidia but retains nearly all viable hyphal fragments. Conidial yields of less than 100 conidia per colony can be determined by this method without interference from hyphal fragments.

Cell-free extracts for enzyme assays. For the assay of cleistothecial laccase (laccase II) confluent surface cultures of yA2-carrying strains were prepared by inoculating 50 ml YGT plates with 10⁵ conidia in 4 ml of YGT liquid medium. After incubation of the plates in the dark and unstacked for various times at 37 °C, the mycelium was peeled from the agar and homogenized in a Ten Broeck tissue homogenizer with one volume of 0.01 M-Tris, pH 8.5, and 1.0 mM-phenylmethylsulphonyl fluoride. After centrifugation the homogenate for 15 min at 26000 g the supernatant was used for the determination of laccase activity by the spectrophotometric method of Clutterbuck (1972) employing the chromogenic substrate N,N-dimethyl-p-phenylenediamine sulphate (DMP) (Sigma product no. D-4142).

For the assay of ornithine transcarbamylase (EC 2.1.3.3; OTC) of mycelia grown under conditions where the enzyme would be repressed, a heavy conidial suspension was inoculated into 250 ml flasks containing 100 ml of liquid MM(Arg) medium. Cultures were grown at 37 °C for 30 h in a shaker bath at 160 r.p.m. When derepression of OTC was required, mycelium grown for 22 h as above was filtered on to sterile nitrocellulose filters (Millipore), washed with MM, resuspended in 100 ml MM and incubated for an additional 8 h on the shaker. Mycelial pellets were harvested by filtration on Millipore filters, washed with cold distilled water and ground in a Ten Broeck homogenizer with one volume of 0.01 M-Tris, pH 8.5, and 1.0 mM-phenylmethylsulphonyl fluoride. After centrifugation of the homogenate at 10000 g for 20 min the supernatant was assayed for OTC activity. OTC activity was assayed spectrophotometrically as the enzymically formed citrulline using the Sigma modification of the Archibald reaction (Sigma product no. 0-2501). For specific activity, protein was determined by the Bio-Rad procedure.

RESULTS

Isolation of an auxotrophic aconidial mutant

The mutant described below was obtained from the parental strain R-21 by UV treatment of conidia. Single, spore-originated colonies growing on YGT complete medium were screened for
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aconidial or other morphologically abnormal phenotypes by visual inspection. Most such variants grew at the normal rate when replated on MM. One aconidial variant, however, did not grow on MM unless the medium was supplemented with arginine. Furthermore, the aconidial phenotype of the mutant on YGT was reversed by supplementing this medium with arginine. The mutant allele will be designated *arg-*12.

In two successive backcrosses of the mutant strain to the parental strain R-21 (*yA2 pabaA1*) the aconidial phenotype exhibited on YGT medium always segregated with the auxotrophic phenotype. One *arg*-12 segregant (WIM-180) from the last backcross was selected for use in experiments in which the yellow-spore phenotype is desirable. A further green-spore, *arg-*12 but otherwise prototrophic segregant (WIM-181) was selected from a cross of WIM-180 with strain R-153 (*wA3; pyroA4*) and is the *arg*-12-carrying strain used in most of the experiments described below.

Reversal of the aconidial phenotype of WIM-181 by arginine is shown quantitatively in Fig. 1. On YGT medium without added arginine very few conidiophores were present and the conidial yield was as low as that of most previously described aconidial mutants (Yager *et al.*, 1982) but increased some 10^4-fold with the addition of arginine to 500 μg ml^-1_. By contrast, the radial growth was not sensitive to arginine addition.

**Pleiotropy of the *arg-*12 mutation**

In addition to their aconidial phenotype, strains which carried the *arg-*12 mutation behaved differently from the R-21 parent in three other respects: (1) confluent lawns of the mutant strains on YGT medium exhibited premature formation of laccase II which, we have shown previously (Hermann *et al.*, 1983), is localized in cleistothecial primordia and the globose hülle cells that surround the primordia of this species; (2) conidia of mutant strains had a very low efficiency of colony formation on MM(Arg) medium; and (3) the conidia of mutant strains which formed colonies on MM(Arg) medium did so with a significant delay relative to the parental strain. Each of these phenotypes, which are demonstrated in Figs 2, 3 and 4, segregated with the arginine auxotrophic phenotype.

The premature development of laccase II by the *arg-*12 mutant is shown in Fig. 2 in which the mutant strain (WIM-180) also carried a mutation in the structural gene for laccase I (*yA*) to avoid interference by the higher level of laccase I. Laccase II activity of the mutant strain reached a maximum at about 45 h, in contrast to the slow increase up to 100 h for the parental strain. Microscopic examination of mutant tissue stained for laccase (Hermann *et al.*, 1983)
Fig. 2. Premature formation of laccase I by WIM-180. Mycelial extracts were prepared as described in Methods from YGT plates inoculated with 10⁵ conidia and incubated at 37 °C. Laccase activity was assayed using the chromogenic substrate DMP as described by Clutterbuck (1972). The mutant strain WIM-180 and the parental strain R-21 both carried the yA2 marker which eliminates the conidial laccase (laccase I).

showed that the source of the activity was profuse masses of hülle cells which were, in fact, the only differentiated structures observable prior to 45 h. At later times, small cleistothecia (about one-third normal diameter) developed in great abundance, but these were completely devoid of ascospores (counted microscopically or as colony forming units) even after two weeks of development. Supplementation of YGT medium with arginine restored early sexual development of the mutant to the normal schedule, but did not correct its sexual sterility. Surprisingly, however, high levels of arginine were found to strongly inhibit ascospore formation by the parental strain R-21 (Table 1).

The efficiency of colony formation by conidia of WIM-181 on MM(Arg) medium varied inversely with the glucose concentration (Fig. 3). At the usual concentration of glucose used in this medium (1%), the efficiency was only 0.01% to 0.1% that of the parental strain R-21, but the conidia produced by the colonies that did develop again had the same low efficiency of colony formation. If, however, glucose was replaced by fructose the efficiency of colony formation was near 100% over a wide concentration range. Nevertheless, as shown, the presence of fructose did not spare the inhibition by glucose, even when glucose was present at levels well below that of fructose. The rare colonies of WIM-181 that did develop on MM(Arg) medium (at the standard glucose concentration) were also smaller than those of the parental strain. As shown in Fig. 4, this size difference was not due to a slower radial growth rate, but rather to a delay in attaining a linear growth rate. Microscopic examination showed that on this medium only a small fraction of the conidia formed germ tubes and those that did, did so very slowly.

<table>
<thead>
<tr>
<th>Arginine added to YGT (µg ml⁻¹)</th>
<th>Viable ascospores per cleistothecium</th>
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<tbody>
<tr>
<td>0</td>
<td>R-21</td>
</tr>
<tr>
<td>50</td>
<td>R-21</td>
</tr>
<tr>
<td>100</td>
<td>R-21</td>
</tr>
<tr>
<td>150</td>
<td>R-21</td>
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<tr>
<td>200</td>
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</tr>
<tr>
<td>300</td>
<td>R-21</td>
</tr>
<tr>
<td>500</td>
<td>R-21</td>
</tr>
</tbody>
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Fig. 3. Efficiency of colony formation by conidia of WIM-181 on MM(Arg) medium containing glucose and/or fructose. The efficiency of colony formation is expressed as a percentage of the colonies formed on MM(Arg) medium containing 0.01% fructose.

Fig. 4. Colony expansion of WIM-181 and the parental strain R-21 on standard MM(Arg) medium (containing 1% glucose). Plates were point-inoculated with 10^6 ml^-1 conidial stocks and incubated at 37 °C. Each point represents an average measurement from four plates.

The metabolic block of arg-12

Cybis et al. (1972) have identified four genetic loci of A. nidulans which determine the last steps of the arginine biosynthetic pathway as follows:

ornithine $\xrightarrow{\text{argB}}$ citrulline $\xrightarrow{\text{argC}}$ argininosuccinate $\xrightarrow{\text{argE}}$ arginine

(ornithine transcarbamylase) (argininosuccinate synthetase) (argininosuccinate lyase)

In this study it was reported that citrulline is unable to restore the growth of an argB mutant even though, from independent evidence, the block appeared to be before citrulline in the biosynthetic pathway. Likewise, argininosuccinate did not restore the growth of argB, argC or argE mutants. In agreement with this earlier work we found that the growth defect of WIM-181 on MM was not corrected by ornithine or citrulline. Presumably this failure is caused by poor penetration of these exogenously supplied compounds, possibly due to the lack of induction of necessary permeases in MM. If this were the case, certain of the arginine precursors should be able to reverse the aconidial phenotype of WIM-181 on YGT medium, and indeed it was found that citrulline, but not ornithine, stimulated conidiation. To determine whether the stimulation of conidiation by citrulline requires its conversion to arginine, an arg-12 argC3 double mutant was constructed by crossing WIM-181 to FGSC-256. For this double mutant citrulline was without effect on conidiation while arginine was still stimulatory.

The pattern of response to arginine precursors suggests that the block of WIM-181 is at the same step as that controlled by the argB locus, namely the conversion of ornithine to citrulline catalysed by ornithine transcarbamylase (OTC). To test this, OTC activity was measured in mycelial extracts of WIM-181, its parent strain R-21 and FGSC-256 (argC3). The results shown in Table 2 confirm that WIM-181 is, in fact, deficient in OTC activity.
Table 2. Ornithine transcarbamylase activity of the parental (arg\textsuperscript{+}) strain R-21 and of two arginine-requiring strains

OTC activity is expressed as μmol citrulline min\textsuperscript{-1} (mg protein)\textsuperscript{-1} and was measured as described in Methods.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Repressed</th>
<th>Derepressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-21 (arg\textsuperscript{+})</td>
<td>0.142</td>
<td>0.152</td>
</tr>
<tr>
<td>FGSC-256 (argC3)</td>
<td>0.144</td>
<td>0.365</td>
</tr>
<tr>
<td>WIM-181 (arg-12)</td>
<td>&lt;0.002</td>
<td>&lt;0.002</td>
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Genetic analysis of arg-12

Complementation analysis with standard arginine markers showed that arginine-independent heterokaryons could be formed between arg-12 and strains carrying argAl and argC3, and that these heterokaryons were strongly conidial. In contrast, arginine-independent heterokaryons between arg-12 and argB2 could not be generated. Further, mitotic and meiotic mapping showed that the mutational site of arg-12 is located on chromosome III, 19.5 recombination units from palA1 and 38.5 recombination units from galAl, closely matching the position of the argB locus (Clutterbuck, 1974). Lastly, crosses of arg-12 with argB2 produced fewer than 1% arg\textsuperscript{+} recombinants. These results, taken together with our finding that arg-12, like argB2, is deficient in OTC activity, show with some certainty that arg-12 is a mutation at the argB locus. According to convention, we will thus designate this mutant argB12.

The only difference in behaviour observed between the strains WIM-181 (argB12) and G034 (argB2; biA1) is the inhibition of germination on glucose MM exhibited by the former strain but not by the latter. To test the biotin-requiring strain G034 it was, of course, necessary to add biotin to the medium, which thus differed in this respect from the medium used in Figs 3 and 4. Indeed, when conidia of WIM-181 were plated on MM(Arg) medium containing as little as 1 pg biotin ml\textsuperscript{-1} no inhibition or delay of germination was observed. Moreover, when the biotin requirement was crossed out of G034, the resulting substrain exhibited inhibition of germination by glucose in the absence of biotin. The inhibition of germination by glucose thus applies to both argB alleles and appears to be specific for argB mutants since neither argA nor argC mutants (in a bi\textsuperscript{+} background) were inhibited.

The period of arginine dependence for conidiation

To identify the period during which the presence of arginine is required for conidiation, colonies of WIM-181 growing on a Miracloth filter pad at 37 °C were shifted from arginine-supplemented YGT agar to unsupplemented YGT agar at various times after spore inoculation, and the conidial yields of the colonies were measured at a later fixed time (70 h). As shown by curve (a) of Fig. 5, a shift to arginine-poor medium at any time prior to 23 h prevented subsequent conidiation, whereas with later shifts the conidial yields progressively increased. The period during which arginine must be present for conidiation to occur thus begins shortly before the onset of conidiation (indicated by the arrow). Before 23 h the presence of arginine is irrelevant to subsequent conidiation.

The time at which colonies become dependent on the presence of arginine for conidiation could be a fixed time independent of the developmental state of the mycelium or could be determined by the fact that conidiation is about to begin. These alternatives can be distinguished by taking advantage of the fact that conidiation is totally suppressed for colonies growing in submerged liquid culture but can be induced at any time by transfer of the colonies to solid medium (Axelrod \textit{et al.}, 1973). It is thus possible to ask whether colonies of WIM-181 growing in submerged culture respond to the presence of arginine (by subsequent conidiation) after 23 h as they do when growing as surface colonies. Curve (b) of Fig. 5 shows the results of an experiment in which conidia of WIM-181 were inoculated into arginine-supplemented YGT
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Fig. 5. Conidiation of colonies of WIM-181 transferred from arginine-rich to arginine-poor medium at various times. (a) Spore-originated colonies were grown on Miracloth filter discs placed on the surface of YGT(Arg) agar. At the times indicated, discs were peeled from the agar, soaked in unsupplemented YGT liquid medium to remove residual arginine and transferred to the surface of an unsupplemented YGT agar plate. Conidial yields of excised colonies were assayed at 72 h (37 °C). (b) Conidia were inoculated into YGT(Arg) liquid medium to give 50 conidia ml⁻¹. After incubation for the indicated times, 0.1 ml of the culture was transferred to unsupplemented YGT plates and the conidial yields of the resulting colonies were determined at 72 h. In both experiments the conidial yield is expressed as the percentage of the yield of colonies grown continuously on YGT(Arg) plates. The arrow indicates the approximate time that conidiation commenced on YGT(Arg) agar.

liquid medium and the resulting colonies were transferred to unsupplemented YGT agar at various times. The conidial yields of these colonies were uniformly low even for transfers as late as 40 h. In summary, the experiments of Fig. 5 show that arginine stimulated conidiation if present after induction but was ineffective before induction. The conidiation process itself, rather than some preparatory preinduction event, thus appears to impose a heavy demand on arginine.

The role of arginine in conidiation

In the course of the above studies it was noted that other mutants of the arginine biosynthetic pathway also conidiated poorly on YGT medium and that conidiation was stimulated by arginine supplementation. Whether these mutants are affected as severely as argB12 and whether they are also aberrant in sexual development like argB12 has not yet been decided because the mutations are in strains of diverse genetic backgrounds. In any case, this observation suggests that arginine itself, or some catabolic product of arginine, has a positive effect on conidiation as opposed to a negative effect due to some metabolic perturbation arising indirectly from the lack of OTC. That arginine itself rather than some catabolic product is the critical factor can be argued from the observation that when the nitrate of MM was replaced by ammonium, which is known to repress the arginine catabolic enzymes (Bartnik & Weglenski, 1974), addition of arginine still strongly stimulated conidiation of WIM-181.

Another indication of the morphogenic role of arginine is the effect of the argB12 mutation on colony morphology of an aconidial fluffy variant (Dorn, 1970) which, in an arg⁺ background, produces a dense growth of aerial hyphae. We constructed a strain which carried argB12 and fluffy. On YGT medium colonies of this strain completely lacked aerial hyphae while on medium supplemented with arginine the fluffy phenotype was restored. This finding suggests that the morphogenic process which requires arginine is the formation of aerial hyphae whether these be differentiated conidiophores or the apparently undifferentiated hyphae of a fluffy mutant.
DISCUSSION

The present study demonstrates the importance of arginine for both asexual and sexual sporulation in *Aspergillus nidulans*. The need for arginine in conidiation is clear-cut: arginine auxotrophs had greatly reduced conidial yields on arginine-poor medium but conidiated normally on arginine-supplemented medium. The effect of arginine auxotrophy on sexual development is, however, more complex. On arginine-poor medium sexual development of *argB12* was initiated prematurely, as judged by the early formation of hülle cells and the associated enzyme laccase, but the cleistothecia completely lacked ascospores. Arginine supplementation suppressed the premature appearance of hülle cells, but did not allow the formation of ascospores and, in fact, prevented ascospore formation by the parental strain. The arginine-induced sexual sterility of the parental strain would explain the failure of arginine to correct the sterility of the *argB12* mutant strain at high arginine concentrations. It is noted, however, that the sexual sterility of *argB12* is not significantly corrected by intermediate arginine concentrations for which arginine-induced sterility is incomplete (Table 1). This suggests that the sexual sterility resulting from the *argB12* mutation is not due to an arginine deficiency *per se* but that some secondary metabolic perturbation is responsible.

Mutants defective simultaneously for both asexual and sexual sporulation are not uncommon (Clutterbuck, 1969), indicating a close coupling between the two processes. In a recent study of thermosensitive but non-auxotrophic aconidial mutants (Yager et al., 1982) we found that eight out of eleven mutants totally lacked hülle cells and cleistothecia while one mutant formed hülle cells but proceeded no further. The sexual phenotype of *argB12* (sterile cleistothecia) is yet a third manifestation of this coupling. In this case it is interesting that the developmental schedule was disrupted: hülle cells appeared for the mutant at about the same time that conidia appeared for the parental strain.

The aconidial phenotype of the *argB12* mutant was also exhibited, at least qualitatively, by mutants affected at the *argA* and *argC* loci. One phenotype of *argB12* (and *argB2*) clearly not exhibited by mutants at other loci is the inhibition of conidial germination by glucose. This inhibition reveals a complex relationship between arginine metabolism, glycolysis and biotin, the elucidation of which is beyond the intent of this study. However, two previously reported similar observations are illuminating (Strigini & Morpurgo, 1961; Maitra, 1971).

Strigini & Morpurgo (1961) found that the conidia of a wild-type strain of *A. nidulans* require a low level of biotin for germination on glucose, but not on fructose, minimal medium. The amount of biotin required was well below the amount required for growth of a biotin-requiring mutant, suggesting a difference in the utilization of exogenous and endogenous biotin as indeed is implied by our results. Our *argB12* mutant thus behaves very similarly to some wild-type strains with regard to the biotin requirement, but the parental strain R-21 does not exhibit this biotin requirement. This can be understood if the *argB12* mutation merely amplifies the need for biotin which all strains may require for germination but to a different degree. For strains which require a very low level of biotin for germination, the need can be satisfied endogenously, as presumably is the case for R-21.

Maitra (1971) reported that a mutant of *Saccharomyces cerevisiae* defective in phosphoglucoisomerase grew on fructose medium but not on medium containing fructose plus glucose. The inhibition by glucose was attributed to the accumulation of toxic concentrations of glucose 6-phosphate.

The observed glucose inhibition of *argB* mutants could similarly be attributed to a block in an early step of glycolysis before fructose enters the pathway, thus mimicking the behaviour of the yeast phosphoglucoisomerase-defective mutant. A likely candidate for the blocked enzyme is phosphofructokinase, a key enzyme in the control of glycolysis which is inhibited by ATP and citrate as well as other allosteric modulators. How this block could result specifically from a lack of ornithine transcarbamylase, however, is not obvious and could be quite indirect. It is noteworthy, nevertheless, that addition of biotin, which eliminates the block, would be expected to decrease the concentration of ATP due to the fact that all of the biotin-requiring carboxylation reactions consume ATP. It should be stressed that the observed glucose inhibition applies to the special case of spores emerging from dormancy for which the relative metabolic activities must
initially be very different from that of actively growing cells. Conceivably, small imbalances, which would be innocuous for growing cells, could abort the germination process.

In considering the role of arginine in conidiation it should be kept in mind that many metabolites, particularly the basic amino acids, are not totally free in the cytoplasm of fungal hyphae but are sequestered to a greater or lesser degree in vacuolar particles. For Neurospora crassa it has been estimated that 90% of the arginine and ornithine pool is contained in rapidly sedimenting particles distinct from mitochondria (Weiss, 1973). Although most of the definitive work has been done in yeast (Wiemken & Nurse, 1973; Nurse & Wiemken, 1974; Wiemken & Durr, 1974) and Neurospora (Weiss, 1973; Cramer et al., 1980), similar vacuoles almost certainly exist in A. nidulans as well. This compartmentation not only protects amino acid pools from metabolic breakdown but also allows ‘packets’ of metabolites to be delivered with high efficiency to sites where they may be in high demand. Brody (1981) has suggested that the vacuoles of N. crassa may be the reservoirs of compounds required for conidiogenesis, and, in particular, that arginine delivered via vacuoles to conidia is the precursor of the glutamic acid found in exceptionally high concentrations in conidia. In view of our finding that the arginine requirement for conidiation is satisfied only if arginine is present after induction, it would be enlightening to re-investigate such mutants for asexual and sexual abnormalities by the quantitative methods now available.

By isolating developmental mutants on minimal medium containing low levels of nutritional supplements the present work could be extended to identify other metabolites or metabolic pathways that are crucial for development. Indeed, in a survey of conidiation mutants Martinelli & Clutterbuck (1971) reported that auxotrophs exist for which partial starvation is more deleterious for conidiation than for growth; these include auxotrophs for methionine, lysine, adenine, pyridoxine and riboflavin (A. J. Clutterbuck, personal communication). It may prove illuminating to re-investigate such mutants for asexual and sexual abnormalities by the quantitative methods now available.

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