Ammonium Regulation in *Aspergillus nidulans*

J. A. Pateman, J. R. Kinghorn, Etta Dunn, and E. Forbes

Department of Genetics, University of Glasgow, Glasgow G11 5JS, Scotland

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L-glutamate uptake, thiourea uptake, and methylammonium uptake and the intracellular ammonium concentration were measured in wild-type and mutant cells of *Aspergillus nidulans* held in various concentrations of ammonium and urea. The levels of L-glutamate uptake, thiourea uptake, nitrate reductase, and hypoxanthine dehydrogenase activity are determined by the extracellular ammonium concentration. The level of methylammonium uptake is determined by the intracellular ammonium concentration. The uptake and enzyme characteristics of the ammonium-derepressed mutants, *meaA8, meaB6, DER3, amrA1, xprD1, and gdhA1*, are described. The *gdhA* mutants lack normal nicotinamide adenine dinucleotide phosphate-glutamate dehydrogenase (NADP-GDH) activity and are derepressed with respect to both external and internal ammonium. The other mutant classes are derepressed only with respect to external ammonium. The mutants *meaA8, DER3, amrA1, and xprD1* have low levels of one or more of the L-glutamate, thiourea, and methylammonium uptake systems. A model for ammonium regulation in *A. nidulans* is put forward which suggests: (i) NADP-GDH located in the cell membrane complexes with extracellular ammonium. This first regulatory complex determines the level of L-glutamate uptake, thiourea uptake, nitrate reductase, and xanthine dehydrogenase by repression or inhibition, or both. (ii) NADP-GDH also complexes with intracellular ammonium. This second and different form of regulatory complex determines the level of methylammonium uptake by repression or inhibition, or both.

A number of enzyme and uptake systems in *Aspergillus nidulans* are regulated by ammonium. The level of all these systems is minimal if wild-type cells are grown, or held, in the presence of ammonium. It is not known whether this effect of ammonium is on protein synthesis, on inhibition of activity, or a combination of both. However, the phenomenon is frequently referred to as ammonium repression and, for convenience, will usually be called so here. Ammonium repression is widespread and very important in the whole area of inorganic and simple organic nitrogen metabolism in *A. nidulans* in an analogous fashion to catabolite repression in carbon metabolism of many prokaryotes. Systems repressed by ammonium include nitrate reductase (12), xanthine dehydrogenase (16), acetamidase and formamidase (6, 7), L-glutamate uptake (8; Pateman and Kinghorn, manuscript in preparation), urea uptake (4), and extracellular protease (2). Mutations in a number of genes can affect the regulation by ammonium of these systems. Arst and Cove (1) found that mutation in two loci, *meaA* and *meaB*, resulted in resistance to the toxic analogue methylammonium and in derepression for nitrate reductase and xanthine dehydrogenase. Cohen (2) isolated a mutant *xprD1* which is derepressed for extracellular protease and other systems. Pateman et al. (manuscript in preparation) have isolated two other classes of derepressed mutants, DER3 and *amrA1*, which have ammonium transport abnormalities.

In this paper we summarize the relevant enzyme and uptake activities and the level of resistance to toxic analogues of all the known classes of ammonium-derepressed mutants. We also present data on the intracellular ammonium concentrations in wild-type and mutant cells held in the presence of various concentrations of ammonium and urea. These data show that there are two types of ammonium-regulated systems. The ammonium uptake system is determined by the intracellular ammonium concentration. The level of the other enzyme and uptake systems previously mentioned is determined by the extracellular ammonium concentration. Only the *gdhA* mutants are derepressed for both types of ammonium regulation, and they also lack normal nicotinamide adenine dinucleotide phosphate-glutamate dehydro-
genase (NADP-GDH) activity. We propose a unifying hypothesis concerning ammonium regulation which accounts for the great variety of experimental observations on the wild type and ammonium-derepressed mutants.

MATERIALS AND METHODS

Strains and genetic techniques. The genetic techniques employed were those described by Pontecorvo et al. (15) and McCully and Forbes (10). The strain used as the wild type with respect to nitrogen metabolism was a translocation-free auxotroph bi1 (Glasgow no. 051). Strains meaA8 and meaB6 (formerly meaA6) are methylammonium-resistant, ammonium-derepressed mutants (1) supplied by H. H. Arst. Strain DER3 is one of a series of ammonium-derepressed mutants obtained by J. A. Pateman, who selected directly for mutants with ammonium derepression of nitrate reductase by using a replica plating technique similar to that of Pateman and Cove (12). Strain amra1 was obtained by J. A. Pateman by selecting for mutants with poor growth on ammonium as a nitrogen source. This strain shows poor growth on nitrate, ammonium, or urea, has ammonium transport abnormalities, and is ammonium derepressed (Pateman et al., manuscript in preparation). Strain xprD1 is an ammonium-derepressed mutant obtained by Cohen (2) by selecting directly for mutants with ammonium derepression of extracellular protease production. Strain gdhA1 is an ammonium-derepressed mutant obtained by selecting for sensitivity to 200 mM ammonium; it lacks NADP-GDH activity (Kinghorn and Pateman, manuscript in preparation).

Media and growth conditions. Media and supplements described by Cove (3) were modified by the method of Pontecorvo et al. (15). N medium is nitrogen-free minimal medium. Mycelium for uptake and enzyme assays was grown in shaken cultures at 25 C for 18 to 20 h, harvested, and, for enzyme assays, extracted as described by Cove (3).

Enzyme assays. NADPH-nitrate oxidoreductase (EC 1.6.6.3) was assayed, as described by Cove (3), after mycelium had been grown in the presence of nitrate (14). NADPH-L-glutamate oxidoreductase (EC 1.4.1.14) was assayed as described by Pateman (11). In hypoxanthine-xanthine dehydrogenase, hypoxanthine and xanthine are both substrates for the A. nidulans enzyme. The enzyme mediates the transfer of electrons to various acceptors, including cytochrome c. The rate of reduction of cytochrome c is increased several-fold by the presence of various electron donors-acceptors of which benzyl viologen is the most convenient for enzyme assay purposes (Pate- man, unpublished results). The reaction mixture contained: hypoxanthine, 50 μg; benzyl viologen, 15 mg; cytochrome c, 4 mg; cell extract, 50 to 100 μlitters; and 100 mM sodium pyrophosphate buffer, pH 9.4, to a final volume of 1.5 ml. The initial rate of reduction of cytochrome c is determined in the presence and absence of hypoxanthine at 35 C by the change in extinction at 551 nm in a Unicam SP800 spectrophotometer. The amount of soluble protein in extracts was estimated by the method of Lowry et al., (9). Specific enzyme activities are given as nanomoles of substrate transformed per minute per milligram of protein.

Plate tests for ammonium derepression. (i) Ammonium, by repressing nitrate reductase synthesis, protects the wild type against chlorate toxicity; poor growth on N medium containing 100 mM KC10, and 10 mM ammonium indicates derepression of nitrate reductase (1). (ii) A green-spored wild type develops yellow conidia in the presence of 2-thioxanthine due to the action of xanthine dehydrogenase. Ammonium prevents the development of yellow conidia by repressing xanthine dehydrogenase. The production of yellow conidia by a genotypically green-spored strain, grown on N medium containing 500 μM 2-thioxanthine and 10 mM ammonium, indicates derepression of xanthine dehydrogenase (1). (iii) Ammonium derepression of protease release is indicated by a milk-clearing halo around a colony growing on N medium containing milk and 10 mM ammonium (2). (iv) Ammonium, by repressing the thiourea uptake system, protects the wild type against thiourea toxicity. Poor growth on N medium containing 5 mM thiourea and 5 mM ammonium tartrate indicates derepression of thiourea uptake (5; Dunn and Pate- man, manuscript in preparation).

Plate test for methylamine resistance. Wild type will not grow on N medium containing 100 mM methylamine chloride; methylamine-resistant strains grow well (1).

Preparation of cells for uptake and intracellular ammonium assays. After growth in shaken flasks at 25 C for 10 to 20 h on N medium containing 0.15% Casamino Acids, cells were harvested on nylon cloth, washed with N medium at 25 C, pressed dry with absorbent paper, and weighed. The cells were resuspended at 10 g/liter and shaken for 4 h at 25 C in N medium (N-free treatment) or N medium plus a nitrogen source specified in the text. When a nitrogen source was added, the cells were transferred to fresh medium five times during the 4-h period to maintain the nitrogen concentration close to the original value. Finally, the cells were harvested and assayed.

L-Glutamate, thiourea, and methylammonium uptake assays. When grown under the conditions described above, A. nidulans is largely in the form of small colonies less than 1 mm in diameter. These colonies can be kept in suspension by shaking, and quantitative samples can be withdrawn from the suspension. After treatment, 1 g of the cells was suspended in 50 ml of N medium containing L-glutamate, thiourea, or methylammonium, in a 250-ml Erlenmeyer flask in a shaking water bath at 25 C. The uptake flask contains: for L-glutamate uptake, L-[14C]- and L-[14C]glutamate to a final concentration of 100 μM and approximately 0.5 μCi of radioactivity; for thiourea uptake, 14C- and 14C-thiourea to a final concentration of 200 mM and approximately 2.5 μCi of radioactivity; for methylammonium uptake, 14C- and 14C-methylammonium to a final concentration of 500 μM and approximately 2.5 μCi of radioactivity. At times 0, 2, 4, 6, 8, and 10 min, 5-ml fractions of the cell suspension were filtered, with two 10-ml washes of water, on a membrane filter (Millipore Corp.) The
resultant pad of cells was weighed and transferred to 5 ml of Bray scintillation fluid, and the radioactivity was measured in a Beckman liquid scintillation spectrometer. The rate of uptake of radioactivity into the cells was linear for all three chemicals for the first 10 min. The uptake capacity of the cells is expressed as nanomoles of substrate taken up per minute per milligram (dry weight) of cells. A full description of each of these uptake systems will be published elsewhere.

**Intracellular ammonium assay.** The perchlorate extraction and the determination of ammonium by using an NADP-L-glutamate dehydrogenase-mediated reaction is based on the method of Grenson and Hou (5) and M. Grenson (personal communication).

**Chemicals.** Analytical-grade chemicals were used whenever possible. Purified NADP-L-glutamate dehydrogenase was obtained from Sigma Chemical Co., St. Louis, Mo. L-[14C]glutamate, 3-C-thiourea, and 14-C-methylammonium were obtained from the Radiochemical Centre, Amersham, England.

**RESULTS**

**Phenotypic characteristics of mutations which affect ammonium regulation.** Table 1 is a summary of the known characteristics of mutant strains carrying mutations which affect in some degree ammonium regulation. There are two general points to note. (i) the number of different combinations of control aberrations—five out of eight mutant classes have unique phenotypes with respect to overall ammonium regulation of the various test systems. (ii) Only one of the six mutant classes with abnormal ammonium regulation lacks normal NADP-GDH activity. This class, gdhA, is the only one with abnormal regulation of methylammonium uptake.

**Intracellular ammonium pool size and ammonium regulation.** Cells of the wild type and various mutants were grown on N medium containing 0.15% Casamino Acids and 10 mM ammonium tartrate as the nitrogen sources, and then held for 4 h in N medium or N medium containing 10 mM ammonium tartrate. The comparison between cells held under nitrogen-free conditions and cells held in 10 mM ammonium was chosen deliberately since the former results in maximal derepression and the latter results in maximal repression for most of the test systems mentioned in Table 1. After such treatments samples of the cells were assayed for thiourea uptake, L-glutamate uptake, and methylammonium uptake, and the concentration of intracellular ammonium was determined (Table 2).

The thiourea and glutamate uptake systems are fully derepressed in cells which have been nitrogen starved. In contrast, cells held in 10 mM ammonium are fully repressed, with the specific activities of the two uptake systems some 10- to 20-fold lower. The concentration of the intracellular ammonium pool is similar, about 100 to 200 μM, after the two treatments. Therefore normal cells can be either fully repressed or fully derepressed with respect to ammonium regulation of the two uptake systems, and yet have the same low level of intracellular ammonium. This indicates that, in some way, normal cells monitor and respond to the extracellular ammonium concentration with respect to the regulation by ammonium of thiourea uptake and of glutamate uptake.

The degree of derepression for thiourea or glutamate uptake varies for the different mutants. For example, the xprD mutant shows 100% derepression (percent derepression = activity on N-free medium/activity on 10 mM ammonium × 100) for glutamate uptake,

**Table 1. Characteristics of ammonium-derepressed mutants**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Nitrate reductase</th>
<th>Chlorate toxicity</th>
<th>Xanthine dehydrogenase</th>
<th>Thioxanthine test</th>
<th>Thiourea toxicity</th>
<th>Glutamate uptake</th>
<th>Extracellular protease</th>
<th>Methylammonium toxicity</th>
<th>200 mM ammonium toxicity</th>
<th>NADP-GDH activity</th>
<th>Chromosome location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>R</td>
<td>Resis</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>Resis</td>
<td>—</td>
</tr>
<tr>
<td>meaA8</td>
<td>D</td>
<td>Sens</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>Sens</td>
<td>D</td>
<td>R</td>
<td>R</td>
<td>Resis</td>
<td>100</td>
</tr>
<tr>
<td>meaB6</td>
<td>D</td>
<td>Sens</td>
<td>D</td>
<td>D</td>
<td>R</td>
<td>Resis</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td>100</td>
</tr>
<tr>
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<td>D</td>
<td>Sens</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>Resis</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>Resis</td>
<td>100</td>
</tr>
<tr>
<td>amrA1</td>
<td>D</td>
<td>Sens</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>R</td>
<td>D</td>
<td>R</td>
<td>R</td>
<td>Resis</td>
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<tr>
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<td>Sens</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>Resis</td>
<td>100</td>
</tr>
<tr>
<td>gdhA1</td>
<td>D</td>
<td>Sens</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>Resis</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

*In the uptake and enzyme assays, R = ammonium repressed or low level, D = ammonium derepressed or high level. In the toxicity tests, Sens = sensitive, Resis = resistant. NADP-GDH is given as a percentage of the wild-type activity.
whereas *amrA1* is about 30% derepressed for this activity. Some mutants have significantly higher intracellular ammonium pools after treatment with 10 mM ammonium than do the normal cells. These variations in intracellular pool size are not correlated with their degree of ammonium derepression for either thiourea or glutamate uptake.

The level of the methylammonium uptake system in wild-type cells after treatment with 10 mM ammonium is about 50% of that found in wild-type cells after the N-free treatment. This shows that, in wild-type cells, repression by 10 mM extracellular ammonium of the methylammonium uptake system—if it exists at all—is slight compared to the repression of the thiourea and glutamate uptake systems. The various mutants also show similar, comparatively slight, effects of 10 mM extracellular ammonium on the level of methylammonium uptake.

The concentration of intracellular ammonium is not affected by 10 mM extracellular ammonium, but it is increased by higher concentrations of external ammonium or urea. The urea taken up by the cells is converted to carbon dioxide and ammonia by the high level of urease activity always found in *A. nidulans* (16). Cells of the wild type and various mutant genotypes were grown on a medium containing 0.15% Casamino Acids and 10 mM ammonium tartrate and then held for 4 hr in media containing a range of concentrations varying from 10 mM to 100 mM ammonium tartrate or urea. The rate of glutamate and methylammonium uptake and the intracellular concentration of ammonium were measured (Table 3 and Fig. 1). It can be seen from Table 3 and Fig. 1 that the concentration of intracellular ammonium rises in the wild type and mutants as the extracellular concentration of ammonium or urea is increased. As the intracellular ammonium concentration increases, the level of methylammonium uptake decreases in the wild type and

### Table 2. Intracellular ammonium concentration and ammonium regulation

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Thiourea uptake (N-free</th>
<th>Thiourea uptake (10 mM NH₄⁺)</th>
<th>L-Glutamate uptake (N-free</th>
<th>L-Glutamate uptake (10 mM NH₄⁺)</th>
<th>Methylammonium uptake (N-free</th>
<th>Methylammonium uptake (10 mM NH₄⁺)</th>
<th>Intracellular ammonium concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>0.97 (0.22) 0.09 1.07 (0.21) 0.08 (0.02) 9.1 (1.1) 5.2 (0.3) 0.13 (0.02) 0.17 (0.04)</td>
<td>0.84 0.83 1.09 0.56 4.5 (1.3) 4.4 0.18 0.15</td>
<td>0.79 0.12 1.2 0.08 12.9 8.6 0.23 0.55</td>
<td>0.71 0.85 1.01 0.89 2.0 (0.48) 1.4 0.20 0.22</td>
<td>0.46 (0.032) 0.14 1.01 0.29 8.5 4.3 0.15 1.4 (0.55)</td>
<td>0.17 (0.014) 0.15 1.08 1.1 4.8 1.7 0.55 1.3 (0.41)</td>
<td>0.20 0.95 1.14 0.31 12.6 8.3 0.10 1.5 (0.51)</td>
</tr>
</tbody>
</table>

* N-free and 10 mM NH₄⁺ refer to 4 hr of incubation in these conditions before the uptake or ammonium assay. Uptake activities are expressed as nanomoles per minute per milligram (dry weight) of cells. Standard deviation in brackets.

### Table 3. Intracellular ammonium concentration and methylammonium uptake

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment*</th>
<th>Methylammonium uptake*</th>
<th>Intracellular ammonium (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>N-free 9.0 (1.10) 10.0</td>
<td>10 mM urea 5.2 (1.10) 1.05</td>
<td>20 mM urea 2.3 (0.98) 1.43</td>
</tr>
</tbody>
</table>

* Treatment refers to 4 hr of incubation in the presence of the nitrogen source before the uptake or ammonium assay.

* Figures in parentheses refer to L-glutamate uptake in these treatments. Uptake activity expressed as nanomoles per minute per milligram (dry weight) of cells.

in the mutants. This shows that, in some way, the cells monitor and respond to the intracellular ammonium concentration with respect to the regulation of methylammonium uptake. It is the level of the intracellular ammonium pool which represses or inhibits, or both, the methylammonium uptake system, which is in fact the ammonium uptake system (13). This contrasts with the regulation of the urea and
glutamate uptake systems by extracellular ammonium.

It has been shown above that the extracellular ammonium concentration regulates the level of thiourea uptake and glutamate uptake. There is also a possibility that high intracellular ammonium concentrations might repress the level of these uptake systems and enzymes such as nitrate reductase. These systems are not affected by intracellular ammonium concentrations of less than 1.5 mM. There is a maximal level of glutamate uptake in cells held in the presence of 20 mM urea with an intracellular concentration of 1.43 mM and about 40% of the maximum level of glutamate uptake in cells with an internal concentration of 3.5 mM ammonium (Table 3). In addition, nitrate reductase is fully induced by nitrate in the presence of 20 mM urea (Pateman, unpublished data).

Wild-type cells efflux ammonium when the intracellular ammonium concentration is greater than 2 mM (13; Pateman and Dunn, manuscript in preparation). Consequently, it is not possible to study the effect of high intracellular ammonium concentrations on systems such as glutamate uptake in the absence of extracellular ammonium. If there is such a regulatory effect of intracellular ammonium, it can only operate under conditions when extracellular ammonium will also be present due to ammonium efflux. Thus, a regulatory effect of intracellular ammonium would appear to be superfluous.

The gdhA mutants, like the wild type, show a decrease in the level of the methylammonium uptake system when the intracellular ammonium pool increases. However, the gdhA mutants show a significantly different degree of response to intracellular ammonium when compared to the wild type and all the other mutant genotypes. Figure 1 shows the relationship between intracellular ammonium pool size and the level of methylammonium uptake for the wild type and the gdhA mutants. When the intracellular ammonium concentration is above 2 to 3 mM ammonium, wild-type cells are repressed, with methylammonium uptake levels of <0.3 nmol per min per mg. When the intracellular ammonium concentration is about 2 to 3 mM, gdhA cells are derepressed with methylammonium uptake levels of about 6.0 nmol per min per mg, and the methylammonium uptake level is greater than 1.0 nmol per min per mg even when the intracellular ammonium concentration is 8 to 9 mM. Thus gdhA mutants are derepressed or deinhibited, or both, to a considerable degree with respect to the regulation by intracellular ammonium of ammonium uptake.

**FIG. 1. The relationship between the rate of methylammonium uptake and the intracellular ammonium concentration in wild type (○) and gdhA mutants (△).**

The gdhA mutants are unique in their response to regulation by intracellular ammonium. The other classes of mutants are similar in this respect to the wild type. It is shown in Table 3 that gdhA mutants develop higher intracellular ammonium concentrations than the wild type in response to extracellular ammonium or urea. It is probable that this is due to their lack of NADP glutamate dehydrogenase activity since the synthesis of glutamic acid is normally the main route for the utilization of ammonium. In spite of their higher intracellular ammonium pool, gdhA cells are derepressed and have higher methylammonium uptake levels than the wild type in the presence of 10 to 100 mM extracellular ammonium or urea.

**Transport abnormalities associated with ammonium regulation.** The levels of thiourea uptake, glutamate uptake, and methylammonium uptake in nitrogen-starved cells of the wild type and mutant genotypes are given in Table 2. Nitrogen starvation is the condition for maximal derepression of all three uptake systems, yet some of the mutant genotypes have lower derepressed levels than the wild type for one or more of the three uptake systems. The meaA8 strain has about 50% and the DER3 strain about 25% of the wild-type level of methylammonium uptake. The amrA1 strain has about 50% of the wild-type level for both thiourea and glutamate uptake. The xprD1
strain has about 20% of the wild-type level for thiourea uptake. Thus various combinations of transport deficiencies and ammonium regulation abnormalities can result from mutation in a number of genes.

**DISCUSSION**

It is clear that the level of thiourea uptake and l-glutamate uptake in wild-type cells of *A. nidulans* is dependent on the extracellular concentration of ammonium. It is probable that the synthesis of the enzymes nitrate reductase and xanthine dehydrogenase is also dependent on the extracellular ammonium concentration. It has been shown that wild-type cells produce nitrate reductase after growth on nitrate plus 10 mM urea, but produce far less enzyme after growth on nitrate plus 10 mM ammonium (12). Similarly, xanthine dehydrogenase is produced after growth on hypoxanthine or uric acid plus 10 mM urea, but not after growth on hypoxanthine plus 10 mM ammonium (14). Furthermore, 10 mM ammonium provides protection from the toxic effects of chlorate as a result of ammonium repression of nitrate reductase, whereas 10 mM urea does not repress and consequently does not relieve chlorate toxicity (12). The plate tests incorporating 2-thioxanthine provide similar evidence for xanthine dehydrogenase (1). In fact there is a good case for assuming that, in all systems in which 10 mM extracellular ammonium results in repression but 10 mM urea does not, it is the extracellular concentration not the intracellular concentration of ammonium which determines the level of the system. In contrast the level of methylammonium uptake i.e., the ammonium uptake system itself, is largely or completely determined by the intracellular ammonium concentration.

The reason for the distinction between ammonium uptake and the other systems with respect to regulation is not clear. The dependence of ammonium uptake upon the intracellular ammonium concentration is a simple, direct, and economical control situation. It is not at all obvious why the level of enzymes such as nitrate reductase and uptake systems for metabolites such as urea and glutamate should be determined by extracellular ammonium. It may be that ammonium is the preferred major source of nitrogen because the least amount of energy overall is required for its uptake and utilization. So, if extracellular ammonium is available, the uptake and utilization of other forms of nitrogen are minimized to the extent where they can be supplementary, but not major, nitrogen sources. Alternatively, if there is no extracellular ammonium available, it may not be advantageous to utilize any single nitrogen-containing metabolite to the exclusion of others. For example, even if sufficient urea is available to act as sole nitrogen source and urease action results in a sizable intracellular ammonium pool, it could still be advantageous to utilize some nitrate or glutamate, or purines if these were also available. Thus these systems would be independent of the intracellular ammonium concentration unless this exceeded 3 or 4 mM, at which level *A. nidulans* effluxes ammonium (Pateman et al., manuscript in preparation).

The material presented in Table 1 shows how widespread are the ramifications of ammonium regulation in *A. nidulans*. A wide variety of mutants has been analyzed, their properties indicating a paradoxical and complex relationship between ammonium transport and the ammonium regulation of various transport and enzyme systems. The correlation of the intracellular ammonium pool size with various regulatory characteristics of normal and mutant cells has led us to a unifying hypothesis. The main points of this hypothesis are as follows. (i) Wild-type cells of *A. nidulans* monitor separately the extracellular and intracellular ammonium concentrations with respect to ammonium regulation. (ii) In wild-type cells, only the extracellular ammonium concentration affects the level of a number of uptake and enzyme systems including nitrate reductase, glutamate uptake, and urea uptake. (iii) In wild-type cells, only the intracellular ammonium concentration affects the level of ammonium uptake. (iv) NADP-glutamic dehydrogenase, in addition to its catalytic function, plays a dual role in ammonium regulation. (v) NADP-glutamate dehydrogenase located in a regulatory site in the cell membrane can complex with extracellular, but not intracellular, ammonium. This special regulatory complex of NADP-GDH and extracellular ammonium determines the ammonium repression or inhibition, or both, of such systems as nitrate reductase, glutamate uptake, and urea uptake. (vi) NADP-glutamic dehydrogenase can combine with intracellular ammonium to form a second type of regulatory complex which determines the ammonium repression or inhibition, or both, of ammonium uptake (Fig. 2).

There are two reasons for proposing the existence of special sites in the cell membrane in which a regulatory protein can interact with extracellular, but not intracellular, ammonium. First, a monitoring mechanism located in the cell membrane itself is the simplest type that can be envisaged for distinguishing between...
external and internal concentrations of a metabolite. Second, a number of genes are known at which mutation can simultaneously result in abnormalities of both regulation and transport. There is no doubt that the various low uptake levels of strains meaA8, DER3, amrA, and xprD are genuine transport defects. Consequently the sites of transport malfunction will be located in the semipermeable cell membrane. Therefore there is a strong reason for supposing that the sites of regulatory malfunction in each class of mutant are also located in the cell membrane. We suggest that in all the known genes in which mutation can cause ammonium regulation abnormalities (except for gdhA) those genes determine cell membrane components. Mutation in such genes would result in production of defective membrane components. Such general membrane defects could reduce the efficiency of a range of unrelated transport sites and simultaneously upset the normal functioning of the ammonium regulatory sites. The xprD1 mutant is a special case in that protease release is not a simple transport function. It is not known, as yet, whether the ammonium control is at the level of protease synthesis or protease release, or both (2). Certainly it is plausible to suggest that the xprD gene determines a membrane component and that mutation in this gene determines a defective membrane component which results in defective protease release, defective urea uptake, and malfunction of the extracellular ammonium regulation sites. Thus, this type of hypothesis provides a plausible explanation for the variety of mutants which exhibit complex syndromes involving both regulatory and transport defects.

The arguments for the existence of a regulatory protein and regulatory sites in the cell membrane are based on the properties of the wild type and the ammonium-derpressed mutants, except for the gdhA class. The arguments for the existence of a regulatory molecule, probably a protein, are general and valid without any indication of the identity of such a regulatory molecule. However, the gdhA mutants lack normal NADP-glutamate dehydrogenase activity, are relieved of control by both extracellular and intracellular ammonium, and have no known transport abnormalities. This immediately suggests that NADP-glutamate dehydrogenase could be the regulatory protein which mediates the action of extracellular ammonium. It also suggests that it has a second regulatory function in mediating the action of intracellular ammonium in controlling the level of ammonium uptake. Finally, the fact that gdhA mutants, unlike the other ammonium-derpressed mutants, are not deficient in any transport function is a natural consequence if our hypothesis is, in a general sense, true.

We consider that the hypothesis we propose clarifies to a worthwhile extent a very involved situation and, equally important, has considerable heuristic value. The detailed catalytic and regulatory properties of a wide range of gdhA mutants and the relationship between NADP-GDH and the cell membrane in the other classes of derepressed mutants are of obvious interest. No doubt investigations on these lines will prove or disprove our hypothesis. However, there are still several matters connected with ammonium regulation about which very little is known. The target(s) for methylammonium toxicity in A. nidulans is not known, nor is the basis of the methylammonium resistance shown by meaA, meaB, and DER3 mutants. The high level of resistance shown by meaA and DER3 may be partly due to a reduced level of methylammonium uptake (Table 2) (Pateman et al., manuscript in preparation; H. H. Arst, personal communication, 1972). The target(s) for ammonium sensitivity in gdhA mutants is unknown. These mutants have high intracellular ammonium concentrations (Table 2) and are unique in being derepressed for ammonium uptake. However other mutants, notably amrA1, also have high ammonium pools but are not ammonium sensitive. The reason for the high intracellular ammonium concentration in gdhA mutants is not known for certain. It is likely that the low level of NADP-GDH activity and the derepression of ammonium uptake are at least partly responsible. The reason for the high intracellular ammonium concentrations in the amrA1 and xprD1 mutants is quite unknown.
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