tRNA plays an important role in this reciprocal positioning. Indeed we have observed that nearly all tRNA species that are most easily aminoacylated by phenylalanyl-tRNA synthetase or valyl-tRNA synthetase possess an A residue at the fourth position from the 3'-terminus, whereas those which are correctly or incorrectly charged by aspartyl-tRNA synthetase usually have a G residue at this position. This can be related with observations made by Shimura et al. (1972) and Hooper et al. (1972) showing that tRNAAsn mutated at this position, or in the terminal part of the amino acid acceptor stem, can be mischarged. This region which seems to be essential for the specificity of the aminoacylation reaction is, however, not important in the recognition step, as we observed that yeast tRNAophe and tRNAVal, degraded up to the seventh and ninth base from the 3'-end, are still able to complex with their cognate aminoacyl-tRNA synthetase. Similar affinities have been found for the complexes of degraded tRNAVal and intact tRNAVal with valyl-tRNA synthetase.

In conclusion we propose that in a system involving a purified tRNA and a purified aminoacyl-tRNA synthetase, the aminoacylation reaction proceeds by a two-step discrimination mechanism involving, first, a more or less specific recognition of the tRNA by the aminoacyl-tRNA synthetase and, secondly, the positioning of the 3'-terminal adenosine on the catalytic site of the enzyme, responsible for the $V_{max}$ of the reaction. In conditions in vivo, the presence of all tRNA species, aminoacyl-tRNA synthetases and amino acids leads to additional correction mechanisms minimizing the aminoacylation errors. As suggested by Yarus (1972a), there are competitive effects which practically eliminate the aminoacylation errors that could take place in spite of the previous discriminations. Alternatively, some error corrections could also occur, as suggested by Eldred & Schimmel (1972) and by Yarus (1972b), by a more rapid enzymic deacylation of mischarged than of correctly charged tRNA species; however, our own experiments do not allow us to consider this last mechanism as a general correction mechanism for mischarging (Bonnet et al., 1972).

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Nicotinamide–Adenine Dinucleotide Phosphate–Linked Glutamate Dehydrogenase Activity and Ammonium Regulation in Aspergillus nidulans

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Aspergillus nidulans can synthesize two L-glutamate dehydrogenases. L-Glutamate NADP oxidoreductase (EC 1.4.1.4) has an aminating function; maximal activity is found in cells utilizing inorganic N and minimal activity is found in cells utilizing L-glutamate (Pateman, 1969). L-Glutamate–NAD oxidoreductase (EC 1.4.1.2) appears to have a deaminating function and optimum activity is found in cells utilizing L-glutamate as a sole C source (Kinghorn & Pateman, 1973a).

Grenson & Hou (1972) have described a mutant in Saccharomyces cerevisiae that lacks NADP-glutamate dehydrogenase activity and is abnormal with respect to the regulation by ammonium of the general amino acid permease. In A. nidulans the
syntheses of a number of enzyme and uptake systems are repressed by ammonia. These systems include nitrate reductase (Pateman & Cove, 1967), hypoxanthine dehydrogenase (Sc accozchio & Darlington, 1968), extracellular protease (Cohen, 1972), acetamidase and formamidase (Hynes & Pateman, 1970a,b), glutamate uptake (Kinghorn & Pateman, 1972) and thiourea uptake (Dunn & Pateman, 1972).

Arst & Cove (1969) found that mutation in two loci, meaA and meaB, resulted in resistance to the toxic ammonium analogue, methylamine and de-repression for many ammonium-repressed systems. Cohen (1972) isolated a mutant xprD1 that was de-repressed for extracellular protease release and other systems. Pateman et al. (1973) have isolated other classes of de-repressed mutants DER-3 and amrA1, which have ammonium transport abnormalities. These various classes of de-repressed mutants have wild-type NADP-glutamate dehydrogenase activity. We have selected mutants (designated gdhA1–A9) lacking this enzyme and found that they are de-repressed for xanthine dehydrogenase, nitrate reductase, glutamate uptake and urea uptake, but repressed for extracellular protease release (Kinghorn & Pateman, 1973b). Also, they exhibit wild type sensitivity to methylamine. The gdhA mutants are unable to utilize inorganic N efficiently for the synthesis of glutamic acid and require a supplement of amino acid for normal growth. The gdhA mutants are sensitive to high concentrations of ammonium. The reason for this is not clear. It may be because gdhA mutants have a higher intracellular ammonium concentration than the wild-type in the presence of extracellular ammonium or urea (Pateman et al., 1973).

The gdhA locus is probably the structural gene for NADP-glutamate dehydrogenase since no other locus is known at which mutation can result in the lack of this enzyme activity.

The fact that the gdhA mutants are de-repressed for a number of enzyme and uptake systems suggests that the NADP-glutamate dehydrogenase protein plays some role in ammonium regulation.

Three main possibilities occur to us concerning the relationship between NADP-glutamate dehydrogenase and ammonium regulation. The first possibility is that NADP-glutamate dehydrogenase is a multi-functional protein which has catalytic activity and also plays a direct role in the repression inhibition of a number of activities. Mutation in the gdhA locus, the structural gene for NADP-glutamate dehydrogenase, would result in both abnormal enzyme and control activity. Secondly, the NADP-glutamate dehydrogenase protein specified by the gdhA locus has only a catalytic function, but low NADP-glutamate dehydrogenase activity itself results in metabolic changes, e.g. ammonium pool size, which then result in de-repression. Thirdly, the gdhA gene specifies a product which has some fundamental, but at present unknown, regulatory function with respect to a number of metabolic systems. Mutations in the gdhA locus can simultaneously result in repression of NADP-glutamate dehydrogenase and derepression of other systems. This is an unlikely explanation for the following reason. A common class of NADP-glutamate dehydrogenase-deficient mutants should be due to mutation in the structural gene(s) for the protein. On this hypothesis such structural gene mutants should have abnormal enzyme activity but normal ammonium regulation. However, all nine known NADP-glutamate dehydrogenase-deficient mutants are also ammonium-de-repressed. Therefore the hypothesis requires the assumption that there are no NADP-glutamate dehydrogenase structural-gene mutations among the nine known NADP-glutamate dehydrogenase-deficient mutants, although such structural-gene mutants should be the most common class exhibiting enzyme deficiency.

At present there is evidence concerning the relationship between ammonium pool size and ammonium regulation which makes the second possibility mentioned above an unlikely one (Pateman et al., 1973). To distinguish between the first and third hypotheses it is necessary to determine if the gdhA locus is in fact a structural gene for NADP-glutamate dehydrogenase.

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**Ammonium Regulation in Aspergillus nidulans**

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A number of enzyme and uptake systems in *Aspergillus nidulans* are regulated by ammonium. The activity of all these systems is minimal if wild-type cells are grown, or kept, in the presence of ammonium. Systems repressed by ammonium include nitrate reductase (Pateman & Cove, 1967), xanthine dehydrogenase (Scazzocchio & Darlington, 1968), acetamidase (Hynes & Pateman, 1970), L-glutamate uptake (Kinghorn & Pateman, 1972; J. A. Pateman & J. R. Kinghorn, unpublished work), urea uptake (Dunn & Pateman, 1972) and extracellular protease (Cohen, 1972). Mutations in a number of genes can affect the regulation by ammonium of these systems. We present information on the intracellular ammonium concentration and the rate of thiourea uptake, L-glutamate uptake and methylammonium uptake in wild-type and mutant cells kept 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. Only the *gdhA* mutants are de-repressed for both types of ammonium regulation and they also lack normal NADP-glutamate dehydrogenase activity. We propose a unifying hypothesis concerning ammonium regulation which accounts for the variety of experimental observations on the wild-type and ammonium-de-repressed mutants. The main points of this hypothesis are: (1) *A. nidulans* monitors separately the extracellular and intracellular ammonium concentrations with respect to ammonium regulation; (2) the extracellular ammonium concentration determines the activities of a number of uptake and enzyme systems including nitrate reductase, glutamate uptake and urea uptake; (3) the intracellular ammonium concentration determines the rate of ammonium uptake; (4) NADP-glutamate dehydrogenase, in addition to its catalytic function, plays a dual role in ammonium regulation; (5) NADP-glutamate dehydrogenase located in a regulatory site in the outer cell membrane can complex with extracellular, but not intracellular, ammonium. This special regulatory complex of NADP-glutamate dehydrogenase and extracellular ammonium determines the ammonium repression and/or inhibition of such systems as nitrate reductase, glutamate uptake and urea uptake; (6) NADP-glutamate dehydrogenase can combine with intracellular ammonium to form a second type of regulatory complex, which determines the ammonium repression and/or inhibition of ammonium uptake.

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