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# Purine Base Transport in *nit-2* Mutants of *Neurospora crassa*

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The *nit-2* mutants possess general purine transport activity. Reduced hypoxanthine uptake in germinated conidia of these mutants may be a consequence of their defective purine metabolism.

Freshly harvested conidia of wild-type *Neurospora* have a general purine transport system which transports adenine, hypoxanthine, guanine, and 6-methyl purine. During germination of conidia, an additional system develops which transports adenine and 6-methyl purine (3). The general purine transport system activity can be assessed in conidia by studying the hypoxanthine uptake directly, since this purine base has a single transport system (2). Alternately, the general purine transport system activity can also be measured by studying the hypoxanthine-inhibitable adenine uptake, since hypoxanthine is a competitive inhibitor of adenine uptake through this system. The hypoxanthine-uninhibited adenine uptake represents activity of the second adenine transport system.

Recently, Tsao and Marzluf reported that ammonium regulation mutants (*amr*) that show a pleiotropic loss of several enzymes related to nitrogen metabolism including some in the purine catabolic pathway (4) also have a low level of hypoxanthine transport, which they have attributed to the lack of the general purine permease (5). The *amr* mutants are allelic to *nit-2* mutants, and the *amr* (*nit-2*) locus was demonstrated to be a major control gene for nitrogen metabolism (4, 5). Interestingly, the general purine transport system is not regulated by ammonium repression, whereas many other activities regulated by the *amr* locus appear to be ammonium repressible (4, 5).

Based on the activities of hypoxanthine and adenine transport in ungerminated and germinated conidia, we report here that *nit-2* mutants possess the general purine transport activity in ungerminated conidia to the same level as the wild type and that, in germinated conidia, only the hypoxanthine transport is affected. We also report here that the second adenine transport system is not affected in the *nit-2* mutants.

*Neurospora* strains, wild-type (74-OR23-1A) and *nit-2* mutants (k-31, nr37a, and nr37A), were obtained from the Fungal Genetics Stock Center, Arcata, Calif. The mutants were pretested for growth on various nitrogen sources as described by Coddington (1). For uptake studies, washed conidia of mutants and wild type were suspended in minimal medium to give an absorbance of 0.3 to 0.35 at 540 nm. At zero time, 0.5 ml of $^{14}$C-labeled purine base was added to 4.5 ml of conidial suspension, and 1-ml samples were taken at 2, 4, 8, and 12 min. When measuring hypoxanthine-inhibited adenine transport, 5 mM unlabeled hypoxanthine was added along with [$^{14}$C]adenine. Each sample of conidia was quickly filtered on Whatman no. 540 filters, washed with ice-cold distilled water, dried under a heat lamp and counted in 15 ml of scintillation fluid containing 2,5-diphenyloxazole and 1,4-bis-[2]-(5-phenyloxazolyl)benzene in toluene. Samples from boiled conidial suspension, identically receiving $^{14}$C-labeled purine base at zero time, were used to estimate the radioactivity adsorbed on the surfaces of conidia and filter paper. Dry weights were obtained from the remaining conidial suspension, and radioactivity found at each sampling time in conidia was converted to give nanomoles of purine transported per milligram of dry weight. The rate of uptake of each of the purines by wild-type and *nit-2* mutants listed in Table 1 was calculated from 0- to 12-min uptake data.

Table 1 lists the general purine transport activity as estimated by [$^{14}$C]hypoxanthine transport and by the hypoxanthine-inhibitable fraction of [$^{14}$C]adenine transport in both ungerminated and germinated conidia. As shown in Table 1, *nit-2* mutants and wild type had very similar general purine transport activity in the ungerminated conidia. In germinated conidia of the mutants, hypoxanthine transport was reduced to 55 to 61% that of wild type. However, the same mutants showed no reduction in [$^{14}$C]-adenine uptake through the general purine transport system in germinated conidia. No difference was found in the rate of adenine uptake through the second adenine transport system (as measured by hypoxanthine-uninhibited adenine uptake) between *nit-2* mutants and wild type.

Since the hypoxanthine transport in unger-

TABLE 1. *Activities of general purine transport system and second adenine transport system in ungerminated and germinated conidia of wild-type and nit-2 mutants of Neurospora crassa*

| Strain | Allele | Rate of uptake of the purine base (nmol/mg per min) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ungerminated conidia[a] | | | Germinated conidia[b] | | |
| | | General purine transport system | | Second adenine transport system | General purine transport system | | Second adenine transport system |
| | | [$^{14}$C]adenine[c] | [$^{14}$C]hypoxanthine[d] | [$^{14}$C]adenine | [$^{14}$C]adenine | [$^{14}$C]hypoxanthine | [$^{14}$C]adenine |
| 74-OR23-1A | Wild type | 0.31 | 0.13 | 0.02 | 0.55 | 0.18 | 0.20 |
| nr37a | *nit-2* | 0.30 | 0.14 | 0.03 | 0.55 | 0.10 | 0.20 |
| nr37A | *nit-2* | 0.31 | 0.13 | 0.02 | 0.54 | 0.11 | 0.20 |
| k31 | *nit-2* | 0.31 | 0.13 | 0.02 | 0.54 | 0.10 | 0.20 |

[a] Freshly harvested conidia, incubated at 30°C for 30 to 45 min in Fries minimal medium, were used for the uptake studies.
[b] Conidia incubated at 30°C for 5.5 h in Fries minimal medium were used for the uptake studies.
[c] [$^{14}$C]adenine: 10 μM; specific activity, 8.3 μCi/μmol.
[d] [$^{14}$C]hypoxanthine: 10 μM; specific activity, 8.3 μCi/μmol.

minated conidia of *nit-2* mutants and adenine transport in both ungerminated and germinated conidia of *nit-2* mutants are identical to those in wild type, the *nit-2* mutants certainly possess the general purine permease, and, therefore, its synthesis is not dependent on the *nit-2* gene product. The reduction in hypoxanthine transport activity in germinated conidia of *nit-2* mutants, therefore, appears to be an indirect consequence of their defect in nitrogen metabolism.

We reported previously that adenine deamination products accumulating intracellularly can inhibit the general purine transport system and affect hypoxanthine transport more severely than adenine transport through this system (3). The *nit-2* mutants are deficient in xanthine dehydrogenase (EC 1.2.3.2), allantoinase (EC 3.5.2.5), allantoicase (EC 3.5.3.4), and uricase (EC 1.7.3.3) in the purine catabolic pathway (4), which might lead to the accumulation of adenine deamination products that could inhibit hypoxanthine uptake.

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