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Nitrogen Regulation of Acid Phosphatase in *Neurospora crassa*

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Neurospora crassa possesses a repressible acid phosphatase with phosphodiesterase activity which appears to permit it to utilize ribonucleic acid as a phosphorus and as a nitrogen source. This acid phosphatase, which is specified by the *pho-3* locus, is derepressed approximately eightfold during nitrogen limitation and to an even greater extent during phosphorus limitation, but is unaffected by sulfur limitation. Derepression of the enzyme did not occur when adenosine 5'-monophosphate was the sole phosphorus or nitrogen source. Synthesis of the acid phosphatase is not under the control of the *nit-2* locus, which regulates the expression of a large number of other nitrogen catabolic enzymes. The structural gene of the acid phosphatase appears to be a member of both the phosphorus and nitrogen regulatory circuits.

The fungus *Neurospora crassa* utilizes ammonia, glutamate, or glutamine as preferred nitrogen sources. This organism is also capable of utilizing nitrogen from a wide spectrum of secondary sources, including nitrate, nitrite, purines, many amino acids, small peptides, and intact proteins. To use these less-preferred sources, a group of nitrogen catabolic enzymes normally present in low levels within the cells are produced in much larger quantities (2, 7). This derepression of nitrogen catabolic enzymes occurs when *Neurospora* either is limited for one of the above-mentioned preferred nitrogen sources or is provided only with one of the secondary nitrogen sources (2, 7). A regulatory mutant, *nit-2*, cannot grow with any of the less-preferred nitrogen sources, but does grow well on ammonia, glutamate, or glutamine. The product of the *nit-2* locus is apparently involved in derepression of the genes that specify the nitrogen-related enzymes (1, 2, 7). One of the enzymes in this group, an extracellular protease, allows *Neurospora* to utilize proteins present in the growth medium as a nitrogen source. Synthesis of this protease is considerably increased during nitrogen limitation. Furthermore, synthesis of this same protease is also "turned on" when *Neurospora* is instead limited for either carbon or sulfur (2). The structural gene that encodes this protease apparently responds to three different regulatory signals (2).

In addition to the family of nitrogen metabolic enzymes, there exist in *Neurospora* other groups of metabolically interrelated enzymes. One of these groups is involved in utilization of secondary sources of phosphorus. Similar to the control of the nitrogen enzymes, the phosphorus catabolic enzymes are coordinately controlled by specific regulatory genes (4). One of the enzymes of this group, a repressible acid phosphatase, has phosphodiesterase activity (6) which apparently allows *Neurospora* to utilize RNA as a source of phosphorus (3). Synthesis of acid phosphatase is greatly increased during phosphorus limitation (6). This enzyme is specified by a gene, designated *pho-3*, a temperature-sensitive mutant of which has been isolated (5).

Since RNA contains considerable nitrogen, it might also serve as a nitrogen source for *Neurospora*. It has been suggested that the acid phosphatase hydrolyzes RNA to mononucleotides (3), which serve as a source of nitrogen (Table 1). Thus, it was of interest to determine whether or not this repressible acid phosphatase was subject to nitrogen regulation in addition to the well-established control of this enzyme by the phosphorus circuit (4).

To test whether *Neurospora* can utilize RNA as a nitrogen source, growth tests with various nitrogen sources were performed with both the wild-type and *nit-2* strains. As shown in Table 1, the wild-type strain grows about one-fourth as well on adenosine or AMP as with ammonia as the nitrogen source. These compounds are enzymatically converted to hypoxanthine, which is then metabolized via the purine catabolic pathway to yield ammonia (7). Adenine also supported growth, but not nearly as well as adenosine or AMP did. Adenine is also converted to hypoxanthine, but by a different pathway which is apparently less efficient. RNA, whose hydrolysis releases mononucleotides, also supported growth (Table 1). No growth was detected when the only available nitrogen source was either

TABLE 1. *Growth of Neurospora on various nitrogen sources*[a]

Nitrogen source[b]	Wild type (%)	*nit-2* (%)
Complete (25 mM NH_4NO_3)	100	100
Adenine (0.1 mg/ml)	8.4	4.3
Adenosine (0.1 mg/ml)	29.0	4.9
5'-AMP (0.1 mg/ml)	22.1	5.3
5'-AMP (0.5 mg/ml)	11.9	0
5'-GMP (0.5 mg/ml)	6.4	4.6
RNA (0.1 mg/ml)	4.5	4.3
ss DNA (0.1 mg/ml)	0.0	1.5
ds DNA (0.1 mg/ml)	0.0	0.6
ds DNA (0.5 mg/ml)	0.0	0.0

[a] Conidia from wild-type and *nit-2* strains were inoculated into growth media containing various nitrogen sources to an optical density at 420 nM of 0.2. Mycelia were grown in standing cultures for 48 and 72 h, collected by filtration, dried, and weighed. Growth on complete medium was defined as 100%, and other values are reported relative to it. AMP and GMP were provided as the sodium salt.

[b] Growth with 0.5 mg of AMP, GMP, and DNA per ml was determined in a separate experiment. Note that a higher AMP concentration did not lead to improved growth. ss, Single-stranded; ds, double-stranded.

single- or double-stranded DNA. The *nit-2* mutant strain grew on all of these nitrogen sources (except DNA) to only a very limited extent and generally utilized these compounds more poorly than did wild type (Table 1). These results suggest that *nit-2* cannot derepress synthesis of the enzymes required for nucleotide metabolism, which is in agreement with earlier work (7).

It was of interest to determine whether or not the synthesis of acid phosphatase is increased by nitrogen limitation. Acid phosphatase was assayed as described by Nelson et al. (5) using bis(*p*-nitrophenyl) phosphate as the substrate. Acid phosphatase activity was elevated 60- to 100-fold in cells grown with limiting phosphorus, as previously reported (6). When *Neurospora* was instead grown under nitrogen-limiting conditions (0.75 mM nitrate), acid phosphatase activity was increased approximately eightfold relative to activity in control cells grown on complete medium (Fig. 1). When cells were instead limited for sulfur, no such increase in enzyme activity was detected (Fig. 1). These same conditions of sulfur starvation did lead to derepression of the sulfur-controlled enzyme, aryl sulfatase (data not shown). Thus, a considerable increase in acid phosphatase synthesis results from nitrogen or phosphorus limitation but not from sulfur limitation. Therefore, acid phosphatase derepression is not a general response to poor growth conditions but results from signals arising from specific nutrient limitations. This result

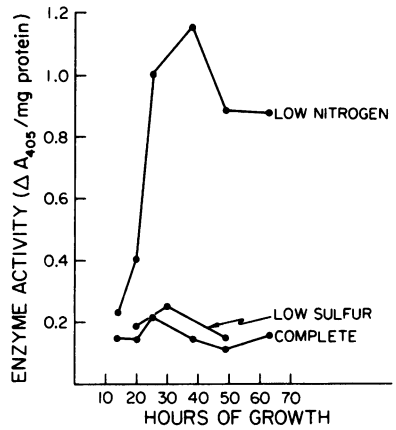

FIG. 1. *Acid phosphatase activity in wild-type mycelia grown under various nutrient conditions. After growth for various times in three different media, mycelia were harvested and homogenized by grinding with sand in a cold mortar and pestle in 0.05 M sodium succinate buffer (pH 5.0). Enzyme activity was assayed as described by Nelson et al. (5). Specific activity is given as absorbance at 405 nm per mg of protein in crude extracts. Complete medium contained 25 mM NH_4NO_3 and 1 mM $MgSO_4$, low-sulfur medium contained 0.1 mM $MgSO_4$, and low-nitrogen medium contained 0.75 mM NH_4Cl.*

is consistent with the fact that the principal substrates of acid phosphatase, such as RNA, can serve as a source of nitrogen or phosphorus, but not of sulfur.

Nelson et al. (5) concluded that the repressible acid phosphatase was the only enzyme present in extracts of *Neurospora crassa* with measurable bis(*p*-nitrophenyl) phosphate phosphodiesterase activity. However, it seemed important to determine whether the increased phosphodiesterase activity that occurred during nitrogen limitation was due to an increase in this same enzyme rather than in some other phosphatase that was turned on by these specific growth conditions. To address this question, we took advantage of the fact that *pho-3* has been shown to be the structural gene that encodes the repressible acid phosphatase (5). A temperature-sensitive *pho-3* mutant is available which specifies a heat-labile form of acid phosphatase. The increased acid phosphatase that accumulates in this mutant during nitrogen limitation should be heat labile if it is indeed the same enzyme. This result was obtained. A wild-type strain and the temperature-sensitive *pho-3* strain were grown on complete medium and on low-nitrogen medium. Extracts from these cultures were incubated for 30 min at 25 and at 50°C and then assayed as described above. For each strain, enzyme activity was five to seven times higher

in extracts of mycelia grown on low-nitrogen medium than in mycelia grown on complete medium (Table 2). After treatment at 50°C, both wild-type extracts possessed about 40% of the activity they displayed after incubation at 25°C. In contrast, about 90% of the activity in both *pho-3* extracts was heat labile at 50°C. These results are consistent with the reported heat stabilities of these enzymes (5) and indicate that the activity derepressed by nitrogen limitation is the enzyme specified by the *pho-3* locus, namely the repressible acid phosphatase.

The ability of the *pho-3* temperature-sensitive mutant to utilize RNA as a nitrogen and as a phosphorus source at an elevated temperature was examined by growth tests. At 25°C, wild type and *pho-3*(Ts) grew to approximately the same extent. However, at 38°C, the highest temperature at which these strains grew well on any medium, the mutant grew only 70% as well as did the wild type when RNA was either the only nitrogen source or the only phosphorus source (data not shown). These results are consistent with the suggestion that the repressible acid phosphatase is responsible for the metabolism of RNA. We did not expect growth to be inhibited completely, since the temperature-sensitive enzyme is only partially labile at this temperature.

The extent of derepression of the acid phosphatase under various growth conditions was determined as shown in Table 3. No inducer seems to be required for increased enzyme synthesis, since acid phosphatase activity was significantly increased in mycelia grown with a limited amount of ammonia (1 mM) or with nitrate, urea, or RNA as the sole nitrogen source. However, unexpectedly, no derepression of enzyme activity occurred when AMP was either the sole nitrogen or the sole phosphorus source. This result suggests that the regulation of acid phosphatase may not be identical to control of other enzymes in the two families. In this context, it was found that acid phosphatase was derepressed by nitrogen limitation to the same extent in the *nit-2* regulatory mutant as it was in wild type (Table 3), whereas nearly all other nitrogen-regulated enzymes cannot be "turned on" in *nit-2*. The results presented here demonstrate that the repressible acid phosphatase is derepressed by nitrogen limitation and suggests that it permits *Neurospora* to utilize RNA as a nitrogen source as well as a phosphorus source. Regulation of *pho-3*, the structural gene that specifies this enzyme, is of particular interest because this gene appears to be a member of at least two distinct regulatory circuits.

TABLE 2. *Acid phosphatase activity in wild-type and pho-3 extracts after preincubation at 25 and 50°C*[a]

Extract	Activity after incubation		% Activity retained at 50°C
	25°C	50°C	
Wild type			
Complete	1.00	0.39	39
Low nitrogen	7.30	2.47	34
pho-3			
Complete	1.00	0.08	8
Low nitrogen	5.15	0.61	12

[a] Both strains were inoculated into appropriate media as described in Table 1. Complete medium contained 25 mM NH_4NO_3; low-nitrogen medium contained 0.75 mM NH_4Cl. Mycelia were grown at 25°C for 30 h. Crude enzyme extracts were preincubated for 30 min at 25 or 50°C before being assayed as described in Fig. 1. Enzyme activity in 25°C samples from mycelia grown in complete medium was normalized to 1.00 for each strain, and all other values are reported relative to them.

TABLE 3. *Acid phosphatase activity in extracts of mycelia grown under various conditions*[a]

Nitrogen source[b]	Enzyme activity[c]	Strain
Complete (25 mM NH_4NO_3)	1.00	Wild type
NH_4Cl (1 mM)	2.21	Wild type
$NaNO_3$ (1 mM)	2.22	Wild type
Urea (1 mM)	2.25	Wild type
RNA (0.5 mg/ml)	3.80	Wild type
AMP (0.5 mg/ml)	0.99	Wild type
AMP (0.5 mg/ml, sole phosphorus source; 25 mM NH_4NO_3)	1.18	Wild type
Complete (25 mM NH_4NO_3)	1.00	*nit-2* (K31)
NH_4Cl (1 mM)	3.33	*nit-2* (K31)
NH_4Cl (1 mM)	2.71	Chl-R≠9[d]

[a] Conidia were inoculated as described in Table 1. Mycelia were grown for 18 h.

[b] Except where noted, phosphorus source was 37.5 mM KH_2PO_4.

[c] Enzyme activity was determined as described in Table 2. Activities are lower in this experiment because mycelia were grown for only 18 h.

[d] A *nit-2* allele isolated in our laboratory.

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