JOURNAL OF BACTERIOLOGY, Feb. 1981, p. 884-888
0021-9193/81/020884-05$02.00/0

Vol. 145, No. 2

Effect of the *gln-1b* Mutation on Nitrogen Metabolite Repression in *Neurospora crassa*

NIGEL S. DUNN-COLEMAN AND REGINALD H. GARRETT*
Department of Biology, University of Virginia, Charlottesville, Virginia 22901

In *Neurospora crassa*, synthesis of the enzymes of nitrate assimilation, nitrate reductase and nitrite reductase, was repressed by the presence of ammonium, glutamate, or glutamine. This phenomenon was a manifestation of the regulatory process termed nitrogen metabolite repression whereby alternative pathways of nitrogen acquisition are not expressed in cells enjoying nitrogen sufficiency. However, the glutamine synthetase mutant *gln-1b* had derepressed levels of the nitrate assimilation enzymes. The inability of glutamine to achieve nitrogen metabolite repression in this mutant militated against its potential role as the direct effector of this regulation.

Their metabolic diversity and relative ease of genetic manipulation render fungi particularly suited for the study of gene expression in lower eucaryotes. The regulation of the assimilation of nitrogen has been extensively studied in several fungi, namely, *Aspergillus nidulans* (2, 25), *Saccharomyces cerevisiae* (8), and *Neurospora crassa* (18). If *N. crassa* is grown in the absence of ammonium, a variety of alternative nitrogen-metabolizing pathways, such as nitrate assimilation, potentially have elevated levels of activity. The ammonium generated by these pathways is used to form glutamate from 2-oxoglutarate by the action of NADP-glutamate dehydrogenase. The glutamate formed is amidated with a second equivalent of ammonium via the action of glutamine synthetase (GS). Recently, it has been shown by several groups that glutamate and glutamine also repress the same pathways as ammonium, and it is believed that the presence of glutamine in some way leads to the repression of these alternative nitrogen metabolic pathways (3, 11, 18, 21). This nitrogen metabolite repression is thought to entail the cessation of positive gene regulation. The positive regulator is believed to be a product of the *nit-2* regulatory gene, since mutation in this gene leads to an inability to utilize a wide variety of nitrogen sources such as purines and nitrate (18). Recently, we suggested that GS, directly or indirectly, is the signal to stop the action of the *nit-2* gene. It was shown that the *gln-1a* mutant has derepressed levels of the nitrate assimilation enzymes nitrate reductase (NaR) and nitrite reductase (NiR) in the presence of ammonium or glutamate but not glutamine (10, 11). The effects of various nitrogen metabolites on NaR and NiR have been examined in a more extreme GS structural gene mutant, *gln-1b*, which is allelic to *gln-1a*. This mutant, like *gln-1a*, has derepressed levels of NaR and NiR in the presence of ammonium or glutamate. In the presence of glutamine, *gln-1b* has levels of NaR and NiR which vary. There is an inverse relationship between the level of derepression of these enzymes and the stage of the mycelial culture. An additional finding is that *gln-1b*, unlike the wild type or *gln-1a*, synthesizes about 30% of the maximal level of GS even under repressing conditions, such as growth with glutamine. GS in eucaryotes typically consists of eight identical subunits. The native enzyme has a molecular weight approaching 400,000 (19). Palacios (20) and Limón-Lason et al. (15) have studied the regulation and biochemistry of GS in *N. crassa* and have reported that the oligomeric structure of this enzyme may be a function of the nitrogen status of the cell. Under conditions of ammonium excess, GS apparently exists as an octamer; when nitrogen is limited, the tetrameric form predominates. More recent results indicate that two similar but distinct monomers, α and β, may be involved in the formation of GS oligomers in *N. crassa* (22). GS from *N. crassa* cultures grown under ammonium-limited conditions is structured in a tetrameric form composed of α-monomers, whereas GS isolated from nitrogen-rich cultures is an octamer of β-polypeptides (4). The results presented in this communication imply that GS has a regulatory role in gene expression and that this role directly or indirectly involves the oligomeric nature of the enzyme.

MATERIALS AND METHODS

Strains and growth conditions. Strain 74-OR23-1A was used as the wild type, and the following mutants were also studied: *gln-1a* (allele *R1015*), *gln-1b* (kindly provided by J. Mora), and *nit-2* (allele *nr37*). Mycelium to be used for enzyme assays was grown for 24 or 40 h at 25°C on Vogel minimal medium (6) with

80 mM NH_4Cl plus 2.5 mM L-glutamine. Mycelium was then transferred (2 to 3 g, wet weight) to the treatment medium, consisting of 100 ml of Vogel minimal medium (6) and the appropriate nitrogen source, and then shaken for 4 or 6 h at 25°C. The mycelium was then harvested by filtration on Büchner funnels, washed several times with distilled water, weighed, and frozen at −70°C until used.

Enzyme extraction. The frozen mycelium was homogenized with extraction buffer. The extraction buffer for NiR and NaR was that described by Greenbaum et al. (14). The GS extraction buffer was buffer A (5 mM phosphate–0.5 mM EDTA–50 mM K_2SO_4, pH 7.5) as described by Dávila et al. (5). The crude homogenate was then centrifuged for 20 min at 27,000 × *g* at 4°C to give a supernatant which was used as the crude extract.

Enzyme assays. NiR was assayed as described by Greenbaum et al. (14). NiR was assayed as described by Garrett and Nason (13). GS activity was determined by the transferase assay as described by Ferguson and Sims (12).

Growth tests. Conidial suspensions were made from each of the strains to be tested and then spotted on solid Fries minimal medium (6) supplemented with the appropriate nitrogen source. The plates were incubated for several days at 24°C before scoring for degree of growth.

Protein determination. The method used was that of Lowry et al. (16), with bovine serum albumin as the standard.

Materials. Analytical-grade chemicals were used throughout.

RESULTS

GS mutant *gln-1b* is nitrogen metabolite derepressed. Table 1 presents the results of plate tests where the growth response of *gln-1b* was compared with that of the wild type and various nitrogen nonutilizing mutants. The *gln-1b* mutant initially grew more poorly than the *gln-1a* mutant on the various nitrogen sources tested, except glutamine. After a time, this initial lag was overcome, and the growth of *gln-1b* was similar to that of *gln-1a*. These results are consistent with the fact that *gln-1b* is a more extreme GS mutant than *gln-1a* (5). Chlorate toxicity is closely associated with NaR levels (1). Thiourea is an analog of urea (9), and aspartic hydroxamate is an analog of asparagine (7). Because ammonium, glutamate, or glutamine repressed nitrate reductase, urea transport, and asparaginase, the wild type is resistant to these analogs. The *gln-1b* mutant, like *gln-1a*, was sensitive to all of these analogs in the presence of ammonium or glutamate. However, in the presence of glutamine, *gln-1b* was initially more sensitive than *gln-1a* to the analog chlorate. After several days, *gln-1b* appeared as resistant as *gln-1a*.

The *nit-2* mutant showed nitrogen-starved growth with 0.5 mM glutamate but normal growth with 5 mM glutamate as the nitrogen source. Similar results were obtained with alanine as the nitrogen source (results not shown). These results demonstrate that the *nit-2* mutation leads to an impaired ability to subsist on limiting amounts of these amino acids.

Mutant *gln-1b* has derepressed levels of nitrate reductase and altered levels of GS. Table 2 shows the effects of various nitrogen sources on the activities of NaR and GS in the wild type, *gln-1a*, and *gln-1b*. As previously established, NaR in the wild type was repressed by ammonium, glutamate, or glutamine. The mutants *gln-1a* and *gln-1b* had substantial levels of NaR in the presence of ammonium or glutamate. Only *gln-1b* had significant levels of NaR in the presence of glutamine. GS levels as assayed by its transferase activity were highest in the wild type in the presence of glutamate and lowest in the presence of glutamine. The mutant *gln-1a* had substantially the same levels of GS activity as the wild type, apart from the finding that *gln-1a* could not achieve the maximal level of GS found in the wild type when grown with glutamate. Under all the conditions tested here, the mutant *gln-1b* consistently produced GS to about 30% of the maximal levels ever found in the wild type.

The effect of the stage of mycelial growth on

TABLE 1. *Growth responses of the wild-type and mutant strains*[a]

Strain	NH_4Cl	L-Glutamate		L-Glutamine	$KClO_3$			Thiourea			Aspartic hydroxamate		
		0.5 mM	5 mM		NH_4Cl	L-Glutamate	L-Glutamine	NH_4Cl	L-Glutamate	L-Glutamine	NH_4Cl	L-Glutamate	L-Glutamine
Wild type	4	4	4	4	R	R	R	R	R	R	R	R	R
gln-1a	2	1	2	4	S	S	R	S	S	R	S	S	R
gln-1b	1	1	1.5	4	S	S	R/S	S	S	R	S	S	R
nit-2	3.5	1	3.5	4	R	R	R	R	R	R	R	R	R

[a] R and S indicate resistant and sensitive growth, respectively, in the presence of the toxic analogs. 1–4 indicate increasing levels of growth. The growth tests were carried out at 25°C on appropriate supplemented minimal medium (6). The carbon source was sucrose (2%, wt/vol). The nitrogen source concentration was 5 mM except in the presence of an analog, where it was 10 mM. The toxic analog concentrations were 100 mM $KClO_3$, 50 mM thiourea, and 4 mM aspartic hydroxamate.

the levels of NaR, NiR, and GS in the wild type, *gln-1a*, and *gln-1b* was examined (Table 3). It was shown in Table 2 that *gln-1b* had substantial levels of NaR even in the presence of glutamine. The level of NaR was found to vary considerably, from a specific activity of 1.0 to 19.7 (results not shown). This level of NaR activity was inversely correlated with the mycelial yields of *gln-1b* cultures. The results shown in Table 3 demonstrate attempts made to quantify this phenomenon. All three strains, wild type, *gln-1a*, and *gln-1b*, had higher NaR activities when grown for 24 instead of 40 h. Only 24-h-old *gln-1b* mycelia had substantial levels of NaR or NiR when grown in the presence of 10 or 20 mM glutamine. Note that the GS levels of *gln-1a* but not *gln-1b* were only 10% of those found in the wild type after 24 h of growth. After 40 h of growth there was a substantially higher yield of mycelia in all three strains. Again, both the wild type and *gln-1a* had very low levels of NaR and NiR in the presence of glutamine. Although *gln-1b* had higher levels of NaR and NiR in the presence of glutamine compared with the wild type and *gln-1a*, these levels were substantially lower than found in 24-h *gln-1b* mycelia. Note that the GS levels in *gln-1a* and *gln-1b* in 40-h mycelia when transferred to nitrate plus glutamine were less repressed than those found in 24-h *gln-1a* or *gln-1b* mycelia.

TABLE 2. *Effect of nitrogen source on the activities of NaR and GS in the wild type, gln-1a, and gln-1b*[a]

Medium nitrogen source	Enzyme activity					
	NaR			GS		
	Wild type	*gln-1a*	*gln-1b*	Wild type	*gln-1a*	*gln-1b*
20 mM $NaNO_3$	25.0 (5)	31.2 (5)	37.6 (5)	19.5 (7)	20.5 (6)	7.6 (6)
10 mM $NaNO_3$ + 20 mM NH_4Cl	0.97 (2)	36.2 (1)	37.1 (3)	13.6 (5)	12.6 (3)	8.0 (4)
10 mM $NaNO_3$ + 20 mM L-glutamate	4.3 (2)	19.0 (2)	38.5 (2)	26.1 (7)	16.6 (5)	8.7 (6)
10 mM $NaNO_3$ + 10 mM L-glutamine	0.05 (5)	0.48 (4)	7.07 (4)	7.3 (7)	6.3 (6)	7.0 (8)

[a] Mycelia were grown for 40 h at 25°C in minimal medium (6) with 3% (wt/vol) cane sugar and 80 mM NH_4Cl + 2.5 mM L-glutamine and then transferred to the indicated media for a 4-h period. The enzyme activities are expressed as nanomoles per minute per milligram of protein. Figures in parentheses indicate number of different mycelial preparations assayed.

TABLE 3. *Effect of time and mycelial yield on NaR and NiR levels in cultures transferred to inducing or repressing conditions*[a]

Growth medium	Strain	Treatment medium	Enzyme activity		
			NaR	NiR	GS
80 mM NH_4Cl + 2.5 mM L-glutamine, 24 h	Wild type	20 mM $NaNO_3$	60.8	9.8	14.2
	gln-1a		66.8	10.3	2.8
	gln-1b		72.7	42.1	3.0
Yield: wild type, 12.0 g	Wild type	10 mM $NaNO_3$ + 10	0.04	0.0	3.9
gln-1a, 1.4 g	*gln-1a*	mM L-glutamine	0.00	0.0	0.3
gln-1b, 3.8 g	*gln-1b*		10.6	8.7	2.6
	Wild type	10 mM $NaNO_3$ + 20	0.0	0.0	4.4
	gln-1a	mM L-glutamine	0.0	0.0	0.5
	gln-1b		8.5	4.7	2.3
80 mM NH_4Cl + 2.5 mM L-glutamine, 40 h	Wild type	20 mM $NaNO_3$	13.8	18.8	18.1
	gln-1a		45.8	19.3	20.8
	gln-1b		68.2	57.3	4.7
Yield: wild type, 24.4 g	Wild type	10 mM $NaNO_3$ + 10	0.0	0.0	9.7
gln-1a, 13.8 g	*gln-1a*	mM L-glutamine	0.20	0.0	6.6
gln-1b, 11.6 g	*gln-1b*		1.12	3.5	8.9
	Wild type	10 mM $NaNO_3$ + 20	0.0	0.0	10.0
	gln-1a	mM L-glutamine	0.29	0.0	6.0
	gln-1b		0.97	2.8	8.8

[a] Mycelia were grown for either 24 or 40 h at 25°C in minimal medium (6) with 3% cane sugar plus 80 mM NH_4Cl plus 2.5 mM L-glutamine and then transferred to the indicated media for a 4-h period. Enzyme activities are expressed as in Table 2.

It is possible that a more valid comparison of the real effects of glutamine might be obtained from a consideration of enzyme levels in 24-h wild-type mycelia (12-g yield) versus enzyme levels in 40-h *gln-1a* (13.8-g yield) and *gln-1b* (11.6-g yield) mycelia, assuming that equivalent yields indicate similar physiological ages between the cultures. Such a comparison nevertheless reveals that *gln-1b* mycelia contained significantly higher levels of the nitrate assimilation enzymes than found in either *gln-1a* or wild-type mycelia in the presence of glutamine (Table 3). It is apparent then that the repressive effect of glutamine on nitrate assimilation in *Neurospora* is substantially diminished in the *gln-1b* strain.

Comparisons of GS activity in the wild type and *gln-1b*. Sánchez et al. (24) have shown that GS from cell-free extracts of *gln-1b* has only 50% of the activity per mole of enzyme compared with the activity per mole of wild-type enzyme. Yet the levels of GS activity in cell-free extracts of *gln-1b* grown with glutamine were similar to those of the wild type (Table 2).

This finding raised the possibility that the regulation of GS activity in *gln-1b* may be altered. To test this possibility, wild-type and *gln-1b* mycelia were grown for 40 h with 80 mM NH_4Cl plus 2.5 mM glutamine and 5 mM glutamate and then transferred to various treatment media. The levels of GS activity in the wild type and *gln-1b* were determined (Table 4). Although the GS of *gln-1b* has a lower intrinsic activity, the levels of GS activity found in cell-free extracts of *gln-1b* were generally comparable to those in the wild type. This finding indicates that *gln-1b* was possibly producing more GS protein than the wild type. Note particularly that, when grown with glutamine, lower levels of GS were found in the wild type than in *gln-1b*.

TABLE 4. *GS activity in the wild type and gln-1b*[a]

Medium nitrogen source	Strain	GS activity
20 mM $NaNO_3$	Wild type	17.8
	gln-1b	14.7
10 mM $NaNO_3$ + 25 mM NH_4Cl	Wild type	9.4
	gln-1b	12.9
10 mM $NaNO_3$ + 20 mM glutamate	Wild type	26.7
	gln-1b	14.5
10 mM $NaNO_3$ + 20 mM glutamine	Wild type	4.7
	gln-1b	7.0
5 mM NH_4Cl	Wild type	13.4
	gln-1b	19.0
5 mM glutamate	Wild type	24.2
	gln-1b	23.4
5 mM glutamine	Wild type	4.4
	gln-1b	9.0

[a] Mycelia were grown initially for 40 h in 80 mM NH_4Cl plus 5 mM glutamate plus 2.5 mM glutamine, transferred to the appropriate treatment medium for 6 h and then harvested. Activity is expressed as in Table 2.

DISCUSSION

The repression of secondary pathways of cellular nitrogen acquisition such as nitrate assimilation by ammonium or the immediate products of ammonium fixation, namely, glutamate and glutamine, is termed nitrogen metabolite repression. This regulation provides an obvious economy to the cell by preventing the elaboration of unneeded metabolic machinery. Genetic and biochemical studies suggest that nitrogen metabolite repression is achieved through the cessation of *nit-2* gene expression (18). The *nit-2* gene is believed to encode a positive gene regulator which acts pleiotropically to permit the expression of any of a variety of alternative nitrogen-metabolizing pathways. A central question then is to determine how the *nit-2* gene is regulated, specifically how the condition of cellular nitrogen sufficiency is transmitted to this locus. Of the pathways subject to *nit-2* control, nitrate assimilation is the optimal system for examination of the regulation since the expression of nitrate assimilation is under very tight control: in the wild type, relief from nitrogen metabolite repression leads to a 500-fold increase in NaR levels (Table 2). In comparison, the enzyme xanthine dehydrogenase of the purine catabolic pathway, which is also subject to *nit-2* regulation, realizes only a sevenfold increase (17). Thus, studies on the regulation of nitrate assimilation offer detection of subtleties of nitrogen metabolite repression.

The effects of chlorate on the growth of the *gln-1b* mutant (Table 1) are subtle but reproducible. These observations reveal that the *gln-1b* mutant shows chlorate sensitivity, albeit transiently, even in the presence of glutamine. The results of Tables 2 and 3 are consistent with this observation in demonstrating that the nitrate assimilation enzymes are expressed in *gln-1b* despite the presence of glutamine. The possibility that glutamine has immediate and direct action upon *nit-2* gene expression, for example as a co-repressor, is seriously questioned by these results. Other elements which would normally be responsive to glutamine levels apparently intervene between the signal of cellular nitrogen sufficiency (glutamine) and *nit-2* gene action. The effects observable in the GS mutants *gln-1a* and *gln-1b* suggest that this enzyme may be a potential component in the regulatory scheme. A model based upon these observations and the known alterations in the oligomeric state of GS in response to fluctuations in the nitrogen status of the cell has been proposed (10). In this

model, octameric GS, the oligomeric form predominant in nitrogen-rich cells, represses *nit-2* gene expression.

GS activity in the wild type is highest in cells grown with glutamate and lowest in cells grown with glutamine. Sánchez et al. (23) have shown that glutamine represses GS-specific mRNA synthesis. This fact accounts for the repressive effects seen on GS levels in wild-type mycelia exposed to glutamine (Table 4). The relatively higher levels of GS activity in *gln-1b* mycelia under the same conditions stand in marked contrast, particularly in light of the intrinsically lesser activity of *gln-1b* GS molecules. Like *gln-1b*, the other GS mutant examined here, *gln-1a*, has lower GS activity per mole of enzyme (24) compared with wild type, but the alteration in GS activity levels in this mutant in response to various nitrogen sources is similar to that of the wild type. Like the wild type, the mutant *gln-1a* can readily form octameric GS when glutamine is present in sufficient amounts. Although the intrinsic activity of the GS enzyme in *gln-1a* is apparently lower than that of the wild type, the specific activity of GS found in *gln-1a* extracts is very similar to that of the wild type. These observations and the results presented in Table 4 would suggest that GS in *gln-1a* and *gln-1b* is produced in greater amounts than in the wild type. This situation is similar to the result found in bacteria, where some GS mutants synthesize GS at a higher rate than the wild type (26). Furthermore, glutamine is apparently less effective in repressing GS in *gln-1b* than in the wild type. A possible explanation for this result is that GS in *N. crassa* is autogenously regulated.

ACKNOWLEDGMENTS

We thank Brian Tomsett for stimulating discussions of our results and Marie Nassiff for technical assistance.

This investigation was supported in part by Public Health Service research grant GM-22738 from the National Institutes of Health.

LITERATURE CITED

1. **Cove, D. J.** 1976. Chlorate toxicity in *Aspergillus nidulans*. Studies of mutants altered in nitrate assimilation. Mol. Gen. Genet. **146**:147–159.
2. **Cove, D. J.** 1979. Genetics studies of nitrate assimilation in *Aspergillus nidulans*. Biol. Rev. **54**:291–327.
3. **Dantzig, A. H., W. K. Zurowski, T. N. Ball, and A. Nason.** 1978. Induction and repression of nitrate reductase in *Neurospora crassa*. J. Bacteriol. **133**:671–679.
4. **Dávila, G., M. Lara, J. Guzmán, and J. Mora.** 1980. Relation between structure and function of *Neurospora crassa* glutamine synthetase. Biochem. Biophys. Res. Commun. **92**:134–140.
5. **Dávila, G., F. Sánchez, R. Palacios, and J. Mora.** 1978. Genetics and physiology of *Neurospora crassa* glutamine auxotrophs. J. Bacteriol. **134**:693–698.
6. **Davis, R. H., and F. J. DeSerres.** 1970. Genetic and microbiological research techniques for *Neurospora crassa*. Methods Enzymol. **17A**:79–143.
7. **Drainas, C., J. R. Kinghorn, and J. A. Pateman.** 1977. Aspartic hydroxamate resistance and asparaginase regulation in the fungus *Aspergillus nidulans*. J. Gen. Microbiol. **98**:493–501.
8. **Dubois, E., S. Vissers, M. Grenson, and J.-M. Wiame.** 1977. Glutamine and ammonia in nitrogen catabolite repression in *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. **75**:233–239.
9. **Dunn, E., and J. A. Pateman.** 1972. Urea and thiourea uptake in *Aspergillus nidulans*. Heredity **29**:129.
10. **Dunn-Coleman, N. S., and R. H. Garrett.** 1980. The role of glutamine synthetase and glutamine metabolism in nitrogen metabolite repression, a regulatory phenomenon in the lower eukaryote *Neurospora crassa*. Mol. Gen. Genet. **179**:25–32.
11. **Dunn-Coleman, N. S., A. B. Tomsett, and R. H. Garrett.** 1979. Nitrogen metabolite repression of nitrate reductase in *Neurospora crassa*: effect of the *gln-1a* locus. J. Bacteriol. **139**:697–700.
12. **Ferguson, A. R., and A. P. Sims.** 1974. The regulation of glutamine metabolism in *Candida utilis*: the role of glutamine in the control of glutamine synthesis. J. Gen. Microbiol. **80**:159–171.
13. **Garrett, R. H., and A. Nason.** 1967. Involvement of a *b*-type cytochrome in the assimilatory nitrate reductase of *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S.A. **58**: 1603–1610.
14. **Greenbaum, P., K. N. Prodouz, and R. H. Garrett.** 1978. Preparation and some properties of homogeneous *Neurospora crassa* assimilatory NADPH-nitrite reductase. Biochim. Biophys. Acta **526**:52–64.
15. **Liñon-Lason, J., M. Lara, B. Resendiz, and J. Mora.** 1977. Regulation of glutamine synthesis in fed-batch cultures of *Neurospora crassa*. Biochem. Biophys. Res. Commun. **78**:1234–1240.
16. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
17. **Lyon, E. S., and R. H. Garrett.** 1978. Regulation, purification and properties of xanthine dehydrogenase in *Neurospora crassa*. J. Biol. Chem. **253**:2604–2614.
18. **Marzluf, G. A.** 1977. Regulation of gene expression in fungi, p. 196–242. *In* J. C. Copeland and G. A. Marzluf (ed.), Regulatory biology. Ohio State University Press.
19. **Meister, A.** 1974. Glutamine synthetase of mammals, p. 699–754. *In* P. D. Boyer (ed.), The enzymes, vol. 10. Academic Press, Inc., New York.
20. **Palacios. R.** 1976. *Neurospora crassa* glutamine synthetase: purification by affinity chromatography and characterization of subunit structure. J. Biol. Chem. **251**: 4787–4791.
21. **Premakumar, R., G. J. Sorger, and D. Gooden.** 1979. Nitrogen metabolite repression of nitrate reductase in *Neurospora crassa*. J. Bacteriol. **137**:119–1126.
22. **Sánchez, F., E. Calva, M. Compomanes, L. Blanco, J. Guzmán, J. L. Saboris, and R. Palaciosa.** 1980. Heterogeneity of glutamine synthetase polypeptides in *Neurospora crassa*. J. Biol. Chem. **255**:2231–2234.
23. **Sánchez, F., M. Compomanes, C. Quinto, W. Hansberg, and J. Mora.** 1978. Nitrogen source regulates glutamine synthetase mRNA levels in *Neurospora crassa*. J. Bacteriol. **136**:880–885.
24. **Sánchez, F., G. Dávila, J. Mora, and R. Palacios.** 1979. Immunochemical characterization of glutamine synthetase from *Neurospora crassa* glutamine auxotrophs. J. Bacteriol. **139**:537–543.
25. **Smith, J. E., and J. A. Pateman.** 1977. Genetics and physiology of *Aspergillus*. Academic Press, Inc., London.
26. **Tyler, B.** 1978. Regulation of the assimilation of nitrogen compounds. Annu. Rev. Biochem. **47**:1127–1162.