Transcriptional Activation of the *Aspergillus nidulans* *gpdA* Promoter by Osmotic Signals†

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A differentially expressed *gpdA* cDNA clone was isolated from NaCl-adapted *Aspergillus nidulans* (FGSC359) and identified as glyceraldehyde-3-phosphate dehydrogenase (*gpdA*) on the basis of its nucleotide sequence. The level of *gpdA* RNA substantially increased in cultures gradually adapted to NaCl but was greatly reduced in cultures exposed briefly to a high concentration of NaCl. A *pyrG* auxotroph of *A. nidulans* (A773) was cotransformed with a *gpdA-uidA* construct and a plasmid containing the *Neurospora crassa pyr4* gene as a selectable marker. One *pyrG*⁺ β-glucuronidase-positive (GUS⁺) transformant was selected, and stable integration of the *gpdA-uidA* construct into the genome was confirmed by Southern blot analysis. Gradual adaptation to increasing concentrations of NaCl resulted in an increase in GUS activity to 2.7-fold. GUS activity was reduced after a 2-h exposure of an unadapted culture to 2 M NaCl but gradually increased to a maximum of twofold after 24 h. GUS activity also increased by 8.4-fold in Na₂SO₄-adapted cultures, 4.9-fold in polyethylene glycol-adapted cultures, and 7.5-fold in KCl-adapted cultures. These results are consistent with the hypothesis that the *A. nidulans gpdA* promoter is transcriptionally activated by osmotic signals.

Aspergillus nidulans is a common model for molecular genetics and the study of gene expression. Several *A. nidulans* promoters have been investigated for expression studies (20). The *gpdA* gene encodes glyceraldehyde-3-phosphate dehydrogenase (GPD) and has a constitutive promoter (12, 13). Multiple copies of GPD-encoding genes have been reported in higher eukaryotes (3, 4), but only a single GPD-encoding gene has been reported in *A. nidulans* (11). GPD is a key enzyme in glycolysis and glucogenesis and constitutes up to 5% of the soluble cellular protein in *Saccharomyces cerevisiae* (8) and *A. nidulans* (12). Two upstream activating sequences have been identified in the *gpdA* promoter by deletion analysis of a promoter-reporter gene fusion (12, 14). Despite extensive use of the *gpdA* promoter for heterologous gene expression (13), no data on transcriptional regulation by environmental signals have been published.

We have been studying the molecular mechanism of NaCl tolerance in *A. nidulans*. Adaptation to a high concentration of NaCl is accompanied by complex changes in gene expression that affect a large number of proteins involved in various cellular processes (15, 16). We were surprised when we found an increased transcript level of *gpdA* in NaCl-adapted cultures, because this gene is constitutively expressed. We tested the *gpdA* promoter's adaptation to growth on lower-water-potential medium (amended with ionic and nonionic osmotica) and to osmotic shock with a transformed strain carrying a *gpdA-uidA* fusion and by measuring β-glucuronidase (GUS) reporter enzyme activity.

MATERIALS AND METHODS

Strains and culture conditions. *A. nidulans* FGSC359 (*pabaA1 wA3*) was gradually adapted to grow in the presence of 2 M NaCl (osmotic potential ψ , -11.3 MPa) or, following growth in normal medium, was transferred to medium amended with 2 M NaCl to produce salt shock (16). Unadapted, NaCl-adapted, and NaCl-shocked cultures were processed as previously described (16). A773 (*pyrG89 wA3 pyrA4*), an auxotrophic strain of *A. nidulans*, and the transformed cultures were grown in YG medium (14) supplemented with 0.12% uracil and 0.12% uridine.

Plasmids. Plasmids pRG-1 and pNOM-102 were used for cotransformation. Plasmid pRG-1 is a 4.9-kb construct containing the *Neurospora crassa pyr4* gene, which can complement the *pyrG89* mutation in *A. nidulans* (1). Plasmid pNOM-102 is a 7.55-kb construct containing a GUS (*uidA*) gene under the control of the *A. nidulans* GPD (*gpdA*) promoter (18).

Transformation. Protoplasts of *A. nidulans* A773 were cotransformed in the presence of polyethylene glycol (PEG) (5). A 3:1 molar ratio of pNOM-102 to pRG-1 was used, and the *pyrG*⁺ GUS⁺ transformants were selected on X-Gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid) medium (100 μg/ml).

Southern and Northern blots. Total DNA was isolated from untransformed and transformed cultures, and a Southern blot (19) was probed with a 1.8-kb *NcoI* fragment from the *uidA* gene of pNOM-102 (18). RNA isolation, electrophoresis, and Northern blot analysis were performed as previously described (15). The DNA inserts of RR294 and *uidA* from pNOM-102 were used as probes. The blot was stripped and reprobed with a cDNA insert from clone RRU1, an unidentified cDNA clone showing constitutive expression under different growth conditions (16).

GUS assay. Frozen mycelium in liquid nitrogen was ground and suspended in GUS extraction buffer (7) and centrifuged at 12,000 × g for 30 min at 4°C. The supernatant was desalted on a Sephadex G-25 column. GUS specific activity was assayed by fluorometry with 4-methylumbelliferyl-β-D-glucuronide as the substrate (7). The reaction mixture was incubated at 37°C, and the production of 4-methylumbelliferone was monitored for 30 min. GUS activity was determined from the slope of the line showing the increase in fluorescence and was expressed in nanomoles of 4-methylumbelliferone produced minute⁻¹ microgram of protein⁻¹ at 37°C. The protein concentration was determined with a Bio-Rad kit (2).

Adaptation to low-water-potential medium and salt shock. One of the selected transformants was gradually adapted (16) to 0.5, 1.0, 1.5, and 2 M NaCl; 10% PEG 4000, 2.0 M KCl; and 2.0 M Na₂SO₄. Unadapted cultures were subjected to osmotic shock with 2 M NaCl for 2, 12, 18, 24, and 30 h.

RESULTS

Expression of *gpdA* under saline conditions. Clone RR294 (≈1.1-kb insert) was isolated from a λ ZapII cDNA library made from the salt-adapted *A. nidulans* culture (15). The nucleotide sequence of the RR294 clone was identical to that of the *A. nidulans gpdA* gene (11). In Northern blot analysis using RR294 as the probe, RR294 hybridized to a ≈1.8-kb transcript

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FIG. 1. Expression of the *gpdA* gene in untransformed cultures and the *uidA* gene in the T1 cultures. Equal amounts of RNA from unadapted (UN), salt-adapted (SA), and salt-shocked (SS) untransformed cultures (A and B) and T1 cultures (C and D) were electrophoresed and transferred to a nylon membrane. The blots were probed with an *EcoRI-XhoI* insert from RR294 (A) and an *NcoI* fragment from pNOM-102 containing the *uidA* gene (C). The blots were re-probed with an *EcoRI-XhoI* fragment from RRU1 to demonstrate equal amounts of RNA in each lane (B and D).

that was constitutively expressed in the unadapted culture. The transcript level was at least fourfold higher in the NaCl-adapted culture than the basal level in the unadapted culture. Transcripts were not detectable in the NaCl-shocked culture (Fig. 1A). Following prolonged exposure of the blot, a faint band could be detected in the salt-shocked culture (data not shown). The RRU1 insert was used as a probe to demonstrate equal amounts of RNA in each lane (Fig. 1B).

Transformation and integration of the *uidA* gene. Cotransformation of A773 with pRG-1 and pNOM-102 resulted in 20 mitotically stable *pyrG*⁺ GUS⁺ transformants. One transformant, T1, was selected for further analysis. Southern blot hybridization confirmed the GUS⁺ phenotype. A distinct hybridization signal of 12.5 kb was found in the DNA of the T1 transformant, indicating the integration of the *uidA* gene in the genome. As expected, no signal for *uidA* was detected in the DNA from untransformed cultures of A773 (result not shown).

Expression of the *uidA* gene under the control of the *gpdA* promoter. Expression of the *uidA* gene under the control of the *gpdA* promoter in transformant T1 was similar to the expression of the *gpdA* gene in the untransformed A773 culture (Fig. 1C). The presence of equal amounts of RNA in each lane is shown in Fig. 1D. The basal level of GUS activity in cell extract of the unadapted cultures of T1 culture was 40 U and increased to 110 U in the NaCl-adapted T1 culture (Table 1). In the NaCl-shocked culture, GUS activity was reduced to 26 U (Table 2). GUS activity declined 10% in cultures adapted to 0.5 M NaCl, but a gradual increase in GUS activity from 1.4- to 2.7-fold was observed in cultures adapted to 1.0, 1.5, and 2.0 M NaCl in the medium, respectively. Adaptation to PEG (a non-ionic osmoticum) and KCl and Na₂SO₄ (ionic osmotica) resulted in significant increases in GUS activity. A maximum increase of 8.4-fold was observed for the Na₂SO₄-adapted culture, whereas 4.9- and 7.5-fold increases in GUS activity were observed in the PEG- and the KCl-adapted cultures, respectively (Table 1).

The unadapted T1 culture was salt shocked by transfer to medium containing 2 M NaCl for 2, 12, 18, 24, and 30 h. Growth of the culture was inhibited by about 90% (data not shown). GUS activity declined after 2 h and then returned to the initial level after 12 and 18 h of osmotic shock before

TABLE 1. GUS specific activity in extracts from the T1 transformant in cultures adapted to different osmotic agents^a

Osmoticum	Osmotic potential of medium (MPa)	Sp act ^b
None	-0.5	40 ± 4
0.5 M NaCl	-3.2	36 ± 6
1.0 M NaCl	-5.9	56 ± 4
1.5 M NaCl	-8.6	72 ± 6
2.0 M NaCl	-11.3	110 ± 9
10% PEG 4000	-7.5	200 ± 15
2.0 M KCl	-10.8	300 ± 12
2.0 M Na ₂ SO ₄	-9.3	340 ± 13

^a Cultures were adapted to YG medium alone or containing osmotica at the indicated concentrations.

^b Values are means ± standard deviations of three independent measurements.

increasing to nearly twice that of the unadapted culture after 24 h of salt shock (Table 2).

DISCUSSION

GPD is a key enzyme in the carbon metabolic pathway and is responsible for oxidation of glyceraldehyde-3-phosphate to 1,3-bisphosphate glyceric acid. The induction of *gpdA* expression in salt-adapted culture was surprising because we have previously shown that *A. nidulans* accumulates glycerol as a compatible osmolyte in salt-adapted cultures (17). Thus, increased GPD activity mobilizes carbon away from glycerol and into the pathway leading to glycolysis and ATP formation. Cellular adjustment to elevated salinity requires additional energy for growth, and expression of several genes involved in mitochondrial ATP formation is known to be induced in salt-adapted cultures (15). GPD plays an important role in carbon utilization and serves to interconnect stress response and ATP formation during growth under saline conditions.

The increase in transcript levels of the *uidA* gene in the salt-adapted T1 culture suggests transcriptional activation of the *gpdA* promoter. Such activation of the *gpd* gene occurs in response to oxidative stress in the rabbit aorta (6) and to heat stress and anaerobic stress in the halophyte *Atriplex nummularia* (10). The *A. nidulans gpdA* promoter has two transcription-activating elements (14), but it is not known whether either of these two elements is responsive to osmotic signals.

Cultures adapted to increasing concentrations of NaCl also had increased levels of GUS activity. In the PEG-, KCl-, or Na₂SO₄-adapted cultures, the relationship between GUS activity and the osmotic potential of the medium was not the same as that seen in cultures adapted to different levels of NaCl. After normalization of GUS activity for the osmotic

TABLE 2. GUS specific activities in extracts from the T1 transformant in salt-shocked cultures^a

Time (h) of incubation	Sp act
0.....	38 ± 4
2.....	26 ± 5
12.....	42 ± 7
18.....	39 ± 8
24.....	76 ± 10
30.....	74 ± 12

^a Unadapted cultures were grown in YG medium, transferred to medium containing 2 M NaCl, and further incubated for the indicated time. Values are the means ± standard deviations of three independent measurements.

potential of the medium, the KCl- and the Na₂SO₄-adapted cultures exhibited 3- and 3.4-fold increases in GUS activity, respectively, while the PEG-adapted culture showed a 2-fold-higher GUS activity compared with that of the NaCl-adapted cultures. These observations imply that the *gpdA* promoter is responsive to osmotic signals and that the level of response is modulated by specific ions. The decline of GUS activity and the *uidA* transcript level in the salt-shocked culture is consistent with the expression of the *gpdA* gene in untransformed culture. The residual GUS activity after 2 h of salt shock may represent GUS protein constitutively synthesized before shock, since GUS is known to be stable (7). Recovery and the subsequent increase in GUS activity suggest that, even without culture growth under salt shock (16), cellular adjustments to elevated salinity result in a response of the *gpdA* promoter similar to that in the NaCl-adapted culture.

The promoter sequence of the *A. nidulans gpdA* gene has been widely used in vector constructions for heterologous gene expression in fungi. Our finding provides the basis for exploitation of osmotic signals to increase the expression of transgenes in filamentous fungi.

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