Nuclear Accumulation of the GATA Factor AreA in Response to Complete Nitrogen Starvation by Regulation of Nuclear Export

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Both the availability and the quality of nutrients affect cellular functions by controlling gene activity. AreA, a member of the GATA family of transcription factors, globally activates expression of genes involved in nitrogen source utilization in Aspergillus nidulans. The quality of the nitrogen source determines the level and activation capacity of AreA through controls at the level of areA mRNA stability and by interaction of AreA with the corepressor NmrA. The availability of potential nitrogen sources also affects the activation capacity of AreA. We show that the complete absence of a nitrogen source results in an enhanced level of AreA-dependent gene expression and that this response is independent of mechanisms regulating AreA activity in response to nitrogen source quality. During nitrogen starvation AreA accumulates in the nucleus, but the presence of a potential nitrogen source or carbon starvation prevents this accumulation. Furthermore, accumulated AreA is rapidly lost from the nuclei of nitrogen-starved cells when a nitrogen source is supplied or when a carbon source is absent, and this accompanies arrest of the AreA-dependent nitrogen starvation response on regulated gene expression. By the generation of a leptomycin B-sensitive mutant, we have been able to show that nuclear exit occurs via the CrmA exportin. We conclude that sensing mechanisms discriminate between starvation and the presence of potential nutrients that can signal to the AreA transcription factor. Nitrogen source availability, but not quality, affects nuclear accumulation by regulating nuclear exit of AreA, providing a rapid response to changes in the supply of nutrients.

Filamentous fungi can use many compounds as sole nitrogen sources. Production of the enzymes and uptake systems required for utilization of nitrogen sources is regulated by a general control mechanism, termed nitrogen metabolite repression, in which expression of the relevant structural genes is low during growth on glutamine or ammonium and elevated under nitrogen-limiting conditions (31). In addition, pathway-specific transcription factors may activate sets of genes involved in catabolism of a particular compound in response to specific low-molecular-weight inducers.

The molecular genetics of nitrogen metabolite repression has been extensively studied in filamentous fungi (31). The key regulatory gene (areA in Aspergillus nidulans and nit-2 in Neurospora crassa) encodes a transcription activator containing a single GATA zinc finger DNA-binding domain (17, 24). Nitrogen catabolic gene expression requires transcription activation by AreA/NIT2, and nitrogen metabolite repression is signaled by nitrogen metabolites generated during growth on preferred nitrogen sources to prevent or diminish this activation. The major repressing metabolite is likely to be glutamine, as mutations blocking conversion of ammonium to glutamine result in loss of repression (30). Loss-of-function mutations in areA and nit-2 confer an inability to use nitrogen sources other than ammonium, and amino acid substitutions in the DNA-binding domain alter the pattern of nitrogen source utilization (24, 38, 42). AreA and NIT2 bind sequences containing the core GATAR sequence, and functional cis-acting sequences containing this motif are found in the 5′ regions of genes subject to nitrogen metabolite repression (7, 12, 19, 33, 38).

In A. nidulans, AreA activity is regulated in response to nitrogen sources. AreA levels are elevated under nitrogen-limiting conditions by autogenous transcription control via GATAR sequences in the areA 5′ region (28). Growth in the presence of ammonium, glutamate, or glutamine results in reduced areA mRNA stability compared with growth in the presence of limiting nitrogen sources (32, 36). This is mediated by sequences in the 3′ untranslated region (3′UTR) of areA, deletion of which results in increased mRNA stability and partial relief of nitrogen metabolite repression. AreA function is also controlled by interaction with the negative-acting regulator NmrA. Deletion of the nmrA gene or C-terminal truncation of AreA results in partial derepression (1, 36). NMR1, the product of the homologous gene in N. crassa, interacts specifically with NIT2 via C-terminal sequences and sequences within the GATA zinc finger domain (41). It is proposed that under nitrogen-sufficient conditions, NmrA interacts with AreA to prevent activation of nitrogen catabolic genes (1). Recent studies show that NmrA interacts with AreA in vitro and suggest that NmrA does not prevent DNA binding by AreA (26, 27). The two mechanisms controlling AreA activity are independent, as deletion of the AreA C-terminal sequence or the nmrA gene combined with deletion of the areA 3′UTR results in a high level of derepression (1, 36). These controls adjust levels of nitrogen catabolic gene expression according to nitrogen source quality.

Complete starvation for nitrogen may not be equivalent to nitrogen limitation. Some nitrogen catabolic enzymes such as acetamidase, histidase, and formamidase, which do not require...
FIG. 1. Regulation of expression of amds-lacZ in response to different nitrogen sources. (A) ArcA was HA tagged and the areA promoter replaced with the gpdA promoter in a two-step strategy (panel 1). 5' areA (−18 to +811) was gene replaced with riboB by transformation of a riboflavin auxotroph with linearized pJAF5224 and selection for Riboll prototrophs (panel 2). areA was reconstructed with modifications by transformation of the areA:riboB(5') strain from panel 1 with linearized pJAF5200 and selection on 10 mM nitrate. pJAF5200 contains gpdA(p)areA truncation at +1475 and confers AreA function only by homologous integration at the areA locus. (B) Strains of the indicated genotypes were grown for 16 h in 1% glucose medium with the nitrogen sources 10 mM ammonium tartrate (NH4), 10 mM l-glutamine (GLN), and 10 mM L-alanine (ALA) or subjected to nitrogen starvation (−N) by transferring mycelium prior to harvesting, extracted, and assayed for β-galactosidase. Specific activities with standard errors for at least three experiments are shown. (C) Wild-type and areA:riboB strains were grown for 16 h in 1% glucose−10 mM ammonium tartrate medium and then transferred to glucose medium lacking a nitrogen source, harvested at the indicated times, and assayed for β-galactosidase. (D) The indicated strains were grown for 16 h in glucose-ammonium medium (0 min), transferred to addition of an inducer for expression, are produced at high levels in response to nitrogen starvation (14, 23, 37). It was thought that this response occurred by the normal mechanisms of AreA activation, with nitrogen starvation resulting in low levels of repressing metabolites. We show here, however, that nitrogen starvation results in an additional mechanism of AreA-dependent gene expression, independent of NmrA and the areA 3' UTR, and is rapidly reversed by addition of a wide variety of nitrogen compounds.

We have found that the response to nitrogen starvation is paralleled by accumulation of AreA in the nucleus and that its reversal by nitrogen compounds is paralleled by the rapid loss of accumulated AreA from the nucleus. The response to nitrogen starvation of enzymes involved in nitrogen source utilization does not occur during carbon starvation (14, 21, 22, 37). This effect is independent of areA-mediated carbon catabolite repression (14). We show here that accumulation of AreA in the nucleus does not occur when cells are starved for both carbon and nitrogen and that AreA is rapidly lost from the nucleus upon carbon starvation. Therefore, in addition to the previously described mechanisms for modulating AreA activity, control at the level of nuclear localization is important for regulating gene expression in response to starvation.

MATERIALS AND METHODS

Generation of areA gene replacement plasmids. Sequences encoding three copies of the hemagglutinin (HA) epitope (YPYDVPDYA) were inserted in frame into areA at the PvuII site (position +156 relative to the start codon). The areA 5' PvuII fragment (−895 to +156) was inserted into the EcoCRI site of the HA epitope vector pSM491 to give pJAF4688. The 3.1-kb PvuII ScaI areA 3' fragment (+157 to +3252) was then inserted into the Smal site of pJAF4688 to give pJAF4689, which encodes AreA with three copies of the HA epitope inserted between residues 52 and 53. pJAF5198 was generated from pJAF4689 by deleting the 3' end of areA after nucleotide +1475 as an Apal fragment. The areA EcoCRI/BglII promoter fragment (−571 to −19) in pJAF5198 was replaced with an EcoRV/XhoI (end-filled) gpdA promoter fragment (nucleotides −1225 to −119 relative to the gpdA start codon) from pAXL215 to give pJAF5200. The areA 5' deletion construct was created by inserting a 2.2-kb BglII/Smal riboB+ fragment from pPL1 (35) into pJAF5198 digested with BglII/SnaBI, replacing nucleotides −18 to +811 of areA to give pJAF5224.

Epitope tagging of areA derivatives. MH9826 (a1 areA217 pabaA1 fmdS-lacZ) was transformed with the areA18 gene on pJAF4689 and transformants selected on 10 mM nitrate and screened by Southern analysis. MH9841 (a1 pabaA1 areA18 fmdS-lacZ [at argB]) was chosen for immunostaining and assays. Simultaneous HA tagging of AreA variants and replacement of the areA promoter with the gpdA promoter involved two steps (Fig. 1A). (i) 5' areA (−18 to +811) was gene replaced with the riboB gene by transformation of MH764 (a3 riboB2 lacB101) with the areA:riboB(5') construct pJAF5224 linearized with Apal and selection for riboflavin prototrophs. An AreA- transformant MH9922 [pJAF5224(areA3:riboB5') riboB2 lacB101] was identified by lack of growth on 10 mM nitrate, and the gene replacement was confirmed by Southern blotting. (ii) areA was reconstructed with modifications by transforming MH9922 with Apal-linearized pJAF5200, containing gpdA(p)areA18A truncated at +1475, and selecting for growth on 10 mM nitrate. Transformants were tested for loss of riboB, and integration was confirmed by Southern blotting. Transformant MH9883 [a3 gpdA(p)areA18A riboB2 lacB101] was outcrossed, and the segregant MH9949 [b1 a1 gpdA(p)areA18A amds-lacZ] was used in immunostaining, Western analysis, and assays. MH9927 [b1 a1 niaA1 amds-lacZ gpdA(p)areA18A] glucose medium containing various nitrogen sources (10 mM) for the indicated times, and then harvested and assayed for β-galactosidase. GLN, l-glutamine; NH4, ammonium tartrate; GLU, l-glutamate; ALA, l-alanine; PRO, l-proline; UREA, urea; ASN, l-asparagine; UA, uric acid; −N, no added nitrogen source.
**RESULTS**

**AreA-dependent gene expression in response to nitrogen starvation.** The *amdS* gene is controlled by AreA-mediated nitrogen metabolite repression (23). Expression of a gene-replaced *amdS-lacZ* reporter gene (11) increased in response to growth on the limiting nitrogen source alanine compared with the repressing nitrogen sources ammonium and glutamine and was further elevated in response to complete nitrogen starvation (Fig. 1B). The elevated response of *amdS-lacZ* levels to nitrogen starvation was *areA* dependent (Fig. 1C) and was retained in the *nmrA* mutant and the *areA 3'UTR* element deletion mutant, each of which showed partial derepression on ammonium and glutamine compared with the limiting nitrogen source alanine (Fig. 1B). Furthermore, simultaneous deletion of *nmrA* and the *areA 3'UTR* element resulted in a high level of derepression of *amdS-lacZ* expression on ammonium compared with alanine but did not prevent the response to nitrogen starvation (Fig. 1B). Regulation of an *fmdS-lacZ* fusion gene present in single copy at the *argB* locus showed a similar pattern of *areA*-dependent expression, with an elevated response to nitrogen starvation compared with levels in the presence of alanine (14) (data not shown). Overall, these data suggest that nitrogen starvation results in additional *areA*-dependent mechanism different from that effected by NmrA or *areA*.

**AreA accumulates in the nucleus in response to nitrogen starvation.** We modified the *areA* genomic loci to encode AreA with the HA epitope inserted between residues 52 and 53 (AreAHA), within a region dispensable for AreA regulation (5, 28) (see Materials and Methods). We also uncoupled transcriptional regulation of *areA* by replacing the native *areA* promoter with the constitutive *gpdA* promoter (Fig. 1A). The pattern of nitrogen metabolite repression and nitrogen starvation response of *amdS-lacZ* (Fig. 1B) and *fmdS-lacZ* (data not shown) was not affected by either the presence of the HA tag or expression of AreA from the *gpdA* promoter. However, overall levels were slightly lower than wild type in the *areA* strain and were increased in the presence of the *gpdA* promoter.

Immunofluorescence microscopy was used to examine the subcellular distribution of AreAHA. Nuclear accumulation of AreAHA occurred within 1 hour of transfer to medium lacking a nitrogen source, and the amount of AreAHA accumulated in the nucleus increased over the course of 4 h of nitrogen starvation. This regulated distribution of AreAHA was apparent whether expression was from the *areA* or *gpdA* promoter (Fig.)
Thus, nuclear accumulation of AreAHA paralleled the increase in AreA-dependent gene expression in response to nitrogen starvation. Furthermore, nuclear accumulation of AreAHA is a specific consequence of nitrogen starvation. AreAHA nuclear accumulation was not observed when mycelium was transferred for 3 h to medium containing the nitrogen source ammonium, glutamine, glutamate, proline, alanine, or uric acid or the ammonium analog methylammonium (Fig. 2C) or when mycelium was grown for 12 h on medium containing ammonium, glutamine, glutamate, proline, alanine, formamide, gamma-aminobutyric acid, urea, or uric acid (data not shown).

Western blot analysis showed that the relative levels of AreAHA in the gpd(p)areA4HA strain did not differ significantly when mycelia were nitrogen starved or grown on ammonium or alanine (Fig. 2D). However, the mobility of AreAHA was reduced in mycelia grown on alanine compared with those grown on ammonium. A further reduction in AreA4HA mobility was observed in nitrogen-starved mycelia. These data indicate that AreA accumulates in the nucleus during nitrogen starvation and is subject to differential posttranslational modification according to both nitrogen source quality and availability.

Nuclear accumulated AreA is rapidly lost in response to nitrogen sources. The effect of addition of nitrogen sources to nitrogen-starved hyphae was examined. Loss of nuclear accumulated AreAHA from the nucleus was observed within 1 minute after transfer of nitrogen-starved cells to ammonium-containing medium (Fig. 3A). Western analysis showed that the AreAHA protein was stable for up to 60 min after transfer to ammonium (Fig. 3B), indicating that rapid loss of AreAHA from the nucleus reflected increased nuclear export and not AreAHA degradation. Furthermore, the reduced mobility of AreAHA on nitrogen starvation medium (Fig. 2D) was maintained for more than 15 min after transfer to ammonium (Fig. 3B). Transfer to glutamine, methylammonium, proline, alanine, or uric acid also resulted in rapid loss of nuclear accumulated AreAHA.
response to nitrogen starvation (Fig. 4A). Western analysis showed that AreAHA was present under carbon starvation conditions and that AreAHA mobility was reduced during nitrogen starvation but not carbon starvation (Fig. 4B). Transfer of nitrogen-starved mycelium to simultaneous nitrogen and carbon starvation conditions resulted in loss of accumulated AreAHA from the nucleus within 15 minutes (Fig. 4C). The AreAHA protein was stable for up to 60 min after transfer (Fig. 4D). Therefore, rapid loss of AreAHA from the nucleus upon carbon starvation paralleled the response of expression of areA-regulated genes to carbon starvation. Transfer of nitrogen-starved mycelium to carbon starvation conditions in the presence of ammonium resulted in loss of accumulated AreAHA from the nucleus within 1 minute (Fig. 4E).

**AreA nuclear export occurs via the CrmA exportin.** The slow nuclear accumulation and rapid loss of AreA suggests that nuclear export is the major control of AreA nuclear accumulation. The AreA protein contains a putative leucine-rich nuclear export signal at residues 703 to 712 (LHGVVRPLSL), conforming to the consensus for nuclear export signals bound to CRM1 exportins (13, 18). *S. pombe* CRM1 is inhibited by the drug LMB, which binds to a specific cysteine residue (C529), resulting in growth sensitivity and inhibition of CRM1-dependent nuclear export (25). *Saccharomyces cerevisiae* LMB resistant, and the equivalent CRM1 residue is threonine, which when mutated to cysteine causes LMB sensitivity (34). Wild-type *A. nidulans* is LMB resistant, and the *A. nidulans* crmA gene specifies a threonine at residue 525, corresponding to *S. pombe* CRM1 C529 (25). We isolated the full-length crmA gene (see Materials and Methods) and altered codon 525 to specify cysteine (Fig. 5A). The crmA<sup>T525C</sup> mutant was sensitive to the presence of 100 ng/ml LMB (Fig. 5B). The crmA<sup>T525C</sup> mutation had no effect on AreA nuclear export in the absence of LMB (Fig. 5C). We examined the effect of LMB on export of AreAHA in the presence and absence of the crmA<sup>T525C</sup> mutation. Rapid nuclear export of AreA<sup>HA</sup> in the presence of LMB occurred in response to ammonium within 1 minute in the control strain, but AreA<sup>HA</sup> was retained in the nucleus in the crmA<sup>T525C</sup> mutant (Fig. 5C). Therefore, CrmA is the major export route from the nucleus for AreA.

**DISCUSSION**

We have shown that *A. nidulans* can distinguish between conditions of nitrogen sufficiency, nitrogen limitation, and nitrogen starvation and adjust accordingly the expression levels of nitrogen catabolic genes. These changes are AreA dependent and reflect alterations in the level and activity of AreA. Furthermore, we have shown that *A. nidulans* can respond to nitrogen starvation by altering the dynamics of nuclear entry and exit of AreA, using regulated nuclear exit to provide a rapid response when nutritional conditions change.

We favor a model in which, in the presence of nitrogen sources, AreA is present in the nucleus at a relatively low level due to a balance between nuclear entry and exit. When repressing metabolites are at high levels (for example, when ammonium or glutamine is the source of nitrogen), the level of transcriptionally active AreA is low due to accelerated areA mRNA turnover and NmrA inhibition of AreA activation ability (1, 32). When poorer (limiting) nitrogen sources are present

**FIG. 3.** Accumulated AreA<sup>HA</sup> is rapidly lost from the nucleus upon addition of a nitrogen source. (A and C) The gdp(p)areA<sup>HA</sup> strain was grown for 12 h in glucose–10 mM ammonium tartrate minimal medium and transferred to nitrogen-free medium (−N) for 4 h (0 min). Hyphae were then transferred to 10 mM ammonium tartrate medium for 1, 2, 5, 10, and 15 min (A). Hyphae were transferred to glucose minimal medium containing 10 mM ammonium tartrate, transferred for 4 h to medium lacking a nitrogen source (0 min), and then transferred to medium containing 10 mM ammonium tartrate for 1, 5, 15, 30, and 60 min (C). (B) AreA<sup>HA</sup> HA strain was grown for 16 h in glucose–10 mM ammonium tartrate minimal medium and transferred to nitrogen-free medium (−N) medium for 1, 5, 10, and 15 min (B). Hyphae were transferred to glucose minimal medium containing 0.2 mM glutamine (GLN), 1 mM methylothammonium chloride (MACI), 10 mM alanine (ALA), 10 mM proline (PRO), and 10 mM uric acid (UA) for 1 and 15 min (C). The hyphae were fixed and immunostained. Representative DAPI-stained nuclei and γ-HA are shown. Scale bars represent 2 μm.

Carbon starvation prevents nuclear accumulation of AreA. Enzymes involved solely in nitrogen source utilization, including formamidase, histidase, and a general amidase, show an areA-dependent response to nitrogen starvation but not during simultaneous carbon starvation (14, 16, 37). Furthermore, transfer of nitrogen-starved mycelium to medium lacking a carbon source results in rapid loss of areA-dependent expression of an amdS-lacZ fusion gene (14). Carbon starvation was found to prevent accumulation of AreA<sup>HA</sup> in the nucleus in
and glutamine levels are reduced, the increased stability of AreA mRNA and diminished NmrA inhibition of AreA activity result in increased expression of AreA-dependent genes (28, 32, 36). Together these controls are sufficient to account for the difference in AreA activation under nitrogen-sufficient and nitrogen-limiting conditions. However, neither of these controls accounts for the elevated level of AreA-dependent gene expression observed under conditions of complete nitrogen starvation. Increased activation of AreA target genes corre-

FIG. 4. Carbon starvation prevents AreA nuclear accumulation. (A) The gpd(p)areA strain was grown for 12 h in glucose minimal medium plus 10 mM ammonium tartrate and transferred to 1% glucose or carbon-free (C) minimal medium containing (NH₄) or lacking (N) 10 mM ammonium tartrate for 4 h. (B) Analysis of AreA during carbon starvation. Western blot analysis with anti-HA antibody of total protein extracts (50 μg) from gpd(p)areA mycelia grown for 16 h in glucose–10 mM ammonium tartrate and transferred to 1% glucose or carbon-free (C) minimal medium containing (NH₄) or lacking (N) 10 mM ammonium tartrate for 2 h. (C) Loss of accumulated AreA in response to carbon starvation. The gpd(p)areA strain was grown for 12 h in glucose–10 mM ammonium tartrate minimal medium, transferred to 1% glucose nitrogen-free minimal medium for 4 h (0 min), and then transferred to minimal medium lacking both a carbon and a nitrogen source (C–N) for 1, 5, and 15 min. A control 15-min transfer to 1% glucose nitrogen-free minimal medium (C–N) was included. (D) AreA is stable after transfer to carbon starvation medium. Western blot analysis with anti-HA antibody of total protein extracts (50 μg) from gpd(p)areA mycelia grown for 16 h in glucose–10 mM ammonium tartrate minimal medium, transferred for 4 h to minimal medium lacking a nitrogen source (0 min), and then transferred to medium lacking both a carbon and nitrogen source (C–N) for 1, 5, 15, and 60 min. (E) Effects of ammonium predominate over effects of carbon starvation. The gpd(p)areA strain was grown for 12 h in glucose minimal medium plus 10 mM ammonium tartrate, transferred to glucose-nitrogen-free minimal medium for 4 h (0 min), and then transferred to minimal medium lacking a carbon source (C) but containing 10 mM ammonium chloride (NH₄) as a nitrogen source for 1, 5, and 15 min. A control 15-min transfer to 1% glucose nitrogen-free medium (N) was included. For panels A, C, and E, hyphae were fixed and immunostained, representative DAPI-stained nuclei (DAPI) and AreA fluorescence (α-HA) in the corresponding regions are shown, and the scale bars represent 2 μm.

FIG. 5. AreA export from the nucleus occurs via the CrmA exportin. (A) Construction of the LMB-sensitive crmA T525C mutant. Transformants were selected for pyrimidine prototrophy due to insertion of pyrG 3' of the crmA gene and screened by Southern analysis for loss of a genomic StuI site (S) associated with the introduced crmA T525C mutation (×). Crossovers 3' of the mutation resulted in gene replacements carrying the crmA T525C mutation and lacking the StuI site, whereas crossovers 3' of the mutation generated gene replacements lacking the desired mutation but retaining the StuI site. (B) The A. nidulans crmA T525C mutant is LMB sensitive. The crmA T525C and control gene replacement strains were tested for growth on 1% glucose–10 mM ammonium tartrate minimal medium with 100 ng/ml LMB dissolved in 100% ethanol (+LMB) or lacking LMB (–LMB) and grown for 3 days at 37°C. (C) AreA nuclear export occurs via the CrmA exportin. The gpd(p)areA and control gpd(p)areA crmA T525C strains were grown in 1% glucose–10 mM ammonium tartrate minimal medium for 12 h and then transferred to medium lacking a nitrogen source (N) for 4 h. LMB (100 ng/ml) or ethanol (control) was then added and left for 5 min prior to treatment with 10 mM ammonium tartrate for 1 min. Samples were fixed and immunostained. Representative DAPI-stained nuclei (DAPI) and AreA fluorescence (α-HA) in the corresponding regions are shown. Scale bars represent 2 μm.
lates with progressive accumulation of AreA in the nuclei of nitrogen-starved cells. Increased concentrations of the AreA transcription factor inside the nucleus may result in high-level expression of target genes, although we cannot exclude the possibility that additional changes to AreA upon nitrogen starvation act to enhance its activity. Addition of a nitrogen source, whether limiting or nonlimiting, results in rapid loss of accumulated AreA and a loss of the response to nitrogen starvation. Furthermore, carbon starvation of nitrogen-starved cells also causes rapid loss of nuclear accumulated AreA and loss of AreA-dependent gene expression. We have shown that nuclear export of AreA via the CrmA exportin is likely to be the main control point for regulation of AreA nuclear accumulation.

Our model predicts that nitrogen starvation leads to modification of AreA resulting in its retention within the nucleus and that exposure to a nitrogen source leads to rapid CrmA-mediated nuclear exit of AreA. We have shown that AreA is posttranslationally modified according to nitrogen source quality and additionally modified during nitrogen starvation. Preliminary studies indicate that AreA is highly phosphorylated under all conditions (unpublished data). We observed no clear difference in AreA11A mobility accompanying rapid loss from the nucleus. The rapid loss of AreA from the nucleus could result from a minor posttranslational modification of AreA, which would not have been resolved in our analysis, or from modification of a protein that interacts with AreA. The AreA-dependent response of gene expression to nitrogen starvation is prevented by carbon starvation (14, 20, 37). This is paralleled by a lack of accumulation of AreA in the nucleus, which may be due to energy-dependent nuclear import being sensitive to carbon starvation. Carbon starvation also results in loss of nuclear accumulated AreA, and this could be by a mechanism different from the nitrogen source mechanism.

In *S. cerevisiae* control of nuclear localization of the GATA DNA-binding activator proteins Gln3 and Gat1/Nil1 is the major mechanism for the response of genes involved in nitrogen acquisition to changes in nitrogen source availability (2, 3, 10, 29). However, the molecular basis of this regulation differs from that in *A. nidulans*. Unlike the situation in *A. nidulans*, there is no reported difference in response to nitrogen starvation compared to limitation, and Gln3 nuclear export via the CrmA homolog Crm1 is not subject to regulation (6). Instead, under conditions of nitrogen excess the product of the *URE2* gene, defined by mutations resulting in insensitivity of gene expression to the nitrogen source, prevents nuclear entry of Gln3 and Gat1. In *A. nidulans*, the closest homolog of *URE2* encodes a glutathione *S*-transferase with no detectable role in nitrogen metabolite repression (15). In the presence of a good nitrogen source, the rapamycin-sensitive Tor kinase complex is thought to phosphorylate Gln3, which complexes with Ure2 in the cytoplasm (2, 4, 10). Nitrogen limitation or starvation leads to dephosphorylation of Gln3, allowing release from Ure2 and nuclear entry. However, recent data indicate that the phosphorylation state of Gln3 is complex and not necessarily correlated with nuclear localization and the nitrogen source present, raising the possibility that Tor activity and nutrient signaling affect Gln3 by different mechanisms (8, 40). The role, if any, of the Tor complex in controlling AreA activity in *A. nidulans* is unknown. In complete contrast to *A. nidulans*, glucose starvation in the presence of ammonium results in nuclear localization of Gln3 (4), but not when glutamine is present (9). These observations have been interpreted as resulting from carbon starvation causing reduced levels of ammonium assimilation into glutamine, the signaling molecule for control of Gln3 localization (9).

The pattern of regulation of genes by AreA would seem to be adapted to the ecology of filamentous fungi, which scavenge a wide variety of nitrogen sources derived from growth in soil and on decaying organic matter. Hyphae are likely to experience nutrient starvation during growth in microenvironments completely lacking particular nutrients and as substrates are exhausted. Increased expression of genes involved in nitrogen source acquisition during nitrogen starvation would allow a rapid response when new nutrients are encountered. However, in the absence of a carbon or energy source, derepression of nitrogen catabolism would not provide the nutrients necessary for growth. The ability of *A. nidulans* to adjust the nucleocytoplasmic distribution of AreA in response to both the carbon and nitrogen status of its environment highlights a previously undiscovered level of regulation of nitrogen catabolic gene expression.

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