

MINIREVIEW

Recent Advances in Nitrogen Regulation: a Comparison between *Saccharomyces cerevisiae* and Filamentous Fungi^{∇†}

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Myriad biological processes are dynamically regulated in response to environmental cues. Transcriptional gene regulation is of fundamental importance, and regulatory mechanisms ensure that genes are expressed at levels appropriate to the cellular state. Microorganisms undergo major shifts in their transcriptional profile in response to changes in the nutrients that are available. In the free-living fungi this is evident when different growth substrates are available or when pathogenic species infect their hosts. Three model ascomycetes in particular, *Aspergillus nidulans*, *Neurospora crassa*, and *Saccharomyces cerevisiae*, have contributed much to the detailed functional analysis of metabolic gene regulation due to their small haploid genome size, excellent genetics, and metabolic versatility. Phylogenetic analysis indicates that the filamentous ascomycetes *A. nidulans* and *N. crassa* and the hemiascomycete *S. cerevisiae* belong to separate lineages within *Ascomycota* (39), which also includes various human and plant pathogens and species with important industrial applications. The increasing availability of fungal genome sequences has enabled comparative studies across species occupying different ecological niches.

Nitrogen is an essential requirement for growth, and fungi are able to use a wide variety of compounds as nitrogen sources. However, the use of different nitrogen compounds is selective, and readily assimilated nitrogen sources such as ammonium and glutamine are preferentially used. Underlying this selective utilization of nitrogen sources are complex controls that operate primarily at the transcriptional level. The global regulation of nitrogen source utilization, known as nitrogen catabolite repression (NCR) in *S. cerevisiae* and nitrogen metabolite repression (NMR) in *A. nidulans* and *N. crassa*, involves the regulation of the synthesis of appropriate catabolic enzymes and permeases in response to the nitrogen status of the cell. For many nitrogen sources, expression of the respective catabolic activities is also subjected to pathway-specific control in response to specific inducers (for reviews see references 12, 23, and 65).

The major transcription factors regulating nitrogen gene expression in all fungal species studied thus far are GATA factors. These transcription factors share a common DNA

binding motif (Cys-X2-Cys-X17-Cys-X2-Cys) that recognizes a core 5'-GATA-3' sequence (87). Despite this, there are significant differences in key aspects of the molecular circuitry that underlies nitrogen regulation in different fungi. In this review we will highlight recent advances made in understanding the nitrogen regulatory systems and explore the evolutionary history of these differences in the fungi as revealed by their genome sequences.

REGULATION OF NITROGEN CATABOLISM BY GATA FACTORS

In *S. cerevisiae*, two positively acting transcription factors, Gln3 and Gat1 (Nil1), control expression of a large number of genes involved in nitrogen metabolism (23). Both Gln3 and Gat1 contain a single zinc finger domain, which is highly similar to the DNA binding domain of mammalian GATA factors, and an amphipathic helix required for transcriptional activation has been identified in Gln3 (69, 74, 96, 97).

Genome-wide expression analysis has identified a large number of genes whose expression is dependent on Gln3 and Gat1 (27, 88). Although these factors bind to a common GATA core sequence in the promoters of target genes, they display overlapping yet distinct roles in the activation of the NCR-regulated genes (20, 96). The relative contributions of Gln3 and Gat1 to activation vary with their target genes and the available nitrogen source, and it has been proposed that Gln3 and Gat1 activities are inactivated in response to high levels of endogenous glutamine and glutamate, respectively (52, 86, 95, 96).

In filamentous ascomycetes, regulation of nitrogen catabolic genes is also mediated by GATA-type transcription factors. The *A. nidulans* AreA and *N. crassa* Nit2 proteins are positively acting GATA factors involved in nitrogen regulation (Fig. 1), and loss-of-function *areA* and *nit-2* mutants are unable to utilize any nitrogen source other than glutamine and ammonium (3, 65). A single AreA orthologue with significant sequence homology throughout the entire protein is found in all other filamentous ascomycetes analyzed. Cross-complementation experiments have shown that many of these orthologues are functionally conserved (18, 19, 41, 42, 47, 63, 76, 77, 90, 107, 117).

Gat1/AreA/Nit2 orthologues are present in all ascomycetes (see Fig. S1 in the supplemental material). In addition to the conserved GATA DNA binding domain, these orthologues contain two highly conserved blocks of sequences, RMENLT

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	GATA Factors						
	Positive		Negative		Ure2	NmrA	MeaB
<i>S.cer</i>	Gln3	Gat1	Dal80	Gzf3	Ure2	-	-
<i>A.gos</i>	+	+	+	+	+	-	-
<i>C.alb</i>	+	+	+	+	+	-	-
<i>A.nid</i>	-	AreA	AreB		3 GST	NmrA	MeaB
<i>N.cra</i>	-	Nit2	Asd4		2 GST	Nmr1	+
<i>U.may</i>	-	+	-		3 GST	-	-
<i>C.neo</i>	-	+	-		1 GST	-	-

FIG. 1. Evolutionary relationships of the key regulators of nitrogen metabolism in fungi. Phylogenetic relationships among the hemiascomycetes (dark shading), filamentous ascomycetes (light shading), and basidiomycetes (no shading) are indicated on the left. Protein sequences of the respective regulators of nitrogen metabolism from *S. cerevisiae* (*S.cer*) and *A. nidulans* (*A.nid*) were analyzed using the *blastp* algorithm to identify putative orthologues in various fungi (1). Sequences were also searched against the genome sequence database of *Ashbya gossypii* (*A.gos*), *Candida albicans* (*C.alb*), *N. crassa* (*N.cra*), *Ustilago maydis* (*U.may*), and *Cryptococcus neoformans* (*C.neo*) using *blastp* and *tblastn* analyses. The presence or absence of orthologous sequences in the genome of the respective fungi is indicated by a plus sign and a minus sign, respectively. Published protein names for the orthologues are indicated where possible, and those that have been shown to be involved in nitrogen regulation are highlighted in bold. Various criteria were followed to assist identification of orthologues of low sequence conservation. Classification of Gln3 orthologues is based on the position of the GATA zinc finger and sequence conservation at both the GATA domain and the C terminus (DLDWLKFGI, where the underlined residue is variable) in the putative protein sequence. For Gat1 orthologues, the position and sequence conservation of the GATA finger as well as the short stretch of conserved sequences (RMENLTWRMM, where underlined residues are less conserved) at the N terminus were used. Protein sequences containing an N-terminal GATA finger, a C-terminal leucine zipper, and the conserved motif (LKTKISELELVTDL, where underlined residues are different in Dal80 and Gzf3) are considered as Dal80/Gzf3 orthologues. Putative Ure2, NmrA, and MeaB orthologues were identified by *blastp* and *tblastn* analyses. GST represents sequences, identified by *tblastn* and *blastp* searches, that have weak but significant similarity to Ure2 and contain GST domains. Other putative sequences of GST are not included. See Table S1 in the supplemental material for accession numbers for the listed orthologues. Alignments used Clustal W (105).

WRMM and WEWLMSL, at the N and C termini, respectively. The C-terminal domain of AreA and Nit2 is important for modulating activities by NmrA and Nmr1, respectively (see below). Intriguingly, *Yarrowia lipolytica* appears to have two sequences orthologous to Gat1/AreA/Nit2, with one relatively more similar to AreA/Nit2 and the other relatively more similar to Gat1 when the C-terminal motifs are compared. Furthermore, the N-terminal motif is also conserved in possible Gat1/AreA/Nit2 orthologues in the basidiomycetes *Cryptococcus neoformans* and *Ustilago maydis* (see Fig. S2 in the supplemental material), where their role in nitrogen regulation is yet to be established. Although deletion of the N-terminal region in AreA does not detectably affect NMR in *A. nidulans* (13), this conservation across distantly related orthologues might imply an undiscovered function for the N-terminal motif. The relatively higher level of conservation shared between orthologues in *C. neoformans* and *U. maydis* and AreA than with Gat1 in this region may indicate AreA as the ancestral form of

the orthologues in the *Ascomycota* lineage. Gln3, on the other hand, appears to be unique to hemiascomycetes including *Debaryomyces hansenii*, *Pichia stipitis*, *Ashbya gossypii*, and *Candida albicans* (Fig. 1). However, the sequences of the orthologues from *D. hansenii*, *P. stipitis*, and *C. albicans* have diverged considerably from *S. cerevisiae* Gln3 with conservation restricted only to the sequence and position of the DNA binding domain and the extreme C-terminal motif (DLDWLKFGI) (see Fig. S3 in the supplemental material). In spite of this, the *C. albicans* Gln3 orthologue is involved in controlling nitrogen metabolism and also has a role that is overlapping with but distinct from that of the *C. albicans* Gat1 (60, 61).

Two other GATA factors, Dal80 (Uga43) and Gzf3 (Nil2 or Deh1), have a negative role in controlling expression of genes involved in nitrogen catabolism in *S. cerevisiae* (23). Both Dal80 and Gzf3 contain a single GATA zinc finger and a putative leucine zipper allowing homodimer as well as heterodimer formation (25, 32, 86, 94, 96, 98). Dal80 and Gzf3 are proposed to antagonize Gln3 and Gat1 activation by competing for binding to a GATA element(s) in the promoter of NCR-regulated genes to fine-tune expression (21, 34, 86, 94, 96). Gln3 and Gat1 can function via a single GATA element but may depend on the presence of an auxiliary site (86). However, not all genes regulated by Gln3 and Gat1 are controlled to the same extent by Dal80 and Gzf3 (24). Dal80 requires dimerization for binding to two GATA sequences in either tail-to-tail or head-to-head orientation, while Gzf3 is able to function via a single GATA site and can potentially form homodimers or heterodimerize with Dal80 (33). The configuration of the GATA elements and/or auxiliary elements in the promoters of different nitrogen catabolic genes is likely the key determinant of the affinities and specificities of Gln3, Gat1, Dal80, and Gzf3 binding. It is noteworthy that the appearance of the two related factors Dal80 and Gzf3 is the result of whole-genome duplication and that only one Dal80/Gzf3 orthologue is present in other hemiascomycetes that did not undergo this event (Fig. 1) (112). It would be interesting to find out whether the Dal80/Gzf3 orthologues share similar DNA binding specificities to Dal80 or to Gzf3 and whether they antagonize the action of Gln3 and Gat1 orthologues to the same extent in these species.

A single GATA factor similar to Dal80 and Gzf3 may also be present in filamentous ascomycetes (Fig. 1). The AreB protein contains an N-terminal GATA zinc finger and a C-terminal leucine zipper domain (22), and *Penicillium chrysogenum* NREB and *N. crassa* Asd4 are orthologues of AreB with a high level of sequence conservation spanning the entire protein. There is a single putative protein sequence similar to AreB/NREB/Asd4 in the genome of all filamentous ascomycetes sequenced thus far. Expression of *areB* and *nreB* is regulated by nitrogen sources (22, 46), and overexpression of *nreB* leads to repression of nitrogen catabolic genes (46). Therefore, AreB and NREB may act in a manner analogous to that of Dal80. However, Asd4 has yet to be shown to be involved in nitrogen regulation, but interestingly a role in sexual development has been reported (37). No orthologue of Dal80/Gzf3/AreB/NREB can be identified in the basidiomycetes *C. neoformans* and *U. maydis* (Fig. 1), suggesting that the involvement of positive and negative GATA factors in nitrogen regulation may be a unique evolutionary feature of the ascomycetes.

DNA BINDING SPECIFICITY OF GATA FACTORS

Sequence analysis of the *S. cerevisiae* genome reveals six additional open reading frames that contain the GATA zinc finger motif. While some of these GATA factors (Gat2, Gat3, and Gat4) have unknown function (27), others have been shown to be involved in diverse processes such as pre-rRNA processing (Srd1) (51), mating-type switching in haploid cells (Ash1) (93), and pseudohyphal growth (Ash1 and Srd2) (14, 17). In *A. nidulans*, additional GATA factors have been identified that control siderophore biosynthesis (SreA) (48) and sexual development (NsdD) (49), with two other GATA proteins being identified as the putative functional homologues of *N. crassa* WC1 (LreA [accession no. 470313]) and WC2 (LreB [accession no. 82072]), which are the regulators of light response and circadian rhythmicity (35, 45).

The presence of multiple GATA factors and their diverse biological roles raises the intriguing question of how DNA binding specificity of these proteins is maintained in vivo. The *N. crassa* GATA factors Nit2, Asd4, WC1, and WC2 can all bind to the same GATA element in vitro (66). However, not all GATA sequences are in vivo functional binding sites for the nitrogen-regulatory GATA factors (24, 65), and some degree of selectivity is based on the interaction between amino acid residues within the DNA binding motif and flanking sequences of the GATA site. In *A. nidulans*, AreA binds to the consensus HGATAR (where H = A, T, and C and R = A and G) and V683L substitution in the DNA binding domain alters the preference of the mutant protein for TGATAG over (A/C)G ATAG (85). Dimerization may also contribute to DNA binding specificity, as in the case of Dal80, where two closely spaced GATA sites are required for DNA binding (33). Although AreA can function with a single GATA element, the DNA binding affinity of AreA and orthologues in other filamentous ascomycetes can be increased when two or more GATA elements are situated in close proximity (12, 24). In at least some cases, the DNA binding specificity and affinity of GATA factors may be dependent upon interaction with pathway-specific transcription factors, as illustrated by the cooperative action of Nit2 and Nit4 in *N. crassa* and AreA and NirA in *A. nidulans* on the expression of genes involved in nitrate assimilation (12, 66). Similarly binding of Gln3 and Gat1 in *S. cerevisiae* requires either several GATA sites or other transcriptional elements, as is the case for the allantoin degradative pathway where interactions between Gln3, Dal82, and Dal83 are required for transcriptional activation (82, 83, 89, 109). AreA is also required for nucleosome repositioning and NirA binding at the *niaD-niiA* bidirectional promoter upon nitrate induction (6, 72, 73). The influence of chromatin structure around the GATA element and/or binding sites of auxiliary factors on in vivo DNA binding by GATA factors is an important area of future research.

REGULATION OF Gln3 AND Gat1 ACTIVITIES IN *S. CEREVISIAE*

In *S. cerevisiae*, expression of *GAT1*, that of *DAL80*, and that of *GZF3* are all regulated by NCR and are subjected to cross-regulation by the other GATA factors with *GAT1* and *DAL80* levels also being modulated by autogenous control (23). In

contrast, *GLN3* expression is independent of the other GATA factors and nitrogen sources. Regulation of Gln3 function by nitrogen sources occurs primarily at the posttranslational level. Gln3 is predominantly cytoplasmic in the presence of a good nitrogen source but enters the nucleus during growth on a poor nitrogen source (Fig. 2A). Consequently, Gln3 activates expression of NCR-regulated genes including *GAT1*, *DAL80*, and *GZF3*, whose gene products act independently or in concert to influence expression of nitrogen catabolic genes (Fig. 2A). Therefore, Gln3 may be the initial regulator that responds to nitrogen availability in the environment and is a major point of control for NCR (24).

Mutations in *URE2* result in elevated expression of NCR-regulated genes under repressing conditions (20, 95, 116). Ure2 is proposed to control the nuclear localization of Gln3 by sequestering it in the cytoplasm under repressing conditions (see below). Interestingly, Ure2 has a structural relationship with glutathione *S*-transferase (GST) with a flexible loop in the GST domain that is specific to this class of GST (10, 11, 108) and has a protective role against heavy metal ions and oxidative damage in the cell (81, 84). However, the physiological role of the GST function of Ure2 in nitrogen regulation is not known. In addition, Ure2 has an N-terminal extension that is required for formation of the prion form of Ure2 [URE3] (66), which can render Ure2 inactive. Although recent findings indicate that the prion domain has a stabilizing effect on the Ure2 protein (88), truncated Ure2 lacking the prion domain is able to complement the *ure2Δ* mutation, albeit less efficiently than the full-length protein (67, 92). Moreover, the N-terminal prion domain of Ure2 orthologues and the ability of Ure2 to form [URE3] have diverged within hemiascomycetes (4, 99), suggesting that the prion property of Ure2 is not critical for function in NCR.

Ure2 orthologues with the N-terminal extension and a highly conserved GST domain can be found in hemiascomycetes that also contain Gln3 (Fig. 1) and are able to complement the *ure2Δ* mutant in *S. cerevisiae* (4, 36). It is possible that Ure2 and Gln3 have coevolved in the hemiascomycete lineage. In filamentous ascomycetes and basidiomycetes, there are various putative protein sequences that contain a GST domain and show weak similarity to Ure2 (Fig. 1). However, these sequences lack the N-terminal extension and the loop region unique to Ure2 (10, 108). There is currently no known role for these in nitrogen regulation, and in one case, deletion, while affecting some xenobiotic resistances, did not have any effect on NMR (40).

Early studies suggested that nuclear localization of Gln3 is controlled by phosphorylation. Nuclear import of Gln3 is mediated by the Srp1 importin, which preferentially interacts with dephosphorylated forms of Gln3; phosphorylation of serine residues within the nuclear localization signal of Gln3 abolished Gln3 nuclear localization (16, 53). The TOR (*target of rapamycin*) protein kinase has been proposed to negatively control NCR-regulated genes by preventing Gln3 nuclear localization through phosphorylating Gln3 (5, 7). TOR resides in multiprotein complexes (TorC1 and TorC2) on membrane structures (55, 62, 113). The Tap42-Sit4 phosphatase complex, which is required for Gln3 dephosphorylation (111), has recently been suggested to be inactive due to its association with membrane-bound TorC1 (118). Inhibition of the TOR kinase

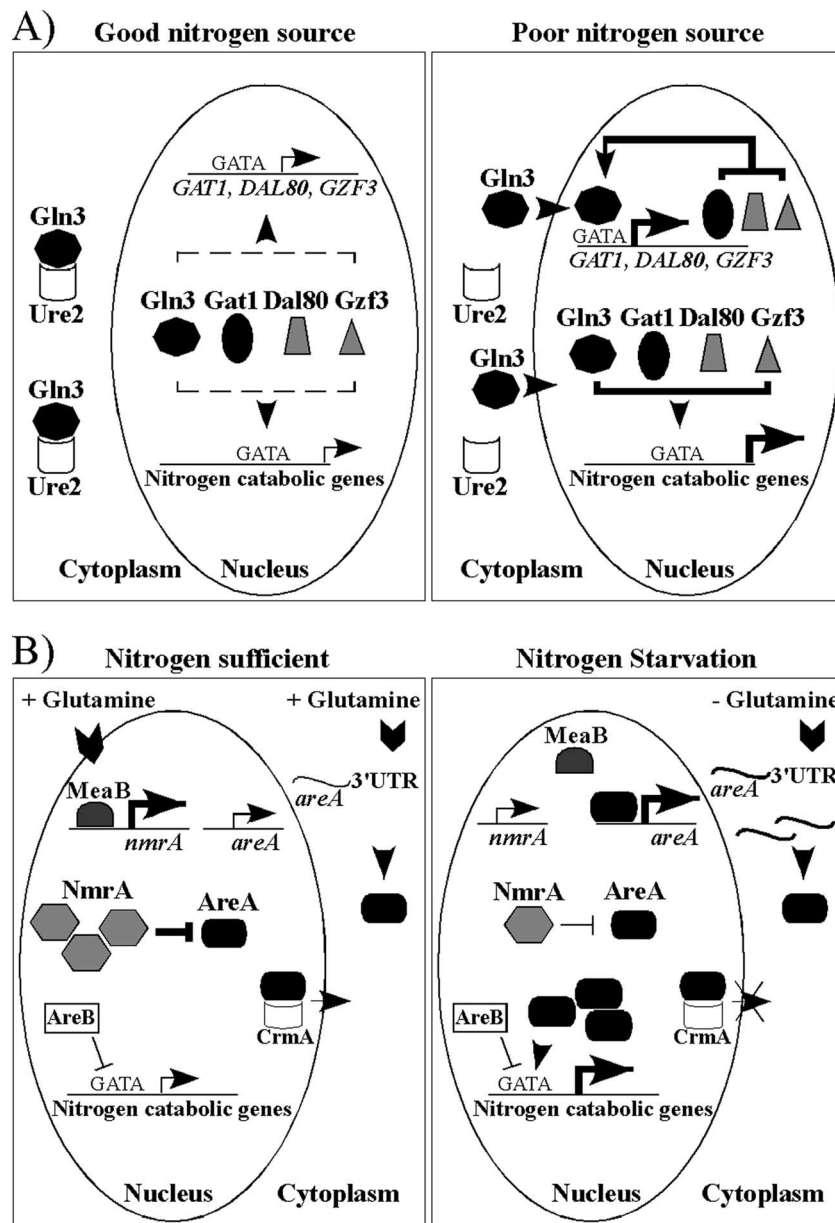


FIG. 2. Nitrogen regulation in ascomycetes. (A) Schematic model of the role of Gln3 in nitrogen catabolic repression in *S. cerevisiae*. During growth in a good nitrogen source, Gln3 is predominantly retained in the cytoplasm by interaction with Ure2 and the levels of Gat1, Dal80, and Gzf3 are low. Consequently, Gln3, Gat1, Dal80, and Gzf3 activities are minimal. On poor nitrogen sources or with nitrogen starvation, Gln3 is released from Ure2 and enters the nucleus. Nuclear Gln3 turns on the expression of NCR-regulated genes including *GAT1*, *DAL80*, and *GZF3*, whose products feed back to influence their own expression and, together with Gln3, modulate gene expression. (B) Schematic model of NMR in *A. nidulans*. In nitrogen-sufficient conditions, AreA is present at a low level as a result of the transcript stability control of *areA* via its 3' UTR. The AreA protein is present in both the cytoplasm and the nucleus, and the expression of *nmrA* is activated by MeaB. The high level of NmrA is sufficient to prevent AreA function. Upon nitrogen starvation, AreA levels are higher as the result of autogenous regulation and increased transcript stability and MeaB-dependent expression of *nmrA* is lost. AreA also accumulates in the nucleus by an unknown mechanism that influences CrmA-mediated nuclear export of AreA. The low level of NmrA is insufficient to inhibit the relatively higher amount of nuclear AreA. Consequently, the activating function of AreA prevails over NmrA inhibition during nitrogen starvation. AreB is proposed to negatively control nitrogen catabolic gene expression by competing with AreA for binding to GATA elements.

by treatment with rapamycin causes dissociation of Tap42-Sit4 complex from TorC1, dephosphorylation, and cytoplasmic-to-nuclear translocation of Gln3 as well as derepression of NCR-regulated genes in the presence of good nitrogen sources—paralleling the responses that are triggered by nitrogen limitation and starvation (5, 7, 8, 15, 50, 88, 118). By using

the glutamine synthetase inhibitor L-methionine sulfoximine (MSX) or a *gln1* mutant that lacks glutamine synthetase activity, it was shown that glutamine or its derivative is the signaling molecule that acts upstream of the TOR pathway for Gln3 localization (29).

The relationship between phosphorylation of Gln3 and Ure2

action is controversial. It has been suggested that Ure2 functions to protect Gln3 from dephosphorylation (7) or that Ure2 preferentially sequesters phosphorylated Gln3 to the cytoplasm (5). Recent findings have raised doubts about the Gln3 phosphorylation/dephosphorylation model as the mechanism for cell responses to nitrogen quality and availability, as the level of Gln3 phosphorylation is not absolutely correlated with its subcellular localization (26, 28, 102). Thus, the nuclear localization of Gln3 in response to rapamycin treatment and growth on poor nitrogen sources may be controlled by different mechanisms inducing short- and long-term responses, respectively. Furthermore, control of nuclear localization of Gln3 by various stresses has been shown to be independent of Ure2 (101). This stress-related control is able to override all known signals for Gln3 nuclear localization and thereby prevent expression of nitrogen catabolic genes in stressful environments.

Compared to Gln3, less is known about the control of Gat1 activity. In addition to transcriptional regulation by Gln3 (see above), Gat1 subcellular localization is also controlled by the quality of nitrogen source (52). Although the situation for Gat1 phosphorylation has been controversial, it appears that the TOR kinase is also involved in modulating Gat1 localization (5, 7, 52). However, nuclear localization of Gln3 and Gat1 appears to be controlled by a different component of the TOR complex (44). Overexpression of *URE2* prevents Gat1 nuclear localization (31), and Ure2 interacts with Gat1 in two-hybrid analysis (92). However, recent studies indicate that cytoplasmic localization of Gat1 is only partially mediated by Ure2 and that an unknown TOR-regulated factor, in addition to Ure2, sequesters Gat1 in the cytoplasm in the presence of a good nitrogen source (43). During nitrogen starvation Gln3 is accumulated in the nucleus, but Gat1 localizes to the nucleus only transiently and it is mostly cytoplasmic at 60 min after nitrogen starvation (52). Glutamine depletion by MSX does not lead to nuclear accumulation of Gat1 (30, 52), which is consistent with the finding that the effect of MSX on the derepression of NCR-regulated genes is mediated by Gln3 alone (30). This may also indicate that endogenous glutamate is sufficient to prevent Gat1 function. Therefore, in addition to the differences in the regulatory mechanisms for the proposed short- and long-term responses with Gln3, it is likely that there are additional pathways for Gat1 regulation in NCR in *S. cerevisiae*.

REGULATION OF AreA ACTIVITY IN *A. nidulans*

Although the core transcription factors involved in nitrogen regulation in *A. nidulans* and *S. cerevisiae* are GATA factors, the regulatory mechanisms controlling their activity are very different. While Gln3 function is controlled primarily at the level of nuclear localization, multiple levels of regulation, including nuclear accumulation in response to nitrogen starvation, exist to modulate AreA activity in response to nitrogen quality and availability in the environment.

Levels of AreA are regulated by nitrogen source via autogenous transcription control of the *areA* promoter (57). The stability of *areA* transcripts is modulated by intracellular levels of glutamine via a 218-bp region in the 3' untranslated region (3' UTR) of the *areA* transcript (70, 71, 78). Deletion of this region leads to an increase in *areA* transcript stability in the

presence of a preferred nitrogen source and, consequently, to partial derepression of various nitrogen catabolic genes (78). An inverted repeat within the regulatory region controlling *areA* transcript stability is also present in the 3' UTR of *areA* homologues in *P. chrysogenum*, *Penicillium roqueforti*, *Penicillium utricae*, and *Aspergillus oryzae* (71, 78, 100). Database searches show that the inverted repeat is also found in the 3' UTR of *areA* homologues from other *Aspergillus* species, and interestingly, only the second half of this sequence is present in the 3' UTR of the *areA* orthologue in *Coccidioides immitis* and *Uncinocarpus reesii*, which are related to *Aspergillus* and *Penicillium* species. However, the sequence is not present in other filamentous ascomycetes including *N. crassa*, and it has been shown that the stability of the *nit-2* mRNA is not regulated (100). Therefore, the regulation of *areA* transcript stability appears to have diverged within the filamentous fungi.

At the protein level, the function of AreA is negatively regulated by the corepressor NmrA. NmrA is highly conserved among all filamentous ascomycetes and is unique to this class of fungi (Fig. 1), and the functions of the orthologues in *N. crassa* (Nmr1) and *Fusarium fujikuroi* (NMR-GF) are also conserved (65, 68). NmrA interacts with the DNA binding domain and the highly conserved C terminus of AreA (2, 78), and mutations in *areA* that disrupt these regions or deletion of the *nmrA* gene leads to partial derepression of nitrogen catabolic genes under nitrogen-sufficient conditions (2, 78, 106). The contributions of differential *areA* transcript stability and NmrA inhibition of AreA to NMR are additive (2, 78, 106), and strains lacking both regulatory elements are fully derepressed (78), suggesting that the activation of genes by AreA is primarily controlled by these two mechanisms. Mutants affecting this repressor (*nmr-1*) were first isolated in *N. crassa*, and mutations in the DNA binding domain and C terminus of Nit2 affect interaction with Nmr1 (75, 80). *nmr-1* mutants, like *nmrA* mutants, are derepressed for a number of nitrogen catabolic genes (65). However, in *N. crassa*, the *nit-2* transcript is subjected neither to autogenous transcription regulation nor to transcript stability control by nitrogen conditions and the stability of Nit2 is not regulated (100). Therefore, Nmr1 may be a more potent inhibitor of Nit2 function than NmrA is in *A. nidulans*.

The mechanism of NmrA inhibition of AreA activity is currently not clear. Unlike Ure2, it does not modulate AreA subcellular localization (106, 115). Therefore, NmrA could either block AreA activation directly or prevent DNA binding. The interaction of NmrA with the DNA binding domain of AreA may imply that NmrA blocks AreA from binding to DNA. The tertiary structure of NmrA conforms to a NAD-binding Rossmann fold, found in a number of other negatively acting transcription regulators like *S. cerevisiae* Gal80 (104), which acts to block the activation domain of Gal4 (58, 59, 91), mammalian C-terminal binding proteins (CtBPs) (54), and the highly conserved Sir2 deacetylase (9). The Rossmann domain of NmrA has been shown to bind NAD and NADH dinucleotides in vitro, with much higher affinity toward NAD (56). The NAD/NADH binding motif is necessary for function in vivo (unpublished data). However, it is at present unclear whether NAD/NADH binding serves a structural role or has a regulatory function as found for CtBPs and Sir2 deacetylase (9, 103, 110, 119). NAD/NADH levels might vary under different ni-

trogen conditions to control NmrA activity. However, recent findings strongly argue against this possibility. Overexpression of NmrA results in loss of AreA activation even under nitrogen-limiting and starvation conditions (56).

Studies of mutants affected in the ammonium assimilation pathway show that the level of glutamine or a derivative is likely to be the signal for NMR in *A. nidulans* (64). Endogenous glutamine levels affect *areA* transcript stability, which together with autogenous control of *areA* impacts on AreA levels (Fig. 2B). The transcript and protein levels of NmrA and of AreA are inversely regulated in response to different nitrogen conditions (115). Therefore, the relative abundance of NmrA and AreA is the important determinant for nitrogen catabolic gene expression.

Recent studies have shown that the conserved bZIP transcription factor MeaB activates *nmrA* expression during nitrogen-sufficient conditions via a highly conserved motif (TTGC ACCAT) in the promoter of *nmrA* (115). This finding reveals a new level of regulation in NMR but at the same time raises the question of how MeaB function is controlled by the nitrogen signal. The levels of *meaB* mRNA and MeaB protein do not vary with the nitrogen source present, and preliminary analysis shows that MeaB is localized to the nucleus under both nitrogen-sufficient and starvation conditions (unpublished data) (115). Remarkably, the basic DNA binding region of MeaB is invariably conserved among all MeaB orthologues of filamentous ascomycetes while a high level of conservation is present in the leucine zipper dimerization motif (115). It is possible that MeaB function is, at least in part, controlled at the level of dimerization. Notably, *meaB* loss-of-function mutants exhibit additional phenotypes that are not consistent with a role solely in *nmrA* regulation (79, 115). Like many other bZIP transcription factors, MeaB may execute different functions when dimerized with other bZIP transcription factors. Endogenous glutamine may promote formation of an activator complex for *nmrA* activation. However, it remains to be shown if *nmrA* expression is mediated by a MeaB homodimer or a heterodimeric complex involving MeaB. This is an important area for further study. The presence of a conserved MeaB orthologue in all filamentous ascomycetes analyzed, including *N. crassa* (Fig. 1), implies that the regulation of *nmrA* expression by MeaB may be a conserved feature of nitrogen regulation in these species.

The expression of some AreA-dependent genes is further up-regulated during complete nitrogen starvation. This nitrogen starvation response correlates with gradual accumulation of AreA in the nucleus (106). Nuclear accumulation of AreA is not a consequence of elevated AreA levels (unpublished data) but is a result of the lack of CrmA-mediated nuclear export of AreA (Fig. 2B) (106). In contrast to *S. cerevisiae*, treatment with rapamycin does not result in nuclear localization of AreA under nitrogen sufficiency (unpublished data), consistent with the finding that the TOR signaling pathway has a minor role, if any, in NMR in *A. nidulans* (38). Despite this, high levels of phosphorylation are found with AreA (114) and may potentially be important in modulating AreA activity. Further study is clearly needed to identify the upstream signaling pathway for NMR in *A. nidulans*.

CONCLUSIONS

Fungi can be found in diverse and often hostile natural habitats, where they are constantly challenged by nutrient availability, and their ability to survive and grow can largely be attributed to their metabolic versatility. Nitrogen source utilization plays an important part in many aspects of fungal biology such as development, secondary metabolite production, and, for some fungi, pathogenesis. This minireview highlights our latest understanding of how the model ascomycetes *S. cerevisiae*, *A. nidulans*, and *N. crassa* deal with the available nitrogen in the environment at the level of transcription and addresses the evolution of nitrogen regulation in the *Ascomycota*.

GATA factors are central to nitrogen regulation in both *S. cerevisiae* and *A. nidulans*, but the regulatory circuits controlling their transcriptional activity have little in common. Nevertheless, both systems ensure appropriate regulated expression of a large set of nitrogen catabolic genes to allow these fungi to respond and adapt to the nitrogen availability in their environment. The common theme of GATA factors at the heart of nitrogen regulation indicates conservation of an ancient evolutionary assignment of transcription factor roles. However, studies thus far suggest that the details of nitrogen regulation are conserved within, but not necessarily between, the filamentous ascomycetes and hemiascomycetes clades. The potential coevolution of Ure2 and Gln3 in hemiascomycetes and the highly conserved NmrA and MeaB orthologues unique to filamentous ascomycetes indicate that regulatory rewiring has occurred after the separation of these two lineages. Much remains to be found out about nitrogen regulation in the well-established systems in *S. cerevisiae*, *A. nidulans*, and *N. crassa*, particularly the nature of the signal(s) and the molecular details of the regulatory interactions. Furthermore, extending these studies to other ascomycetes as well as basidiomycetes, where nothing is known, is certain to provide fascinating new insights into the mechanisms and evolution of nitrogen regulation in fungi.

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