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Abstract
The major nitrogen metabolite repression control genes of Aspergillus nidulans and Neurospora crassa are the areA and nit-2 genes respectively. Both genes encode transcriptional activators with highly conserved C2-X17-C2 zinc finger DNA binding domains which recognize similar WGATAR DNA sequences (Kudla et al., 1990 EMBO J. 9: 1355-1364; Fu and Marzluf, 1990 Proc. Natl. Acad. Sci USA 87: 5331-5335). We have shown previously that the N. crassa nit-2 gene can fully complement an areA loss-of-function mutation in A. nidulans, restoring growth on a wide variety of nitrogen sources. However, the nit-2 transformants are partially derepressed (Davis and Hynes, 1987 Proc. Natl. Acad. Sci. USA 84: 3753-3757).

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Why are nit-2 transformants of Aspergillus nidulans partially derepressed?

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The major nitrogen metabolite repression control genes of Aspergillus nidulans and Neurospora crassa are the areA and nit-2 genes respectively. Both genes encode transcriptional activators with highly conserved C2-X17-C2 zinc finger DNA binding domains which recognize similar WGATAR DNA sequences (Kudla et al., 1990 EMBO J. 9: 1355-1364; Fu and Marzluf, 1990 Proc. Natl. Acad. Sci USA 87: 5331-5335). We have shown previously that the N. crassa nit-2 gene can fully complement an areA loss-of-function mutation in A. nidulans, restoring growth on a wide variety of nitrogen sources. However, the nit-2 transformants are partially derepressed (Davis and Hynes, 1987 Proc. Natl. Acad. Sci. USA 84: 3753-3757).

We have considered a number of possible explanations for the apparent inability of the Nit-2 protein to respond fully to nitrogen control in A. nidulans.

1. One possibility is that the Nit-2 protein is overexpressed in A. nidulans and that elevated levels of Nit-2 lead to derepression. Expression of nit-2 is from the N. crassa promoter and transformants were generated by random integration into the A. nidulans genome. However, no correlation was observed between plasmid copy number and the degree of derepression of individual transformants. In addition, we have generated random multicopy areA transformants and found that these retain nitrogen control. The fact that overexpression of AreA protein does not lead to equivalent derepression also argues against this possibility.

2. A second possibility is that the Nit-2 protein is not interacting efficiently with the product of the A. nidulans homolog of the nmr-1 gene. In N. crassa, there is clear evidence that the Nmr-1 protein is required for repression. nmr-1 mutants are derepressed and the Nit-2 and Nmr-1 proteins have been shown to interact physically via sequences in the DNA-binding region and the C-terminal regions of Nit-2 (Xiao et al., 1995 Biochemistry 34:8861-8868). The nit-2 and areA genes show considerable divergence outside these regions and the overall conformation of the two proteins may differ such that the presumptive A. nidulans Nmr-1 equivalent protein may not interact effectively with Nit-2 to prevent its activity. We have found that this possibility is also not a sufficient explanation for the partial derepression phenotype. We have co-transformed the N. crassa nmr-1 gene, together with the nit-2 gene, into areA mutants of A. nidulans and find no difference between transformants which contain nmr-1 and those that do not contain nmr-1.

3. A third possibility is that the nit-2 clone used in the initial experiments lacks key sequences in the 3’ untranslated region (3’UTR) of the gene. Recent studies (Platt et al., 1996 EMBO J. 14:2791-2801) have shown that the areA gene encodes sequences present in the 3’UTR of the areA mRNA which have a role in determining rates of mRNA turnover. Deletion of these sequences results in a more stable mRNA and a partially derepressed phenotype. The original nit-2 clone used was a 6kb EcoRI clone (pni-2). Inspection of the nit-2 sequence shows an EcoRI site only 170bp downstream from the translation stop codon and hence the 3’ end of the nit-2 gene may have been truncated with respect to these key sequences.
While neither Nit-2 overexpression nor Nit-2/Nmr-1 protein interactions appear to be sufficient explanation for the partial derepression of nit-2 transformants, the possibility of differences in RNA turnover needs further investigation. The primary experiment would be to transform an *A. nidulans areA* loss of function mutant with a longer nit-2 clone. If the results of this experiment support the prediction that such transformants would regain full nitrogen control, a number of important conclusions can be drawn. The results would indicate that *areA* and *nit-2* share this additional level of control. Furthermore, it would help to localise the key sequences as they must lie downstream of the 3’ *EcoRI* site of the *nit-2* gene. Platt *et al.* (1996) identified a region of conserved sequence 3’ to the *A. nidulans areA* gene and the *Penicillium chrysogenum nre* gene. Comparison of this sequence with the 3’ untranslated sequences of *nit-2* suggests there may be a less well conserved sequence (Figure 1) downstream of the *EcoRI* site (nt 4389). Conserved sequences in the 3’ UTR region of these genes may therefore indicate functionally important sites for nitrogen source-regulated turnover of both *areA* and *nit-2* mRNAs.

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**Figure 1.** Alignment of *nit-2* (Nit2UTR) and *areA* (areAUTR) 3’ sequences using Clustal W (Thompson *et al.* 1994 Nucl. Acid Res. 22: 4673-4680) accessed via ANGIS (Australian National Genome Information Service).