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 depression 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 *Eco*RI clone (pnit-2). Inspection of the *nit-2* sequence shows an *Eco*RI 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' *Eco*RI 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 *Eco*RI 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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Nit2UTR   4454 TGCTTTTTACTCTGGCCTTTTTAAACGGTACGGCTGTTGAAGCTGTCAAGTTTCGA
areAUTR   3035 TGTGCTTGACT-TGTGATTTTTGCTTGATACAACCGC---GTCTGTCCGGTACCTG
               **   ** *** **   *****    * ***  * *      *****  **  *

Nit2UTR        AGGACAGCTTTTCTAAACATTTCTCCCCCGCACGTCTTATTTCCTGGTACACAAAG
areAUTR        GCCTTCGTCTGTCCGG-TACCTGGCCTTCGTCTGCTTTTTTTGTTTGTGTTTCATG
                     *  * **     *  *  **  **   *  ** ***  * **     * *

Nit2UTR        CGTTGATAGAAGAAGGAGCCGGGCTGATGAGGATGGGAAGAGGGGCCCAGGAG
areAUTR        TGTT-ATGTTTGTTGTTGCATTCGAGGTCAGGCCGGGCAGAAGTGCGCATCTC
                *** **    *  *  **      * * ***  *** *** * ** **
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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).

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