Functional analysis of TamA, a coactivator of nitrogen-regulated gene expression in *Aspergillus nidulans*

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Abstract The *tamA* gene of *Aspergillus nidulans* encodes a 739-amino acid protein with similarity to Uga35p/Dal81p/DurLp of *Saccharomyces cerevisiae*. It has been proposed that TamA functions as a co-activator of AreA, the major nitrogen regulatory protein in *A. nidulans*. Because AreA functions as a transcriptional activator under nitrogen-limiting conditions, we investigated whether TamA was also present in the nucleus. We found that a GFP-TamA fusion protein was predominantly localised to the nucleus in the presence and absence of ammonium, and that AreA was not required for this distribution. As the predicted DNA-binding domain of TamA is not essential for function, we have used a number of approaches to further define functionally important regions. We have cloned the *tamA* gene of *A. oryzae* and compared its functional and sequence characteristics with those of *A. nidulans tamA* and *S. cerevisiae UGA35/DAL81/DURL*. The *Aspergillus* homologues are highly conserved and functionally interchangeable, whereas the *S. cerevisiae* gene does not complement a *tamA* mutant when expressed in *A. nidulans*. Uga35p/Dal81p/DurLp was also found to be unable to recruit AreA. The sequence changes in a number of *tamA* mutant alleles were determined, and altered versions of TamA were tested for *tamA* complementation and interaction with AreA. Changes in most regions of TamA appeared to destroy its function, suggesting that the overall conformation of the protein may be critical for its activity.

Keywords Nitrogen · Gene regulation · areA · UGA35/DAL81/DURL · Aspergillus oryzae · Filamentous fungi

Introduction

In the filamentous fungi *Aspergillus nidulans* and *Neurospora crassa*, the catabolism of complex nitrogen sources is tightly controlled. The transcription of many genes encoding enzymes required for nitrogen source utilization is activated only under nitrogen-limiting conditions, and in many cases, subject to substrate availability. The response to nitrogen limitation is mediated by a positively acting transcription factor, AreA in *A. nidulans* or NIT2 in *N. crassa*, which binds via a single GATA-type zinc finger to the promoters of regulated genes to activate their expression under nitrogen-limiting conditions (Fu and Marzluf 1990; Kudla et al. 1990). Loss-of function *areA* or *nit-2* mutants are unable to grow on most nitrogen sources other than ammonium, and the functional similarity of AreA and NIT-2 is confirmed by the ability of *N. crassa nit-2* to complement an *A. nidulans areA* mutant (Arst and Cove 1973; Hynes 1975; Marzluf 1981; Davis and Hynes 1987). Homologues of *areA* and *nit-2* have been identified in a number of filamentous fungi (Haas et al. 1995; Froelinger and Carpenter 1996; Christensen et al. 1998; Tudzynski et al. 1999), and in the budding yeast *Saccharomyces cerevisiae* two positively acting GATA transcription factors, Gln3p and Nil1p/Gat1p, have been identified as regulators of gene expression under nitrogen-limiting conditions. Gln3p and Nilp/Gat1p have overlapping but not identical DNA binding specificities, and the expression of different structural genes may be more or less dependent on one of these factors (Minehart and Magasanik 1991; Coffman et al. 1996; Stanborough and Magasanik 1996).

A role for the *tamA* gene in nitrogen regulation in *A. nidulans* was previously indicated by the phenotype of *tamA* mutants, which show resistance to a number of toxic nitrogen source analogues and reduced levels of a number of nitrogen catabolic enzymes. *tamA* mutants also express low levels of the major nitrogen assimilatory enzyme, NADP-glutamate dehydrogenase and grow more poorly on ammonium than wild-type strains...
(Kinghorn and Pateman 1975; Davis et al. 1996). We have proposed that the product of the 

tamA gene of 

_A. nidulans_ functions as a co-activator of nitrogen-regulated transcription. TamA fused to the DNA-binding domain of either of the regulatory proteins FacB and AmdR was found to recruit ArcA to activate expression of FacB- and AmdR-regulated genes, respectively (Small et al. 1999). A co-activator of Gln3p and Nil1p/Gat1p has recently been identified in a genetic screen for mutants showing poorer growth on a number of different nitrogen sources. The _GAI1_ gene was found to encode a component of the Adalp/Gen5p transcriptional co-activator complex (Soussi-Boudekou and Andre 1999). Despite some phenotypic similarities to the _gai1_ mutant, sequence comparisons have shown that _tamA_ is more similar to the _UGA35/DAL81/DURL_ gene of _S. cerevisiae_ (Bricmont et al. 1991; Davis et al. 1996). _uga35_ mutants also exhibit reduced expression of a number of genes involved in nitrogen catabolism (Yoo et al. 1985; Gembauke and Cooper 1986; Vissers et al. 1990), and both _TamA_ and _Uga35p/Dal81p/DurLp_ contain a Zn(II)_2-

Cys_6_ cluster-type DNA-binding motif that is not essential for known functions. Both proteins also contain a region similar to the central domain identified in a number of _Zn(II)_2-

Cys_6_ cluster proteins (Chasman and Kornberg 1990; Schjerling and Holmberg 1996). The function of the central domain is unclear, although certain mutations within this region have been shown to result in proteins with altered activation capacity (Stone and Sadowski 1993; des Etages et al. 1996; Ding and Johnston 1997). Therefore, _TamA_ and _Uga35p/Dal81p/DurLp_ may have similar functions in the regulation of nitrogen source utilization in their respective species.

To further investigate the function of _TamA_, we determined the molecular basis of a number of _tamA_ mutations and investigated the ability of various mutant _TamA_ proteins to recruit _ArcA_ when fused to the DNA-binding domain of AmdR. We found that changes other than in the N-terminal and extreme C-terminal segments of _TamA_ interfere with both _tamA_ complementation and _ArcA_ recruitment functions. We have also cloned and sequenced the _A. oryzae tamA_ homologue and found that it is highly conserved relative to _A. nidulans tamA_. In contrast, the _S. cerevisiae UGA35/DAL81/DURL_ gene is more diverged and does not complement _A. nidulans tamA_ mutations, or function in an _ArcA_ recruitment assay when expressed in _A. nidulans_. The proposed coactivator function of _TamA_ suggests that it may act in the nucleus. We have found that Green Fluorescent Protein (GFP)-tagged _TamA_ expressed in _A. nidulans_ is capable of _ArcA_-independent entry into the nucleus under nitrogen-sufficient and nitrogen-limiting conditions.

**Materials and methods**

_Asperrillus_ strains, media, and growth conditions

_Asperrillus_ media and growth conditions were as described by Cove (1966). Nitrogen sources were added at a final concentration of 10 mM and carbon sources at 1% (w/v). Genetic manipulations were carried out using techniques described by Clutterbuck (1974). The _A. nidulans_ strains used in this study are listed in Table 1. Gene symbols have been described previously (Clutterbuck 1974).

Selection of _tamA_ mutants

_tamA_ mutants were isolated by nitrosoguanidine (NTG) mutagenesis of MH54 ( _tamA10_), MH288 ( _tamA24_ and _MH295_ ( _tamA21_)) strains (Polkinghorne 1979) followed by selection for stronger growth on 10 mM histidine. MH288 and MH295 carry the _arcA102_ mutation and the _sarA_ or _sarA10_ mutations, respectively. _sarA_ mutations are suppressors of _arcA102_ and reduce growth on histidine and various other nitrogen sources (Polkinghorne and Hynes 1975). _tamA_ mutations enable increased growth on histidine to occur in this background.

**Aspergillus** transformation and enzyme assays

_A. nidulans_ strains were transformed according to the method of Andrianopoulos and Hynes (1988). Transformants from cotransformation experiments were selected using the _ribob_’’ selectable marker plasmid pPL3 (Oakley et al. 1987) on media lacking riboflavin. Southern analysis was used to confirm that assayed cotransformants contained the plasmids of interest. No effects of copy number on phenotypes were observed. _β-Galactosidase_ assays of protein extracts from _A. nidulans_ were performed as described in Davis et al. (1988).

Single-strand conformation polymorphism analysis and sequencing of _tamA_ mutants

Single-strand conformation polymorphism (SSCP) analysis was based on methods used by Orita et al. (1989). Fragments of _tamA_ were amplified from genomic DNA using TAM3 (5’-TCCGCA-GTCGCCCAACCTG-3’) and TAM4 (5’-TGTTTAGACGGG-ACATGATG-3’), TAM5 (5’-GACTATCTGACTGTATCT-GCC-3’) and TAM6 (5’-CTGTTCTGACGCTGCGCCG-3’) in the presence of 60 µCi of [α-32P]dATP, and digested with appropriate restriction enzymes. Digested samples were then denatured and electrophoresed on a 5% acrylamide gel at constant power (3 W). Gels were fixed in 10% acetic acid, 20% methanol and dried before being exposed to film at −70°C. For fragments where mutation caused a difference in DNA conformation compared to wild type, at least two PCR fragments from each of two independent PCRs were cloned into the pGEM-T vector (Promega). DNA was prepared using the High Pure Plasmid kit (Boehringer) and sequenced using the PRISM Dye Primer Cycle Sequencing Ready Reaction Kit (Applied Biosystems) or by the Australian Genome Research Facility.

Cloning of altered _tamA_ constructs

Constructs containing 3’ truncations of the _tamA_ coding region were made by deleting as far as the _XhoI_ (pMD3430), _XbaI_ Table 1 _A. nidulans_ strains used in the present study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
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<tbody>
<tr>
<td>MH54</td>
<td>biA1, nilA4</td>
</tr>
<tr>
<td>MH6121</td>
<td>biA1, arcA102, tamA24, riboB2</td>
</tr>
<tr>
<td>MH6209</td>
<td>biA1, amdR44, jacB::BleR, amdS::lacZ, riboB2, prn309</td>
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<td>MH8272</td>
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<td>MH288</td>
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</tr>
<tr>
<td>MH295</td>
<td>biA1, puA2, arcA102, sarA10</td>
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