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Induction of *jlbA* mRNA synthesis for a putative bZIP protein of *Aspergillus nidulans* by amino acid starvation

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Abstract The *jlbA* (*j*un-*l*ike *b*ZIP) gene of *Aspergillus nidulans* was isolated. The deduced amino acid motif of the C-terminal region of *jlbA* encodes a putative DNA-binding site composed of a basic amino acid domain and an adjacent leucine zipper motif. This region shares highest similarities to the C-terminal DNA-binding domain and the basic zipper (bZIP)-motifs of transcription factors like CPCA from *A. niger*, Gcn4p from *Saccharomyces cerevisiae*, human JUNB and c-JUN. The putative *jlbA* protein contains a PEST-rich region (an instability region rich in the amino acids proline, glutamic acid, serine and threonine) described to be implicated in protein stability. The *jlbA* mRNA formation is elevated up to 40-fold upon amino acid starvation induced by the addition of the false feedback inhibitor 3-amino-1,2,4-triazole. This induction is partially dependent and partially independent on the presence of the transcription factor CPCA. Therefore *jlbA* is a novel gene of *A. nidulans* which is transcriptionally activated by amino acid starvation conditions.

Keywords Transcription factor · *jun*-like-bZIP protein · Amino acid starvation · *Aspergillus*

Introduction

The "cross-pathway control" (cpc) of the filamentous fungi *Aspergillus nidulans* (Sachs 1996) and *Neurospora crassa* (Carsiotis and Jones 1974; Carsiotis et al. 1974; Paluh et al. 1988) and the "general control of amino acid biosynthesis" (gc) of the budding yeast *Saccharomyces cerevisiae* (Hinnebusch 1988, 1992) are equivalent regulatory networks that act upon amino acid starvation. Activated by starvation for at least one single amino acid, this control system finally leads to an increased synthesis of a transcriptional activator protein which binds to specific DNA sequence elements present in the upstream regions of more than 50 biosynthetic target genes (Hinnebusch 1985; Arndt and Fink 1986). The expression of the yeast transcription factor *GCN4* is mainly regulated at the translational level. The primary signal for *GCN4* activation seem to be uncharged t-RNAs which are recognised by the bi-functional Gcn2p protein located at ribosomes. Activation of this sensor kinase leads to increased translation of the final effector protein Gcn4p (Lopinski et al. 2000). The complete signal transduction pathway from Gcn2p to the *GCN4* translational control requires more than 12 genes of the gc network. Genes of this network are either *GCN* (gc non-derepressible) or *GCD* (gc derepressible). Finally Gcn4p and its homologue CPCA from *A. nidulans* and *A. niger* act as a transcriptional activator and increase the expression of more than 50 target genes.

The *CpcA* protein of *A. nidulans* and *A. niger*, its yeast homologue Gcn4p and the eukaryotic transcription factors JUNB and c-JUN are members of the basic zipper (bZIP)-type proteins (Landschulz et al. 1988; Vinson et al. 1989; Hurst 1994), a subgroup of the AP-1 family of proteins. bZIP-type proteins are characterised by a DNA-binding domain which interacts with the target sequences via basic amino acid residues (Ellenberger et al. 1992) and an adjacent C-terminal leucine zipper motif at the end of the protein, which functions in the dimerisation of two bZIP-monomers. The leucine zipper motif is characterised by a helical heptad repeat of 3–5 leucine residues (Landschulz et al. 1988), but some unusual amino acids have also been described in the zipper motif (Wanke et al. 1997).

Beside the bZIP-domain, two distinct activation regions [a central acidic activation domain (CAAD) and a N-terminal activation domain (NTAD)] are often present in the N-terminal part of these transcription factors. Additionally in the yeast Gcn4p, an instability region rich in the amino acids proline (P), glutamic acid (E),

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serine (S) and threonine (T), the PEST region, has been described (Kornitzer et al. 1994). This region overlaps the two activation domains and is proposed to function in the regulation of protein stability and proteolytic degradation.

All *jun*-like bZIP transcription factors seem to be part of a general cellular protection system against damage and/or harmful environmental influences or stress conditions in general. For example, ultraviolet (UV) irradiation activates the transcription of the yeast *GCN4* and the mammalian *c-JUN*, and resistance to UV irradiation is correlated to the level of Gcn4p in yeast (Zimmermann et al. 1999). Expression of *JunD*, *JunB*, *c-Jun* and *Fos* are also involved in cell-death processes (Woodgate et al. 1999).

Here we present an additional member of the bZIP-type protein family from *A. nidulans* of unknown function, which is transcribed in response to amino acid starvation. The deduced amino acid sequence of *jlbA* codes for a protein containing a basic amino acid domain and a leucine zipper motif. Beside this DNA-binding domain, an adjacent PEST region is present. Comparison of the *jlbA* protein sequence shows highest homology to other eukaryotic bZIP proteins, like JUNB from the fish *Cyprinus carpio*. The mRNA expression of *jlbA* is strongly induced by the addition of 3-amino-1,2,4-triazole (3AT) to the growth medium and induces an increase (up to 40-fold) of the *jlbA* mRNA level.

Materials and methods

Strains and media

A. nidulans strain A234 (*yA2, pabaA1; veA1*) was provided from the Fungal Genetic Stock Center (FGSC, University of Kansas, USA). Strain GR5 (*wA3; pyrG89; pyroA4; veA1*) was obtained from G. May (Houston, USA). Strains AGB10 (*pyrG89; pyroA4*) (Eckert et al. 2000) and AGB13 (*wA3; pyrG89; pyroA4*) are descendants from a cross between GR5 (phle resistance) and FGSC A4 (Eckert et al. 1999). Mutant strain AGB51 (*yA2, pabaA1; veA1; cpcAΔ::phle^R*) is a derivative from A234 (Hoffmann and Braus, unpublished results). A chromosome-specific recombinant DNA library from *A. nidulans* (Brody et al. 1991) was obtained from the FGSC.

All strains were grown in minimal liquid medium or plated on minimal medium (Bennett and Lasure 1991) supplemented as described by Käfer (1977). Amino acid starvation in growth media was induced as follows. Strains were grown in minimal liquid medium for 24 h at 30 °C. Mycelium was shifted to fresh medium and incubated for another 2 h, to a certain logarithmic growth. Mycelium was harvested again and distributed equally into fresh minimal medium without and with 10 mM 3AT. Cultures were then incubated for 2 h, 4 h and 8 h, respectively. Finally, mycelium was harvested, frozen in liquid nitrogen and stored at –80 °C until further use.

Recombinant DNA techniques

Transformation of *A. nidulans* was performed according to Punt and van den Hondel (1992). Transformants were selected on minimal medium containing 20 μg phleomycin/ml and were screened, using three independent primers in parallel PCR experiments.

DNA sequencing of chromosomal and cDNA was performed by automated, dye-labeled terminator DNA sequencing (Rosenblum et al. 1997), using an ABI 310 sequencer (PE Applied Biosystems, Weiterstadt, Germany) and custom oligonucleotides (Gibco BRL, Eggenstein-Leopoldshafen, Germany). Sequencing of the coding and non-coding strand between positions –250 and +1,000 (see Fig. 1B) was performed using 16 independent primers and was repeated at least twice each for functional analysis. Commercial sequencing was performed by MWG Biotech (Ebersberg, Germany).

Standard enzyme-restriction analysis and Southern blot analysis were performed as described by Sambrook et al. (1989). For Northern blot analysis, total RNA was isolated from harvested mycelium, using the TRIzol reagent (Gibco BRL, Karlsruhe, Germany). Total RNA (20 μg) was separated on an agarose/formaldehyde gel, electroblotted onto a membrane filter (Biodyne B; Pall, Portsmouth, UK) and hybridised with ^{32}P-radiolabelled DNA (Feinberg and Vogelstein 1984). Radiolabelled signals were quantified with a IPR 1000 bio-imaging analyser (Fuji Photo Film Co., Tokyo, Japan). A 0.16–1.77-kb RNA-ladder (Gibco BRL, Karlsruhe, Germany) was used as size standard. The 5′ rapid amplification of cDNA ends (5′RACE) was performed using a 5′/3′RACE kit, according to the distributor's manual (Boehringer, Mannheim, Germany). *jlbA*-cDNA was synthesised from total RNA by reverse transcription, using the gene specific primer junD (5′-CCCGACGAAGAAGCAGGATCC-3′). After mRNA degradation by RNase H activity, single stranded cDNA was tailed, using terminal transferase in the presence of dATP and TdT. Finally, the 5′ end of the *jlbA* transcript was amplified by PCR, with the oligo dT-Anchor primer supplemented by the distributor and a gene specific primer SP3 (5′-GAGTTCGGGTCGAGGTCAACGGG-3′). A distinct amplicon of about 400 bp was isolated, cloned into pBSK and sequenced. This procedure was repeated twice, but the length of the amplicons did not change.

Results

Identification of a *jun*-like bZIP encoding gene in *A. nidulans*

The *cpcA* gene of *A. niger* encodes the bZIP transcription factor, which is required for the activation of amino acid biosynthetic genes (Wanke et al. 1997). Similar genes had not yet been described in *A. nidulans*. To identify such proteins, we screened partially digested DNA from *A. nidulans* in a heterologous Southern hybridisation experiment. Radiolabelled *cpcA* from *A. niger* was used as probe. A 3.0-kb genomic *Bam*HI DNA fragment of *A. nidulans* was detected and subcloned into pBluescript (pBSK). The DNA sequence was determined and revealed an open reading frame which includes a conserved region in the 5′ end of the cloned DNA. This conserved part of the gene encodes a putative DNA-binding site composed of a basic amino acid-rich domain and an adjacent leucine zipper motif. Upstream of the DNA-binding site, a putative instability region (PEST region) is localised. Since the cloned fragment did not include the 5′ region of the cloned gene, a second 2.1-kb DNA fragment containing the upstream DNA region was cloned, using *Eco*RI/*Xho*I-digested genomic *A. nidulans* DNA (Fig. 1A). The putative DNA-binding site and the adjacent leucine zipper show highest similarities with JUN-proteins, suggesting that this gene corresponds to a *jun* homologue. Therefore, this gene was designated *jlbA* (*jun-l*ike-*b*ZIP). The GeneBank accession number assigned to this sequence is AF361222.

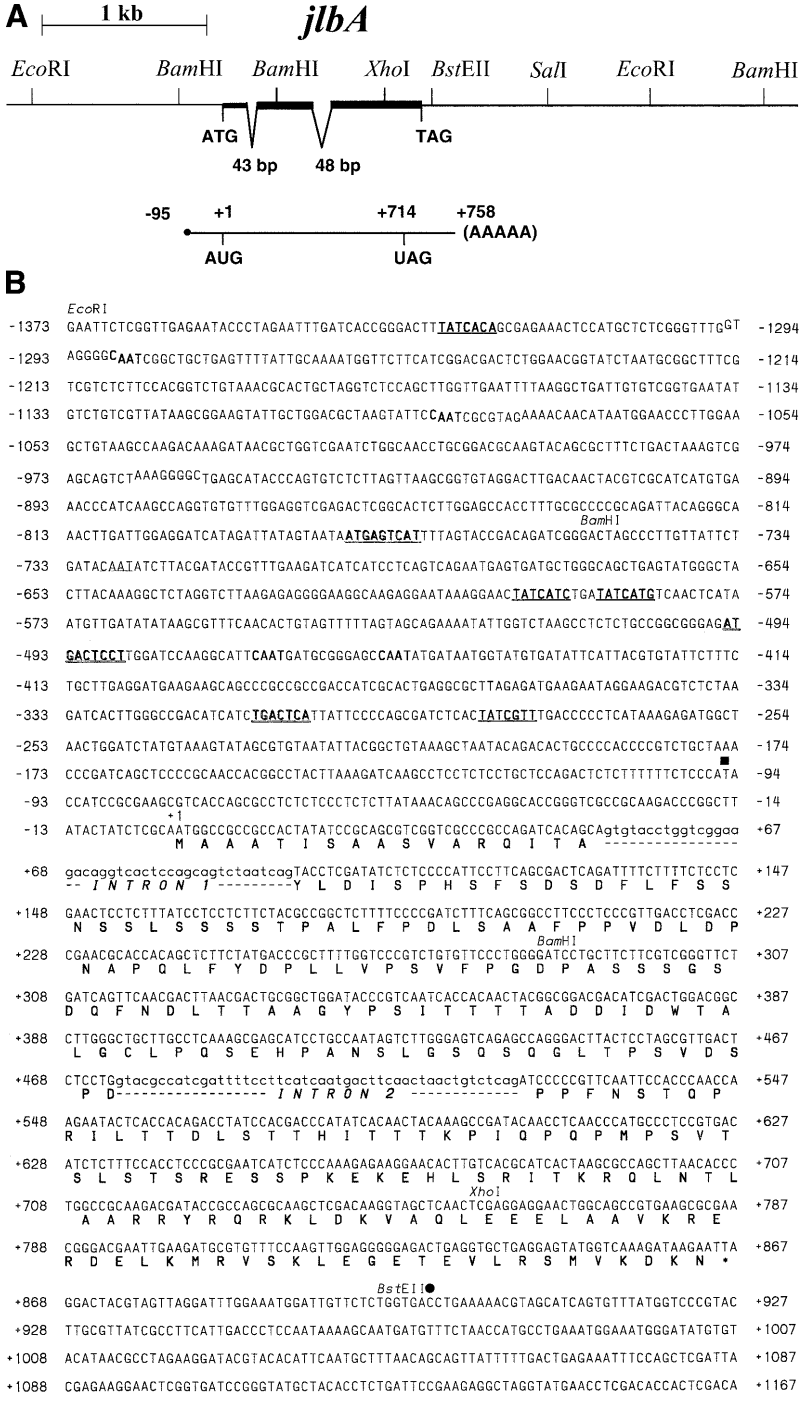

Fig. 1 A,B The *jlbA* gene of *Aspergillus nidulans*. **A** Schematic view of the chromosomal gene locus of the *j*un-*l*ike-*b*ZIP gene, *jlbA*, of *A. nidulans*. Two introns, of 43 bp and 48 bp, are indicated. *Below*, a schematic draw of the *jlbA* mRNA is shown. A transcriptional start site was identified at –95. Additionally, translational start and stop sites and the position of the poly(A)tail are given. **B** DNA sequence and deduced amino acid sequence of *jlbA* of *A. nidulans*. Putative CT boxes are in *bold*, putative boxes homologous to the NIT2p-binding site are in *bold and underlined*, putative GCRE-binding sites are in *bold, underlined and shaded*. Two putative stress response elements are given in *uppercase letters*, a putative StuAp-binding site is given in *lowercase letters*. The major transcriptional start site of *jlbA* is headed by a *black square*, the identified poly(A)-site is headed by a *dot*. Restriction sites used for cloning of the chromosomal DNA and referred to in the text are indicated. The amino acid sequence of a putative JLBA protein is indicated in *bold* and is interrupted by two marked introns

The *jlbA* gene encodes a transcript with two putative intervening sequences

In a Northern hybridisation experiment, *jlbA* mRNA was characterised using total RNA and poly(A)-enriched RNA, respectively. The length of the *jlbA* mRNA was determined as about 1.0 kb, using a *Bam*HI / *Bst*EII DNA fragment of the conserved region as probe (Fig. 2A). To determine the precise 5′ end of the *jlbA* mRNA, a 5′RACE was performed. A cDNA was synthesised, isolated, cloned into pBSK and sequenced. This experiment identified a thymidine at –95 as the major start point of *jlbA* transcription (Fig. 1B).

The first ATG codon is located 95 nt downstream of this transcriptional start site. The DNA sequence suggests an open reading frame of 714 bp, which is interrupted by two introns (Fig. 1A, B). Starting with a commonly used GTG (Unkles 1992) at +52, the first intron includes 43 bp, whereas the second intron (at position +474) includes 48 bp. The two GTCs at positions +58 and +73 are also possible intron start sites, resulting in introns with lengths of 34 bp and 22 bp,

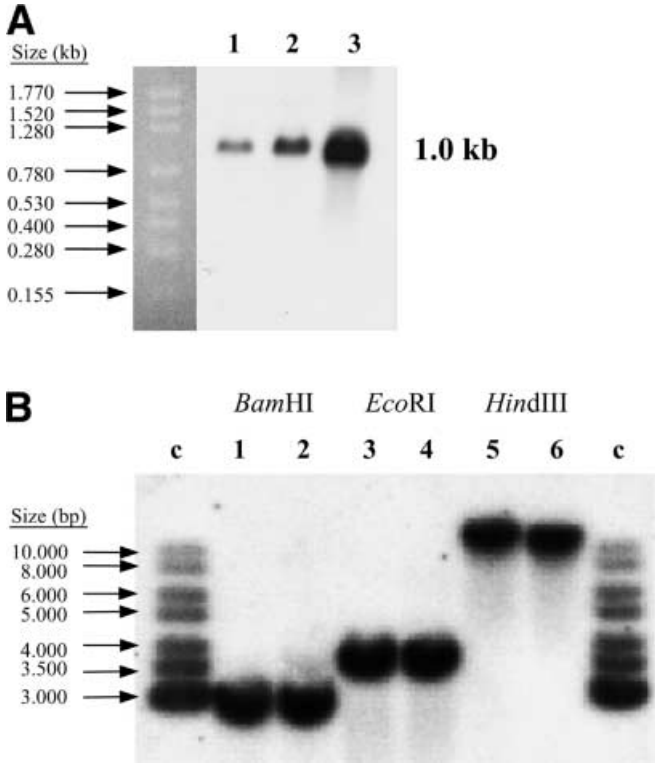

Fig. 2 A,B Transcript length and Southern blot analysis of the *jlbA* gene. **A** In a Northern blot analysis, different amounts of *jlbA* total RNA (*lanes 1, 2*) and poly(A) enriched RNA (*lane 3*) of *A. nidulans* were separated on an agarose/formaldehyde gel and hybridised with a *Bam*HI/*Bst*EII chromosomal DNA fragment containing the 3′ conserved region of the *jlbA* gene. RNA size markers (in nucleotides) are indicated *on the left*. The mRNA transcript length was determined to about 1.0 kb. **B** Chromosomal copy number of *jlbA* was determined in a Southern blot analysis, using DNA of the related *A. nidulans* strains A234 (*lanes 1, 3, 5*) and GR5 (*lanes 2, 4, 6*). DNA was digested with *Bam*HI, *Eco*RI and *Hin*dIII, respectively and hybridised using a 600-bp *Bam*HI/*Bst*EII fragment of the gene. Size of the DNA marker (*lane c*) is indicated *on the left*

respectively. The usage of these splice sites seem to be unlikely, because introns smaller than 36 bp have not been described yet (Unkes 1992).

In silico analysis of the promoter region of the gene revealed five putative CT-boxes (5′-CAAT-3′) at nucleotide positions –1,288, –1,090, –729, –471 and –456, respectively (Fig. 1B). CT boxes have been demonstrated to function in transcriptional start-site selection (Punt et al. 1990) as well as transcription initiation (Adams and Timberlake 1990; Unkles 1992). Three putative *C*pcA *p*rotein *r*ecognition *e*lements (CPREs) at nucleotide positions –780, –495 and –321, respectively, are also present (Fig. 1B). These sequence elements share high identity with the 5′-(A)TGA(G/C)TCA(T)-3′ consensus sequence found for the CpcA homologue protein Gcn4 of the yeast *S. cerevisiae* (Arndt and Fink 1986; Hollenbeck and Oakley 2000). Other transcription factor-binding sites are also present within the *jlbA* promoter. Four sites homologous to the target of the positive-acting NIT2p from *N. crassa* and *A. nidulans* (Fu and Marzluf 1990) are present at positions –284, –590, –600 and –1,329, respectively. Two sequences similar to the binding sites of the stress-response elements (STRE; Schueller et al. 1994) are located at positions –965 and –1,293. Beside these, one StuAp-specific binding site is present at pos. –1,089 (Dutton et al. 1997).

To identify the exact 3′ end of the *jlbA* mRNA, we screened for a *jlbA* cDNA. An inducible cDNA expression library of *A. nidulans* (Krappmann et al. 1999) was screened by colony hybridisation. A 600-bp *Bam*HI/*Bst*EII radiolabelled fragment from the conserved region of the gene was used as a probe. We isolated and sequenced a shortened cDNA clone. Comparison of genomic and cDNA sequence revealed the second intron to be located as postulated. The exact 3′ end of the *jlbA* mRNA and a poly(A)-tail was identified at +911, 41 bp downstream of the translational stop TAG (Fig. 1B). Conserved poly(A)-signals, as described for other genes from *A. nidulans* (Sienko and Paszewski 1999), are not present in the identified 3′ sequences of *jlbA*.

The *jlbA* protein contains a DNA-binding region that shares similarities with other proteins with a bZIP DNA-binding motif

The deduced amino acid sequence of JLBA encodes for a bZIP-type protein of 238 amino acids, with a calculated molecular mass of approximately 20 kDa (Fig. 3A). Comparison of the deduced amino acid sequence to other proteins revealed identities only in the C-terminal part. JLBA shares similarities with the bZIP-type family of transcription factors, like Gcn4 from *S. cerevisiae*, CPCA from *A. nidulans* and the JUN protein from vertebrates. JunB of the fish *C. carpio* was identified as the most related protein (AdvancedBlast at NCBI). The conserved region of the *jlbA* gene codes for a basic DNA-binding domain and an adjacent C-terminal leucine zipper motif with four leucines and one valine. The identities in this part of the protein are small when compared to the eukaryotic JUNB from the fish *C. carpio* (31.9%) or human (28.4%), but similarities are higher (51.3% and 52.6%, respectively). Compared to some other transcriptional activator proteins (Fig. 3B) the identities in the conserved part of JLBA range from 27.1% (c-JUN) to 37.3% (Gcn4p). The proposed N-terminus of JLBA shares no homology to any other proteins described so far.

The basic amino acid-rich region of jlbAp, which probably functions in DNA-binding and recognition, is conserved and shares a typical KR-NT-AAR-RK amino acid motif. This part of the protein contains only one atypical Asp at the end of the domain (D197, Fig. 3A, B). The adjacent C-terminal zipper motif allows the dimerisation of two proteins. Zipper motifs are characterised by at least three repeats of seven amino acids (designated *a–g*) forming an α-helical coil with the *d* positions often occupied by leucines (Landschulz et al. 1988). JLBA is conserved in all *d* positions sharing hydrophobic amino acid residues (e.g. leucines) beside the

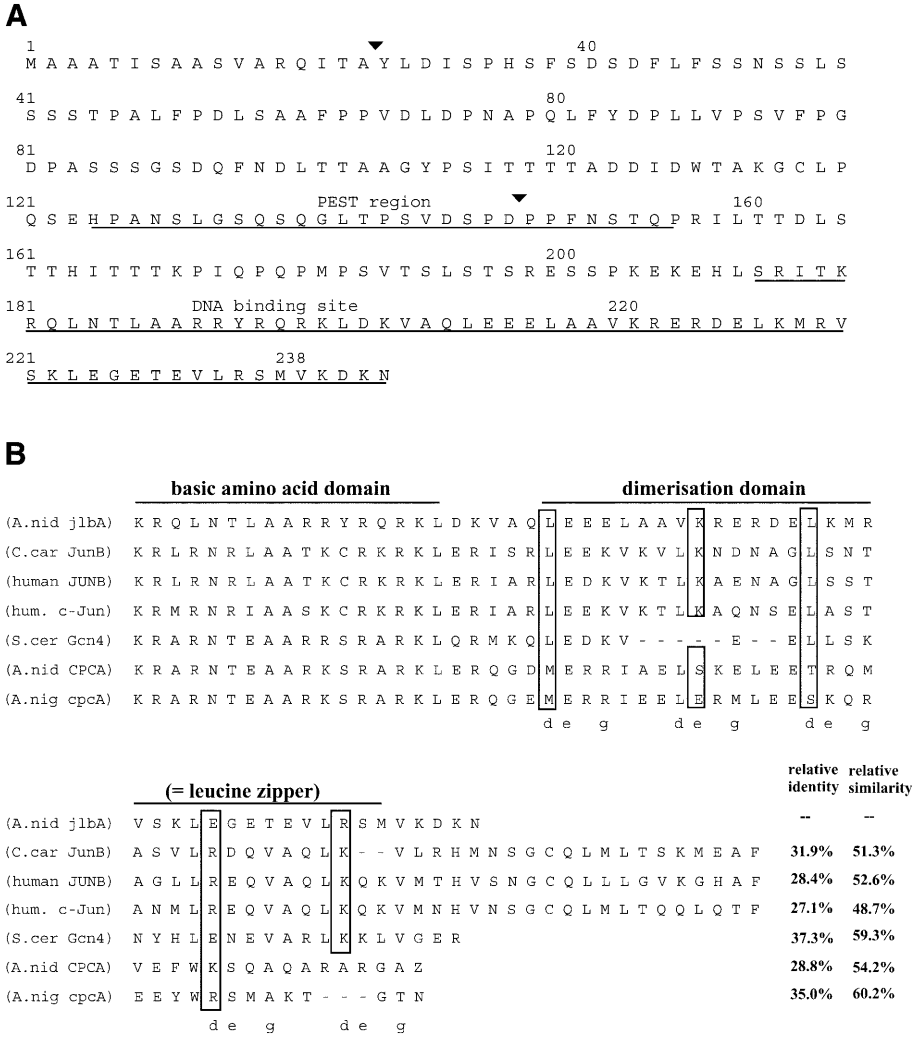

Fig. 3 A,B Putative amino acid sequence of JLBA. **A** Schematic view of the putative amino acid sequence of JLBA and its functional domains. *Numbers above* represent the numbers of the deduced JLBA protein. *Triangles* mark the positions of two introns referred to in the text. Amino acid sequence of an identified PEST region is given in *bold*. Conserved amino acids of the DNA-binding site are also given in *bold*. **B** The lower scheme shows a partial comparison of the JLBA DNA-binding domain to other related DNA-binding domains. Above the sequence, the identified subdomains of the DNA-binding site are headed by *lines*. The conserved positions of the basic amino acid domain and the dimerisation domain are marked in *bold*. Below the sequence, the conserved *d*, *e* and *g* positions of the leucine zipper motif are indicated in *lowercase*. Partial amino acids are given from *A. nidulans* (*A. nid*), *Cyprinus carpio* (*C. car*), human (*human*, *hum.*), *Saccharomyces cerevisiae* (*S. cer*) and *A. niger* (*A. nig*)

second *d*, which is occupied by a valine. The *e* and *g* positions of zipper motifs are characterised by charged amino acid residues (Alber 1992), just as in the deduced leucine zipper motif of JLBA.

PEST regions have been described to function in rapid protein turnover and degradation (Kornitzer et al. 1994; Salama et al. 1994; Roth et al. 1998) and in protein–protein interactions (Chu et al. 1996). A putative PEST region of JLBA, rich in the amino acids proline, glutamic acid, serine and threonine, could be identified using PESTfind (EMBnet, Austria). The identified region has a PESTfind score of +6.12. Values bigger than +5.0 mark real PEST regions (Rogers et al. 1986; Rechsteiner and Rogers 1996).

Neither a CAAD nor a NTAD, as described for the proteins CPCA (Wanke et al. 1997) and Gcn4p (Hope and Struhl 1986; Hope et al. 1988), is present in JLBA.

jlbA is a single-copy gene and is localised on chromosome VII

To determine the copy number of the *jlbA* gene, chromosomal DNAs from *A. nidulans* strain A234 and the related background of strain GR5 were digested with *Bam*HI, *Eco*RI and *Hin*dIII, respectively. In Southern hybridisation analysis, the DNAs were hybridised using a *jlbA* gene fragment as radiolabelled probe. This resulted in a single band for each restriction and demonstrated that the *jlbA* gene is a single-copy gene in both *A. nidulans* strains (Fig. 2B).

To identify the chromosomal localisation of the *jlbA* gene, pools of chromosomes I–VIII of a chromosome-specific recombinant DNA library from *A. nidulans* (Brody et al. 1991) were used as templates for PCR, using two different pairs of *jlbA*-specific oligonucleotides. This experiment resulted in single amplicons of appropriate length only in reactions using chromosomal DNA of pool VII as template (data not shown). Therefore, the *jlbA* gene of *A. nidulans* is localised on chromosome VII.

The *jlbA* transcription level is strongly induced by addition of the amino acid analog 3AT

The promoter region of *jlbA* contains five putative CT boxes and two sequence motifs similar to the yeast

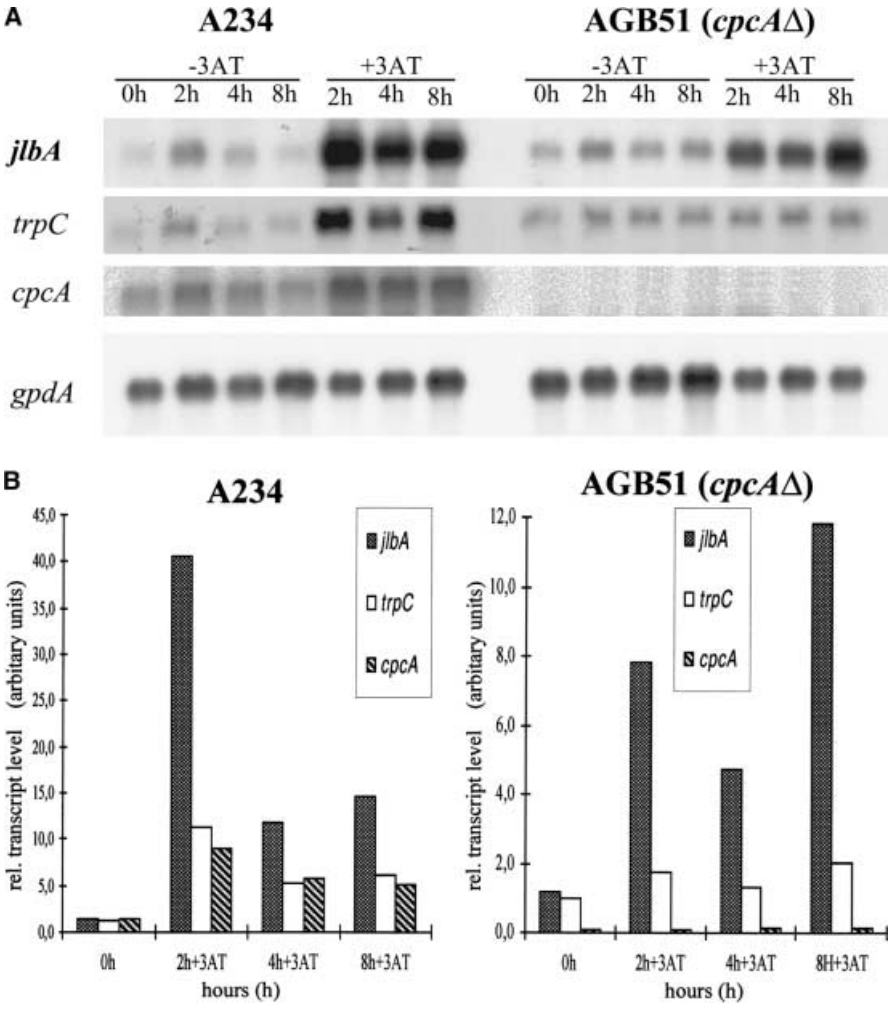

Fig. 4 A,B Induction of the *jlbA* mRNA expression under amino acid starvation conditions and Northern analysis of total RNA isolated from *A. nidulans* strains A234 and AGB51 (*cpcA*Δ) grown in minimal medium. At the indicated time (*0 h*) mycelium was harvested and distributed equally to fresh minimal medium with and without 10 mM 3-amino-1,2,4-triazole (*3AT*). RNA was isolated 2 h, 4 h and 8 h after induction. Steady-state levels of the *gpdA* transcript gene were used as internal standard. **A** Strong induction of the *jlbA* mRNA expression under amino acid starvation conditions after addition of 3AT. The *trpC* and *cpcA* mRNA expressions were used as controls regulated by the general cross-pathway control network. **B** Quantification of the *jlbA*, *trpC* and *cpcA* mRNA levels normalised with respect to the *gpdA* levels, using a Phosphoimager. The induction of the mRNA level of strains A234 and AGB51 are indicated in two diagrams, each representing the average of three independent measurements. Standard deviation did not exceed 15%

GCRE consensus sequence 5′-TGA(G/C)TCA-3′ upstream of the putative transcriptional start sites. GCREs function as Gcn4p recognition elements and are located in promoter regions of genes transcriptionally regulated by gc (Hinnebusch 1992). In addition, these conserved sequence motifs are present not only in promoter regions of regulated genes, but also in the *cpcA* promoter of *A. niger* (Wanke et al. 1997) and in other cpc regulators. This prompted us to test whether *jlbA* expression is regulated by amino acid starvation. Starvation for one single amino acid is sufficient to induce the regulatory network of amino acid biosynthesis. Therefore we tested the expression of the *jlbA* gene in the presence of 10 mM 3AT, which induces starvation for the amino acid histidine. When compared to total RNA isolated from identical liquid cultures without 3AT, the *jlbA* mRNA level of the *A. nidulans* wild-type strain A234 increased about 40-fold after 2 h of exposure to 3AT (Fig. 4A). Further, the inductive effect was approximately 80-fold in strain GR5 (data not shown). After 4 h of exposure to 3AT, the induction in strain A234 was around 10-fold and reached a 15-fold level after 8 h (Fig. 4B). The *trpC* mRNA level of this strain showed a similar induction pattern. After 2 h of exposure to 3AT, transcription of *trpC* mRNA was induced 10-fold and showed a 6-fold induction after 8 h of exposure to 3AT. These values are coincident to *trpC* values obtained from Busch et al. (2001).

In mutant strain AGB51 (*cpcA*Δ), the induction of *jlbA* mRNA was 8-fold after 2 h, 5-fold after 4 h and finally reached a 10-fold induction level after 8 h exposure to 3AT. In contrast, *trpC* mRNA level of the mutant strain AGB51 was not effected under starvation conditions. Whereas *trpC* mRNA induction was fully dependent on the *cpcA* gene product, *jlbA* mRNA levels were partially increased, even in the absence of CPCA. They therefore have additional regulation.

Discussion

The cpc of amino acid biosynthesis is a conserved mechanism in cellular and filamentous fungi. Induction of mRNA expression of genes regulated by this system under amino acid starvation conditions have been shown in *S. cerevisiae* (Braus 1991; Hinnebusch 1992, 1997; Zaman et al. 1999), *N. crassa* (Sachs 1996), *A. niger* (Wanke et al. 1997) and in *A. nidulans* (Piotrowska 1980; Hoffmann et al. 2000).

We have identified a new gene that is strongly induced by the addition of 3AT to growth medium, starving cells for histidine. Our results demonstrated an induction of up to 40-fold for the *jlbA* mRNA transcription level after 2 h of exposure to 3AT in strain A234. This transient induction peak in the mRNA expression of the *jlbA* gene is similar to the results obtained for the expression of the *GCN4* homolog *cpcA* in *A. niger*, although the induction effect for this gene was not so strong and did not exceed three-fold (Wanke et al. 1997). Interestingly, the mutant strain AGB51 (*cpcA*Δ) also showed a significant induction of the *jlbA* mRNA expression. Despite the absence of CPCA, the mRNA level of *jlbA* still was partially induced after 2 h of incubation in the presence of 3AT and finally reached a ten-fold level after 8 h of exposure to 3AT. These values are similar to the measured values obtained from strain A234. In contrast, the *trpC* mRNA level varied only weakly in strain AGB51. The *trpC* mRNA expression is regulated in response to the protein level of the transcription factor CPCA, probably because of the CPCA-binding sites within the promoter region of the *trpC* gene (Hoffmann et al. 2000; Busch et al. 2001). Disruption of *cpcA* leads to a constant *trpC* mRNA level, indicating a strong dependence of the induction on the protein levels of CPCA. Because of two deduced GCRE-binding sites within the promoter region of the *jlbA* gene, results obtained for the *jlbA* mRNA levels in the *cpcA* mutant strain are surprising. Obviously, the *jlbA* expression is also regulated independently of CPCA. This suggests that starvation using the analogue 3-AT results in the induction of cpc genes and an additional induction process not yet described. Whether this effect is caused by a changed basal transcription or by other transcriptional activators, like NIT2p (Fu and Marzluff 1990), StuAp (Dutton et al. 1997) or factors binding to the STRE-related elements (Schueller et al. 1994), remains to be tested.

The DNA-binding domain of *jlbA* codes for a typical basic amino acid domain and an adjacent leucine zipper motif. This part of the deduced protein seems to be conserved, when compared to other transcriptional activator proteins. In the middle of the putative JLBA protein, a PEST region was identified. This region might function in degradation processes of JLBA, as described for other members of the bZIP-type protein family (Salama 1994; Roth 1998) or may be necessary for phosphorylation to alter protein-protein interaction (Chu et al. 1996). The N-terminal part of JLBA shares no homology to other proteins described so far. Therefore, the JLBA protein might be a new member of the bZIP-type protein family. The function of JLBA in *A. nidulans* remains unknown. Neither the inactivation of *jlbA* mRNA by an antisense RNA (Eguchi et al. 1991), nor the overexpression of *jlbA* displayed any significant phenotype. The drastic induction of *jlbA* expression upon amino acid starvation suggests that JLBA might have a yet unknown role in the response to the starvation signal, perhaps in combination with the CPCA transcription factor.

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