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The *Aspergillus nidulans* sulphur regulatory gene *sconB* encodes a protein with WD40 repeats and an F-box

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Abstract The *Aspergillus nidulans* gene *sconB*, one of the four identified genes controlling sulphur metabolite repression, was cloned and analysed. It encodes a polypeptide of 678 amino acids containing seven WD repeats characteristic of the large WD40 family of eukaryotic regulatory proteins. The SCONB protein has nuclear localisation signals and is very similar to the *Neurospora crassa* SCON2 and *Saccharomyces cerevisiae* Met30 proteins, both of which are involved in the regulation of sulphur metabolism. The *N. crassa scon-2* gene complements the *sconB2* mutation. All three proteins also contain a newly identified motif, the F-box, found in a number of eukaryotic regulatory proteins. This motif is responsible, at least in some cases, for ubiquitin-mediated proteolysis. The *sconB* transcript is derepressed under sulphur limitation conditions and partly repressed by high methionine.

Key words *sconB* · *Aspergillus nidulans* · Sulphur regulation · F-box · WD repeats

Introduction

Aspergillus nidulans is able to utilise a wide range of compounds as sole sulphur source (for a review, see Paszewski et al. 1994). Sulphur uptake and assimilation in *A. nidulans*, as in other fungi, are carried out by a set of enzymes encoded by coordinately expressed structural genes. There is a strong selective advantage in the expression of these genes only when a suitable substrate is available. The phenomenon known as sulphur metabolite repression leads to the repression of genes involved in the utilisation of sulphur sources other than preferentially utilised organic sulphur compounds like methionine. These include primarily genes coding for enzymes of the sulphate assimilation pathway, sulphohydrolases and, to a lesser extent, enzymes of the alternative pathway of cysteine synthesis. Therefore, under repression conditions (in the presence of a high concentration of methionine) the cells are resistant to toxic analogues of sulphate (chromate and selenate), which are taken up by the sulphate permease.

In *Neurospora* sulphur metabolite repression is abolished by mutations in the *scon*^{c} gene (Burton and Metzenberg 1972), now designated *scon-1*, and in the *scon-2* gene. The latter gene has been cloned and sequenced (Paietta 1990; Kumar and Paietta 1995). It codes for a protein very similar to the *Saccharomyces cerevisiae* Met30 protein, which is also involved in sulphur regulation (Thomas et al. 1995). On the basis of epistatic relationships and gene expression analysis, Paietta (1990) proposed a tentative regulatory hierarchy in *Neurospora*. The model comprises the two *scon* genes, which control expression of the *cys-3* locus. The latter, a major positive regulator of sulphur-related structural genes (Marzluf and Metzenberg 1968), encodes a DNA-binding protein (Fu et al. 1989; Fu and Marzluf 1990).

In *A. nidulans* sulphur metabolism is controlled by four genetically defined *trans*-acting, apparently inhibitory *scon* (*A*, *B*, *C*, *D*) regulatory genes. The *scon* mutations suppress lesions in the cysteine to homocysteine pathway (*metA*, *metB*, and *metG* loci) owing to the derepression of the alternative pathway of homocysteine synthesis involving homocysteine synthase (Nadolska-Lutyk et al. 1989; Natorff et al. 1993). Mutations in *scon* genes, which phenotypically resemble the *Neurospora scon* mutations, lead to constitutive derepression of several enzymes of sulphur metabolism, including sulphate permease, rendering the strains sensitive to selenate and chromate, even in the presence of methionine. Nothing is known about the mechanism of action of these genes or the possible interactions between their protein products.

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We report the cloning and characterisation of the *A. nidulans sconB*$^+$ gene. Analysis of the deduced amino acid sequence revealed the presence of seven highly conserved tandem repeats of the so-called WD40 motif. The SCONB protein shows a high degree of similarity with *S. cerevisiae* Met30 (Thomas et al. 1995) and *Neurospora crassa* SCON2 (Kumar and Paietta 1995) proteins, which are both involved in the regulation of sulphur metabolism. In addition, the *sconB* and *scon-2* genes reciprocally complement each other in heterologous transformations (see below, and J. Paietta, personal communication). All three proteins belong to an expanding family of regulatory proteins (the WD40 protein family) known to mediate a diverse array of cellular functions (Neer et al. 1994). In addition, they contain a newly identified motif, the F-box, found in a number of regulatory proteins, including some involved in the control of the cell cycle (Bai et al. 1996).

Materials and methods

Strains

The following strains of *A. nidulans* from our collection, which carry standard markers (Clutterbuck 1994; Martinelli 1994), were used: *pyroA4 yA2*; *argB2 nicA2 biA1*; and *pyrG89 metA17 pyroA4 pabaA2*. The *scon* strains used were: *sconB2 argB2 nicA2 biA1*; *sconA25 pyroA4 yA2; sconB2 pyroA4 yA2*; *sconC3 pyroA4 yA2*; and *sconD6 pyroA4 yA2*. The wild-type strains used as a reference in the experiments were *pyroA4 yA2*; and *anA1 biA1 phenA2*.

The *Escherichia coli* strains used were DH5α, and XL1-Blue (Stratagene).

Media and growth conditions

The *A. nidulans* strains were grown on solid and liquid media: minimal medium (MM), containing 2 mM sulphate (Paszewski and Grabski 1974), and MM−S (minimal minus sulphate) in which sulphates were replaced by the corresponding chlorides. The latter was supplemented with L-methionine at 5 mM (high sulphur), 0.25 mM (low sulphur) or 0.05 mM (limiting sulphur). For transformation, strains were grown in complete medium (Martinelli 1994). Cultures were started by inoculation of 100 ml medium with 3–5 ml of a heavy conidial suspension and grown with shaking (200 rpm) at 37° C for 16 h. Mycelia were harvested by filtration, washed with distilled water and immediately used for protoplast preparation or nucleic acid isolation.

Strains of *E. coli* were grown in standard media, LB, 2 × TY or NZY (Sambrook et al. 1989), as required. Antibiotics were used at the following concentrations: ampicillin, 50 μg/ml; kanamycin, 50 μg/ml; tetracycline, 35 μg/ml.

Plasmids, libraries and synthetic primers

pUC19 and pBluescript II KS and SK were from Stratagene. The pGM32 plasmid carrying the *N. crassa pyr-4* gene (as a nonhomologous selectable marker in *A. nidulans*) was obtained from G. Turner. The pHELP1 and ARp1 plasmids (Gems et al. 1991), obtained from J. Clutterbuck, contain the AMA1 sequence, which enables autonomous replication. The ARp1 plasmid also contains the *argB*$^+$ gene. Co-transformation with these plasmids results in as much as 200-fold increase in transformation efficiency (Gems and Clutterbuck 1993). The pIC19R plasmid containing a fragment of the *A. nidulans* γ-actin gene (Fidel et al. 1988) was obtained from R. Bradshaw. The *A. nidulans* chromosome-specific cosmid pWE15 and pLORIST2 gene libraries (Brody et al. 1991) and the *A. nidulans* λZapII 24 h developmental cDNA library constructed by R. Aramayo were obtained from the Fungal Genetics Stock Center, Kansas City, Kansas, USA. The pscon2 plasmid containing the entire *scon-2* gene from *N. crassa* was kindly provided by J. V. Paietta.

The following oligonucleotides were used for primer extension:
B2L 5′ TCCAGAACTGACACCATTCGCCTCGCTTGAT 3′
B4L 5′ CAGTGACCGAAGACGAACAAGGAGCGAAGC 3′

Transformation and strategy for *sconB* gene selection

Protoplasts of the *sconB2 argB2 nicA2 biA1* strain were obtained after Novozyme 234 treatment. Mycelium (1 g of wet weight) was resuspended in 10–15 ml of 0.6 M KCl buffered at pH 6.0 with 10 mM phosphate buffer; 2 mg/ml Novozyme 234 and 2 mg/ml helicase were added. The mixture was incubated on an orbital shaker at 37° C for 90 min. Protoplasts were separated from mycelial debris by filtration through a Buchner funnel with a sintered glass disc (pore size 16–40 μm). The protoplasts were centrifuged at 3500 × g for 10 min, washed twice in 0.6 M KCl and once in 1.2 M sorbitol, 50 mM $CaCl_2$, 10 mM TRIS-HCl, pH 7.5. Then the protoplasts were resuspended in the same solution to a final concentration of 1–5 × 10^8/ml.

Selection for *sconB*$^+$ transformants relied on the sensitivity of *scon* strains to selenate under repressive (i.e. high methionine) conditions. Selenate, a toxic analogue of sulphate is transported into the cell by sulphate permease. Expression of the enzyme is repressed in the methionine-grown wild-type strain which, unlike *scon*$^-$ mutants is thus resistant to selenate in the presence of this amino acid. Therefore, *sconB*$^+$ transformants were selected on the basis of their selenate resistance on high methionine medium.

Protoplasts of the *sconB2 argB2* strain were cotransformed to the wild-type phenotype (*scon*$^+$ *arg*$^+$) with DNA isolated from the fraction of the *A. nidulas* cosmid gene library covering chromosome I (divided into subpools), and the ARp1 helper plasmid. Transformation mixes (1–5 × 10^7 protoplasts and 1–5 μg DNA per plate) were transferred to 1 ml 25% polyethylene glycol in 50 mM $CaCl_2$, 10 mM TRIS-HCl, pH 7.5, then diluted with 3 ml 1.2 M sorbitol in 50 mM $CaCl_2$, 10 mM TRIS-HCl, pH 7.5, supplemented with 4 ml selective medium and overlaid on MM−S plates containing 5 mM L-methionine and 1.2 M sorbitol. After 24 h incubation the plates were overlaid with 5 ml of MM−S solid medium (melted and cooled to 55° C) supplemented with 5 mM methionine and 1 mM sodium selenate. The final concentration of selenate was 0.2 mM. Under these conditions only *arg*$^+$ *scon*$^+$ transformants that have become prototrophic for arginine and resistant to selenate can grow. The same transformation mix (or a suitable dilution) was applied to MM−S plus methionine medium to estimate the transformation frequency to *arg*$^+$. The subpool that gave *scon*$^+$ *arg*$^+$ transformants was further subdivided in order to identify which of the 96 clones (Metzenberg and Kang 1987) transforms the recipient strain to selenate resistance. Selenate-resistant transformants were subsequently tested to check whether they had regained wild-type regulation of the tester enzyme arylsulphatase, which is derepressed in the *scon*$^-$ mutants (Natorff et al. 1993).

Transformation of *E. coli* was by the standard calcium chloride method (Sambrook et al. 1989).

Cloning of *sconB* cDNA

A cDNA copy of *sconB* was isolated from the λZAPII library by probing of about 5 × 10^5 phage clones with the digoxygenin-labeled 0.7-kb *Nru*I-*Cla*I fragment from the 5′ end of the *sconB* gene. This was done according to the Stratagene protocol.

DNA sequencing

Subclones of the *sconB* gene in pUC19 or pBluescript KS and SK vectors were sequenced on both strands using an ALF automatic

sequencer (Pharmacia), or manually, using the Sequenase Version 2.0 system (USB) and appropriate primers and synthetic oligonucleotides as required.

RNA isolation

A. nidulans mycelia were harvested by filtration and blotted on filter paper. Some 500 mg of wet mycelium was ground in a mortar with an equal amount of baked glass powder (obtained by grinding broken glass in a mortar) and 5 ml of TRI Reagent (Molecular Research Center; Chomczyński 1993). The subsequent steps of total RNA extraction and mRNA isolation on oligo(dT) columns (MRC) were carried out according to the supplier's instructions.

Mapping of the 5′ and 3′ mRNA termini of *sconB*

The *sconB* transcription initiation sites were mapped by primer-extension analysis. The oligonucleotides B4L and B2L (complementary to the sense strand at positions −157 to −187 and −439 to −472 relative to the initiator AUG, respectively) were hybridized to 5 μg of total RNA isolated from the wild-type strain grown in minimal medium. The primer was then extended with 200 U of reverse transcriptase (BRL or Promega) at 42° C for 2 h, using [α-^{32}P]dCTP (according to the protocol for first-strand cDNA synthesis using Superscript II RT, Gibco BRL). The reaction product was fractionated by 8% denaturing gel electrophoresis in parallel with DNA sequencing reactions initiated with the same primer. The 3′ end of *sconB* mRNA was determined by sequencing of two *sconB* cDNA clones.

Northern blot analysis

A sample containing 3–4 μg poly(A) RNA in FORMAzol (Molecular Research Center) was mixed with an equal amount of loading buffer (4.4 M formaldehyde, 40 mM MOPS, 10 mM sodium acetate, 2 mM EDTA) and denatured by heading at 55° C for 15 min, followed by immediate cooling on ice. The mRNA was fractionated by gel electrophoresis in 1.2% (or 1.4%) agarose containing 1.1% formaldehyde. After electrophoresis, the mRNA was transferred to a nylon membrane (Hybond N, Amersham) by overnight capillary blotting in 20×SSC and fixed to the membrane by UV cross-linking for 1.5 min. Filters were prehybridized for 4 h at 42° C in 50% formamide, 5×SSC, 100 μg/ml denatured herring sperm DNA, 5×Denhardt's (0.1% BSA, 0.1% Ficoll 400, 0.1% polyvinyl pyrrolidone 40,000), 0.5% SDS. The DNA probes were α-^{32}P-labelled using the random primed labelling kit (MegaPrime or RediPrime Amersham) and hybridization was allowed to proceed for 40 h at 42° C. Membranes were washed two times (10 min each) in 2×SSC, 0.1% SDS at room temperature, 0.1×SSC, 0.1% SDS at room temperature and 0.1×SSC, 0.1% SDS at 42° C and subjected to autoradiography at −80° C.

Computer sequence analysis

The following analyses were done with GCG software (version 8.1) on a Silicon Graphics Challenge computer. Homology searches were carried out against the GenBank (release 95.0), EMBL (release 47.0), Swiss-Prot (release 33.0) and RIR (release 48.0) databases using BLAST (Altschul et al. 1990) and FASTA (Wisconsin package, version 8.0.1). Motifs and features of the polypeptide were searched with PROSITE (release 13.0; Appel et al. 1994) and the cellular location of the protein was predicted with PSORT (Nakai and Kanehisa 1992). The ImageQuant version 3.3 program (Molecular Dynamics) was used for quantification of hybridization signals.

Results

Isolation of *sconB*-complementing clone

The selection strategy (see Materials and methods) led to identification of one 16-kb cosmid clone, designated L17:A10, that transformed the *sconB2* strain to selenate resistance on high methionine. Interestingly, only 20% of these transformants did not stain for the tester enzyme arylsulphatase, i.e. had regained complete wild-type sulphur regulation.

The cosmid was digested with a series of restriction endonucleases and the restriction fragments obtained were used along with the ARp1 helper plasmid to transform the *sconB2 argB2* strain to the *sconB*$^+$ *argB*$^+$ phenotype. A 2.6-kb *Bam*HI-*Eco*RI fragment was the smallest fragment found to complement the *sconB2* mutation. Further deletions at either end of this fragment abolished the complementing ability.

We have also shown that the *A. nidulans sconB2* mutant recovers the wild-type phenotype after transformation with heterologous pscon2 plasmid containing the entire *scon-2* gene from *N. crassa*.

Sequence and organisation of the *sconB*$^+$ gene

The *Bam*HI-*Eco*RI fragment containing the *sconB*$^+$ gene was subcloned in pUC19 (psconB) and sequenced on both strands. The DNA sequence contains a single open reading frame (ORF) of 2081 bp, interrupted at its 5′ end by one small intron of 47 bp, as determined by comparison of *sconB*$^+$ genomic and cDNA sequences (Figs. 1, 2). In order to confirm that we had cloned the *sconB* gene, one of the most phenotypically extreme *sconB* alleles (*sconB9*) was sequenced. It was found that the mutation changed the translational initiation codon *A*TG to *G*TG.

Transcriptional initiation sites were mapped by primer-extension analysis. For this purpose we had to subclone an additional 2-kb *Bgl*II fragment of the primary cosmid that overlaps the 2.6-kb *Bam*HI-*Eco*RI fragment by 0.6 kb. This gave us about 1.7 kb of sequence upstream of the initiation ATG of the *sconB* gene. Primer-extension products gave strong bands corresponding to initiation sites at positions −238 and −338 and weaker bands at −443 and −514 (Fig. 3). The transcriptional start at position −338 is in good agreement with the size of the transcript, estimated to be about 2.7 kb by denaturing gel electrophoresis. The longest cDNA copy isolated and sequenced from the library had a 5′ UTR (untranslated region) of 268 nucleotides, indicating that at least some transcripts start upstream of the −238 position. If transcription starts at the fourth and third uppermost initiation sites the long leader formed contains three or two short ORFs, respectively. As shown in Fig. 2 there are nine consensus sequences recognized by the *N. crassa* CYS3 major

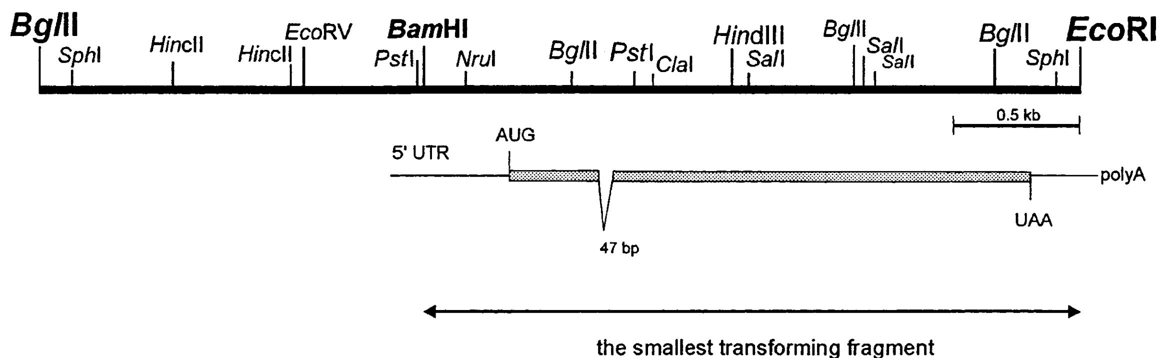

Fig. 1 Restriction map of the 4-kb *Bgl*II-*Eco*RI fragment containing the entire *sconB* gene. Organisation of the mRNA as deduced from cDNA sequencing and primer extension studies is shown below. The exons are indicated as *grey boxes*. The AUG start and UAA termination codons of the 2081-bp $sconB^+$ open reading frame (ORF) are indicated. The smallest DNA fragment (*Bam*HI-*Eco*RI) that complements the *sconB2* mutation is shown

sulphur regulatory protein (Li and Marzluf 1996) in the *sconB* promoter. The promoter also contains five potential binding sites for the *A. nidulans* AREA regulatory protein (Kudla et al. 1990; Caddick 1994) and six for the CREA factor (Kelly 1994).

The 3′ end of *sconB* mRNA occurs 223 or 226 bp downstream from the translation termination site as determined by sequencing of two independent cDNA clones of the *sconB* gene (Fig. 2).

Sequence analysis of the SCONB protein

The $sconB^+$ gene encodes a polypeptide of 678 amino acids and a calculated molecular mass of 76.07 kDa. The C-terminal part of the SCONB protein contains seven internal repeats of about 40 amino acids. Each repeat exhibits a characteristic pattern of residues highlighted by strongly conserved Gly-His (GH) in part A and Try-Asp (WD) dipeptides in part B, separated by regions that are variable in both sequence and length (Fig. 4a), (van der Voorn and Ploegh 1992). This repeated motif, named WD-40 (or β-transducin repeat), was first found in the β-subunit of heterotrimeric GTP-binding proteins (Fong et al. 1986). The WD40 repeats of SCONBp are arranged tandemly. In repeat 5 the A part is separated from the B part by a spacer of 63 amino acids (Fig. 4b).

The SCONB protein shows high similarity/identity to the *N. crassa* SCON2 (74%/55.6%, respectively) and *S. cerevisiae* Met30 (63%/43.9) proteins, involved in the regulation of sulphur metabolism (Paietta 1990; Thomas et al. 1995) (Fig. 5). In addition, the SCONB protein exhibits a strongly conserved domain, placed N-terminal to the WD40 repeats, that defines a subset of WD proteins, including SCON2, Met30 and also yeast CDC4, *Xenopus* BTrCP and mouse MD6 (Kumar and Paietta 1995). This domain comprises a newly described motif of 41 amino acids, called the F-box, found in a number of eukaryotic regulatory proteins (see Discussion).

The results of PSORT analysis revealed the presence of three nuclear localisation signals (NLS) in the SCONB protein giving a high probability (the score was 0.98) that it is localised in the nucleus.

Analysis of $sconB^+$ gene expression

mRNA was isolated from *A. nidulans* wild-type and *sconA25*, *B2*, *C3*, *D6* strains grown on high and low levels of sulphur (i.e., repressing and derepressing conditions). Northern blots were probed with the ^{32}P-labelled 1.1-kb *Bgl*II cDNA fragment of the *sconB* gene. The constitutively expressed γ-actin gene was used as a control. In the wild type, the *sconB* transcript is present at all levels of sulphur. Its level is twofold increased under sulphur limitation (i.e. when methionine at a concentration of 0.05 mM is the only sulphur source) as compared with low sulphur conditions (0.25 mM methionine, or 2 mM sulphate, as a sole sulphur source) and somewhat reduced in the presence of high methionine (Fig. 6a).

The transcript is present in the *sconA25*, *sconB2* and *sconD6* strains at levels comparable to those of the wild type grown under the same conditions, with the exception of the *sconC3* strain, where it is about threefold decreased (Fig. 6b).

▶

Fig. 2 Nucleotide sequence of the $sconB^+$ gene and deduced amino acid sequence of its product. The sequence is numbered relative to the initiator ATG codon. The coding sequence is given in *bold*, *uppercase* letters. The $sconB^+$ transcriptional initiation sites (at positions −238, −338, −443 and −514) are indicated with *vertical arrows* (↓). The *thin bar* at position −268 indicates the 5′ end of the longest cDNA clone. Palindromic sequences (10 bp in length) are marked by *converging horizontal arrows* (→←). Sequences similar to the *Neurospora crassa* CYS-3 transcription factor binding site are *boxed*. Sequences homologous to the AREA recognition site are *underlined twice*. Sequences potentially binding CREA protein are in *italic* and *bold*. The start codons of additional short uORFs present in the 5′ UTR are marked in *bold* (*atg*) and stop codons are *underlined* (*tga*, *taa*). The 3′ end sequence obtained from sequencing of two independent *sconB* cDNA clones is shown in *italic*. Polyadenylation sites are marked by (*A*). Polyadenylation signal is in *italic* and *underlined*. The sequence of the above 2.6-kb *Bam*HI-*Eco*RI fragment has been deposited in the EMBL/GenBank data base under accession number U21220

Disruption of the *sconB* gene

Plasmid pGMBC was designed as a vector for targeted disruption of the *A. nidulans sconB* gene via homologous recombination. It was constructed by inserting a 0.9-kb *Bam*HI-*Cla*I fragment of the *sconB* gene into the pGM32 plasmid (containing the *N. crassa pyr-4* gene, which is able to complement the *A. nidulans pyrG89* mutation). Disruption of the *sconB* gene was done by transformation of the *pyrG89 metA17 pyroA4 pabaA2* strain with the pGMBC plasmid (Fig. 7). It led to the separation of the coding region from the promoter. All *pyr*$^{+}$ transformants obtained were found to be simultaneously *met*$^{+}$, i.e. the disruption of the *sconB* gene led to the *sconB* phenotype mainfested by suppression of the *metA17* mutation. This was confirmed genetically by crossing two disruptant strains with the wild type. Disruption of the *sconB* gene was confirmed by Southern hybridisation (data not shown).

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agatctgcctgagcggcattggtacggtcgattgagctcttcagcgtcgagtgaagctcctctacgccccgctcatccacaaagtcgtgtagactcttgggttcctcaccctcagggcga  -1666
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                                                                                 -514
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         -338                                                                      -268                             -238
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acgaagcgatagaaagaacgaaatccaacgattgacctcgcgctgcataacggtcggccgatacagacctggaacggtcgttgagccaagccgacagcaaggaaaATGTCTACCGAGGAT  15
                                                                                                           M  S  T  E  D    5
AATCACGATTCGCAAATTTTGACGGCAAGACATCGCTCGGACGCCAGCGAGCAGTCGTTCAAATCCCTGTTCGGCGGCCCATCGAGTGAAGACGGAAAAGAGACGGAGCCCGATACCCAC  135
N  H  D  S  Q  I  L  T  A  R  H  R  S  D  A  S  E  Q  S  F  K  S  L  F  G  G  P  S  S  E  D  G  K  E  T  E  P  D  T  H    45
GATCATAACCATAGCTTCAGCAACGCAAAGGCGCCCGCAAAGTTTGCAAACCAGAATGTCGCACCCTTCCTCGCCAGACACATCCCTGAACAATATGCACCCTTAGGTGCTCAGTCGATT  255
D  H  N  H  S  F  S  N  A  K  A  P  A  K  F  A  N  Q  N  V  A  P  F  L  A  R  H  I  P  E  Q  Y  A  P  L  G  A  Q  S  I    85
CTACCAGCAGATCTCAGCAGCGCAAACTCCAAATACTGTTATCGCCATCGCCCAGATCAGAAATGTAGACGGCAAGCCGACGAGCCATCTATGGATAAGCTACAGCGGgtgagtaacccc  375
L  P  A  D  L  S  S  A  N  S  K  Y  C  Y  R  H  R  P  D  Q  K  C  R  R  Q  A  D  E  P  S  M  D  K  L  Q  R                121
gcttcactcaatggtggttgtgtgctaattagtagGAACTGGAATCGCTTCCTCAAGGTGATCAGCAGAGCATATCACATGTCTGGTCCTTGTTCTCTGCTGCGCCTGCCAAACATCGGA  495
                                   E  L  E  S  L  P  Q  G  D  Q  Q  S  I  S  H  V  W  S  L  F  S  A  A  P  A  K  H  R  K  150
AACTCATTCTGCAGGGAATCATGGCTCAATGCTGTTTCCCGCAACTCTCTTACATCTCCGCCACCGTCCGTGACCTCATCCGAATCGATTTCATCACCGCGCTCCCTCCCGAGATTGCTT  615
 L  I  L  Q  G  I  M  A  Q  C  C  F  P  Q  L  S  Y  I  S  A  T  V  R  D  L  I  R  I  D  F  I  T  A  L  P  P  E  I  A  F  190
TCAAAATCCTTTGCTATCTGGATACAACCTCTCTGTGCAAGGCTTCCCAGGTCTCGCGCGGATGGCGTGCGCTAGCCGATGATGATGTAGTTTGGCACAGAATGTGTGAACAACATATTC  735
 K  I  L  C  Y  L  D  T  T  S  L  C  K  A  S  Q  V  S  R  G  W  R  A  L  A  D  D  D  V  V  W  H  R  M  C  E  Q  H  I  H  230
ATCGCAAGTGCAAAAAGTGTGGTTGGGGCTTACCGCTTCTGGATCGCAAACGATTGCGTGAGTCGAAGCGTGAGATTGAACTTCGTGCTACAACGTGGGACAAGGGAGTGGTGGGCCCAC  855
 R  K  C  K  K  C  G  W  G  L  P  L  L  D  R  K  R  L  R  E  S  K  R  E  I  E  L  R  A  T  T  W  D  K  G  V  V  G  P  R  270
GATCCCCTGATGCTTCGGCAGAGTCACCTcCTTCGGGCAAACGAAAGCTTGAGGATGACGAGGTTGCTGTTGTTAAGCGGCACTGTTCATCGCTCGGGTCAGACGCAGGAGTCGACAAAG  975
 S  P  D  A  S  A  E  S  P  P  S  G  K  R  K  L  E  D  D  E  V  A  V  V  K  R  H  C  S  S  L  G  S  D  A  G  V  D  K  D  310
ATAGTGATTTCTTCAAGACCCGCTACCGGCCATGGAAGGAAGTGTACAAAGATCGCTTCAAGGTCGGCACGAACTGGAAATACGGGCGATGCTCGATCAAAACATTTAAGGGACATACTA  1095
 S  D  F  F  K  T  R  Y  R  P  W  K  E  V  Y  K  D  R  F  K  V  G  T  N  W  K  Y  G  R  C  S  I  K  T  F  K  G  H  T  N  350
ATGGAGTCATGTGTCTGCAATTCGAGGATAACATTCTTGCCACTGGCTCCTACGACACTACAATCAAAATCTGGGACACTGAAACCGGGGAGGAGCTGCGCACACTCCGTGGACACGAGT  1215
 G  V  M  C  L  Q  F  E  D  N  I  L  A  T  G  S  Y  D  T  T  I  K  I  W  D  T  E  T  G  E  E  L  R  T  L  R  G  H  E  S  390
CTGGCATCCGTTGCTTACAGTTTGACGACACCAAGTTGATCAGCGGCAGCATGGACCGCACTATCAAGGTCTGGAACTGGCGCACTGGGGAGTGCATTTCCACATATACTGGTCACCGCG  1335
 G  I  R  C  L  Q  F  D  D  T  K  L  I  S  G  S  M  D  R  T  I  K  V  W  N  W  R  T  G  E  C  I  S  T  Y  T  G  H  R  G  430
GGGGAGTCATTGGGCTTCACTTTGATGCTTCGATTCTCGCCTCTGGCTCGGTTGACAAGACGGTCAAGATCTGGAACTTCGAGGATAAGTCGACTTTCTCCCTGCGTGGACACACAGATT  1455
 G  V  I  G  L  H  F  D  A  S  I  L  A  S  G  S  V  D  K  T  V  K  I  W  N  F  E  D  K  S  T  F  S  L  R  G  H  T  D  W  470
GGGTCAATGCAGTCAGAGTCGACACTTCCTCTCGAACTGTCTTCTCAGCATCTGATGACTGTACTGTTCGCCTTTGGGACTTGGACACGAAAACCTGCATTCGGACTTTCCATGGACACG  1575
 V  N  A  V  R  V  D  T  S  S  R  T  V  F  S  A  S  D  D  C  T  V  R  L  W  D  L  D  T  K  T  C  I  R  T  F  H  G  H  V  510
TGGGTCAAGTGCAGCAGGTTGTTCCTCTGCCCCGCGAATTTGAGTTCGAAGAACATGATGCCGAGTGCGAGAACGACGATCTCAGTACCACCTCCGGCGACGCGAACCCGCCGTCAATCC  1695
 G  Q  V  Q  Q  V  V  P  L  P  R  E  F  E  F  E  E  H  D  A  E  C  E  N  D  D  L  S  T  T  S  G  D  A  N  P  P  S  I  Q  550
AGGCTTCGATGGGTTTGGAACCCAACGCAGCGTACAGCCAATCCTCGGCCTTTGGGACTTCGTTCGATAACGGCCGTGCCGCGCCGCCACGATACATGGTTACAAGTGCGCTGGATTCTA  1815
 A  S  M  G  L  E  P  N  A  A  Y  S  Q  S  S  A  F  G  T  S  F  D  N  G  R  A  A  P  P  R  Y  M  V  T  S  A  L  D  S  T  590
CGATCCGCCTCTGGGAAACCACCACCGGCCGCTGCTTGCGTACGTTCTTCGGCCACTTGGAGGGAGTATGGGCGCTGGGAGCCGACACTCTCCGCATCGTCTCTGGCGCTGAAGACCGTA  1935
 I  R  L  W  E  T  T  T  G  R  C  L  R  T  F  F  G  H  L  E  G  V  W  A  L  G  A  D  T  L  R  I  V  S  G  A  E  D  R  M  630
TGATCAAGATCTGGGACCCCAGAACTGGTAAATGCGAGCGTACTTTCACCGGCCACTCTGGCCCTGTCACTTGCATTGGCCTTGGTGACAGTCGATTCGCTACTGGCAGCGAGGACTGCG  2055
 I  K  I  W  D  P  R  T  G  K  C  E  R  T  F  T  G  H  S  G  P  V  T  C  I  G  L  G  D  S  R  F  A  T  G  S  E  D  C  E  670
AAGTACGGATGTACAGCTTTCAGAGTtaatttcttcttcctttcttggcatgatcttattatttttaatgacttgtattttatggacggataccctttgagctggcgttttgattttgctg  2175
 V  R  M  Y  S  F  Q  S  *                                                                                                    678
ggtttagttccgttagcatgcaattccggcgttggatggactatcaggctcgaagctattcctcaacgcatagcaaggacactgtttttttgtttgaattagctgtcagagtaatgaattc  2295

attttctaaatt(A)atc(A)
```

Fig. 3 Mapping the 5′ end of the *sconB* mRNA by primer extension analysis. Using the synthetic primers B4L and B2L, three strong bands and one weaker band were obtained (*right lanes*). A parallel sequencing reaction with the same primers mapped transcriptional initiation sites at positions −238, −338, −443, and −514

Discussion

The *sconB* sulphur regulatory gene described here codes for a protein belonging to the extended family of so-called WD40 proteins, which are characterized by highly conserved repeating motifs usually ending with Trp-Asp (WD). These proteins, found almost exclusively in eukaryotes, play diverse cellular functions, being involved in signal transduction, cell cycle regulation, vesicular trafficking, mRNA processing and regulation of gene expression (for a review see Neer et al. 1994). Those involved in signal transduction are almost entirely composed of WD40 repeats, while other proteins, like SCONB, also contain N-terminal or (less frequently) C-terminal extensions. Although the function of WD40 motifs is unknown, it is postulated that they play a role in protein-protein interactions.

Analysis of the *sconB* promoter revealed the presence of nine CYS3 recognition sites. Two are in excellent agreement with the CYS3 consensus sequence (9/10 and 10/10) and are near each other. The presence of a number of CREA-and AREA-responsive elements is also noteworthy. Whether these motifs have regulatory significance remains to be determined.

The *sconB* mRNA has an unusually long 5′ UTR that may contain three or two short upstream open reading frames (uORFs) if transcription starts from the uppermost initiation site. The longest uORF might encode a protein of 126 amino acids. Other *A. nidulans* regulatory genes with long UTRs have been described (*stuA*, *creA*, *bimG* and the TATA binding protein-encoding gene); some of these also contain short uORFs (Kucharski and

Fig. 4a Consensus sequence of WD40 repeats according to Neer et al. (1994). **b** WD40 repeats of the SCONB protein

a

```
            {[GH (27±2 aa) WD] (11±2 aa)}      x 4-8
                 core length       variable length   number of repeats

         (A)                              (B)
Lx[GHxxxIxxLxF  (x6±2)     LxSGGxDxxLxLWD]   (x9±2)
F  S    L  I Y             I TAA    I IFN
   A    V  V W             V  SS    V VYR
   V    F  F L             F        F F K
        M  M I             M        M M
```

b

```
                            (A)                               (B)
Repeat 1 (345)  FKGHTNGVMCLQF  (EDNI--)LATGSYDTTIKIWD  (TETGEELRT)  (384)
Repeat 2 (385)  LRGHESGIRCLQF  (DDTK--)LISGSMDRTIKVWN  (WRTGECIST)  (424)
Repeat 3 (425)  YTGHRGGVIGLHF  (DASI--)LASGSVDKTVKIWN  (FEDKSTFS-)  (463)
Repeat 4 (464)  LRGHTDWVNAVRV  (DTSSRT)VFSASDDCTVRLWD  (LDTKTCIRT)  (505)
Repeat 5 (506)  FHGHVGQVQQVVP
(LPREFEFEEHDAECENDDLSTTSGDANPPSIQASMGLEPNAAYSQSSAFGTSFDNGRAAPPRY)
                                       MVTSALDSTIRLWE  (TTTGRCLRT)  (604)
Repeat 6 (605)  FFGHLEGVWALGA  (DTLR--)IVSGAEDRMIKIWD  (PRTGKCERT)  (644)
Repeat 7 (645)  FTGHSGPVTCIGL  (GDSR--)FATGSEDCEVRMYS  (FQS------)  (678)
```

```
                                                                                         NLS
SCONBP  MSTE-------DNHDSQILTARHRSDASE-------QSFKSLFGGPSSEDGKETEPDTHDHNHSFSNAKAPAKFANQNVAPFLARHIPEQYAPLGAQSIL   86
SCON2P  MSS--------------VLMSK------------------------------------------------------TVTPFLREHIPSIYAPIGKPGNQ   31
MET30P  MRRERQRMMSFEDKDKDDLDNSNSNNSSEMTDTAMMPPLKRLLITGSSDDLAQGSSGKKKITMATRSPSSSPDLATNDSGTRVQPLPEYNFT--------   92
        *.          * ..                                                                  . .. .     ..

SCONBP  -PADLSSANSKYCYRHRPDQK-------CRRQADEPSMDKLQRELESLPQGDQQSISHVWSLFSAAPAKHRKLILQGIMAQCCFPQLSYISATVRDLIRI  178
SCON2P  ETARAENPNSKYCYRHHPDSK-------CRRAADKAKMVMIQSELDKLTSADQQAVTHVWSLFSAAPARHRDLMLQGILSQLCFPQLSFVSREVNEALKI  124
MET30P  ----------KFCYRHNPDIQFSPTHTACYKQ-DLKRTQEINANIAKLPLQEQSDIHHIISKYSNSNDKIRKLILDGILSTSCFPQLSYISSLVTHMIKI  181
                  *.****.** .      * .. *  .   .. ....*.  .* .. *. * .*.. .. *.*.*.**..  ******..*  *  . ..*

                   F-box                                           NLS
SCONBP  DFITALPPEIAFKILCYLDTTSLCKASQVSRGWRALADDDVVWHRMCEQHIHRKCKKCGWGLPLLDRKRLRESKREIELR---ATTWDKGVVGPRSPDAS  275
SCON2P  DFISALPVELAQKVLCYLDTVSLTKAAQVSQRWRTLADSDAVWVRNCEQHVNRKCTKCGVGLPLLERKKLRNYTRQRQLAKGGPQGRVTELADSHDSQDR  224
MET30P  DFISILPQELSLKILSYLDCQSLCNATRVCRKWQKLADDDRVWYHMCEQHIDRKCPNCGWGLPLLHMKRAR----------------------------  252
        ***. ** *.. *.*.***  **  .*..*.. *. ***.* **  .****..***  .** *****..*. *

          NLS                                                                                WD-1
SCONBP  AESPPSGKRKLE-DDEVAVVKRHCSSLGSDAGVDKDSDFFKTRYRPWKEVYKDRFKVGTNWKYGRCSIKTFKGHTNGVMCLQFEDNILATGSYDTTIKIW  374
SCON2P  SVNQHGKRPAAEAEEEDPIKKRQCM-----AAAEASKAVTQPKTRSWKAVYRDRWQVSYNWKNSRYKLSVLKGHENGVTCLQLDDNILATGSYDTTIKIW  319
MET30P  -------------------IQQNSTGSSSNADIQTQTT------RPWKVIYRERFKVESNWRKGHCRIQEFKGHMDGVLTLQFNYRLLFTGSYDSTIGIW  327
                         ....       *.  .  ..       *.** .*..*..*. **. .....  ****  .**  **.. ..* *****.** **

                          WD-2                                     WD-3
SCONBP  DTETGEELRTLRGHESGIRCLQFDDTKLISGSMDRTIKVWNWRTGECISTYTGHRGGVIGLHFDASILASGSVDKTVKIWNFEDKSTFSLRGHTDWVNAV  474
SCON2P  NIETEECIRTLVGHTAGIRALQFDDSKLISGSLDHTIKVWNWHTGECLSTFAAHTDSVISVHFDGHLLASGSSDKTVKIFDFNSKETYCLKGHSDWVNST  419
MET30P  DLFTGKLIRRLSGHSDGVKTLYFDDRKLITGSLDKTIRVWNYITGECISTYRGHSDSVLSVDSYQKVIVSGSADKTVKVWHVESRTCYTLRGHTEWVNCV  427
        .  *.. .* * **..*.. * *** ***.**.*.**.***. ****.**. .*  ..*....      ...*** *****... .... . *.**..*** .

              WD-4             WD-5A
SCONBP  RVDTSSRTVFSASDDCTVRLWDLDTKTCIRTFHGHVGQVQQVVPLP-REFE--FEEH----DAECENDDLSTTSGDANPPSIQASMGLEPNAAYSQSSA-  566
SCON2P  HVDIKSRTVFSASDDTTIKLWDLDTRQVIRTYEGHVGHVQQVLILP-PEYE--PDEEVLNGASQDNQDAMSVSSGGSGSPSMSHAQIERAGSPGSHSSSH  516
MET30P  KLHPKSFSCFSCSDDTTIRMWDIRTNSCLKVFRGHVGQVQKIIPLTIKDVENLATDNTSDGSSPQDDPTMTDGADESDTPSNEQETVLDENIPY------  521
        ... .* . ** *** *...**. *.   .... ****.**... *.   . *   ...     ..  .. ... .......**    .       . .

                           WD-5B                         WD 6
SCONBP  -----------------FGTSF--DNGRAAPPRYMVTSALDSTIRLWETTTGRCLRTFFGHLEGVWALGADTLRIVSGAEDRMIKIWDPRTGKCERTFTG  647
SCON2P  NLLPSSLPSGDEDVRHLYGSAFVADESRPLPPRYFMTGGLDSTMRLWDSATGRCLRTLFGHLEGVWSLAGDTIRVISGANDGMVKTWEPRSGKCDATYTG  616
MET30P  -------------------------------PTHLLSCGLDNTIKLWDVKTGKCIRTQFGHVEGVWDIAADNFRIISGSHDGSIKVWDLQSGKCMHTFNG  590
                                       * .... .**.*..**.  **.*.** ***.****....*..*..**..*  .*.*.  ..***   *..*

                          WD-7
SCONBP  H-----------------SGPVTCIGLGDSRFATGSEDCEVRMYSFQS--   678
SCON2P  H-----------------CGPVTCVGLSDSLMASGSEDGTIRLHSFKPCR   649
MET30P  RRLQRETQHTQTQSLGDKVAPIACVCIGDSECFSGDEFGCVKMYKFDL-N   639
        .                 .*..*. ..**   .*.*   .....*.
```

Fig. 5 Alignment of the *Aspergillus nidulans* SCONB, *N. crassa* SCON2 and *Saccharomyces cerevisiae* Met30 proteins. The F-box, WD40 repeats, and nuclear localisation signals (NLS) are indicated

Bartnik 1997 and references therein). Such leader ORFs might play a role in the regulation of translation (for a review see Kozak 1991; Hinnebusch 1988; Oliviera and McCarthy 1995).

Computer analysis of the SCONB protein strongly suggests nuclear localisation. This is consistent with its regulatory role, direct or indirect, in gene expression which, by analogy with the *N. crassa* SCON2 protein (Paietta 1990) must occur at the transcriptional level. The SCONB protein shows a high similarity to the *S. cerevisiae* Met30 and *N. crassa* SCON2 proteins. The fact that the *scon-2*$^{+}$ gene complements the *sconB2* mutation and the *sconB* gene complements the *scon-2* mutation (J. Paietta, personal communication) indicates that this similarity extends to their physiological function.

All three proteins are involved in the regulation of sulphur metabolism, which might suggest that they form a subfamily of WD40 proteins specific for this area of metabolism, at least in fungi. These proteins share a strongly conserved N-terminal domain with the yeast CDC4, *Xenopus* BTrCP and mouse MD6 proteins (Kumar and Paietta 1995). This domain contains a newly described motif, the F-box (Bai et al. 1996) found in some cell cycle regulators such as human cyclin F and yeast CDC4; the latter also belongs to the WD40 family. Evidently the WD and F-box families overlap, in that there are proteins that contain both motifs. Bai et al. (1996) found that cyclin F and CDC4 bind, through the

Fig. 6a, b Northern blot analysis of the *sconB* transcript. **a** The wild-type strain grown in minimal minus sulphate medium supplemented with 0.05, or 0.25 or 5 mM L-methionine as a sole sulphur source. **b** The wild-type and *scon*-strains grown in minimal medium plus sulphate (2 mM) without (–), or with (+) the addition of 5 mM L-methionine

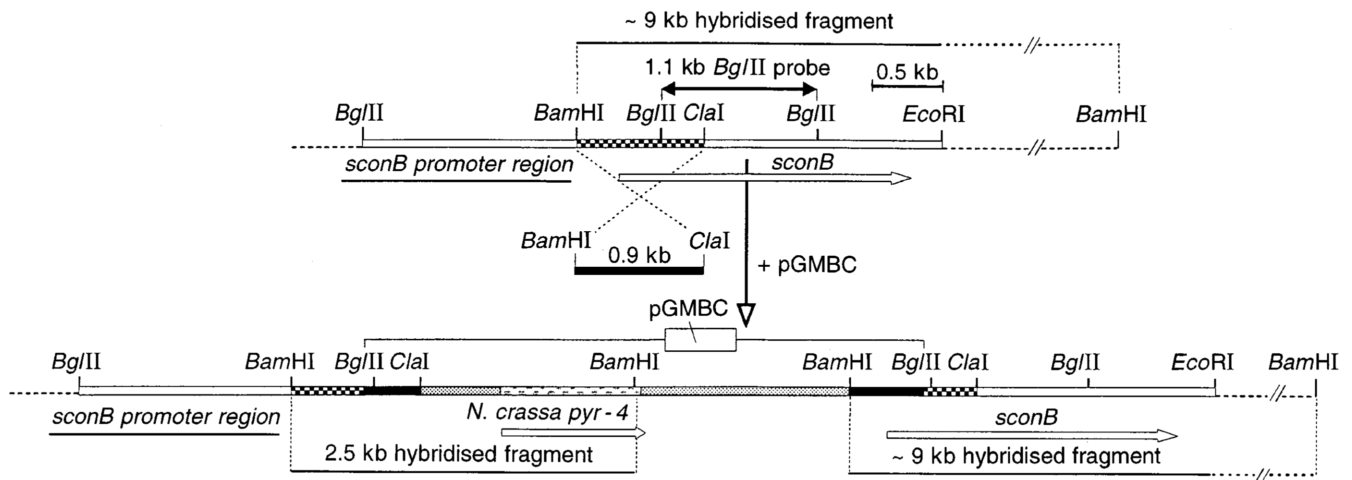

Fig. 7 Diagrammatic representation of the homologous recombination of plasmid pGMBC at the chromosomal *sconB* locus. The restriction map around the *sconB* locus with and without an integrated pGMBC plasmid is shown. The *Bam*HI restriction fragments that were detected by the use of an α-^{32}P-labelled 1.1-kb *Bgl*II fragment as a probe are indicated as hybridised fragments. The part of plasmid pGMBC (*Bam*HI-*Cla*I) homologous to the chromosomal *sconB* gene is marked by a *filled black bar*, and the corresponding region of the chromosomal *sconB* gene by a *checked bar*. *Arrows* indicate the orientations of genes

F-box, the Skp1 protein, which leads to ubiquitin-mediated destruction of the former. However, the authors suggest on the basis of available data that there may be Skp 1 associations with F-box proteins that result in events unrelated to proteolysis. In this connection it is worth mentioning that the *A. nidulans sconC*$^+$ gene, which we have recently cloned (Piotrowska et al., in preparation) encodes a protein homologous to Skp1. It is, therefore, tempting to speculate that the *sconB* and *sconC* gene products interact. This suggestion is supported by the fact that the plasmid carrying the *sconB* gene transforms the *sconC3* mutant to the wild-type phenotype (our unpublished results). This may be due to overproduction of the SCONB protein.

It is also possible that the SCONB, SCON2 and Met30 proteins have a larger range of action than regulation of sulphur metabolism, given that the screening methods with which the mutants were isolated might not have uncovered other phenotypes. In this connection it is worth noting that the Met30 and Skp1 genes are essential in *S. cerevisiae* (Thomas et al. 1995; Bai et al. 1996). The finding that the *sconB9* mutant, which lacks a translational initiation codon, and the *sconB* disruptant both act as suppressors of the *metA17* mutation strongly suggests that the *sconB* gene is non-essential for *A. nidulans*. The conclusion is supported by the properties of the newly isolated mutation, which leads to methionine auxotrophy. This mutation is epistatic to *sconB2* and no *sconB* transcript was detected in such a strain (unpublished results).

In spite of the structural and functional similarity between the *sconB* and *scon-2* genes there are marked differences in the regulation of their expression. The *sconB* gene is expressed under all sulphur conditions, while no *scon-2* transcript was found when cells were grown under repressing (i.e. high methionine) conditions (Paietta 1990). It is possible that there are differences in the organisation of the sulphur regulatory systems between *Aspergillus* and *Neurospora* that account for the observed differences in the regulation of *sconB* and *scon-2* gene expression. Some evidence supports this hypothesis. In *Neurospora* the *cys-3* gene has been identified (Marzluf and Metzenberg 1968), mutations in which lead to cysteine auxotrophy. Its product is a positive regulator of a set of structural genes involved in sulphur metabolism. In spite of many efforts and various experimental approaches, neither mutants equivalent to the *Neurospora cys-3* gene nor *cys-3*-homologous sequences have been isolated so far in *Aspergillus* (our unpublished results, and G. Turner, personal communication) although, interestingly, there are sequences in the *sconB* promotor strongly resembling CYS3-binding sites.

Another observation that suggests differences in sulphur regulatory systems between the two fungi is the finding of Katz et al. (1996) that the extracellular protease is not regulated by any of the *Aspergillus scon* genes, which contrasts with *Neurospora* where the synthesis of the enzyme is under the control of the *cys-3* and, indirectly, *scon-2* genes (Hanson and Marzluf 1973; Marzluf 1975).

Until other *Aspergillus scon* genes have been characterised, we shall not attempt to present a model of the sulphur regulatory system. In *Neurospora* it has been postulated that three regulatory genes, *scon-1*, *scon-2*, and *cys-3*, act in a hierarchical order in which the *scon-1* is a positive regulator of the *scon-2* gene, which, in turn, is a negative regulator of the *cys-3* gene (Kumar and Paietta 1995). The fact that *cys-3* mRNA is not detectable under repressing conditions in the wild-type strain, but can be detected in the *scon-2* mutant is consistent with this model. What does not seem consistent with the model is the fact that *scon-2* gene transcription is

repressed in mycelia grown in high sulphur medium. Under these conditions the *scon-2* gene product should be present to prevent expression of the *cys-3* gene. Thus there may be yet another gene in the sulphur regulatory cascade in *Neurospora*, situated between the *scon-2* and *cys-3* genes, which is a negative regulator of the latter and is itself positively regulated by the SCON2 protein. The existence of at least four *scon*-type genes in *Aspergillus* gives some support to this hypothesis.

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Appel RD, Bairoch A, Hochstrasser DF (1994) A new generation of information retrieval tools for biologists: the example of the ExPASy WWW server. Trends Biochem Sci 19:258–260

Bai C, Sen P, Hofmann K, Ma L, Goebl M, Harper JW, Elledge SJ (1996) SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. Cell 86:263–274

Brody H, Griffith J, Cuticchia AJ, Arnold J, Timberlake WE (1991) Chromosome-specific recombinant DNA libraries from the fungus *Aspergillus nidulans*. Nucleic Acids Res 19:3105–3109

Burton EG, Metzenberg RL (1972) Novel mutation causing derepression of several enzymes of sulfur metabolism in *Neurospora crassa*. J. Bacteriol 109:140–151

Caddick MX (1994) Nitrogen metabolite repression. Prog Ind Microbiol 29:323–354

Chomczyński P (1993) TRI Reagent – RNA, DNA protein isolation reagent. Manufacturer's protocol, Molecular Research Center, Cincinnati, Ohio

Clutterbuck AJ (1994) Linkage map and locus list. Prog Ind Microbiol 29:791–824

Fidel S, Doonan JH, Morris NR (1988) *Aspergillus nidulans* contains a single actin gene which has unique intron locations and encodes γ-actin. Gene 70:283–293

Fong HKW, Hurley JB, Hopkins RS, Miake-Lye R, Johnson MS, Doolittle RF, Simon MI (1986) Repetitive segmental structure of the transductin β subunit: homology with the CDC4 gene and identification of related mRNAs. Proc Natl Acad Sci USA 83:2162–2166

Fu YH, Marzluf GA (1990) *cys-3* the positive-acting sulfur regulatory gene of *Neurospora crassa*, encodes a sequence-specific DNA-binding protein. J Biol Chem 265:11942–11947

Fu YH, Paietta JV, Monnix DG, Marzluf GA (1989) *Cys-3*, the positive-acting sulfur regulatory gene of *Neurospora crassa* encodes a protein with a putative leucine zipper DNA binding protein. Mol Cell Biol 9:1120–1127

Gems DH, Clutterbuck JA (1993) Co-transformation with autonomously-replicating helper plasmids facilitates gene cloning from an *Aspergillus nidulans* gene library. Curr Genet 24:520–524

Gems DH, Johnstone IL, Clutterbuck AJ (1991) An autonomously replicating plasmid transforms *Aspergillus nidulans* at high frequency. Gene 98:61–67

Hanson MA, Marzluf GA (1973) Regulation of a sulfur-controlled protease in *Neurospora crassa*. J Bacteriol 116:785–789

Hinnebusch AG (1988) Novel mechanism for translational control in *Saccharomyces cerevisiae*. Trends Genet 4:169–174

Katz ME, Flynn PK, vanKuyk PA, Cheetham BF (1996) Mutations affecting extracellular protease production in the filamentous fungus *Aspergillus nidulans*. Mol Gen Genet 250:715–724

Kelly JM (1994) Carbon catabolite repression. Prog Ind Microbiol 29:355–368

Kozak M (1991) Structural features in eukaryotic mRNAs that modulate the initiation of translation. J Biol Chem 266:19867–19870

Kucharski R, Bartnik E (1997) The TBP gene from *Aspergillus nidulans* – structure and expression in *Saccharomyces cerevisiae*. Microbiology 143:1263–1270

Kudla B, Caddick MX, Langdon T, Martinez-Rossi NM, Bennett CF, Sibley S, Davies RW, Arst HN (1990) The regulatory gene *areA* mediating nitrogen metabolite repression in *Aspergillus nidulans*. Mutations affecting specificity of gene activation alter a loop residue of a putative zinc finger. EMBO J 9:1355–1364

Kumar A, Paietta JV (1995) The sulphur controller-2 negative regulatory gene of *Neurospora crassa* encodes a protein with β-transducin repeats. Proc Natl Acad Sci USA 92:3343–3347

Li Q, Marzluf GA (1996) Determination of the *Neurospora crassa* CYS3 sulphur regulatory protein consensus DNA-binding site: amino-acid substitutions in the CYS3 bZIP domain that alter DNA-binding specificity. Curr Genet 30:298–304

Martinelli SD (1994) Gene symbols. Media. Prog Ind Microbiol 29:825–833

Marzluf GA (1975) Regulation of gene expression in fungi. In: Copeland JC, Marzluf GA (eds) Regulatory biology. Ohio State University Press, Columbus, pp 196–242

Marzluf GA, Metzenberg RL (1968) Positive control by the *cys-3* locus in regulation of sulfur metabolism in *Neurospora*. J Mol Biol 33:423–437

Metzenberg RL, Kang S (1987) A simple strategy for identifying a single complementing clone in a 96-well microtiter dish. Fungal Genet Newslett 34:44–45

Nadolska-Lutyk J, Balińska M, Paszewski A (1989) Interrelated regulation of sulphur-containing amino-acid biosynthetic enzymes and folate-metabolizing enzymes in *Aspergillus nidulans*. Eur J Biochem 181:231–235

Nakai K, Kanehisa M (1992) A knowledge base for predicting protein localisation sites in eukaryotic cells. Genomics 14: 897–911

Natorff R, Balińska M, Paszewski A (1993) At least four regulatory genes control sulphur metabolite repression in *Aspergillus nidulans*. Mol Gen Genet 238:185–192

Neer EJ, Schmidt CJ, Nambudripad R, Smith TF (1994) The ancient regulatory – protein family of WD-repeat proteins. Nature 371:297–300

Oliviera CC, McCarthy JE (1995) The relationship between eukaryotic translation and mRNA stability. A short upstream open reading frame strongly inhibits translational initiation and greatly accelerates mRNA degradation in the yeast *Saccharomyces cerevisiae*. J Biol Chem 270:8936–8943

Paietta JV (1990) Molecular cloning and analysis of the *scon-2* negative regulatory gene of *Neurospora crassa*. Mol Cell Biol 9:3630–3637

Paszewski A, Grabski J (1974) Regulation of S-amino acids biosynthesis in *Aspergillus nidulans*. Mol Gen Genet 132:307–320

Paszewski A, Brzywczy J, Natorff R (1994) Sulphur metabolism. Prog Ind Microbiol 29:299–319

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Thomas D, Kuras L, Barbey R, Cherest H, Blaiseau P-L, Surdin-Kerjan Y (1995) Met30p, a yeast transcriptional inhibitor that responds to S-adenosylmethionine, is an essential protein with WD40 repeats. Mol Cell Biol 15:6526–6534

van der Voorn L, Ploegh HL (1992) The WD-40 repeat. FEBS Lett 307:131–134