Cloning and Characterization of scon-3
t, a New Member of the
Neurospora crassa Sulfur Regulatory System

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The sulfur regulatory system of Neurospora crassa consists of a group of sulfur-regulated structural genes (e.g., arylsulfatase) that are under coordinate control of the CYS3 positive regulator and sulfur controller (SCon) negative regulators. Here we report on the cloning of scon-3
t, which encodes a polypeptide of 171 amino acids and is a Skp1 family homolog. Repeat-induced point mutation of scon-3
t resulted in a phenotype of constitutive expression of arylsulfatase, a phenotype consistent with other sulfur controller mutants. Northern analysis indicated that, unlike other members of the sulfur regulatory system, expression of scon-3
t is not under the direct control of the CYS3 transcriptional activator. In particular, scon-3
t mRNA was detectable under sulfur repressing or derepressing conditions in a Δcys3 mutant. In yeast, Skp1p and an F-box protein binding partner are core constituents of a class of E3 ubiquitin ligases known as SCF complexes. The N. crassa negative regulator SCON2 contains an F-box motif essential for the operation of the sulfur regulatory system and suggests a role for an SCF complex in the N. crassa sulfur regulatory system. A crucial set of experiments, by using a yeast two-hybrid approach with confirming coimmunoprecipitation assays, demonstrated that SCON3 interacts with SCON2 in a manner dependent upon the F-box motif of SCON2. The protein-protein interaction detected between SCON2 and SCON3 represents the initial demonstration in a filamentous fungus of functional interaction between putative core components of a SCF complex.

The sulfur regulatory system of Neurospora crassa is composed of a set of trans-acting regulatory genes and a group of structural genes that encode enzymes used in the uptake and assimilation of a variety of sulfur compounds (20, 27). When N. crassa cultures are grown under conditions of sulfur limitation (i.e., derepressing conditions), then the entire group of sulfur-related genes is coordinately expressed (20). The structural genes involved encode for arylsulfatase, choline sulfatase, choline sulfate permease, methionine permease, sulfate permeases I and II, and an extracellular protease (20, 27). Essentially, the sulfur regulatory system monitors the cellular sulfur status and expresses the sulfur structural genes as needed to ensure an adequate internal supply of sulfur, while subjecting the sulfur-related genes to repression under conditions of sulfur sufficiency.

The positive regulator of sulfur-controlled gene expression in N. crassa is CYS3, a basic region-leucine zipper protein that functions as a DNA-binding transcriptional activator (20, 24, 25). cys-3
t gene expression is controlled in part by a positive feedback loop involving the CYS3 protein and in part by the negative regulatory sulfur controller genes, scon-1
t and scon-2
t (25, 26). scon-1 and scon-2 mutants show constitutive expression of cys-3
t and the sulfur structural genes (26). scon-2
t has been cloned and found to be expressed only under conditions of sulfur limitation (16, 26). The SCON2 protein contains two domains implicated in protein-protein interactions: (i) a region of six WD40 (or β-transducin) repeats and (ii) a motif that we originally termed the “N-terminal domain” (16). The N-terminal domain is now referred to as the F-box motif (1, 12).

F-box motifs are found in a large number regulatory proteins (12, 14, 28). In Saccharomyces cerevisiae, F-box proteins have been shown to assemble with Skp1p,Cdc53p, and Rbx1p to form a complex known as the SCF (for Skp1p/Cdc53p/F-box) (28, 38). SCF complexes act as E3 ubiquitin ligases to target proteins for ubiquitin-mediated proteolysis. SCF complexes have been implicated in diverse processes, including cell cycle progression, glucose sensing, and developmental processes (7, 10, 12, 14, 28, 43). The F-box protein component is responsible for SCF complex target specificity, with the F-box motif thought to be responsible for the interaction with Skp1p (1, 29, 38). In yeast, Met30p is the F-box protein that is homologous to SCON2 (41).

Previous work has demonstrated that the F-box domain of SCON2 is required for SCON2’s role as a regulator within the N. crassa sulfur control system (17). The ability of SCON2 to function as a negative regulator of sulfur-related gene expression may be due to the ability of SCON2 to form a functional SCF complex in N. crassa via protein-protein interactions between the F-box motif of SCON2 and the N. crassa homolog of yeast Skp1p. Skp1 homologs, both designated sconC, have been found in Aspergillus nidulans (31) and Microsporum canis (GenBank AF408428) but have not been examined for possible molecular interactions with other putative SCF complex constituents.

We report here the isolation and analysis of scon-3
t, a new member of the N. crassa sulfur regulatory system. scon-3
t encodes a protein showing strong homology to the Skp1p family of proteins. Repeat-induced point mutation (RIP) of the scon-3
t locus resulted in the production of mutants having a phenotype of constitutive expression of arylsulfatase, confirm-
ing the putative role of scon-3 in sulfur gene regulation. Importantly, we found that SCON3 interacted with SCON2 in vivo as detected by two-hybrid assays and in vitro by using coimmunoprecipitation. Further, the interaction between SCON3 and SCON2 was found to be dependent upon the F-box motif of SCON2. The data presented supports a role for an SFC complex partially comprised of SCON2 and SCON3 as an crucial component required for the regulated expression of sulfur-related genes in \( N. \) \textit{crassa}.

**MATERIALS AND METHODS**

**Strains and culture conditions.** The \( N. \) \textit{crassa} strains \( \Delta gnt-3 (18–4) \) and scon-2 (PSD272) have been described previously (25, 26). 74OR32-1a was used as the wild-type strain for these studies. \( N. \) \textit{crassa} cultures were grown at 25°C on minimal Vogel medium (6) with supplements as required. Cells were grown under sulfur-repressing and sulfur-depressing conditions by using Vogel-minus-sulfur medium supplemented with 5.0 mM methionine and 0.25 mM methionine, respectively (25). The assay of arylsulfatase activity, which is normally derepressed under sulfur-limited growth conditions (27), was used to confirm the presence of sulfur derepression or repression. Arylsulfatase assays were performed by monitoring \( p \)-nitrophenol liberation at 405 nm from \( p \)-nitrophenyl sulfate according to standard methods (21, 27).

Crosses were carried out with cornmeal agar or Westergard-Mitchell medium (6). Homokaryons were isolated by growth on Westergard-Mitchell medium with 1 mM iodate, harvesting of the microconidia, and filtration through 5-μm (porosity) Millipore filters (8). For the \( \Delta gnt-3 \) strain, mating was confirmed by the formation of yeast colonies on sulfur minimal media (SD-Leu-Trp) to select for expression of TRP1 and LEU2 (2). Double recombinants were then isolated and screened for induction of HIS3 expression, as measured by growth on minimal media (SD-Leu-Trp-His) containing 20 mM 3-amino-1,2,4-triazole. Double recombinants were also assayed for expression of \( lacZ \) by growth on minimal medium (SD-Leu-Trp) containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) with sucrose as the sole carbon source.

**In vitro Transcription and Translation.** PCR was used to produce templates for transcription that carried a T7 promoter and fused coding region specifying an epitope for later use in coimmunoprecipitation experiments. The oligonucleotide primers 5'-AAATTGTAATACGACTCACTATAGGGGCGAGCCGCCCA CATCTGAGGAAGCAAGAAGCTGATCTCGCCGACAGGGC-3' and 5'-CCCGTCGACCCTCACTGCCGACAGGGC-3' were used to amplify the \( \Delta gnt-3 \) gene under previously described conditions (16). The resulting hybrid was subcloned into pGEM7Zf(+) containing the \( \Delta gnt-3 \) gene under previously described conditions (16). The resulting hybrid was extended by use of the Klenow fragment of Escherichia coli DNA polymerase I, and the extended primer was cut with Apol. The resulting 23-bp probe was hybridized to 20 μg of \( N. \) \textit{crassa} poly(A)-mRNA. The probe-mRNA hybrids were then digested with 100 U of \( S. \) \textit{cerevisiae} nuclease at 30°C as described previously (16).

**Transcription start site determination.** The \( 5' \) end of the \( \Delta gnt-3 \) transcript was mapped by primer extension and \( S1 \) nuclease analysis. For primer extension, the \( \Delta gnt-3 \) oligonucleotide \( 5'-GATTGTGGGCGATGTTCTGCGTTGAGG-3' \) was used as a probe in a PCR amplified from \( S. \) \textit{cerevisiae} cDNA by PCR with \( \Delta gnt-3 \) as a template, in a PCR amplification reaction. The resulting PCR fragment was cloned into the yeast two-hybrid activation domain (AD) vector pGBD-CAM to create pBD-scon3. The oligonucleotide primers 5'-CCGGAATTCATGTCGTCCGTCCTCAT-3' and 5'-AAC ACTATAGGGCGAGCCGCCACCATGTACCCATACGACGTTCCAGATTACGAGGGCACAGGTGACGGGC-3' were used to amplify the \( \Delta gnt-3 \) gene under previously described conditions (16). The resulting hybrid was extended by use of the Klenow fragment of Escherichia coli DNA polymerase I, and the extended primer was cut with Apol. The resulting 23-bp probe was hybridized to 20 μg of \( N. \) \textit{crassa} poly(A)-mRNA. The probe-mRNA hybrids were then digested with 100 U of \( S. \) \textit{cerevisiae} nuclease at 30°C as described previously (16).

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precipitated, washed, electrophoresed, and fixed according to the manufacturer’s specification (Clontech).

Nucleotide sequence accession number. The genomic DNA sequence of *scon-3/H11001* has been deposited in GenBank (AF402682). The nucleotide sequence of the RIP-generated 12b-1 allele of *scon-3/H11001* has also been deposited as AY135642.

RESULTS

Sequence and organization of the *scon-3* gene. The *scon-3* gene was isolated by using mixed oligonucleotide primed PCR amplification to generate a partial gene segment, followed by hybridization screening of a *N. crassa* genomic library to identify a clone containing the entire gene. The isolated and sequenced *scon-3* gene and flanking 5' region contained within a 2,581-bp genomic segment is presented in Fig. 1. The *scon-3* gene encodes a polypeptide of 171 amino acids with a molecular mass of 19.9 kDa. Isolation and sequencing of *scon-3/H11001* cDNA clones revealed that the coding region is interrupted by two introns of 80 and 98 bp. Nucleotide sequences at the exon-intron boundaries, as well as the internal lariat sequence conform to established *N. crassa* consensus sequences.
Sequence analysis of SCON3. Database searches with SCON3 revealed highly significant matches to the members of the Skp1 protein family. An alignment of SCON3 with the *M. canis* and *A. nidulans* SCONC proteins, as well as other Skp1 family homologs, is shown in Fig. 3. SCON3 shows the highest homology (87%) to SCONC of *M. canis* (GenBank no. AF408428). The conservation of residue identity relative to *N. crassa* for the Skp1 family proteins presented in Fig. 3 ranges from 70 to 87%, excluding *S. cerevisiae*. *S. cerevisiae* Skp1p differs from the others in the grouping by having an interior 32-amino-acid segment that is not observed in the other proteins. As with all of the proteins shown in Fig. 3, SCON3 contains a PEST sequence (33), which would allow for targeting of the protein for degradation and rapid turnover. An ATP/GTP-binding motif, termed the P-loop (35), is also present in SCON3 and other members of the Skp1 protein family (Fig. 3).

Induction of *scon-3* mutants by RIP. RIP can be used to efficiently induce targeted mutations in *N. crassa* (4, 6, 36). Multiple point mutations in a target sequence can be obtained by inserting into a wild-type strain an extra copy of the gene for which a functional disruption is required and then crossing the transformant to the wild type (36). A RIP experiment was conducted for the *scon-3* locus by introducing one or more copies of *scon-3* into the wild type by transformation and then crossing the transformant with the wild type. The progeny were then analyzed for any phenotypic alterations compared to the wild type. To ensure that no general class of sulfur regulatory mutants were overlooked, methionine supplementation was used for the germination and culturing of cross-progeny. Screening of the progeny revealed no sulfur auxotrophs but did reveal progeny showing a phenotype of constitutive expression of arylsulfatase (Table 1).

Constitutive expression of sulfur-related genes (e.g., arylsulfatase) is the typical phenotype of mutants given the sulfur controller designation (see Table 1). The RIP progeny were examined for any variations from wild-type in terms of (i) growth or morphology (i.e., variations in colony size or shape and variations in hyphal or conidial morphology) and (ii) fertility in crosses, but no mutant phenotypes were observed. The observed RIP phenotype is therefore similar to that seen in *N. crassa scon-1* (36-18), *scon-2* (PSD272) (see Table 1), and *A. nidulans* sconC mutants (31). Only conditional yeast mutants (skp1-11 and skp1-12) have been found that show defective sulfur-related regulation of MET25 (homocysteine synthase) (29) but have an pleiotropic range of effects (e.g., cell cycle arrest) (1, 5, 29). Finally, when the *scon-3* RIP mutants were transformed with the wild-type *scon-3* gene normal sulfur gene regulation was restored (Table 1), demonstrating the specificity of the RIP effect on the *scon-3* gene.

To further analyze the RIP-generated mutant, the molecular nature of the 12b-1 allele was determined by cloning and subsequent nucleotide sequencing. The nucleotide sequence of 12b-1 (GenBank no. AY135642) demonstrates clear evidence of RIP-induced mutations, which include a Gln78Stop mutation within the coding region, as well as Gln40Lys, Gly43Ser, and Arg63Gln mutations occurring prior to the introduced stop codon. The RIP-introduced stop codon results in a predicted truncated protein of 77 amino acids compared to the full length of 171 amino acids for SCON3. Although the mutations severely alter the protein and specifically generate the sulfur-related phenotype, we cannot rule out the possibility of partial functionality, particularly since there is *scon-3* transcript detectable by Northern blot analysis (data not shown).

Analysis of *scon-3* gene expression. *scon-3* transcript levels were initially assayed in wild-type *N. crassa* grown on high and low levels of sulfur (i.e., repressing and derepressing conditions, respectively). Northern blots were prepared and probed with the cloned *scon-3* gene. In the blot shown in Fig. 4 (lanes 1 and 2), a 1.1-kb message showed hybridization to the *scon-3* probe and was clearly detectable under both sulfur repressing and derepressing conditions. The steady-state level of *scon-3* transcript, however, showed a substantially higher level under sulfur derepressing conditions (approximately twofold more compared to the sulfur repressing condition based on phosphorimager quantitation of the blot). Typically, we have found tightly regulated expression for the other compo-

**FIG. 2.** Determination of the transcription start site for *scon-3*.

(A) S1 nuclease analysis. The size of the S1 nuclease-protected fragment was determined by comparison with a DNA sequencing reaction generated with the same primer and template used to produce the S1 probe. An arrow indicates the protected fragment corresponding to a start site at nucleotide −72 (Fig. 1). (B) Primer extension. Primer extension products were sized by comparison against a deoxy sequencing reaction generated with the same primer and with pSCON3 as a template. An arrow indicates the major primer extension product corresponding to a transcriptional initiation site at −72.
The components of the sulfur regulatory system that have been examined (e.g., ars-1/H11001, cys-3/H11001, and scon-2/H11001) (26). The scon-2/H11001 gene included as a control and used to reprobe the blot in Fig. 4 demonstrates the expected high expression under low-sulfur conditions and no expression under high-sulfur conditions. A further experiment examined the level of scon-3/H11001 transcript in the cys-3 mutants grown under sulfur derepressing and repressing conditions. The scon-3/H11001 transcript was detectable under derepressing conditions in cys-3 as lane 3 in Fig. 4 demonstrates, but the level of scon-3/H11001 was twofold lower under derepressing conditions compared to wild-type based on the phosphorimager data. In sharp contrast, the level of scon-3/H11001 transcript was highly elevated in cys-3 grown under repressing conditions compared to wild-type (an approximate twofold increase). The unusual pattern of expression for scon-3/H11001 transcript levels in cys-3 was consistently observed between mRNA preparations, and the high sulfur response is unlike that observed for other known genes in the N. crassa sulfur regulatory system (i.e., cys-3 mutants show highly reduced transcript levels of sulfur-related genes under both derepressing and repressing conditions).

All of the Northern blot experiments conducted used the constitutively expressed am/H11001 gene as a control probe for the

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<td>scon-3up(12b-1)</td>
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*Expressed as nanomoles per minute per milligram of total protein.

a High-sulfur medium with 5.0 mM methionine.
b Low-sulfur medium with 0.25 mM methionine.
c Isolate of scon-3up(12b-1) transformed with scon-3H11001.
Northern blots to ensure that the bulk mRNA levels in the samples were comparable.

**Protein-protein interaction between SCON2 and SCON3.** The SCON2 and SCON3 proteins represent major constituents of a hypothetical SCF complex involved in sulfur-related gene regulation in *N. crassa*. Initial screening for in vivo interactions between SCON2 and SCON3 were carried out by using the yeast two-hybrid system with BD and AD derived from the GAL4 system (2, 30). Full-length SCON3 and full-length SCON2, as well as truncated versions of SCON2 with either a deletion of the F-box domain or WD-40 domain, were assayed for interactions. A vector, pBDscon3, encoding the wild-type SCON3 protein was tested against pAD vectors containing the F-box to interact with Skp1 homologs, whereas the WD-40 eliminated the interaction between SCON2 and SCON3. The result is consistent with the putative role of the F-box to interact with Skp1 homologs, whereas the WD-40 domain is thought to be involved in the protein-protein interaction with the particular target protein of the SCF complex. The entire range of control combinations tested were negative in these experiments (Fig. 5).

A second set of assays was done by using in vitro coimmunoprecipitation (30) to provide confirmation of the interactions observed between SCON2 and SCON3 in the yeast two-hybrid system. For these experiments, a T7 promoter and tag sequence encoding an epitope (either Myc or HA) were added to the SCON2 and SCON3 coding sequence by PCR with appropriately designed primers. The PCRs produced the templates used for coupled transcription and translation. SCON3 was generated in its full-length with a HA tag, whereas SCON2 was generated in full-length, ΔF-box, and ΔWD-40 forms all with a Myc tag. In each case, proteins of the expected sizes were produced with the transcripts translated separately and examined by polyacrylamide gel electrophoresis. After transcription and translation, the SCON2 and SCON3 products were incubated in various combinations, and antibodies to the HA and Myc epitopes were used to assay whether the proteins were interacting and could be coimmunoprecipitated or not. In the experiments shown in Fig. 6, coimmunoprecipitation was seen only in the following combinations: (i) full-length SCON3 plus full-length SCON2 and (ii) full-length SCON3 plus SCON2 with WD-40 region deleted. No coimmunoprecipitation was seen when the F-box was deleted from SCON2 and tested against SCON3. In addition, all control combinations were negative for coimmunoprecipitation demonstrating the specificity of the the assay. The protein-protein interactions detected by the coimmunoprecipitation assays agreed exactly with the yeast two-hybrid assays.

**FIG. 5.** In vivo association of SCON2 and SCON3. The GAL4 AD, an AD fusion with full-length SCON2 (residues 1 to 650), an AD fusion with the N-terminal domain of SCON2 (residues 1 to 250), or an AD fusion with the C-terminal domain of SCON2 (residues 265 to 650) were tested for their ability to interact with a GAL4 BD fusion with SCON3 in the yeast two-hybrid assay. Each patch represents an independent transformation of the yeast YRG-2 host strain expressing the indicated proteins. The various pAD-scon2 constructs or the pAD control were cotransformed with pBD-scon3 into the YRG-2 yeast host strain. Interaction between fusion proteins was assayed by their ability to induce expression of β-galactosidase on SD-Leu-Trp media augmented with X-Gal and having sucrose as the sole carbon source. Note that YRG-2 cotransformed with pBD-scon3 and either pAD-scon2 or pAD-scon2ΔWD40 tested positive for expression of lacZ, indicating the presence of protein-protein interaction. In contrast, yeast cotransformed with pBD-scon3 and pAD-scon2Δbox tested negative for induction of lacZ expression. Controls, including pAD with pBD (not shown), were all negative for interactions in the assays.
of Skp1 are known and result in cell cycle arrest in G1 phase, whereas other alleles result in cell cycle arrest in G2 phase (1, 5, 29). The conditional yeast Skp1 mutants, skp1-11 and skp1-12, show a range of pleiotropic effects, including defective sulfur gene regulation (e.g., MET25) (29). Within the Skp1 family, however, the only currently known mutations that result solely in a phenotype of altered expression of sulfur-related genes, as represented here by arylsulfatase activity levels, are at the sconC locus of A. nidulans (31) and the N. crassa RIP-generated mutants of scon-3 that we report here. The RIP-induced scon-3 mutant phenotype (which may not represent the null phenotype) is similar to the observed phenotype of other sulfur controller mutants (e.g., scon-1 and scon-2) (Table 1). The lack of pleiotropic effects may indicate an evolutionary divergence of function, but we must include a caveat that other more severe or lethal phenotypes may not have been recoverable from the screenings. In this regard, many eukaryotes have multiple versions of skp1-like proteins (e.g., Cae-

exhibits a high degree of protein sequence conservation (Fig. 3). SCON3 is most similar to two fungal homologs: SconC in the dermatophyte M. canis (87%; GenBank no. AF408428) and SCONC in A. nidulans (31) (85%). Members of the Skp1 family have been shown to be essential in various cellular functions ranging from glucose sensing to cell cycle progression (12, 13, 18, 34, 44). In S. cerevisiae, only conditional alleles of Skp1 are known and result in cell cycle arrest in G1 phase, whereas other alleles result in cell cycle arrest in G2 phase (1, 5, 29). The conditional yeast Skp1 mutants, skp1-11 and skp1-12, show a range of pleiotropic effects, including defective sulfur gene regulation (e.g., MET25) (29). Within the Skp1 family, however, the only currently known mutations that result solely in a phenotype of altered expression of sulfur-related genes, as represented here by arylsulfatase activity levels, are at the sconC locus of A. nidulans (31) and the N. crassa RIP-generated mutants of scon-3 that we report here. The RIP-induced scon-3 mutant phenotype (which may not represent the null phenotype) is similar to the observed phenotype of other sulfur controller mutants (e.g., scon-1 and scon-2) (Table 1). The lack of pleiotropic effects may indicate an evolutionary divergence of function, but we must include a caveat that other more severe or lethal phenotypes may not have been recoverable from the screenings. In this regard, many eukaryotes have multiple versions of skp1-like proteins (e.g., Cae-

FIG. 6. Coimmunoprecipitation of SCON2 and SCON3. The proteins were transcribed and translated in vitro from PCR products. Prior to transcription and translation, c-Myc epitope and T7 promoter sequences were added upstream of the scon-3 coding region via PCR, whereas an HA epitope tag and T7 promoter region were added upstream of the scon-2 constructs via PCR. [35S]methionine was included in the translation mixture to generate the radiolabeled products: SCON3-Myc, SCON2-HA, SCON2 WD40-HA, and SCON2 Fbox-HA. After translation, coimmunoprecipitation was carried out as described in Materials and Methods. After elution from the protein A-beads, 10 μl of the immunoprecipitate was loaded onto a sodium dodecyl sulfate–15% polyacrylamide gel. Lane 1, SCON3 plus c-Myc antibody; lane 2, SCON3 plus HA antibody; lane 3, SCON2 plus c-Myc antibody; lane 4, SCON2 plus HA antibody; lane 5, SCON2 WD40 plus c-Myc antibody; lane 6, SCON2 WD40 plus HA antibody; lane 7, SCON2 Fbox plus c-Myc antibody; lane 8, SCON2 Fbox plus HA antibody; lane 9, SCON3 plus SCON2 plus c-Myc antibody (note that both proteins were precipitated); lane 10, SCON3 plus SCON2 plus HA antibody (note that both proteins were precipitated); lane 11, SCON3 plus SCON2 WD40 plus c-Myc antibody (note that both proteins were precipitated); lane 12, SCON3 plus SCON2 WD40 plus HA antibody (note that both proteins were precipitated); lane 13, SCON3 plus SCON2 Fbox plus c-Myc antibody (note that only the SCON2 F-box band is present). Lanes 9 to 12 demonstrate coimmunoprecipitation and protein-protein interactions between SCON3 and SCON2, whereas lanes 13 and 14 demonstrate the necessity of an F-box in SCON2 for the interaction with SCON3 to occur.

norhabditis elegans has 21 skp1-like proteins) (23). Extensive searches of the recently available N. crassa genome (release 3; http://www-genome.wi.mit.edu) have revealed, however, only the presence of the Skp1 homolog reported in the present study.

In A. nidulans, sconC transcript levels are not significantly affected by sulfur concentration in the wild type (31). A. nidulans metR1 mutants show an increased level of sconC transcript under sulfur derepressing conditions (31). Based upon these data it has been suggested that, in A. nidulans, METR may act as a negative regulator of sconC expression (31). Given the fact that the sulfur regulatory systems of N. crassa and A. nidulans show many similarities (22), including homology between the positive regulators CY3S and METR, we investigated the possibility that CY3S might influence the expression of SCON3 in N. crassa. We observed detectable expression of scon-3+ under repressing and derepressing conditions in both wild-type and Δcys-3 strains. In wild-type N. crassa the steady-state level of scon-3+ transcript was substantially higher under sulfur derepressing conditions, an expression pattern similar to what has been observed for other components of the N. crassa sulfur regulatory system and perhaps suggesting positive transcriptional control of scon-3+ by CY3S. However, in repeated experiments, the Δcys-3 strain demonstrated an unusual pattern of scon-3+ expression. Under low-sulfur conditions, expression
was severely reduced, whereas under high-sulfur conditions expression was elevated. As a control, reprobing the blots with scon-2+ (Fig. 2) demonstrated the expected regulated expression for that gene. The Δcys-3 data are not in agreement with the expected pattern of expression for a gene under control of CYS3, nor do they agree with a model where regulation of CYS3 acts as a negative regulator of scon-3− expression. The unusual expression pattern suggests several possibilities, where scon-3+ may (i) encode a more stable component of the SCF complex; (ii) be subject to posttranscriptional controls; or (iii) be involved in other, as-yet-undefined, cellular functions.

Skp1p is a major component in two evolutionarily conserved ubiquitin-conjugating E3 complexes: the anaphase-promoting complex and SCF complexes (14, 14, 28). SCF complexes were first identified in S. cerevisiae and have subsequently been identified in a number of eukaryotic organisms (14). SCF complexes are composed of at least three common subunits: Skp1p, Cdc53p, and Rbx-1p. SCF complexes also contain a modular ubiquitin-conjugating E3 complexes: the anaphase-promoting complex (31). SCF complexes were thought to target specific substrates for ubiquitin-mediated proteolysis (38). An SCF complex with Grr1p as the F-box component has been shown to target Cln2p for rapid degradation (18, 39). Another SCF complex, with MET30 as the F-box constituent is involved in the repression of the Met25 gene (29, 40). The yeast Met30 gene (41) is a homolog of the Aspergillus nidulans F-box constituent is involved in the repression of the Met25 gene expression was elevated. As a control, reprobing the blots with Cdc53p, and Rbx-1p. SCF complexes also contain a modular subunit, an F-box protein, which provides the SCF complex with substrate specificity (12, 28). Each SCF complex is thought to target specific substrates for ubiquitin-mediated proteolysis. SCF complexes were first identified in S. cerevisiae and have subsequently been identified in a number of eukaryotic organisms (14). SCF complexes are composed of at least three common subunits: Skp1p, Cdc53p, and Rbx-1p. SCF complexes also contain a modular subunit, an F-box protein, which provides the SCF complex with substrate specificity (12, 28). Each SCF complex is thought to target specific substrates for ubiquitin-mediated proteolysis. An SCF complex with Grr1p as the F-box component has been shown to target Cln2p for rapid degradation (18, 39). Another SCF complex, with MET30 as the F-box constituent is involved in the repression of the Met25 gene (29, 40). The yeast Met30 gene (41) is a homolog of the Aspergillus nidulans F-box constituent is involved in the repression of the Met25 gene

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