Cloning and Characterization of *scon-3* <sup>+</sup>, 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* <sup>+</sup>, which encodes a polypeptide of 171 amino acids and is a Skp1 family homolog. Repeat-induced point mutation of *scon-3* <sup>+</sup> 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* <sup>+</sup> is not under the direct control of the CYS3 transcriptional activator. In particular, *scon-3* <sup>+</sup> mRNA was detectable under sulfur repressing or derepressing conditions in a Δcys-3 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-<sup>+</sup> 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* <sup>−</sup> and *scon-2* <sup>−</sup> (25, 26). *scon-1* and *scon-2* mutants show constitutive expression of cys-<sup>+</sup> and the sulfur structural genes (26). *scon-2* <sup>+</sup> 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* <sup>+</sup>, a new member of the *N. crassa* sulfur regulatory system. *scon-3* <sup>+</sup> encodes a protein showing strong homology to the Skp1p family of proteins. Repeat-induced point mutation (RIP) of the *scon-3* <sup>+</sup> locus resulted in the production of mutants having a phenotype of constitutive expression of arylsulfatase, confirm-
ing the putative role of scon-3\textsuperscript{+} 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 SCF complex partially comprised of SCON2 and SCON3 as an crucial component required for the regulated expression of sulfur-related genes in *N. crassa*.

**MATERIALS AND METHODS**

**Strains and culture conditions.** The *N. crassa* strains Δcys-3 (18–4) and scon-2 (PSD272) have been described previously (25, 26). 74OR23-1a was used as the wild-type strain for these studies. *N. crassa* cultures were grown at 25°C on minimal Vogel medium (6) with supplements as required. Cells were grown under sulfur-repressing and sulfur-derepressing conditions by using Vogel-mimosulfur 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-limiting 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*-nitrophenylsulfate according to standard methods (21, 27).

Crosses were carried out with congeneric agar or Westergard-Mitchell medium (6). Homokaryons were isolated by growth on Westergard-Mitchell medium with 1 mM isoleucine, harvesting of the microconidia, and filtration through 5-μm (pore size) Millex filters (8).

**Plasmid constructs and gene cloning.** PCR with the primers 5′-AAAGAATTC

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\begin{align*}
\text{TTATGAGTGGTAGCAGTGTGAGGTCG-3′} & \quad \text{and} \quad 5′-\text{AAGGAATTCRTNCGCCCATCTTIRTCCG(C/G)G(C/G)C(G)-3′} \\
\text{(R is purine and Y is pyrimidine) and wild-type N. crassa chromosomal DNA as a template was used to} & \quad \text{ampify a 300-bp portion of the scon-3 gene. The primers were designed by using} \\
\text{highly conserved regions determined from alignments of available Skp} & \quad \text{family protein sequences (i.e., *S. cerevisiae*, *A. nidulans*, and *Homo} \\
\text{sapiens*), as well as incorporation of established N. crassa codon preferences.} & \quad \text{The PCR product was digested with EcoRI and inserted into pSPORT1 to generate pSC3.} \\
\text{pSC3 was used to probe a} & \quad \text{The following constructs were used to determine the cDNA sequence of} \\
\text{J1 genomic library (obtained from the Fungal} & \quad \text{scon-3 and generate fusion constructs for the analysis of interactions between} \\
\text{Genetics Stock Center, Kansas City, Kans.) by plaque hybridization and a hy} & \quad \text{SCON3 and SCON2. The oligonucleotide primers 5′-CGGAATTCTAGGCGCCCGAGAGAACGACGAACGTGCC-3′} \\
\text{bridizing clone, designated} & \quad \text{were used to amplify scon-3 as bended from} \\
\text{Δhscon-3 was isolated. Subsequently,} & \quad \text{inserted into pCB1004 to make pSCON3, which was used for primer} \\
\text{Δhscon-3 was} & \quad \text{extension and RIP-related experiments. pSCON3 has been} \\
\text{sequenced by the dideoxy method.} & \quad \text{isolated and sequenced in a 1:1 mixture of phenol-chloroform-isoamyl alcohol (49:49:2)} \\
\text{Total RNA was isolated by the} & \quad \text{and 12b-1 was subsequently cloned by PCR and sequenced by the dideoxy method.} \\
\text{RNA isolation and Northern blot analysis. Total RNA was isolated by the} & \quad \text{PCR-amplified fragment was used to determine the cDNA sequence, as previously} \\
\text{phenol extraction procedure of Reintert et al. (32) as modified by Paietta (26).} & \quad \text{described (15).}
\end{align*}
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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 predicted polypeptide sequence is given below the nucleotide sequence in single-letter code. The 5' splice sites, the 3' splice sites, and the internal lariat sequences within the introns are underlined. The sequence corresponding to the N. crassa transcriptional initiation site consensus is boxed. A vertical arrow indicates the major transcriptional initiation site at -72. A potential CYS3 binding site within the scon-3* promoter region is bracketed.

FIG. 1. Nucleotide and predicted amino acid sequence of the scon-3* gene. The sequence is shown from 1,547 nucleotides upstream of the translation start codon to 339 nucleotides downstream of the stop codon (indicated by an asterisk). The nucleotides are numbered relative to the initiator ATG codon. The predicted polypeptide sequence is given below the nucleotide sequence in single-letter code. The 5' splice sites, the 3' splice sites, and the internal lariat sequences within the introns are underlined. The sequence corresponding to the N. crassa transcriptional initiation site consensus is boxed. A vertical arrow indicates the major transcriptional initiation site at -72. A potential CYS3 binding site within the scon-3* promoter region is bracketed.

Nucleotide sequence accession number. The genomic DNA sequence of scon-3* has been deposited in GenBank (AF402682). The nucleotide sequence of the RIP-generated 12b-1 allele of scon-3* has also been deposited as AY135642.
An arrow indicates the protected fragment corresponding to a transcriptional initiation site at nucleotide −72 (Fig. 2). The initiation site is found immediately adjacent to the sequence TCACATAG, which closely matches the transcription initiation site consensus determined for N. crassa (3). The promoter region does not contain any sequences exactly matching the established consensus binding site for the sulfur regulatory system transcription activator, CYS3 (19, 37). A single site, at −1100, has a two-base mismatch versus the consensus sequence (ATGRYRYCAT) and represents, based on our prior binding site studies (37), a weak potential site for CYS3. In comparison, the N. crassa scon-2” regulatory gene, which is clearly under CYS3 control based on Northern blot analysis, has four putative CYS3 binding sites (17).

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 a 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 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”RIP 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 two-fold 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-
nents 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 /H9004 cys-3 mutant grown under sulfur derepressing and repressing conditions. The scon-3/H11001 transcript was detectable under derepressing conditions in /H9004 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 /H9004 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 /H9004 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., /H9004 cys-3 mutants show highly reduced transcript levels of sulfur-related genes under both derepressing and repressing conditions). scon-2/H11001, again acting as a control, demonstrates no expression in /H9004 cys-3 as expected (Fig. 4).

All of the Northern blot experiments conducted used the constitutively expressed am/H11001 gene as a control probe for the...
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 three versions of SCON2(wild-type, ΔF-box, and ΔWD-40). Two significant interactions, detectable by the expression of lacZ activity, were observed: (i) pBDscon3 (wild type) showed interaction with pADscon2 (wild type), and (ii) pBDscon3 (wild type) showed interaction with pADscon2ΔWD40 (Fig. 5). In demonstration of the importance of the F-box domain for SCON2-SCON3 interactions, the experiments showed no interaction present between pBDscon3(wild-type) and pADscon2ΔF-box. Although deletion of the WD-40 domain in SCON2 had no detectable effect on the interaction present, the deletion of the SCON2 F-box 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 confirm 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 assay. The protein-protein interactions detected by the coimmunoprecipitation assays agreed exactly with the yeast two-hybrid assays.

**DISCUSSION**

We report here on the cloning and characterization of *sulfur controller-3*, which encodes a protein that acts as a negative regulator in the *N. crassa* sulfur regulatory system and belongs to the Skp1 gene family. The Skp1 grouping, including SCON3,
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 A. nidulans, 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., Caenorhabditis 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 (31). Based upon these data it has been suggested that, in N. crassa, 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 (31). Based upon these data it has been suggested that, in N. crassa, 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 (31). Based upon these data it has been suggested that, in N. crassa, 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 (31). Based upon these data...
was severely reduced, whereas under high-sulfur conditions expression was elevated. As a control, reprobing the blots with scon-2* (Fig. 4) 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 initially 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 Rbx1p. 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 (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 N. crassa scon-2* gene. Further, the N-terminal F-box domain of SCON2 is required for normal sulfur-mediated gene regulation in N. crassa (17). We have hypothesized that SCON2’s ability to function as a repressor of sulfur-mediated gene expression is dependent upon the ability of SCON2 to form a functional SCF complex with CYS3 as the most likely target. To date, a functional test of the predicted molecular interactions in an SCF complex in filamentous fungi had not been carried out. A crucial test of the hypothetical role of SCON3 was in confirming an interaction with SCON2. The two-hybrid experiments presented here show that full-length SCON2 and SCON3 interact with each other. The interaction between SCON2 and SCON3 was clearly dependent on the F-box domain. When the F-box domain was deleted from SCON2, there was no detectable interaction with SCON3. In support of the specificity of the effect was the finding that if only the WD-40 repeats were removed from SCON2, then the interaction with SCON3 could still be detected. Communoprecipitation experiments confirmed these results. These experiments provide the initial functional evidence for interactions between putative SCF components in filamentous fungi and are an important step in defining the N. crassa sulfur control system.

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REFERENCES


regulation of the transcriptional activator Met4 is triggered by the SCF Met30 complex. EMBO J. 19:282–294.