Both KH and non-KH domain sequences are required for polyribosomal association of Scp160p in yeast

Ai-min Li, Claudia A. Vargas, Melissa A. Brykailo¹, Kimberly K. Openo, Anita H. Corbett² and Judith L. Fridovich-Keil*

Department of Human Genetics, ¹Graduate Program in Genetics and Molecular Biology and ²Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322, USA

Received February 20, 2004; Revised July 21, 2004; Accepted August 19, 2004

ABSTRACT

Scp160p is a 160 kDa RNA-binding protein in yeast previously demonstrated to associate with specific messages as an mRNP component of both soluble and membrane-bound polyribosomes. Although the vast majority of Scp160p sequence consists of 14 closely spaced KH domains, comparative sequence analyses also demonstrate the presence of a potential nuclear localization sequence located between KH domains 3 and 4, as well as a 110 amino acid non-KH N-terminal region that includes a potential nuclear export sequence (NES). As a step toward investigating the structure/function relationships of Scp160p, we generated two truncated alleles, FLAG.SCP160ΔN1, encoding a protein product that lacks the first 74 amino acids, including the potential NES, and FLAG.SCP160ΔC1, encoding a protein product that lacks the final KH domain (KH14). We report here that the N-truncated protein, expressed as a green fluorescent protein fusion in yeast, remains cytoplasmic, with no apparent nuclear accumulation. Biochemical studies further demonstrate that although the N-truncated protein remains competent to form RNPs, the C-truncated protein does not. Furthermore, polyribosome association is severely compromised for both truncated proteins. Perhaps most important, both truncated alleles appear only marginally functional in vivo, as demonstrated by the inability of each to complement scp160/eap1 synthetic lethality in a tester strain. Together, these data challenge the notion that Scp160p normally shuttles between the nucleus and cytoplasm, and further implicate polyribosome association as an essential component of Scp160p function in vivo. Finally, these data underscore the vital roles of both KH and non-KH domain sequences in Scp160p.

INTRODUCTION

One of the fundamental questions in molecular biology concerns the role of RNA-binding proteins in mediating the processes of post-transcriptional gene expression in eukaryotes (1). Recent studies in a variety of experimental systems have identified large numbers of RNA-binding proteins, many of which have been conserved across broad expanses of evolutionary time (1,2). Nonetheless, the precise biological roles and structure/function relationships for many of these proteins remain unknown (2). We report here a significant step toward uncovering the structure/function relationships of Scp160p, a 160 kDa RNA-binding protein from Saccharomyces cerevisiae.

First identified in 1995, Scp160p was originally hypothesized to function in the maintenance of ploidy in yeast, due in large part to the null phenotype, which includes abnormal cell size and shape, increased DNA content and aberrant segregation of genetic markers through meiosis (3). Biochemical studies from our laboratory and others quickly challenged this hypothesis, however, demonstrating that Scp160p binds selectively in vitro to ribohomopolymers and to rRNA, but not to tRNA (4), and that it associates in vivo with a specific subset of mRNAs and other proteins to form large mRNP complexes (5–8). Some of these mRNPs remain free in the cytosol, but most co-fractionate with soluble or membrane-bound polyribosomes (5–8). In addition, loss or mutation of EAP1, which encodes an eIF4E binding protein involved in both translation initiation and spindle pole body function, is synthetically lethal in combination with loss of SCP160 (8). Finally, Gou et al. (9) recently identified Scp160p as a potential effector of Gα-mediated signal transduction in yeast, although the mechanism and the extent of this function remain unclear. Combined, these data implicate Scp160p for a role in mediating the post-transcriptional regulation of specific mRNAs, as well as the function of specific mRNP proteins. The scp160-null phenotype may therefore reflect the downstream effects of aberrant expression or function of Scp160p’s target mRNAs and proteins, rather than a direct role of Scp160p in many distinct cellular processes.

Although little direct structural information regarding Scp160p has been reported, sequence alignment studies have revealed the presence of 14 predicted hnRNP K homology, or KH domains (4,10,11). KH domains, each spanning about 70 amino acids in length, and including a conserved pattern of hydrophobic residues, an invariant Gly-X-X-Gly segment, and a variable loop, are one of the most common motifs found in the RNA-binding proteins of both prokaryotes...
and eukaryotes, including humans (11,12). Of the 14 KH domains in Scp160p, seven [2, 8–12 & 14] are classical, containing the Gly-X-Y-Gly sequence, and seven [1, 3–7 & 13] are divergent, with insertions or deletions interrupting the Gly-X-Y-Gly sequence (4). Recently, Baum et al. (13) probed the significance of the final four KH domains of Scp160p by testing the functionality of alleles missing either KH domains 13 and 14 or 11–14. Consistent with the results reported here, both truncated proteins failed to associate with polyribosomes in yeast.

In large part because of its KH domains, Scp160p demonstrates significant sequence homology (~23% amino acid sequence identity and ~40% similarity) to a growing family of multiple KH-domain proteins collectively known as vigilins. First identified in chicken (14), vigilin homologs have now been reported in species ranging from Neurospora crassa (Genpept #7899383) to humans (15). Although all vigilins appear to bind nucleic acid, the nature and specificity of the ligands, as well as the proposed cellular functions, remain diverse (14,16–24). Whether these disparities represent true evolutionary divergence, or the vagaries of different experimental systems and approaches, remains unclear.

In addition to its many KH domains, Scp160p also contains a potential nuclear localization sequence (NLS) positioned between KH domains 3 and 4, and a 110 amino acid non-KH domain N-terminal region including a potential nuclear export sequence (NES; amino acids 52–61) (4,10). Neither the NLS nor the NES has been functionally confirmed. Nonetheless, the potential presence of an NLS and an NES raises the possibility that Scp160p may be a nuclear/cytoplasmic shuttle protein, as has been seen for a number of other KH domain shuttle proteins, e.g. FMRP (25). Other than the 110 amino acid N-terminal region, the largest stretch of non-KH domain sequence in Scp160p is 8 amino acids.

As a step toward elucidating the structure/function relationships of Scp160p, we have generated two truncated alleles, FLAG.SCP160ΔN1, whose protein product lacks the first 74 amino acids, including the putative NES, and FLAG.SCP160ΔC1, whose protein product lacks the final KH domain (KH14), and expressed both proteins in yeast. Our results demonstrate that the N-truncated protein, expressed as a green fluorescent protein (GFP)-fusion, continues to localize to the cytoplasm, with no apparent nuclear accumulation. Considering that the putative NLS remains intact in this truncated protein, which now lacks an NES, these results clearly challenge the assumption that wild-type Scp160p is a nuclear-cytoplasmic shuttle protein.

Size fractionation studies demonstrate that the N-truncated protein remains competent to form RNPs of normal size, although the C-truncated protein does not. Interestingly, polyribosome association is severely compromised in both, with the N-truncated protein demonstrating no detectable polyribosome association, and the C-truncated protein demonstrating a biphasic distribution with only a small fraction remaining associated with polyribosomes. Perhaps most important, both truncated alleles appear only marginally functional, as measured by the inability of each to complement scp160/eampl synthetic lethality in a tester strain. Together, these data further implicate polyribosome association as an essential component of Scp160p function, and underscore the vital roles of both the KH and non-KH domain sequences in Scp160p.

### MATERIALS AND METHODS

#### Yeast strains and manipulation

All yeast manipulations were performed according to standard protocols (26). The strains utilized in this study are listed in Table 1 and as indicated, all were derived either from the haploid parent strain J52 [MATα gal7Δ102 ura3-52 trp1-289 ade1 lys1 leu2-3,112, a gift from Dr M. Parthun and Dr J. Jaehning (University of Colorado Health Sciences Center)] or from the haploid parent strain W303 [MATα ade2-1 his3-11,15 leu2-3,112 ura3-1 trpl-1 can1-100 RAD5, a gift from Dr R. Rothstein (Columbia University, NY)].

#### Construction and expression of the SCP160ΔN1 allele

The FLAG-SCP160ΔN1 allele was generated by cutting a wild-type subclone Pst–ApaI, dropping out the ~200 bp N-terminal fragment, and ligating in the annealed

---

**Table 1. Yeast strains used in this study**

<table>
<thead>
<tr>
<th>Strain code</th>
<th>Relevant genomic alleles</th>
<th>Plasmid(s)</th>
<th>Comments (parent strain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JFY4740</td>
<td>FLAG.SCP160ΔN1GFP</td>
<td>None</td>
<td>For microscopy only (W303)</td>
</tr>
<tr>
<td>JFY4743</td>
<td>FLAG.SCP160ΔN1GFP</td>
<td>None</td>
<td>For microscopy only (W303)</td>
</tr>
<tr>
<td>JFY0023</td>
<td>Wild-type (untagged) SCP160</td>
<td>None</td>
<td>Expresses wild-type, untagged Scp160p (J52)</td>
</tr>
<tr>
<td>JFY1511</td>
<td>FLAG.SCP160</td>
<td>None</td>
<td>Expresses wild-type FLAG.Scp160p (J52)</td>
</tr>
<tr>
<td>JFY4619</td>
<td>FLAG.SCP160ΔN1</td>
<td>Wild-type SCP160 URA3 (JF3116, 2μ)</td>
<td>Expresses only FLAG.Scp160ΔN1p once plasmid cured on 5-FOA (J52)</td>
</tr>
<tr>
<td>JFY4634</td>
<td>HA.BFR1 Δscp160</td>
<td>FLAG.SCP160ΔN1 URA3 (JF4390, CEN)</td>
<td>Expresses only FLAG-Scp160ΔC1p and HA.Bfr1p (J52)</td>
</tr>
<tr>
<td>JFY4834</td>
<td>FLAG.SCP160ΔC1</td>
<td>None</td>
<td>Expresses only FLAG-Scp160ΔC1p (W303)</td>
</tr>
<tr>
<td>JFY3362</td>
<td>HA.BFR1</td>
<td>None</td>
<td>Expresses HA.Bfr1p and wild-type, untagged Scp160p (J52)</td>
</tr>
<tr>
<td>JFY4247</td>
<td>Δscp160 Δeampl</td>
<td>SCP160 URA3 (JF3116)</td>
<td>For quantitative test of FLAG-SCP160ΔN1 and FLAG.SCP160ΔC1 function in vivo (W303)</td>
</tr>
</tbody>
</table>
oligonucleotides SCPATF1 (5'-GGATCCTAAATGGACTACAAAGGACGCAGAGAAGGGCC-3') and SCPATR1 (5'-TTGTCGGTCGCAGTCCTTTTGCTACCTGAGC-3'). This manipulation resulted in loss of the first 74 codons from the SCP160 open reading frame (ORF), with the concomitant substitution of sequences encoding a starting methionine followed by a FLAG epitope (Asp-Tyr-Lys-Asp-Asp-Asp-Lys). The PstI restriction site immediately upstream of the ORF had been introduced by prior manipulation of the wild-type sequence. Strains of yeast expressing either FLAG-tagged wild-type Scp160p or FLAG-tagged Scp160AN1p in place of the wild-type protein were generated by two-step gene replacement, as described previously (5,6,26). As indicated in Table 1, strain JFy4619, which expresses FLAG.Scpx160AN1p from a genomic allele, was functionally covered by a URA3 plasmid-borne wild-type allele, which was eliminated from the cells by 5FOA selection just prior to analysis.

C-terminal GFP tags were also added to both the wild-type and Scp160AN1p sequences, as described previously (6). The GFP-tagged alleles (JF2140 and JF2592, respectively) were confirmed by DNA sequencing, linearized with BstEII, and integrated into the SCP160 locus for expression. Strains expressing GFP-tagged isoforms of Scp160p were further confirmed by western-blot analysis using an affinity-purified anti-GFP polyclonal antibody (SIGMA G1544) at 0.1 μg/ml, as recommended by the manufacturer. Yeast expressing FLAG-Scpx160p.GFP or FLAG.Scpx160AN1p.GFP were used for microscopy (Figure 2), but not for any of the other experiments reported here.

Construction and expression of the SCP160ΔC1 allele

The FLAG.SCP160ΔC1 allele was generated initially using one-step gene replacement in the haploid strain JFy4493, which already encoded a FLAG-tag at the N-terminus of SCP160. In brief, the C-terminus of this allele was modified by insertion of a PCR-amplified kanMX cassette flanked by the appropriate SCP160 sequences (26). The oligonucleotides scpdelKH14kan2 (5'-TGATACGTCTGTTAAGTGTGAAGAAAGAATTGCGCAACCATCTGCTACATAGGttttgccgctcccc-3') and scpdelKH14kan2 (5'-TTATATTAGTTAAGCCAAATTCATATAGTTAAGAAAGAAATTTGGTTCTCTACTTTTGCTTTGTTTGAAGCTTGTggagggcgcgggtagtagcg-3') were used as primers to generate this integrating fragment. Following selection on G418 plates, colonies were confirmed by genomic PCR using the primers scpKH10f1 (5'-TGGAGAACCGACATAAGATGGT-3') and scpR5 (5'-CACCAGCCTTTATACAGAAGAC-3'), followed by direct sequencing of the PCR product. The resultant strain was designated JFy4834 (Table 2).

To obtain a plasmid-borne allele, we applied a gap-repair strategy using as gapped backbone the plasmid pYCPlac.SCP160 (JF4688) cut AflII/EagI, and as insert a PCR fragment generated using the primers scpKH10f1 (5'-TGGAGAACCGACATAAGATGGT-3') and scpR5 (5'-CACCAGCCTTTATACAGAAGAC-3') with genomic DNA from yeast JFy4834 as template. As above, positive clones were confirmed by PCR followed by DNA sequencing of relevant regions. The confirmed plasmid clone that was used in subsequent studies was designated JF4544.

<table>
<thead>
<tr>
<th>Test plasmid</th>
<th>Relative plasmid loss (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pJF2803 (2μ) SCP160</td>
<td>100.00 ± 30.64 (29)</td>
</tr>
<tr>
<td>pJF1102 (2μ) SCP160AN1</td>
<td>0.06 ± 0.08 (64)</td>
</tr>
<tr>
<td>pJF2542 (2μ) SCP160AN1</td>
<td>9.62 ± 5.82 (12)</td>
</tr>
<tr>
<td>pJF4648 (CEN) SCP160</td>
<td>100.00 ± 22.22 (2)</td>
</tr>
<tr>
<td>pJF1906 (CEN) SCP160ΔC1</td>
<td>0.00 ± 0.00 (4)</td>
</tr>
<tr>
<td>pJF4544 (CEN) SCP160ΔC1</td>
<td>3.30 ± 4.68 (6)</td>
</tr>
</tbody>
</table>

As reported previously (8), high plasmid loss values in this system indicate that a given test plasmid is sufficient to relieve the essential nature of the SCP160 maintenance plasmid in the eap1/scp160 double null strain, while low plasmid loss values indicate that a given test plasmid is not sufficient to complement SCP160 maintenance plasmid loss in this strain. All values were normalized to the degree of plasmid loss seen using the corresponding positive control plasmid (JF2803 for 2μ or JF4648 for CEN).

Fluorescence microscopy

Prior to microscopy, cells expressing either FLAG, Scp160p.GFP or FLAG.Scpx160AN1p.GFP were grown to mid-log phase in synthetic medium with 2% dextrose. Cells were stained by incubation in 5 μM Hoechst dye (SIGMA# B2261, Bis Benzamide Hoechste #33342) for 30 min. Cells were viewed using an Olympus BX-60 epifluorescence microscope with a GFP optimized filter set (Chroma). Images viewed at 100x magnification in the GFP, Hoechst and DIC channels were captured in digital form using IPLab Spectrum software. The DIC images presented in Figure 2 were brightened using Adobe Photoshop, but no there was no digital enhancement of any of the other images.

Biochemical analysis of Scp160p

All cell lysis, subcellular fractionation and anti-FLAG affinity isolation procedures were performed as described previously (6,7). Western-blot analyses were also performed as described previously (6) using the monoclonal antibodies M2 (Roche) to detect FLAG-tagged proteins, HA-11 (Covance) to detect hemagglutinin (HA)-tagged proteins and the anti-GFP affinity-purified polyclonal antibody from Sigma (#G1544) to detect GFP-tagged proteins, as cited in the text.

Analysis of in vivo function of the FLAG.SCP160AN1 and FLAG.SCP160ΔC1 alleles

The test of SCP160 in vivo function was performed as described previously (8), using the scp160/eap1 double null tester strain JFy427, transformed with the test plasmids pJF2542, which encodes FLAG.Scp160AN1p, and pJF4544, which encodes FLAG.Scpx160ΔC1p. As described previously (8), plasmids encoding wild-type Scp160p (pJF2803 for 2μ, pJF4648 for CEN) versus no Scp160p (pJF1102 for 2μ, pJF1906 for CEN) were also included as positive and negative controls, respectively.

RESULTS

Expression of FLAG-tagged Scp160AN1p and Scp160ΔC1p in yeast

As a first step toward defining a structure/function map for Scp160p, we generated both an N-terminally truncated allele,
**FLAG.SCP160ΔN1p**, in which the first 74 codons, including the potential NES (open inverted triangles, Figure 1A), were removed and replaced by a FLAG tag, and a C-terminally truncated allele, **FLAG.SCP160ΔC1**, in which the final KH domain was removed. All 14 KH domains (shaded squares, Figure 1A), as well as the potential NLS (filled inverted triangles, Figure 1A), remained intact in **FLAG.SCP160ΔN1p**.

Western-blot analysis of yeast expressing FLAG-tagged wild-type Scp160p, FLAG-tagged Scp160ΔN1p, or FLAG-tagged Scp160ΔC1p demonstrated comparable levels of protein, although as expected, the migration patterns of both truncated proteins differed slightly from that of the full-length protein (Figure 1B, filled arrow). Yeast expressing native, untagged Scp160p served as a negative control, and an ~100 kDa cross-reacting band of unknown identity (Figure 1B, open arrow) served as a convenient internal control for the loading of lanes.

**Figure 1.** Expression of FLAG.Scp160ΔN1p and FLAG.Scp160ΔC1p in yeast. (A) Cartoons illustrating the domain structures of FLAG.Scp160p, FLAG.Scp160ΔN1p and FLAG.Scp160ΔC1p. The asterisk (*) represents the FLAG epitope, the circle represents the 110 amino acid N-terminal region, the open triangle represents the potential NES and the filled triangle represents the potential NLS. Each box represents one KH domain. (B) Anti-FLAG western-blot analysis of lysates from yeast expressing either untagged Scp160p (control), FLAG-Sscp160p, FLAG.Scp160ΔN1p or FLAG-Sscp160ΔC1p, as indicated. The filled arrow indicates the full-length and truncated Scp160p proteins; the open arrow indicates a ~100 kDa anti-FLAG cross-reacting protein of unknown identity (5,6) that served as an internal control for loading of lanes.

**Subcellular localization of FLAG.Scp160ΔN1p**

To explore the subcellular localization of FLAG.Scp160ΔN1p, we generated a C-terminally GFP-tagged isoform, and integrated the corresponding allele into the normal genomic locus, as described previously for the wild-type allele (6). As reported previously (3,6,27), GFP signal in the wild-type cells was predominantly if not completely cytoplasmic, with clear enrichment around the nuclear periphery, the site of the yeast endoplasmic reticulum (ER). Hoescht staining of these cells confirmed the position of the nucleus, and the corresponding DIC images revealed the overall outline of each cell (Figure 2). Like their wild-type counterparts, yeast expressing the truncated FLAG.Scp160ΔN1p, GFP fusion protein showed GFP signal that was predominantly, if not completely cytoplasmic. No evidence of nuclear accumulation of the truncated protein was seen, suggesting that the potential NES in the deleted region is not normally responsible for the cytoplasmic localization of the full-length protein. These data therefore further suggest that wild-type Scp160p may not normally shuttle between the nucleus and the cytoplasm of yeast.

**Figure 2.** Fluorescence microscopy of yeast expressing either full-length FLAG.Scp160p.GFP or FLAG.Scp160ΔN1p.GFP. All images were viewed at 100x magnification. The left-most panel in each row presents the GFP signal, indicating distribution of the tagged Scp160p protein, the middle panel in each row presents the corresponding Hoescht dye signal, indicating nuclear location, and the right-most panel in each row presents the DIC image. As discussed in the text, no nuclear accumulation was observed in the truncated Scp160p protein despite loss of the putative NES.
even in the context of untreated lysates (Figure 3, third panel),

demonstrating that this protein did not remain competent to
form normal RNP complexes.

As a next step to probe the composition of the
FLAG.Scp160DN1p containing RNP complexes, we applied
a previously described anti-FLAG affinity purification proce-
dure (5–8) to isolate these complexes from yeast also expres-
sing HA-tagged Bfr1p. Prior studies identified Bfr1p as one of
many proteins that co-purify with Scp160p-containing mRNP
complexes in yeast (6). As shown in Figure 4 (upper panels),
and as reported previously for full-length FLAG.Scp160p
(6), HA.Bfr1p co-purified with FLAG.Scp160DN1p. Also
like its wild-type counterpart, the association between
FLAG.Scp160DN1p and HA-Bfr1p was sensitive to RNase
pre-treatment (Figure 4, middle set of panels). Finally, no
HA-Bfr1p signal was detected following the mock-isolation
of complexes from yeast expressing HA-tagged Bfr1p in
conjunction with native, untagged Scp160p, demonstrating
the specificity of the isolation procedure (Figure 4, lower
panels). These data demonstrate that the FLAG.Scp160Δ
N1p protein retained the ability to form at least a subset of
its normal macromolecular interactions.

Both FLAG.Scp160ΔN1p and FLAG.Scp160ΔC1p are
severely compromised in their ability to associate
with polyribosomes

To probe the ability of each truncated protein to associate with
polyribosomes, soluble cell lysates were size-fractionated by
sucrose gradient centrifugation, as described previously (5,6).
Lysates from yeast expressing the FLAG-tagged wild-type
protein were analyzed in parallel as a control. By their nature,
sucrose gradients enable separation of much larger complexes
than do S300 columns, allowing the distinction of free RNPs
from ribosomal subunits, monosomes and polysomes, as
shown in Figure 5. Western-blot analyses of the fractions
from these gradients (Figure 5) demonstrated that although
the wild-type FLAG.Scp160p protein localized predominantly
to the bottom of the gradient, co-fractionating as expected with
polyribosomes, the FLAG.Scp160ΔN1p protein localized
predominantly to the lighter fractions. Furthermore, the
FLAG.Scp160ΔC1p protein localized predominantly to
the very top of the gradient, although a small amount of this
protein continued to co-fractionate with polyribosomes.
Differential centrifugation of cell lysates to separate soluble from membrane-associated pools also demonstrated a notable distinction between the full-length and truncated Scp160p proteins. As reported previously (4,6,27), wild-type FLAG.Scp160p localized predominantly to the membrane pellet, while both the FLAG.Scp160ΔN1p and FLAG.Scp160ΔC1p proteins were almost evenly distributed between the soluble and membrane-associated fractions (Figure 6A and B). This significant shift of both the FLAG.Scp160ΔN1p and FLAG.Scp160ΔC1p signals from the membrane-associated pool to the soluble pool is fully consistent with our previous observation that treatments which disrupt Scp160p association with polyribosomes also shift signal from the membrane-associated pellet to the soluble fraction (6).

**In vivo** function of both truncated **FLAG.SCP160ΔN1** and **FLAG.SCP160ΔC1** alleles is severely compromised

To assess functional capacity of both the **FLAG.SCP160ΔN1** and **FLAG.SCP160ΔC1** alleles in vivo, we quantified the ability of each to complement **scp160**Δ**cap1** synthetic lethality in a double null tester strain, as described previously (8). In brief, plasmid-borne copies of each truncated **SCP160** allele were introduced into yeast genomically null for both **scp160** and **cap1**, covered by a **URA3** maintenance plasmid encoding wild-type **SCP160**. Following growth for a fixed number of generations in the absence of uracil selection, transformants were plated to medium with and without 5FOA. The fraction of cells that could grow in the presence of 5FOA, which kills only

**URA3** cells, versus in its absence, served as an indicator of how many cells had lost their **URA3** plasmid. Yeast transformed with either empty plasmid backbone, or plasmid carrying a second wild-type copy of **SCP160**, served as negative and positive controls, respectively. For comparison, the degree of **URA3** plasmid loss in each strain was scaled relative to that seen in the corresponding positive control, which was set to 100%. The final values calculated, therefore, no longer directly reflected plasmid retention or loss, but rather indicated how effectively, relative to the wild-type allele, the test sequence in question could enable cells to remain viable despite loss of their **URA3** **SCP160** maintenance plasmid. Since this maintenance plasmid was the same in all strains, issues of plasmid replication efficiency or distribution, independent of **SCP160** sequence, should have been cancelled out. As shown in Table 2, according to this procedure, the **FLAG.SCP160ΔN1** allele retained <10% wild-type function, and the corresponding **FLAG.SCP160ΔC1** allele retained <5% wild-type function.

**DISCUSSION**

The experiments presented here were designed to ask three questions: (i) is the N-terminal region of Scp160p, including the potential NES, responsible for the cytoplasmic localization...
of Scp160p; (ii) is the non-KH domain N-terminal region important for Scp160p macromolecular interaction or function; and (iii) is the final KH domain important for Scp160p macromolecular interaction or function? In effect, we have compared the relative functional contributions of KH and non-KH domain sequences in SCP160. Our results clearly demonstrate that the N-terminal region and NES are not required for cytoplasmic localization of Scp160p, or for assembly of Scp160p into RNPs, although this region is required for association of Scp160p with polyribosomes. In contrast, the final KH domain is required for both RNP assembly and association of Scp160p with polyribosomes. Perhaps most important, both the non-KH domain N-terminus and the C-terminal KH domain are essential for function of Scp160p in vivo. Together, these data underscore the importance of both KH and non-KH domain sequences in Scp160p, and further implicate polyribosome association as a key component of Scp160p function.

Subcellular localization

All studies reported to date addressing the subcellular localization of Scp160p have placed the protein firmly in the cytoplasm, with enrichment around the nuclear envelope/ER (3–8,24,27). Nonetheless, the identification of both putative NLS and NES sequences in Scp160p (4,10) raised the possibility that Scp160p might be a nuclear/cytoplasmic shuttle protein with a steady-state distribution favoring the cytoplasm. The results presented here, demonstrating that loss of the putative NES does not result in nuclear accumulation of the truncated Scp160p, clearly challenge this hypothesis, and further suggest that the remaining putative NLS also may be non-functional. Where in the cell, and at what stage of complex assembly the Scp160p protein finds and associates with its target mRNAs, therefore remains an open question, although presumably these interactions occur within the cytoplasm.

Macromolecular interactions

The data presented here demonstrate that the FLAG.Scp160ΔN1p truncated protein was able to form a subset of its normal biochemical interactions, including association with HA-Bfr1p and assembly into RNPs, although it was severely compromised in its ability to associate with polyribosomes. Whether these data reflect a direct versus indirect role of the N-terminal sequence in polyribosome association remains unclear, as does whether the full N-terminal region is required for polyribosome association, versus a smaller sub-domain. Furthermore, beyond the presence of Bfr1p, the precise nature and composition of the RNP complexes formed by FLAG.Scp160ΔN1p remains to be explored. At minimum, however, the fact that at least some normal biochemical interactions involving FLAG.Scp160ΔN1p remain intact implies that the truncated protein is not globally misfolded. The observation that RNP formation and polyribosome association can be uncoupled further implies that polyribosome association is not an obligate first step in the process of Scp160p mRNP formation.

The data presented here also demonstrate that the FLAG.Scp160ΔC1p truncated protein is compromised with regard to both RNP formation and polyribosome association. This result is striking for at least three reasons. First, it demonstrates either that Scp160p requires all 14 KH domains to function properly, or else that KH14 is special in some way. This is a testable hypothesis that is currently under investigation. Second, although Baum et al. (13) also reported recently that deletion of the final two KH domains from Scp160p disrupted polyribosome association, those authors concluded that sequences within KH13 were essential for this process, and never checked the impact of KH14-loss alone. Our results clearly demonstrate that the loss of KH14 alone is sufficient to disrupt Scp160p-polyribosome association, although the mechanism of that impact remains unclear. While the possibility of partial or global unfolding of the C-truncated protein cannot formally be excluded, the fact that this protein remains largely soluble (Figure 6) and is detected in lysates at levels comparable to that of the wild-type protein (Figure 1B), argues against this possibility. Finally, these results demonstrate that although the N-terminus may be essential for polyribosome association of Scp160p, it is not sufficient.

Function in vivo

One of the most important observations reported here is that both the FLAG.SCP160ΔN1 and FLAG.SCP160ΔC1 were significantly compromised with regard to function in vivo (Table 2). These data demonstrate that although the non-KH domain sequences in Scp160p may be minimal, they are not insignificant. These data further demonstrate that despite the presence of 13 other KH domains, KH14 is essential. Finally, as mentioned earlier, these data implicate polyribosome association as an essential component of Scp160p function in vivo.

ACKNOWLEDGEMENTS

We are grateful to Kristen Riehman, who helped to generate the FLAG-SCP160ΔN1 allele, to Brian Lang, who performed some of the early characterization of this allele, and to Alice Watson, who helped to generate earlier versions of some of the strains and plasmids utilized in this study. This work was supported by funds from the National Science Foundation (Award 0112911 to J.L.F.-K.).

REFERENCES


