Sscp160p, an RNA-binding, Polysome-associated Protein, Localizes to the Endoplasmic Reticulum of Saccharomyces cerevisiae in a Microtubule-dependent Manner*

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Scp160p is an RNA-binding protein containing 14 tandemly repeated heterogenous nuclear ribonucleoprotein K-homology domains, which are implicated in RNA binding. Scp160p interacts with free and membrane-bound polysomes that are dependent upon the presence of mRNA. Despite its presence on cytosolic polysomes, Scp160p is predominantly localized to the endoplasmic reticulum (ER). Accumulation of Scp160p-ribosome complexes at the ER requires the function of microtubules but is independent of the actin cytoskeleton. We propose that the multi-K-homology-domain protein Scp160p functions as an RNA binding platform, interacting with polysomes that are transported to the ER.

RNA localization and spatial restriction of translation are important mechanisms in biological processes (reviewed in Refs. 1 and 2). The process of mRNA localization is initiated by association of mRNA with one or more RNA-binding proteins through a targeting signal most commonly located in the 3′-untranslated region of the transcripts. This association may occur in the nucleus soon after transcription, resulting in the formation of ribonucleoprotein particles (3), which are then exported into the cytosol (4). Alternatively, such complexes may form in the cytosol. RNP particles migrate along the cytoskeleton to their final destinations, where they are anchored and translated (reviewed in Ref. 5). The multistep process of RNA localization depends on specific trans-acting proteins. One common feature of these factors is repeats of different RNA binding domains (6). These RNA-binding proteins include members of the double-stranded RNA-binding protein family (7, 8), homologues of the zipcode-binding protein (9, 10), and members of the double-stranded RNA-binding protein family RNA localization and spatial restriction of translation are important mechanisms in biological processes (reviewed in Refs. 1 and 2). The process of mRNA localization is initiated by association of mRNA with one or more RNA-binding proteins through a targeting signal most commonly located in the 3′-untranslated region of the transcripts. This association may occur in the nucleus soon after transcription, resulting in the formation of ribonucleoprotein particles (3), which are then exported into the cytosol (4). Alternatively, such complexes may form in the cytosol. RNP particles migrate along the cytoskeleton to their final destinations, where they are anchored and translated (reviewed in Ref. 5). The multistep process of RNA localization depends on specific trans-acting proteins. One common feature of these factors is repeats of different RNA binding domains (6). These RNA-binding proteins include members of the double-stranded RNA-binding protein family (7, 8), homologues of the zipcode-binding protein (9, 10), and members of the heterogenous nuclear RNP1 family (11–13).

One of the most common protein motifs involved in RNA binding is the K-homology (KH) domain, originally described in the protein heterogenous nuclear RNP-K (14). A KH domain consists of ~70 amino acids and includes a conserved hydrophobic core, an invariant GXXG motif, and an additional variable segment. NMR structural studies of individual KH domains revealed a conserved βαβαβα fold (15, 16).

In yeast, Scp160p, a protein containing 14 copies of the KH domain, has been identified (17). Scp160p localizes to the cytosol with an enrichment of Scp160p at the ER (17). scp160Δ mutants are viable, but display defects in cell morphology and nuclear segregation, resulting in cells with increased size and DNA content per cell (17, 18). The mechanism by which this complex phenotype is established in Δscp160 cells is unknown. In Drosophila, a functional homologue of Scp160p has also been identified. This protein, DDPI, binds dodeca satellite repeat regions of centromeric heterochromatin in embryonic and larval cell nuclei (19, 20). Overexpression of the DDPI protein complements the cell morphology and nuclear segregation defects in Δscp160 mutants (19). Similar multi KH-domain proteins are found ubiquitously in all eukaryotic cells. In vertebrate species this protein is known as vigilin (21–23). A clear picture of the cellular function and of specific RNA targets of these proteins has not yet emerged. Northwestern blot analyses with Scp160p demonstrated RNA binding activity in vitro with low sequence specificity (24). RNA gel mobility shift assays and mRNA affinity column chromatography showed that Xenopus vigilin binds specifically to the 3′-untranslated region of vitellogenin mRNA (23, 25), stabilizing the RNA by blocking cleavage by an endonuclease (26). In contrast to these results, Kruse et al. (27, 28) propose that human vigilin may be involved in binding and transport of tRNA.

Recently, Scp160p was reported to be associated with cytosolic polysomes as a component of a messenger RNP complex containing poly(A)-binding protein, which is released upon EDTA treatment (29). These observations are contradictory to the previously reported localization of Scp160p predominantly at the ER membrane (17). To resolve this apparent discrepancy, we decided to investigate the intracellular distribution of Scp160p more carefully and to ask specifically whether Scp160p interacts with both cytosolic and membrane-bound polysomes. We could show that Scp160p is enriched at the ER membrane and is associated with polysomes in a mRNA-dependent manner. Interestingly, we could demonstrate that this localization requires intact microtubules.

EXPERIMENTAL PROCEDURES

Plasmids and Strains—To replace SCP160 with an engineered gene encoding Scp160-GFP, we amplified by PCR a DNA fragment encoding the SCP160 gene from +2465 to the stop codon using 5′-CACCAGTC-CAAAAGCCCTCA-3′ and 5′-CCGctgagATCTTCTTAAGGATTTCAAAA-CC-3′ primers and a genomic fragment containing the SCP160 gene (YEP13/6 (17)) as template. The resulting fragment was cloned first into TOPOII vector (TOPO TA cloning kit, Invitrogen), re-excised with XhoI and used for transformation of a S288c-derived diploid yeast strain. Ura− transformants were selected and checked by fluorescence

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microscopy for expression of Scp160p-GFP. To confirm the functional replacement, the diploid strain was sporulated, and Scp160p-GFP-expressing spores were compared with wild-type spores. Growth of Scp160p-GFP cells at all temperatures was indistinguishable from wild-type cells. No increase of cell or nuclear size was observed in Scp160p-GFP cells.

To replace SCP160 with an engineered gene encoding GFP-SCP160, we cloned a DNA fragment encoding the SCP160 gene from 625 to +3, amplified by PCR using 5'-CggactcGGATCTTCCCTCTTCTTCATTTAATTGTTG-3' and 5'-CactagtCCTTTGTAATGACTAAATAGATTC-3' primers. The PCR product was subcloned in pBluescript SK- plasmid (Stratagene) and ligated into the unique BamHI and EcoRI sites of pRS314 (31), resulting in pMS346. A linear 4.4-kilobase BamHI-EcoRI fragment was generated by restriction digest and ligated into a haploid S288c-derived yeast strain. A HIS3 promotor upstream of GFP-TUB1 (33), with kilobase position 2, was generated by subcloning a DNA fragment encoding the lisKKRKLVADC-CONH2 and CPIRKYAIKV-COOH, respectively, from SCP160 plasmid we cloned the rabbits, using standard techniques (34), against the peptides VA-

Expression of Scp160p was previously reported to be associated with cytosolic polysomes (28). However, indirect immunofluorescence revealed that the majority of Scp160p is localized at the ER membrane (17), and this large fraction was not analyzed with respect to polysome association. Therefore, we decided to investigate the intracellular distribution of Scp160p by whole cell fractionation to clarify whether Scp160p is predominantly associated with free or membrane-bound ribosomes. To analyze the entire cellular pool of Scp160p, we generated a total lysate from cycloheximide-treated wild-type yeast cells by glass bead lysis in LS buffer containing 100 mM potassium acetate. From this lysate, we obtained, by consecutive centrifugation fractions enriched in ER membranes (P6), ribosomes (P18 and P200) and a ribosome-free cytosol (S200). As shown in Fig. 1, half of the luminal Kar2p was present in P6, and the other half was found in S200, due to the release of Kar2p from the ER as a result of mechanical damage to the microsomes by glass bead lysis. Almost all of the ER membrane marker Sec61p was found in P6, with a minor fraction in P18. The cytosolic marker protein glucose-6-phosphate-dehydrogenase (Zwf1p) was only present in S200. As revealed by immunofluorescence of the small and large ribosomal proteins Rps3p and Rpl35p, respectively, about half of all ribosomes was found in the P200 fraction. A smaller proportion was found in P6 and P18. Taken together, this indicates that P6 is enriched in ER membranes, and P200 is enriched in membrane-free (cytosolic) ribosomes. P18 contains a small amount of ER and heavy polysomes.

RESULTS

Scp160p Cofractionations with Free and Membrane-bound Polysomes—Scp160p was previously reported to be associated with cytosolic polysomes (28). However, indirect immunofluorescence revealed that the majority of Scp160p is localized at the ER membrane (17), and this large fraction was not analyzed with respect to polysome association. Therefore, we decided to investigate the intracellular distribution of Scp160p by whole cell fractionation to clarify whether Scp160p is predominantly associated with free or membrane-bound ribosomes. To analyze the entire cellular pool of Scp160p, we generated a total lysate from cycloheximide-treated wild-type yeast cells by glass bead lysis in LS buffer containing 100 mM potassium acetate. From this lysate, we obtained, by consecutive centrifugation fractions enriched in ER membranes (P6), ribosomes (P18 and P200) and a ribosome-free cytosol (S200). As shown in Fig. 1, half of the luminal Kar2p was present in P6, and the other half was found in S200, due to the release of Kar2p from the ER as a result of mechanical damage to the microsomes by glass bead lysis. Almost all of the ER membrane marker Sec61p was found in P6, with a minor fraction in P18. The cytosolic marker protein glucose-6-phosphate-dehydrogenase (Zwf1p) was only present in S200. As revealed by immunofluorescence of the small and large ribosomal proteins Rps3p and Rpl35p, respectively, about half of all ribosomes was found in the P200 fraction. A smaller proportion was found in P6 and P18. Taken together, this indicates that P6 is enriched in ER membranes, and P200 is enriched in membrane-free (cytosolic) ribosomes. P18 contains a small amount of ER and heavy polysomes.
A large proportion of Scp160p sedimented with the membrane-containing fraction (Fig. 1); a second, smaller pool was found in the cytosolic ribosome fraction (P18 and P200). ribosome-depleted cytosol (S200) was almost free of Scp160p. Therefore, Scp160p is clearly enriched in ER membrane-containing fractions.

Next we analyzed the association of Scp160p with cytosolic ribosomes by density gradient centrifugation on linear 10–40% sucrose gradients. All Scp160p present in the membrane-depleted fraction comigrated with polysomes (Fig. 2A). Scp160p-containing complexes smaller than monosomes could not be detected. Additional density centrifugation with gradients from 10 to 50% sucrose showed an accumulation of Scp160p in fractions containing heavy polysomes (data not shown). In our analysis, the comigration of Scp160p with cytosolic polysomes was very labile. Complete comigration of Scp160p with polysomes was only observed in lysates processed immediately. In lysates from frozen cells, the amount of polysomes was reduced, and some slower migrating Scp160p complexes appeared (data not shown). The cofractionation of Scp160p with polysomes is salt-sensitive, indicating an ionic interaction between Scp160p and ribosomes. After treatment with 500 mM potassium acetate, most Scp160p was found in the ribosome-free fractions on top of the gradient (Fig. 2B). Removal of Mg$^{2+}$ ions by the addition of EDTA leads to the dissociation of 80 S ribosomes and polysomes into partially disassembled and unfolded ribosomal subunits and the release of mRNA and 5 S RNA (38, 39).

After EDTA treatment, all Scp160p was shifted into a slower sedimenting complex (Fig. 2C, fractions 1–5), which peaked in fractions 2–4, suggesting that EDTA treatment causes the release of a Scp160p-containing complex that sediments at similar position as the 40 S ribosomal subunit. A similar sized messenger RNP particle, which contains Scp160p and is formed in the presence of EDTA has been described previously (28).

To distinguish whether mRNA contributes to the association of Scp160p with polysomes or whether Scp160p interacts directly with naked ribosomes, we selectively digested mRNA by mild treatment with micrococcal S1 nuclease. After mRNA digestion, ribosomes migrated as monosomes in the sucrose gradient (Fig. 2D, fractions 6–9). The vast majority of Scp160p was found in fractions 1 and 2; smaller amounts comigrated with ribosomal subunits and monosomes (Fig. 2D, fractions 3–7), indicating that the association of Scp160p with ribosomes requires the presence of mRNA. Treatment with higher concentrations of S1 nuclease caused an additional shift of Scp160p into the top fractions of the gradient (data not shown). The increased amount of fractionated rRNA seen after EDTA and S1 nuclease treatment (Fig. 2C and D) corresponds to disassembled heavy polysomes that pellet in the absence of these agents.

To investigate whether Scp160p from the membrane-enriched fraction (P6) also interacts with polysomes, we resuspended P6 in LS buffer containing the nonionic detergent Nikkol and separated the solubilized material from the
unsolubilized fraction by recentrifugation at 6,000 × g. Under low salt conditions, half of Scp160p and more than half of the Sec61p and ribosomes were solubilized (Fig. 3A). Due to the low salt concentration present during the Nikkol treatment, the solubilization of membranes was not complete.

Solubilized Scp160p cosedimented with ribosomes at high speed centrifugation (20 min; 200,000 × g; Fig. 3A, lanes 7 and 8), suggesting that the Scp160p present at the ER membrane is also bound to polysomes. To confirm this, we subjected Nikkol-solubilized P6 to sucrose density gradient centrifugation. Solubilized ribosomes migrated as a major peak containing monosomes (Fig. 3B, fraction 6) and as smaller peaks containing polysomes (Fig. 3B, fractions 7–10). The vast majority of Sec61p cofractionated with ribosomes, suggesting that the binding of ribosomes to the Sec61p complex is maintained. Some of the Scp160p migrated in fractions 2–5; however, the majority of Scp160p remained in the mono- and polysome-containing fractions (Fig. 3B, fractions 6–10). This indicates that Scp160p associates with membrane-bound polysomes.

Dissociation of Ribosomes or Digestion of mRNA Releases Scp160p from the Rough ER—To compare the interaction of Scp160p with cytosolic polysomes and membrane-bound ribosomes, we treated P6 either with high salt or EDTA or digested the mRNA. A control incubation for 15 min at 23 °C in LS buffer did not release a significant amount of Scp160p from P6. Incubation with 500 mM potassium acetate or removal of Mg²⁺ ions by EDTA led to the extraction of more than two-thirds of the Scp160p from P6 (Fig. 4A); similar to the results with cytosolic ribosomes (Fig. 2B). This indicates that 500 mM potassium acetate abolished the interaction of Scp160p with membrane-bound ribosomes. EDTA treatment released Scp160p and ribosomes from the membrane, indicating again a ribosome-mediated binding of Scp160p to the ER membrane. Digestion of mRNA by S1 nuclease led to the release of some Scp160p. However, compared with the incubation with EDTA, the release of Scp160p and ribosomes was less efficient. The release of the nascent chain from translating ribosomes by puromycin/GTP at low salt did not cause the release of additional Scp160p from P6. To investigate whether the interaction of Scp160p with solubilized membrane ribosomes is mRNA-dependent, we solubilized P6 from untreated lysate and from S1-nuclease-treated lysate and analyzed the material by density gradient centrifugation. Half of Scp160p from the solubilized, untreated P6 fraction comigrated with the ribosome peaks (Fig. 4B, fraction 5–10). Digestion of mRNA reduced the amount of membrane-bound ribosomes that stay attached to the membrane (compare ribosome peak in fraction 5 and 6; Fig. 4, B and C). Ribosomes that were not directly associated with the Sec61p complex or with other proteins of the ER membrane were removed. Digestion of mRNA also abolished the cofrac-

FIG. 3. Scp160p binds to ER membranes. A, the membrane-containing fraction was resuspended in LS buffer (lanes 1 and 2) or LS buffer containing 2% Nikkol (lanes 3–8) and incubated for 15 min at 23 °C. Samples were centrifuged again (20 min; 6,000 × g) to separate unsolubilized material (lanes 1 and 3) from solubilized material (lanes 2 and 4). The solubilized material was centrifuged again for 20 min at 6,000 × g (lanes 5 and 6) or 200,000 × g (lanes 7 and 8). The distribution of Scp160p, Kar2p, Sec61p, and ribosomes was analyzed by immunodetection. B, solubilized P6 was centrifuged for 20 min at 6,000 × g, and the supernatant was subjected to 10–40% sucrose density gradient centrifugation. The distribution of ribosomes, Scp160p, and Sec61p was analyzed by immunodetection. The state of ribosomes was monitored by the A₂₅₄ profile.

FIG. 4. Scp160p interacts with membrane-bound ribosomes. A, the membrane-containing fraction (P6) was incubated at 23 °C for 15 min in LS buffer (mock) in buffer with 500 mM potassium acetate (HS), in LS buffer with 8 mM EDTA (EDTA), in LS buffer with 25 units/ml micrococcal S1 nuclease (S1), or in LS buffer with 1 mM puromycin and 2 mM GTP (puromycin). Samples were centrifuged again (20 min; 6,000 × g) to reisolate the membrane-containing fractions. Distribution of Scp160p, Kar2p, Sec61p, and ribosomes after the indicated treatment was analyzed by immunodetection. B and C, P6 isolated after mock treatment (B) or treatment with S1 nuclease (C) was resuspended in LS buffer containing 2% Nikkol and incubated at 23 °C for 15 min. Solubilized material was separated by centrifugation (20 min; 6,000 × g) and subjected to sucrose density gradient centrifugation. The distribution of ribosomal proteins and Scp160p was assayed as in Fig. 3.
tionation of Scp160p with ribosomes (Fig. 4C). The vast majority of Scp160p was found in fractions 1 and 2, indicating that this association of Scp160p with membrane-bound ribosomes is dependent on mRNA.

**GFP-tagged Scp160p Is Enriched at the ER of Living Yeast Cells**—To investigate the dynamic distribution of Scp160p in living cells, gene fusions of SCP160 were created that encode the entire Scp160p fused to a bright derivative of GFP (40). The GFP moiety was fused either at the N or C terminus of Scp160p. These constructs were then introduced into the yeast genome to completely replace wild-type Scp160p, making the GFP fusion protein the only functional copy in the cell. This approach allowed us to study the distribution of Scp160p at its normal expression levels.

An immunoblot probed with anti-Scp160p antibodies confirmed that the GFP fusions are the only version of Scp160p present in the cells (Fig. 5A). Furthermore, the levels of the tagged forms of Scp160p present in the cells were similar to that of untagged Scp160p in wild-type cells. Fluorescence microscopy showed that GFP-tagged Scp160p is concentrated around the nucleus and in patches close to the periphery of the cell (Fig. 5C), reminiscent of ER staining. To correlate this more directly, we transformed cells with a plasmid encoding Sec63p-GFP. Sec63p-GFP fluorescence showed a broadly similar pattern to GFP-tagged Scp160p (Fig. 5B). However, compared with Sec63p-GFP, more GFP-Scp160p signal was seen at regions typical for cortical ER. GFP-Scp160p and Sec63p-GFP seem to be excluded from the nucleus, but some diffuse cytoplasmic staining of GFP-tagged Scp160p was also visible. The partial overlapping localization of Scp160p with the integral ER membrane protein Sec63p confirmed the localization of Scp160p at the ER membrane.

**Limited Binding Sites for Scp160p at the ER Membrane**—Next, we localized GFP-tagged Scp160p in cells overexpressing wild-type Scp160p from a low copy plasmid. Quantitation from immunoblotting indicated an approximately 4-fold overexpression (data not shown). The increase in Scp160p levels induced a significant redistribution of GFP-Scp160p from the ER to the cytosol (Fig. 6A), consistent with a limited number of binding sites. To investigate whether GFP-Scp160p behaves similarly as the endogenous Scp160p, we prepared a 6,000 × g supernatant, treated the pellet with Nikkol, and subjected both frac-
tions to density centrifugation. GFP-Scp160p present in the supernatant derived from cells transformed with an empty CEN plasmid cofractionated preferentially with mono- and polysome-containing fractions (Fig. 6B, top panel, fractions 6–10). This indicates that the GFP-tagged Scp160p behaves similarly with respect to its binding to polysomes as the endogenous Scp160p (Fig. 2). The distribution of GFP-Scp160p changed dramatically in cells overexpressing Scp160p; GFP-Scp160p was shifted from the polysome-containing fractions into fractions 2–4 (Fig. 6B, bottom panel). This complex, which emerged only at a high concentration of Scp160p, has a similar size as the complex generated by EDTA treatment (Fig. 2). The majority of the membrane-bound GFP-Scp160p cofractionated with ribosomes (Fig. 6C, top panel, fractions 6–10) and behaved similar to the endogenous Scp160p (Fig. 3B). Overexpression of Scp160p abolished the cofractionation of GFP-Scp160p and membrane-bound ribosomes. Increased levels of Scp160p caused the accumulation of a ribosome-free Scp160p pool (Fig. 6C, bottom panel, fractions 2–4). The total amount of GFP-Scp160p bound to ribosomes remained roughly constant (Fig. 6, B and C, bottom panel, fractions 6–10). Taken together, these results suggest a specific, saturable binding of Scp160p-ribosome complexes at the ER membrane.

Microtubule-depolymerizing Drugs Abolish Scp160p Accumulation at the ER—The majority of Scp160p-ribosome complexes were found at the ER membrane. To analyze how these complexes get to the ER, we localized GFP-Scp160p in cells where the cytoskeleton was disassembled. We depolymerized the actin network or the microtubules by treatment with the drugs latrunculin A (41) and benomyl (42), respectively. Treatment with 30 μg/ml latrunculin A, which induces a rapid depolymerization of actin (Fig. 7A, panels d and h), did not change the localization of GFP-tagged Scp160p (Fig. 7A, panels a–e). In contrast, depolymerization of microtubules by benomyl induced a complete redistribution of GFP-Scp160p from the ER to the cytosol within 15 min (Fig. 7B, panels a–e). Complete depolymerization of microtubules after benomyl treatment is shown by localization of GFP-Tub1p. Spindle depolymerization was first visible after 15 min and was complete after 2 h; all cells were arrested with dot-like GFP-tubulin fluorescence (Fig. 7B, panel k). Treatment with nocodazol, a drug also known to depolymerize microtubules, induced a similar redistribution of...
GFP-Scp160p (data not shown). The perinuclear staining of Sec63-GFP after treatment with either benomyl or latrunculin A indicated that the ER structure was not affected (Fig. 7, A, panels c and g, and B, panels d and i). The loss of ER localization after benomyl treatment only occurred in the presence of ongoing protein synthesis. Elongation arrest induced by cycloheximide abolished the benomyl-dependent redistribution of Scp160p from the ER into the cytosol (Fig. 7B, panels b and g). In the absence of benomyl, active translation was not required to accumulate GFP-Scp160p at the ER, as cells incubated in cycloheximide showed a similar concentration of Scp160p at the perinuclear and cortical ER as control cells (Fig. 7B, panel c and h). Taken together, these data suggest that (i) the accumulation of Scp160p at the ER depends on microtubules, (ii) translation is required to release Scp160p from the ER to the cytosol, and (iii) the anchoring of Scp160p at the ER is independent of microtubules. Therefore, we propose a microtubule-dependent transport of Scp160p-ribosome complexes to the ER.

DISCUSSION

Scp160p Bound to Polysomes Accumulates at the ER—Here, we report that the majority of the multi KH-domain protein Scp160p is located at the ER, as indicated by fluorescence microscopy in living cells with functional, GFP-tagged Scp160p and by cell fractionation. This distribution is consistent with the results of Wintersberger et al. (17), who show by indirect immunofluorescence that Scp160p colocalized with the ER marker Kar2p. Our cell fractionation experiments clearly demonstrate that this large pool of membrane-bound Scp160p is associated with polysomes. We could also demonstrate that the minor cytosolic fraction of Scp160p is similarly associated with polysomes, consistent with a previous report (29). Like these authors, we could show a salt-sensitive interaction of Scp160p with ribosomes. Importantly, we now show in addition that this interaction is dependent upon mRNA and is saturable. EDTA treatment released Scp160p from polysomes as a complex partially comigrating with, but distinct from, 40 S subunits. This complex contains mRNA (data not shown) and shares characteristics of the previously described messenger RNP complex, which was released from cytosolic polysomes upon EDTA treatment (29).

The cofractionation of Scp160p with large polysomes implies that Scp160p may either associate with actively translating ribosomes or with mRNAs carrying stalled polysomes. Binding of Scp160p to the ribosome is sensitive to low concentrations of S1 nuclease, which degrades mRNA but leaves rRNA largely intact. This suggests that the specificity of ribosome binding is dependent on the interaction of Scp160p with defined mRNAs and not on the interaction of KH domains with rRNA per se. This is consistent with the fact that binding of Scp160p to polysomes becomes saturated upon mild overexpression (Fig. 6) where the concentration of Scp160p is still substoichiometric to the concentration of ribosomes (data not shown).

The localization of Scp160p by fluorescence microscopy showed a clear concentration of GFP-tagged Scp160p at the ER. Complexes containing Scp160p, ribosomes, and mRNA could be solubilized from membranes. Furthermore, ribosomes were required to maintain the association of Scp160p with the ER, as suggested by the EDTA-dependent release of Scp160p from the membrane fraction. Interestingly, mRNA digestion only partially released Scp160p from the membrane, although sucrose density gradient analysis after mRNA digestion and solubilization showed that Scp160p was no longer associated with ribosomes. This suggests that other membrane-bound factors are involved in the association of Scp160p with the membrane.

The accumulation of GFP-tagged Scp160p at the ER diminished upon overexpression of untagged Scp160p. One possible explanation is a requirement of specific mRNAs for the targeting of Scp160p ribosome complexes to the ER. Alternatively, a cytosolic factor required for binding of Scp160p at the membrane may be limiting.

Overexpressed Scp160p was found in particles with a similar size as the complex, which was released from polysomes upon EDTA treatment (29). The functional relevance of these complexes remains obscure; we do not even know whether such complexes exist in a normal growing yeast cell.

Accumulation of Scp160p at the ER Requires Microtubules—Most interestingly, we found a microtubule-dependent localization of Scp160p-bound polysomes. Until now, it was hypothesized that smaller cells like yeast rely only on the actin network to transport and/or anchor localized mRNAs (43, 44). Microtubule-dependent long distance transport is known for large cells such as neurons, oligodendrocytes, and oocytes. For example, Staufen mediates microtubule-dependent localization and translational control of Oskar mRNA at the posterior of Drosophila oocytes (45, 46) and anchoring of Bicoid mRNA at the anterior of the egg (47). Microtubules are important for the transient interaction of mRNA with the cytoskeleton during the transport phase as well as for the attachment of mRNAs at their final destinations. Our findings suggest that cytosolic Scp160p-ribosome complexes are transported to the ER membrane dependent on intact microtubules. The high steady state concentration of Scp160p at the ER reflects the final destination of such localized complexes. Few examples of mRNA transport to the ER are known. Recently, mammalian Staufen, which is expressed in most tissues, was implicated in the transport of large RNPs to the ER. Staufen interacts with microtubules and polysomes and colocalizes with markers of the rough ER (48–50). However, it remains unclear whether the observed Staufen-containing particles represent vesiculated ER-Staufen complexes or whether these complexes themselves are involved in mRNA transport. The zipcode-binding protein expressed in Xenopus oocytes promotes microtubule-dependent transport of Vg1 mRNA to a subcompartment of the ER (51). Another example exists in plant cells, where mRNAs encoding rice seed storage proteins are targeted to a subdomain of the ER, known as prolamalin protein bodies (52).

Intracellular Targeting of Scp160p-bound Polysomes—The large number of RNA binding domains in Scp160p (14 KH domains) provides different surfaces to form simultaneous contacts with mRNAs and rRNAs, allowing selective binding to polysomes. Since Scp160p is associated with polysomes, it is conceivable that the protein plays a dual role: (i) positioning specific mRNAs at the ER and (ii) regulating their translation at this site. In summary, our data show a mRNA-dependent association of Scp160p with membrane-bound polysomes and microtubule-dependent accumulation of these complexes at the ER. These results encourage us to speculate that Scp160p may function as an mRNA binding platform involved in the targeting of a subset of mRNAs to the ER. Identification of specific mRNA substrates will help us to further define the cellular function of Scp160p.

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