The Multi-KH Domain Protein of Saccharomyces cerevisiae Scp160p Contributes to the Regulation of Telomeric Silencing

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Multi-KH domain proteins are highly evolutionarily conserved proteins that associate to polyribosomes and participate in RNA metabolism. Recent evidence indicates that multi-KH domain proteins also contribute to the structural organization of heterochromatin both in mammals and Drosophila. Here, we show that the multi-KH domain protein of Saccharomyces cerevisiae, Scp160p, contributes to silencing at telomeres and at the mating-type locus, but not to ribosomal silencing. The contribution of Scp160p to silencing is independent of its binding to the ribosome as deletion of the last two KH domains, which mediate ribosomal binding, has no effect on silencing. Disruption of Scp160p increases cell ploidy but this effect is also independent of the contribution of Scp160p to telomeric silencing as strong relief of silencing is observed in ∆scp160 cells with normal ploidy and, vice versa, ∆scp160 cells with highly increased ploidy show no significant silencing defects. The TPE phenotype of ∆scp160 cells associates to a decreased Sir3p deposition at telomeres and, in good agreement, silencing is rescued by SIR3 overexpression and in a ∆sirf2-∆rfo2 mutant. Scp160p shows a distinct perinuclear localization that is independent of its ability to bind ribosomes. Moreover, telomere clustering at the nuclear envelope is perturbed in ∆scp160 cells and disruption of the histone deacetylase RPD3, which is known to improve telomere clustering, rescues telomeric silencing in ∆scp160 cells. These results are discussed in the context of a model in which Scp160p contributes to silencing by helping telomere clustering.

Multi-KH domain proteins are highly evolutionarily conserved proteins that have been described in all eukaryotic organisms analyzed to date. These proteins are characterized by the presence of multiple KH domains that are organized in tandem. The KH domain is a single-stranded nucleic acid binding motif that, first identified in the RNA-binding protein hnRNPK, has been found in a number of proteins binding single-stranded nucleic acids (1). Consistent with this molecular organization, several multi-KH domain proteins that associate to polyribosomes and participate in RNA regulation of mRNA translation and protein synthesis (2–5). In particular, multi-KH domain proteins contribute to the structural and functional properties of heterochromatin (2, 19–21). Here, we show that the multi-KH domain protein of Saccharomyces cerevisiae, Scp160p, contributes to silencing at telomeres and at the mating-type locus, but not ribosomal silencing. Our results also indicate that the contribution of Scp160p to silencing is independent of its binding to the ribosome or of its contribution to chromosome segregation.

EXPERIMENTAL PROCEDURES

Yeast Strains and Plasmids—Strains in these experiments are described in Table 1. Deletion of SCP160 was carried out by transforming the corresponding parental strains with either a NotI-NotI fragment obtained from plasmid pASC160::HIS3, which was constructed by cloning a 4276-bp PCR fragment of the SCP160 locus into pGEM-T (Promega) and replacing the Nrp1-KpnI fragment by the HIS3 marker, or with a DNA fragment obtained from plasmid YDP-L (22) by amplification with primers: ∆SCP160-UP (5′-ATGTCCTGAGAAGAAA-CGGCTATGGACTCTCCCTCCCTCCAGCCCGGCTTTAAC-3′) and ∆SCP160-LO (5′-TCGGACACGCTTTGTAAGTCTCATTGATGAGAACAATCATCATCGGGCTTTGATTAGC-3′). Deletion of RIF1 was carried out by transforming the corresponding parental strains with a NotI-NotI fragment obtained from plasmid pARIF1::TRP1, which was constructed by cloning a 6846-bp PCR fragment of the RIF1 locus into pGEM-T (Promega) and replacing the Nhel-Aval fragment by the TRP1 marker. Deletion of Rif2 was carried out by trans-

\[\text{interaction with specific mRNAs (1, 8) to tRNA export and the general regulation of mRNA translation and protein synthesis (9–12). In particular, in S. cerevisiae, Scp160p is found associated to both soluble and membrane-bound polyribosomes (13–15). Binding of Scp160p to ribosomes requires the C-terminal region so that, a truncated protein missing the last two KH domains is not capable of binding ribosomes (16, 17). Also in human cells, vigilin associates to ribosomes through its C-terminal domain (18) and localizes to the rough endoplasmic reticulum (RER) (19). Consistent with an association to ribosomes of the RER, Scp160p shows a perinuclear localization (6, 15).}]

Multi-KH domain proteins also contribute to the regulation of chromosome structure and function. Functional analysis in S. cerevisiae showed that disruption of SCP160 results in cells with increased ploidy (6) and, in Drosophila, ddp1 mutations showed defects on chromosome condensation and segregation (20, 21). Several indications suggest that, both in mammals and Drosophila, multi-KH domain proteins contribute to the structural and functional properties of heterochromatin (2, 19–21). In particular, ddp1 mutations suppress heterochromatin-induced gene silencing (PEV) in Drosophila (20). In this article, we show that, in S. cerevisiae, a ∆scp160 deletion relieves silencing both at telomeres and at the mating-type locus, but not ribosomal silencing. Loss of telomeric silencing is associated to a decreased Sir3p deposition and telomere clustering is perturbed in ∆scp160 cells. Our results also indicate that the contribution of Scp160p to silencing is independent of its binding to the ribosome or of its contribution to chromosome segregation.

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3. **Multi-KH domain proteins are highly evolutionarily conserved proteins that have been described in all eukaryotic organisms analyzed to date. These proteins are characterized by the presence of multiple KH domains that are organized in tandem. The KH domain is a single-stranded nucleic acid binding motif that, first identified in the RNA-binding protein hnRNPK, has been found in a number of proteins binding single-stranded nucleic acids (1). Consistent with this molecular organization, several multi-KH domain proteins that associate to polyribosomes and participate in RNA regulation of mRNA translation and protein synthesis (2–5). In particular, multi-KH domain proteins contribute to the structural and functional properties of heterochromatin (2, 19–21). Here, we show that the multi-KH domain protein of Saccharomyces cerevisiae, Scp160p, contributes to silencing at telomeres and at the mating-type locus, but not ribosomal silencing. Our results also indicate that the contribution of Scp160p to silencing is independent of its binding to the ribosome or of its contribution to chromosome segregation.**

4. **EXPERIMENTAL PROCEDURES**

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forming the corresponding parental strains with a NotI-NotI fragment obtained from plasmid pΔRIF2::LEU2, which was constructed by cloning a 1424-bp PCR fragment of the RIF2 locus into pGEM-T (Promega) and replacing the BspTI-BspTI fragment with the RIF2inga1424-bp PCR fragment of the marker. The extent of silencing was determined by plating 200/ H9262 purifying, and PCR reactions were performed as described elsewhere (25).

FACS Analysis—For FACS analysis, cells were grown to an optical density of 0.5, fixed with ethanol, treated with RNase, and stained with propidium iodine. FACS analysis was performed in a Coulter Epics cytometer with a blue argon laser (488 nm, 15 milliwatts). Fluorescence was detected at 665–685 nm.

Western Analysis—For Western analysis, whole cell protein extracts from 10⁶ cells were obtained as described in Ref. 27. Proteins were separated on a 10% SDS-PAGE gel and blotted to a reinforced cellulose nitrate membrane (OPTITRAN BA-S 85, Schleicher and Schuell). Western blots were analyzed with a monoclonal αHA 3F10 antibody (Roche Applied Science) at a 1:500 dilution with rabbit αScp160p antiserum at 1:5000 dilution, and detected by ECL (Amersham Biosciences).

Immunofluorescence Microscopy Analysis—Immunofluorescence microscopy analysis was performed according to Ref. 28. Briefly, cells were grown to a density of 10⁹ cells/ml and then fixed for 2 h at room temperature with 3.7% formaldehyde in 100 mM phosphate buffer (pH 6.5). After fixation, cells were harvested, treated with Lyticase (ICN Biomedicals) and attached to poly-L-lysine-coated slides. Cells were then incubated for 30 min at room temperature with rat monoclonal αHA 3F10 antibody (Roche Applied Science) at a 1:500 dilution or with rabbit αScp160p antiserum at 1:5000 dilution, and detected by ECL (Amersham Biosciences).

Results

A Δscp160 Deletion Relieves Silencing at Telomeres and the Mating-type Locus, but Not at the Ribosomal Locus—Fig. 1 shows the effects of a Δscp160 deletion on telomeric silencing. For these experiments, disruption of SCP160 was carried out in three different strains carrying telomeric insertions of a URA3 marker. UCC506 (29) and YDS21U (30)
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Strains carry the reporter URA3 gene inserted at a similar position in chromosome V-R, at 2.1 kb from the telomere end, but correspond to two different genetic backgrounds. On the other hand, in AYH2.45 strain (26), the URA3 marker is located on chromosome VII-L immediately adjacent to the telomeric repeats. In all three cases, deletion of SCP160 relieves telomeric silencing as judged by the decreased growth observed in the presence of FOA. This TPE phenotype shows an incomplete penetrance with about 65% of the Δscp160 isolates showing significant relief of telomeric silencing (rows labeled + in Fig. 1). The TPE phenotype of Δscp160 isolates is maintained upon serial growing for up to more than 80 generations indicating that it is mitotically stable. A similar situation is observed in mutants of some telomere components, such as KU70/80, where two mitotically stable states of silencing also coexist (31).

Silencing at the mating-type locus is also impaired in Δscp160 cells (Fig. 2A). In this case, deletion of SCP160 was performed in two different strains, YLS409 and YLP19, which carry an ADE2 marker inserted at the HMR locus (32, 33). In the absence of adenine, silencing of the ADE2 gene results in pink-colored colonies as the consequence of the accumulation of adenine precursors (Fig. 2A, WT). YLP19 shows weaker silencing because it carries a hmrΔA mutation that corresponds to deletion of the HMR-E silencer. In both strains, deletion of SCP160 relieves silencing of the ADE2 marker as judged by the increased white color of the colonies (Fig. 2A, Δscp160). In this case, penetrance of the mutation is high as significant derepression of the ADE2 marker was observed in all the Δscp160 isolates analyzed.

Contrary to the effects on silencing observed at telomeres and the mating-type locus, deletion of SCP160 shows no significant relief of ribosomal silencing (rDNA) (Fig. 2B). For these experiments, two strains were used that carry a URA3 gene inserted at the NTS1 or the NTS2 region of the rDNA locus, strains JS125 and JS128, respectively (34). At these positions, silencing of the URA3 marker is not significantly affected in Δscp160 cells (Fig. 2B, Δscp160).

Deposition of Sir3p at Telomeres Is Reduced in Δscp160 Cells—In S. cerevisiae, Sir proteins are essential for heterochromatin-induced gene silencing (for a review see Ref. 35). In particular, Sir3p is required
for heterochromatin formation and silencing at telomeres and the mating-type locus, but not at the rDNA locus. ChiP experiments were performed to determine the effects of a Δscp160 mutation on the deposition of Sir3p at telomeres (Fig. 3). For these experiments, deletion of SCP160 was carried out in the AYH2.45 strain described above that, in addition to the telomeric URA3 insertion, carries a HA-tagged form of Sir3p. Cross-linked material was immunoprecipitated with αHA antibodies and analyzed by PCR using a set of primers that amplify a 250-bp long fragment corresponding to the telomeric URA3 insertion on chromosome VII-L (URA3-Tel) and, on the same PCR reaction, a second set of primers that amplify a 395-bp long control fragment corresponding to the endogenous ura3-52 locus (ura3-52). (Fig. 3A). The extent of Sir3p deposition at telomeres was then determined as the ratio between the URA3-Tel and ura3-52 fragments (Fig. 3B). Three out of the five independent Δscp160 isolates analyzed showed a strong decrease on the URA3-Tel/ura3-52 ratio (Fig. 3B, compare column WT with columns Δscp160) to values similar to that obtained with an untagged SIR3 strain (Fig. 3B, column NO-HA). As judged by Western analysis, the level of Sir3p expression is not significantly affected in Δscp160 cells (Fig. 3C), indicating that reduced telomeric deposition of Sir3p is not the consequence of a decreased Sir3p expression. The two other Δscp160 strains analyzed showed only a moderate decrease on the URA3-Tel/ura3-52 ratio though they also show significant TPE, indicating that there is not a strict correlation between the intensity of TPE and the extent to which telomeric Sir3p deposition is affected.

These results indicate that the TPE phenotype observed in Δscp160 cells is likely the consequence of a reduced Sir3p deposition. Consistent with this interpretation, overexpression of SIR3 restores telomeric silencing in Δscp160 cells (Fig. 4A). In this experiment, a Δscp160 strain derived from UCC506 and showing a strong TPE phenotype was transformed with plasmid pHR67-23, expressing Sir3p (23), or with a similar empty plasmid, pRS425 (24). Robust silencing is observed upon Sir3p overexpression (Fig. 4A, row pHr67-23 on the right panel), that is not significantly different from that observed when overexpression is carried out in the wild-type UCC506 strain (Fig. 4A, row pHr67-23 on the left panel). Transformation with the empty plasmid shows no significant effect on silencing (Fig. 4A, rows pRS425). Moreover, the TPE phenotype of the Δscp160 mutation is not efficiently established when deletion of SCP160 is carried out in cells transformed with pHr67-23 and, therefore, overexpressing Sir3p (Fig. 4B, left panel). In this case, only one out of six different Δscp160 isolates showed any significant derepression of the telomeric URA3 marker, which corresponds to a much lower penetrance (17%) than that observed in the absence of Sir3p overexpression (65%) (Fig. 1). However, upon growing on complete medium to induce loss of the pHr67-23 plasmid, penetrance of the Δscp160 deletion is restored with four isolates (67%) showing a significant TPE phenotype (Fig. 4B, right panel).

In good agreement with these results, establishment of the TPE phenotype of the Δscp160 mutation is impaired in rif1/rif2 mutants (Fig. 5). Rif1p/Rif2p are negative regulators of Sir3p deposition as they compete with Sir3p for binding to the C-terminal domain of Rap1p, which is responsible for recruitment of Sir3p to telomeres (35). In a Δrif1Δrif2 double mutant, telomeric deposition of Sir3p increases though the actual levels of Sir3p expression are not affected (36, 37). Deletion of SCP160 in a Δrif1Δrif2 background shows no TPE phenotype as no significant relief of silencing was observed in any of the eight Δscp160Δrif1Δrif2 isolates analyzed (Fig. 5C, rows Δscp160Δrif1Δrif2). Establishment of the TPE phenotype associated to the Δscp160 deletion is also impaired in single Δrif1 and Δrif2 mutants (Fig. 5, A and B), though to a lower extent than in the Δrif1Δrif2 double mutant. Pen-
Entrance of the TPE phenotype in a \( \text{TPE} \) background is significantly lower (25%) than that observed in wild-type cells (67%). On the other hand, in a \( \text{TPE} \) mutant, penetrance of the TPE phenotype is only slightly lower (50%) than in the wild-type condition. These results correlate well with previous work by others showing that a \( \text{TPE} \) mutation has only weak telomeric phenotypes but that, together with a \( \text{TPE} \) mutation, shows strong synergistic effects (37). Altogether, these results strongly suggest that the TPE phenotype of \( \text{TPE} \) cells arises from defective Sir3p deposition at telomeres.

Nuclear Organization Is Perturbed in \( \text{TPE} \) Cells—\( \text{TPE} \) cells show altered nuclear morphology and organization. Nuclei of \( \text{TPE} \) cells are significantly larger and more irregular in shape and size, than nuclei of wild-type cells (6) (not shown). In \( \text{S. cerevisiae} \), telomeres cluster to the nuclear envelope forming 4–8 discrete foci, which are enriched in Rap1p, Sir2p, Sir3p, and Sir4p, and facilitate silencing (reviewed in Ref. 38). As shown in Fig. 6, telomere clustering is perturbed in \( \text{TPE} \) cells. In these experiments, the pattern of distribution of Sir3p was determined in wild-type and \( \text{TPE} \) cells.
carrying HA-tagged Sir3p by immunolocalization using αHA-specific antibodies. In ∆scp160 cells, Sir3p foci are distributed more irregularly, and are less well defined, than in wild-type cells. In fact, the percentage of cells containing at least three Sir3p foci is reduced by about 30% in ∆scp160 cells (total number of cells analyzed is 669) when compared with wild-type cells (total number of cells analyzed is 562). This difference is statistically significant (p < 0.0001). No significant αHA reactivity was observed in control cells carrying no HA-tagged protein (not shown).

These results indicate that deletion of SCP160 affects telomere clustering at the nuclear envelope. In agreement with this interpretation, the TPE phenotype of ∆scp160 cells is rescued in a rpd3 mutant background. Disruption of the histone deacetylase RPD3 is known to improve anchoring of telomeres to the nuclear membrane (39) and, therefore, silencing (30, 40). As shown in Fig. 7A, disruption of RPD3 restores silencing in a ∆scp160 strain derived from UCC506 showing a strong TPE phenotype (Fig. 7A, row ∆scp160). As discussed below, ∆scp160 cells show increased ploidy (2, 6) so that the ∆scp160 strain used in these experiments was diploid for the RPD3 locus. Disruption of just one copy of the RPD3 gene showed no significant effect on silencing (Fig. 7A, row ∆scp160RPD3/rpd3) but when both copies were mutated silencing was restored (Fig. 7A, rows ∆scp160rpd3/rpd3). Similarly, the TPE phenotype of the ∆scp160 mutation is not efficiently established in a rpd3 mutant (Fig. 7B). In this case, all twelve ∆scp160rpd3 isolates analyzed showed strong silencing similar to that of the rpd3 strain.

**FIGURE 6.** Telomere clustering is perturbed in ∆scp160 cells. A, immunolocalization patterns of HA-Sir3p in wild-type (wt) and ∆scp160 cells are presented. In B, a few representative cases are shown at a higher magnification. Arrows indicate distinct Sir3p foci.

**FIGURE 7.** Disruption of RPD3 rescues the silencing defect of a ∆scp160 mutation. A, disruption of RPD3 was performed in a ∆scp160 strain, derived from UCC506, showing strong TPE phenotype and diploid for the RPD3 locus. A single ∆scp160RPD3/rpd3 and several ∆scp160rpd3/rpd3 isolates were analyzed. B, deletion of SCP160 was performed in an rpd3 strain derived from UCC506. Several ∆scp160rpd3 isolates were analyzed. Cells were grown in complete medium to a final density of A600 = 1.0 and plated as serial 10th-fold dilutions (lanes 1–5) onto selective medium with or without 5-FOA. WT corresponds to the parental UCC506 strain. For each isolate, the intensity of the effect on silencing with respect to the rpd3 strain is shown on the right: (+), indicates isolates that, in the presence of FOA, show at least a 10-fold reduction of growth with respect to WT; (−), indicates isolates showing no significant growth defects.
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The TPE Phenotype of the Δscp160 Mutation Is Not Associated to an Increased Ploidy—It was shown earlier that deletion of SCP160 results in cells of increased ploidy (2, 6). As for the TPE phenotype, the ploidy phenotype of Scp160p shows an incomplete penetrance. About 10% of the Δscp160 isolates analyzed showed normal FACS profiles undistinguishable from that of a wild-type strain. Several observations indicate that the TPE phenotype of the Δscp160 mutation is not directly related to its ploidy phenotype. On one hand, some Δscp160 isolates of normal ploidy showed significant TPE (Fig. 8, Δscp160 row 3) and vice versa Δscp160 cells of highly increased ploidy did not show any significant TPE phenotype (Fig. 8, Δscp160 row 1). Moreover, disruption of RPD3 or overexpression of SIR3 rescues the TPE phenotype (Figs. 4A and 7A) but not the ploidy phenotype of Δscp160 cells (not shown). In addition, overexpression of SIR3 strongly prevents establishment of the TPE phenotype (Fig. 4B) but not of the ploidy phenotype, which was established in all the isolates analyzed. Similarly, in a rpd3 mutant background, deletion of SCP160 increases ploidy although it does not affect silencing (Fig. 7B). Interestingly, penetrance of the ploidy phenotype is significantly reduced in a rpd3 mutant (35%) when compared with a wild-type strain (90%).

Deletion of the C-terminal Region of Scp160p, Which Mediates Binding to the Ribosome, Does Not Affect Telomeric Silencing or Perinuclear Localization of Scp160p—Scp160p is known to associate to polyribosomes (13–15). The ability of Scp160p to bind to the ribosome resides in its C-terminal region so that, a C-terminal deletion to E1081 that involves the last two KH domains, fully abolishes binding to the ribosome (16, 17). A similar behavior was described for the human homolog, vigilin (18). Also in this case, vigilin binds ribosomes and the last two KH domains are required for ribosomal binding. The TPE phenotype of a Δscp160 mutation is, however, independent of binding of Scp160p to ribosomes as deletion of the last two KH domains does not have any significant effect of telomeric silencing (Fig. 9). A scp160ΔKH13–14 mutation, missing the last two KH domains (KH13 and KH14), shows similar telomeric silencing as that observed in the wild-type strain. In this case, eleven different scp160ΔKH13–14 isolates were analyzed and none of them showed any significant relief of telomeric silencing. Moreover, as judged by FACS analysis, ploidy of scp160ΔKH13–14 cells is not increased (not shown), which is in good agreement with previous work reported by others (17).

Scp160p shows a distinct perinuclear localization that was proposed to reflect its association to ribosomes of the RER (6, 15). However, as shown in Fig. 10, perinuclear localization of Scp160p is independent of its ability to bind ribosomes. In these experiments, both full-length Scp160p and a truncated Scp160pΔKH13–14 form, missing the last two KH domains involved in ribosomal binding, were HA-tagged and their cellular distribution analyzed by immunolocalization using αHA-specific antibodies. In good agreement with previously reported results (6, 15), a clear perinuclear αHA-signal is observed in cells carrying wild-type HA-Scp160p together with a diffuse cytoplasmic reactivity (Fig. 9A). A similar immunolocalization pattern is observed in cells carrying the truncated HA-Scp160pΔKH13–14 protein (Fig. 9B) though, in this case, the cytoplasmic signal is more intense than in wild-type HA-Scp160p cells. No significant αHA-reactivity was observed in control cells carrying no HA-tagged protein (not shown).
DISCUSSION

In this article, we report results indicating that deletion of SCP160 relieves silencing at telomeres and the mating-type locus (HMR), but not at the ribosomal locus (rDNA). This differential effect is not surprising as rDNA silencing involves a different mechanism that silencing at telomeres and the HMR locus (for a review see Ref. 35). Most of the factors involved in silencing at telomeres and HMR are common (i.e. Rap1p, Sir2p, Sir3p, and Sir4p) and, in both cases, anchoring to the nuclear membrane facilitates silencing. On the other hand, rDNA silencing requires the deacetylase activity of Sir2p but it is not dependent on Sir3p, Sir4p, or Rap1p, while it is affected by mutations in multiple genes involved in DNA replication. In fact, our results indicate that the TPE phenotype of Δscp160 cells is associated to a reduced deposition of Sir3p, a factor that has no effect on rDNA silencing.

There is abundant experimental evidence favoring a role of Scp160p in mRNA metabolism. In particular, it was reported that Scp160p binds to ribosomes (13–15) and associates to a small subset of specific mRNAs (8), suggesting a contribution to their stabilization, processing and/or translation. The TPE phenotype of Δscp160 cells may, therefore, reflect the contribution of Scp160p to the post-transcriptional regulation of specific mRNAs. In particular, it might be argued that, in Δscp160 cells, decreased Sir3p deposition is a consequence of the contribution of Scp160p to the stabilization and/or processing of specific mRNAs encoding for factors required for Sir3p deposition or for Sir3p itself. Several indications, however, make this possibility unlikely. On one hand, our results indicate that expression of Sir3p is not significantly affected in Δscp160 cells. Moreover, both in a rpd3 mutant as well as in a Δrf1Δrf2 double mutant, a Δscp160 mutation has no effect on telomeric silencing indicating that, in these genetic backgrounds, telomeric deposition of Sir3p is as efficient in the absence of Scp160p as in its presence. In addition, deletion of the C-terminal domain of Scp160p, which mediates binding to the ribosome (16, 17), does not affect telomeric silencing. Altogether, these results strongly suggest that the TPE phenotype of the Δscp160 mutation is independent of the contribution of Scp160p to mRNA metabolism.

Δscp160 cells show increased ploidy. However, our results indicate that the TPE phenotype of the Δscp160 mutation is independent on its ploidy phenotype since there is not a strict correlation between both phenotypes as: (i) Δscp160 isolates of normal ploidy show significant TPE and, vice versa, isolates showing no TPE have a highly increased ploidy, and (ii) the ploidy phenotype of Δscp160 cells is not rescued, or prevented, by mutations that restore telomeric silencing or prevent its relief (i.e. overexpression of SIR3 and disruption of RPD3).

Penetration of the TPE phenotype of a Δscp160 deletion is incomplete but, once established, it is mitotically stable. Interestingly, a similar situation is observed in mutants of ku70/80 (31, 41), a DNA repair factor that localizes to telomeric repeats and contributes to clustering of telomeres at the nuclear membrane (42). Our results indicate that telomere clustering is altered in Δscp160 cells. It is uncertain, however, whether Scp160p actually localizes at telomeres as ChIP-experiments performed with a tagged HA-Scp160p protein provided no evidence for its association with telomeric DNA (not shown). In addition, Scp160p shows a distinct perinuclear localization that is not exclusively mediated by binding to ribosomes of RER as a truncated form missing the last two KH domains, which mediate ribosome binding, also shows perinuclear localization. Consistent with these results, biochemical analysis showed that the vast majority of Scp160p remains bound to membranes after RNase treatment though, under these conditions, binding of Scp160p to...
ribosomes is fully abolished (15). Moreover, perinuclear localization of Scp160p is microtubule-dependent (15), but not actin-dependent as more usual for an RNP component in yeast (43, 44). In accordance to these results, a Δscp160 deletion is hypersensitive to the microtubule-destabilizing drug benomyl (not shown). Taken together, these observations suggest that, at least in part, Scp160p is an integral component of the nuclear envelope that is involved in telomere clustering. Tethering of telomeres to the nuclear envelope is mediated by, at least, two different partially redundant pathways (38). One of these pathways is mediated by the Sir proteins and requires the contribution of Esc1p, an integral component of the nuclear envelope that is known to interact with Sir4p. A second pathway, which is Sir-independent, is known to be mediated by heterodimeric Ku70/80. Components of the nuclear envelope involved in anchoring of Ku70/80 remain unknown. Interestingly, the human homolog of Scp160p, vigilin, was recently shown to interact with Ku70/86, the human counterpart of yeast Ku70/80 (21). Therefore, it is possible that, through the interaction with Ku70/80, Scp160p helps clustering of telomeres at the nuclear envelope. Consistent with this hypothesis is the fact that disruption of RPD3, which is known to improve Sir-dependent anchoring (39), rescues the silencing defect of Δscp160 cells. Moreover, a Δesc1Δscp160 double mutant shows strong TPE phenotype although a single Δesc1 mutant shows only a very slight effect on telomeric silencing (45) (not shown). In addition, this model provides a reasonable interpretation for the incomplete penetrance of Δscp160 cells as it might simply reflect maintenance of the Sir-dependent pathway of telomere clustering and silencing. 

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