Genetic Interaction between a Chaperone of Small Nucleolar Ribonucleoprotein Particles and Cytosolic Serine Hydromethyltransferase*

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Srp40p is a nonessential yeast nucleolar protein proposed to function as a chaperone for over 100 small nucleolar ribonucleoprotein particles that are required for rRNA maturation. To verify and expand on its function, genetic screens were performed for the identification of genes that were lethal when mutated in a Srp40p null background (srp40Δ). Unexpectedly, mutation of both cytosolic serine hydromethyltransferase (SHM2) and one-carbon tetrahydrofolate synthase (ADE3) was required to achieve synthetic lethality with srp40Δ. Sm2p and Ade3p are cytoplasmic enzymes producing 5,10-methylene tetrahydrofolate in convergent pathways as the primary source for cellular one-carbon groups. Nonetheless, point mutants of Sm2p that were catalytically inactive (i.e. failed to rescue the methionine auxotrophy of a shm2Δ ade3Δ strain) complemented the synthetic lethal phenotype, thus revealing a novel metabolism-independent function of Sm2p. The same Sm2p mutants exacerbated a giant cell phenotype observed in the shm2Δ ade3Δ strain suggesting a catalysis-independent role for Sm2p in cell size control, possibly through regulation of ribosome biogenesis via Srp40p. Additionally, we show that the Sm-like protein Lsm5p, which as part of Lsm complexes participates in cytosolic and nuclear RNA processing and degradation pathways, is a multiplicity suppressor of the synthetic lethality and of the specific depletion of box H/ACA snoRNAs from the srp40Δ shm2Δ ade3Δ strain. Finally, rat Nopp140 restored growth and stability of box H/ACA snoRNAs after genetic depletion of Srp40p in the synthetic lethal strain indicating that it is indeed the functional homolog of yeast Srp40p.

Biogenesis of vertebrate rRNA involves the modification of ~200 nucleotides (~100 in yeast) by pseudouridylation and 2′-O-methylation. Although apparently nonessential, most of these modifications occur in functionally important regions of the ribosome suggesting a role in translation (1). The nucleotides to be modified are selected by site-specific base pairing with a similar number of small nuclear RNAs (snRNAs)3 of the box H/ACA and box C/D class for rRNA pseudouridylation and 2′-O-methylation, respectively (2–5). The same four core proteins associate with each box H/ACA snoRNA to form separate box H/ACA small nucleolar ribonucleoprotein particles (snoRNPs) and another set of four core proteins forms box C/D snoRNPs together with each box C/D snoRNA. One of the core proteins is the pseudouridylase and the methylase, respectively, catalyzing the modification. In yeast, individual depletion of most of the snoRNP core proteins leads to instability of the particle and the respective class of snoRNAs (6–9). Although little is known about the maturation of the snoRNPs themselves or their organization while modifying rRNA, they are concentrated in the nucleolus and Cajal (coiled) bodies of vertebrate cells. Cajal bodies generally are enriched in small nuclear RNAs and may be involved in their maturation, but their function essentially remains elusive (10).

Vertebrate Nopp140 is the only protein to date that associates with both classes of snoRNPs, although it is not an integral component of either particle (11). Like the snoRNPs, Nopp140 is concentrated in the nucleolus and Cajal bodies (12, 13). In fact, a dominant negative Nopp140 construct specifically chases snoRNPs out of these subnuclear structures indicating an in vitro interaction and a role for Nopp140 in snoRNP localization (14). The interaction of Nopp140 with snoRNPs is reversible and controlled by its unusually high degree of phosphorylation (15). These observations characterized Nopp140 as a chaperone of snoRNPs.

Based on sequence homology and nucleolar localization, Srp40p is the closest Nopp140 relative in yeast (16, 17). Originally identified in genetic screens as a multicopy suppressor of temperature-sensitive mutations in genes involved in rRNA transcription (18, 19), Srp40p concentrates in a nucleolar substructure, the nucleolar body, together with box C/D snoRNAs (20). These and other data indicate that Srp40p, like Nopp140, interacts with snoRNPs. Deletion of the nonessential Srp40 leads to the loss of the nucleolar body similar to the dispersal of Cajal bodies by the dominant negative Nopp140 construct (14, 20). These similarities between Srp40p and Nopp140 prompted us to exploit yeast genetics to learn more about the role of these proteins in ribosome and snoRNP biogenesis. Specifically, we report on the unexpected findings in a screen for genes that are synthetically lethal with an Srp40 deletion.

EXPERIMENTAL PROCEDURES

Yeast Strains, Media, and Genetic Techniques—Strains used in this work are shown in Table I. Yeast were grown in 1% yeast extract, 2% bactopeptone, and 2% dextrose media (YPD) or in synthetic complete media (SC) supplemented with the appropriate amino acids and carbon source of 2% glucose or 2% raffinose, 2% galactose, and 2% sucrose. 5-FOA plates contained 1 mg/ml 5-fluoroorotic acid (Research Products International Corp., Prospect, IL) in SC medium. Yeast transformations were performed using lithium acetate (21), and general genetic manipulations were conducted using standard procedures (22).
dilution assays, yeast cells were grown in galactose-containing medium, washed twice in water, spotted on glucose-containing medium at 10-fold serial dilutions and incubated for 2 days at 30°C. Escherichia coli strain DH5α was used as the bacterial host for all plasmids and was manipulated using standard methods (23).

Synthetic Lethal Screens—To delete SRP40, the SRP40 open reading frame including 130 nucleotides 5’ and 455 nucleotides 3’ in pTM32 (17) was replaced with the HIS3-containing EcoRI-XhoI fragment of pRS313 generating pYY1. The srp40::HIS3-containing ApaI-XhoI fragment of pYY1 served to delete the genomic copy of SRP40 by integrative transformation of YYX2 and YYX3 generating YYY2 and YYX3, respectively. YYX2 and YYX3 were transformants of YCH125 and YCH126, respectively (24). Proper integration of the HIS3 construct at the SRP40 locus was verified by PCR on genomic DNA with the appropriate primers. These SRP40-deleted strains were used in the synthetic lethal screens after transformation with pYY5 (SRP40 ADE3 URA3 CEN) to generate YYX12 and YYX14. pYY5 was constructed by subcloning the pYY60. The URA3-containing 678-bp I fragment in pYY45 and pYY75, respectively, was replaced by a NotI fragment in pYY67 into pYY71 to disrupt SRP40. For the construction of a conditional GAL::srp40 strain, pYY38 (pGAL-SRP40) (11) was transformed into the synthetic lethal strain YYX34. To create YYX31, the GAL::srp40 strain generated by mating two haploids obtained from sporulation and dissection of YYX34, to create YYX3. Precise deletion of the SM2 and ADE3 open reading frames was achieved by replacement with templates that contained oligonucleotides with complementary flanking sequences of the target gene (27). For the construction of a conditional GAL::srp40 strain, pYY38 (pGAL-SRP40) (11) was transformed into the synthetic lethal strain YYX34 which was used as the bacterial host for all plasmids and was manipulated using standard methods (23).

Identification and Molecular Analysis of the Synthetic Lethal Gene

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**Table 1**

| Strain | Genotype |
|--------|----------|
| W303a  | Mata ADE3 ade2 his3 leu2 trp1 ura3 lys2 can1 |
| Y190   | Mata ura3 his3 lys2 ade2 trp1 leu2 gal4D gal80A cyt230 cyh2 lys2::GAL1-HIS3-HIS3 URA3::GAL1-GAL1- lacZ |
| YCH125 | Mata trp1 lys2 ade2 ade3 ura3 his3 can1 |
| YCH128 | Mata trp1 lys2 ade2 ade3 ura3 his3 3' |
| YYY1   | Mata trp1 LYS2 ade2 ade3 ura3 leu2 his3 1 can1 + pRS316A (ADE3 URA3 CEN) |
| YYY2   | Mata trp1 LYS2 ade2 ade3 ura3 leu2 his3 1 can1 + pRS316A (ADE3 URA3 CEN) |
| YYY4   | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY5   | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY7   | Mata srp40::HIS3 shm2::LEU2 TRP1 lys2 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY8   | Mata srp40::HIS3 shm2::LEU2 TRP1 lys2 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY9   | Mata srp40::HIS3 shm2::LEU2 TRP1 lys2 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY10  | Mata srp40::HIS3 shm2::LEU2 TRP1 lys2 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY11  | Mata srp40::HIS3 shm2::LEU2 TRP1 lys2 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY12  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY13  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY14  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY15  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY16  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY17  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY18  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY19  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY20  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY21  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY22  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY23  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY24  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY25  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY26  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY27  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY28  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY29  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY30  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY31  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY32  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY33  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY34  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY35  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |
| YYY36  | Mata srp40::HIS3 trpl lys2 ade3 ade3 ura3 leu2 his3 1 can1 + pY5 (SRP40 ADE3 URA3 CEN) |

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For the construction of a conditional GAL::srp40 strain, pYY38 (pGAL-SRP40) (11) was transformed into the synthetic lethal strain YYX34 which was used as the bacterial host for all plasmids and was manipulated using standard methods (23).
TTCC-3’ that generated an in-frame XbaI site in front of the SHM2 stop codon (pYY69 and pYY79, respectively). A triple c-Myc tag from C3003 (gift from Pascal Chartrand, University of Montreal, Montreal, Canada) was subcloned into these XbaI sites generating SHM2-myc (pYY70) and SHM2 (K248Q/K393A)-myc (pYY80), respectively.

GAL4 DNA binding (GBD) and activation domain (GAD) fusion proteins were constructed by subcloning the respective amplified proteins and/or fragments thereof into pAS2 and pACT2 (Clontech Laboratories, Inc., Palo Alto, CA). Thus, the following constructs were generated: GBD-Srp40p (pY72), GBD-Shm2p (pY74), GAD-Srp40p (pY75), GAD-Shm2p (pY73), GAD-NoppA (amino acids 60–704 of Nopp140, pTM64), GAD-NoppRΔ (60–94, pTM63). The other Nopp140 constructs were as described previously (14). GAD-Lsm5p (pAE70) was a gift from Jean Beggs (28).

RNA Analysis—Srp40p and Chb5p depletion experiments were performed essentially as described (8, 11). Briefly, strains were grown in galactose-containing medium to mid-log phase before switching to glucose-containing medium in which they were maintained in log phase by dilution. At 0 and 24 h in glucose, total RNA was prepared (29). For Northern blotting, gel loading of RNA was adjusted to yield approximately equal quantities of U4 snRNA in each lane of 8% polyacrylamide gels. SnRNAs were detected by hybridization with the following 32P-labeled oligonucleotides: snR10, 5′-ATTGGTATGTCTCATTCGGAT-3′; snR42, 5′-CGAGGAAAGTTGCCTACTACGGACA-3′; snR50, 5′-CGAGGAAAGTTGCCTACTACGGACA-3′; snR50, 5′-CGAGGAAAGTTGCCTACTACGGACA-3′; snR50, 5′-CGAGGAAAGTTGCCTACTACGGACA-3′; and those published previously (11). Blots were quantitated using a PhosphorImager (Amersham Biosciences).

**Results**

Identification of SHM2 and ADE3—Deletion of SRP40 causes slight growth retardation and lack of box C/D snRNA retention in nucleolar bodies but no other apparent deficiencies (17, 20). We took advantage of these facts by performing synthetic lethal screens in an srp40Δ deletion strain to identify genes that were functionally related to SRP40. For this purpose, we used a colony-sectoring approach looking for colonies whose growth depended on the presence of an SRP40 ADE3 URA3 plasmid, which turned the colonies uniformly red and rendered them inviable on medium containing 5-FOA (34). In two independent screens, the srp40Δ strain was mutagenized either by random lacZ LEU2 insertions through transformation with a mutagenized genomic yeast library (26) or by exposure to EMS. Although 100,000 and 177,000 mutagenized colonies were screened, respectively, only one each contained a mutation in a single gene that conferred lethality in conjunction with the srp40Δ deletion. After plasmid rescue, sequencing from the inserted lacZ gene identified the disrupted gene as cytosolic serine hydroxymethyltransferase, SHM2, formerly referred to as LSE2 (lethal with srp40Δ; see Ref. 11). The insertion occurred at amino acid 119 out of 469 for the full-length protein and generated a stop codon amino acids downstream. The EMS mutagenized gene was identified by complementation with a yeast genomic library as the only predicted full-length open reading frame in a 3.8-kb insert corresponding to SHM2. Indeed, SHM2 and SRP40 restored growth to the synthetic lethal strain (srp40Δ shm2Δ pSRP40 URA3) on 5-FOA-containing medium when provided under their own promoters on LEU2 plasmids (Fig. 1A). Thus both screens identified one and the same gene, SHM2.

Cytosolic serine hydroxymethyltransferase converts THF into 5,10-methylene THF by transfer of a hydroxymethyl group from serine while liberating glycine and water (Fig. 1B). 5,10-Methylene THF is the major one-carbon source for methionine, thymidylate, and purine synthesis. Although identified in two independent screens, no connection between this cytoplasmic enzyme and the nucleolar SRP40 was apparent. To confirm further the synthetic lethal relationship between these two genes, therefore, we deleted one of the genomic copies of SHM2 by replacement with the URA3 gene in an SRP40-deleted diploid strain (srp40Δ/srp40Δ). Sporulation and tetrads with the resulting shm2Δ/SHM2 srp40Δ/srp40Δ strain yielded only two viable spores each, all uracil auxotroph, confirming the synthetic lethality (Fig. 1C).
this finding, we deleted both genomic copies of SRP40. The genomic copies of SRP40 edited no major growth defects. However, after deletion of one of the corresponding residues in the E. coli enzyme alone abolished its activity with no apparent impact on its tertiary structure (35, 36). To confirm the catalytic inactivation of the yeast enzyme by these point mutations, their ability was tested to complement a strain rendered methionine-auxotrophic by disruption of the genomic copies of SHM2 and ADE3 (37). Although wild type SHM2 supported growth of the srp40Δ ade3 strain on medium lacking methionine, the single (K248Q and R393A) and double (K248Q/R393A) point mutants failed to confer growth strongly suggesting that they were catalytically inactive (Fig. 2C). Surprisingly, when these apparently inactive constructs were transformed into the synthetic lethal strain, they, like wild type SHM2, complemented growth on 5-FOA-containing medium (Fig. 2D). However, a construct truncated at amino acid 203, SHM2ΔC, failed to restore growth in both cases demonstrating the specificity of the effect of the point mutations (Fig. 2, C and D). We conclude that a block in folate-mediated one-carbon metabolism did not cause the synthetic lethality and, consequently, that Shm2p serves two cellular functions, a catalytic and a noncatalytic one. These findings further imply that Ade3p too may harbor an additional, noncatalytic function linking it in some manner to Srp40p and Shm2p.

Effects of Shm2p Expression on Localization and Cell Size—Shm2p has been characterized as a cytoplasmic protein, mainly based on the subcellular fractionation of its orthologs in other species. The unexpected synthetic lethal relationship of Shm2p with the nucleolar Srp40p led us to reevaluate its localization. In particular, it was interesting to examine if the catalytically inactive Shm2p, freed of its predicted cytoplasmic duty, localized to the nucleolus. For this purpose, Myc-tagged copies of SHM2 or SHM2 (K248Q/R393A) were transformed into an null strain and observed by indirect immunofluorescence (Fig. 2, E and F). Both constructs were situated in the cytoplasm and appeared excluded from nuclei (left panels), which were identified by DNA staining with 4,6-diamidino-2-phenylindole (DAPI) staining (right panel). F, as in E but with the catalytically inactive Shm2p. Bar, 5 μm.

5,10-Methylene THF, the product of Shm2p, can also be generated from formate and THF by the cytoplasmic trifunctional C1-THF synthase, Ade3p (Fig. 1B). Coincidentally, both our synthetic lethal strains were ade3 mutants as required for screening with the colony sectoring assay (34). Therefore, we tested if ADE3 also was involved in the synthetic lethal phenotype. Indeed, like SRP40 and SHM2, ADE3 restored growth to the synthetic lethal strain on 5-FOA-containing medium when provided on a LEU2 plasmid (Fig. 1A). To corroborate this finding, we deleted both genomic copies of SRP40 and SHM2 in an ADE3 wild type diploid strain. This strain exhibited no major growth defects. However, after deletion of one of the genomic copies of ADE3 from this strain, sporulation and tetrad dissection yielded only two viable spores in each case, confirming the participation of ADE3 in the synthetic lethal phenotype (Fig. 1D). Thus, our synthetic lethal strains exhibit triple synthetic lethality between SRP40, SHM2, and ADE3.

A Noncatalytic Function for SHM2—The surprising identification of two major cytosolic enzymes involved in one-carbon metabolism in a synthetic lethal relationship with a nucleolar chaperone suggested that the metabolites could be part of this genetic interaction. We addressed this possibility in two ways, by the complementation of the lethal phenotype by addition of either metabolites or catalytically inactive SHM2. Addition of increased concentrations of serine, glycine, or methionine to the 5-FOA-containing medium failed to rescue growth of the synthetic lethal strain (Fig. 2A). Additionally all media were supplemented with adenine. These data suggested that the growth defect was not caused by a simple lack of metabolites.

To inactivate Shm2p, we targeted two evolutionary conserved amino acid residues required for internal aldime formation with its cofactor pyridoxal phosphate and for binding of the substrate carboxyl group. Specifically, the lysine at position 248 of Shm2p was mutated to a glutamine (K248Q) and arginine 393 to alanine (R393A), individually and combined. Mutation of each of the corresponding residues in the E. coli enzyme alone abolished its activity with no apparent impact on its tertiary structure (35, 36). To confirm the catalytic inactivation of the yeast enzyme by these point mutations, their ability was tested to complement a strain rendered methionine-auxotrophic by disruption of the genomic copies of SHM2 and ADE3 (37). Although wild type SHM2 supported growth of the shm2Δ ade3 strain on medium lacking methionine, the single (K248Q and R393A) and double (K248Q/R393A) point mutants failed to confer growth strongly suggesting that they were catalytically inactive (Fig. 2C). Surprisingly, when these apparently inactive constructs were transformed into the synthetic lethal strain, they, like wild type SHM2, complemented growth on 5-FOA-containing medium (Fig. 2D). However, a construct truncated at amino acid 203, SHM2ΔC, failed to restore growth in both cases demonstrating the specificity of the effect of the point mutations (Fig. 2, C and D). We conclude that a block in folate-mediated one-carbon metabolism did not cause the synthetic lethality and, consequently, that Shm2p serves two cellular functions, a catalytic and a noncatalytic one. These findings further imply that Ade3p too may harbor an additional, noncatalytic function linking it in some manner to Srp40p and Shm2p.

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Although the localization of the wild type and the catalytically inactive Shm2p appeared identical, we noted a significant increase in the size of the cells expressing mutant Shm2p (Fig. 2, compare E and F). This striking phenotype was investigated by quantitative analysis of cell size in images of logarithmically growing strains (Fig. 3). First, the effect of the genetic background of the strains with respect to the three synthetic lethal genes was tested. As recently reported (38), single disruption of SHM2, SRP40, and ADE3 had no effect on cell size (not shown). However, double disruption of SHM2 and ADE3 caused a near doubling in cell size (Fig. 3, compare A to B) whereas disruption of SHM2 in conjunction with SRP40 had no effect (Table II). Because cell diameter is reported here (Table II), cell volume actually increased 7–8-fold. The size increase was unlikely caused by changes in osmotic behavior because it even occurred in the presence of 1 M sorbitol (not shown). Exogenous expression of wild type Shm2p rescued the giant cell phenotype of the
Shm2p, via Lsm5p, may display some distant relationship to four core proteins of box H/ACA snoRNPs (8, 41). Therefore, the interaction between Shm2p and Lsm5p (39). As part of Sm-like proteins, Lsm5p participates in the assembly of box H/ACA snoRNPs. The catalytically inactive Shm2p (K248Q/R393A) increased the cell size from 200 to 250% of wild type (Fig. 3, A and D). In contrast, the stability of the box C/D snoRNAs U3, U14, U24, and the spliceosomal RNA U4 was unaffected (Fig. 4C, lane 4). Overexpression of LSM5 in this GAL::srp40 strain in glucose-containing liquid medium stabilized the tested box H/ACA snoRNAs sn3 and snR10 (Fig. 4C, lane 6). Restoration of growth under these conditions contrasted that of the synthetic lethal strain with the genomic copy of SRP40 deleted, growth of which in liquid medium was not rescued by LSM5 overexpression (data not shown). This apparent discrepancy is most likely explained by residual expression of minute amounts of Srp40p in the GAL::srp40 strain, even in the presence of glucose, as we observed previously (11) for a GAL::xb5 strain. Nevertheless, these results supported a genetic link between Lsm5p and box H/ACA snoRNPs.

**Rat Nopp140 Is a Functional Homolog of Yeast Srp40p**—Based on sequence comparison and nucleolar localization, we previously established that yeast Srp40p was the closest homolog to rat Nopp140 (17). The generation of a yeast strain conditional for SRP40 allowed us to test if Nopp140 also was a functional homolog. For this purpose, we tested if Nopp140 rescued growth on glucose of our srp40∆ shm2 ade3 strain carrying a plasmid copy of GAL::srp40. Indeed, Nopp140 restored growth unlike a vector control (Fig. 5A). Nopp140 consists of three major domains, the unique amino and carboxyl termini separated by the signature central repeat domain containing 11 half-acidic and half-basic repeats (Fig. 5B). The carboxyl terminus is most closely related to yeast Srp40p with 59% sequence identity between the last 50 amino acids, whereas the repeat domain is structurally related to the rest of Srp40p (17). Surprisingly, deletion of the conserved carboxyl terminus had no effect on the ability of Nopp140 to restore growth on glucose (Fig. 5A, NoppC). Similarly, a deletion of the amino terminus did not impair the complementation ability of Nopp140 (NoppN), and the amino terminus alone appeared insufficient for complementation, although it is uncertain to what extent the latter construct was expressed (Fig. 5A, A and C, NoppN). Furthermore, the conserved carboxyl terminus of Nopp140 alone failed to complement, whereas the repeat domain alone fully restored growth on glucose (Fig. 5A, NoppC and NoppR). Even the first four Nopp140 repeats alone were sufficient to fully restore growth, whereas a single repeat was less efficient (NoppR∆ and NoppR∆∆, respectively). To ensure that these results were not a mere reflection of expression levels of the heterologous proteins in yeast, their expression was verified by Western blotting. With the exception of NoppN, all constructs expressed detectable amounts of proteins that migrated at their expected positions (Fig. 5C). Although the expression levels varied, they were unrelated to the ability of the constructs to complement growth on glucose (Fig. 5, compare A and C).
Depletion of Srp40p by growth in glucose-containing medium led to a specific loss of box H/ACA but not C/D snoRNAs (11). To test if, like the growth defect, this phenotype was complemented by Nopp140 or its repeat domain, Northern blots of total RNA from the corresponding strains were probed for snoRNAs (Fig. 5D). The box H/ACA snoRNAs snR3, snR10, snR11, and snR42 were depleted after 24 h of growth in glucose-containing medium in the presence of vector control, whereas the box C/D snoRNAs U3, U14, and snR190 and the spliceosomal snoRNA U4 remained unaffected (Fig. 5D, lane 4). Complementation with full-length Nopp140 restored the levels of all RNAAs tested (Fig. 5D, lane 6). However, despite complementation of growth on solid medium (Fig. 5A), in liquid medium the repeat domain alone barely restored growth (not shown) and only marginally stabilized box H/ACA snoRNAs (Fig. 5D, lane 8). Nevertheless, these data suggest that Nopp140 can functionally replace Srp40p and that this occurs mostly via its repeat domain but not its conserved carboxyl terminus.

**DISCUSSION**

We identified a triple synthetic lethal relationship between SRP40, SHM2, and ADE3. This unexpected connection between a nucleolar protein involved in ribosome biogenesis and two cytosolic enzymes required for basic metabolism was independent of the enzymatic activity of Shm2p. Therefore, Shm2p exhibited a novel noncatalytic function and thereby joined a growing number of proteins playing roles in addition to their previously established functions (42).

Shm2p has been highly conserved throughout evolution and has a well-established role in one-carbon metabolism, in addition, our studies now suggest that it acquired a noncatalytic function linking it to ribosome biogenesis. The point mutations selected for inactivation of Shm2p were based on mutations previously shown to abolish serine hydroxymethyltransferase activity in the bacterial ortholog (35, 36). They affect its substrate carboxyl-binding site and the internal aldimine formation with its prosthetic group pyridoxal phosphate. These residues have been conserved from bacteria to man at the amino acid level as well as in their position within the crystal structure (43, 44). The lack of complementation of the methionine auxotrophy of an shm2Δ ade3 strain by Shm2p with the equivalent mutations (K248Q/R393A) suggested that these positions in the yeast enzyme serve the same functions and that their mutation indeed inactivated the enzyme. Therefore, the complementation of the synthetic lethality by the mutant enzyme was likely caused by an additional catalysis-independent function of the protein.

A function independent of amino acid and folate substrate binding has also been reported for the human Shm2p ortholog (45). Thus, human cytoplasmic serine hydroxymethyltransferase inhibits translation of its own mRNA by binding to the 5′-untranslated region. Such an activity has not been docu-
ment for yeast Shm2p but illustrates that this metabolic enzyme can perform other functions. Although we did not test if enzymatically impaired Ade3p would rescue the synthetic lethality, the results with mutant Shm2p and the lack of complementation by metabolites suggest that it too might exhibit a noncatalytic function. In fact, Ade3p plays a noncatalytic role of Shm2p (37, 53, 54). Indeed all strains used in this study contained a wild type copy of SHM1. Because Shm1p can contribute to the cellular need of one-carbon units in the absence of Shm2p, these data further support our observation that the synthetic lethality is not based on impaired metabolism but on a defect noncatalytic role of Shm2p (37, 53, 54).

The synthetic lethality in our strains strictly required the simultaneous disruption of all three genes, SRP40, SHM2, and ADE3. Disruption of SHM2 together with ADE3 impaired growth on medium lacking methionine. These findings are in conflict with recent results showing synthetic lethality between shm2 and ade3 alone (55). This discrepancy could be explained by different genetic backgrounds of the strains used in these studies. Regardless, our studies are consistent with at least two previous reports (37, 54) showing that shm2 ade3 strains are methionine auxotrophs.

The most striking phenotype of the shm2Δ ade3 strain was its increase in cell size. This phenotype was reversed by expression of wild type Shm2p but exaggerated by catalytically inactive Shm2p suggesting a role for Shm2p in cell growth. Based on these observations, the effect of Shm2p was apparently catalysis-independent and not due to a dominant negative mechanism. It is interesting to note that mammalian serine hydroxymethyltransferases were recently identified as Myc targets in the regulation of cell growth bolstering the role of these enzymes in this signaling pathway (56). One potential mechanism for the regulation of cell size during growth is the control of ribosome biogenesis (57, 58). Srp40p, through snoRNPs, could provide a link between Shm2p, ribosome biogenesis, and consequently cell size. This is consistent with the fact that the same Shm2p mutant increased cell size and rescued the triple synthetic lethality. Additionally, overexpression of SRP40 increased cell size in the triple synthetic lethal background suggesting a role for Srp40p in the regulation of cell size. Although these connections are highly speculative, they
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are tantalizing. Further investigation is required to sort out which, if any, of these interactions are involved in the control of cell size.

The synthetic lethal strain provided us for the first time with the opportunity to engineer an SRP40 conditional strain by placing its expression under the conditional GAL promoter. We had previously taken advantage of this fact to demonstrate that Srp40p depletion in the synthetic lethal background leads to the specific depletion of box H/ACA but not box C/D snoRNAs. Because box H/ACA snoRNA snoR30 is essential for rRNA processing and consequently viability of yeast (59), its depletion is the likely cause for growth arrest after Srp40p depletion. Indeed, depletion of Srp40p caused a defect in early pre-rRNA cleavages at sites A0, A1, and A2 that is characteristic for the repeat domain was strong on solid but only partial in liquid medium. This is reminiscent of the fact that yeast only when grown on solid medium exhibits a nucleolar body, which contains Srp40p, snoRNAs, and the cap methylase Tgs1p (20, 61).

This nucleolar body, which may be related to the mammalian Cajal body, is lost in an srp40 null background. Therefore, it is interesting to speculate that the nucleolar body may be involved in the NoppR complementation mechanism. Whether Nopp140 in mammalian cells also exhibits a connection to cytosolic serine hydroxymethyltransferase awaits further experimentation.

Acknowledgments—We are indebted to the following people for providing reagents used in this study: Jean Beggs, Andy Bognar, Amy Chang, Pascal Chartrand, Mike Snyder, Patrick Stover, Susan Wente, and Ian Willis. We thank Charles Query, Susan Smith, and Jon Warner for critical reading of the manuscript.

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