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

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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 *SRP40* null background (*srp40*Δ). Unexpectedly, mutation of both cytosolic serine hydroxymethyltransferase (*SHM2*) and one-carbon tetrahydrofolate synthase (*ADE3*) was required to achieve synthetic lethality with *srp40*Δ. Shm2p 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 Shm2p that were catalytically inactive (*i.e.* failed to rescue the methionine auxotrophy of a *shm2*Δ *ade3* strain) complemented the synthetic lethal phenotype, thus revealing a novel metabolism-independent function of Shm2p. The same Shm2p mutants exacerbated a giant cell phenotype observed in the *shm2*Δ *ade3* strain suggesting a catalysis-independent role for Shm2p in cell size control, possibly through regulation of ribosome biogenesis via *SRP40*. 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 multicopy suppressor of the synthetic lethality and of the specific depletion of box H/ACA small nucleolar RNAs from the *srp40*Δ *shm2 ade3* strain. Finally, rat Nopp140 restored growth and stability of box H/ACA snoRNAs after genetic depletion of *SRP40* 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 nucleolar RNAs (snoRNAs)[1] 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 vivo* 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). For

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[1] The abbreviations used are: snoRNA, small nucleolar RNA; 5-FOA, 5-fluoroorotic acid; EMS, ethylmethane sulfonate; GAD, GAL4 activation domain; GBD, GAL4 DNA binding domain; SC, synthetic complete; snoRNP, small nucleolar ribonucleoprotein particle; THF, tetrahydrofolate; snRNA, small nuclear RNA; HA, hemagglutinin.

TABLE I
Strains used in this study

Strain	Genotype
W303a	*Mat**a** ADE3 ade2 his3 leu2 trp1 ura3 LYS2 can1*
Y190	*Mat**a** ura3 his3 lys2 ade2 trp1 leu2 gal4D gal80Δ cyhr2 LYS2::GAL1-HIS3-HIS3 URA3::GAL1-GAL1-lacZ*
YCH125	*Mat**a** trp1 LYS2 ade2 ade3 ura3 leu2 his3 can1*
YCH128	*Matα TRP1 lys2 ade2 ade3 ura3 leu2 his3 can1*
YYY2	*Mat**a** trp1 LYSZ ade2 ade3 ura3 leu2 his3 can1* + pRS316A (*ADE3 URA3 CEN*)
YYY3	*Matα TRP1 lys2 ade2 ade3 ura3 leu2 his3 can1* + pRS316A (ADE3 URA3 CEN)
YYY12	*Mat**a** srp40Δ::HIS3 trp1 LYS2 ade2 ade3 ura3 leu2 his3 can1* +pYY5 (*SRP40 ADE3 URA3 CEN*)
YYY14	*Matα srp40Δ::HIS3 TRP1 lys2 ade2 ade3 ura3 leu2 his3 can1* +pYY5 (SRP40 ADE3 URA3 CEN)
SL1	*Matα srp40Δ::HIS3 shm2::LEU2 TRP1 lys2 ade2 ade3 ura3 leu2 his3 can1* + pYY5 (*SRP40 ADE3 URA3 CEN*)
SL2	*Matα srp40Δ::HIS3 shm2 TRP1 lys2 ade2 ade3 ura3 leu2 his3 can1* + pYY5 (*SRP40 ADE3 URA3 CEN*)
YYY206	*Matα srp40Δ::HIS3 shm2 TRP1 lys2 ade2 ade3 ura3 leu2 his3 can1* + pYY38 (*GAL10-SRP40 LYS2 CEN*)
YYX30	*Mat**a**/α srp40Δ::HIS3/srp40::HIS3 LYS2/lys2 TRP1/trp1 ade2/ade2 ade3/ade3 leu2/leu2*
YYX31	*Mat**a**/α srp40Δ::HIS3/srp40::HIS3 shm2Δ::URA3/SHM2 LYS2/lys2 TRP1/trp1 ade2/ade2 ade3/ade3 leu2/leu2*
YYX32	*Mat**a**/α srp40Δ::URA3/srp40::URA3 ade2/ade2 his3/his3 leu2/leu2 trp1/trp1 ura3/ura3 can1/can1*
YYX33	*Mat**a**/α srp40Δ::URA3/srp40::URA3 shm2Δ::TRP1/SHM2 ade2/ade2 his3/his3 leu2/leu2 trp1/trp1 ura3/ura3 can1/can1*
YYX34	*Mat**a**/α srp40Δ::URA3/srp40Δ::URA3 shm2Δ::HIS3/SHM2 ade2/ade2 his3/his3 leu2/leu2 trp1/trp1 ura3/ura3 can1/can1*
YYY342	*Mat**a** srp40Δ::URA3 shm2Δ::HIS3 ade2 his3 leu2 trp1 ura3 can1*
YYX38	*Mat**a**/α srp40Δ::URA3/srp40Δ::URA3 shm2Δ::HIS3/shm2Δ::His3 ura3/ura3 his3/his3 leu2/leu2 trp1/trp1 ura3/ura3 LYS2/LYS2 can1/can1*
YYX39	*Mat**a**/α srp40Δ::URA3/srp40Δ::URA3 shm2Δ::HIS3/shm2Δ::HIS3 ade3Δ::TRP1/ADE3 ura3/ura3 his3/his3 leu2/leu2 trp1/trp1 ura3/ura3 LYS2/LYS2 can1/can1*
YYY336	*Matα shm2Δ::URA3 TRP1 lys2 ade2 ade3 leu2 his3 can1*

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 *Eco*RI-*Xho*I fragment of pRS313 generating pYY1. The *srp40*Δ::*HIS3*-containing *Apa*I-*Xba*I fragment of pYY1 served to delete the genomic copy of *SRP40* by integrative transformation of YYY2 and YYY3 generating YYY4 and YYY5, respectively. YYY2 and YYY3 were transformants of YCH125 and YCH128, 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 YYY12 and YYY14. pYY5 was constructed in two steps. First, *ADE3* was cloned from pSW198 (24) into pRS316 (25) as a *Bam*HI-*Sal*I fragment resulting in pRS316A. Second, the *SRP40* gene including 1029-nucleotide 5′- and 475-nucleotide 3′-untranslated region, isolated by PCR amplification using yeast genomic DNA as template, was subcloned into the *Sal*I site of pRS316A yielding pYY5. YYY12 and YYY14 produced colonies that exhibited a high degree of sectoring when grown on nonselective medium and were used in a colony-sectoring screening assay. For this purpose, they were mutagenized by random *lacZ LEU2* insertions by transformation with a mutagenized genomic yeast library (26) or by exposure to ethylmethane sulfonate (EMS) (24). Colonies were screened for a nonsectoring phenotypes, and candidates were restreaked on YPD plates to confirm the lack of sectoring and on 5-FOA plates to determine that growth indeed depended on the presence of pYY5 (*SRP40 ADE3 URA3 CEN*). Both screens yielded a single clone that satisfied all criteria.

Identification and Molecular Analysis of the Synthetic Lethal Gene SHM2—The gene mutated by insertional mutagenesis was identified by inverse PCR of the DNA adjacent to the LEU2 transposon (26). Briefly, genomic yeast DNA was digested by *Rsa*I, ligated, and transformed into *E. coli*. Transformants containing the LEU2 transposon with the *E. coli* origin of replication and the ampicillin resistance gene were rescued. The genomic DNA adjacent to the LEU2 transposon was amplified by inverse PCR using primers 5′-TAAGTTGGGTAACGCCAGGGTTTTC-3′ and 5′-TGTTGCCACTCGCTTTAATG-3′ and *SHM2* was identified by DNA sequencing followed by data base comparison.

The synthetic lethal gene mutated by EMS was identified by complementation analysis with a yeast genomic library in a centromeric LEU2 vector (gift from Ian Willis, Albert Einstein College of Medicine, Bronx, NY). Transformants were screened for the ability to sector after 7 days of growth at 23 °C on SC medium lacking leucine. Candidates were restreaked on 5-FOA-containing medium to lose pYY5 leading to the isolation of 11 plasmids. Eight contained *SRP40* and the others a 3.8-kb genomic fragment with only one intact open reading frame, *SHM2*.

For complementation and deletion purposes, *SHM2* including 494 nucleotides upstream and 289 nucleotides downstream was amplified from genomic DNA using oligonucleotides 5′-CGGGATCCTCAAAGAGCATAGCTCAATTGC-3′ and 5′-GCGGCCGCGAAAACGGTGAAAAAGTGAACAATG-3′ and inserted into the *Bam*HI and *Not*I sites of pGEM-T (Promega, Madison, WI) to generate pYY59. pYY45 was constructed by subcloning the *SHM2*-containing *Not*I fragment from pYY59 into pRS315 (25). Nucleotides 115–793 were deleted from the 1407-bp *SHM2* coding region in pYY45 by replacement with the URA3-containing 678-bp *Cla*I-*Bgl*II fragment from pRS316 (25) to generate pYY60. The *shm2*Δ::*URA3*-containing *Not*I fragment was used to disrupt *SHM2* in YYX30 to create YYX31.

Precise deletion of the *SHM2* and *ADE3* open reading frames was achieved by replacement with the *HIS3* and *TRP1* markers, respectively. For this purpose, the markers were PCR-amplified using pRS313 and pRS314 (25) as templates with oligonucleotides that contained complementarity to the flanking regions of the target gene (27). The following oligonucleotides were used for *HIS3*, 5′-TGTTTTATATATAATGCAGAACTTGCTTCCCTTAGTTTGCGTTCACAGATTGTACTGAGAGTGCA-3′ and 5′-TAATGTTTATTTTTTTGGGATTAAGTGTCGTTGTGGTGATTTCTCATCTGTGCGGTATTTCACA-3′, and for *TRP1*, 5′-CCAGGTAACGAGACGAACACAACTTTACAAGTCAAATAAGAAATCCAGATTGTACTGAGAGTGCA-3′ and 5′-TGTCTTAGAACAGGCCATCGATCTCACCGTCATCATCGACTTCCCATCTGTGCGGTATTTCACA-3′. In this way *SHM2* was deleted in YYX32, a *srp40Δ/srp40*Δ strain generated by mating TMY20 with TMY21 (17), to create YYX33 and YYX34. *ADE3* was deleted in YYX38, an *srp40Δ/srp40*Δ *shm2Δ/shm2*Δ strain generated by mating two haploids obtained from sporulation and dissection of YYX34, to create YYX39. Correct insertion of the markers was tested by PCR on yeast colonies.

For the construction of a conditional *GAL*::*srp40* strain, pYY38 (pGAL-SRP40) (11) was transformed into the synthetic lethal strain SL1 while shuttling out plasmid pYY5 by growth on 5-FOA-containing medium thus producing YYY206. In the process and for unknown reasons, YYY206 lost its *LEU2* marker from the transposon in *SHM2*.

Plasmids—Site-directed mutagenesis was performed according to the QuickChange™ protocol (Stratagene, La Jolla, CA) using the following primers for PCR amplification, 5′-GGTGTCGCTATTGG**A**GCTCCAGCCATGA-3′ and 5′-TGGAGC**T**CCAATAGCGACACCACCTGGA-3′ for the R393A mutation and 5′-ACTCACCAGTCTTTGAGAGGTCC**C**CGTGGTGCTATGA-3′ and 5′-ACCACG**G**GGACCTCTCAAAGACTGGTGAGTGGTGGT-3′ for the K248Q mutation of *SHM2* generating pYY67 and pYY71, respectively. The underlined nucleotides were introduced to obtain the point mutations, and the nucleotides in boldface reflected silent changes to generate new restriction sites, *Sac*I and *Dra*III, respectively. The K248Q/R393A double mutation of *SHM2* was generated by inserting the *Bgl*II-*Xba*I fragment of pYY67 into pYY71 to create pYY75.

To epitope tag *SHM2* and *SHM2* (K248Q + K393A), the 5′-terminal *Sal*I fragment in pYY45 and pYY75, respectively, was replaced by a fragment amplified with the oligonucleotides 5′-CCAAGGTCGACGAAGGCT-3′ and 5′-ACGCGTCGACTTATCTAGACACAGCCAATGGGTA-

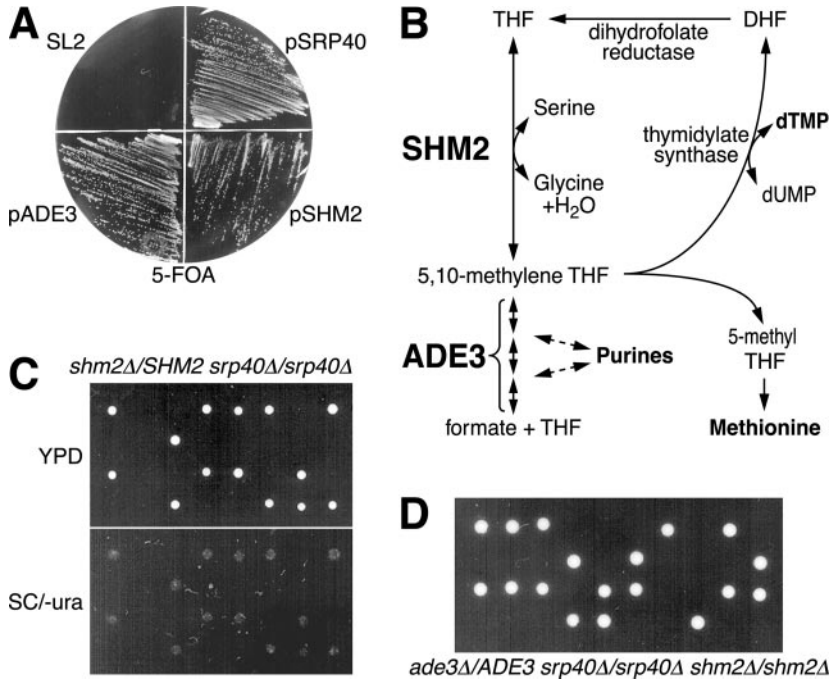

FIG. 1. **Synthetic lethality between *SRP40*, *SHM2*, and *ADE3*.** *A*, each of the three genes rescues the synthetic lethal phenotype on 5-FOA-containing medium when provided under its own promoter on a CEN plasmid. *B*, pathway of cytosolic folate-mediated one-carbon metabolism in yeast. *DHF*, dihydrofolate. *C*, tetrad dissections of a diploid strain with one deleted copy of the *SHM2* gene (*shm2*Δ::*URA3*/*SHM2 srp40*Δ/*srp40*Δ *ade3*/*ade3*). Note all spores growing on YPD medium (*upper panel*) were uracil-auxotrophic (*lower panel*). *D*, tetrad dissection of a diploid strain with one deleted copy of the *ADE3* gene (*ade3*Δ::*TRP1*/*ADE3 srp40*Δ/*srp40*Δ *shm2*Δ/*shm2*Δ).

TTCG-3′ that generated an in-frame *Xba*I 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 *Xba*I 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 (*pYY52*), GBD-Shm2p (*pYY54*), GAD-Srp40p (*pYY51*), GAD-Shm2p (*pYY53*), GAD-NoppΔN (amino acids 60–704 of Nopp140, *pTM64*), GAD-NoppRΔ (60–241, *pTM81*), and GAD-NoppRΔΔ (60–94, *pTM83*). The other Nopp140 constructs were as described previously (14). GAD-Lsm5p (*pAEM70*) was a gift from Jean Beggs (28).

RNA Analysis—Srp40p and Cbf5p 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. SnoRNAs were detected by hybridization with the following ^{32}P-labeled oligonucleotides: snR10, 5′-CATGGGTCAAGAACGCCCCGGAGGGG-3′; snR30, 5′-GCCGTTGTCCGAAGCGCC-3′; snR42, 5′-TCAAACAATAGGCTCCCTAAAGCATCACAA-3′; snR190, 5′-CGAGGAAAGAAGAGACACCATTATC-3′; U24, 5′-ATTGGTATGTCTCATTCGGATCTCAAAGTTCCATCTGA-3′; U4, 5′-CGAATCCTCACTGATATG-3′; and those published previously (11). Blots were quantitated using a PhosphorImager (Amersham Biosciences).

Immunochemical Methods and Microscopy—Indirect immunofluorescence microscopy was performed essentially as described (17, 30) using mouse monoclonal antibodies 9E10 (31) against the Myc epitope (culture supernatant at a 1:3 dilution). Total protein extracts were prepared according to standard methods (32), separated by 9% PAGE, and transferred to nitrocellulose. The blots were probed with 12CA5 anti-HA ascites fluid (33) at a 1:5000 dilution.

For cell size analysis, strains were grown in SC media to log phase. The pelleted cells were resuspended in 0.1 M potassium phosphate buffer by sonication in a water bath sonicator for 5 min. The cells were fixed with 3.7% formaldehyde and observed on an inverted Nikon microscope equipped with a JVC KY55B color CCD camera. Cell size was determined manually on digitized images by measuring the maximal diagonal length of at least 100 cells using NIH Image software.

RESULTS

Identification of SHM2 and ADE3—Deletion of *SRP40* causes slight growth retardation and lack of box C/D snoRNA retention in nucleolar bodies but no other apparent deficiencies (17, 20). We took advantage of these facts by performing synthetic lethal screens in an *srp40*Δ 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 *srp40*Δ 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 *srp40*Δ deletion. After plasmid rescue, sequencing from the inserted *lacZ* gene identified the disrupted gene as cytosolic serine hydroxymethyltransferase, *SHM2*, formerly referred to as *LES2* (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 5 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 (*srp40*Δ *shm2* pSRP40 URA3) on 5-FOA-containing medium when provided under their own promoters on *LEU2* plasmids (Fig. 1*A*). 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. 1*B*). 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 housekeeping gene 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 (*srp40*Δ/*srp40*Δ). Sporulation and tetrad dissection of the resulting *shm2*Δ/*SHM2 srp40*Δ/*srp40*Δ strain yielded only two viable spores each, all uracil auxotroph, confirming the synthetic lethality (Fig. 1*C*).

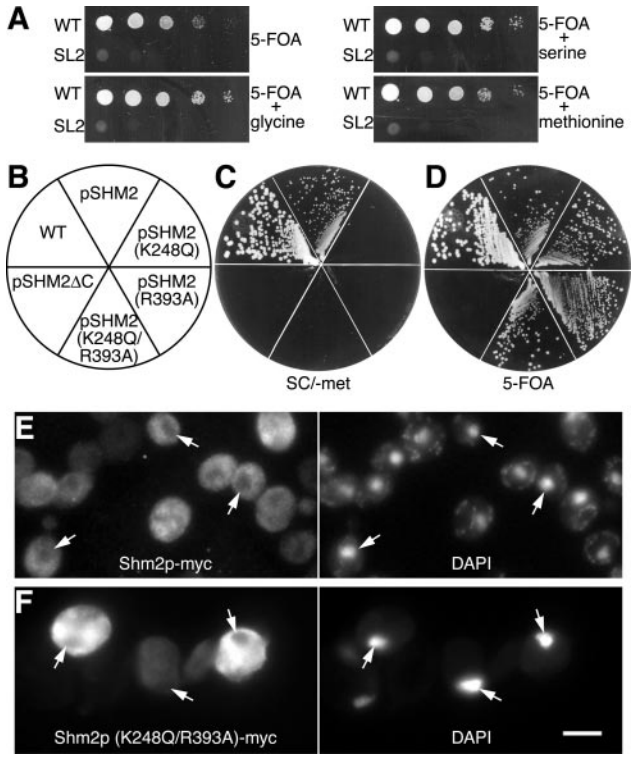

FIG. 2. **A noncatalytic function for Shm2p.** *A*, 10-fold dilution series of the logarithmically growing synthetic lethal (SL2) and its parental strain (*WT*, YCH128) on 5-FOA-containing medium supplemented with glycine, serine, and methionine. *B*, key indicating which plasmid-borne proteins are expressed in the respective strains in *C* and *D*. *C*, growth of *shm2*Δ *ade3* strains carrying the plasmids indicated in *B* on SC medium lacking methionine. *D*, growth of the synthetic lethal strain SL2 carrying the plasmids indicated in *B* on 5-FOA-containing medium. *E*, indirect immunofluorescence of Myc-tagged Shm2p expressed in an *shm2*Δ strain detected by fluorescein isothiocyanate-labeled antibodies against the Myc epitope (*left panel*). Note Shm2p appeared excluded from the nucleus (*arrows*) identified by 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 C_1-THF synthase, Ade3p (Fig. 1*B*). 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. 1*A*). 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. 1*D*). 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. 2*A*). 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 aldimine 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 *shm2*Δ *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. 2*C*). 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. 2*D*). However, a construct truncated at amino acid 203, *SHM2*Δ*C*, 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 *shm2*Δ 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 (*right panels*). Although, these data did not exclude the possibility of a minor fraction of wild type or mutant Shm2p making its way into nuclei, it demonstrated the same localization for both constructs, which was clearly distinct from that of the nucleolar Srp40p (11).

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

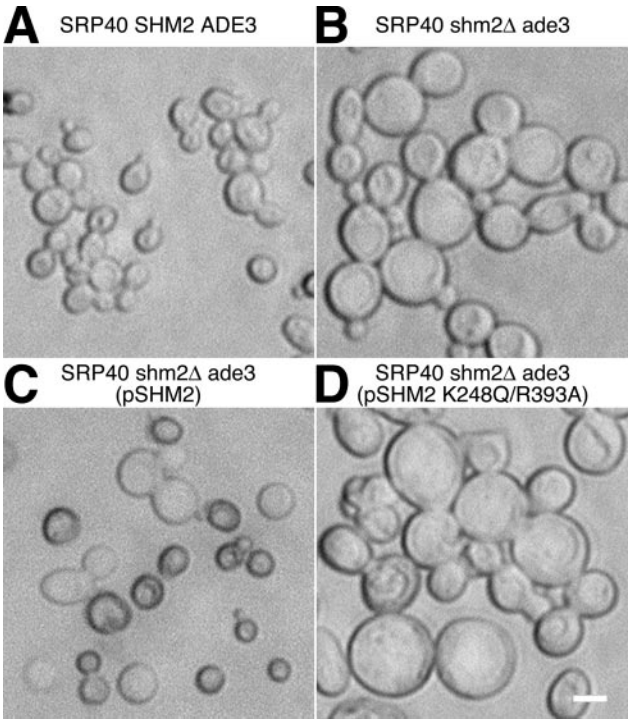

FIG. 3. **Giant cell phenotype.** Phase contrast images of strains (relevant genotype indicated on top) carrying plasmid-borne copies of the *LEU2* marker and the wild type (*C*) or mutant *SHM2* (*D*) grown in SC medium lacking leucine. Note the increase in cell size of strains with mutant (*B* and *D*) compared with wild type *SHM2* (*A* and *C*). *Bar*, 5 μm.

*shm2*Δ *ade3* strain, whereas that of the catalytically inactive Shm2p (K248Q/R393A) increased the cell size from ~200 to ~250% of wild type (Fig. 3, *C* and *D*; Table II). In fact, when compared with vector control, expression of mutant Shm2p (K248Q/R393A) consistently and significantly enlarged cell size in a variety of strains (Table II). Thus, the exaggeration of the giant cell phenotype was apparently caused by a catalysis-independent function of this basic metabolic enzyme. Additionally, the impact on cell size of the mutant Shm2p (K248Q/R393A) was not based on a dominant negative effect because it occurred even in the absence of wild type Shm2p, *i.e.* in *shm2*Δ strains (Fig. 3*D*; Table II). To test if *SRP40* played a role in the giant cell phenotype, cell size of the synthetic lethal strain was analyzed when *SRP40* on plasmids was expressed from its own promoter and in addition from the *GAL10* promoter. Although the former situation was meant to mimic the *SRP40 shm2*Δ *ade3* strain, its cell size was not increased (Fig. 3*B* and Table II). However, overexpression of Srp40p in that background significantly enlarged the cells suggesting its participation in the phenotype (Table II). These data, together with the fact that the catalytically inactive Shm2p (K248Q/R393A) increased cell size and rescued the synthetic lethality, were consistent with similar signaling pathways controlling both cell size and synthetic lethal phenotypes.

LSM5 Is a Multicopy Suppressor of the Triple Synthetic Lethality—A genome-wide two-hybrid analysis revealed an interaction between Shm2p and Lsm5p (39). As part of Sm-like complexes, Lsm5p has been implicated in several nuclear activities including normal processing of pre-tRNAs (40). Pre-tRNAs, unlike mature tRNAs, are often localized to nucleoli through a poorly understood process involving Cbf5p, one of the four core proteins of box H/ACA snoRNPs (8, 41). Therefore, Shm2p, via Lsm5p, may display some distant relationship to box H/ACA snoRNAs, which are specifically depleted in the synthetic lethal strain (11). For these reasons, we tested if overexpression of Lsm5p rescued the *srp40*Δ *shm2 ade3* synthetic lethality on 5-FOA-containing medium. Indeed, providing additional copies of *LSM5* under the *ADH1* promoter on a multicopy plasmid partially restored growth on solid medium (Fig. 4*A*). This was consistent with the reported Shm2p-Lsm5p interaction between full-length Lsm5p and the carboxyl terminus of Shm2p (39), although the two full-length proteins failed to interact in a two-hybrid assay (data not shown).

Nevertheless, we further investigated the *LSM5-SHM2* connection in our *srp40*Δ *shm2 ade3* strain rendered conditional for growth on galactose by carrying a plasmid copy of *GAL*::*srp40*. Indeed, extra copies of *LSM5* partially restored growth to this strain on glucose (Fig. 4*B*). As reported previously (11), growth arrest of this conditional *SRP40* strain was at least partially caused by the specific depletion of box H/ACA snoRNAs, because after 24 h snR3, snR10, snR11, snR42, and the essential snR30 were depleted (Fig. 4*C* and 5*C, lanes 4*). In contrast, the stability of the box C/D snoRNAs U3, U14, U24, and the spliceosomal snRNA U4 was unaffected (Fig. 4*C, lane 4*). Overexpression of *LSM5* in this *GAL*::*srp40* strain in glucose-containing liquid medium stabilized the tested box H/ACA snoRNAs snR3 and snR10 (Fig. 4*C, 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*::*cbf5* 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*Δ *shm2 ade3* strain carrying a plasmid copy of *GAL*::*srp40*. Indeed, Nopp140 restored growth unlike a vector control (Fig. 5*A*). 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. 5*B*). 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. 5*A*, *Nopp*Δ*C*). Similarly, a deletion of the amino terminus did not impair the complementation ability of Nopp140 (NoppΔN), and the amino terminus alone appeared insufficient for complementation, although it is uncertain to what extent the latter construct was expressed (Fig. 5, *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. 5*A*, *NoppC* and *NoppR*). Even the first four Nopp140 repeats alone were sufficient to fully restore growth, whereas a single repeat was less efficient (NoppRΔ and NoppRΔΔ, 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. 5*C*). 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*).

TABLE II
Yeast cell diameters

Plasmid	Relevant genotype	Cell diameter in μm	
		Average ± S. D.[a]	*n*
	SRP40 SHM2 ADE3	3.82 ± −0.62	107
Control	*SRP40 SHM2 ADE3*	3.91[b] ± −0.86	113
Shm2p (K248Q/R393A)	*SRP40 SHM2 ADE3*	5.34[c] ± 1.54	111
	SRP40 shm2Δ ade3	7.30[b] ± 1.94	94
Shm2p	*SRP40 shm2Δ ade3*	4.40 ± −1.31	136
Shm2p (K248Q/R393A)	*SRP40 shm2Δ ade3*	9.66[c] ± 2.32	108
Control	*srp40Δ shm2Δ ADE3*	3.78[b] ± 0.77	146
Shm2p (K248Q/R393A)	*srp40Δ shm2Δ ADE3*	4.31[c] ± 1.05	124
Srp40p	*srp40Δ shm2 ade3*	4.24[b] ± 0.69	118
Srp40p + Srp40p[d]	*srp40Δ shm2 ade3*	5.67[c] ± 1.16	113

[a] Student's *t* test analysis of the data sets within each strain indicated that value (*b*) was significantly different from value (*c*) ($p < 0.001$).
[d] Srp40p was overexpressed from the GAL10 promoter.

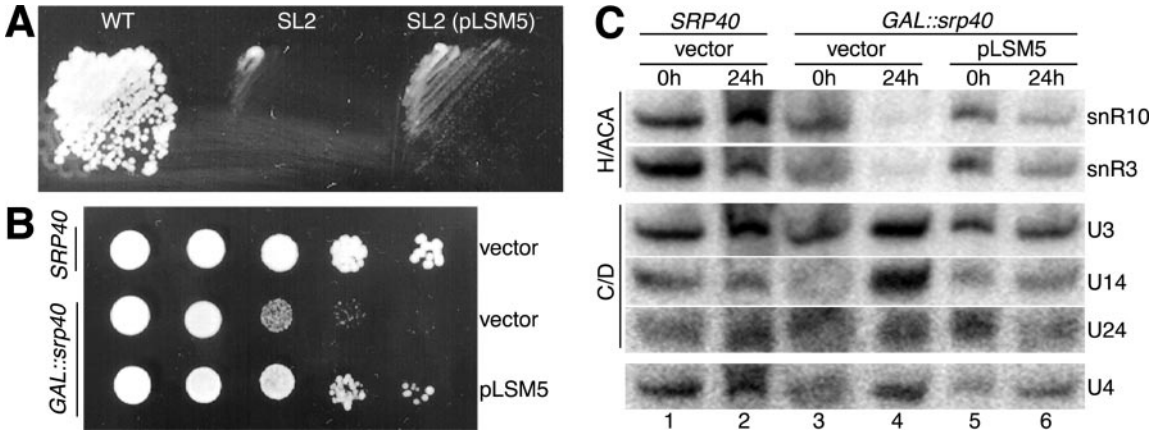

FIG. 4. ***LSM5* is a multicopy suppressor of the synthetic lethality.** *A,* growth of the synthetic lethal strain (SL2) and its parental strain (WT, YCH128) on 5-FOA-containing medium. Expression of extra copies of Lsm5p (pLSM5) partially rescued the synthetic lethality. *B*, 10-fold dilution series of growth on glucose of wild type (*SRP40*) or conditional *SRP40* strains (*GAL::srp40*). Unlike the vector alone, extra copies of Lsm5p (pLSM5) rescue the Srp40p depletion in the synthetic lethal background. *C,* Northern blots of total RNA isolated from the same three strains as in *B* after growth for 0 and 24 h in glucose-containing medium. The blot was probed with ^{32}P-labeled oligonucleotides complementary to the indicated small nuclear RNAs and autoradiographed. Note Lsm5p was expressed as a GAD-fusion protein and the vector controls expressed GAD alone.

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 small nuclear RNAs (Fig. 5*D*). 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 snRNAs U3, U14, and snR190 and the spliceosomal snRNA U4 remained unaffected (Fig. 5*D, lane 4*). Complementation with full-length Nopp140 restored the levels of all RNAs tested (Fig. 5*D, lane 6*). However, despite complementation of growth on solid medium (Fig. 5*A*), in liquid medium the repeat domain alone barely restored growth (not shown) and only marginally stabilized box H/ACA snoRNAs (Fig. 5*D, 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-

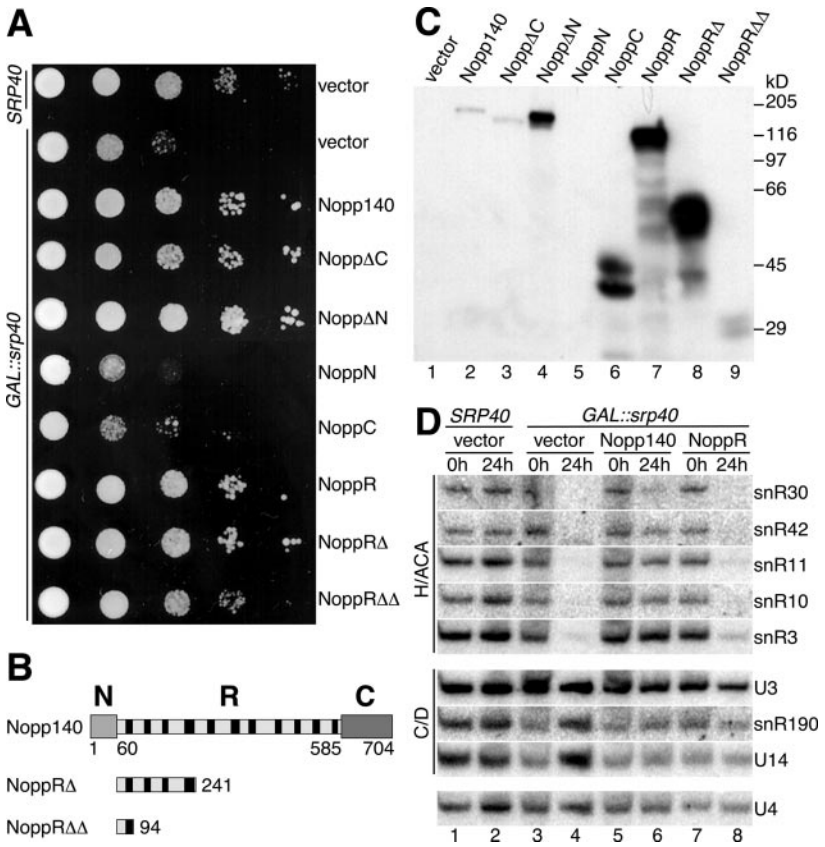

FIG. 5. **Rat Nopp140 functionally complements depletion of yeast Srp-40p.** *A*, 10-fold dilution series of growth on glucose of wild type (*SRP40*) or conditional *SRP40* strains (*GAL::srp40*) harboring plasmid copies of the indicated rat Nopp140 constructs. *B*, schematic of rat Nopp140 and its domains with the *black bars* representing the acidic serine stretches of the repeat domain (*R*). NoppRΔ and NoppRΔΔ are also depicted. Numbers refer to amino acid positions in the full-length protein. *C*, Western blot of total protein extracts from the conditional *SRP40* strains as in *A* probed for expression of the rat constructs with anti-HA antibodies and detected by enhanced chemiluminescence. *D*, Northern blots of total RNA isolated from some of the strains as in *A* after growth for 0 and 24 h in glucose-containing medium. The blot was probed with ^{32}P-labeled oligonucleotides complementary to the indicated small nuclear RNAs and quantitated by PhosphorImaging. Although barely visible, the amounts of box H/ACA snoRNAs detected in *lane 8* compared with those in *lane 4* were 0.7–3-fold increased when normalized to the amounts of U4. Note the rat constructs were expressed as GAD fusion proteins containing an HA tag, and the vector controls expressed GAD alone.

mented 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 in *de novo* purine biosynthesis (46). Thus, Shm2p and Ade3p may display a nonmetabolic interaction, which may be indirect and which may link them both to Srp40p. What exactly constitutes the noncatalytic function of Shm2p remains to be determined, but insight might be gained from the analysis of interacting proteins such as Lsm5p.

We identified Lsm5p as a candidate communicator between Shm2p and Srp40p. Apparently, the latter two proteins did not interact directly because we failed to detect any physical interaction in two-hybrid and coimmunoprecipitation assays (data not shown). This was supported by the localization of the two proteins in separate cellular compartments. Lsm5p, however, was present in both cytoplasmic and nuclear complexes and interacted with Shm2p in a genome-wide two-hybrid screen (28, 39, 47, 48). Indeed overexpression of *LSM5* stabilized box H/ACA snoRNAs, which were specifically depleted from the synthetic lethal strain, and presumably thereby rescued the synthetic lethality. Although depletion of Lsm proteins affects the stability of pre-rRNA and rRNAs, it does not impact snoRNAs (49). Therefore, the effect of *LSM5* overexpression on the stability of box H/ACA snoRNAs was likely indirect and could have been mediated via Srp40p, thus closing the chain between *SHM2* and *SRP40*.

In addition to the cytosolic serine hydroxymethyltransferase, eukaryotes contain a mitochondrial enzyme that is about 60% identical, encoded by *SHM1* in yeast (50–52). Shm1p is part of a parallel system for interconversion of one-carbon units in mitochondria. Due to its sequestration in mitochondria, it is unlikely that Shm1p participates in the synthetic lethality. 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 defunct 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 *shm2*Δ *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

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 snR30 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[2] that is characteristic for the depletion of box H/ACA snoRNAs (8, 59, 60).

Growth and stability of box H/ACA snoRNAs of the conditional *SRP40* strain could be restored by expression of rat Nopp140 suggesting that it is indeed the functional homolog of Srp40p. It was the central repeat domain of Nopp140 and not its evolutionary most highly conserved carboxyl terminus, which complemented the Srp40p depletion. Although surprising at first, this may reflect the capacity of the repeat domain to interact with box H/ACA snoRNPs in a phosphorylation-dependent manner (14, 15). Complementation of growth by 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.

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[2] D. Lafontaine, personal communication.