The Survival Motor Neuron Protein of *Schizosaccharomyces pombe*

CONSERVATION OF SURVIVAL MOTOR NEURON INTERACTION DOMAINS IN DIVERGENT ORGANISMS*

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Spinal muscular atrophy is a common often lethal neurodegenerative disease resulting from deletions or mutations in the survival motor neuron gene (SMN). SMN is ubiquitously expressed in metazoan cells and plays a role in small nuclear ribonucleoprotein assembly and pre-mRNA splicing. Here we characterize the *Schizosaccharomyces pombe* orthologue of SMN (yeast SMN (ySMN)). We report that the ySMN protein is essential for viability and localizes in both the cytoplasm and the nucleus. Like human SMN, we show that ySMN can oligomerize. Remarkably, ySMN interacts directly with human SMN and Sm proteins. The highly conserved carboxyl-terminal domain of ySMN is necessary for the evolutionarily conserved interactions of SMN and required for cell viability. We also demonstrate that the conserved amino-terminal region of ySMN is not required for SMN and Sm binding but is critical for the housekeeping function of SMN.

Spinal muscular atrophy (SMA)¹ is a common autosomal recessive neurodegenerative disease that is one of the leading hereditary causes of infant mortality (1). SMA is characterized by degeneration of motor neurons of the anterior horn of the spinal cord resulting in muscle weakness and atrophy (2). SMA results from reduced levels of expression or mutations in the survival motor neuron protein (SMN). The SMN gene is duplicated as an inverted repeat on human chromosome 5 at 5q13 (3, 4), and the telomeric copy of SMN (SMN1) is deleted or mutated in over 98% of SMA patients, although they retain at least one copy of centromeric SMN (SMN2), which produces mostly carboxyl-terminally truncated SMN protein (5). The SMN protein is expressed in all tissues of metazoan organisms, mostly carboxyl-terminally truncated SMN protein (5). The least one copy of centromeric SMN is essential for viability and localizes in both the cytoplasm and the nucleus. Like human SMN, we show that ySMN can oligomerize. Remarkably, ySMN interacts directly with human SMN and Sm proteins. The highly conserved carboxyl-terminal domain of ySMN is necessary for the evolutionarily conserved interactions of SMN and required for cell viability. We also demonstrate that the conserved amino-terminal region of ySMN is not required for SMN and Sm binding but is critical for the housekeeping function of SMN.

The SMN gene encodes a 294-amino acid protein, which is part of a multiprotein complex. Gemin2 (formerly SIP1) associates with SMN directly (8). In addition, SMN interacts with Gemin3 and with several Sm proteins, and it is capable of homotypic interactions (8–12). SMN, Gemin2, Gemin3, and Gemin4, another component of the complex that does not interact with SMN directly (13), are located in the cytoplasm and the nucleus of somatic cells. In the nucleus, these proteins are highly concentrated in gems, nuclear bodies that are similar in size, number, and are often found associated with coiled bodies (8, 9, 12, 13). In higher eukaryotes both SMN and Gemin2 play a crucial role in the assembly of snRNPs (14, 15, 16). This process takes place in the cytoplasm, where the snRNAs combine with Sm proteins, and the assembled snRNPs are subsequently imported to the nucleus where pre-mRNA splicing occurs (17–19). SMN also plays a role in the nucleus, where it is required for maintaining an active pre-mRNA splicing machinery likely by regenerating functional snRNPs after splicing (14). The essential roles of SMN were further demonstrated using mouse and *Caenorhabditis elegans* models (20–24). Mice with homozygous SMN disruption display massive cell death during early embryonic development (20). Reduction of SMN protein levels in the spinal cord of SMN heterozygous mice leads to motor neuron degeneration resembling SMA type III (22). Using a mouse model it was also shown that SMA is caused by insufficient SMN production by the SMN2 gene (24).

A candidate orthologue of SMN was identified by sequence analysis of the *Schizosaccharomyces pombe* genome (hypothetical protein Yab8) (25). The genetic approaches to which *S. pombe* is amenable makes it an attractive system for the study of SMN function. Here we characterize the *S. pombe* orthologue of SMN (ySMN). We show that ysmn− is essential for viability of *S. pombe*. We demonstrate that ySMN can interact with SMN and Sm proteins indicating the remarkable conservation of SMN functional domains in *S. pombe*.

**EXPERIMENTAL PROCEDURES**

**Strains and Media**—To construct a diploid strain for use in the disruption of *smn*−, the two haploid strains FY261 (ATCC; h− ura4-D18 leu1−32 ade6-M120 can1−1) and KG554 (ATCC; h+ ura4-D18 leu1−32 ade6-M216 his3-D1) were crossed to produce the diploid C2. *S. pombe* strains were grown at 30 °C in YES or EMM medium lacking the appropriate auxotrophic supplements for marker selection (26). For preparing plates, the media were solidified by adding 2% agar. Medium containing 5-fluoro-orotic acid (5-FOA) was prepared as described previously (27). *Escherichia coli* cells were grown in LB broth or LB agar (28) and supplemented with ampicillin or kanamycin at 100 µg/ml.

**Genetic Manipulations**—To generate a null allele of *smn*−, most of the coding region was replaced by the hisG-ura4−hisG cassette, which can be used repeatedly for constructing gene disruptions and/or for sequentially introducing a ura4− marker plasmid (29). The starting plasmid was pDM291 (a gift from Leonard Guarente, Massachusetts Institute of Technology). The 5′-flanking region was obtained by genomic PCR using the following oligonucleotides: 5′-CCGTTGAAGAT-

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1 The abbreviations used are: SMA, spinal muscular atrophy; SMN, survival motor neuron; snRNP, small nuclear ribonucleoprotein; 5-FOA, 5-fluoro-orotic acid; PCR, polymerase chain reaction; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; hSMN, human SMN; ySMN, yeast SMN.
The obtained fragment was inserted into pDM291, which was then used to clone the 3'-flanking region. The 3'-flanking region was obtained by PCR using the following oligonucleotides: 5'-ATACTGATTTACGGCGAAG-3' and 5'-TACGGAATCTCTACGCTGATCGGTT-3'.

The plasmid was digested with BglII and transformed into the C2 diploid strain using the lithium acetate protocol (30). Transformed cells were spread on EMM medium -Ura (26) and incubated for 5 days. Individual colonies were streaked on the same media again, and 20 single colony isolates were checked by genome PCR to determine the correct integration of the inserted cassette, as described previously (31). A diploid strain with one chromosomal copy of smn+ replacement with the knockout cassette was then transformed with pSP1/SMN, which had smn+ as hemagglutinin epitope amino-terminal fusion under the control nmt1 promoter of different strengths (pSP1/SMN173, pSP1/SMN273, or pSP1/SMN373). Random spore analysis was performed again to get the haploid strain Δsmn (h+ urad1-18 leu1-32 ade6-M210 can1-1 smn::ura4+) (pSP1/SMN, smn+). The Δsmn (h+ urad1-18 leu1-32 ade6-M210 can1-1 smn (pSP1/SMN, smn+, urad1)) strain was produced by transforming Δsmn with pSLF/SMN plasmids (pSLF73/SMN, pSLF273/SMN, or pSLF373/SMN) expressing ySMN1. The urad1+ selectable marker was excised from the disruption allele by selecting the Δsmn strain on 5-FOA medium. Transformants were streaked on nonselective YES medium, and individual colonies were plated on EMM-Ura and -Leu plates. The clones, which could grow on -Ura medium but could not grow on -Leu media, were referred to as Δsmn1.

To check the ability of hSMN to substitute for smn+ we used the following method. The tester strain Δsmn was maintained on smn+ on pSP1/SMN plasmids (pSP1/SMN173, pSP1/SMN273, or pSP1/SMN373) carrying LEU2 selectable marker. The urad1+ selectable marker was excised by plating cells on medium containing 5-FOA so that we could later transform them with pSLF/SMN plasmids (pSLF73/SMN, pSLF273/SMN, or pSLF373/SMN) carrying urad1+. Transformants were streaked on nonselective YES medium, and 500 individual colonies were plated on EMM-Ura plates selective for smn+ or -Leu plates selective for hSMN.

The plasmid shuffle technique was used to test the ability of deletion mutants of smn+ to substitute for wild type smn+ as described previously (32). The tester strain Δsmn1 carried smn+ on pSLF/SMN plasmids (pSLF73/SMN, pSLF273/SMN, or pSLF373/SMN). The wild type allele of smn was deleted in Δsmn1 to generate ΔsmnΔnmt1. The ΔsmnΔnmt1 was transformed with pSP1/N47 plasmids, which could grow on 5-FOA plates.

**Production of Proteins in Vitro**—The [35S]methionine-labeled proteins were produced by in vitro coupled transcription-translation reactions (Promega Biotech) in the presence of [35S]methionine (Amersham Pharmacia Biotech). 6His-tagged fusion proteins were produced from a pET28 bacterial expression system in the E. coli strain BL21(DE3) and then purified on glutathione-Sepharose (Novagen) according to the manufacturer's protocol. The glutathione S-transferase (GST)-ySMN, GSThSMN, and GSThSMN fusion proteins were expressed from a GST expression vector, pGEX-5X-3 (Amersham Pharmacia Biotech), in the E. coli strain BL21 and purified on glutathione-Sepharose (Amersham Pharmacia Biotech) according to the manufacturer's protocol.

**Evolutionarily Conservation of SMN Interactions**

To replace one chromosomal copy of smn+, the final plasmid 23842 was used to produce PCR fragments carrying HAG-smn and ySMN at different levels. The obtained fragment was inserted into pDM291, which was then transformed with pSP1/SMN, which had smn+ as hemagglutinin epitope amino-terminal fusion under the control nmt1 promoter of different strengths (pSP1/SMN173, pSP1/SMN273, or pSP1/SMN373). Random spore analysis was performed again to get the haploid strain Δsmn (h+ urad1-18 leu1-32 ade6-M210 can1-1 smn::ura4+) (pSP1/SMN, smn+). The Δsmn (h+ urad1-18 leu1-32 ade6-M210 can1-1 smn (pSP1/SMN, smn+, urad1)) strain was produced by transforming Δsmn with pSLF/SMN plasmids (pSLF73/SMN, pSLF273/SMN, or pSLF373/SMN) expressing ySMN1. The urad1+ selectable marker was excised from the disruption allele by selecting the Δsmn strain on 5-FOA medium.

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**Production of Anti-ySMN Monoclonal Antibody**—Anti-ySMN antibody 1G6 was produced by immunizing Balb/C mice with ySMN protein fused to GST. Hybridoma production, screening, and ascites fluid production were performed as described previously (35) using 6His-tagged ySMN protein.

**Gel Filtration Chromatography**—Purified recombinant His-tagged ySMN or ySMNTD4 (40 mg) proteins were applied to a TSK-GEL G3000SW column (Tosoh, Montgomeryville, PA) equilibrated in a buffer containing 50 mM Hepes, pH 7.5, 100 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol. Fractions were collected at a 0.25 ml/min flow rate, pooled as indicated, and analyzed by SDS-PAGE and Western blotting with an anti-T7 tag monoclonal antibody (Novagen).

**Immunofluorescence on S. pombe**—Immunofluorescence on S. pombe was performed as described by Hagan and Hyams (36). Laser confocal fluorescence microscopy was performed with a Leica TCS 4D (Germany) confocal microscope, and images from each channel were recorded separately.

**RESULTS**

**The SMN Gene Is Essential for Viability of S. pombe**—We began the analysis of ySMN by determining whether smn+ is essential for S. pombe. We constructed a cassette for disruption of a chromosomal copy of the smn+ gene. In this cassette, most of the coding sequence of smn+ was replaced with hisG:ura4+·hisG·G after transformation of the cassette and urad1+ selection, random spore analysis was performed to assess the haploid phenotype. None of the examined individual haploid clones was able to grow on EMM-Ura medium selective for the knockout allele (data not shown). This demonstrates that the knockout of the SMN gene is lethal. To obtain a knockout of the SMN gene in a haploid strain, it was necessary to express ySMN from a plasmid. To do so, we transformed diploid cells heterozygous for smn+ (pLEU2 plasmid carrying a wild type copy of the smn+ gene) with a LEU2 plasmid carrying a wild type copy of the smn+ gene. To obtain haploid clones with a knocked out allele of smn+ and a plasmid carrying normal smn+, we performed random spore analysis. To confirm that the smn+ gene is essential we checked the stability of the plasmid carrying the normal smn+ allele. All 500 of the tested clones retained the plasmid indicating smn+ demonstrating that the smn+ gene is essential for S. pombe.

The importance of ySMN for survival of S. pombe is demonstrated that ySMN plays a fundamental role in cell physiology and suggests that the functions performed by SMN are evolutionarily conserved from S. pombe to mammals.

hSMN Cannot Replace smn+—We tested whether hSMN...
Evolutionarily Conservation of SMN Interactions

Intracellular Localization of the ySMN Protein—To further study the ySMN protein we produced an anti-ySMN monoclonal antibody (1G6). Western blot analysis shows that the 1G6 monoclonal antibody does not cross-react with Xenopus laevis and human SMNs but specifically recognizes the purified ySMN recombinant protein and a single band of similar size in total yeast extract (Fig. 2A). Endogenous ySMN migrates at a size corresponding to about 32 kDa instead of the predicted size of 18 kDa. We note that hSMN also migrates at higher molecular weight than is predicted from its molecular mass (9). To further confirm that antibody 1G6 reacts specifically with the ySMN protein, we performed immunoprecipitation of ySMN produced by transcription and translation in vitro. 1G6 antibody, but not the nonspecific SP2/0 antibody, efficiently immunoprecipitates ySMN (Fig. 2B). The 1G6 antibody was further used to localize ySMN by confocal immunofluorescence microscopy. ySMN is found in both the nucleus and the cytoplasm (Fig. 3, A and B). A similar distribution was also observed upon expression of a green fluorescent protein-ySMN fusion protein (Fig. 3, C and D). This indicates that the subcellular localization of SMN is evolutionary conserved. In the nucleus of mammalian cells, SMN is highly concentrated in gems (9). However, we were unable to discern whether ySMN is concentrated in similar subnuclear structures because of the much smaller size of S. pombe nuclei.

ySMN Oligomerizes and Interacts Directly with Human SMN and Sm Proteins—Human SMN interacts with itself, Gemin2, Gemin3, and the spliceosomal core Sm proteins (8–12). If, in S. pombe, ySMN performs similar functions to its human orthologue, it would be expected to display similar interactions. To test this possibility, we performed in vitro protein binding assays with ySMN as well as with hSMN, hGemin2, and the human Sm proteins B, D1, D2, D3, E, F, and G (37–39). We used human Sm proteins for these experiments because they are similar to their yeast counterparts (45–85% similarity), readily available, and because we were interested in determining the degree of conservation of the interactions between SMN and Sm proteins. ySMN was first produced as a fusion protein with GST. Purified GST or GST-ySMN immobi-

FIG. 1. Amino acid sequence alignment of human SMN (hSMN, GenBankTM Q16637), X. laevis SMN (XeSMN, GenBankTM AF156887.1), and S. pombe SMN (ypSMN, GenBankTM 09808). Light gray boxes indicate similar amino acids, and dark gray boxes indicate identical amino acids. The conserved Y/G bracket indicates the amino and carboxyl termini of the ySMNΔN47 and ySMNΔYG mutants, respectively.

FIG. 2. A, Western blot analysis using the anti-ySMN monoclonal antibody 1G6 on total cell lysates of X. laevis XL177, human HeLa and S. pombe, and on 6His-tagged yeast- (ySMN) or hSMN-purified recombinant proteins (10 ng each). The position of the molecular mass markers in kDa is indicated on the left. B, 6His-tagged ySMN was in vitro translated in rabbit reticulocyte lysate in the presence of [35S]methionine, immunoprecipitated using either 1G6 or nonspecific SP2/0 antibodies, and resolved by SDS-PAGE and autoradiography. The left panel shows 10% of the input. IP, immunoprecipitate.
Evolutionarily Conservation of SMN Interactions

An Evolutionarily Conserved Domain of ySMN Mediates Its Interactions with SMN and with Sm Proteins—The evolutionary conservation of SMN interactions despite the low overall sequence similarity between the hSMN and ySMN proteins prompted us to analyze the role of the conserved domains of ySMN in oligomerization and binding to the human Sm proteins. Two short regions of SMN appear to be particularly highly conserved through evolution (Fig. 1). In hSMN the conserved amino terminus is involved in binding Gemin2 and nucleic acids, whereas the conserved Y/G box near the carboxy terminus (25) plays a crucial role in oligomerization and Sm and Gemin3 binding (8, 10, 11, 12, 15). We therefore generated two deletion mutants of ySMN, ySMNΔN47 and ySMNΔYG, which lack the amino-terminal or carboxy-terminal conserved domains, respectively (Fig. 1). Immobilized GST, GST-ySMN, or GST-hSmB fusion proteins were incubated with in vitro translated [35S]methionine-labeled full-length ySMN, ySMNΔN47, or ySMNΔYG. Fig. 6A shows that full-length ySMN and ySMNΔN47, but not ySMNΔYG, bound specifically to GST-ySMN and GST-hSmB. No binding to GST alone was observed. Similarly, the deletion of the conserved Y/G box of hSMN completely abolished binding to GST-hSMN and GST-SmB (Fig. 6B). We conclude that the Y/G box in ySMN and hSMN is necessary for both SMN oligomerization and for its binding to Sm proteins.

To examine directly the state of oligomerization of ySMN and the ySMNΔYG deletion mutant, we performed gel filtration experiments using purified recombinant ySMN or ySMNΔYG. Fig. 6C shows that ySMN forms large oligomers of up to a molecular mass corresponding to ~440 kDa. We note that no monomeric ySMN is detectable. In contrast, the deletion mutant ySMNΔYG is unable to oligomerize. Therefore, ySMN like its human counterpart efficiently oligomerizes, and the Y/G box is required for oligomerization.

The Conserved Domains of ySMN Are Essential for Viability—In light of the findings described above we asked whether the SMNΔN47 and SMNΔYG deletion mutants could functionally substitute for full-length ySMN protein in vivo. To do so, we used the plasmid shuffle technique (32). Because the smn<sup>+</sup> gene is essential for viability, our tester Δsmn1 strain carried the smn<sup>−</sup> gene on a ura4 plasmid. The wild type ySMN and the deletion mutants, SMNΔN47 and SMNΔYG, expressed from the nmt1 promoters of different strengths, were transformed into the tester strain on a second plasmid bearing the LEU2 selectable marker. The LEU<sup>+</sup> transformants were plated on medium containing 5-FOA. Only cells that have lost the ura4<sup>+</sup> plasmid expressing smn<sup>−</sup> can grow on 5-FOA plates. Thus, the transformants will not grow unless the deletion mutants of ySMN can functionally substitute for ySMN. As shown in Fig. 7, the test strain transformed with a LEU2 plasmid expressing ySMN lacking either the carboxy- or amino-terminal domains failed to grow on 5-FOA-containing medium. This demonstrates that these conserved domains of SMN are essential for ySMN function and cell viability.

Effect of Overexpression of Wild Type and Mutant SMN on Cell Growth—Interestingly, we found that smn cells containing a plasmid carrying smn<sup>−</sup> under the control of a strong nmt1 promoter formed small colonies and grew significantly slower than cells containing smn<sup>−</sup> expressed under the control of medium or weak promoters (Fig. 8). This effect did not depend on strain background, because overexpression of ySMN in wild type strain FY261 also slowed down growth (data not shown).

**Fig. 3. Intracellular localization of ySMN.** A, laser confocal image of indirect immunofluorescence on *S. pombe* cells using the anti-ySMN monoclonal antibody 1G6. B, DAPI-4,6-diamidino-2-phenylindole staining of the field shown in A. C, laser confocal image of *S. pombe* cells expressing the green fluorescent protein-ySMN fusion protein. D, DAPI-4,6-diamidino-2-phenylindole staining of the field shown in C. Scale bars indicate 2 μm.

**Fig. 4. ySMN interacts in vitro with human Sm proteins as well as with yeast and human SMN proteins.** In vitro translated [35S]methionine-labeled 6His-ySMN, Myc-hSMN, Myc-hGemin2, and Myc-hSm proteins B, D1, D2, D3, E, F, and G were incubated with purified GSTySMN or GST alone as described under “Experimental Procedures.” Bound proteins were analyzed by SDS-PAGE and fluorography. The in vitro translation panel shows 10% of the input. The position of the molecular mass markers in kDa is indicated on the left.
analyzed by SDS-PAGE and Western blotting with anti-His-tag antibody. The input lane shows 10% of 6His-tagged proteins. Bound proteins were incubated with purified GST in vitro (hSmB).

A, in vitro purified mutant Myc-tagged hSMN proteins were incubated with purified GST-hSmB, or GST as indicated. Bound proteins were analyzed by SDS-PAGE and Western blotting with anti-His-tag antibody.

B, in vitro purified recombinant His-tagged ySMN and GST-hSmB, or GST as indicated. Bound proteins were analyzed by SDS-PAGE and fluorography. The in vitro translation panel shows 20% of the input. The interaction of SMN with SmB is weaker than with itself and the exposure time was 3-fold longer in Sm.

D, ySMN interacts directly with itself and human SmB (hSmB) in vitro. Purified 6His-tagged ySMN or hSmB recombinant proteins were incubated with purified GST-ySMN or GST alone. The input lane shows 10% of 6His-tagged proteins. Bound proteins were analyzed by SDS-PAGE and Western blotting with anti-His-tag antibody.

This indicates that overexpression of ySMN is disadvantageous to cells. Cells expressing SMNΔYG under a strong promoter did not have the same phenotype (Fig. 8).

We also found that cells overexpressing the ySMNΔN47 allele under control of the strong nmt1 promoter did not grow (Fig. 8). This effect did not depend on the background level of smn expression. We conclude that overexpression of ySMNΔN47 is particularly toxic to these cells and suggests that it has a dominant negative phenotype on cell growth. We have recently determined that hSMN deleted of the first 27 amino acids (hSMNΔN27) has a dominant negative phenotype (14). Fig. 6B shows that hSMNΔN27 has similar binding avidity for hSMN and SmB as wild type hSMN. Thus, both hSMNΔN27 and ySMNΔN47 maintain the ability to interact with SMN and Sm proteins and display a dominant negative phenotype.

**DISCUSSION**

A putative S. pombe orthologue of mammalian SMN, Yab8, was previously identified by sequence similarity (25). However, the extent of primary sequence similarity between Yab8 open reading frame and hSMN is only 24% and is restricted to the amino-terminal and carboxyl-terminal regions of these proteins. This raised a question as to whether the Yab8 is a genuine orthologue of hSMN. The findings reported here support the conclusion that Yab8 is indeed an orthologue of hSMN, and we therefore refer to it here as ySMN.

The knockout of the smn1 strain is maintained with pSLF373/ySMN, which carries smn1 under the control of nmt1 (weak) promoter. This strain was transformed with plasmids expressing wild type ySMN, GST-ySMN, or GST as indicated. Bound proteins were analyzed by SDS-PAGE and Western blotting with anti-His-tag antibody.

The conserved YG box of ySMN is necessary for oligomerization and interaction with Sm proteins. A, in vitro translated [35S]methionine-labeled wild type and mutant ySMN proteins were incubated with purified GST-ySMN, GST-hSmB, or GST as indicated. Bound proteins were analyzed by SDS-PAGE and fluorography. The in vitro translation panel shows 20% of the input. The interaction of SMN with SmB is weaker than with itself and the exposure time was 3-fold longer in Sm binding experiments. The shorter forms of hSMN and hSMNΔYG correspond to products translated from the endogenous AUG codon of the hSMN open reading frame. C, purified recombinant His-tagged ySMN and ySMNΔYG proteins were analyzed individually by high pressure liquid chromatography gel filtration. Fractions were pooled as indicated and analyzed by SDS-PAGE and Western blotting with anti-T7 tag monoclonal antibody.

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We also found that cells overexpressing the ySMNΔN47 allele under control of the strong nmt1 promoter did not grow (Fig. 8). This effect did not depend on the background level of smn expression. We conclude that overexpression of ySMNΔN47 is particularly toxic to these cells and suggests that it has a dominant negative phenotype on cell growth. We have recently determined that hSMN deleted of the first 27 amino acids (hSMNΔN27) has a dominant negative phenotype (14). Fig. 6B shows that hSMNΔN27 has similar binding avidity for hSMN and SmB as wild type hSMN. Thus, both hSMNΔN27 and ySMNΔN47 maintain the ability to interact with SMN and Sm proteins and display a dominant negative phenotype.

**DISCUSSION**

A putative S. pombe orthologue of mammalian SMN, Yab8, was previously identified by sequence similarity (25). However, the extent of primary sequence similarity between Yab8 open reading frame and hSMN is only 24% and is restricted to the amino-terminal and carboxyl-terminal regions of these proteins. This raised a question as to whether the Yab8 is a genuine orthologue of hSMN. The findings reported here support the conclusion that Yab8 is indeed an orthologue of hSMN, and we therefore refer to it here as ySMN.

The knockout of the smn1 strain is maintained with pSLF373/ySMN, which carries smn1 under the control of nmt1 (weak) promoter. This strain was transformed with plasmids expressing wild type ySMN, GST-ySMN, or GST as indicated. Bound proteins were analyzed by SDS-PAGE and Western blotting with anti-His-tag antibody.

The conserved YG box of ySMN is necessary for oligomerization and interaction with Sm proteins. A, in vitro translated [35S]methionine-labeled wild type and mutant ySMN proteins were incubated with purified GST-ySMN, GST-hSmB, or GST as indicated. B, in vitro translated [35S]methionine-labeled wild type and mutant Myc-tagged hSMN proteins were incubated with purified GST-hSMN, GST-hSmB, or GST as indicated. Bound proteins were analyzed by SDS-PAGE and fluorography. The in vitro translation panel shows 20% of the input. The interaction of SMN with SmB is weaker than with itself and the exposure time was 3-fold longer in Sm binding experiments. The shorter forms of hSMN and hSMNΔYG correspond to products translated from the endogenous AUG codon of the hSMN open reading frame. C, purified recombinant His-tagged ySMN and ySMNΔYG proteins were analyzed individually by high pressure liquid chromatography gel filtration. Fractions were pooled as indicated and analyzed by SDS-PAGE and Western blotting with anti-T7 tag monoclonal antibody.

This indicates that overexpression of ySMN is disadvantageous to cells. Cells expressing SMNΔYG under a strong promoter did not have the same phenotype (Fig. 8).

We also found that cells overexpressing the ySMNΔN47 allele under control of the strong nmt1 promoter did not grow (Fig. 8). This effect did not depend on the background level of smn expression. We conclude that overexpression of ySMNΔN47 is particularly toxic to these cells and suggests that it has a dominant negative phenotype on cell growth. We have recently determined that hSMN deleted of the first 27 amino acids (hSMNΔN27) has a dominant negative phenotype (14). Fig. 6B shows that hSMNΔN27 has similar binding avidity for hSMN and SmB as wild type hSMN. Thus, both hSMNΔN27 and ySMNΔN47 maintain the ability to interact with SMN and Sm proteins and display a dominant negative phenotype.
similar interactions i.e. oligomerization and binding to Sm proteins. Indeed, we found that ySMN displayed similar interactions to the vertebrate SMN protein in that it can oligomerize and bind Sm proteins. These findings strongly suggest that Yab8p is the S. pombe orthologue of hSMN.

YSMN and hSMN interact with each other and ySMN interacts with human Sm proteins. Considering the evolutionary divergence between ySMN and hSMN proteins (24% similarity) and between S. pombe Sm proteins and human Sm proteins (45–85% similarity), these results indicate a remarkable conservation of SMN interactions. This prompted us to examine further the role of the conserved domains of ySMN in oligomerization and binding to Sm proteins. Our binding experiments demonstrate that the Y/G box of ySMN and hSMN is necessary for both SMN self-association and for interaction with Sm proteins.

Studies with SMN deletion constructs indicate that both the amino-terminal and carboxyl-terminal domains of ySMN are essential for cell viability. The fact that SMNΔN47 has similar avidity for ySMN and human Sm proteins as the wild type protein suggests that other interactions, in addition to SMN oligomerization and Sm binding, are likely essential for the housekeeping function of SMN. The most likely candidate for such interactions would be the S. pombe orthologue of human Gemin2, which interacts with the conserved amino-terminal region of hSMN (8, 14).

The central region of human SMN (amino acids 96–160) shows amino acid sequence similarity to a Tudor domain (40). The specific function of this domain is still an open issue. It has been proposed that the Tudor domain might represent an RNA-binding module (40, 114) and/or protein binding domain, and it has recently been suggested that the Tudor domain of SMN in higher eukaryotes is essential for cell viability. The fact that SMN oligomerization is required for high affinity interaction with human Sm proteins. Indeed, we found that ySMN displayed similar interactions to the vertebrate SMN protein in that it can oligomerize and bind Sm proteins. These findings strongly suggest that Yab8p is the S. pombe orthologue of hSMN.

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