Molecular Biology of the Cell
Vol. 20, 90–101, January 1, 2009

Gemin3 Is an Essential Gene Required for Larval Motor Function and Pupation in *Drosophila*

Karl B. Shpargel,* Kavita Praveen,† T. K. Rajendra,† and A. Gregory Matera*†

*Department of Genetics, School of Medicine, Case Western Reserve University, Cleveland, OH 44106-4955; and †Departments of Biology and Genetics, Program in Molecular Biology and Biotechnology, Curriculum in Genetics and Molecular Biology, and Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599-3280

Submitted January 10, 2008; Revised September 17, 2008; Accepted October 7, 2008
Monitoring Editor: Karsten Weis

The assembly of metazoan Sm-class small nuclear ribonucleoproteins (snRNPs) is an elaborate, step-wise process that takes place in multiple subcellular compartments. The initial steps, including formation of the core RNP, are mediated by the survival motor neuron (SMN) protein complex. Loss-of-function mutations in human *SMN1* result in a neuromuscular disease called spinal muscular atrophy. The SMN complex is comprised of SMN and a number of tightly associated proteins, collectively called Gemins. In this report, we identify and characterize the fruitfly ortholog of the DEAD box protein, Gemin3. *Drosophila* Gemin3 (dGem3) colocalizes and interacts with dSMN in vitro and in vivo. RNA interference for dGem3 codepletes dSMN and inhibits efficient Sm core assembly in vitro. Transposon insertion mutations in *Gemin3* are larval lethals and also codeplete dSMN. Transgenic overexpression of dGem3 rescues lethality, but overexpression of dSMN does not, indicating that loss of dSMN is not the primary cause of death. *Gemin3* mutant larvae exhibit motor defects similar to previously characterized *Smn* alleles. Remarkably, appreciable numbers of *Gemin3* mutants (along with one previously undescribed *Smn* allele) survive as larvae for several weeks without pupating. Our results demonstrate the conservation of Gemin3 protein function in metazoan snRNP assembly and reveal that loss of either *Smn* or *Gemin3* can contribute to neuromuscular dysfunction.

INTRODUCTION

Spinal muscular atrophy (SMA) is an autosomal recessive genetic disease with a carrier frequency of 1 in 50 unrelated individuals and is distinguished by degeneration of spinal motor neurons and severe atrophy of skeletal muscle (Pearn *et al.*, 1978; Ogino and Wilson, 2004). The *Survival Motor Neuron 1* gene (*SMN1*) was identified by positional cloning as the gene responsible for ~95% of SMA cases (Lefebvre *et al.*, 1995). Because of the observed variability in phenotypic severity, at least three classes of SMA have been established (Pearn, 1980; Ogino and Wilson, 2004). SMA type I, also known as Werdnig-Hoffman disease, is the most common and the most severe form of the disease, with an age of onset at <6 mo. SMA type I patients do not survive and typically die within the first 24 mo. SMA type II is an intermediate form, with an age of onset in the first 18 mo, and these patients often survive well into their teens. SMA type III, or Kugelberg-Welander syndrome, is characterized by late onset (after 18 mo) and chronic muscle weakness without a significant decrease in lifespan. All three classes of SMA are allelic, caused by mutations in *SMN1* (Lefebvre *et al.*, 1995).

Interestingly, the human genome contains a second locus, *SMN2*, which produces reduced amounts of full-length SMN protein and cannot fully compensate for the loss of *SMN1* (Lorson *et al.*, 1999; Monani *et al.*, 1999). Complete loss of *Smn* function results in early embryonic lethality in mice (Schrank *et al.*, 1997); animals that carry low-copy *SMN2* transgenes survive embryogenesis but die postnatally, yet those with high-copy transgenes are completely viable (Hsieh-Li *et al.*, 2000; Monani *et al.*, 2000). Thus, SMA can be viewed as a "protein-dosage" disease, an interpretation that correlates well with the fact that SMA severity is inversely proportional to SMN protein levels (Coovert *et al.*, 1997; Lefebvre *et al.*, 1997).

SMN is part of a large, oligomeric protein complex that is essential for a number of distinct steps in the biogenesis of metazoan Sm-class small nuclear ribonucleoproteins (snRNPs; reviewed in Matera *et al.*, 2007). SMN localizes diffusely throughout the cytoplasm, with intense nuclear signals corresponding to Cajal bodies (Liu and Dreyfuss, 1996; Matera and Frey, 1998). Based on the known protein–protein interactions, organization of the complex centers around SMN, which directly interacts with itself, Gemin2, Gemin3, Gemin5, and Gemin8 (Liu *et al.*, 1997; Lorson *et al.*, 1998; Charroux *et al.*, 1999; Meister *et al.*, 2000; Baccon *et al.*, 2002; Gubitz *et al.*, 2002; Pellizzoni *et al.*, 2002a; Carissimi *et al.*, 2006a; Battle *et al.*, 2007; Otter *et al.*, 2007). Gemin8 is thought to recruit Gemin6, Gemin7, and unr-interacting protein (UNRIP/STRAP), whereas Gemin3 brings Gemin4 into the complex (Charroux *et al.*, 2000; Baccon *et al.*, 2002; Carissimi *et al.*, 2005, 2006b). The SMN complex binds directly to the snRNA and to Sm proteins in order to coordinate snRNP assembly (Fischer *et al.*, 1997; Liu *et al.*, 1997; Pellizzoni *et al.*, 2002b; Yong *et al.*, 2002; Battle *et al.*, 2006). We previously demonstrated by RNA interference (RNAi) knockdown that SMN, Gemin2, Gemin3, and Gemin4 are each required for efficient snRNP assembly in HeLa cells (Shpargel and Matera,

This article was published online ahead of print in *MBC in Press* (http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E08–01–0024) on October 15, 2008.

Address correspondence to: A. Gregory Matera (agmatera@email.unc.edu).

 © 2008 by The American Society for Cell Biology

2005). Current theories suggest that Gemins and associated proteins function together to mediate the various steps of snRNP biogenesis (Shpargel and Matera, 2005; Feng *et al.*, 2005; Girard *et al.*, 2006; Lemm *et al.*, 2006). However, despite the excellent correlation between SMN protein levels and disease phenotype, mutations in other members of the SMN complex have not been associated with human disease.

Genetic analysis in model organisms provides a unique opportunity to study factors contributing to disease pathogenesis. *Drosophila* SMN (dSMN) has been identified on the basis of sequence and functional conservation, and null mutations within the gene are larval lethal in the second and third instar stages (Chan *et al.*, 2003; Rajendra *et al.*, 2007). These larvae exhibit motor and neuromuscular defects. We have also generated an adult model for *Drosophila* SMA. A hypomorphic mutation, called Smn^{E33}, was created by imprecise excision of a P-element residing in the upstream control region (Rajendra *et al.*, 2007). Smn^{E33} homozygotes exhibit reduced dSMN protein levels in the thorax of the adult fly. This deficiency leads to severe neuromuscular defects, including flightlessness, all of which can be rescued by expression of a YFP-*Smn* transgene (Rajendra *et al.*, 2007). Notably, SMN is a sarcomeric protein in both flies and mice, and because snRNPs are absent from myofibrils, SMN likely performs a tissue-specific function in muscle (Rajendra *et al.*, 2007). Other members of the *Drosophila* SMN complex have not been described.

Here, we identify and characterize dGemin3 (dGem3) as a member of the *Drosophila* SMN complex. Like its human counterpart, dGem3 interacts directly with dSMN in vitro and in vivo. Furthermore, these two proteins colocalize in the *Drosophila* Cajal body and are required for efficient assembly of Sm snRNPs. Previously uncharacterized transposon insertions in *Gemin3* and *Smn* exhibit larval motor defects, developmental delay, and a failure to pupate. Our results demonstrate the conservation of Gemin3 function in the fruitfly SMN complex and establish its essential role in various aspects of *Drosophila* development.

MATERIALS AND METHODS

DNA Constructs

Smn and *Gemin3* full-length cDNAs were PCR amplified with appropriate primers flanked by Gateway recombination sequences (Invitrogen, Carlsbad, CA). These products were recombined initially into pDONR221 (Invitrogen) before entry into glutathione *S*-transferase (GST)-tagged pDEST15, His-tagged pDEST17, green fluorescent protein (GFP)-tagged pAGW, or myc-tagged pAMW vectors (Invitrogen and the T. Murphy collection, housed at the *Drosophila* Genome Resource Center, Bloomington, IN).

Recombinant Protein Expression and S2 Cell Transfections

GST-dSMN and His-dGemin3 were expressed in BL21-star bacteria (Invitrogen) by 1 mM IPTG induction for 3 h. Lysate was extracted by sonication and passed over glutathione or Ni-agarose beads. S2 cells were transfected using Cellfectin as directed (Invitrogen).

Antibodies

GST (Santa Cruz Biotechnology, Santa Cruz, CA; 1:1000), His (Lab Vision, Fremont, CA; 1:1000), GFP (Roche, Indianapolis, IN; 1:1000), myc (Santa Cruz, 1:1000), SMN (Transduction Laboratories, Lexington, KY; 1:5000), SNF (U2B″, 1:1000), and tubulin (anti-rabbit; Sigma-Aldrich, St. Louis, MO) antibodies were used for Western blotting. Anti-myc (Santa Cruz, 1:40) was used for immunofluorescence. Anti-myc antibodies or Flag-conjugated agarose beads (Sigma) were used for immunoprecipitation in modified RIPA buffer.

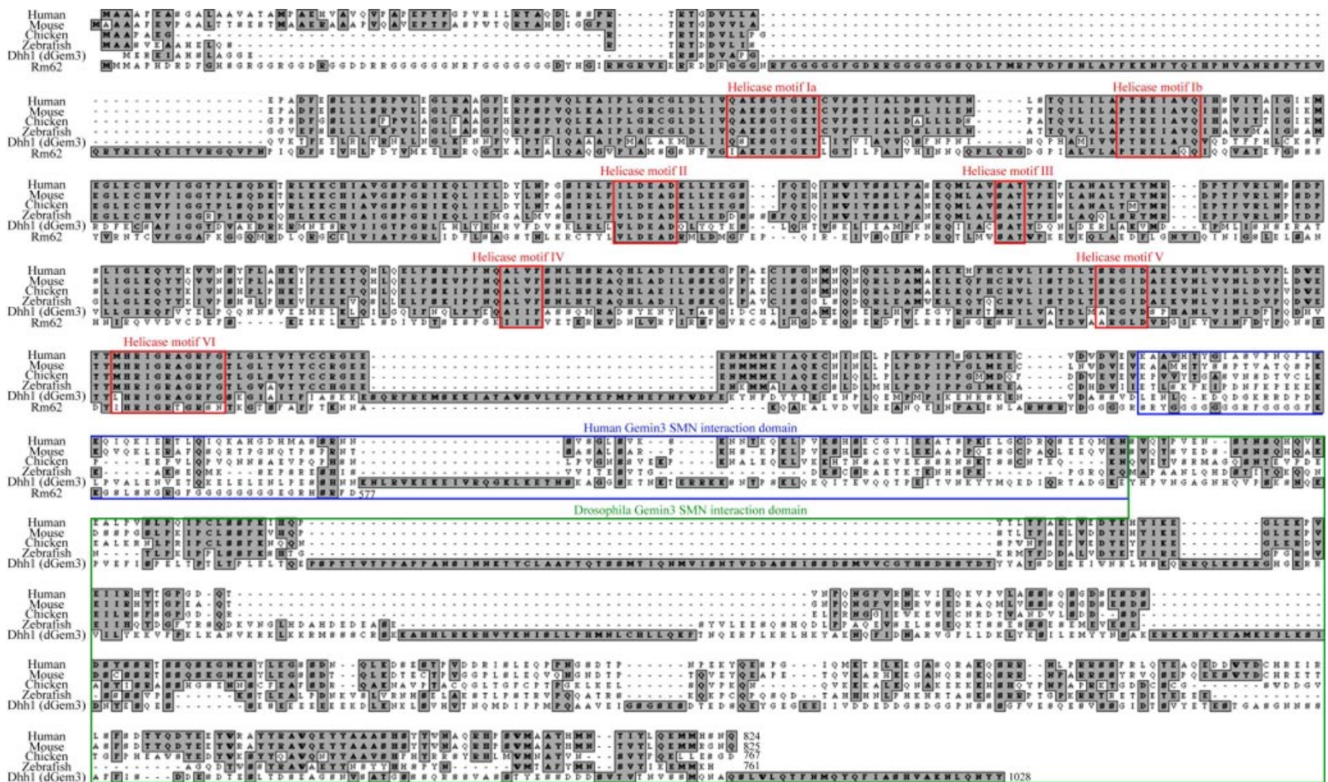


Figure 1. *Drosophila* Gemin3 is conserved throughout its helicase motifs. Gemin3 sequences from human, mouse, chicken, and zebrafish are aligned, along with two *Drosophila* proteins Dhh1 (dGem3) and Rm62. The seven helicase motifs are boxed in red, the Human SMN interaction domain is boxed in blue, and the *Drosophila* dSMN interaction domain is boxed in green.

Sm Assembly Assay

Smn, Gemin3, and *LacZ* dsRNAs were transcribed in vitro from PCR products flanked with T7 promoters. *Drosophila* S2 cells were placed in SF-900 media containing 14 μg/ml double-strand RNA (dsRNA). Extracts were generated 3 d after transfection using the Ne-Per nuclear/cytoplasmic extraction kit as directed (Pierce, Rockford, IL) and dialyzed in reconstitution buffer (20 mM HEPES-KOH, pH 7.9, 50 mM KCl, 5 mM $MgCl_2$, and 0.2 mM EDTA (Pellizzoni *et al.,* 2002a). Forty micrograms of cytoplasmic extract was loaded on a gel for Western blotting analysis to confirm knockdown. For the assembly assay, wild-type U1 snRNA and U1 snRNA containing a deletion of the Sm assembly site were in vitro transcribed from PCR products in the presence of P^{32}-rUTP and m7G cap analogue (Promega). Equivalent amounts of radiolabeled U1 snRNA (n = 100,000 cpm) were incubated in 100 μg of cytoplasmic extract at 22°C for 40 min in reconstitution buffer. Assembled snRNPs were precleared with protein G beads before immunoprecipitation with 15 μl (1.5 μg) Y12 antibody in RSB-100 buffer (600 mM NaCl, 20 mM Tris-HCl, pH 7.4, 2.5 mM $MgCl_2$, and 0.01% NP40). Immunoprecipitation products were denatured in formamide loading buffer, run on a 6% acrylamide TBE-urea gel, and exposed to a phosphorimager.

Fly Stocks

Smn^{A} (Smn^{73Ao}, G202S; Chan *et al.,* 2003), Smn^{B} (S201F; Chan *et al.,* 2003), Smn^{C} (PBac{WH}Smn^{f05960}; Thibault *et al.,* 2004), Smn^{D} (PBac{WH}Smn^{f01109}; Thibault *et al.,* 2004), and Smn^{F} (PBac{PL}Smn^{00733}; Häcker *et al.,* 2003) were maintained over TM3, P{ActGFP}JMR2, Ser[1] or TM6B, P{Ubi-GFP.S65T}PAD2, Tb[1] balancer chromosomes. $Gem3^{A}$ (Pbac{RB}$Gem3^{e03688}$; Thibault *et al.,* 2004) and $Gem3^{B}$ (P{PZ}$Gem3^{rL562}$; Spradling *et al.,* 1999) were maintained on TM6B, Tb balancer chromosomes. A deletion removing the *Gemin3* region, Df(3L)ED4457, was obtained from the Bloomington Stock Center (Bloomington, IN). Alleles were recombined to create multiple insertions on a single chromosome. $Gem3^{B-rev}$ was created by precise excision of $Gem3^{B}$. Timed matings were allowed to proceed for 6 h, and larvae were collected for phenotypic analyses on subsequent days. For the transgenic construct, the Flag tag was added to the *Drosophila Gemin3* cDNA by PCR amplification. The *Flag-Gemin3* product was cloned into *pUAST* and sent to BestGene, (Chino Hills, CA) for embryo injection and transgene screening. The YFP-*Smn* transgene (a gift from J. Gall, Carnegie Institution of Washington, Baltimore, MD) has been previously characterized (Liu *et al.,* 2006; Rajendra *et al.,* 2007).

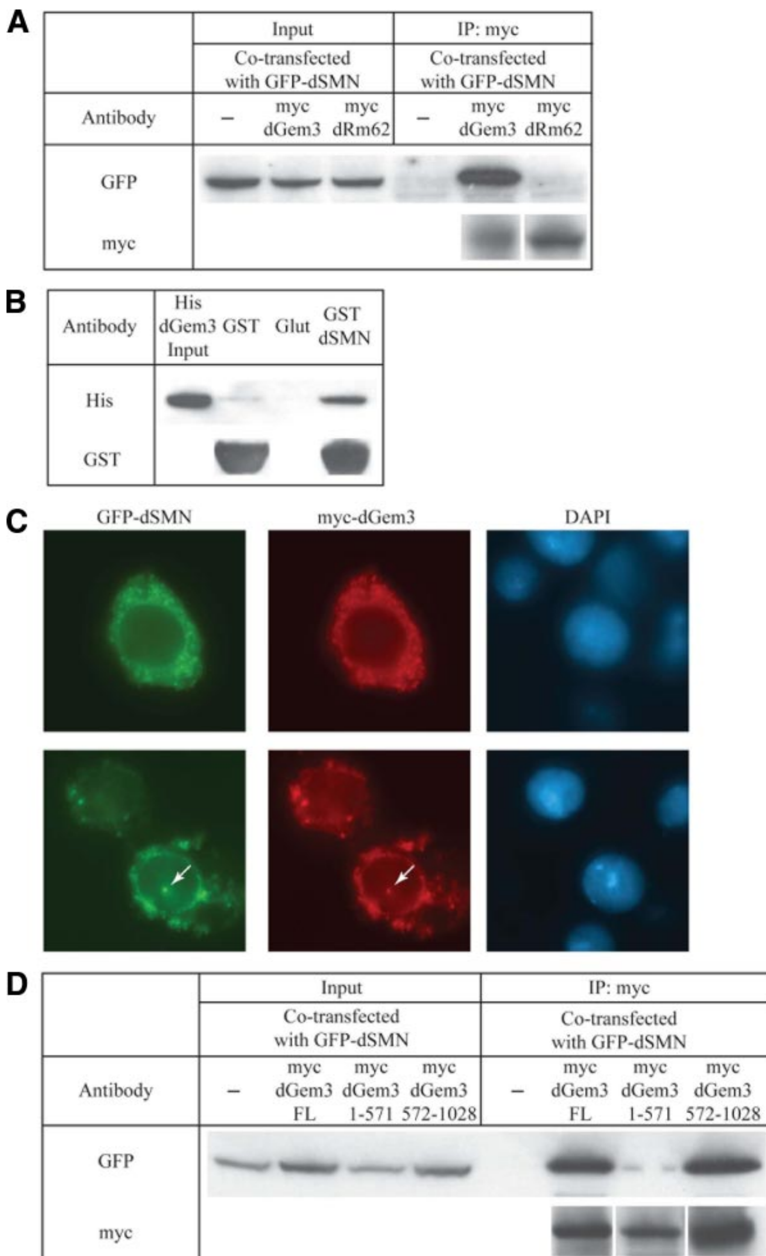


Figure 2. dGemin3 interacts with dSMN in vitro and in vivo. (A) *Drosophila* S2 cells were transfected with GFP-dSMN alone or cotransfected with myc-dGemin3 (dGem3, Dhh1) or myc-dRm62. Immunoprecipitation with anti-myc antibodies coprecipitated GFP-dSMN only when myc-Gem3 was present. Inputs represent 10% of immunoprecipitation reactions. (B) Bacterially expressed His-dGem3 was passed over GST beads, glutathione beads (Glut), or GST-dSMN beads in a pulldown reaction. Western blotting demonstrated a specific, direct interaction with GST-dSMN. (C) GFP-dSMN and myc-dGem3 colocalize throughout the cytoplasm and in nuclear Cajal bodies (arrow). (D) dSMN interacts with the dGemin3 C-terminus. Myc-dGem3 deletion constructs were generated and transfected into S2 cells, repeating the experiment in A. GFP-dSMN is coimmunoprecipitated by myc-dGem3 amino acids 572-1028.

RESULTS

Drosophila "Dhh1" Encodes a Putative Gemin3 Ortholog

BLAST searches using human Gemin3 to probe the *Drosophila* proteome identified a number of potential orthologues, including the DEAD box proteins Dhh1 (CG6539) and Rm62 (CG10279). Alignment of Dhh1 and Rm62 with vertebrate orthologues of Gemin3 demonstrated strong conservation of the N-terminal helicase motifs among each of these proteins, with Dhh1 scoring slightly higher (Figure 1, red boxes). Although the C-terminal domain of Dhh1 is extensive, it bears little resemblance to vertebrate Gemin3 proteins; the C-terminus of Rm62 is truncated, and thus is also not conserved (Figure 1). A genome-wide *Drosophila* yeast two-hybrid analysis has been published, along with an accompanying searchable online database (Giot *et al.*, 2003). Examination of dSMN (CG16725) in this database identified a high confidence interaction with Dhh1, but not with Rm62. Although dSMN did not interact with Rm62 in the yeast interaction screen, it is difficult to assign orthology on the basis of two-hybrid analysis and amino acid similarity alone. Human Gemin3 is characterized by its direct interaction and colocalization with SMN throughout the cytoplasm and in nuclear Cajal bodies, and by its function in efficient assembly of the Sm core snRNP. To identify the fruitfly Gemin3 ortholog, full-length *Dhh1* and *Rm62* cDNAs were cloned into myc-tagged expression vectors and cotransfected into S2 cells with GFP-dSMN.

Coimmunoprecipitation analyses revealed that Dhh1 (myc-dGem3), but not Rm62 (myc-dRm62), forms complexes with GFP-dSMN in vivo (Figure 2A). The physical interaction between dSMN and Dhh1 (dGem3) is direct, as evidenced by in vitro GST-pulldown assays using purified recombinant proteins (Figure 2B). Furthermore, immunofluorescence of S2 cells cotransfected with dSMN (GFP-dSMN) and Dhh1 (myc-dGem3) constructs revealed complete colocalization throughout the cytoplasm and in nuclear Cajal bodies (Figure 2C). Interestingly, dSMN overexpression formed cytoplasmic aggregates, similar to its human ortholog (Shpargel *et al.*, 2003). On the basis of these and other experiments (see below), we conclude that Dhh1 is the *Drosophila* ortholog of Gemin3. Because the well-studied yeast Dhh1 protein, a component of P-bodies, is actually orthologous to another *Drosophila* protein, called Me31B (CG4916), we have adopted the nomenclature dGemin3 (dGem3) for fruitfly Dhh1, to avoid confusion.

We generated myc-tagged dGem3 truncation constructs to assess the functional conservation of the C-terminal domain for its ability to interact with dSMN. Cotransfection with GFP-dSMN revealed that myc-dGem3 interacts with dSMN via amino acids 572-1028, a C-terminal region that is more

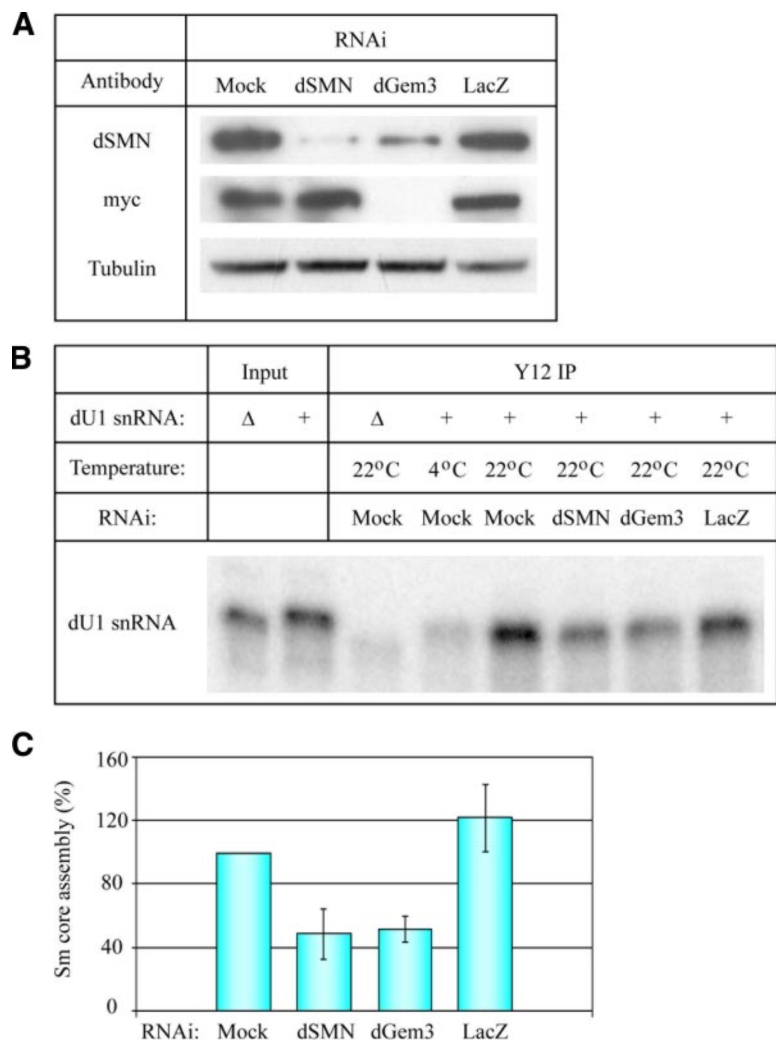
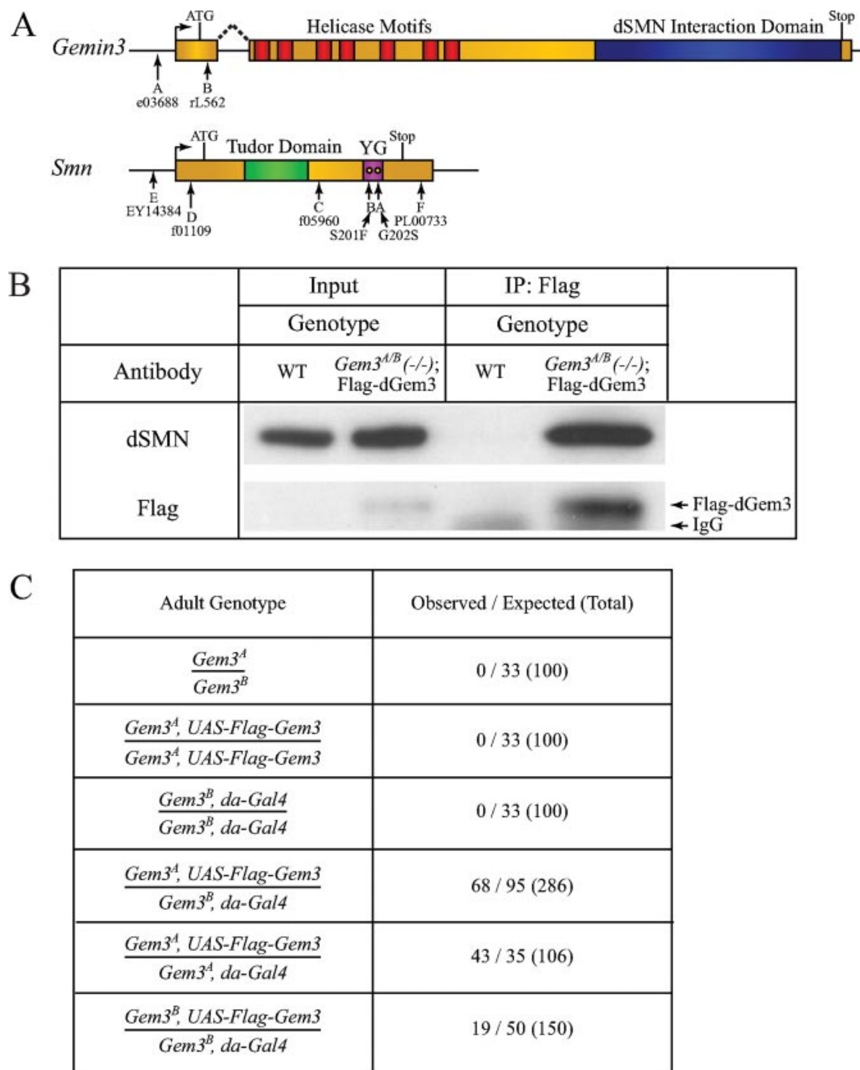


Figure 3. *Drosophila* SMN and Gemin3 are required for efficient snRNA Sm core assembly. (A) *Drosophila* S2 cells were left untreated (Mock) or incubated with either *Smn*, *Gem3*, or *LacZ* dsRNAs for 24 h. Cells were then transfected with a myc-dGemin3 reporter construct and cytoplasmic extracts were collected after 3 additional days of incubation with the dsRNAs. Western blotting confirmed efficient knockdown of dSMN and dGem3 relative to the Tubulin loading control. (B) A radiolabeled U1 snRNA transcript was incubated in cytoplasmic extract and immunoprecipitated with Y12 (anti-Sm) antibody to assay for Sm core assembly. The U1 snRNA containing a deletion of the Sm protein assembly site (Δ) or incubating a wild-type U1 snRNA (+) at a nonpermissive temperature (4°C) serve as negative controls in the experiment. RNAi of dSMN and dGem3 significantly disrupted Sm core assembly compared with Mock and LacZ dsRNA treatments. (C) Quantification of Sm core assembly assays from three separate experiments. The results, normalized relative to the Mock control, were significant ($p < 0.005$). Approximately 50% reduction in Sm core assembly was observed for dSMN and dGem3 knockdown. LacZ RNAi had no significant effect ($p > 0.2$).



Adult Genotype	Observed / Expected (Total)
Gem3^A / *Gem3^B*	0 / 33 (100)
Gem3^A, UAS-Flag-Gem3 / *Gem3^A, UAS-Flag-Gem3*	0 / 33 (100)
Gem3^B, da-Gal4 / *Gem3^B, da-Gal4*	0 / 33 (100)
Gem3^A, UAS-Flag-Gem3 / *Gem3^B, da-Gal4*	68 / 95 (286)
Gem3^A, UAS-Flag-Gem3 / *Gem3^A, da-Gal4*	43 / 35 (106)
Gem3^B, UAS-Flag-Gem3 / *Gem3^B, da-Gal4*	19 / 50 (150)

Figure 4. Flag-dGem3 interacts with dSMN and rescues *Gemin3* mutant larval lethality. (A) Schematic illustrating names and locations of the various *Gemin3* and *Smn* alleles used in this study. (B) dGem3 interacts with dSMN in vivo. Adult fly lysates were prepared from either wild-type (WT) or *Gem3^{A/B}* transheterozygotes expressing a Flag-dGem3 transgene driven by *daughterless*-Gal4 expression. Immunoprecipitation of Flag-dGem3 copurified dSMN. (C) Both *Drosophila Gemin3* alleles are lethal, fail to complement each other, and can be rescued by exogenous transgene expression. The table illustrates the crosses performed (left) along with the adult progeny scored for the given genotype (right). The total numbers of flies analyzed are in parentheses. One-third of the total progeny resulting from the various heterozygous intercrosses (over balancer chromosomes) are expected to have the desired adult genotype.

distal to the equivalent SMN interaction domain in the human protein (Figure 2D). Thus, human and *Drosophila* Gemin3 proteins interact with SMN through divergent domains (Figure 1, blue and green boxes).

Drosophila Gemin3 Is Required for Efficient snRNP Assembly

SMN and Gemin3 are essential for formation of the Sm protein core RNP in human cells (Shpargel and Matera, 2005). We utilized an Sm core assembly assay to investigate whether dGem3 plays a similar conserved role in flies. As described in Rajendra *et al.* (2007), this assay uses cytoplasmic extracts prepared from S2 cell lysates depleted for individual components by RNAi. Radiolabeled U1 snRNA was incubated in these lysates, and its assembly with Sm proteins was assayed by coimmunoprecipitation with anti-Sm antibodies (mAb Y12). We performed dsRNA-mediated RNAi on S2 cells to deplete dSMN and dGem3 proteins (Figure 3A). Western blotting of lysates derived from untransfected S2 cells (mock) or S2 cells treated with *LacZ* (control), *Smn*, or *Gemin3* dsRNA demonstrated efficient and specific knockdown of dSMN and dGem3 compared with the Tubulin loading control. In each case, the cells were transfected with myc-dGem3 to monitor levels of dGem3 knockdown, because of the unavailability of an antibody targeting the endogenous protein. Interestingly, RNAi of dGem3 resulted in a moderate codepletion of dSMN (Figure 3A). As shown in Figure 3B, cytoplasmic extracts were incubated with either radiolabeled wild-type U1 snRNA (+) or mutant U1 snRNA (Δ), which lacks the Sm-binding site. Extracts were incubated at nonpermissive (4°C) or permissive (22°C) temperatures for the assembly assay (Figure 3B). Depletion of dSMN and dGem3 significantly reduced Sm core assembly ($p < 0.005$) relative to the mock or LacZ controls. Quantification of three separate experiments verified a 50% reduction in Sm core assembly activity when dSMN and dGem3 were depleted (Figure 3C). We conclude that the function of *Gemin3* in snRNP assembly is conserved in invertebrates.

Gemin3 Is an Essential Gene in the Fly

To investigate the function of *Gemin3* at the organismal level, we obtained two transposon insertion lines, designated e03688 and rL562 (Figure 4A). The e03688 allele is a piggyBac insertion (PBac{RB}*Gem3*^{e03688}) and will be referred to as *Gem3*^{A} in the text; the rL562 allele contains a P-element insertion (P{PZ}*Gem3*^{rL562}) and will be termed *Gem3*^{B}. The *Gem3*^{A} insertion is located in the *Gemin3* pro-

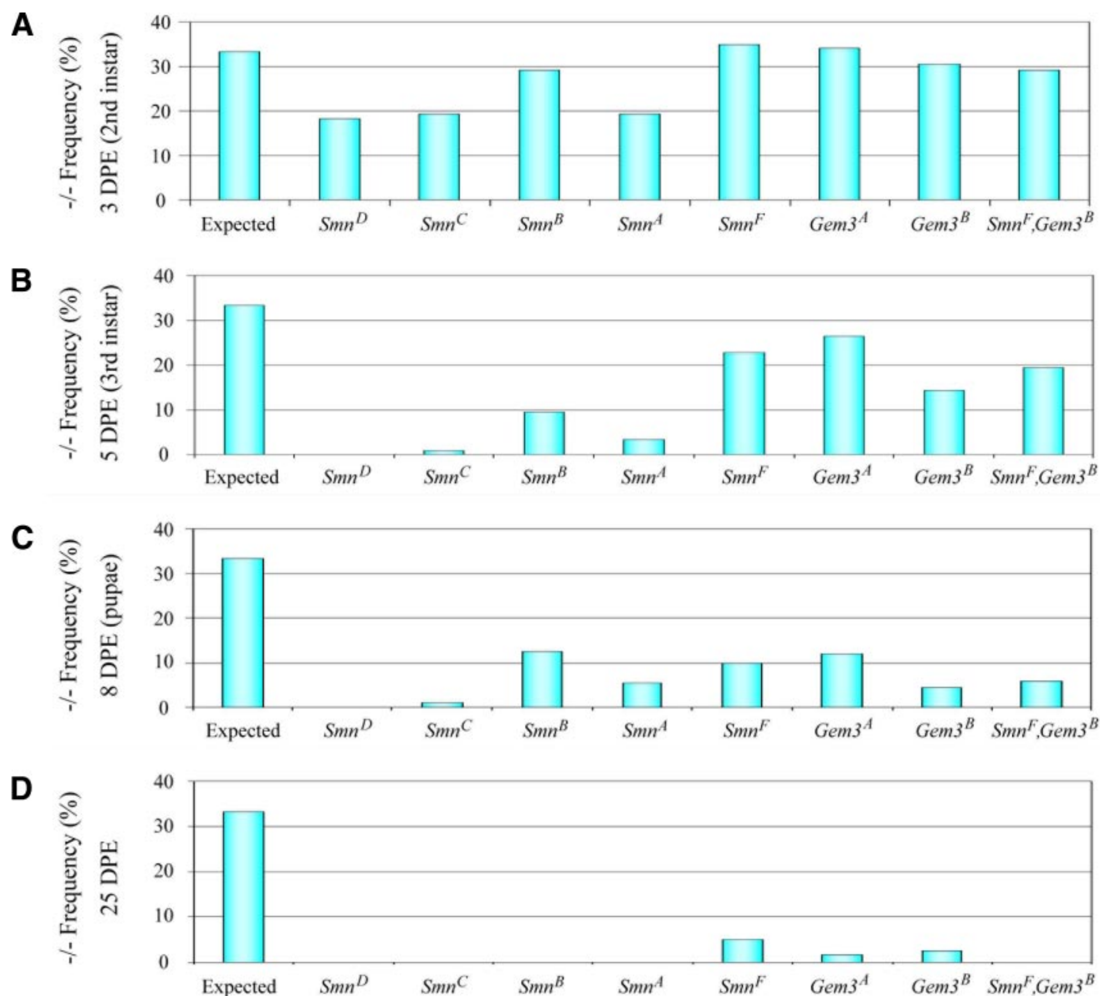


Figure 5. *Smn* and *Gemin3* mutant larvae exhibit viability and pupation defects. (A–D) *Smn* and *Gemin3* mutant larvae exhibit viability and pupation defects. Graphs illustrate the percentage of collected homozygous larvae at 3 DPE (A, second instar stage), 5 DPE (B, third instar stage), 8 DPE (C, pupation stage), or 25 DPE (D). γ^2 analysis was performed for each genotype at each developmental time point. The expectation is that heterozygous intercrosses over balancer chromosomes will produce 33% homozygous mutant progeny ($n \geq 100$ larvae scored for each genotype). Three days after egg laying, the numbers of Smn^D, Smn^C, and Smn^A larvae were significantly reduced ($p < 0.001$); p for remaining genotypes was >0.3. At day 5, p for all genotypes was <0.004. At day 8, p for all genotypes was $<3 \times 10^{-5}$. Note that at 8 DPE, several *Smn* and *Gemin3* mutants survived as larvae for extended periods, but failed to pupate (see text).

moter region and the $Gem3^B$ insertion is located immediately downstream of the translation start site (genomic insertion sites were verified by sequencing; see Flybase for details).

Similar to all previously described *Smn* null alleles, homozygous mutants of both $Gem3^A$ and $Gem3^B$ die before pupation. Notably, these two *Gemin3* alleles fail to complement each other and the recessive lethality can be rescued by ubiquitous expression of a Flag-*Gemin3* transgene (Figure 4B,C). Additionally, when crossed over a deletion encompassing the *Gemin3* region, Df(3L)ED4457, the $Gem3^A$ and $Gem3^B$ mutations failed in complementation tests and exhibited phenotypes indistinguishable from the individual homozygous mutations (data not shown). The pUAST-Flag-*Gemin3* transgene was expressed ubiquitously by the *Daughterless*-GAL4 (*da*-GAL4) driver. Transgenic expression of Flag-dGem3 rescued the larval lethality and produced viable and fertile adults; the degree of rescue depended on the underlying combination of *Gemin3* mutations, but was significant in each case (Figure 4C). As expected, no rescue was observed with the driver-only (*da*-Gal4) or responder-only (UAS-Flag-*Gemin3*) crosses (Figure 4C). Immunoprecipitation of Flag-dGem3 from total adult *Drosophila* lysates copurified dSMN (Figure 4B). Therefore, the dGem3 and dSMN interaction observed in S2 cells (Figure 2) is also maintained within the organism. The *Gemin3* lethal phenotype was reverted by transposon excision repair of the disruption in $Gem3^B$ (data not shown). Taken together with the transgenic rescue experiments, these data lead us to conclude that the transposon insertions in the *Gemin3* gene cause the observed lethal phenotype.

Long-lived Smn and Gemin3 Mutants Fail to Pupate

In an effort to draw correlations with SMN complex function, we compared $Gem3^A$ and $Gem3^B$ mutants to four previously characterized *Smn* alleles (Smn^A to Smn^D; Chan *et al.*, 2003; Rajendra *et al.*, 2007) and one previously uncharacterized allele, PBac{PL}Smn^{00733}, which we designate Smn^F (Figure 4A; Häcker *et al.*, 2003). Similarly, each of the *Smn* and *Gemin3* mutants are larval lethals and no homozygous pupae or adult flies were observed (data not shown). To better establish the critical lethal phases of the *Smn* and *Gemin3* mutants, we performed a temporal analysis of het-

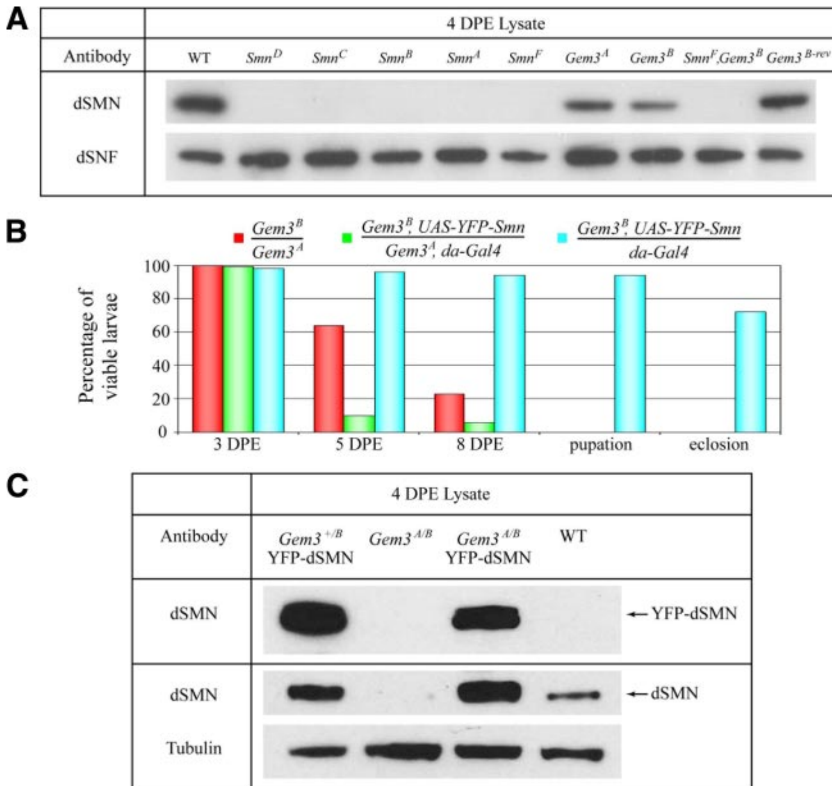


Figure 6. YFP-dSMN overexpression fails to rescue *Gemin3* mutant phenotypes. (A) Western blotting of larval lysates at 4 DPE. At this time point, all of the *Smn* alleles were essentially protein nulls, whereas the two *Gemin3* alleles displayed reduced dSMN levels compared with the controls (WT and $Gem3^{B\text{-}rev}$). Anti-SNF (anti-U2B″) antibodies recognize *Drosophila* Sans fille, a homolog of this snRNP-specific protein, and were used as the loading control. (B) $Gem3^{B/A}$ transheterozygous larvae, $Gem3^{B/A}$ larvae with a YFP-*Smn* transgene, or $Gem3^{B/+}$ larvae also bearing the YFP-*Smn* transgene were isolated at 2 DPE and scored for viability respective to the starting population at 3, 5, and 8 DPE. $Gem3^{B/A}$ larvae exhibited significant lethality at 5 and 8 DPE even in the presence of ectopic YFP-dSMN. Although a small percentage of these larvae survive to extended time points (i.e., well beyond 8 DPE), they failed to pupate and reach adulthood. n = 100–150 larvae scored for $Gem3^{B/A}$, and $Gem3^{B/A}$, and YFP-*Smn* larvae per time point. n = ~50 larvae for $Gem3^{+/B}$ and YFP-*Smn* at each time point. (C) Western blot of lysate from $Gem3^{B/A}$, $Gem3^{B/A}$ with YFP-*Smn*, $Gem3^{B/+}$ larvae with YFP-*Smn*, or WT larvae. Expression of the YFP-*Smn* transgene rescued endogenous dSMN protein in $Gem3^{B/A}$ larvae. Note that for dSMN antibody detection, the membrane was cut and exposed separately to detect YFP-dSMN and endogenous dSMN because YFP-dSMN expression was much greater in intensity.

erozygous intercrosses. These experiments revealed that a certain fraction of the Smn^D, Smn^C, and Smn^A homozygous larvae die by 3 d post egg laying (DPE; corresponding to the second instar larval stage in control animals, γ^2 $p < 0.001$; Figure 5A). Smn^D and Smn^C appeared to be the most severely affected, with very few larvae surviving past day 5 (third instar; Figure 5B). Notably, whereas Smn^B and Smn^F exhibited moderate viability defects at the third instar time point (γ^2 $p < 0.0002$), approximately one-third of the homozygous larvae (i.e., 10–12% of the total) survived beyond day 8, a period wherein the control wild-type and heterozygous larvae have already pupated (Figure 5C). Incredibly, a fraction of the Smn^F larvae survived for more than 3 wk without pupating (Figure 5D). Although none of the Smn^B homozygotes survived to day 25 (Figure 5D), a large fraction of them survived to day 8 (Figure 5C); some of these animals formed pseudopupae before dying (data not shown). On the basis of these and other phenotypic analyses, we conclude that the *Smn* alleles described to date (Chan *et al.*, 2003; Rajendra *et al.*, 2007; this study) can be ranked in order of decreasing severity as follows: D > C > A > B > F > E33.

A temporal analysis of *Gemin3* mutant intercrosses indicated that significant larval lethality also occurs during the second-third instar time points (Figure 5B; third instar γ^2 $p < 0.004$). Similar to the results of the Smn^F intercross, 30% of $Gem3^A$ and 15% of $Gem3^B$ homozygous larvae survived to day 8 after egg laying, but failed to pupate (Figure 5C). Comparable to Smn^B, $Gem3^A$ mutants occasionally formed pseudopupal cases (data not shown). Unlike the stronger *Smn* alleles (A–D), but similar to Smn^F, a small fraction of $Gem3^A$ and $Gem3^B$ homozygotes survived to day 25 (Figure 5D).

Double homozygotes for the $Gem3^B$ and Smn^F insertions (illustrated as $Gem3^B$, Smn^F in Figure 5) displayed a phenotype similar to that of the individual mutations. In other words, homozygous loss of both genes did not significantly enhance the phenotype, except at the longest time point (Figure 5D). Thus, although dSMN and dGem3 work together in snRNP assembly, complete loss of function of both genes is equivalent to the loss of either one of them. Notably, a *Gemin3* revertant allele, $Gem3^{B\text{-}rev}$, recovered the ability to pupate and is fully viable (data not shown). Crossing $Gem3^A$ and $Gem3^B$ to the Df(3L)ED4457 deletion did not enhance the larval lethality phenotype (data not shown). We conclude that *Smn* and *Gemin3* are essential for larval viability and pupation.

YFP-dSMN Overexpression Fails to Rescue Gemin3 Phenotypes

Because depletion of dGem3 by RNAi in S2 cells resulted in codepletion of dSMN (Figure 3A), we compared dSMN levels in the *Gemin3* mutants to those of the five characterized *Smn* alleles. Larval lysates (4 DPE) were prepared and analyzed by Western blotting with anti-dSMN antibodies. As reported previously (Rajendra *et al.*, 2007), the mutant *Smn* larvae expressed little or no dSMN during the phenocritical stage (Figure 6A). A certain degree of variability in the levels of dSMN was observed for the Smn^A and Smn^B alleles from preparation to preparation (Figure 6A and Rajendra *et al.*, 2007). Interestingly, $Gem3^A$ and $Gem3^B$ homozygotes also expressed reduced levels of dSMN protein (Figure 6A), reminiscent of the results obtained in cell culture (Figure 3A). Importantly, transgenic expression of Flag-dGem3 in the *Gemin3* mutant background (Figure 4B, compare dSMN input lanes) or reversion of the lethal phenotype by excision repair (Figure 6A) were each able to rescue dSMN levels. Thus, mutations in *Gemin3* result in a corresponding depletion of dSMN, which may contribute to the phenotype.

To test the hypothesis that dSMN function is compromised by loss of *Gemin3* activity and that the observed

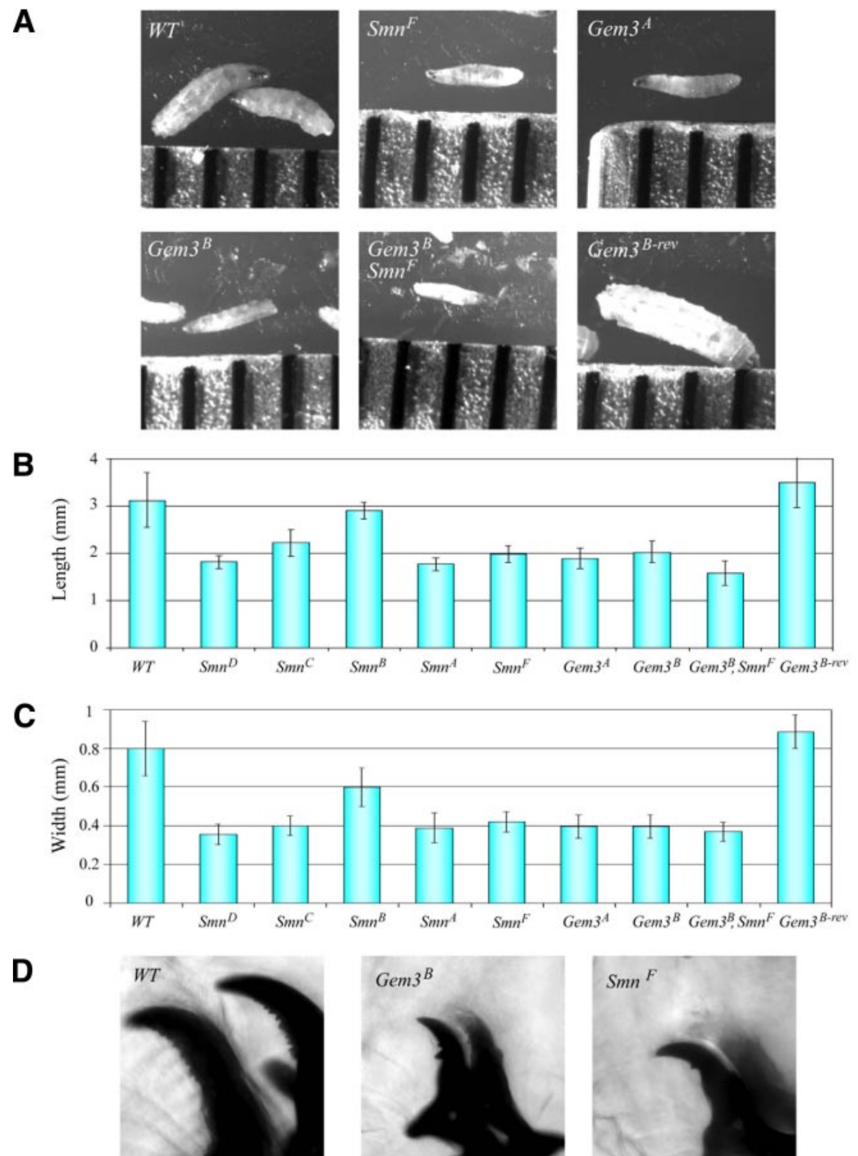


Figure 7. *Smn* and *Gemin3* mutants exhibit growth defects. (A) Images of *Smn* and *Gemin3* second-third instar larvae (4 DPE) compared with WT and revertant ($Gem3^{B\text{-}rev}$) controls. Tick marks on the ruler are 1 mm apart. (B) Graph of average length of 4-d-old larvae (n = 20 larvae scored for each genotype). Mutant length $p < 0.0001$ except for Smn^B with a $p > 0.3$. (C) Graph of average width of 4-d-old larvae (mutant width $p < 0.002$). (D) *Smn* and *Gemin3* mutants exhibit developmental delay. Four days after egg laying, the mouth hooks of *Smn* and *Gemin3* larvae correspond to second instar larvae, whereas WT larvae have entered the third instar larval stage.

lethality of *Gemin3* mutants might be due to codepletion of dSMN, we overexpressed YFP-dSMN in the *Gemin3* mutant background. The YFP-*Smn* transgene is fully functional and capable of rescuing *Smn* mutant phenotypes (Rajendra *et al.*, 2007). To identify $Gem3^B/Gem3^A$ transheterozygotes in the control cross, individual mutations were maintained over TM3, P{ActGFP} balancers, thus nonfluorescent larvae were selected as compound heterozygotes. In the YFP-dSMN rescue crosses, mutations were maintained over nonfluorescent TM3 balancers and the desired larvae were identified by fluorescent YFP-dSMN expression. Larvae of the desired genotypes were isolated at 2 DPE and scored for survival at 3, 5, and 8 DPE. Interestingly, although $Gem3^B/Gem3^A$ transheterozygotes displayed a similar lethality profile to individual homozygotes (Figure 6B vs. Figure 5, A–C), expression of YFP-dSMN in this background did not rescue the larval lethality (Figure 6B). Furthermore, YFP-dSMN expression in the $Gem3^B/Gem3^A$ larvae failed to rescue pupation, whereas $Gem3^{+/B}$, YFP-dSMN control animals reached adulthood normally (Figure 6B). Notably, overexpression of YFP-dSMN appeared to stabilize endogenous dSMN protein levels (Figure 6C). Because *Gemin3* mutant phenotypes persist when dSMN levels are restored, we conclude that dGem3's essential function in larval viability and pupation is not limited to regulating dSMN protein levels.

Smn and Gemin3 Mutant Larvae Exhibit Growth Defects

Although a fraction of the $Gem3^A$, $Gem3^B$, and Smn^F homozygotes survived as larvae beyond 25 DPE, the mutants were by no means normal. In fact, by day 4, *Smn^F and Gemin3* mutant larvae appeared runted in size (Figure 7A). Wild-type or $Gem3^{B\text{-}rev}$ control larvae measured >3.0 mm in length and averaged ~0.8 mm in width. Conversely, *Smn* and *Gemin3* mutant alleles generally averaged only 1.5–2.0 mm in length and 0.4 mm in width (Figure 7, B and C; except for Smn^B, all $p < 0.0001$). Smn^B homozygotes were intermediate in size, averaging only 0.6 mm in width (a significant reduction, $p < 0.002$), but were of normal length (2.9 mm, $p > 0.3$). Analysis of the larval mouth hooks at 4 DPE revealed that the Smn^F and $Gemin3^B$ homozygotes are more similar to second instar

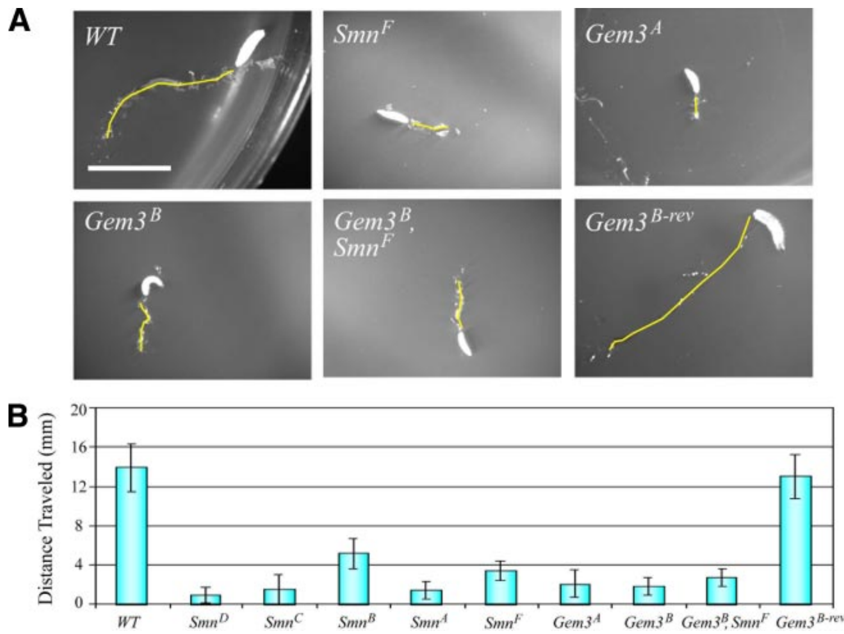


Figure 8. *Smn* and *Gemin3* mutant larvae exhibit defects in motor function. (A) Control (WT or *Gem3*$^{B\text{-}rev}$), *Smn*, or *Gemin3* homozygous larvae were prodded with a needle to stimulate movement and traced over 20 s (yellow line). Scale bar, 5 mm. (B) Graph of average distance traveled over 20 s (n = 10 larvae scored for each genotype). Larval movement was impaired for *Smn* and *Gemin3* mutants relative to WT controls ($p < 5 \times 10^{-6}$).

larvae, because similarly staged wild-type larvae have entered the third instar and have more highly serrated mouth hooks (Figure 7D). Therefore *Smn* and *Gemin3* mutant larvae are significantly smaller than controls and appear to be developmentally arrested.

Smn and Gemin3 Are Required for Proper Motor Function

Smn^A and *Smn*^{B} were previously characterized as having defects in larval motility (Chan *et al.*, 2003). To analyze the motor dysfunction of *Smn* and *Gemin3* mutants, we placed second-third instar larvae on plates and stimulated movement with a needle. The distance they traveled over the next 20 s was traced and measured (Figure 8, A and B). Wild-type or *Gem3*$^{B\text{-}rev}$ larvae traveled an average of ~14 mm during the 20 s; *Smn* and *Gemin3* mutant larvae traveled at most 5 mm over the same interval (mutant $p < 5 \times 10^{-6}$). As shown in Figure 8B, *Smn*^{B} again displayed the least severe phenotype. Thus, the motor defects originally observed in *Smn*^{A} and *Smn*^{B} larvae (Chan *et al.*, 2003) are also recapitulated in the other *Smn* and *Gemin3* mutants.

DISCUSSION

To definitively demonstrate homologous *Gemin3* activity in the fruitfly, we have shown that dGem3 interacts with dSMN in vitro and in vivo. Additionally, dGem3 and dSMN colocalize to *Drosophila* Cajal bodies and are required for efficient Sm core snRNP assembly in S2 cells. In human cells, Gemin3 interacts strongly with Gemin4 and forms a subcomplex with this protein (Charroux *et al.*, 2000). Database searches have failed to identify putative Gemin4 orthologues in nonvertebrate species (Kroiss *et al.*, 2008). Similarly, Gemins6–8, form distinct subcomplexes in human cells (Carissimi *et al.*, 2006b; Battle *et al.*, 2007; Otter *et al.*, 2007), but orthologues of these proteins have not been identified in the *Drosophila* genome (Kroiss *et al.*, 2008). With the possible exception of *Gemin2* (Liu *et al.*, 1997), budding yeast genomes do not appear to contain any of the known SMN complex proteins. Fission yeast, however, encode clear *Smn* and *Gemin2* orthologues (Hannus *et al.*, 2000; Owen *et al.*, 2000). Despite these facts, there is little evidence for a role for *Smn* in snRNP assembly in *Schizosaccharomyces pombe* (Hannus *et al.*, 2000; Paushkin *et al.*, 2000) or even for a cytoplasmic phase for Sm core assembly in *Saccharomyces cerevisiae* (Murphy *et al.*, 2004; Boon *et al.*, 2007). These and other findings (see below) suggest that *Drosophila* contains a primitive version of the mammalian SMN complex.

The Drosophila SMN Complex and Ecdysone Signaling

Although database searches suggest that many of the SMN complex proteins are not conserved in the fly, putative orthologues of Gemin2 (dGemin2, CG10419), Gemin5 (rigor mortis/dGemin5; CG30149), and UNRIP/STRAP (wmd; CG3957) can be identified. Several lines of evidence suggest that these proteins function together. We have shown that endogenous dSMN copurifies with Flag-dGemin3 (Figures 2 and 4) and Flag-dGemin2 (K. Praveen and A.G. Matera, unpublished results). While this manuscript was under revision, Kroiss *et al.* (2008) also reported that dSMN forms complexes with dGem3 in S2 cells. However, dGem3 appears to be weakly or transiently associated with dSMN, as this protein was not recovered when Flag-dSMN or Flag-dGemin2 were used for the purification pulldowns. Thus it is possible that dGem3 is present in substoichiometric amounts relative to dSMN and dGem2. Despite the relative dearth of biochemical purification data linking these three factors into a single complex, we found that dGem3 is required for Sm core assembly in vitro (Figure 3). Moreover, RNAi knockdown of dGem3 in S2 cells and transposon insertions in the *Gemin3* locus in vivo resulted in codepletion of dSMN (Figures 3 and 5). Importantly, overexpression of YFP-*Smn* in the *Gemin3* null mutant background failed to rescue the lethality (Figure 6). Thus, although dGem3 may function to stabilize dSMN, it may have a separate function inside or outside of the SMN complex. Additional experiments will be required to distinguish among these possibilities.

Evidence supporting a role for the WD-repeat protein rigor mortis (rig/dGem5) in SMN complex function comes

from phenotypic analyses. *rigor mortis* is an essential gene, and mutants therein display significant larval lethality; animals that escape the initial wave of larval lethality are developmentally delayed and fail to pupate (Gates *et al.*, 2004). These phenotypes are strikingly similar to those of the *Smn*^{F} and *Gemin3* alleles described in this work. Thummel and colleagues have shown that rig/dGem5 interacts with several members of the ecdysone signaling pathway required for initiation of puparium formation (Gates *et al.*, 2004). Mammalian Gemin5 is also involved in signal transduction (Kim *et al.*, 2007). Similarly, UNRIP/STRAP, another WD repeat protein is an exclusively cytoplasmic member of the SMN complex (Carissimi *et al.*, 2005; Grimmler *et al.*, 2005) and is involved in intracellular signaling (Datta *et al.*, 1998; Datta and Moses, 2000; Anumanthan *et al.*, 2006). In the future, it will be interesting to determine whether *rigor mortis* interacts genetically and physically with other members of the *Drosophila* SMN complex.

Gemin3, Smn, and Neuromuscular Function

Irrespective of potential roles for the SMN complex in signal transduction, our results demonstrate the essential role that *Gemin3* plays in organismal development. During manuscript revision of this article, Mouillet *et al.* (2008) showed that the murine ortholog of *Gemin3* (*Dp103/Ddx20*) is essential for early embryonic development in mammals. Loss-of-function mutations in *Gemin3* have not been described in other organisms. To date, several *Smn* and *Gemin2* alleles have been characterized. Null mutations in mouse *Smn* and *Gemin2* are also early embryonic lethals (Schrank *et al.*, 1997; Jablonka *et al.*, 2002). Expression of a low-copy human *SMN2* transgene rescues the embryonic lethality, but the mice die shortly after birth and display severe motor neuron degeneration and muscular atrophy phenotypes (Monani *et al.*, 2000). Depletion of Smn in zebrafish embryos by morpholino injection elicits defects in motor axon outgrowth, although the primary versus secondary nature of the reported Smn phenotypes is unclear and the results seem to depend on the extent of depletion (McWhorter *et al.*, 2003; Winkler *et al.*, 2005; Carrel *et al.*, 2006; McWhorter *et al.*, 2007). Interestingly, depletion of Gemin2 is reported to have conflicting effects on motor axon development, possibly because of differences in the levels of gene inhibition or in the methods of phenotypic analysis (Winkler *et al.*, 2005; McWhorter *et al.*, 2007).

The connection between snRNP biogenesis and SMA is certainly complicated and is not well understood. We have shown that mutation of two members of the *Drosophila* SMN complex, *Smn* and *Gemin3*, causes defects in larval motor function. In addition to larval *Smn* mutants, our laboratory has previously reported SMA-like phenotypes in adult flies containing a hypomorphic *Smn*^{E33} mutation (Rajendra *et al.*, 2007). Thus, although it is clear that perturbations in the SMN complex can indeed result in neuromuscular dysfunction, the contribution that snRNP biogenesis plays in the etiology of these phenotypes remains a subject of ongoing investigation (Shpargel and Matera, 2005; Wan *et al.*, 2005; Winkler *et al.*, 2005; Gabanella *et al.*, 2007). Further complicating interpretation of the various SMA models is the fact that the SMN complex appears to function in tissue-specific pathways involved in both neuronal (McWhorter *et al.*, 2003; Zhang *et al.*, 2006; Bowerman *et al.*, 2007) and muscular development (Shafey *et al.*, 2005; Rajendra *et al.*, 2007). Clearly, animal models will play an important role in future research aimed at distinguishing among the various functions of the SMN complex.

ACKNOWLEDGMENTS

We thank A. Spradling (Carnegie Institution of Washington, Baltimore, MD), H. Bellen (Baylor College of Medicine, Houston, TX), and U. Häcker (Lund University, Lund, Sweden), as well as the Bloomington and Harvard/Exelixis stock centers for fly lines. We also thank J. Zhou (University of Massachusetts Medical School, Worcester, MA) for anti-dSMN antibodies and members of the Matera lab for helpful discussions and critical reading of the manuscript. This work was supported by National Institutes of Health (NIH) grants R01-GM53034 and R01-NS41617 (A.G.M.). K.B.S. was supported in part by an NIH predoctoral traineeship (T32-GM08613). K.P. was supported in part by an American Heart Association predoctoral fellowship.

REFERENCES

Anumanthan, G., Halder, S. K., Friedman, D. B., and Datta, P. K. (2006). Oncogenic serine-threonine kinase receptor-associated protein modulates the function of Ewing sarcoma protein through a novel mechanism. Cancer Res. *66*, 10824–10832.

Baccon, J., Pellizzoni, L., Rappsilber, J., Mann, M., and Dreyfuss, G. (2002). Identification and characterization of Gemin7, a novel component of the survival of motor neuron complex. J. Biol. Chem. *277*, 31957–31962.

Battle, D. J., Kasim, M., Wang, J., and Dreyfuss, G. (2007). SMN-independent subunits of the SMN complex. Identification of a small nuclear ribonucleoprotein assembly intermediate. J. Biol. Chem. *282*, 27953–27959.

Battle, D. J., Lau, C. K., Wan, L., Deng, H., Lotti, F., and Dreyfuss, G. (2006). The Gemin5 protein of the SMN complex identifies snRNAs. Mol. Cell *23*, 273–279.

Boon, K. L., Grainger, R. J., Ehsani, P., Barrass, J. D., Auchynnikava, T., Inglehearn, C. F., and Beggs, J. D. (2007). prp8 mutations that cause human retinitis pigmentosa lead to a U5 snRNP maturation defect in yeast. Nat. Struct. Mol. Biol. *14*, 1077–1083.

Bowerman, M., Shafey, D., and Kothary, R. (2007). Smn depletion alters profilin II expression and leads to upregulation of the RhoA/ROCK pathway and defects in neuronal integrity. J. Mol. Neurosci. *32*, 120–131.

Carissimi, C., Baccon, J., Straccia, M., Chiarella, P., Maiolica, A., Sawyer, A., Rappsilber, J., and Pellizzoni, L. (2005). Unrip is a component of SMN complexes active in snRNP assembly. FEBS Lett. *579*, 2348–2354.

Carissimi, C., Saieva, L., Baccon, J., Chiarella, P., Maiolica, A., Sawyer, A., Rappsilber, J., and Pellizzoni, L. (2006a). Gemin8 is a novel component of the survival motor neuron complex and functions in small nuclear ribonucleoprotein assembly. J. Biol. Chem. *281*, 8126–8134.

Carissimi, C., Saieva, L., Gabanella, F., and Pellizzoni, L. (2006b). Gemin8 is required for the architecture and function of the survival motor neuron complex. J. Biol. Chem. *281*, 37009–37016.

Carrel, T. L., McWhorter, M. L., Workman, E., Zhang, H., Wolstencroft, E. C., Lorson, C., Bassell, G. J., Burghes, A. H., and Beattie, C. E. (2006). Survival motor neuron function in motor axons is independent of functions required for small nuclear ribonucleoprotein biogenesis. J. Neurosci. *26*, 11014–11022.

Chan, Y. B., Miguel-Aliaga, I., Franks, C., Thomas, N., Trulzsch, B., Sattelle, D. B., Davies, K. E., and van den Heuvel, M. (2003). Neuromuscular defects in a *Drosophila* survival motor neuron gene mutant. Hum. Mol. Genet. *12*, 1367–1376.

Charroux, B., Pellizzoni, L., Perkinson, R. A., Shevchenko, A., Mann, M., and Dreyfuss, G. (1999). Gemin3, a novel DEAD box protein that interacts with SMN, the spinal muscular atrophy gene product, and is a component of gems. J. Cell Biol. *147*, 1181–1194.

Charroux, B., Pellizzoni, L., Perkinson, R. A., Yong, J., Shevchenko, A., Mann, M., and Dreyfuss, G. (2000). Gemin4, A novel component of the SMN complex that is found in both gems and nucleoli. J. Cell Biol. *148*, 1177–1186.

Coovert, D. D. *et al.* (1997). The survival motor neuron protein in spinal muscular atrophy. Hum. Mol. Genet. *6*, 1205–1214.

Datta, P. K., Chytil, A., Gorska, A. E., and Moses, H. L. (1998). Identification of STRAP, a novel WD domain protein in transforming growth factor-beta signaling. J. Biol. Chem. *273*, 34671–34674.

Datta, P. K., and Moses, H. L. (2000). STRAP and Smad7 synergize in the inhibition of transforming growth factor beta signaling. Mol. Cell. Biol. *20*, 3157–3167.

Feng, W., Gubitz, A. K., Wan, L., Battle, D. J., Dostie, J., Golembe, T. J., and Dreyfuss, G. (2005). Gemins modulate the expression and activity of the SMN complex. Hum. Mol. Genet. *14*, 1605–1611.

Fischer, U., Liu, Q., and Dreyfuss, G. (1997). The SMN-SIP1 complex has an essential role in spliceosomal snRNP biogenesis. Cell *90*, 1023–1029.

Gabanella, F., Butchbach, M. E., Saieva, L., Carissimi, C., Burghes, A. H., and Pellizzoni, L. (2007). Ribonucleoprotein assembly defects correlate with spinal muscular atrophy severity and preferentially affect a subset of spliceosomal snRNPs. PLoS ONE *2*, e921.

Gates, J., Lam, G., Ortiz, J. A., Losson, R., and Thummel, C. S. (2004). *rigor mortis* encodes a novel nuclear receptor interacting protein required for ecdysone signaling during *Drosophila* larval development. Development *131*, 25–36.

Giot, L. *et al.* (2003). A protein interaction map of *Drosophila melanogaster.* Science *302*, 1727–1736.

Girard, C., Neel, H., Bertrand, E., and Bordonne, R. (2006). Depletion of SMN by RNA interference in HeLa cells induces defects in Cajal body formation. Nucleic Acids Res. *34*, 2925–2932.

Grimmler, M., Otter, S., Müller, F., Peter, C., Chari, A., and Fischer, U. (2005). Unrip, a factor implicated in cap-independent translation, associates with the cytosolic SMN-complex and influences its intracellular localization. Hum. Mol. Genet. *14*, 3099–3111.

Gubitz, A. K., Mourelatos, Z., Abel, L., Rappsilber, J., Mann, M., and Dreyfuss, G. (2002). Gemin5, a novel WD repeat protein component of the SMN complex that binds Sm proteins. J. Biol. Chem. *277*, 5631–5636.

Häcker, U., Nystedt, S., Barmchi, M. P., Horn, C., and Wimmer, E. A. (2003). piggyBac-based insertional mutagenesis in the presence of stably integrated P elements in *Drosophila.* Proc Natl. Acad Sci. USA *100*, 7720–7725.

Hannus, S., Buhler, D., Romano, M., Seraphin, B., and Fischer, U. (2000). The *Schizosaccharomyces pombe* protein Yab8p and a novel factor, Yip1p, share structural and functional similarity with the spinal muscular atrophy-associated proteins SMN and SIP1. Hum. Mol. Genet. *9*, 663–674.

Hsieh-Li, H. M., Chang, J. G., Jong, Y. J., Wu, M. H., Wang, N. M., Tsai, C. H., and Li, H. (2000). A mouse model for spinal muscular atrophy. Nat. Genet. *24*, 66–70.

Jablonka, S., Holtmann, B., Meister, G., Bandilla, M., Rossoll, W., Fischer, U., and Sendtner, M. (2002). Gene targeting of Gemin2 in mice reveals a correlation between defects in the biogenesis of U snRNPs and motoneuron cell death. Proc. Natl. Acad Sci. USA *99*, 10126–10131.

Kim, E. K., Noh, K. T., Yoon, J. H., Cho, J. H., Yoon, K. W., Dreyfuss, G., and Choi, E. J. (2007). Positive regulation of ASK1-mediated c-Jun NH(2)-terminal kinase signaling pathway by the WD-repeat protein Gemin5. Cell Death Differ. *14*, 1518–1528.

Kroiss, M., Schultz, J., Wiesner, J., Chari, A., Sickmann, A., and Fischer, U. (2008). Evolution of an RNP assembly system: a minimal SMN complex facilitates formation of UsnRNPs in *Drosophila melanogaster.* Proc. Natl. Acad Sci. USA *105*, 10045–10050.

Lefebvre, S. *et al.* (1995). Identification and characterization of a spinal muscular atrophy-determining gene. Cell *80*, 155–165.

Lefebvre, S., Burlet, P., Liu, Q., Bertrandy, S., Clermont, O., Munnich, A., Dreyfuss, G., and Melki, J. (1997). Correlation between severity and SMN protein level in spinal muscular atrophy. Nat. Genet. *16*, 265–269.

Lemm, I., Girard, C., Kuhn, A. N., Watkins, N. J., Schneider, M., Bordonne, R., and Luhrmann, R. (2006). Ongoing U snRNP biogenesis is required for the integrity of Cajal bodies. Mol. Biol. Cell *17*, 3221–3231.

Liu, J. L., Buszczak, M., and Gall, J. G. (2006). Nuclear bodies in the *Drosophila* germinal vesicle. Chromosome Res. *14*, 465–475.

Liu, Q., and Dreyfuss, G. (1996). A novel nuclear structure containing the survival of motor neurons protein. EMBO J. *15*, 3555–3565.

Liu, Q., Fischer, U., Wang, F., and Dreyfuss, G. (1997). The spinal muscular atrophy disease gene product, SMN, and its associated protein SIP1 are in a complex with spliceosomal snRNP proteins. Cell *90*, 1013–1021.

Lorson, C. L., Hahnen, E., Androphy, E. J., and Wirth, B. (1999). A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. Proc. Natl. Acad Sci. USA *96*, 6307–6311.

Lorson, C. L., Strasswimmer, J., Yao, J. M., Baleja, J. D., Hahnen, E., Wirth, B., Le, T., Burghes, A. H., and Androphy, E. J. (1998). SMN oligomerization defect correlates with spinal muscular atrophy severity. Nat. Genet. *19*, 63–66.

Matera, A. G., and Frey, M. R. (1998). Coiled bodies and gems: Janus or gemini? Am. J. Hum. Genet. *63*, 317–321.

Matera, A. G., Terns, R. M., and Terns, M. P. (2007). Non-coding RNAs: lessons from the small nuclear and small nucleolar RNAs. Nat. Rev. Mol. Cell Biol. *8*, 209–220.

McWhorter, M. L., Boon, K. L., Horan, E. S., Burghes, A. H., and Beattie, C. E. (2008). The SMN binding protein gemin2 is not involved in motor axon outgrowth. Dev. Neurobiol. *68*, 182–194.

McWhorter, M. L., Monani, U. R., Burghes, A. H., and Beattie, C. E. (2003). Knockdown of the survival motor neuron (Smn) protein in zebrafish causes defects in motor axon outgrowth and pathfinding. J. Cell Biol. *162*, 919–931.

Meister, G., Buhler, D., Laggerbauer, B., Zobawa, M., Lottspeich, F., and Fischer, U. (2000). Characterization of a nuclear 20S complex containing the survival of motor neurons (SMN) protein and a specific subset of spliceosomal Sm proteins. Hum. Mol. Genet. *9*, 1977–1986.

Monani, U. R., Lorson, C. L., Parsons, D. W., Prior, T. W., Androphy, E. J., Burghes, A. H., and McPherson, J. D. (1999). A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. Hum. Mol. Genet. *8*, 1177–1183.

Monani, U. R. *et al.* (2000). The human centromeric survival motor neuron gene (SMN2) rescues embryonic lethality in Smn(-/-) mice and results in a mouse with spinal muscular atrophy. Hum. Mol. Genet. *9*, 333–339.

Mouillet, J. F., Yan, X., Ou, Q., Jin, L., Muglia, L. J., Crawford, P. A., and Sadovsky, Y. (2008). DEAD-box protein-103 (DP103, Ddx20) is essential for early embryonic development and modulates ovarian morphology and function. Endocrinology *149*, 2168–2175.

Murphy, M. W., Olson, B. L., and Siliciano, P. G. (2004). The yeast splicing factor Prp40p contains functional leucine-rich nuclear export signals that are essential for splicing. Genetics *166*, 53–65.

Ogino, S., and Wilson, R. B. (2004). Spinal muscular atrophy: molecular genetics and diagnostics. Expert. Rev. Mol. Diagn. *4*, 15–29.

Otter, S., Grimmler, M., Neuenkirchen, N., Chari, A., Sickmann, A., and Fischer, U. (2007). A comprehensive interaction map of the human survival of motor neuron (SMN) complex. J. Biol. Chem. *282*, 5825–5833.

Owen, N., Doe, C. L., Mellor, J., and Davies, K. E. (2000). Characterization of the *Schizosaccharomyces pombe* orthologue of the human survival motor neuron (SMN) protein. Hum. Mol. Genet. *9*, 675–684.

Paushkin, S., Charroux, B., Abel, L., Perkinson, R. A., Pellizzoni, L., and Dreyfuss, G. (2000). The survival motor neuron protein of *Schizosaccharomyces pombe.* Conservation of survival motor neuron interaction domains in divergent organisms. J. Biol. Chem. *275*, 23841–23846.

Pearn, J. (1980). Classification of spinal muscular atrophies. Lancet *1*, 919–922.

Pearn, J. H., Gardner-Medwin, D., and Wilson, J. (1978). A clinical study of chronic childhood spinal muscular atrophy. A review of 141 cases. J. Neurol. Sci. *38*, 23–37.

Pellizzoni, L., Baccon, J., Rappsilber, J., Mann, M., and Dreyfuss, G. (2002a). Purification of native survival of motor neurons complexes and identification of Gemin6 as a novel component. J. Biol. Chem. *277*, 7540–7545.

Pellizzoni, L., Yong, J., and Dreyfuss, G. (2002b). Essential role for the SMN complex in the specificity of snRNP assembly. Science *298*, 1775–1779.

Rajendra, T. K., Gonsalvez, G. B., Walker, M. P., Shpargel, K. B., Salz, H. K., and Matera, A. G. (2007). A *Drosophila melanogaster* model of spinal muscular atrophy reveals a function for SMN in striated muscle. J. Cell Biol. *176*, 831–841.

Schrank, B., Gotz, R., Gunnersen, J. M., Ure, J. M., Toyka, K. V., Smith, A. G., and Sendtner, M. (1997). Inactivation of the survival motor neuron gene, a candidate gene for human spinal muscular atrophy, leads to massive cell death in early mouse embryos. Proc. Natl. Acad. Sci. USA *94*, 9920–9925.

Shafey, D., Cote, P. D., and Kothary, R. (2005). Hypomorphic Smn knockdown C2C12 myoblasts reveal intrinsic defects in myoblast fusion and myotube morphology. Exp. Cell Res. *311*, 49–61.

Shpargel, K. B., and Matera, A. G. (2005). Gemin proteins are required for efficient assembly of Sm-class ribonucleoproteins. Proc. Natl. Acad Sci. USA *102*, 17372–17377.

Shpargel, K. B., Ospina, J. K., Tucker, K. E., Matera, A. G., and Hebert, M. D. (2003). Control of Cajal body number is mediated by the coilin C-terminus. J. Cell Sci. *116*, 303–312.

Spradling, A. C., Stern, D., Beaton, A., Rhem, E. J., Laverty, T., Mozden, N., Misra, S., and Rubin, G. M. (1999). The Berkeley *Drosophila* Genome Project gene disruption project: single P-element insertions mutating 25% of vital *Drosophila* genes. Genetics *153*, 135–177.

Thibault, S. T. *et al.* (2004). A complementary transposon tool kit for *Drosophila melanogaster* using P and piggyBac. Nat. Genet. *36*, 283–287.

Wan, L., Battle, D. J., Yong, J., Gubitz, A. K., Kolb, S. J., Wang, J., and Dreyfuss, G. (2005). The survival of motor neurons protein determines the capacity for

snRNP assembly: biochemical deficiency in spinal muscular atrophy. Mol. Cell. Biol. *25*, 5543–5551.

Winkler, C., Eggert, C., Gradl, D., Meister, G., Giegerich, M., Wedlich, D., Laggerbauer, B., and Fischer, U. (2005). Reduced U snRNP assembly causes motor axon degeneration in an animal model for spinal muscular atrophy. Genes Dev. *19*, 2320–2330.

Yong, J., Pellizzoni, L., and Dreyfuss, G. (2002). Sequence-specific interaction of U1 snRNA with the SMN complex. EMBO J. *21*, 1188–1196.

Zhang, H., Xing, L., Rossoll, W., Wichterle, H., Singer, R. H., and Bassell, G. J. (2006). Multiprotein complexes of the survival of motor neuron protein SMN with Gemins traffic to neuronal processes and growth cones of motor neurons. J. Neurosci. *26*, 8622–8632.