Report

FUS-SMN Protein Interactions
Link the Motor Neuron Diseases ALS and SMA

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SUMMARY

Mutations in the RNA binding protein FUS cause amyotrophic lateral sclerosis (ALS), a fatal adult motor neuron disease. Decreased expression of SMN causes the fatal childhood motor neuron disorder spinal muscular atrophy (SMA). The SMN complex localizes in both the cytoplasm and nuclear Gems, and loss of Gems is a cellular hallmark of fibroblasts in patients with SMA. Here, we report that FUS associates with the SMN complex, mediated by U1 snRNP and by direct interactions between FUS and SMN. Functionally, we show that FUS is required for Gem formation in HeLa cells, and expression of FUS containing a severe ALS-causing mutation (R495X) also results in Gem loss. Strikingly, a reduction in Gems is observed in ALS patient fibroblasts expressing either mutant FUS or TDP-43, another ALS-causing protein that interacts with FUS. The physical and functional interactions among SMN, FUS, TDP-43, and Gems indicate that ALS and SMA share a biochemical pathway, providing strong support for the view that these motor neuron diseases are related.

INTRODUCTION

Mutations in at least ten genes cause ALS, but the disease mechanisms are not yet understood. Approximately 10% of ALS cases are familial and the rest are sporadic (Boillée et al., 2006; Valdmanis and Rouleau, 2008). Mutations in the RNA binding protein FUS are the cause of a subset of familial and sporadic ALS cases (Kwiatkowski et al., 2009; Vance et al., 2009). FUS has features in common with the RNA binding protein

TDP-43, and mutations in TDP-43 also cause ALS (Gitcho et al., 2008; Kabashi et al., 2008; Sreedharan et al., 2008). FUS and TDP-43 are nuclear proteins at steady state and shuttle between the nucleus and cytoplasm. Both proteins function in transcription, splicing, mRNP transport, and other processes in the nucleus and cytoplasm (Liu-Yesucevitz et al., 2011). These and other observations suggest a relationship between RNA metabolism and motor neuron disease. For example, the childhood motor neuron disease spinal muscular atrophy (SMA) is caused by deficiency in the SMN protein (Lefebvre et al., 1995). SMN is a component of the SMN complex, which functions in snRNP biogenesis (Battle et al., 2006), and has been implicated in other RNA-related roles, such as mRNP transport (Fallini et al., 2012b). RNA metabolism defects may also explain the pathogenicity of C9ORF72, which causes ALS via a repeat expansion in the first intron (DeJesus-Hernandez et al., 2011; Renton et al., 2011). This expansion forms nuclear aggregates that can titrate crucial RNA binding proteins (DeJesus-Hernandez et al., 2011).

In most cell types, including motor neurons, the SMN complex localizes in the cytoplasm and in nuclear Gems (Battle et al., 2006; Gubitz et al., 2004; Liu and Dreyfuss, 1996). Loss of Gems is a cellular hallmark of SMA. Of interest, studies have shown that Gems are also lost from motor neurons in a TDP-43 knockout mouse (Shan et al., 2010), and mutations in SOD1, which account for a large fraction of familial ALS cases, also lead to Gem deficiency in mouse models (Gertz et al., 2012; Kariya et al., 2012). These mouse model studies suggest that ALS and SMA may be related diseases, but the biochemical pathways involved are not known. These studies have generated considerable interest in understanding how RNA-related proteins cause motor neuron disease. Here, we report that FUS interacts both physically and functionally with SMN. We show that SMN-containing nuclear Gems are lost from FUS knockdown HeLa cells. Strikingly, Gem loss also occurs in HeLa cells transfected with a FUS construct bearing a severe ALS-causing
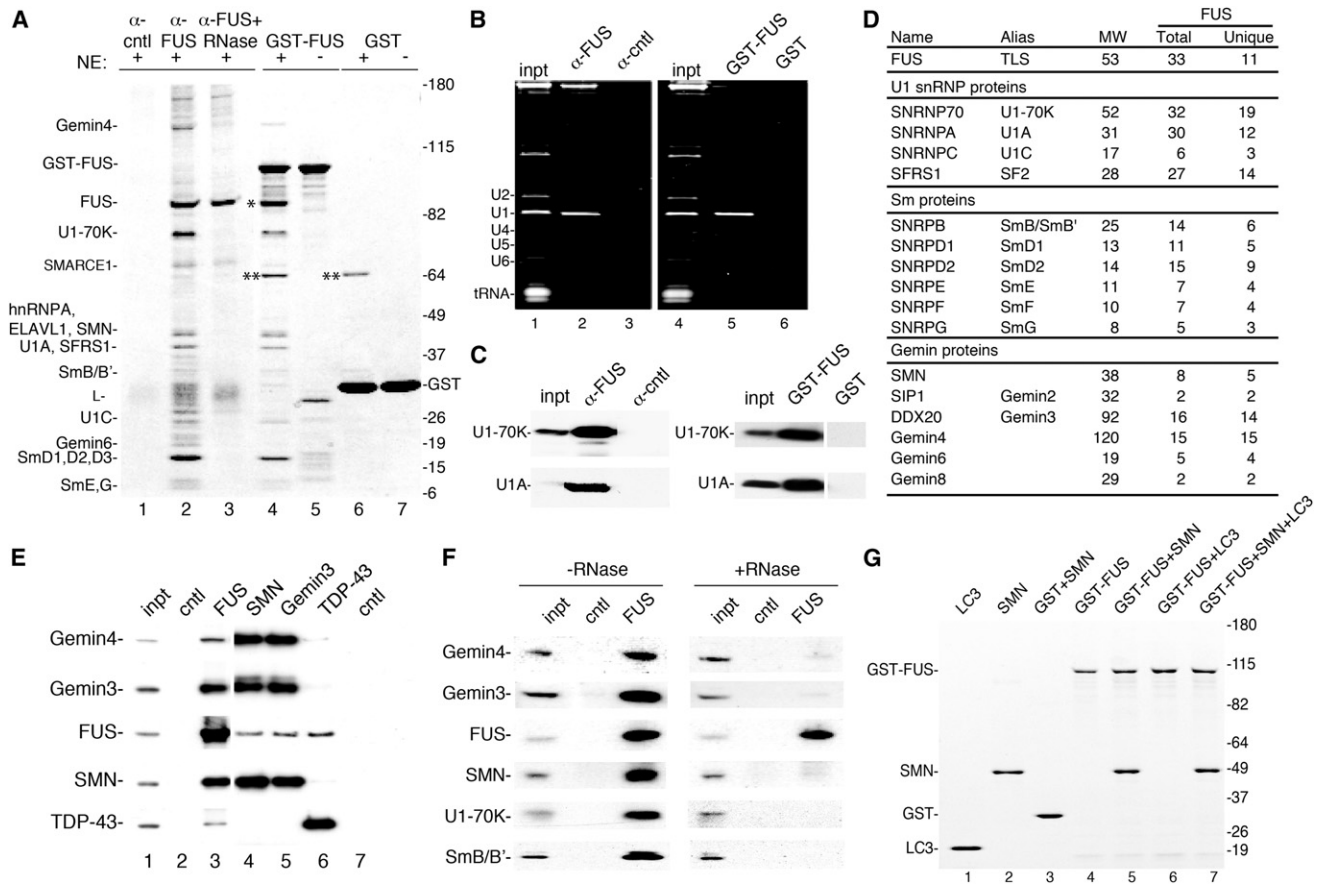
Figure 1. The SMN Complex and U1 snRNP Associate with FUS

(A) IP was carried out with HeLa nuclear extracts using FUS or negative control antibodies (lanes 1–3). In lane 3, nuclear extract was incubated with RNase A prior to IP. GST-FUS (lanes 4 and 5) or GST (lanes 6 and 7) was used for pulldowns from nuclear extract (lanes 4 and 6) or buffer alone (lanes 5 and 7). Proteins were run on a 4%–12% sodium dodecyl sulfate (SDS) gradient gel and detected by Coomassie staining. Indicated proteins were identified by MS. L, antibody light chain; *, FUS; **, a nonspecific band.

(B) Total RNA from IP and GST pulldown samples used in (A); 30% input (inpt) was loaded. RNAs were detected with ethidium bromide.

(C) Protein was isolated from samples in (B) followed by western blotting with the indicated antibodies; 15% input (inpt) was loaded.

(D) Table showing MS data for the indicated proteins from FUS IP. The number of total peptides (Total) and total unique peptides (Unique) identified by MS is shown.

(E) IP/western blotting with the indicated antibodies. The negative control for the FUS and TDP-43 antibodies was a rabbit polyclonal antibody (lane 2, SAP130), and the negative control for the monoclonal antibodies (SMN and Gemin3) was a monoclonal against HA (lane 7).

(F) Same as in (E) except that the nuclear extract was treated or not treated with RNase prior to the IP.

(G) FUS interacts directly with SMN. The indicated purified proteins (2 μg) were mixed in the presence of RNase A followed by GST pulldowns. Proteins were separated on a 4%–12% SDS-gradient gel and detected by Coomassie staining. Molecular weight markers in kDa are indicated.

See also Figure S1.

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mutation and in ALS patient fibroblasts bearing FUS or TDP-43 mutations. Together, these observations suggest that a common biochemical pathway involving SMN, FUS, TDP-43, and Gems links the motor neuron diseases SMA and ALS.

RESULTS

FUS Associates with the SMN Complex and U1 snRNP

To investigate the mechanisms by which mutations in RNA binding proteins cause ALS, we focused on FUS. Antibodies raised against GST-FUS detect one main band on a western blot, and immunoprecipitate FUS from HeLa nuclear extracts

(Figure S1). To examine the FUS interactome, we used the FUS antibody for immunoprecipitation (IP), and GST-FUS for pulldowns from nuclear extract, and analyzed the proteins on a Coomassie gel (Figure 1A). Mass spectrometry (MS) of bands excised from the gel showed that U1 snRNP components were among the most abundant proteins associated with FUS (Figure 1A, lanes 2 and 4). Moreover, U1 small nuclear RNA (snRNA) was abundant in the FUS immunoprecipitate and pulldown (Figure 1B), and western analysis confirmed the presence of U1 snRNP proteins in the FUS immunoprecipitate and pulldown (Figure 1C). The interaction between FUS and U1 snRNP is specific, because U1 snRNP components were not

immunoprecipitated by the FUS antibody in FUS knockdown nuclear extracts (Figure S1).

The FUS IP and pulldown revealed that FUS also associates with components of the SMN complex, including SMN and Gemins 4 and 6 (Figure 1A). This result is significant because an association between FUS and SMN raises the possibility that ALS and SMA are caused by defects in a shared biochemical pathway. To pursue this possibility, we analyzed the total proteins present in the FUS immunoprecipitate by MS. U1 snRNP components and Sm proteins were the most abundant proteins in the data set (Figure 1D; Table S1). In addition, all of the nuclear components of the SMN complex, except for the smallest (Gemin 7, 14 kDa), were present (Figure 1D; Table S1). FUS reciprocally coimmunoprecipitated with SMN and the Gemins, confirming the specificity of the association between the SMN complex and FUS (Figure 1E). Although previous work showed that FUS associates with TDP-43 (Kim et al., 2010; Ling et al., 2010), we found that the level of TDP-43 was not significantly above background in our FUS immunoprecipitate (Table S1). However, IP/western analysis revealed that TDP-43 and FUS do coimmunoprecipitate in nuclear extract (Figure 1E). In contrast, TDP-43 does not coimmunoprecipitate with SMN complex components (Figure 1E). Thus, although FUS and TDP-43 interact, the two proteins have distinct molecular associations. Indeed, TDP-43 associates with components of the miRNA processing machinery (Ling et al., 2010; Sephton et al., 2011).

Most of the proteins in the FUS immunoprecipitate associate with FUS in an RNA-dependent manner (Figure 1A, lane 3), including the SMN complex and U1 snRNP (Figure 1F). Consistent with this observation, analysis of FUS deletion mutants revealed that the RNA-recognition motif is required for the association of FUS with the SMN complex, U1 snRNP proteins, and U1 snRNA (Figure S1). The SMN complex binds directly to U1 snRNA (Yong et al., 2002). Thus, U1 snRNA may mediate the RNA-dependent binding of the SMN complex to FUS. The GST-pulldown data also revealed that FUS associates with itself, because endogenous FUS in the nuclear extract binds to GST-FUS (indicated by the asterisk in Figure 1A, lane 4). An amino-terminal region on FUS (1-111) is necessary and sufficient for the FUS-FUS interaction (Figure S1).

To further characterize the association between FUS and SMN, we carried out GST-FUS pulldowns using purified proteins in the presence of RNase. This analysis revealed that GST-FUS interacted efficiently and directly with SMN, but not with negative control proteins (GST and LC3; Figure 1G, lanes 1–7). We conclude that the associations among FUS, the SMN complex, and U1 snRNP are mediated by U1 snRNA and also by a direct interaction between FUS and SMN.

FUS Is Required for Gem Formation in HeLa Cells

We next asked whether the physical association between FUS and SMN is functionally significant. Accordingly, we targeted FUS with small hairpin RNA (shRNA) in HeLa cells and examined the distribution of SMN and FUS by immunofluorescence (IF). IF showed that FUS was efficiently knocked down, and, as expected, FUS localized in the nucleus in control knockdown cells (Figure 2A). Remarkably, the number of SMN-stained nuclear bodies was dramatically reduced in the FUS knockdown cells

(Figure 2A), and diffuse nuclear staining of SMN was observed in a subset of the cells (Figure 2A, arrowheads). Loss of Gems was also observed when antibodies to Gemins 3 and 4 were used (Figure 2B), indicating that Gems are the nuclear bodies lost in FUS knockdown cells. Consistent with this conclusion, Gems were detected when antibodies to Gemin3 or SMN were used for double IF (Figure S2). Of importance, however, the levels of SMN and the other Gemin proteins were not affected by the FUS knockdown in total cell lysates (Figure 2C). We conclude that FUS knockdown results in loss of Gems without affecting the overall levels of SMN/Gemins.

To further examine the role of FUS in Gem formation, we asked whether the loss of Gems in FUS knockdown cells could be rescued by expression of exogenous SMN. FUS knockdown or control cells were transfected with SMN-GFP and then analyzed for SMN-GFP expression and by IF for Gemin3. Their colocalization was used to identify Gems. As shown in Figure 2D, Gems were lacking in FUS knockdown cells that did not express SMN-GFP or that express GFP alone. In contrast, Gems were efficiently restored in FUS knockdown cells expressing SMN-GFP. In control knockdown cells, the Gem levels were similar in nontransfected cells and in cells transfected with SMN-GFP or GFP alone (Figure 2D). We conclude that increased levels of SMN can bypass the requirement for FUS in Gem formation, which suggests that SMN acts downstream of FUS in a shared pathway required for Gem formation.

Expression of an ALS-Causing FUS Mutation Causes Loss of Gems

Many of the mutations in FUS that cause ALS are found in the nuclear localization sequence (NLS) and result in varying degrees of mislocalization of FUS to the cytoplasm (Dormann et al., 2010). Because these ALS mutations are dominant, we next asked whether transfection of a construct bearing the FUS R495X ALS mutation affects Gem levels in HeLa cells. This mutation, in which the NLS is lacking, causes a severe form of ALS (Bosco et al., 2010; Waibel et al., 2010). When wild-type (WT) FUS was expressed in HeLa cells, FUS properly localized to the nucleus (Figure 3A). In contrast, high levels of FUS R495X were detected in the cytoplasm (Figure 3A). Strikingly, Gem levels were dramatically reduced in the cells containing FUS R495X compared with cells transfected with WT FUS (Figures 3A and 3B). We conclude that normal Gem levels require nuclear localization of FUS. The NLS of FUS is not required for SMN binding (Figure S3). Thus, it is possible that FUS R495X sequesters SMN in the cytoplasm to an extent that results in loss of Gems. Alternatively, FUS R495X may inhibit the normal function of FUS in Gem formation by acting as a dominant negative via the FUS-FUS interaction.

Gems Are Deficient in ALS Patient Fibroblasts Bearing FUS or TDP-43 Mutations

Previous work showed that Gems are lost from SMA patient fibroblasts (Coovert et al., 1997). In addition, Gems were shown to be deficient in both TDP-43 and SOD1 ALS mouse models (Gertz et al., 2012; Kariya et al., 2012; Shan et al., 2010). We therefore asked whether Gem levels are affected in ALS patient fibroblasts. Costaining with SMN and Gemin antibodies was
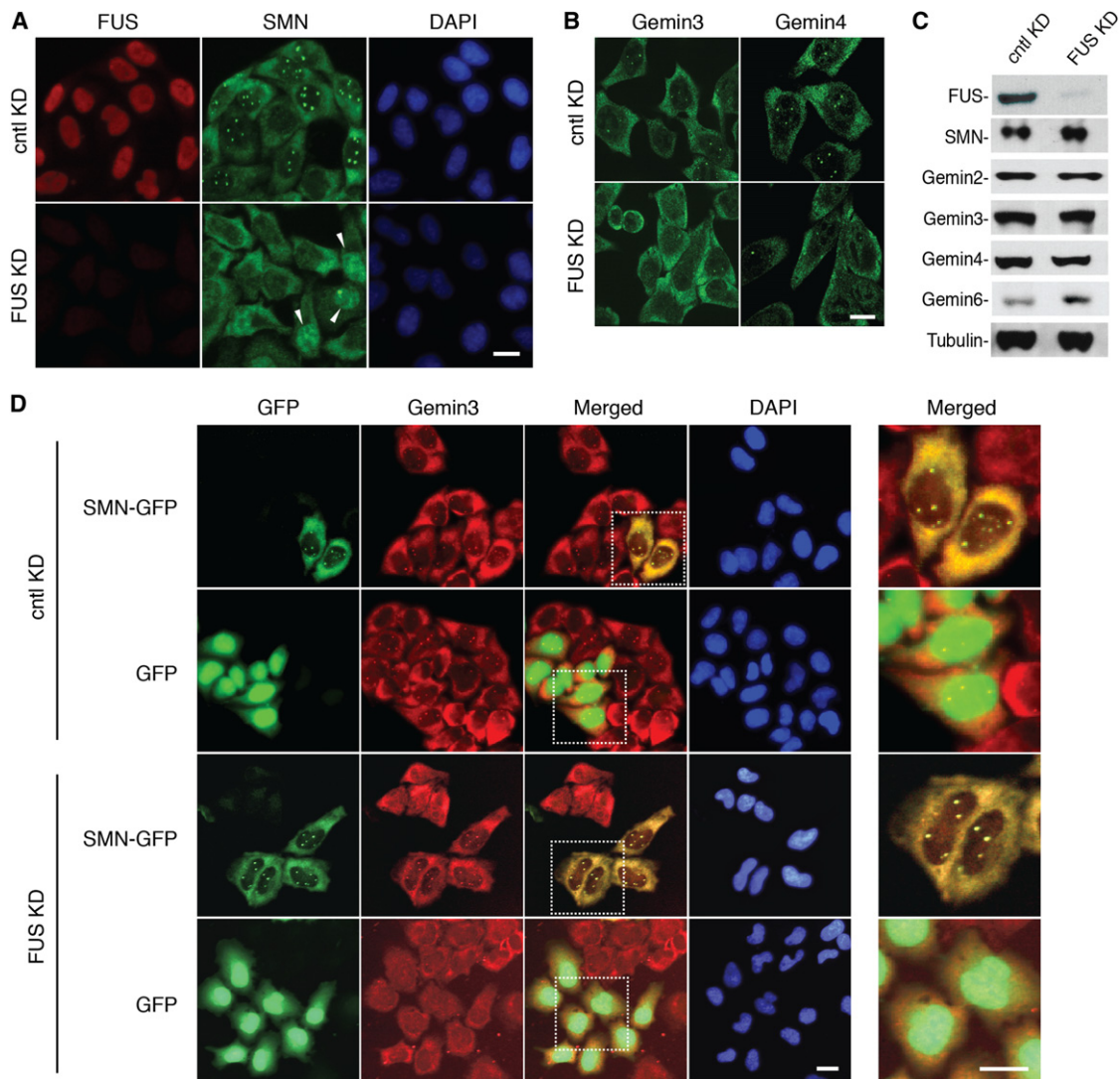
Figure 2. FUS Is Required for Gem Formation in HeLa Cells

(A) FUS or control knockdown (KD) HeLa cells were used to detect Gems (green in nucleus) and FUS (red) by IF with SMN and FUS antibodies, respectively. FUS was knocked down using an shRNA against FUS. Scrambled shRNA was used as a negative control. DAPI shows the nucleus. Scale bar, 20 μm.

(B) IF staining of FUS or control KD HeLa cells was carried out with the indicated antibodies. Scale bar, 20 μm.

(C) Western analysis of FUS and control KDs in HeLa cells using the indicated antibodies. Tubulin was used as the loading control.

(D) SMN-GFP or GFP was expressed in control or FUS KD HeLa cells. Gems were detected with SMN-GFP and costaining with the Gemin3 antibody. DAPI shows the nucleus. Scale bar, 20 μm. Right panels (merged) show high magnification of the dashed squares indicated in the left panels.

See also Figure S2.

used to verify the detection of Gems in the fibroblasts (Figure S4). We first examined two ALS patient fibroblast lines, one bearing a FUS R521C mutation and another bearing a TDP-43 M337V mutation. Strikingly, a Gem deficiency was observed in both FUS and TDP-43 patient fibroblasts compared with age- and sex-matched fibroblasts from unaffected individuals (Figures 4A and 4B). To extend these results, we used an automated system to collect images from three biological replicates of each fibroblast line and then counted the Gems in at least 800 cells for each cell line tested. We also used this system to examine fibroblasts from additional patients carrying a FUS

(R514G) or TDP-43 (G298S) mutation, as well as five unaffected individuals. These data revealed that the average Gem number was 2- to 3-fold lower in the FUS fibroblasts and 1.8-fold lower in the TDP-43 fibroblasts relative to controls (Figure 4C). We conclude that a single dominant amino acid substitution in FUS or TDP-43 results in Gem deficiency in these ALS patient fibroblasts. Both of the FUS patient mutations that we examined were in the NLS, and we observed significant mislocalization of FUS to the cytoplasm in these fibroblast lines relative to the controls (Figure S4). Thus, the decreased level of Gems may be explained by the decrease in levels of nuclear FUS.
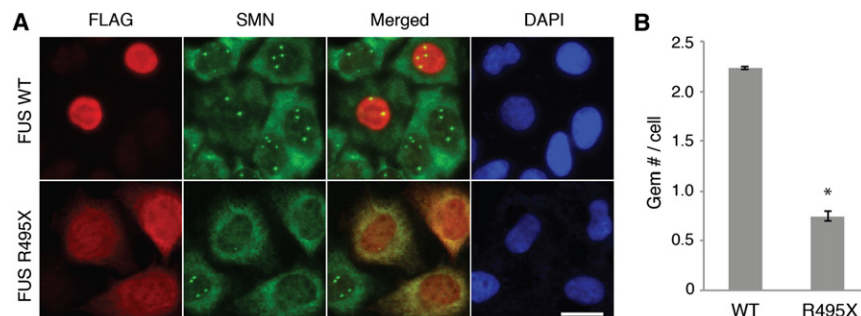
Figure 3. Gems Are Lost in HeLa Cells Transfected with the ALS-Causing R495X FUS Mutation

(A) Representative images showing the expression of FLAG-tagged FUS or FUS R495X in HeLa cells (red). Gems were detected using SMN antibodies (green in nucleus). Scale bar, 20 μm.

(B) Quantitation of Gem levels in HeLa cells expressing the indicated proteins. The mean and standard deviation of Gem numbers per cell were calculated from three independent experiments. At least 100 cells were observed in each experiment. The p values were calculated by comparison with three controls ($*p < 0.01$, Student's t test). See also Figure S3.

Previous work showed that Gems are decreased in a TDP-43 knockout mouse model (Shan et al., 2010). Consistent with this conclusion and our patient fibroblast data, Gems were significantly decreased when TDP-43 was knocked down in HeLa cells (Figure S4). We found that TDP-43 was properly localized in the nucleus in both of the ALS patient fibroblast lines containing TDP-43 mutations (Figure S4). We conclude that these mutations in TDP-43 or knockdown of TDP-43 affect the normal levels of Gems.

DISCUSSION

Here we report several independent lines of evidence indicating that ALS and SMA are motor neuron diseases linked by a common molecular pathway. Specifically, we show that FUS, which is mutated in ALS, interacts with SMN, the protein that is deficient in SMA. SMN is a component of the SMN complex (Battle et al., 2006), and we show that FUS associates with this complex. The SMN complex functions in snRNP biogenesis, and we found that U1 snRNP is abundantly associated with FUS. In the nucleus, the SMN complex is present in Gems, and we found that FUS is required for Gem formation. Furthermore, we found that Gems are lost from HeLa cells transfected with an ALS-causing FUS mutation. Previous work showed that Gems are lacking in SMA patient fibroblasts (Coovert et al., 1997). Strikingly, we found that single dominant mutations in FUS result in decreased Gem levels in ALS patient fibroblasts. FUS is known to interact directly with TDP-43 (Ling et al., 2010) and FUS acts downstream of TDP-43 in shared genetic pathways that are required for normal survival and motor function in *Drosophila* and zebrafish (Kabashi et al., 2011; Wang et al., 2011). As observed for SMN and FUS, TDP-43 is required for normal Gem levels (Shan et al., 2010), and we found that Gems are significantly decreased in ALS patient fibroblasts bearing TDP-43 point mutations. Taken together, these observations suggest a model in which TDP-43 functions upstream of FUS, which in turn is required for assembly of SMN into Gems. Thus, SMA and ALS share a common pathway involving TDP-43, FUS, SMN, and Gems. Of interest, Gems were recently shown to be deficient in SOD1 mouse models of ALS (Gertz et al., 2012; Kariya et al., 2012). Thus, disruption of the Gem pathway may be a common feature of SMA and multiple types of familial ALS. Our data showing that FUS associates with itself may explain why FUS mutations are dominant. By interacting with WT FUS, mutant FUS may inhibit the normal function(s) of FUS or sequester normal FUS, forming aggregates that are toxic to motor neurons. Similar self-interactions have been observed for TDP-43, which may also explain the dominance of these mutations (Da Cruz and Cleveland, 2011).

Our studies also led to important insights into Gem formation. Specifically, we found that Gems are lost in FUS knockdown cells even though the cells contain normal levels of SMN and the Gemin proteins. These data indicate that the requirement for FUS in Gem formation is not due to an effect on SMN/Gemin levels. However, our data revealed that the FUS requirement for Gem formation could be bypassed by overexpression of SMN, suggesting that the role of FUS in Gem formation may be to associate with SMN and increase its effective concentration and/or its association with other Gem components. Our observation that Gems are deficient in HeLa cells transfected with FUS R495X, which lacks the NLS, indicates that the nuclear localization of FUS is required for normal Gem levels. Many of the known patient mutations in FUS that cause ALS are located in the NLS (Dormann et al., 2010), including both of the mutations we analyzed in FUS patient fibroblast lines. Our data show that these lines display significant mislocalization of FUS to the cytoplasm and Gem deficiency. Together, these data raise the possibility that other ALS patients with mutations in the FUS NLS may have the Gem deficiency phenotype. When we analyzed Gem levels in a few examples of ALS patient fibroblasts with unknown mutations, we detected no obvious Gem phenotype (T.Y. and R.R., unpublished). Therefore, a large number of ALS patient fibroblast lines must be examined before the generality of the phenotype can be determined. TDP-43, FUS, and SMN also have other functions. For example, both TDP-43 and FUS are nucleocytoplasmic shuttling proteins that are present in cytoplasmic axonal mRNP transport granules together with the SMN complex (Fallini et al., 2012a, 2012b; Liu-Yesucevitz et al., 2011). Thus, mutant FUS, TDP-43, and SMN may cause motor neuron disease by disrupting axonal transport of mRNAs encoding proteins that are essential for motor neuron function. At present, it remains to be determined which function(s) of FUS, TDP-43, and SMN is mechanistically involved in SMA/ALS and which may be a signature of these diseases.

We observed an ∼2- to 3-fold decrease in Gem levels in ALS patient fibroblasts, whereas Gem levels in SMA patient fibroblasts are reduced by ∼20-fold in severe type I disease and by ∼3- to 4-fold for the less-severe SMA types II and III (Coovert
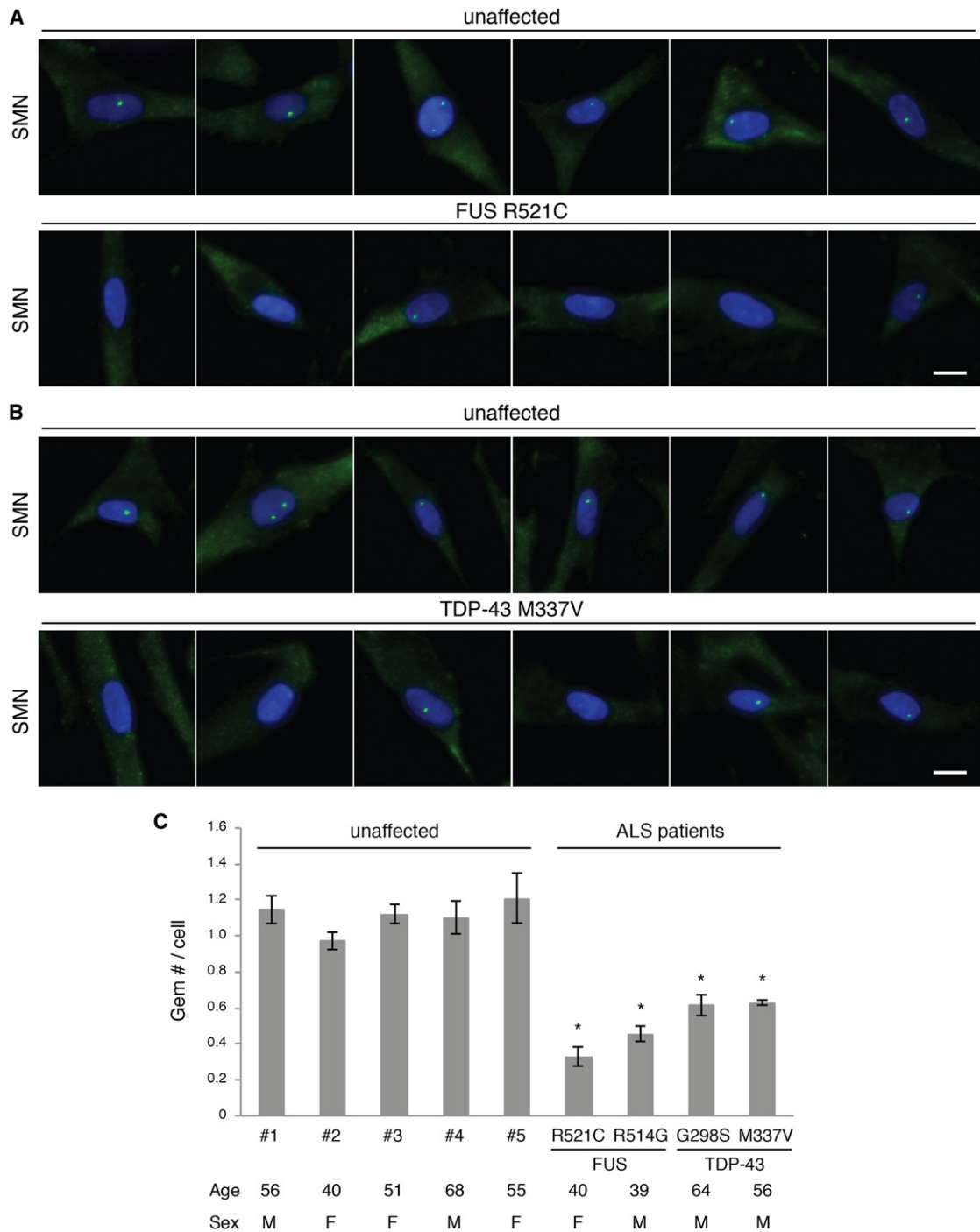
Figure 4. Gems Are Deficient in FUS and TDP-43 ALS Patient Fibroblasts

(A) IF using the SMN antibody was used to detect Gems in fibroblasts from an unaffected individual and an ALS patient carrying a FUS R521C mutation. DAPI shows the nucleus. Scale bar, 20 μm.

(B) Same as in (A), except that fibroblasts bearing a TDP-43 M337V mutation were used.

(C) Graph showing Gem levels in ALS patients and unaffected individuals. The mean and standard deviation of Gem numbers per cell were calculated from three independent experiments. At least 150 cells were analyzed in each experiment. The p values were calculated by comparison with three controls (*p < 0.01, Student's t test). The age (years) and sex (M, male; F, female) of the individuals are indicated.

See also Figure S4.

et al., 1997). The observation that Gem levels are deficient in ALS patient fibroblasts raises the interesting possibility that Gem levels can be used as a rapid diagnostic marker. For example, Gem levels may be potentially useful for subtyping FUS and TDP-43 forms of the disease. However, further ALS patient fibroblasts containing FUS, TDP-43, or other mutations must be analyzed in future work to determine the generality of the Gem phenotype. The observation that both SMA and ALS (at least some subtypes) have a Gem phenotype also raises the possibility that drug candidates identified for SMA may be efficacious for ALS. Our observation that overexpression of SMN rescues Gem levels in FUS knockdown cells, and recent work showing that overexpression of SMN delays disease onset in an SOD1 mouse ALS model (Kariya et al., 2012) provide a rationale for testing SMA therapeutics that both increase Gem levels and rescue motor neuron defects.

The multiple links identified among FUS, TDP-43, SOD1, the SMN complex, U1 snRNP, and Gems provide strong support for the view that defects in RNA metabolism are involved in the pathogenesis of motor neuron disease. In future work, it will be important to assess components of these RNA complexes for mutations that may be candidates for ALS or SMA susceptibility genes or risk factors.

EXPERIMENTAL PROCEDURES

Plasmids, Proteins, and Antibodies

His-SMN and His-LC3 proteins were obtained from Enzo Life Sciences. The SMN-GFP expression plasmid was obtained from Origene. Rabbit polyclonal antibodies were raised against GST-FUS (Covance). We obtained antibodies to SMN (2B1), Sm (Y12), and Gemin3 (12H12) from Abcam; U1-70K (9C4.1) and Gemin2 (2E17) from Millipore; TDP-43 from Proteintech; U1A (BJ-7), HA, Tubulin, Gemin4 (E-8), and Gemin6 (20H8) from Santa Cruz; and FLAG from Sigma. SAP130 and HA were used as negative controls for polyclonal and monoclonal antibodies, respectively.

IP, GST Pulldown, and MS

IP and GST pulldowns were performed as previously described (Das et al., 2007). Gel samples were trypsin digested and peptides were analyzed by liquid chromatography–tandem MS (LC-MS/MS). FUS and control immunoprecipitates were TCA precipitated and analyzed by LC-MS/MS. Keratin and likely contaminants (e.g., desmoplakin, actin, tubulin, myosin, and translation proteins) were not included in Table S1. Proteins found in the negative control immunoprecipitate were not included in Table S1 if the total amount of peptides was <3-fold lower than in the FUS immunoprecipitate. Proteins that were larger than 300 amino acids and contained ≤7 peptides as identified by MS were not included in Table S1. Proteins with <2 unique peptides were also omitted.

RNAi

Lentiviruses were made by transfecting 293FT cells with three packaging plasmids (pLP1, pLP2, and pLP/VSVG) using the method provided by the manufacturer (ViraPower Lentiviral Expression System, Invitrogen). Infected cells were selected with 3 μg/ml puromycin (Sigma). For small interfering RNAs (siRNAs), cells were treated with Lipofectamine 2000 (Invitrogen) for 48 hr according to the manufacturer's protocol.

IF and Gem Imaging

IF was carried out with the use of FUS (1:1000), TDP-43 (1:1000), SMN (1:400), Gemin3 (1:400), Gemin4 (1:400), and FLAG (1:1000) antibodies. HeLa cells were fixed with 4% paraformaldehyde in PBS for 15 min, and fibroblasts were fixed with methanol and acetone (1:1) for 15 min. Cells were permeabilized with 0.1% Triton X-100 in PBS for 15 min. For IF, cells were incubated

in 1° antibody overnight at 4°C. After three washes in PBS, 2° antibody was added for 1 hr at room temperature, followed by three washes in PBS. Then, 1° antibodies were diluted in 10% calf serum in PBS. The 2° antibodies were mouse Alexa-488 and rabbit Alexa-647 diluted 1:1,000 in 10% calf serum in PBS. HeLa cells images were captured with a Nikon TE2000U inverted microscope with a PerkinElmer ultraview spinning disk confocal and a 20× objective using Metamorph software (Molecular Devices, Sunnyvale, CA). See Extended Experimental Procedures for details about the methods used in this work.

REFERENCES

Battle, D.J., Kasim, M., Yong, J., Lotti, F., Lau, C.K., Mouaikel, J., Zhang, Z., Han, K., Wan, L., and Dreyfuss, G. (2006). The SMN complex: an assembly machine for RNPs. Cold Spring Harb. Symp. Quant. Biol. 71, 313–320.

Boillée, S., Vande Velde, C., and Cleveland, D.W. (2006). ALS: a disease of motor neurons and their nonneuronal neighbors. Neuron 52, 39–59.

Bosco, D.A., Lemay, N., Ko, H.K., Zhou, H., Burke, C., Kwiatkowski, T.J., Jr., Sapp, P., McKenna-Yasek, D., Brown, R.H., Jr., and Hayward, L.J. (2010). Mutant FUS proteins that cause amyotrophic lateral sclerosis incorporate into stress granules. Hum. Mol. Genet. 19, 4160–4175.

Coovert, D.D., Le, T.T., McAndrew, P.E., Strasswimmer, J., Crawford, T.O., Mendell, J.R., Coulson, S.E., Androphy, E.J., Prior, T.W., and Burghes, A.H. (1997). The survival motor neuron protein in spinal muscular atrophy. Hum. Mol. Genet. 6, 1205–1214.

Da Cruz, S., and Cleveland, D.W. (2011). Understanding the role of TDP-43 and FUS/TLS in ALS and beyond. Curr. Opin. Neurobiol. 21, 904–919.

Das, R., Yu, J., Zhang, Z., Gygi, M.P., Krainer, A.R., Gygi, S.P., and Reed, R. (2007). SR proteins function in coupling RNAP II transcription to pre-mRNA splicing. Mol. Cell 26, 867–881.

DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.A., Flynn, H., Adamson, J., et al. (2011). Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72, 245–256.

Dormann, D., Rodde, R., Edbauer, D., Bentmann, E., Fischer, I., Hruscha, A., Than, M.E., Mackenzie, I.R., Capell, A., Schmid, B., et al. (2010). ALS-associated fused in sarcoma (FUS) mutations disrupt Transportin-mediated nuclear import. EMBO J. *29*, 2841–2857.

Fallini, C., Bassell, G.J., and Rossoll, W. (2012a). The ALS disease protein TDP-43 is actively transported in motor neuron axons and regulates axon outgrowth. Hum. Mol. Genet. *21*, 3703–3718.

Fallini, C., Bassell, G.J., and Rossoll, W. (2012b). Spinal muscular atrophy: the role of SMN in axonal mRNA regulation. Brain Res. *1462*, 81–92.

Gertz, B., Wong, M., and Martin, L.J. (2012). Nuclear localization of human SOD1 and mutant SOD1-specific disruption of survival motor neuron protein complex in transgenic amyotrophic lateral sclerosis mice. J. Neuropathol. Exp. Neurol. *71*, 162–177.

Gitcho, M.A., Baloh, R.H., Chakraverty, S., Mayo, K., Norton, J.B., Levitch, D., Hatanpaa, K.J., White, C.L., 3rd, Bigio, E.H., Caselli, R., et al. (2008). TDP-43 A315T mutation in familial motor neuron disease. Ann. Neurol. *63*, 535–538.

Gubitz, A.K., Feng, W., and Dreyfuss, G. (2004). The SMN complex. Exp. Cell Res. *296*, 51–56.

Kabashi, E., Valdmanis, P.N., Dion, P., Spiegelman, D., McConkey, B.J., Vande Velde, C., Bouchard, J.P., Lacomblez, L., Pochigaeva, K., Salachas, F., et al. (2008). TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. Nat. Genet. *40*, 572–574.

Kabashi, E., Bercier, V., Lissouba, A., Liao, M., Brustein, E., Rouleau, G.A., and Drapeau, P. (2011). FUS and TARDBP but not SOD1 interact in genetic models of amyotrophic lateral sclerosis. PLoS Genet. *7*, e1002214.

Kariya, S., Re, D.B., Jacquier, A., Nelson, K., Przedborski, S., and Monani, U.R. (2012). Mutant superoxide dismutase 1 (SOD1), a cause of amyotrophic lateral sclerosis, disrupts the recruitment of SMN, the spinal muscular atrophy protein to nuclear Cajal bodies. Hum. Mol. Genet. *21*, 3421–3434.

Kim, S.H., Shanware, N.P., Bowler, M.J., and Tibbetts, R.S. (2010). Amyotrophic lateral sclerosis-associated proteins TDP-43 and FUS/TLS function in a common biochemical complex to co-regulate HDAC6 mRNA. J. Biol. Chem. *285*, 34097–34105.

Kwiatkowski, T.J., Jr., Bosco, D.A., Leclerc, A.L., Tamrazian, E., Vanderburg, C.R., Russ, C., Davis, A., Gilchrist, J., Kasarskis, E.J., Munsat, T., et al. (2009). Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science *323*, 1205–1208.

Lefebvre, S., Bürglen, L., Reboullet, S., Clermont, O., Burlet, P., Viollet, L., Benichou, B., Cruaud, C., Millasseau, P., Zeviani, M., et al. (1995). Identification and characterization of a spinal muscular atrophy-determining gene. Cell *80*, 155–165.

Ling, S.C., Albuquerque, C.P., Han, J.S., Lagier-Tourenne, C., Tokunaga, S., Zhou, H., and Cleveland, D.W. (2010). ALS-associated mutations in TDP-43 increase its stability and promote TDP-43 complexes with FUS/TLS. Proc. Natl. Acad. Sci. USA *107*, 13318–13323.

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

Liu-Yesucevitz, L., Bassell, G.J., Gitler, A.D., Hart, A.C., Klann, E., Richter, J.D., Warren, S.T., and Wolozin, B. (2011). Local RNA translation at the synapse and in disease. J. Neurosci. *31*, 16086–16093.

Renton, A.E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J.R., Schymick, J.C., Laaksovirta, H., van Swieten, J.C., Myllykangas, L., et al; ITALSGEN Consortium. (2011). A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron *72*, 257–268.

Sephton, C.F., Cenik, C., Kucukural, A., Dammer, E.B., Cenik, B., Han, Y., Dewey, C.M., Roth, F.P., Herz, J., Peng, J., et al. (2011). Identification of neuronal RNA targets of TDP-43-containing ribonucleoprotein complexes. J. Biol. Chem. *286*, 1204–1215.

Shan, X., Chiang, P.M., Price, D.L., and Wong, P.C. (2010). Altered distributions of Gemini of coiled bodies and mitochondria in motor neurons of TDP-43 transgenic mice. Proc. Natl. Acad. Sci. USA *107*, 16325–16330.

Sreedharan, J., Blair, I.P., Tripathi, V.B., Hu, X., Vance, C., Rogelj, B., Ackerley, S., Durnall, J.C., Williams, K.L., Buratti, E., et al. (2008). TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science *319*, 1668–1672.

Valdmanis, P.N., and Rouleau, G.A. (2008). Genetics of familial amyotrophic lateral sclerosis. Neurology *70*, 144–152.

Vance, C., Rogelj, B., Hortobágyi, T., De Vos, K.J., Nishimura, A.L., Sreedharan, J., Hu, X., Smith, B., Ruddy, D., Wright, P., et al. (2009). Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. Science *323*, 1208–1211.

Waibel, S., Neumann, M., Rabe, M., Meyer, T., and Ludolph, A.C. (2010). Novel missense and truncating mutations in FUS/TLS in familial ALS. Neurology *75*, 815–817.

Wang, J.W., Brent, J.R., Tomlinson, A., Shneider, N.A., and McCabe, B.D. (2011). The ALS-associated proteins FUS and TDP-43 function together to affect Drosophila locomotion and life span. J. Clin. Invest. *121*, 4118–4126.

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