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RESEARCH ARTICLE

Fam118B, a newly identified component of Cajal bodies, is required for Cajal body formation, snRNP biogenesis and cell viability

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ABSTRACT

Cajal bodies are specialized and dynamic compartments in the nucleus that are involved in the biogenesis of small nuclear ribonucleoproteins (snRNPs). Because of the dynamic and varied roles of Cajal bodies, it is of great interest to identify the components of Cajal bodies to better understand their functions. We performed a genome-wide screen to identify proteins that colocalize with coilin, the marker protein of Cajal bodies. In this study, we identified and characterized Fam118B as a newly discovered component of Cajal bodies. Fam118B is widely expressed in a variety of cell lines derived from various origins. Overexpression of Fam118B changes the canonical morphology of Cajal bodies, whereas depletion of Fam118B disrupts the localization of components of Cajal bodies, including coilin, the survival of motor neuron protein (SMN) and the Sm protein D1 (SmD1, also known as SNRPD1). Moreover, depletion of Fam118B reduces splicing capacity and inhibits cell proliferation. In addition, Fam118B associates with coilin and SMN proteins. Fam118B depletion reduces symmetric dimethylarginine modification of SmD1, which in turn diminishes the binding of SMN to this Sm protein. Taken together, these data indicate that Fam118B, by regulating SmD1 symmetric dimethylarginine modification, plays an important role in Cajal body formation, snRNP biogenesis and cell viability.

KEY WORDS: Cajal body, Coilin, Fam118B, SMN, Symmetric dimethylarginine

INTRODUCTION

Cajal bodies, one of the specialized structures in the nucleus, range from 0.1 to 1.0 µm in diameter (Matera, 1998). As with other subnuclear domains, and in contrast to cytoplasmic organelles, Cajal bodies are not surrounded by phospholipid membranes and, hence, components can flux into and out of the Cajal body from the surrounding nucleoplasm (Sleeman et al., 2003). Cajal bodies are highly dynamic mobile structures that disassemble during mitosis and reassemble in mid-G1-phase (Matera and Frey, 1998; Cioce and Lamond, 2005), and they are best characterized for their role in small nuclear ribonucleoprotein (snRNP) maturation (Hebert, 2010). Specifically, Cajal bodies are thought to be the location of the modification (by 2′-*O*-methylation and pseudouridylation) of the small nuclear RNA (snRNA) component of snRNPs. These modifications are guided by small Cajal-body-specific RNAs (scaRNAs) (Darzacq et al., 2002; Matera and Shpargel, 2006). Mature snRNPs then localize to speckles for storage or to sites of transcription where the splicing of pre-mRNA (to produce mature mRNA) takes place (Gall et al., 1995). Moreover, Cajal bodies are required for telomerase assembly in human cells, although the exact role of Cajal bodies in this process is still elusive (Venteicher et al., 2009). In order to better understand the dynamic and varied roles of Cajal bodies, a comprehensive identification of components that localize to this subnuclear domain is needed.

Both coilin and the survival of motor neuron protein (SMN) are highly enriched in Cajal bodies and are indispensable for the canonical formation of these subnuclear domains (Tucker et al., 2001; Girard et al., 2006; Walker et al., 2009). Coilin contains the N-terminal self-association domain that is sufficient for localization to Cajal bodies (Hebert and Matera, 2000). Recent studies have shown that, apart from its structural role in Cajal bodies, coilin also possesses specific RNase activity on U2 and hTR transcripts (Broome and Hebert, 2012; Broome and Hebert, 2013). The localization of SMN to Cajal bodies is coilin-dependent, and is modulated by the symmetrical dimethylation of arginine in the C-terminal RG dipeptide motif of coilin (Hebert et al., 2001; Hebert et al., 2002). SMN is part of a large complex that is required for the ordered assembly of the Sm core of spliceosomal uridine-rich (U) snRNPs *in vivo* (Fischer et al., 1997). Mutations or deletions of the survival of motor neurons 1 (*SMN1*) gene have been found in over 98% of spinal muscular atrophy (SMA) patients. SMA is the leading genetic cause of infant mortality and is characterized by the progressive loss of motor neurons from the spinal cord (Coovert et al., 1997).

The assembly of the SMN–Sm core is a stepwise and tightly regulated process; the SMN complex, firstly preloaded with Sm proteins, transfers the Sm proteins to the Sm binding site on snRNA (Battle et al., 2006). Although isolated Sm proteins can bind to U snRNA spontaneously *in vitro*, the proper assembly *in vivo* requires the SMN complex (Bühler et al., 1999; Raker et al., 1999). SMN binds directly to Sm proteins. Three of the seven Sm proteins (SmD1, SmD3 and SmB, also known as SNRPD1, SNRPD3 and SNRPB, respectively) are post-translationally

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modified to contain symmetric dimethylarginine (sDMA) (Friesen et al., 2001b), which strongly enhances the affinity of their binding to SMN (Friesen et al., 2001a). Interestingly, disruption of this interaction by specific antibodies inhibits U snRNP assembly, and several SMN mutants found in SMA patients are defective in Sm-protein binding, indicating that defects in SMN–Sm core assembly might play a role in the pathogenesis of SMA (Bühler et al., 1999).

In this study, we identified and characterized Fam118B as a newly discovered component of Cajal bodies. Fam118B associates with coilin and SMN. Depletion of Fam118B not only leads to the disassembly of canonical Cajal bodies but also results in the redistribution of SmD1 proteins to the nucleolus. In addition, we demonstrate that Fam118B depletion reduces splicing efficiency and cell viability. Moreover, Fam118B is important in regulating the sDMA modification of SmD1 and the binding of SmD1 to SMN.

RESULTS

Fam118B is a newly identified component of Cajal bodies and is expressed in a variety of cell lines

Fam118B is an uncharacterized protein and was first identified as a potential component of Cajal bodies in a proteomic screen that we performed recently (Fong et al., 2013). To confirm the expression and localization of Fam118B in cells, we generated rabbit polyclonal antibodies against Fam118B. To verify the specificity of the antibody, we used siRNA against *FAM118B* to knock down endogenous Fam118B. The Fam118B antibody recognized a major band migrating at about 34 kDa, which corresponds to the predicted molecular mass of endogenous Fam118B (Fig. 1A). The 34-kDa band was greatly reduced after siRNA-mediated Fam118B depletion, but it was unaffected by coilin depletion (Fig. 1A), suggesting that our anti-Fam118B antibody specifically recognizes endogenous Fam118B. We detected Fam118B in a variety of cancer cell lines from different tissue origins (Fig. 1B), suggesting that Fam118B might have a general function in the cell.

Next, we performed immunofluorescent staining to determine Fam118B localization. Fam118B localizes mainly in the nucleoplasm and concentrates at a few foci inside the nucleus, where it colocalizes with coilin (Fig. 1C). To better visualize Fam118B at Cajal bodies, we used Triton X-100 to extract the cytosolic and nucleoplasmic proteins, and the colocalization of Fam118B and coilin was obvious (Fig. 1C). Moreover, in a number of cancer cell lines, we detected bright Fam118B-stained foci, which colocalized with coilin in the nucleus (Fig. 1D). These data indicate that Fam118B is a component of Cajal bodies and is expressed in a broad range of tissues.

Fam118B associates with components of Cajal bodies

It is well known that both coilin (the marker protein of Cajal bodies) and the SMN protein complex are highly enriched in Cajal bodies (Tucker et al., 2001), and that they are required for the *de novo* formation of these subnuclear domains (Kaiser et al., 2008). Because Fam118B localizes in Cajal bodies, we examined whether Fam118B would interact with these two main components of Cajal bodies. Indeed, coilin and SMN co-immunoprecipitated with endogenous Fam118B (Fig. 2A). However, in the reciprocal experiment, in which coilin was immunoprecipitated, we were able to detect SMN, but not Fam118B (Fig. 2A). To further confirm the interaction between Fam118B and SMN, we also immunoprecipitated SMN and observed a strong interaction between SMN and Fam118B, whereas we barely detected coilin in SMN immunoprecipitates (Fig. 2B). To address whether or not coilin indeed interacts with Fam118B, we performed *in vitro* pull-down experiments and showed that bacterially expressed MBP-tagged Fam118B associated with GST-tagged coilin, but not with GST alone (Fig. 2C). This result suggests that Fam118B binds directly to coilin.

It is assumed that coilin provides a scaffold for the assembly of the other Cajal body components. To test whether the localization of Fam118 at Cajal bodies is dependent on coilin, we used siRNA to knock down coilin and checked for the formation of Fam118B foci. Fam118B concentrated in Cajal bodies in control cells, but was diffusely localized in the nucleoplasm in coilin-depleted cells (Fig. 2D). These results suggest that the foci formation of Fam118B is coilin dependent.

Residues 190–232 of Fam118B are necessary for its localization in Cajal bodies

Fam118B is a recently identified protein without any functional reports. Based on the prediction of the Conserved Domain Database (CDD), we found that Fam118B contains a conserved SIR2-like domain between residues 159 and 301 (Fig. 3A). To determine which region of Fam118B is required for targeting to Cajal bodies, we constructed several HA-FLAG-tagged truncation mutants of Fam118B (Fig. 3A); M1 (containing residues 1–113), M2 (residues 114–232), M3 (residues 233–351), M4 (residues 1–189) and M5 (residues 114–351). The expression of these mutants was confirmed by western blotting (Fig. 3B), and their subcellular localization was examined by using immunofluorescent staining. We found that mutants M2 and M5 colocalized with coilin, but that the M1, M3 and M4 mutants did not (Fig. 3C,E). Because residues 190–232 within M2 and M5 mutants are highly conserved from fish to mammals (Fig. 3A; supplementary material Fig. S1), we further examined whether this region was required for Cajal body targeting. To this end, we constructed an additional mutant of Fam118B (M6), harboring an internal deletion of residues 190–232 (Fig. 3A). We showed that this mutant failed to target to Cajal bodies (Fig. 3C), suggesting that this region of Fam118B is required for its localization to Cajal bodies. Interestingly, the M6 mutant of Fam118B localized to the nucleolus (Fig. 3D). Of note, both M1 and M4 mutants also displayed nucleolar localization, as determined by their colocalization with fibrillarin (Fig. 3D). Based on these results, we speculated that the N-terminus of Fam118B might contain a putative nucleolar localization signal (NoLS). This region might allow Fam118B to shuttle between the nucleolus and Cajal bodies.

Depletion of Fam118B leads to the destruction of Cajal bodies

It is known that the depletion of Cajal body components, such as coilin (Tucker et al., 2001), SMN (Girard et al., 2006), hCINAP [also known as AK6, (Zhang et al., 2010)], FLASH [also known as CASP8AP2 (Barcaroli et al., 2006)] or ZPR1 [also known as ZNF259 (Gangwani et al., 2005)], leads to the disassembly of Cajal bodies. Therefore, we were eager to determine whether depletion of Fam118B would also result in defects in Cajal body formation. Fam118B protein levels were reduced to ~20% following the transfection of cells with Fam118B-specific siRNA (Fig. 4A). Depletion of Fam118B did not change the total protein levels of most of the Sm proteins and other Cajal body

Journal of Cell Science (2014) 127, 2029–2039 doi:10.1242/jcs.143453

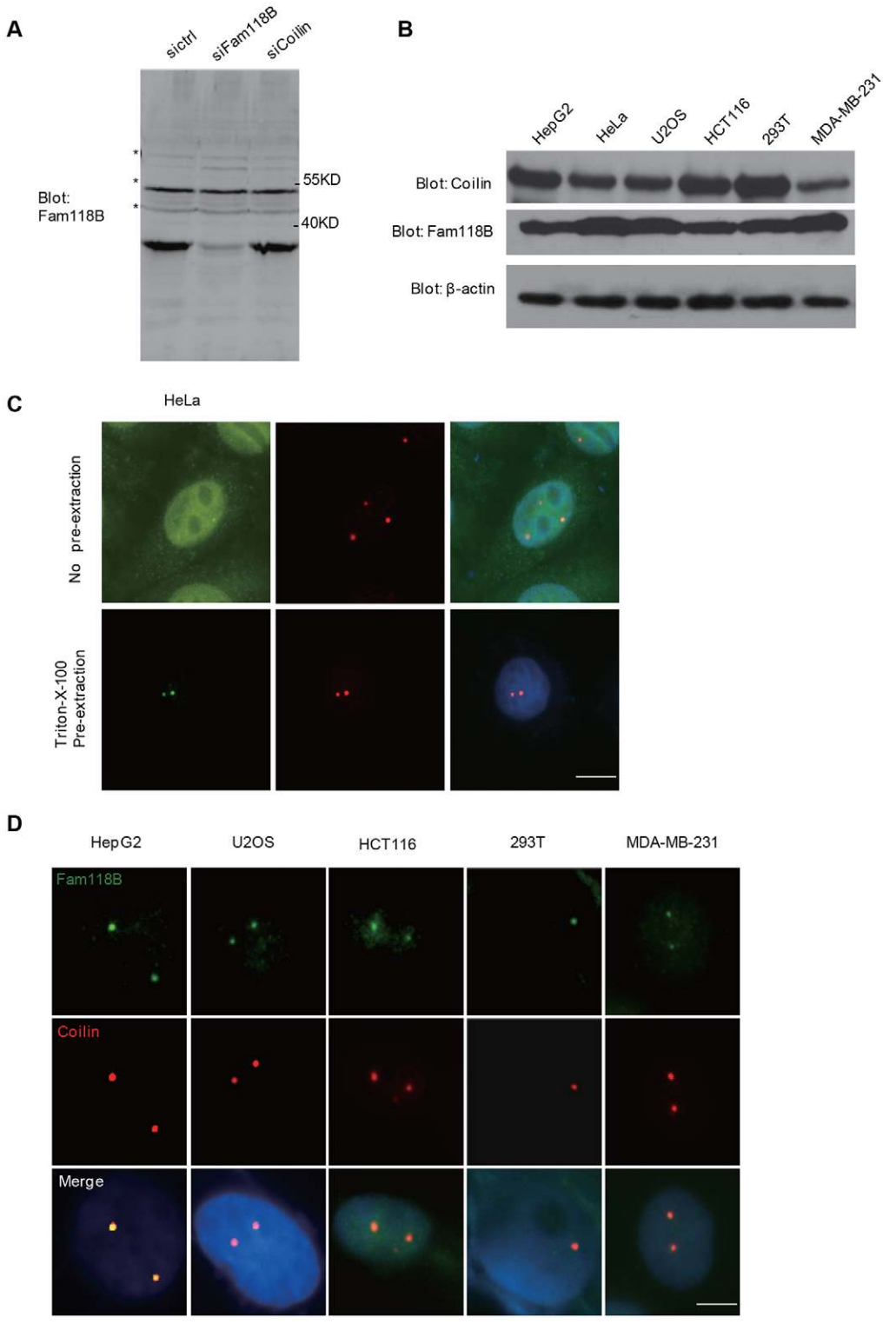

Fig. 1. Fam118B is a newly identified component of Cajal bodies. (A) The specificity of the Fam118B polyclonal antibody was confirmed by western blot analysis. HeLa cells were subjected to two rounds of treatment with the indicated siRNA and the cell lysates were analyzed by western blot analysis with the anti-Fam118B antibody. Asterisks indicate non-specific bands; siCtrl, control siRNA. (B) Fam118B is expressed in different cancer cell lines. Cell lysates from HepG2, HeLa, U2OS, HCT116, 293T and MDA-MB-231 cells were collected. Western blot analysis was performed to detect the protein levels of endogenous Fam118B and coilin. β-actin is included as a loading control. (C) Fam118B concentrates in Cajal bodies. HeLa cells were treated with Triton X-100 or left untreated, and were subjected to immunofluorescent staining using the indicated antibodies recognizing Fam118B (green) or coilin (red). Scale bar: 10 μm. (D) Fam118B colocalizes with coilin in various cancer cell lines. The indicated cancer cell lines were pre-extracted with Triton X-100 and stained with antibodies against Fam118B (green) and coilin (red). Scale bar: 5 μm. In C and D, the nuclei were stained with DAPI (blue).

components, such as coilin and SMN, but it caused a modest reduction in SmD1 and SmB protein levels (Fig. 4A; supplementary material Fig. S2). In cells transfected with control siRNA, we observed that 92.3% of cells contained canonical coilin foci, whereas this number dropped to 2.5% in Fam118B-depleted cells (Fig. 4B,C). Instead, in Fam118B-depleted cells, coilin was found to be dispersed, appearing as tiny dots in the nucleoplasm (Fig. 4B), which is reminiscent of a phenotype resulting from the depletion of other Cajal body components.

Because SMN can form foci termed ‘Gemini of Cajal bodies’ without coilin (Hebert et al., 2001), we next tested whether Fam118B depletion would also affect the formation of SMN foci. Surprisingly, SMN staining became diffuse throughout the nucleoplasm upon Fam118B depletion, and we did not detect the formation of any SMN foci (Fig. 4D). A quantitative analysis

Journal of Cell Science (2014) 127, 2029–2039 doi:10.1242/jcs.143453

showed that the percentage of cells containing SMN foci decreased from 34.6% in control cells to 1.5% in Fam118B-depleted cells (Fig. 4E).

SmD1 is one of seven proteins in the Sm core that bind to snRNA during snRNP biogenesis. The snRNP complex partially assembles in the cytoplasm and then is imported to Cajal bodies in the nucleus for snRNA modification and possibly for binding to additional snRNP-specific proteins (Matera et al., 2007). In accordance with a previous report, we observed that SmD1 colocalized with coilin in the control cells (Sleeman et al., 2001). Upon Fam118B depletion, we could not detect discrete SmD1 foci in the nucleus (Fig. 4F). Instead, SmD1 was redistributed to nucleoli (Fig. 4F). In addition, we found that SmD1 colocalized with fibrillarin, a marker of the nucleolus, upon Fam118B knockdown (Fig. 4F). A quantitative analysis indicated that SmD1 redistributed to nucleoli in 95.6% of Fam118B-depleted cells compared with 2.2% in control cells (Fig. 4G). Taken together, these results suggest that Fam118B is required for the assembly of Cajal bodies.

Depletion of Fam118B causes diminished splicing capacity and a reduction in cell proliferation

It has been previously reported that variable organization of Cajal bodies can influence splicing capacity (Whittom et al., 2008). Because Fam118B depletion caused the disassembly of Cajal bodies, we examined whether Fam118B deficiency would also affect splicing efficiency. We used siRNA to reduce the levels of endogenous Fam118B or coilin protein to <10% of their levels in control cells (Fig. 5A). As a control, we introduced siRNA-resistant Fam118B constructs into Fam118B-depleted cells and were able to restore the expression of Fam118B to a physiological level (Fig. 5A). An artificial splicing reporter, pSI, was transfected into these cells to challenge the splicing machinery. Different primer sets were used to amplify the unspliced or spliced mRNAs (Fig. 5B). As reported previously (Whittom et al., 2008), the depletion of coilin led to a reduction in splicing efficiency to ~58% of that in control cells (Fig. 5C,D). In agreement with the finding that Fam118 is required for the formation of Cajal bodies, we observed a similar reduction in splicing activity (to ~55%) in Fam118B-deficient cells (Fig. 5C,D). Restoring Fam118B expression rescued this splicing defect (Fig. 5C,D), ruling out a role for potential off-target effects of Fam118B siRNA. To further validate the roles of Fam118B in pre-mRNA splicing under physiological conditions, we selected eight endogenous genes (DPP8, DDX20, NOSIP, ACP1, CSDA, CD44, STK6 and ZNF207) and used quantitative real-time PCR (qRT-PCR) to evaluate the abundance of the spliced mRNA as described previously (Fong et al., 2013). We found that the depletion of Fam118B reduced the levels of spliced mRNA by 10–63% whereas the depletion of coilin resulted in a similar reduction of 26–67% (Fig. 5E). Introducing wild-type Fam118B into siFam118B-treated cells largely restored the mRNA-splicing deficiency observed in Fam118-depleted cells (Fig. 5E), indicating that Fam118 is involved in mRNA splicing.

Coilin-deficient cells are viable but have a proliferation defect (Lemm et al., 2006). Therefore, we examined the proliferation of

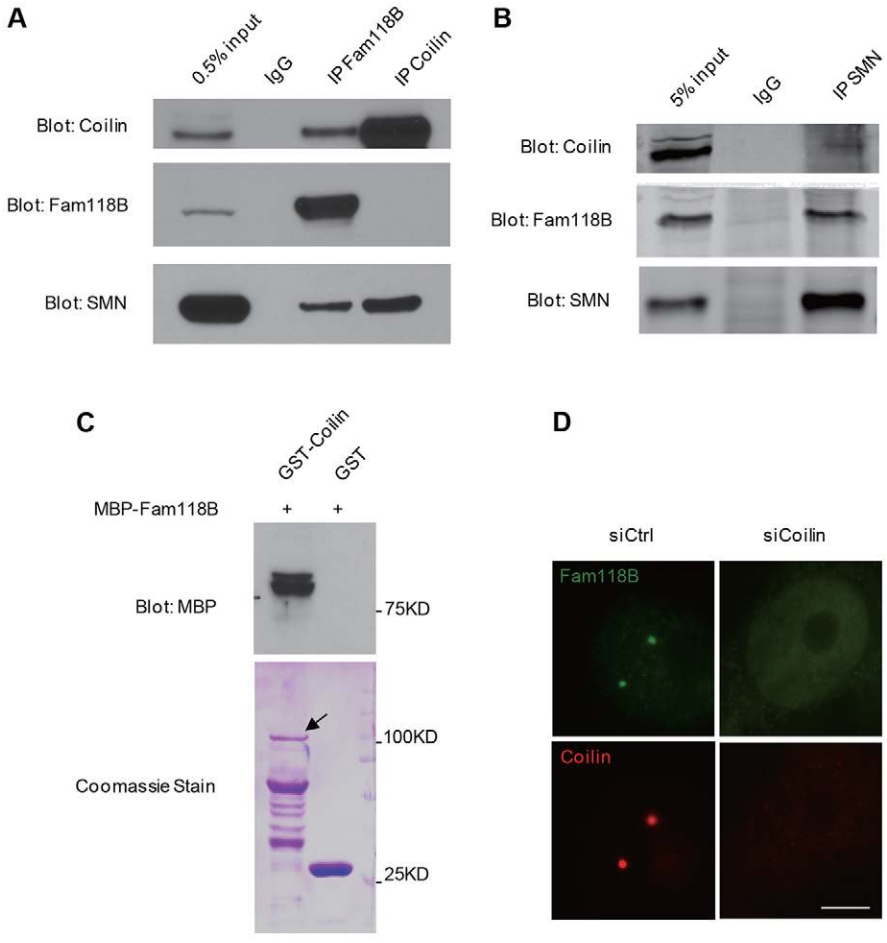

Fig. 2. Fam118B associates with the major components of Cajal bodies. (A) Fam118B interacts with coilin and SMN. HeLa lysates were immunoprecipitated (IP) by using polyclonal anti-Fam118B or anti-coilin antibodies, and were analyzed by western blot analysis with the indicated antibodies. (B) Immunoprecipitation using control IgG or anti-SMN antibody was performed in extracts prepared from HeLa cells. The presence of coilin or Fam118B in these immunoprecipitates was evaluated by immunoblotting with their respective antibodies. (C) A GST pull-down assay was performed using immobilized control GST or GST–coilin fusion protein on agarose beads followed by incubation with bacterially purified MBP-tagged Fam118B protein. The *in vitro* interaction of Fam118B with coilin was assessed by immunoblotting with the anti-MBP antibody. The arrow shows the migrating band of GST–coilin. (D) The formation of Fam118B foci is coilin dependent. HeLa cells depleted of coilin (siCoilin) were immunostained with polyclonal anti-Fam118B (green) and anti-coilin (red). Scale bar: 5 μm.

Journal of Cell Science (2014) 127, 2029–2039 doi:10.1242/jcs.143453

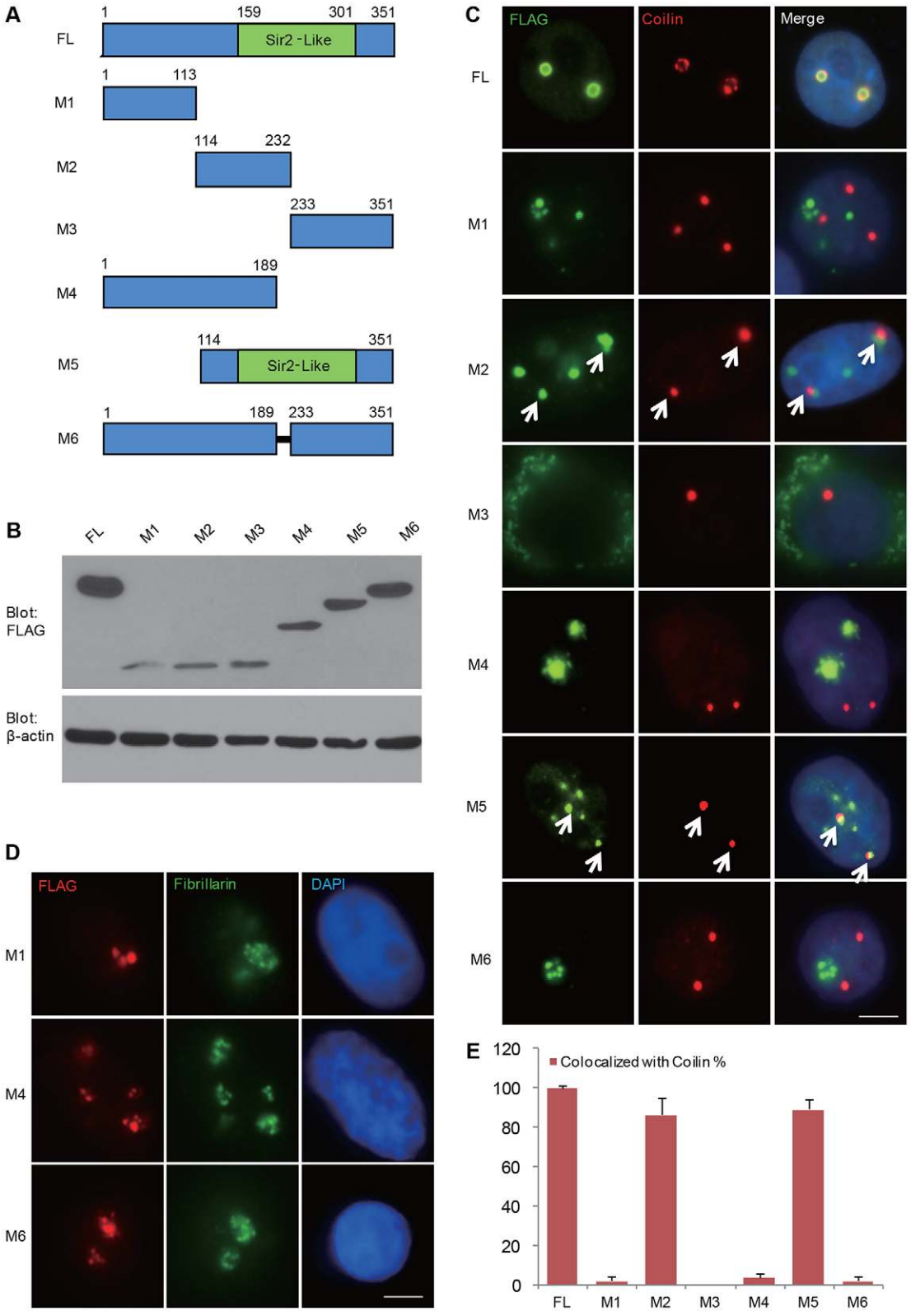

Fig. 3. Amino acid residues 190–232 of Fam118B are required for targeting to Cajal bodies. (A) Schematic representations of the functional domain of Fam118B based on CDD and the mutant constructs used in this study. FL, full length. (B) The expression of wild-type (FL) and mutant Fam118B proteins was examined by western blot analysis with the anti-FLAG antibody. (C) HeLa cells were transiently transfected with constructs encoding the indicated Fam118B wild-type or mutant proteins and were stained with anti-FLAG (green) and anti-coilin (red) antibodies. Arrows indicate the foci in which FLAG-tagged Fam118B colocalizes with coilin. (D) M1, M4 and M6 mutants localize in the nucleoli. HeLa cells were transiently transfected with constructs encoding the M1, M4 or M6 mutants of Fam118, fixed and stained with anti-FLAG (red) and anti-fibrillarin (green) antibodies. In C and D, the nucleus was stained with DAPI (blue). Scale bars: 5 μm. (E) The percentage of Fam118B wild-type or mutant protein that colocalized with coilin is presented. The data show the mean±s.d. of three independent experiments; 50 cells per experiment.

Journal of Cell Science (2014) 127, 2029–2039 doi:10.1242/jcs.143453

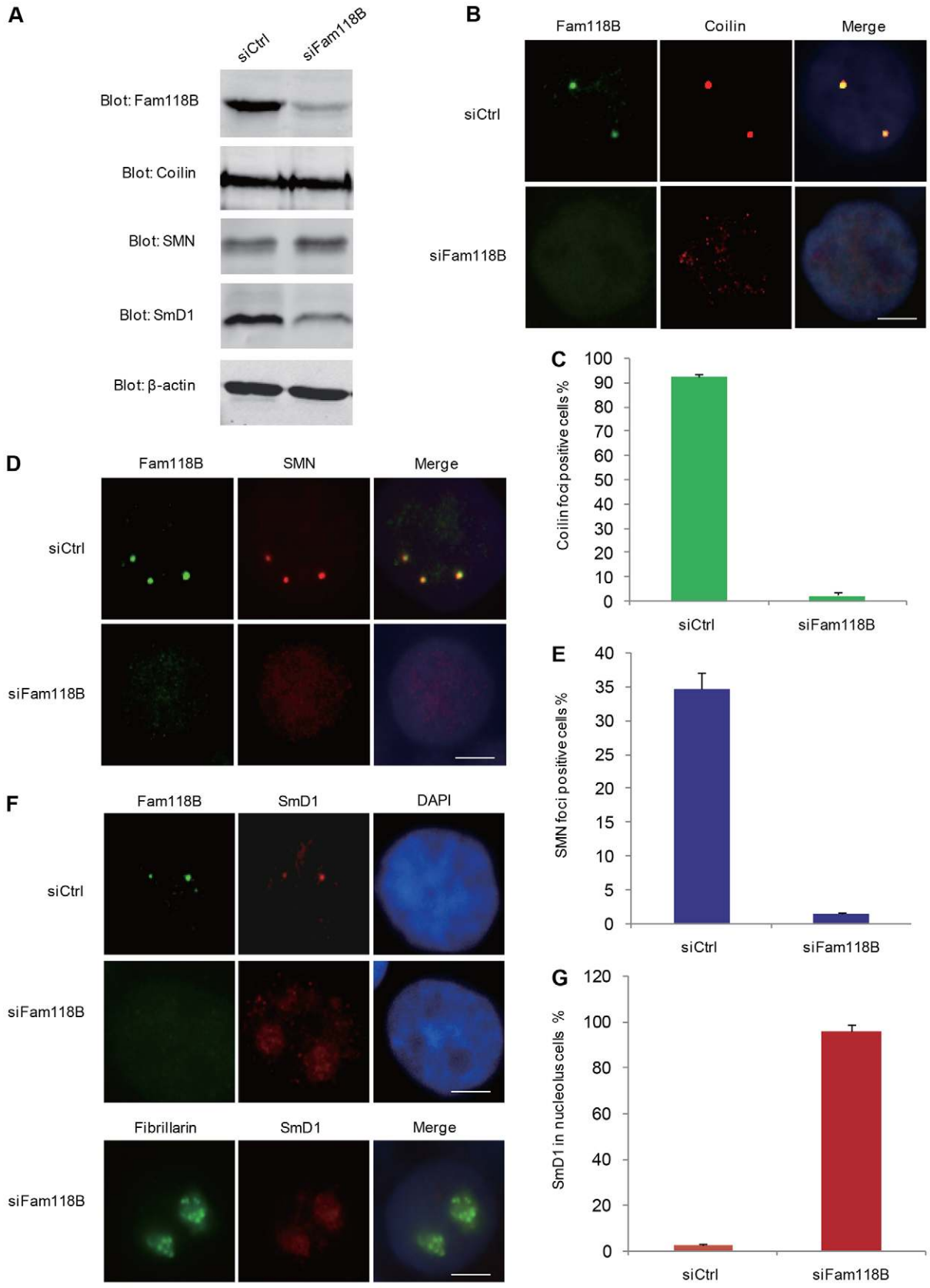

Fig. 4. See next page for legend.

Fig. 4. Depletion of Fam118B causes the disassembly of Cajal bodies. (A) The depletion of Fam118B did not affect the protein level of coilin or SMN, but resulted in a reduction in SmD1 protein levels. HeLa cells depleted of Fam118B (siFam118B) were analyzed by western blotting with the indicated antibodies. (B) The depletion of Fam118B disrupted canonical Cajal body formation. HeLa cells transfected with control siRNA (siCtrl) or Fam118B siRNA were immunostained with polyclonal anti-Fam118B (green) and anti-coilin (red) antibodies. (C) The percentage of cells with canonical coilin foci is shown. (D) The depletion of Fam118B disrupted SMN foci. Cells were immunostained with anti-Fam118B (green) and anti-SMN (red) antibodies. (E) The percentage of cells with SMN foci in the nucleus is shown. (F) The depletion of Fam118B caused SmD1 to be redistributed to the nucleolus. Cells were immunostained with anti-Fam118B (green) and anti-SmD1 (red) antibodies (upper panel), or with anti-fibrillarin (green) and anti-SmD1 (red) antibodies (lower panel). (G) The percentage of cells with SmD1 in the nucleolus is shown. In B, D and F, the nucleus was stained with DAPI (blue). Scale bars: 5 μm. In C, E and G, the data show the mean±s.d. of three independent experiments; 50 cells per experiment.

Fam118B-deficient cells and found that they exhibited a similar growth defect (Fig. 5F). Again, this proliferation defect observed in Fam118B-depleted cells was rescued by the expression of exogenous Fam118B (Fig. 5F). These data indicate that Fam118B plays an important role in splicing and cell proliferation.

Fam118B is required for the SMN–Sm core interaction in the nucleus

Next, we investigated the mechanism by which Fam118B participates in Cajal body assembly. It was previously reported that ongoing U snRNP biogenesis is required for the assembly of Cajal bodies (Lemm et al., 2006). In particular, SMN plays a crucial role in the stepwise assembly of U snRNPs; the SMN complex associates with Sm core proteins and subsequently recruits the snRNA precursor to assemble the SMN–snRNP complex (Yong et al., 2010; Zhang et al., 2011). Given that Fam118B associates with SMN (Fig. 2A,B), we investigated whether Fam118B is involved in the association of SMN with the Sm core proteins. We immunoprecipitated ectopically expressed HA-FLAG-tagged SmD1 from control or Fam118B-depleted cells. SMN could be co-immunoprecipitated with SmD1 in control cells; however, depletion of Fam118B dramatically decreased the amount of SMN that associated with SmD1 (Fig. 6A,B).

It is known that SmD1 undergoes sDMA modification (Brahms et al., 2000; Friesen et al., 2001b). Moreover, SMN binds preferentially to methylated SmD1 protein (Friesen et al., 2001a; Briese et al., 2006). Thus, we examined whether the depletion of Fam118B could decrease the amount of sDMA modification of endogenous SmD1 protein, thus affecting its interaction with SMN. By using the SYM10 antibody, which recognizes sDMA-modified SmD1 (~16 kDa), we detected a dramatic decrease in the amount of modified SmD1 in siFam118B-treated cells (Fig. 6C,D). We obtained similar results for the sDMA modification of ectopically expressed SmD1 (Fig. 6A). To further examine whether Fam118B plays a general role in the sDMA modification of Sm proteins, we also checked the sDMA modification of other two Sm proteins (SmB and SmD3). Similarly, the amount of sDMA modification of SmB or SmD3 was reduced in Fam118B-depleted cells compared with that of cells treated with control siRNA, whereas the levels of control SmE protein remained the same (supplementary material Fig. S3A). We did not detect any significant difference in the amount of SmD1 or SmB that co-immunoprecipitated with endogenous SmD3 in control and Fam118B-depleted cells (supplementary material Fig. S3B,C), suggesting that Fam118B does not affect the assembly of the Sm protein complex. Taken together, these data suggest that Fam118B is important for the regulation of the sDMA modification of Sm proteins and for the SMN–Sm interaction, but not for the assembly of the Sm protein complex.

DISCUSSION

In this study, we identified Fam118B as a Cajal body component that interacts with coilin and SMN. In addition, we showed that Fam118B is required for Cajal body assembly, splicing and cell proliferation, suggesting that Fam118B not only localizes to Cajal bodies, but is likely an important and active component thereof.

Interestingly, we found that overexpression of wild-type Fam118B changed the morphology of canonical Cajal bodies to a cap-like structure (Fig. 3C). It is known that coilin localizes to perinucleolar caps in cells treated with transcriptional inhibitors (Raška et al., 1990). However, the cap-like Cajal bodies observed upon Fam118B overexpression were not formed around the nucleolus, suggesting that this morphological change in Cajal bodies might reflect an unknown status of these structures. Nevertheless, previous studies have revealed a close crosstalk between nucleoli and Cajal bodies, because many components shuttle between these two structures, including coilin, fibrillarin and Nopp140 (Isaac et al., 1998; Tapia et al., 2010). In line with these observations, we showed that although residues 190–232 of Fam118B are required for the targeting of Fam118B to Cajal bodies, Fam118B mutants (M2, M4 and M6) without this motif translocate to nucleoli. We speculate that there is a hidden NoLS within the N-terminus of Fam118B, which might facilitate the targeting of this protein to nucleoli. However, when we deleted a putative NoLS that was predicted by a NoLS detector (http://www.compbio.dundee.ac.uk/www-nod/) at residues 22–42 of M6, Fam118B still localized to nucleoli (data not shown). Additional experiments are needed to further test this hypothesis.

In our study, we found that Fam118B could co-immunoprecipitate coilin, but not vice versa. It is likely that coilin interacts with a plethora of complexes and that Fam118B only interacts with a fraction of coilin in one of the coilin-containing subcomplexes. We also observed that only a small fraction of coilin could be co-immunoprecipitated with SMN, whereas relatively more Fam118B is associated with SMN. It is possible that Fam118B forms a more stable complex with SMN than with coilin in the nucleus.

Because coilin is the fundamental scaffold for Cajal bodies, we investigated whether the depletion of coilin would also affect the formation of Fam118B foci. Indeed, we found that Fam118B distributed evenly in the nucleus upon coilin depletion. Interestingly, depletion of Fam118B also led to coilin dispersal to tiny dots, similar to the phenotypes that result from the knockdown of TGS1, SMN or PHAX (Lemm et al., 2006). This observation suggests that Fam118B might be involved in U snRNP assembly, which is required for the integrity of Cajal bodies. However, we could not rule out the possibility that Fam118B could also serve as a structural component in Cajal bodies that interacts with coilin and stabilizes these structures.

The most striking finding is that the depletion of Fam118B influences the sDMA modification of Sm proteins (Fig. 6C,D; supplementary material Fig. S3A), and that the reduction of sDMA on SmD1 diminished its binding to SMN (Fig. 6A,B). Notably, SMN–snRNP assembly takes place in the cytoplasm (Massenet et al., 2002), whereas most of the Fam118B protein

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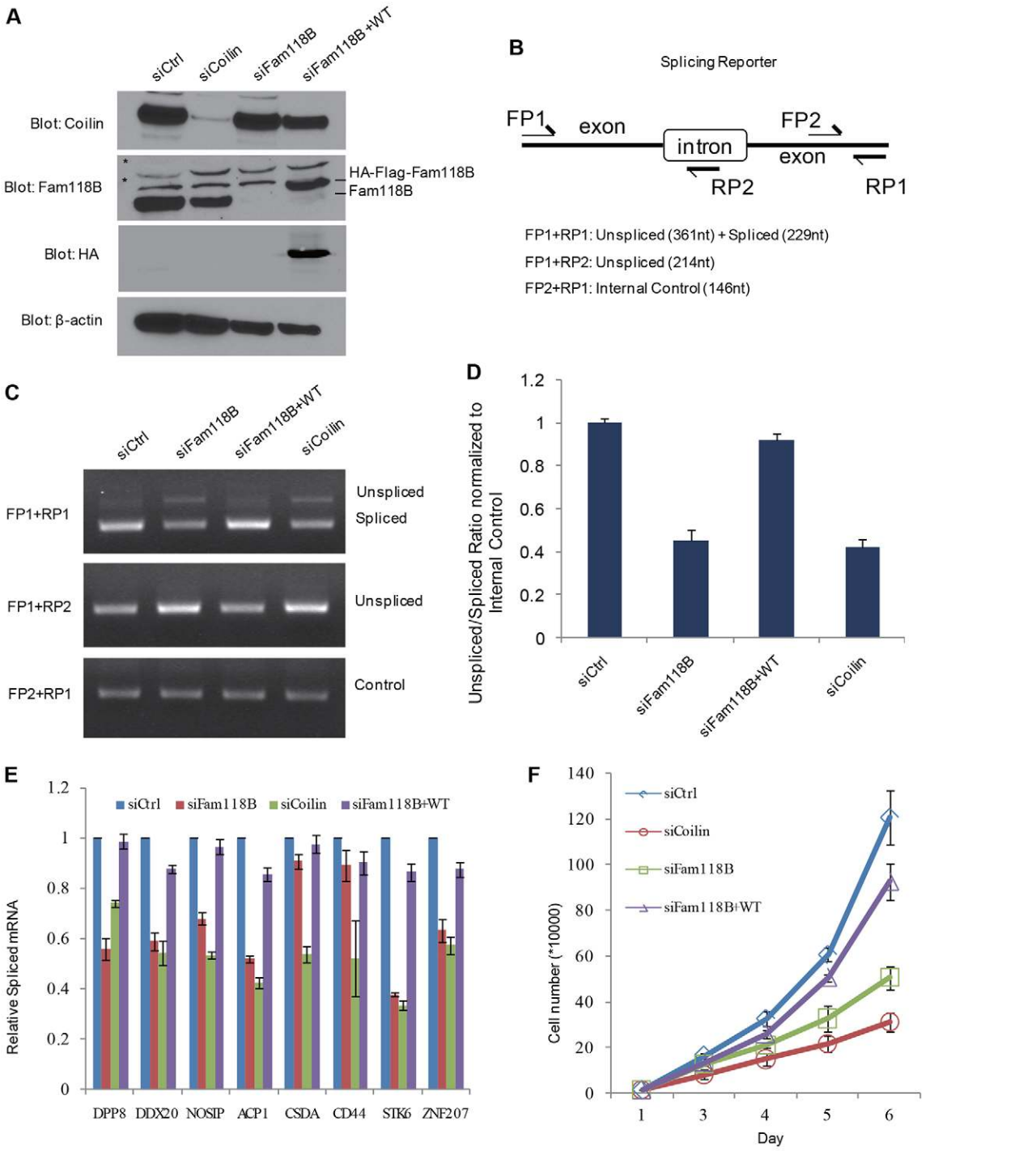

Fig. 5. Depletion of Fam118B causes a reduction in splicing capacity and cell growth. (A) HeLa cells or HeLa cells stably expressing RNAi-resistant wild-type Fam118B (+WT) were subjected to two rounds of treatment with Fam118B (siFam118B) or coilin (siCoilin) siRNA, followed by western blot analysis using the indicated antibodies. Asterisks indicate non-specific bands. siCtrl, control siRNA. (B) A schematic representation of the artificial splicing reporter. The locations of the primers and the expected sizes of PCR products are shown. FP, forward primer; RP, reverse primer. (C) The depletion of Fam118B influenced the splicing of the artificial reporter. HeLa cells or HeLa cells stably expressing RNAi-resistant wild-type Fam118B were subjected to two rounds of treatment with Fam118B or coilin siRNA, followed by pSI transfection. The isolated RNA was subjected to RT-PCR to amplify the pSI message by using the indicated primers. (D) The ratio of unspliced to spliced PCR products normalized to the internal control was compared between the samples. (E) The abundance of spliced mRNA for eight endogenous genes was quantified by qRT-PCR, using primers spanning a particular splice junction. Levels of splicing efficiency are given relative to control (mock siRNA-treated cells, siCtrl) and are normalized to the levels of *NEAT1* RNA, which served as an internal control. (F) The depletion of Fam118B resulted in a reduction in cell proliferation. HeLa cells were seeded and subjected to transfection with Fam118B siRNA or coilin siRNA on the first day after seeding. Fam118B+WT cells were transiently infected with retroviral supernatant to restore Fam118B expression on the second day, and then were counted for the following 4 days. In D–F, the data show the mean±s.d. of three independent experiments.

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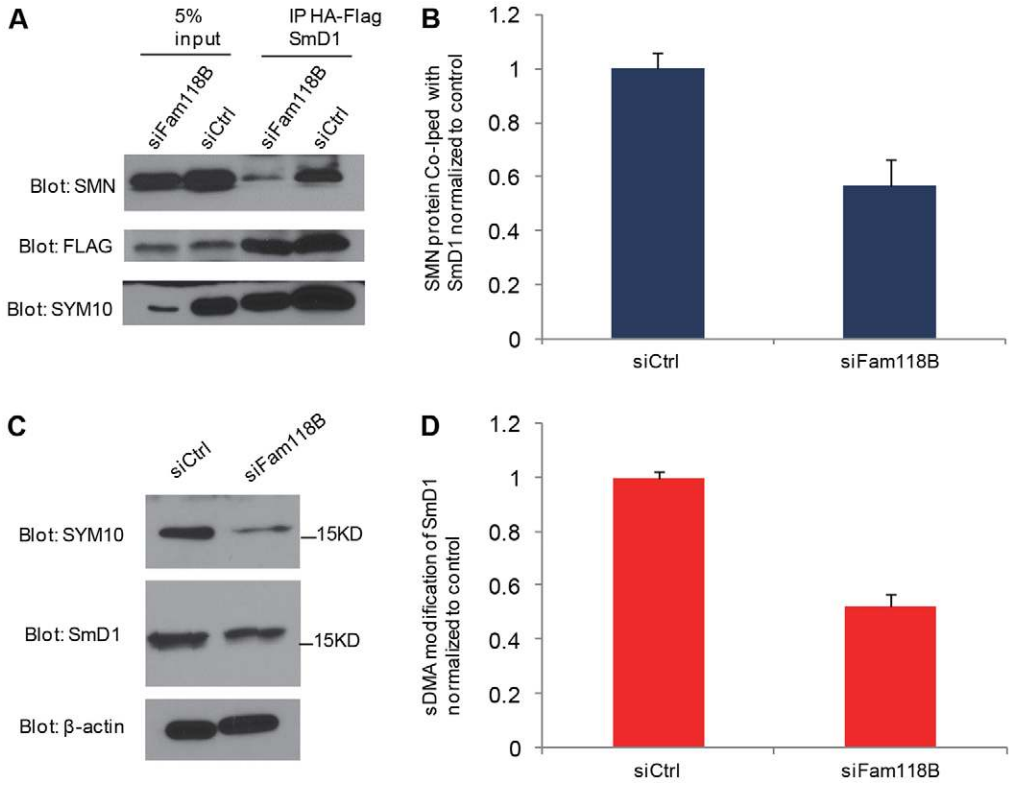

Fig. 6. Depletion of Fam118B decreases the affinity of SmD1 for SMN and reduces the amount of sDMA modification of SmD1. (A) HeLa cells that stably expressed an SmD1 fusion protein containing the HA-FLAG tag were subjected to transfection with control (siCtrl) or Fam118B (siFam118B) siRNA. Ectopically expressed SmD1 protein was immunoprecipitated by using M2 beads and was detected by using anti-FLAG antibody. The amount of co-precipitated SMN was detected by western blotting using the anti-SMN antibody. The sDMA modification of SmD1 was detected by using the anti-SYM10 antibody. (B) Quantification of the amount of SMN protein that was co-immunoprecipitated with ectopic SmD1 (normalized to control) in control or Fam118B-depleted cells. (C) HeLa cells were subjected to transfection with control or Fam118B siRNA and were harvested. Western blot analysis was performed with indicated antibodies. (D) Quantification of the amount of sDMA-modified endogenous SmD1 (normalized to control) in control or Fam118B-depleted cells. In B and D, the data show the mean±s.d. of three independent experiments.

resides in the nucleus (Fig. 1C). Therefore, it is unlikely that Fam118B would influence the initial assembly of the Sm core complex. Indeed, the interaction between various Sm core proteins was not disrupted or altered in Fam118B-depleted cells (supplementary material Fig. S3B,C). We speculate that Fam118B is in close proximity to the SMN–Sm core complex and stabilizes the sDMA modification of Sm proteins; however, the underlying mechanism remains unclear. Interestingly, several SMA-causing mutations in the *SMN1* gene prevent the binding of SMN to Sm proteins (Bühler et al., 1999; Pellizzoni et al., 1999). This raises the possibility that Fam118B, which facilitates the SMN–Sm core interaction, might also play a role in SMA pathogenesis. Therefore, it is of interest to examine whether any SMA patient harbors mutations in the *FAM118B* gene.

In summary, we have identified Fam118B as a newly discovered component of Cajal bodies, which functions in Cajal body formation, snRNP biogenesis and cell proliferation. We suspect that Fam118B carries out these functions at least in part through its ability to stabilize the SMN–Sm core complex and the sDMA modification of Sm proteins.

MATERIALS AND METHODS

Cell lines, cell culture and transfection

HeLa and HEK293T cells (American Type Culture Collection, Manassas, VA) were maintained in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Plasmid transfection was performed by using polyethylenimine reagent. To generate HeLa cells that ectopically expressed HA-FLAG-tagged SmD1, SmD3 and SmB, constructs encoding HA-FLAG-tagged SmD1, SmD3 or SmB were packaged into retroviruses by co-transfection with the packaging plasmids pPCG/P and pVSVG into 293T cells. At 48 h post-transfection, the supernatant was collected and used for the infection of HeLa cells. Infection was repeated twice with an interval of 24 h to achieve maximal infection efficiency. Infected cells were selected with medium containing puromycin (2 μg/ml).

DNA constructs

The pDONOR223 entry clones of Fam118B, SmD1 and SmB were obtained from the human ORFeome v5.1 collection. SmD3 was amplified from HeLa cDNA by PCR and cloned into the pDONOR201 entry clone. pDONOR223 Fam118B, SmD1 and SmB and pDONOR201 SmD3 were subsequently transferred to a gateway-compatible destination vector containing the HA-FLAG tag for protein expression.

Antibodies

The anti-Fam118B antibody was raised by immunizing rabbits using bacterially expressed and purified MBP–Fam118B fusion protein. Antisera were affinity-purified by the use of the AminoLink Plus Immobilization and Purification kit (Pierce). Mouse monoclonal anti-α-tubulin, anti-β-actin and anti-FLAG (M2) antibodies, and anti-FLAG (M2) affinity gel were from Sigma; rabbit polyclonal anti-coilin (H-300), rabbit polyclonal anti-SmD1 (A-9), mouse monoclonal anti-SmG

(K-15) and goat polyclonal anti-SmE (L-17) antibodies were from Santa Cruz Biotechnology; mouse monoclonal anti-SMN and anti-coilin antibodies were from BD Biosciences; rabbit polyclonal anti-fibrillarin antibody was from Abcam; rabbit polyclonal anti-SmD3 antibody was from Bethyl; rabbit polyclonal anti-SmB antibody was from GeneTex; and mouse monoclonal anti-SYM10 antibody was from Millipore.

RNA interference

HeLa or HEK293T cells were transfected twice at 24-h intervals with the indicated small interfering RNA (siRNA) using oligofectamine (Invitrogen) according to the manufacturer's instructions. The Stealth siRNAs targeting Fam118B and coilin were synthesized as 25mers from Invitrogen with the following sequences: siFam118B, 5′-CGACUGACAUGUGAGAUCUCCACAA-3′; siCoilin, 5′- AGCAUUGGAAGAGUCGAGAGAACAA-3′. The RNAi negative control (medium GC duplex) was also purchased from Invitrogen. The siRNA-resistant Fam118B constructs were generated by changing ten nucleotides within the siRNA targeting region (C946A, A948G, C949T, G951A, A954G, T957C, G960A, C963T, C966A and A969G substitutions).

Immunofluorescent staining

Cells grown on coverslips were pre-extracted with Triton X-100 for 5 min on ice and then fixed in 3% paraformaldehyde in phosphate-buffered saline (PBS) at room temperature for 15 min. After fixation, cells were subjected to further extraction for 10 min and then blocked with 5% bovine serum albumin (BSA) in PBS for 1 h at room temperature. Coverslips were immunostained with primary antibodies in 5% goat serum for 1 h. Coverslips were then washed and incubated with secondary antibodies conjugated to Rhodamine or FITC for another 1 h. Cells were then stained with DAPI to visualize nuclear DNA. The coverslips were mounted onto glass slides with anti-fade solution. Images were captured with the use of a Nikon ECLIPSE E800 fluorescence microscope equipped with a Nikon Plan Fluor 40× oil objective lens (NA 1.30) and a SPOT camera (Diagnostic Instruments). Images were analyzed with the use of Adobe Photoshop CS4.

Immunoprecipitation

Briefly, HeLa or HEK293T cells were lysed with NETN buffer (20 mM Tris-HCl pH 8.0, 100 mM NaCl, 1 mM EDTA and 0.5% Nonidet P-40, with protease inhibitors) on ice for 30 min. After centrifugation, the soluble fractions were collected and incubated with the indicated anti-Fam118B or anti-coilin antibodies for 2 h at 4℃, followed by incubation with Protein-A–agarose beads for another 2 h. The beads were then washed and the bound proteins were eluted by boiling in 2× SDS loading buffer and separated by SDS-PAGE.

GST pull-down assay

5 µg of purified GST–coilin or control GST protein was immobilized on agarose beads and incubated with 5 µg of purified MBP–Fam118B fusion protein at 4℃ for 2 h. Bound MBP–Fam118B protein was washed five times with NETN buffer, subjected to SDS-PAGE and detected by using an anti-MBP antibody.

Splicing assay using an artificial reporter

At 72 h after siRNA transfection, the pSI artificial reporter was introduced into the cells by using Lipofectamine 2000. After 24 h, cells were harvested and total RNA was extracted with the use of Trizol (Invitrogen). The resulting RNA was subsequently digested by DNaseI (Sigma), followed by reverse transcription using primer RP1. Next, primers FP1 and RP1 were used to amplify both the spliced and unspliced mRNAs, giving products of different sizes. Primers FP1 and RP2 were used to amplify the intron-containing fragment that was only present in unspliced mRNAs. Primers FP2 and RP1 were used to amplify a common fragment in both spliced and unspliced message as the internal loading control between different samples. The PCR products were separated on a 2% agarose gel. The resulting gel image was exported in a tiff format. Quantity One software (Bio-Rad) was used to quantify the intensity of the bands. The primer sequences were as follows: FP1, 5′-AGGCTTTTGCAAAAAGCTTGATTCTTCTGACACAACAG-3′; FP2, 5′-GTGTCCACTCCCAGTTCAATTACAGCTCTTAAG-3′; RP1, 5′-CTCATCAATGTATCTTATCATGTCTGCTCGAAGCG-3′; RP2, 5′-GTGGAGAGAAAGGCAAAGTGG-3′.

qRT-PCR

Total RNA from siRNA-treated cells was extracted by using Trizol (Invitrogen). Next, 2 µg of RNA was reverse transcribed with the use of the iScript cDNA Synthesis kit (Bio-Rad). cDNAs were subjected to real-time PCR with the use of Power SYBR Green PCR Master Mix (Applied Biosystem), according to the manufacturer's protocol. The primer sequences were as follows: NEAT1 forward, 5′-CAATTACTGTCGTTGGGATTTAGAGTG-3′; NEAT1 reverse, 5′-TTCTTACCATACAGAGCAACATACCAG-3′; DPP8 forward, 5′-TCTATTACCTTGCCATGTCTGGTG-3′; DPP8 reverse, 5′-AATACATTCCATAGTCCAGTGTTG-3′; NOSIP forward, 5′-CTGGAGAAGCCGTCCCGCACGGTG-3′; NOSIP reverse, 5′-CACGGCACACACGTAGCGCTCGCT-3′; DDX20 forward, 5′-TTAAGTACCCAGATTTTGATCTTG-3′; DDX20 reverse, 5′-AAGTCTGGTTTTGTCTTGTGATAA-3′; ACP1 forward, 5′-CGTGCTGTTTGTGTGTCTGGGTAA-3′; ACP1 reverse, 5′-TGTCTCATACCCGGAAGTTGCCGCGC-3′; CSDA forward, 5′-TGGGATATGGATTTATAAATCGAAAT-3′; CSDA reverse, 5′-AAAACCACATCAAACTCTACAGTTTC-3′; CD44 forward, 5′-CTGACAGAATCCCTGCTACCACTTTG-3′; CD44 reverse, 5′-TGAAGATGATTCTTTGACTCTGATG-3′; STK6 forward, 5′-GAGCCCCTGCCATCGGCACCTGAAAA-3′; STK6 reverse, 5′-GGCGACCAATTTCAAAGTCTTCCA-3′; ZNF207 forward, 5′-GTTCTTGAACAGAAAACACAAGAAAG-3′; ZNF207 reverse, 5′-CA AATATAACCTTGCTGAGGTTGAAC-3′.

Cell proliferation assays

Briefly, a total of 1.6×10^4 cells were seeded onto a 6-well plate in triplicate and were transfected with siRNA by using Lipofectamine RNAIMAX (Invitrogen) according to the manufacturer's protocol (day 1). At 24 h after transfection, siFam118B+WT cells were transiently infected with retroviral supernatant to restore Fam118B expression (day 2). At 48 h after seeding, the cells were trypsinized and counted in triplicate for each sample (day 3). The counting was repeated for 3 more days until day 6.

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Competing interests

The authors declare no competing interests.

Author contributions

Y.L. and K.W.F. designed and carried out most of the experiments; M.T., X.H., Z.G. and W.M. provided technical support; M.H., Z.S. and J.C. advised on the design of the experiments; Y.L., K.W.F., M.H., Z.S. and J.C. were responsible for the preparation of the manuscript.

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Supplementary material

Supplementary material available online at
http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.143453/-/DC1

References

Barcaroli, D., Dinsdale, D., Neale, M. H., Bongiorno-Borbone, L., Ranalli, M., Munarriz, E., Sayan, A. E., McWilliam, J. M., Smith, T. M., Fava, E. et al.

(2006). FLASH is an essential component of Cajal bodies. *Proc. Natl. Acad. Sci. USA* **103**, 14802-14807.
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.
Brahms, H., Raymackers, J., Union, A., de Keyser, F., Meheus, L. and Lührmann, R. (2000). The C-terminal RG dipeptide repeats of the spliceosomal Sm proteins D1 and D3 contain symmetrical dimethylarginines, which form a major B-cell epitope for anti-Sm autoantibodies. *J. Biol. Chem.* **275**, 17122-17129.
Briese, M., Richter, D. U., Sattelle, D. B. and Ulfig, N. (2006). SMN, the product of the spinal muscular atrophy-determining gene, is expressed widely but selectively in the developing human forebrain. *J. Comp. Neurol.* **497**, 808-816.
Broome, H. J. and Hebert, M. D. (2012). In vitro RNase and nucleic acid binding activities implicate coilin in U snRNA processing. *PLoS ONE* **7**, e36300.
Broome, H. J. and Hebert, M. D. (2013). Coilin displays differential affinity for specific RNAs in vivo and is linked to telomerase RNA biogenesis. *J. Mol. Biol.* **425**, 713-724.
Bühler, D., Raker, V., Lührmann, R. and Fischer, U. (1999). Essential role for the tudor domain of SMN in spliceosomal U snRNP assembly: implications for spinal muscular atrophy. *Hum. Mol. Genet.* **8**, 2351-2357.
Cioce, M. and Lamond, A. I. (2005). Cajal bodies: a long history of discovery. *Annu. Rev. Cell Dev. Biol.* **21**, 105-131.
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.
Darzacq, X., Jády, B. E., Verheggen, C., Kiss, A. M., Bertrand, E. and Kiss, T. (2002). Cajal body-specific small nuclear RNAs: a novel class of 2′-O-methylation and pseudouridylation guide RNAs. *EMBO J.* **21**, 2746-2756.
Fischer, U., Liu, Q. and Dreyfuss, G. (1997). The SMN-SIP1 complex has an essential role in spliceosomal snRNP biogenesis. *Cell* **90**, 1023-1029.
Fong, K. W., Li, Y., Wang, W., Ma, W., Li, K., Qi, R. Z., Liu, D., Songyang, Z. and Chen, J. (2013). Whole-genome screening identifies proteins localized to distinct nuclear bodies. *J. Cell Biol.* **203**, 149-164.
Friesen, W. J., Massenet, S., Paushkin, S., Wyce, A. and Dreyfuss, G. (2001a). SMN, the product of the spinal muscular atrophy gene, binds preferentially to dimethylarginine-containing protein targets. *Mol. Cell* **7**, 1111-1117.
Friesen, W. J., Paushkin, S., Wyce, A., Massenet, S., Pesiridis, G. S., Van Duyne, G., Rappsilber, J., Mann, M. and Dreyfuss, G. (2001b). The methylosome, a 20S complex containing JBP1 and pICln, produces dimethylarginine-modified Sm proteins. *Mol. Cell. Biol.* **21**, 8289-8300.
Gall, J. G., Tsvetkov, A., Wu, Z. and Murphy, C. (1995). Is the sphere organelle/coiled body a universal nuclear component? *Dev. Genet.* **16**, 25-35.
Gangwani, L., Flavell, R. A. and Davis, R. J. (2005). ZPR1 is essential for survival and is required for localization of the survival motor neurons (SMN) protein to Cajal bodies. *Mol. Cell. Biol.* **25**, 2744-2756.
Girard, C., Neel, H., Bertrand, E. and Bordonné, R. (2006). Depletion of SMN by RNA interference in HeLa cells induces defects in Cajal body formation. *Nucleic Acids Res.* **34**, 2925-2932.
Hebert, M. D. (2010). Phosphorylation and the Cajal body: modification in search of function. *Arch. Biochem. Biophys.* **496**, 69-76.
Hebert, M. D. and Matera, A. G. (2000). Self-association of coilin reveals a common theme in nuclear body localization. *Mol. Biol. Cell* **11**, 4159-4171.
Hebert, M. D., Szymczyk, P. W., Shpargel, K. B. and Matera, A. G. (2001). Coilin forms the bridge between Cajal bodies and SMN, the spinal muscular atrophy protein. *Genes Dev.* **15**, 2720-2729.
Hebert, M. D., Shpargel, K. B., Ospina, J. K., Tucker, K. E. and Matera, A. G. (2002). Coilin methylation regulates nuclear body formation. *Dev. Cell* **3**, 329-337.
Isaac, C., Yang, Y. and Meier, U. T. (1998). Nopp140 functions as a molecular link between the nucleolus and the coiled bodies. *J. Cell Biol.* **142**, 319-329.
Kaiser, T. E., Intine, R. V. and Dundr, M. (2008). De novo formation of a subnuclear body. *Science* **322**, 1713-1717.
Lemm, I., Girard, C., Kuhn, A. N., Watkins, N. J., Schneider, M., Bordonné, R. and Lührmann, R. (2006). Ongoing U snRNP biogenesis is required for the integrity of Cajal bodies. *Mol. Biol. Cell* **17**, 3221-3231.
Massenet, S., Pellizzoni, L., Paushkin, S., Mattaj, I. W. and Dreyfuss, G. (2002). The SMN complex is associated with snRNPs throughout their cytoplasmic assembly pathway. *Mol. Cell. Biol.* **22**, 6533-6541.
Matera, A. G. (1998). Of coiled bodies, gems, and salmon. *J. Cell. Biochem.* **70**, 181-192.
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. and Shpargel, K. B. (2006). Pumping RNA: nuclear bodybuilding along the RNP pipeline. *Curr. Opin. Cell Biol.* **18**, 317-324.
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.
Pellizzoni, L., Charroux, B. and Dreyfuss, G. (1999). SMN mutants of spinal muscular atrophy patients are defective in binding to snRNP proteins. *Proc. Natl. Acad. Sci. USA* **96**, 11167-11172.
Raker, V. A., Hartmuth, K., Kastner, B. and Lührmann, R. (1999). Spliceosomal U snRNP core assembly: Sm proteins assemble onto an Sm site RNA nonanucleotide in a specific and thermodynamically stable manner. *Mol. Cell. Biol.* **19**, 6554-6565.
Raška, I., Ochs, R. L., Andrade, L. E., Chan, E. K., Burlingame, R., Peebles, C., Gruol, D. and Tan, E. M. (1990). Association between the nucleolus and the coiled body. *J. Struct. Biol.* **104**, 120-127.
Sleeman, J. E., Ajuh, P. and Lamond, A. I. (2001). snRNP protein expression enhances the formation of Cajal bodies containing p80-coilin and SMN. *J. Cell Sci.* **114**, 4407-4419.
Sleeman, J. E., Trinkle-Mulcahy, L., Prescott, A. R., Ogg, S. C. and Lamond, A. I. (2003). Cajal body proteins SMN and Coilin show differential dynamic behaviour in vivo. *J. Cell Sci.* **116**, 2039-2050.
Tapia, O., Bengoechea, R., Berciano, M. T. and Lafarga, M. (2010). Nucleolar targeting of coilin is regulated by its hypomethylation state. *Chromosoma* **119**, 527-540.
Tucker, K. E., Berciano, M. T., Jacobs, E. Y., LePage, D. F., Shpargel, K. B., Rossire, J. J., Chan, E. K., Lafarga, M., Conlon, R. A. and Matera, A. G. (2001). Residual Cajal bodies in coilin knockout mice fail to recruit Sm snRNPs and SMN, the spinal muscular atrophy gene product. *J. Cell Biol.* **154**, 293-308.
Venteicher, A. S., Abreu, E. B., Meng, Z., McCann, K. E., Terns, R. M., Veenstra, T. D., Terns, M. P. and Artandi, S. E. (2009). A human telomerase holoenzyme protein required for Cajal body localization and telomere synthesis. *Science* **323**, 644-648.
Walker, M. P., Tian, L. and Matera, A. G. (2009). Reduced viability, fertility and fecundity in mice lacking the cajal body marker protein, coilin. *PLoS ONE* **4**, e6171.
Whittom, A. A., Xu, H. and Hebert, M. D. (2008). Coilin levels and modifications influence artificial reporter splicing. *Cell. Mol. Life Sci.* **65**, 1256-1271.
Yong, J., Kasim, M., Bachorik, J. L., Wan, L. and Dreyfuss, G. (2010). Gemin5 delivers snRNA precursors to the SMN complex for snRNP biogenesis. *Mol. Cell* **38**, 551-562.
Zhang, J., Zhang, F. and Zheng, X. (2010). Depletion of hCINAP by RNA interference causes defects in Cajal body formation, histone transcription, and cell viability. *Cell. Mol. Life Sci.* **67**, 1907-1918.
Zhang, R., So, B. R., Li, P., Yong, J., Glisovic, T., Wan, L. and Dreyfuss, G. (2011). Structure of a key intermediate of the SMN complex reveals Gemin2's crucial function in snRNP assembly. *Cell* **146**, 384-395.