The Tudor domain is an ~60-amino acid structure motif in search of a function. Herein we show that the Tudor domains of the spinal muscular atrophy gene product SMN, the splicing factor 30 kDa (SPF30), and the Tudor domain-containing 3 (TDRD3) proteins interacted with arginine-glycine-rich motifs in a methylarginine-dependent manner. The Tudor domains also associated with methylarginine-containing cellular proteins, providing evidence that methylated arginines represent physiological ligands for this protein module. In addition, we report that spliceosomal small nuclear ribonucleoprotein particles core Sm proteins accumulated in the cytoplasm when arginine methylation was inhibited with adenosine dialdehyde or in the presence of an excessive amount of unmethylated arginine-glycine-rich peptides. These data provide in vivo evidence in support of a role for arginine methylation in the proper assembly and localization of spliceosomal Sm proteins.

Protein modules that bind specific post-translational modifications are known to play key roles from signaling cascades to gene expression (1). Methylation of arginine residues was discovered over 30 years ago but is now receiving renewed attention (2). The enzymes responsible for protein arginine methylation (PRMT) have been classified into two groups. Both type I (PRMT1, PRMT3, CARM1 (PRMT4), and PRMT6) and type II (PRMT5 and PRMT7) methyltransferases induce monomethylation of arginine residues as a reaction intermediate, but type I protein arginine methyltransferases generate asymmetrical N{\textsuperscript{1}}G,N{\textsuperscript{3}}G-dimethylated arginine (aDMA) residues, whereas type II PRMTs catalyze the formation of symmetrical N{\textsuperscript{2}}G,N{\textsuperscript{4}}G-dimethylated arginines (sDMA) residues (2–5). The presence of aDMA in proteins has been implicated in several cellular processes, including protein localization, signal transduction, and transcription (2, 4, 6). Interestingly, arginine methylation has also been shown to regulate specific protein–protein interactions (7–10). For example, arginine methylation in the Sam68-proline-rich domains regulates Sam68 nuclear localization (11) and prevents its interaction with homology 3 domain binding partners, including Fyn, Lck, and Itk, without altering the affinity to the WW domains of the formin-binding proteins (7). However, a protein module that recognizes methylarginines has remained elusive.

PRMT5 was identified in a yeast two-hybrid screen as a JAK2-interacting protein and shown to be an arginine methyltransferase that methylylates histones (12, 13). Indeed PRMT5 is known to be a transcriptional regulator (9, 14–18). PRMT5 also exists as part of a 20 S cytoplasmic complex, termed the “methylosome,” with pICln and MEP50 (19–21). The methylosome has been shown to function in the cytoplasmic assembly of Sm proteins and U small nuclear RNAs into small nuclear ribonucleoprotein particles (snRNPs) (22–27). Interestingly, Sm proteins B, B′, D1, and D3 harbor sDMA in vivo, and the introduction of this modification in their arginine-glycine (RG)-rich C-terminal domains renders them better substrates for the survival of motor neuron (SMN) complex (19, 25, 28–30). Deletions or loss-of-function mutations in the SMN gene lead to spinal muscular atrophy (SMA), an autosomal recessive disease that is among the leading genetic cause of infant death (31–33). SMA is characterized by a degeneration and loss of spinal cord motor neurons resulting in progressive muscle atrophy, paralysis, and death from respiratory distress (34). The level of functional SMN gene product in patients correlates with the severity of the disease (35, 36). SMA patients who do not have a deleted Smn1 gene often display mutations in conserved regions of the SMN protein (37, 33). One such region in SMN for which the physiological relevance is highlighted genetically by SMA-causing mutations (38–40) is the Tudor domain encoded by exon 3. The Tudor domain is an ~60-amino acid conserved domain of unknown function that was initially found to be present in proteins that associate with nucleic acids (41). However, x-ray crystallography structural analysis of the SMN Tudor fold reveals a barrel-like structure composed of β-sheets forming a hydrophobic pocket surrounded by negatively charged residues that more likely constitute a protein–protein interaction surface (42). Indeed, the Tudor domain mediates several of the interactions between SMN and its RG-containing binding partners (29, 42–45).

Maurer-Stroh et al. (48) have uncovered a common structural fold between the Tudor domain and the HP1 Chromo domain, a motif that is known to interact with methylated lysines in histones (46, 47), which led them to propose that methyl-substrate binding might be a general feature of these domains and their common ancestor. By using nuclear magnetic resonance analysis, it was recently shown that a cluster of conserved aromatic residues in the SMN Tudor domain contact
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sDNA residues (49) and methyl-dependent binding of SMN to Sm proteins, p80-coilin, and Epstein-Barr virus nuclear antigen 2 (EBNA2) is thought to be mediated through the SMN Tudor domain (29, 43, 50). However, there are conflicting reports regarding whether or not methylation of the RG-rich region in substrate proteins is required for their interactions with Tudor domains (45, 51, 52). This prompted us to address the capacity of the Tudor domain to function as a general methylarginine-binding protein module. We report here that the Tudor domain of SMN interacted directly, in a methyl-dependent fashion, with several endogenous sDNA-containing proteins. We also show that the Tudor domains of SPF30 and TDRD3 also bind proteins containing methylated arginines. Our data demonstrate that methylated arginines represent physiological ligands for this protein module and suggest that the Tudor domain may be a general methyl-binding module.

MATERIALS AND METHODS

DNA Constructs—The human SMN Tudor domain was amplified from the SMN expression vector (53) by using PCR with oligonucleotides 5'-CGG GGA TCC AAA CAG TGG AAA GTT GG-3' (Harbing HamH1 and EcoRI sites of pGEX-4T2 (Amersham Biosciences). GST-fusion proteins were eluted from the beads with 60 mM glutathione and the labeled proteins were visualized by fluorography using [3H]methionine (Sigma) or adenosine-2',3'-dialdehyde (AdOx) (Sigma), each at a final concentration of 250 μM. The treated cells were lysed in lysis buffer and incubated with the GST-Tudor fusion proteins as described above. The beads were washed with SDS-PAGE, transferred to nitrocellulose, and immunoblotted with anti-SYM10, -SYM11, or -Sam68 antibodies (Upstate Biotechnology, Lake Placid, NY), anti-SmB/D monoclonal antibody (ICN Pharmaceuticals, Costa Mesa, CA), anti-SmE (Santa Cruz Biotechnology, Santa Cruz, CA), or anti-actin. The secondary antibodies were goat anti-mouse or goat anti-rabbit conjugated to horseradish peroxidase (ICN Pharmaceuticals), and chemiluminescence was used for protein detection (PerkinElmer Life Sciences).

RESULTS

Tudor Blot Overlay Assay—HeLa cell lysates were prepared with lysis buffer as described above, and the cellular proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked for 1 h at room temperature in Tris-buffered saline containing 0.5% Tween 20 with 2% nonfat dry milk. The membranes were then incubated with 1 μg/ml purified GST-SMN-Tdr in the same buffer for 1 h at 4 °C. The filters were washed in Tris-buffered saline containing 0.5% Tween 20, and the bound GST-SMN-Tdr was visualized by immunoblotting with anti-GST antibodies. The anti-GST antibodies were raised in rabbits using purified GST as an antigen. The secondary antibody was a goat anti-rabbit conjugated to horseradish peroxidase (ICN Pharmaceuticals), and chemiluminescence was used for protein detection (PerkinElmer Life Sciences).

Peptide Binding Assays—Biotin was added at the N terminus of each peptide using EZ-Link N-hydroxysuccinimido (Pierce). For the peptide/GST-Tudor binding assays the peptides were bound to streptavidin-agarose (Sigmoda) to generate peptide affinity columns, which were then incubated for 30 min at 4 °C with increasing concentrations (10–40 ng) of purified GST-Tudor proteins in lysis buffer. The beads were washed with lysis buffer, and the bound GST-Tudor proteins were separated by SDS-PAGE and detected by immunoblotting with anti-GST antibodies.

Cell-permeable Peptides and Immunofluorescence Staining—HeLa cells were plated on glass coverslips and incubated for 3 h with 20 μM (RG)1 or (R153G)1 peptides coupled at their N termini with the Antennapedia internalization sequence peptide (rhodamine-RQIKIWFQPGRMK) (Pierce Endogen)). The cells were then washed with 4% paraformaldehyde in 1× PBS, pH 7.4, and permeabilized with 0.5% Triton X-100 in 1× PBS. The peptide intake by the cells was visualized directly using the rhodamine fluorescence tag on the peptide. Indirect immunofluorescence staining was performed using anti-SmB/D antibodies at 1:100 in 1× PBS. The secondary antibody (1:300) was an Alexa Fluor 488 goat anti-mouse antibody from Invitrogen. The nuclei were counterstained with 4,6-diamidino-2-phenylindole, and the cells were visualized by fluorescence microscopy.

Tudor Domains Bind Endogenous Methylated Proteins—We generated a recombinant fusion protein consisting of GST fused to amino acids 90–149 of SMN encompassing the Tudor domain (GST-SMN-Tdr). The fusion protein was coupled to glutathione-agarose and incubated with extracts prepared from HeLa cells grown in the presence of L-[methyl-3H]methionine and translation inhibitors, a procedure that results in the labeling of endogenously methylated proteins (11). Interestingly, GST-SMN-Tdr bound several methylated cellular proteins that were not bound by GST alone (Fig. 1A). To determine whether this observation could be generalized, we used two additional Tudor domains for our analysis: one from SPF30 and the other from TDRD3. A proteomic analysis of the complete repertoire of the mammalian splicing machinery identified SPF30 (also known as SMNrp) and TDRD3 (55–57). SPF30 has been characterized biochemically and shown to be an essential splicing factor (58–60). In contrast, TDRD3 (RFSEQ, NM_030794.1), which has a predicted molecular mass of 73 kDa, is known to be expressed based on the presence of several expressed sequence tags but is uncharacterized. To determine whether the Tudor domains of SPF30 and TDRD3 also interact with methylated proteins, GST pull-down experiments were performed as above, with lysates prepared from in vitro [3H]methionine-labeled extracts. Similar to GST-SMN-Tdr, GST-SPF30-Tdr and GST-
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Tudor Domains of SMN, SPF30, and TDRD3 Contact Directly sDMA-containing Polypeptides—Our in vivo labeling experiment demonstrated that Tudor domains predominantly bound methylated proteins in the cell but did not address whether the proteins contained methylated arginines. HeLa cell extracts were incubated with GST-Tudor domains or GST alone, and the bound proteins were immunoblotted with the sDMA-specific antibodies SYM10 and SYM11 (61, 62). Each Tudor domain bound several cellular sDMA-containing proteins from the subset of polypeptides recognized by SYM10/11 (Fig. 2A, lanes 3–5). It is known that SMN binds methylated p80-coilin and Sm proteins (29, 30, 43). Indeed p80-coilin, SmB/B’, and SmD1/SmD3 were confirmed by immunoblotting with anti-p80-coilin and Sm antibodies (Fig. 2B). As predicted from the structural analysis of the Tudor domain (42) the E134K amino acid substitution that was introduced within GST-SMN-Tdr abolished the binding to sDMA-containing interactors (Fig. 2A, lane 6).

Arginine methylation was shown previously to enhance the binding of Sm proteins to the SMN complex (29, 30). To ask whether the methyl mark was required for the binding of Sm proteins to the isolated Tudor domains, we performed GST-pull-down assays with protein extracts prepared from HeLa cells grown in the presence or not of 250 μM MTA, a competitive inhibitor of methyltransferases. This treatment resulted in a reduction of ~50% of the methylated epitopes recognized by SYM10 (Fig. 2C, compare lanes 1 and 2), whereas the levels of Sam68 protein remained equivalent and served as a loading control (Fig. 2C, lanes 3 and 4). Strikingly, the amount of SmB/D proteins bound by all three GST-Tdr fusion proteins was less in the MTA-treated samples (Fig. 2D, compare lanes 5 and 6, 7 and 8, 9 and 10). These findings suggest that methylation is indeed required for stable interaction with Tudor domains.

To determine whether the Tudor domains recognize methylated SmB/B’ and SmD proteins in the context of mature snRNP complexes, we examined whether SmE, a core Sm protein known not to be methylated (28, 62), was associated with the GST-Tudor domains (Fig. 2E). The presence of SmE in addition to SmB and SmD proteins was detected using the SMN or the SPF30 GST-Tudor proteins as an affinity matrix (Fig. 2E, lanes 3 and 4). In contrast, the TDRD3 GST-Tudor protein interacted strongly with methylated SmB/B’ and SmD but not with SmE (Fig. 2E, lane 5). These results suggest that...
the Tudor domains of SMN and SPF30 likely interact with assembled snRNPs, whereas the Tudor domain of TDRD3 might bind unassembled methylated Sm proteins.

To examine whether the Tudor domains bound sDMA directly, in vitro binding reactions were used with (RG)₄-biotinylated peptides containing either no modification or sDMA (Fig. 3A). This motif represents a shortened version of the C-terminal RG-rich domain of SmD1, which is known to harbor sDMA in vivo (28) but also represents a common motif found in many PRMT5 substrates (9, 14, 19, 28, 29, 50, 62). GST-SMN-Tdr bound the sDMA peptide in a dose-dependent manner but did not bind the unmethylated peptide in this assay (Fig. 3A, compare lanes 5–7 with lanes 2–4, respectively). This binding was significantly reduced with the introduction of the E134K mutation in the SMN-Tudor domain (Fig. 3A, GST-E134K-Tdr). GST-TDRD3-Tdr bound to the sDMA-methylated peptide and weakly to the nonmethylated peptide at higher protein concentrations. A similar profile was obtained for the GST-SPF30-Tdr protein. These data show that Tudor domains from three different proteins can bind directly to sDMA-containing peptides. To further confirm direct methyl-dependent interactions with cellular proteins, we performed a blot overlay on cell extracts that were treated or not with AdOx, a general inhibitor of S-adenosylmethionine-dependent methyltransferases. AdOx reduced methylation levels by ~50% as assessed by immunoblotting with SYM10 (Fig. 3B, lanes 3 and 4). GST-SMN-Tdr bound directly to several distinct proteins, and this binding was absent or reduced in AdOx-treated extracts (Fig. 3B, lanes 1 and 2). Immunoblotting with anti-actin antibodies confirmed equal loading (Fig. 3B, lanes 5 and 6). Taken together, these results strongly suggest that the Tudor domains of SMN, but also those of SPF30 and TDRD3, are methyl-binding protein modules.

Arginine Methylation Is Required for Spliceosomal snRNP Proteins Accumulation in the Nucleus—SMN, coordinately with the sDMA-generating methylosome, was shown to promote cytoplasmic assembly of spliceosomal snRNPs, as well as
their import back into the nucleus (22, 63–65). To address whether the driving force behind snRNP assembly is indeed methylation, we used the general methylase inhibitor AdOx and determined the localization of endogenous Sm proteins by indirect immunofluorescence staining (Fig. 4A). HeLa cells were grown in the presence of MeSO or 250 μM AdOx for 20 h. We have previously observed drastic effects on intracellular protein localization using a similar dose of AdOx (11), even though we are able to detect only an ~50–60% decrease in methylation by immunoblotting. The cells were fixed and immunofluorescence performed to visualize the snRNP proteins with an anti-SmB/D antibody (ana128). Sm proteins accumulated in the cytoplasm in the presence of AdOx (Fig. 4A, compare b and e), showing that methylation is important for the proper nuclear localization of the Sm proteins in vivo. To determine whether the observed effect was due to an inhibition of Sm protein arginine methylation, HeLa cells were grown in the presence of excess amounts (20 μM) of nonmethylated or sDMA-containing peptides mimicking the C-terminal tail of SmD1 coupled to the Antennapedia internalization sequence (66). The cell-permeable peptides localized throughout the cell with accumulation in the nucleolus (Fig. 4B, b and e), a common feature of positively charged peptides (67). The cellular distribution of snRNP proteins in the presence of excess amounts of the methylated (sDMA-G)4 peptide was exclusively nuclear with extranuclear staining (Fig. 4B, f), as observed in untreated cells (Fig. 4A, b). In contrast, the nonmethylated RG-rich peptide caused a partial accumulation of snRNPs or free Sm proteins within the cytoplasm (Fig. 4B, c). These findings suggest that a fine balance between nonmethylated versus methylated Sm proteins is crucial for normal assembly and nuclear import of snRNPs.

DISCUSSION

The Tudor Domain of SMN Interacts with Several Endogenous Methylated Proteins—The Tudor domain is essential for the function of SMN in the cytoplasmic assembly of spliceosomal snRNPs (68). This is thought to be mediated at least in part through direct interaction of the Tudor domain with Sm proteins (42, 68). Further evidence for the functional relevance of this domain came recently, as a number of new Tudor domain point mutations were uncovered in SMA type I patients (38, 40). However, other reports suggest that the Tudor domain is not sufficient for interaction and that the highly conserved C terminus of SMN is also required (23, 69–73). Our results lend support to the notion that the isolated Tudor domain of SMN is sufficient to bind directly and preferentially to sDMA-containing RG motifs. In addition to Sm proteins and p80-coilin, we

FIG. 3. Tudor domains contact directly sDMA-containing polypeptides. A, biotinylated (RG)4 peptides containing either unmethylated arginine or sDMA were bound to streptavidin-agarose and used as affinity columns to measure the binding of purified GST fusion SMN, SMN E134K, TDRD3, and SPF30 Tudor domains. The bound GST-Tudor fusion proteins were resolved by SDS-PAGE, transferred to nitrocellulose, and detected by immunoblotting with anti-GST antibodies. Input represents 40 ng of purified protein. B, total protein extracts were prepared from cells grown in the presence or not of the methylase inhibitor AdOx, resolved by SDS-PAGE, and transferred to a nitrocellulose filter. The filters were either blot overlaid with GST-SMN-Tdr followed by immunoblotting with anti-GST antibodies (lanes 1 and 2) or with anti-SYM10 (lanes 3 and 4) or anti-actin antibodies (lanes 5 and 6).

FIG. 4. The role of arginine methylation in regulating snRNP localization. A, HeLa cells grown in the presence of the methylase inhibitor AdOx were prepared for immunofluorescence with anti-SmB/D antibodies followed by an Alexa Fluor 488 (green)-conjugated goat anti-mouse antibody (b and e). The nuclei were stained blue with the nuclear stain 4’,6-diamidino-2-phenylindole (DAPI) (a and d). The composite views represent the merging of a and b (c) and the merging of d and e (f). The cells were visualized by fluorescence microscopy. DMSO, dimethyl sulfoxide. B, HeLa cells were incubated with cell-permeable peptides at 20 μM for 3 h. The peptides were rhodamine (Rho)-Antennapedia (AP) internalization sequence (AP)-RG4 or RhoAP-(R4DMA)4. The cells were prepared for immunofluorescence as described in A. The nuclei are visualized in a and d. The rhodamine peptides are observed in b and e, and the localization of the snRNP with ana128 (green) is observed in c and f.

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show that the SMN Tudor domain also interacts with several endogenous sDMA-containing proteins and that the SMA-causing E134K mutation abrogates these interactions. Selenko et al. (42) have demonstrated previously that the E134K mutation does not affect the proper folding of the SMN Tudor domain but rather alters the charge distribution within the postulated binding surface. These results suggest that methyl-dependent interactions are likely to be central for the cellular roles of SMN.

It is intriguing that others have not observed differential binding of SMN to certain RG-containing substrates in the presence of arginine methylation (45, 51). For example, asymmetrical dimethylation of GAR1 and fibroblastin have been reported as not influencing binding to the SMN Tudor domain (45). Using surface plasmon resonance, Young et al. (51) have observed an increase in the dissociation rate between SMN and a Ewing sarcoma-derived sDMA-containing peptide, albeit using full-length SMN protein. It is also worth noting that Ewing sarcoma was shown to harbor sDMA in vivo (74, 75) and thus might not represent a natural sDMA-containing substrate motif. Alternatively, Tudor domains may be able to distinguish between asymmetrical and symmetrical dimethylation and may have different affinities for the two types of modifications. The (RG)_n motif employed in our study is found in SmD1 and SmD3 as well as p80-coilin and thus represents a true physiological binding site for the Tudor domain of SMN.

Tdr Domains as General Methyl-binding Modules?—Using nuclear magnetic resonance, Sprangers et al. (49) have uncovered residues in the SMN Tudor domain that are in close proximity to the sDMA moiety in the substrate peptides. This binding pocket comprises a cluster of aromatic residues including Trp-102, Tyr-109, Tyr-127, and Tyr-130. Interestingly, an alignment of the Tudor domains utilized in the present study illustrates that these aromatic residues are either perfectly conserved or replaced by a different aromatic residue in SPF30 and TDRD3 (Fig. 5). This is consistent with the fact that we found both of these Tudor domains to bind preferentially to sDMA-containing polypeptides. The only other Tudor domain to have been characterized biochemically is found in the DNA repair protein, 53BP1 (76). 53BP1 harbors a “Tudor tandem,” a new structural motif composed of two tightly packed Tudor repair protein, 53BP1 (76). 53BP1 harbors a “Tudor tandem,” a sDMA-containing polypeptides. The only other Tudor domain found both of these Tudor domains to bind preferentially to conserved or replaced by a different aromatic residue in SPF30 illustrates that these aromatic residues are either perfectly alignment of the Tudor domains utilized in the present study

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with the SMN, SPF30, and TDRD3 in the present study. Strikingly, this same Tudor tandem was recently found to interact with methylated Lys-79 of histone H3 (77). Because only a fraction of the whole repertoire of methylated SMN-Tudor-interacting proteins were identified as being sDMA-containing (compare Figs. 1 and 2), it suggests that the Tudor domains may also recognize other methylated residues.

Tudor Domains in Pre-mRNA Splicing—SPF30 is present in early spliceosomal complexes (57) and is thought to mediate U4/U5/U6 tri-snRNP docking to the A complex during spliceosome assembly (58, 59). In our recent proteomic survey of arginine-methylated protein complexes, we have identified several splicing factors that are potentially methylated and harbor RG-rich motifs (61). The nuclear pool of SMN is also thought to play a role in pre-mRNA splicing (78–80), although the precise molecular mechanism involved remains unclear. SMN is not a bona fide spliceosome component, and it was proposed that it might act at the level of snRNP recycling following a round of splicing (78). Our data demonstrating that two spliceosomal factors (SPF30 and TDRD3) can bind specifically to methylated proteins might provide a molecular mechanism for our previous observation that hypomethylated nuclear extracts display reduced pre-mRNA splicing activity (62).

Arginine Methylation as a Driving Force for snRNP Assembly and Nuclear Import—The PRMT5 methylosome catalyzes the formation of sDMA in the RG-rich C-terminal tail of Sm proteins (19, 20, 80). According to the current model, this triggers the transfer of Sm proteins to the SMN complex, which then serves as a molecular scaffold or chaperone, to assemble Sm proteins onto the incoming U small nuclear RNAs. This properly assembled Sm core, the 5’-m3G-cap structure, and 3′ end processing of the small nuclear RNAs are prerequisites for U snRNPs import back to the nucleus (81–86). SMN is known to form a cytoplasmic, pre-import RNP complex with the import adaptor snurportin1, suggesting that nuclear import of snRNPs and SMN is coupled (64, 65). We report here that Sm proteins (we cannot distinguish between individual Sm proteins and assembled snRNPs in this experiment) accumulate in the cytoplasm in the presence of a general methylation inhibitor (Fig. 4A). This result can be interpreted as an indication that snRNP assembly is hindered by the absence of PRMT5 methylation activity. This in turn would prevent SMN and/or snurportin recruitment, and nuclear import.

We reasoned that introducing excess peptides corresponding to the methylated C-terminal domain of Sm proteins should saturate the assembly process and result in an accumulation of Sm proteins in the cytoplasm. The fact that we actually saw no effect with the sDMA-containing peptide but rather observed the expected Sm cytoplasmic accumulation with the nonmethylated RG peptide was somewhat surprising and we think could be reconciled as follows. In the methylsome, pICln interacts with Sm proteins through their conserved Sm domains, whereas PRMT5 interacts with the C-terminal RG domains
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with preference for the unmethylated proteins (19, 21). In addition, another member of this complex, MEP50, also binds a subset of the Sm proteins (87). Hence, it is likely that the nonmethylated peptides interfere with sDMA assembly by saturating the available PRMT5 complex, generating a pool of Sm proteins that cannot be methylated, which in turn prevents their efficient transfer to the SMN complex. This would also indicate that the amount of the cytoplasmic PRMT5 complex is limited in comparison with the SMN complex because a fraction of the cytoplasmic SMN complex is still capable of mediating sDMA assembly.

In summary, we show that several endogenous proteins harboring sDMA are natural ligands for the Tudor domain of SMN. The identification of SPF30 and TDRD3 as methyl-binding proteins also demonstrates that the methyl-binding properties of the Tudor domain may be generalized to at least certain other Tudor domains. Finally, we provide in vivo evidence supporting a role for arginine methylation in the proper localization of spliceosomal sRNA Sm proteins.

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