The TUDOR domain of SMN is an H3K79me1 histone mark reader

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Summary Blurb

Spinal Muscle Atrophy (SMA) is caused by mutation or deletion of SMN1 gene. Survival Motor Neuron (SMN) protein associates with histone H3 mono-methylated on lysine 79 (H3K79\(^{\text{me1}}\)) through its central TUDOR domain. SMA-linked mutations occur within the TUDOR domain and prevent association with histone H3.

Abstract

Spinal Muscle Atrophy (SMA) is the leading genetic cause of infant mortality and results from the loss of functional Survival Motor Neuron (SMN) protein by either deletion or mutation of the SMN1 gene. SMN is characterized by a central TUDOR domain, which mediates the association of SMN with arginine methylated (R\(^{\text{me}}\)) partners, such as COILIN, FIBRILLARIN, and RNApolII. Herein, we biochemically demonstrate that SMN also associates with histone H3 monomethylated on lysine 79 (H3K79\(^{\text{me1}}\)), defining SMN as the first known H3K79\(^{\text{me1}}\) histone mark reader, and thus the first histone mark reader to recognize both methylated arginine and lysine residues. Mutational analyses provide evidence that SMN\(^{\text{TUDOR}}\) associates with H3 via an aromatic cage. Importantly, most SMN\(^{\text{TUDOR}}\) mutants found in SMA (SMN\(^{\text{ST}}\)) patients fail to associate with H3K79\(^{\text{me1}}\).

Introduction

Although the loss of SMN1 was found in 1995 as responsible for the monogenic pathology SMA (1), it took until 2019 to develop a gene therapy treatment approved by the FDA (2). However, long-term efficacy of such treatment remains unknown and not appropriate to all patients [reviewed (3,4)]. It is thus critical to continue basic research on SMN biochemistry for SMA patients and for general knowledge, which is likely to impact on other neurological disorders with common biochemical pathways, such as amyotrophic lateral sclerosis (ALS) and Fragile X syndrome.

SMN, the protein, also known as GEMIN1, is principally recognized as a marker of membrane-less nuclear structures called Cajal bodies, first identified in neuronal tissues by Santiago Ramón y Cajal (5) and recently found to phase separate (6). SMN most documented cellular function is to assemble snRNPs and thus regulate RNA metabolism and splicing [reviewed in (7,8)]. Essentially, SMN cellular functions are associated with post-translationally modified or unmodified proteins (14,15). Previous work from our team suggests that SMN associates with histone H3 and localizes with damaged centromeres in a DOT1L methyltransferase-dependent manner (16). Although DOT1L is often depicted as the only histone H3 lysine 79 (H3K79) methyltransferase (17,18), Dot1f\(^{\text{−/−}}\) knockout cells retain H3K79\(^{\text{me2}}\) albeit at extremely low levels (0.5% in Dot1f\(^{\text{−/−}}\) versus 3.3% H3K79\(^{\text{me2}}\) in wild-type cells) (19), suggesting that there may be other methyltransferase(s) capable of modifying H3K79. There are indeed a few studies suggesting that NSD1 and NSD2 methyltransferases could mono- and di-methylate H3K79 (20,21).

Histones and histone post-translational modifications (histone marks) are central to chromatin signalling pathways. Essentially, genomic DNA is wrapped around small basic proteins called histones to form nucleosomes, a repetitive unit constituting the chromatin framework, which regulates access to genetic information. Generally, histone modifications such as H3K4\(^{\text{me3}},\) H3K9\(^{\text{me1}},\) and H3K79\(^{\text{me1}}\) mark the chromatin for access to the genetic information, while modifications such as H3K9\(^{\text{me3}}\) and H3K27\(^{\text{me3}}\) marks restrict access to the genetic information (22). Regarding H3K79\(^{\text{me1}}\), the mark correlates with alternative splicing patterns between cell lines (23), in agreement with the positioning of H3K79\(^{\text{me1}}\)-marked nucleosomes on exons (24). More precisely, the H3K79\(^{\text{me1}}\) and H3K79\(^{\text{me2}}\) marks are found at alternative 3′ and 5′ splice sites (25). The H3K79\(^{\text{me1}}\) mark is the most prominent state of methylation on H3K79 in mouse ES cells (19). Notably, the mouse model of Dot1f\(^{\text{−/−}}\) is embryonic lethal while Dot1f\(^{\text{−/−}}\)-derived cells harbor alternative lengthening of telomere (ALT) phenotype (19). Moreover, DOT1L plays an important role in neuronal development (26).

Herein, using biochemical approaches, purified recombinant proteins, and recombinant nucleosomes, we define SMN\(^{\text{TUDOR}}\) as the first H3K79\(^{\text{me1}}\) mark reader, and also the first histone mark reader capable of reading both R\(^{\text{me}}\) and K\(^{\text{me}}\) states. Importantly, SMA-linked SMN mutations (SMN\(^{\text{ST}}\)) prevent SMN-H3 interactions, suggesting the involvement of chromatin signalling pathways in SMA genetic pathology.
Results

Purified recombinant SMN interacts directly with histone H3 in vitro

We previously reported that SMN associates with damaged centromeres in a DOT1L-dependent fashion (16), suggesting that SMN could associate with histone H3 methylated on lysine 79. Indeed, initial investigations suggested that SMN associates with H3K79me2 peptides (16). Herein, we thus aimed to further characterize biochemically this potential interaction. Using recombinant human SMN purified from *Escherichia coli* by a GST affinity purification scheme, GST-SMN was subjected to pulldown assays in the presence of calf thymus histones (CTH), a classic source of modified histones (27). In agreement with previous work (16), GST-SMN was capable to associate directly with histone H3 while the GST alone control failed to associate with histones (Figure 1A). Similar experiments were conducted with the PHD domain of the H3K4me3 reader ING3 (ING3 PHD) and a characterized aromatic cage mutant known to be unable to associate with H3 (ING3W385A) (28,29), as positive and negative controls, respectively. These pulldowns were analyzed by immunoblotting against core histones and histone variants. As expected, ING3 PHD associated with H3, while the aromatic cage mutant ING3W385A failed to do so (Figure 1B). As seen in Figure 1A, we observed that GST-SMN associates predominantly with H3 (Figure 1B). Extended immunoblots for each pulled down proteins are provided in supplementary materials (Figure S1A). These experiments validate the association of SMN with histone H3.

An intact TUDOR domain is required for SMN to interact with H3

As SMN harbors a TUDOR domain, which is found in several other histone mark readers (30), we then set out to define the minimal region of SMN required for H3-binding and generated a panel of truncated forms (Figure 2A). Using these, we found that deletion of either the amino terminus (SMN\text{\text{N\text{N}}}) or the carboxy terminus (SMN\text{\text{C\text{C}}}) affected the association with H3 minimally (Figure 2B). However, the TUDOR domain on its own (SMN\text{TUDOR}) failed to associate with H3 as well as the full-length form of SMN, but seemed to be required for the interaction since the amino terminus (SMN\text{\text{N\text{N}}}) and carboxy terminus (SMN\text{\text{C\text{C}}}), which lack the TUDOR domain, only bound weakly to H3 (Figure 2B). Thus, we conclude that the TUDOR domain is required in vitro, but not sufficient for SMN to associate with H3. We then extended the TUDOR domain on both sides, with actual SMN wild-type sequences, and found that an extension by 25 amino acid residues on its amino terminal side restored to some extent the association of SMN with H3 (Figure 2C). Extension on the carboxy terminal end of SMN\text{TUDOR} by 5, 10, or 15 residues did not appear to improve the association of SMN to histone H3 (Figure S1B). We conclude that SMN\text{TUDOR} is necessary and sufficient for association with histone H3, but requires additional residues outside the classical defined TUDOR domain.

The solution structure of SMN bound to R\text{\text{N\text{N}}} residue displays an aromatic cage composed of W102, Y109, Y127, and Y130 (31). Aromatic cages are broadly found in histone mark readers and involved in sensing methylation states (30). To demonstrate the importance of the TUDOR domain in mediating the interaction between SMN and H3, we have mutated W102, Y109, Y127, and Y130 aromatic cage sites (SMN\text{\text{AC\text{\text{N\text{N}}}}}) individually to alamines and performed pulldown assays to assess the interaction of SMN mutants with H3. Unexpectedly, unlike other readers such as ING4 (32), HP1\text{\text{C\text{\text{C}}}C}, or MPP8 (33), single mutations to alanine within the aromatic cage did not appear to alter SMN\text{\text{AC\text{\text{N\text{N}}}}} binding to H3 and retained the capacity to associate with H3 (Figure 3A). We thus mutated these four residues in various combinations and found that W102 and Y130 seemed to be the most important residues of the aromatic cage as all the SMN forms that had reduced binding affinity to H3 contained W102A and Y130A mutations (Figure 3B-C). Together, these results demonstrate that SMN associates directly with H3 and requires an intact TUDOR domain aromatic cage.

SMA-linked SMN\text{TUDOR} mutants fail to associate with H3

In about 10% of SMA cases, *SMN1* is not deleted, but mutated. These mutations aggregate mostly in the dimerization domain or within the TUDOR domain [reviewed in (4)]. Given that SMN associates with H3 through an aromatic cage within its TUDOR domain (Figures 2-3), we investigated whether SMA-linked TUDOR mutations (SMN\text{\text{ST}}) impact on the association of SMN with histone H3. Experiments with the aromatic cage mutant SMNY109C and the other SMA-linked mutant SMNE134K seemed to show that E134K minimally impact the capacity of SMN to interact with H3 (Figure 4A). We thus expanded our panel to include all known SMN\text{\text{ST}} (4). Interestingly, SMN\text{\text{ST}} V94S, G95R, A111G, and I116F had impaired capacity to associate with histone H3, while V94G, Y109C, Y130C, E134K, and Q136E retained approximately wild-type level of binding to H3 (Figure 4B). These results suggest that SMA may arise in some cases from impaired SMN-H3 interactions or other interactions requiring an intact SMN\text{TUDOR}.

SMN\text{TUDOR} reads H3K79me1
Defining SMN as the first H3K79me1 reader

Previous work suggests that SMN may associates with H3 sequences surrounding the lysine 79 methylation site (16). We thus analyzed GST-SMN pulldowns by immunoblotting against methylated H3K79 forms and found that the H3 species that associate with SMN are predominantly the H3K79me1 and H3K79me2 forms (Figure 5A). Although controversial, another TUDOR domain protein, 53BP1, was also reported to associate with methylated H3K79 (34,35). We thus investigated how SMN associates to histones compared to 53BP1 and found that SMN bound to H3 with the H3K79me2 mark and modestly to H4 with the H4K20me2 modification at least as well as 53BP1 if not better (Figure 5B). We then used the H3K4me3 reader ING3PHD as a control to assess the enrichment of the H3K79 methyl marks by SMN. We found that the H3 pulled down by SMN was enriched with the H3K79me1 mark compared to ING3PHD (Figure 5C), suggesting that SMN may associate with this mark (i.e. H3K79me1). Hitherto, our results show that SMN associates with H3 harbouring the H3K79me1/me2 marks, but does not demonstrate that SMN associates with the H3K79me1/me2 marks themselves.

As mentioned above, although SMN associates with H3 methylated on lysine 79 (Figures 4 and 5), our results do not demonstrate that SMN associates directly with the H3K79 methylated marks. We thus performed pulldowns using synthetic biotinylated peptides, which are either unmodified, mono-, di-, or tri-methylated on H3K79 and recombinant SMN. While performing various contraptions using peptide pulldown assays, we found that SMN associates preferentially with H3K79me1, while 53BP1 bound to the H3K79me2 peptide (Figure 5D). We thus conclude so far that SMN associates directly with H3K79me1, at least in vitro using biochemical assays.

Interestingly, the tyrosine residues (Y109, Y127, and Y130) of the aromatic cage are reported to be phosphorylated (36). Tyrosine phosphorylation within aromatic cages is hypothesized to regulate reader-mark interactions (33). Conversion of Y109, Y127, or Y130 to aspartic acid, to mimic the negative charge of the phosphate moiety, appears to reduce to binding of SMN to H3K79me1 (Figure 5E), suggesting that Yphos of SMNTUDOR could regulate the association of SMN with methylated partners, such as H3. Our in vitro biochemical characterization identifies SMN as the first known H3K79me1 histone mark reader.

As we defined SMN as an H3K79me1 reader, we thus assessed the impact of SMNTUDOR on binding to H3 methylated on K79. Similarly to H3 (Figure 4B), W92S, Y109C, A111G, and I116F had an impact on the association of SMN with H3K79me1, while V94G, G95R, Y130C, E134K, and Q136E had no apparent effect on the binding of SMN (Figure 5F).

The H3K79me1 mark is, unlike histone modifications, such as H3K4me3 and H3K36me3, not found on the unstructured histone tail, but within the histone H3 core region (37). Thus, the nucleosome inherent structure may impact on the accessibility of H3K79me1 to potential readers, such as SMN. To assess this possibility, we generated recombinant nuclear core particles (rNCP) containing histone H3 containing mutations C110A and K79C (K C79) to specifically modify K C79 with a monomethyl-nucleoside analog. Unmodified H3K C79 lacks histone tails, but within the histone H3 core region (37). Thus, the nucleosome inherent structure may impact on the accessibility of H3K79me1 to potential readers, such as SMN. To assess this possibility, we generated recombinant nuclear core particles (rNCP) containing histone H3 containing mutations C110A and K79C (K C79) to specifically modify K C79 with a monomethyl-nucleoside analog. Unmodified H3K C79 was therefore used as a control to assess the enrichment of the H3K79 methyl marks by SMN. We found that the H3 pulled down by SMN was enriched with the H3K79me1 mark compared to unmodified rNCP-H3KC79me0 form (Figure 5G). Moreover, most SMA-linked SMNTUDOR mutants failed to associate with H3K C79me1 nucleosomes (Figure 6). Specifically, W92S, Y109C, A111G, and I116F had an impact on the SMN-H3K79me1 interaction (Figure 5D). Interestingly, the association of SMN and SMNTUDOR with free H3, peptides, and rNCP varied slightly (Table S1). More precisely, G95R bond H3K79me1-marked H3, but not the rNCP-H3KC79me1 nucleosome, while Y109C and Y130C bound total H3 from calf thymus, but not rNCP-H3KC79me1.

Discussion

Unlike histone tail modified residues, such as H3K4, H3K9, or H3K27, histone H3 lysine 79 is found within the core of histone H3 with the side chain of K79 sticking out like a broken bicycle wheel spoke. Specifically, methylation of H3K79 (H3K79me2) alters the surface of the nucleosome (38).

Although H3K79me2 can be weakly recognized by the tandem TUDOR domain (TTD) of 53BP1 (34), 53BP1-TTD prefers H4K20me2 with a K D of 20 μM compared to 2 mM for H3K79me2 (35). Another study found that H3K79me2 can be recognized in vitro by the WD40 domain of EED with low affinity (K D of > 400 μM) [PDB 3JZH (39)]. Although the TUDOR domain protein Fragile X Mental Retardation Protein (FMRP) associates with H3K79me0me3 with a K D of 135 nM, it also binds to H3K4me2me3, H3K9me3, H3K27me1me2me3, and H3K36me2me3 (40). However, the FMRP family members FXR1 and FXR2 fail to associate with H3K79me3, but rather have affinity for H4K20me3 (41). Finally, there is also HDGF2 PWWP that can recognize H3K79me3 (42). Regardless of all these readers that can recognize H3K79me2/me3, no reader for H3K79me1 has been identified so far. Previous work from our laboratory described a DOT1L-dependent relocation of SMN in response to damaged centromeres that required an intact TUDOR domain (16), suggesting that...
SMNTUDOR reads H3K79me1

SMNTUDOR may interact with methylated H3K79. We herein define using multiple assays and approaches that SMNTUDOR associates with H3K79me1.

Interestingly, SMA-linked SMNTUDOR mutants (SMNST) impact differently the interaction of SMN with free histone H3, K79me1-modified H3, and H3K79me1 rNCP (summarized in Table S1). We can only speculate on the nature of the discrepancies between the different histone contexts (i.e. peptides, free histones, rNCP), but likely oligomerization and/or nucleotide-binding properties of SMN play a role.

Although several readers recognize dual marks, such as ZMYND8 and SETDB1 interacting with H3K4me3 and H3K9me3 respectively (43) and SETDB1-H3K9me3 and K14ac (44), while others have affinity for a handful of sites, such as PHD that recognize unmodified histone H3, H3K4me1, or H3K9me (45,46), few readers recognize both methylated arginine and lysine residues indiscriminately. For instance, SPIN1 recognizes H3R8me2a with TUDOR-like SPIN repeat 1 and H3K4me3 via SPIN repeat 2 (47), but also H3K9me3 and H3K4me3 (48). Interestingly, the overall structures of H3K4me3R8me2a- and H3K4me3K9me1-bound SPIN1 remain similar, except for a small rotation of W72 and F251 side chains in the aromatic cage of SPIN1 repeat 1 to accommodate either H3K9me3 or H3R8me2a (48).

We conclude that SMNTUDOR likely conforms like SPIN1 to be able to associate with H3K79me1 as well as RmeGG-containing proteins (e.g. COIL, FBL).

Conclusions

SMNTUDOR is herein identified as the first reader of the H3K79me1 mark, which is found on exons and linked to alternative splicing events. Importantly, SMA-linked SMNTUDOR mutants (SMNST) impact profoundly interactions with histones. Since the TUDOR royal family has always been promiscuous, it is interesting to discover that SMNTUDOR is binding with both methylated arginines and methylated lysines.

Methods

Recombinant protein expression. The cDNA of human full-length SMN and truncations were inserted in pGEX-6P-1 (GE Healthcare) using BamHI and XhoI. Aromatic cage mutants were generated by site-directed mutagenesis using Pfu Turbo (Stratagene) followed by DpnI (NEB) digestion. Constructs were sequence-verified (GATC Biotech AG or Biofidal) and transformed into BL21 DE3 cells (Stratagene). BL21 cells were grown overnight with ampicillin selection at 37°C. The following day, cultures were scaled up in 250 mL LB (Sigma) and grown at 37°C until OD600 ~0.6. Then, expression of recombinant GST proteins was induced with 0.2 mM IPTG for 2.5-3 hours at 37°C. Cells were harvested by centrifugation, resuspended in lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.05% NP-40, supplemented Complete EDTA-free [Roche]). After a brief sonication, lysates were cleared by centrifugation, and incubated with glutathione-sepharose (GE Healthcare) at 4°C on a tumbler wheel. After extensive washing, GST proteins were eluted with 10 mM reduced glutathione (Sigma) in 50 mM Tris pH 8.0.

Antibodies. The α-H3 (ab1791), α-H4 K20me2 (ab9052), and HRP-conjugated α-GST (ab3416) antibodies were obtained from Abcam. The α-H2A (07-146), α-H2B (07-371), and α-H4 (62-141-13) were obtained from Millipore. Methyl-specific H3K79me1 (pAb-082), H3K79me2 (pAb-051), and H3K79me3 (pAb-068) antibodies were purchased from Diagenode. The α-H2AZ antibody was described elsewhere (49).

Histone interactions. GST pulldowns were performed with 25 μg calf thymus histones (Worthington) and ~1 μg recombinant GST or GST-SMN in freshly made 25 mM bis tris propanol (Sigma B6755) pH 6.8 buffer with 1 M NaCl and 0.05% NP-40. Glutathione-sepharose beads (GE17-5132-01) were added for an hour, then washed 4 times with 1 mL bis tris propanol buffer.

Peptide pulldowns. Peptide pulldowns were performed with 1 μg biotinylated H3 peptides (16) and ~1 μg recombinant GST or GST-SMN in freshly prepared 25 mM bis tris propanol buffer with 200 mM NaCl and 0.05% NP-40. Streptavidin-sepharose beads (GE17-5113-01) were added for an hour, then washed 4 times with 1 mL bis tris propanol buffer.

H3K79 recombinant nucleosomes. Bacterial expression vectors for histones H2A and H2B were purchased from Addgene (42634 and 42630, respectively). Plasmids to express X. laevis H3 (xH3) in pET-3d and xH4 in pET-3a were obtained from Professor Arrowsmith (University of Toronto, Canada). Introduction of C110A and K79C mutations in xH3 were done by site-directed mutagenesis using QuikChange (Stratagene) and the plasmid was sequence-verified. Recombinant histones were purified from E. coli, and modified where indicated, prior to octamer assembly and subsequent refolding of recombinant nucleosome core particle (rNCP) with a 151 base pair 601 Widom DNA as previously described (50,51). Briefly, the histones were purified from inclusion bodies under denaturing conditions on a 5 mL HiTrap SP FF (GE Healthcare) cation exchange column on a Next Generation Chromatograph (NGC, Bio-Rad). Fractions containing the purified histone were pooled and dialyzed 3 times into 4 L of water and 2 mM β-mercaptoethanol before lyophilization. The four histones were then unfolded into 20 mM Tris pH 7.5, 7 M Guanidine-HCl and 10 mM DTT and mixed in equimolar ratios prior to octamer refolding into 2 M NaCl, 10 mM Tris pH 7.5, 1 mM EDTA. Octamers were then purified on a Superdex 200 HiLoad 16/600 size exclusion column (GE Healthcare) and wrapped with the 151 base pair 601 Widom DNA to obtain rNCPs. Native gel analysis was used to validate the quality of the reconstitution.
**Histone labeling.** The installation of a monomethyl-lysine analog at the mutated cysteine of the H3K79 (C110A) histone was done as described (52) using the 2-chloro-N-methyl ethylamine hydrochloride (Toronto Research Chemicals C428323) to generate H3K79me1 (C110A). The installation of the analog was confirmed by electrospray ionization mass spectrometry (ESI-MS) on a LC-ESI-QTOF Agilent 6538 mass spectrometer and immunoblotting against H3K79me1 (Figure S2).

**Figure Legends**

**Figure 1:** SMN associates with histone H3. (A) GST alone or GST-tagged SMN were used in GST-pulldown assays in the presence of calf thymus histones (CTH). Pulldowns were analyzed by SDS-PAGE followed by Coomassie staining. (B) As in panel A, but pulldowns were analyzed by immunoblotting using the indicated antibodies.

**Figure 2:** The TUDOR domain is required but not sufficient for SMN to associate with H3. (A) Schematic representation of SMN truncated forms used to assess the region of SMN responsible for the association with H3. Post-translational modification (methyl-arginine [Rme], methyl-lysine [Kme], phosphotyrosine [Yphos], and SUMOylated lysine [KSUMO]) sites are highlighted. The aromatic cage within the TUDOR is marked in purple and the dimerization YG-box highlighted in green at the carboxy terminus. (B) GST-pulldowns were performed as in Figure 1 and analyzed by immunoblotting with α-GST and α-H3 antibodies. (C) As in panel B, but with amino terminal extensions on recombinant SMN-TUDOR.

**Figure 3:** The aromatic cage within SMN-TUDOR is critical for SMN-H3. (A) GST-pulldowns were performed as in Figure 1 and analyzed by immunoblotting with α-GST and α-H3 antibodies. (B) As in panel A, but GST-ING3PHD and ING3W385A were used as positive and negative controls, respectively. (C) The level of H3 was measured using Image Lab (Bio-Rad Laboratories) from 3 independent experiments.

**Figure 4:** SMA-linked SMN-TUDOR mutants impact SMN interaction with H3. (A) GST-pulldowns were performed with full-length recombinant GST-SMN and SMA-linked mutants GST-SMNY109C and GST-SMNE134K. (B) As in panel A, but with a complete panel of TUDOR mutants. The pulldowns were analyzed by immunoblotting against histone H3.

**Figure 5:** SMN is a H3K79me1 reader. (A) GST-pulldowns with CTH were analyzed immunoblotting using α-H3K79me1, H3K79me2, or H3K79me3 antibodies. (B) As in panel A, but GST-53BP1-TUDOR-UDR was used as a positive control known to associate with H3K79me2 (34) and H4K20me2 (35). (C) As in panel A, but GST-ING3PHD, a H3K4me3 reader, was used as a negative control. Ratios of ING3:GST and SMN:ING3 signals are indicated under the immunoblots. (D) Biotinylated synthetic histone peptides were pulled down using streptavidin-agarose in the presence of GST, GST-SMN, or GST-53BP1-TUDOR-UDR. Pulldowns were analyzed by immunoblotting using an α-GST antibody. (E) As in panel D, but biotin H3K79 peptides were pulled down in the presence of GST-SMN aromatic cage mutants. (F) As in Figure 4, panel B, but the same samples were analyzed with an α-H3K79me1 antibody.

**Figure 6:** SMN associates with H3K79me1 nucleosomes. Recombinant nucleosomes core particles were assembled and chemically modified on lysine 79 (nNCP-H3K79me0 and nNCP-H3K79me1). Indicated GST-tagged proteins were pulled down using glutathione-agarose in the presence of either nNCP and analyzed by immunoblotting using an α-H3 antibody.

**Supplementary Material Legends**

**Figure S1:** SMN associates with histone H3. (A) Samples from Figure 1B, but pulled down GST levels are shown for each immunoblot. (B) GST alone or GST-tagged indicated amino and carboxy terminal extensions on recombinant SMN-TUDOR were used in GST-pulldown assays in the presence of calf thymus histones (CTH). Pulldowns were analyzed by immunoblotting using the indicated antibodies.

**Figure S2:** Analysis of nNCPs reconstitution with H3/Kc29me1 (C110A) or H3/Kc29me2 (C110A). Histone H3 nNCP-H3c29me1 (C110A) either unmodified or chemically modified with a monomethyl lysine analog, were analyzed by (A) immunoblotting using α-H3K79me1 or α-H3 and by (B) electrospray ionization mass spectrometry (ESI-MS) analysis. Total mass shift due to Kc79 monomethylation: calculated +37; observed +33. (C) Octamers refolded the indicated histone H3 were separated on a 15 % SDS-PAGE and stained with Coomassie Brilliant Blue (CBB). (D) Reconstituted nNCP were run on native 6% retardation gel and sequentially stained by SYBR Green and InstantBlueTM (ISB1L, Sigma). Free 151 bp 601 DNA is used as a control.

**Table S1.** Summary of SMN association with various forms of histone H3. Qualitative estimates of the association between SMN and histone H3 (from CTH; Figure 4B), H3 monomethylated on K79 (from CTH; Figure 5F), and nNCP-H3K79me1 (Figure 6).

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References


Figure 2

A

B

C

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Figure 3

**A**

Coomassie

**B**

α-GST

**C**

Relative H3 binding

<table>
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<tr>
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<td>0.8</td>
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See also: bioRxiv preprint doi: https://doi.org/10.1101/2022.10.06.511070; this version posted October 11, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY-NC-ND 4.0 International license.
Figure 4

A

CTH input (5%)

α-H3

α-GST

B

CTH input (5%)

α-H3

Ponceau

CTH input (5%)

α-H3

Ponceau
Figure 5

A

B

C

D

E

F

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<table>
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<th>Input (1%)</th>
<th>GST-ING3PHD</th>
<th>GST-SMNW102A/Y130A</th>
<th>GST-SMNG95R</th>
<th>GST-SMNW92S</th>
<th>GST-SMNE134K</th>
<th>GST-SMNA111G</th>
<th>GST-SMNQ136E</th>
<th>GST-SMNY130C</th>
<th>GST-SMNI116F</th>
<th>GST-SMNY109C</th>
<th>GST-SMNQ136E</th>
<th>GST-SMNQ136E</th>
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<td>Total H3</td>
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</tbody>
</table>
FIGURE S2

A

Histone
H3KC79
(C110A)

me0  me1

α-H3

α-H3K79me1

B

H3KC79me0 (C110A)
Av. MW: 15257.20 Da

C

Octamer
H3KC79 (C110A)

me0  me1

H3

H2B

H2A

H4

CBB

D

rNCP
H3KC79
(C110A)

Free DNA

me0  me1

SYBR Green

Instant Blue

6% PAGE

Av. MW: 15290.24 Da

rNCP

free DNA