Tudor staphylococcal nuclease (Tudor-SN) participates in snRNP assembly via interacting with symmetrically dimethylated Sm proteins

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SUPPLEMENTAL DATA

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Plasmid Construction—GST-Prp8-2.1 and 2.2 plasmids were constructed by inserting PCR products corresponding to amino acids 303-435 (domain 2.1) and 303-768 (domain 2.2) into the pGEX-4T-1 vector with *Eco*RI and *Not*I. The DNA sequence of U5-116 (amino acids 1-531) was cloned into *Sal*I/*Mun*I sites of pEGFP-CI vector to generate the GFP-U5-116 constructs. All PCR products were sequenced.

Cell-free Translation *in Vitro* and GST Pull-down Assay—The cell-free *in vitro* translation of full-length U5-116 and Tudor-SN protein was carried out in a nuclease-treated rabbit reticulocyte lysate (RRL) system (Promega BioSciences) according to the manufacturer's recommendations. Proteins were labeled with L-[^{35}S]-methionine (Amersham Biosciences) and incubated with beads-bound GST fusion proteins in binding buffer (12.5 mM HEPES, pH 7.4, 0.1 mM EDTA, 0.05%NP-40, 1 mM DTT, 1 mM phenylmethylsulfonyluoride, 1 mM Na_3VO_4, 2 mg/ml aprotinin, and 0.5% bovine serum albumin). After washing, the bound proteins were separated by SDS-PAGE and visualized by autoradiography.

SUPPLEMENTAL RESULTS

The conserved Prp8 protein in U5 snRNP occupies a central position in the catalytic core of the spliceosome (S1). Our previous study demonstrated that the N-terminal region of Prp8 (amino acids 303-768) interacts with the TSN domain of Tudor-SN protein (S2), while U5-116 could also associate with this region of Prp8. Thus, to further delineate the interaction domain of Prp8 protein in the ternary complex, GST pull-down assays were performed using GST fusion proteins containing Prp8-2.1 domain (amino acids 303-435) or Prp8-2.2 domain (amino acids 303-768) (**Supplemental Fig.S1 *A***). The Prp8-2.2 domain interacted with the *in vitro*

translated U5-116, while the Prp8-2.1 or GST protein alone did not (**supplemental Fig.S1 *B*, *upper panel***). On the other hand, both Prp8 2.1 and 2.2 domains associated with *in vitro* translated Tudor-SN (**supplemental Fig.S1 *B*, *lower panel***).

In addition, the specificity of the interaction of Tudor-SN with Prp8 and U5-116 was further investigated in stringent condition. The GST-TSN fusion protein was incubated with total cell lysate of HeLa cells in 700 mM NaCl. As shown in **supplemental Fig.S2 *A***, after washing, the bound proteins were eluted from the fusion protein with high salt elution buffer (1M NaCl), then desalted and separated by SDS-PAGE gel and detected with silver staining as previously reported (S3). As arrow indicated, two bands were strongly precipitated with TSN protein, which corresponded to the molecular weight of Prp8 and U5-116. To verify that these two proteins were indeed Prp8 and U5-116, the eluted proteins were also detected by blotting with anti-Prp8 or anti-U5-116 antibody (**supplemental Fig.S2 *B*)** respectively, which present the consistent result.

The RNA-binding protein immunoprecipitation (RIP) assay was performed to verify the association of Tudor-SN and U snRNAs. As shown in Fig. 1*B*, U1, U2, U4/6, U5 snRNAs can be detected in anti-Tudor-SN, anti-TMG-cap or anti-SmB precipitation, whereas no PCR products were detected in IgG negative control. Quantitative real-time PCR assay was also performed to detect the amount of bound U1, U2, U4/6, U5 snRNAs with anti-Tudor-SN and anti-TMG RIP. As shown in **supplemental Fig.S3**, more than 80-fold of U snRNAs co-immunoprecipitated with anti-Tudor-SN antibody were enriched efficiently, while over 160-fold of bound U snRNAs were detected in the anti-TMG-cap positive controls. These results confirmed the *in vivo* association of Tudor-SN with U1, U2, U4/6, U5 snRNAs.

SUPPLEMENTAL REFERENCES

S1. Boon KL, Norman CM, Grainger RJ, Newman AJ, and Beggs JD. (2006) *RNA.* **12,** 198-205.

S2. Yang, J., Välineva, T., Hong, J., Bu, T., Yao, Z., Jensen, ON., Frilander ,M.J., and Silvennoinen, O. (2007) *Nucleic Acids Res.***35,**4485-4494.

S3. Shaw, N., Zhao,M., Cheng C, Xu H, Saarikettu J, Li Y, Da Y, Yao Z, Silvennoinen O, Yang J, Liu ZJ, Wang BC, and Rao Z.(2007) *Nat. Struct. Mol.Biol.* **14,** 779-784.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental FIG.S1 Interaction domain of Prp8 in the Tudor-SN/ U5-116/ Prp8 complex

***A*,** Schematic diagram representing positions of Prp8 2.1 and 2.2 domains and other functional regions, including the locations of the putative NLS (nuclear localization

signal), RRM (RNA-recognition motif), and a known MPN (Mpr-1,Pad-1,N-terminal) domain in Prp8 protein. ***B*,** GST alone, GST-Prp8 2.1, and GST-Prp8 2.2 proteins were individually incubated with ^{35}S-labeled *in vitro* translated U5-116 (*upper panel*) or Tudor-SN protein (*lower panel*). Bound proteins were separated by SDS-PAGE and detected by autoradiography. Approximately 10% of the *in vitro* translated proteins were included as a control.

Supplemental FIG.S2 Tudor-SN, U5-116, and Prp8 protein form a stable ternary complex

***A*,** The beads-bound GST protein alone or GST-TSN fusion protein was incubated with the total cell lysate of HeLa cells with 700 mM NaCl. After stringent washing, the precipitated proteins were eluted with high salt elution buffer (1M NaCl). The elution was desalted and concentrated with microcon columns (30 kDa NMWL, Millipore), then separated by SDS-PAGE gel and detected with silver stain. ***B*,** The eluted coprecipitated proteins were separated by SDS-PAGE gel and blotted with anti-Prp8 antibody (*upper panel*), or anti-U5-116 antibody (*lower panel*) respectively. The input was equal to 20% of lysates. **C,** The loading control of GST-TSN and GST proteins.

Supplemental FIG.S3 Tudor-SN binds U1,U2,U4/6,U5 snRNA *in vivo*.

RNA-binding protein immunoprecipitation assay was performed using HeLa cells with anti-Tudor-SN, anti-TMG-cap and rabbit IgG antibody, respectively. The co-precipitated U1, U2, U4, U5 and U6 snRNAs were reverse-transcribed to cDNA with random hexamer primers. And then quantitative real-time PCR assay was performed to detect the relative fold changes of precipitated U1, U2, U4, U5 and U6 snRNA with anti-Tudor-SN and anti-TMG RIP.

SUPPLEMENTAL TABLE

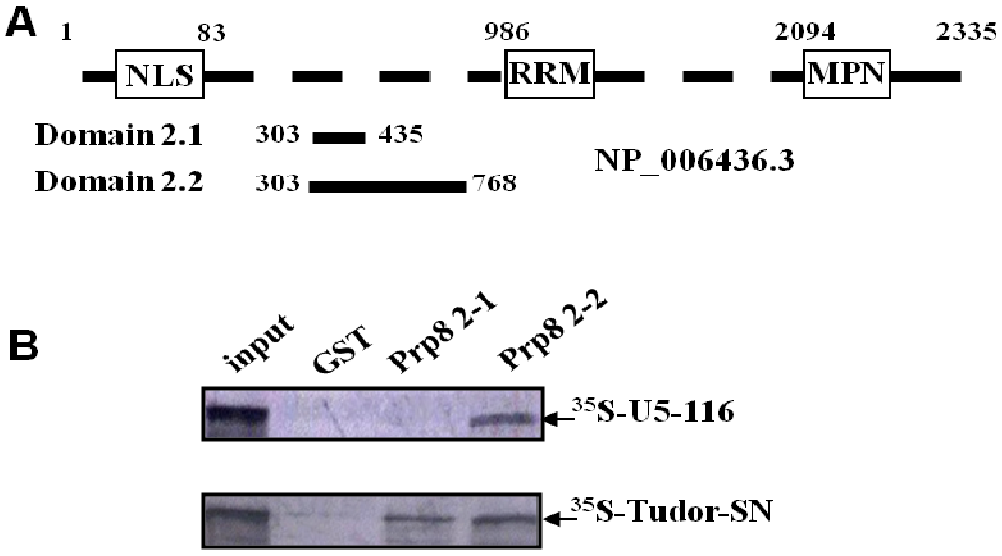
Supplemental tableS 1 RNase Mixture (100ul) Used In Protein Binding Assay

component	volumn	concentration	Function
Rnase A/Ti (#EN0551)	60ul	A : 2ug/ul	Specifically hydrolyzes RNA at C and U residues
		Ti :5U/ul	Specifically hydrolyzes RNA at G residues
Rnase H (#EN0202)	10ul	5U/ul	Specifically degrades the RNA strand in RNA-DNA hybrids.
Rnase I (#EN0602)	30ul	10U/ul	Preferentially hydrolyses single-stranded RNA

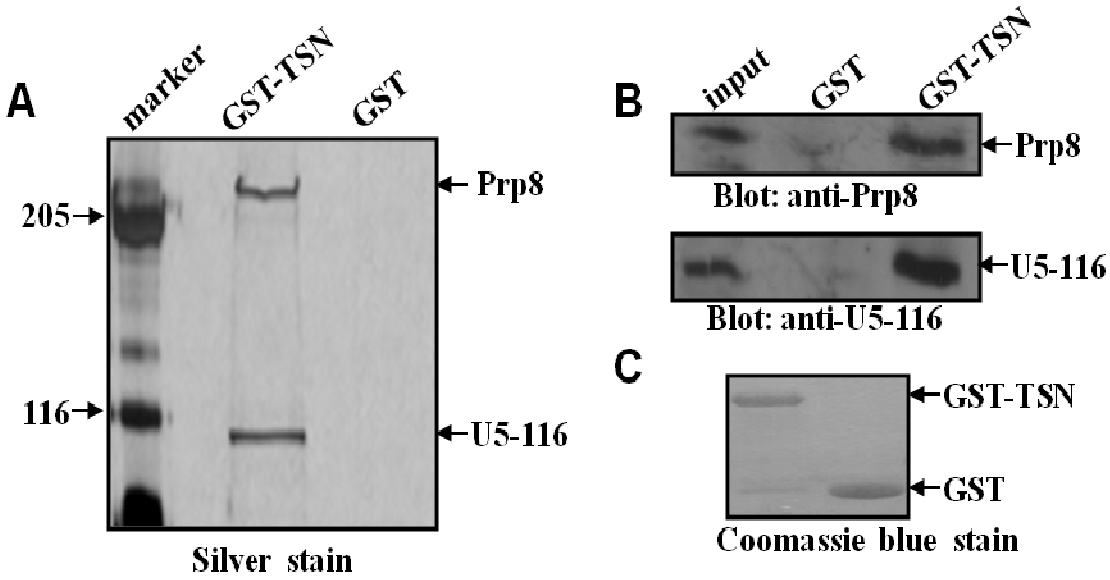
Supplemental tableS 2 Information On The Primer Used In This Study

gene	forward primer (5'-3')	reverse primer (5'-3')	NCBI Reference Sequence (PCR product size)
5S rRNA	ACGGCCATACCACC CTGAA	AGGCGGTCTCCCA TCCAAG	NR_023379.1 (96bp)
5.8S rRNA	CTTAGCGGTGGATC ACTCGG	ACGCTCAGACAGG CGTAGCC	NR_003285.2 (147bp)
GAPDH	GCACCGTCAAGGCT GAGAAC	TGGTGAAGACGCC AGTGGA	NM_002046.3 (138bp)

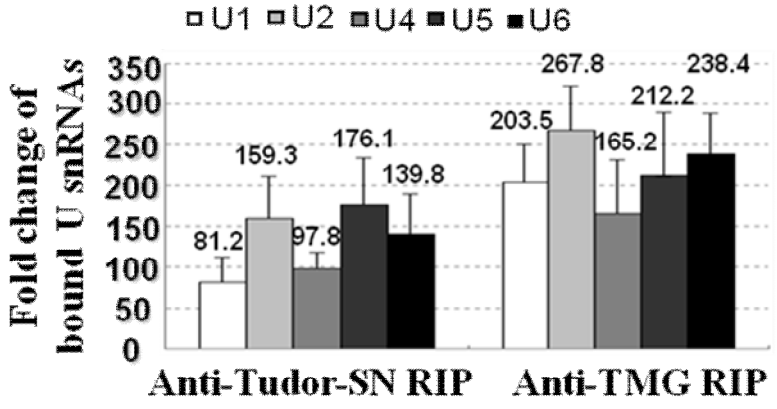
SUPPLEMENTAL FIGURE



Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3