

GENETICS

Supporting Information

<http://www.genetics.org/cgi/content/full/genetics.109.106955/DC1>

Spp382p Interacts With Multiple Yeast Splicing Factors, Including Possible Regulators of Prp43 DExD/H-Box Protein Function

**Shatakshi Pandit, Sudakshina Paul, Li Zhang, Min Chen, Nicole Durbin,
Susan M. W. Harrison and Brian C. Rymond**

Copyright © 2009 by the Genetics Society of America

DOI: 10.1534/genetics.109.106955

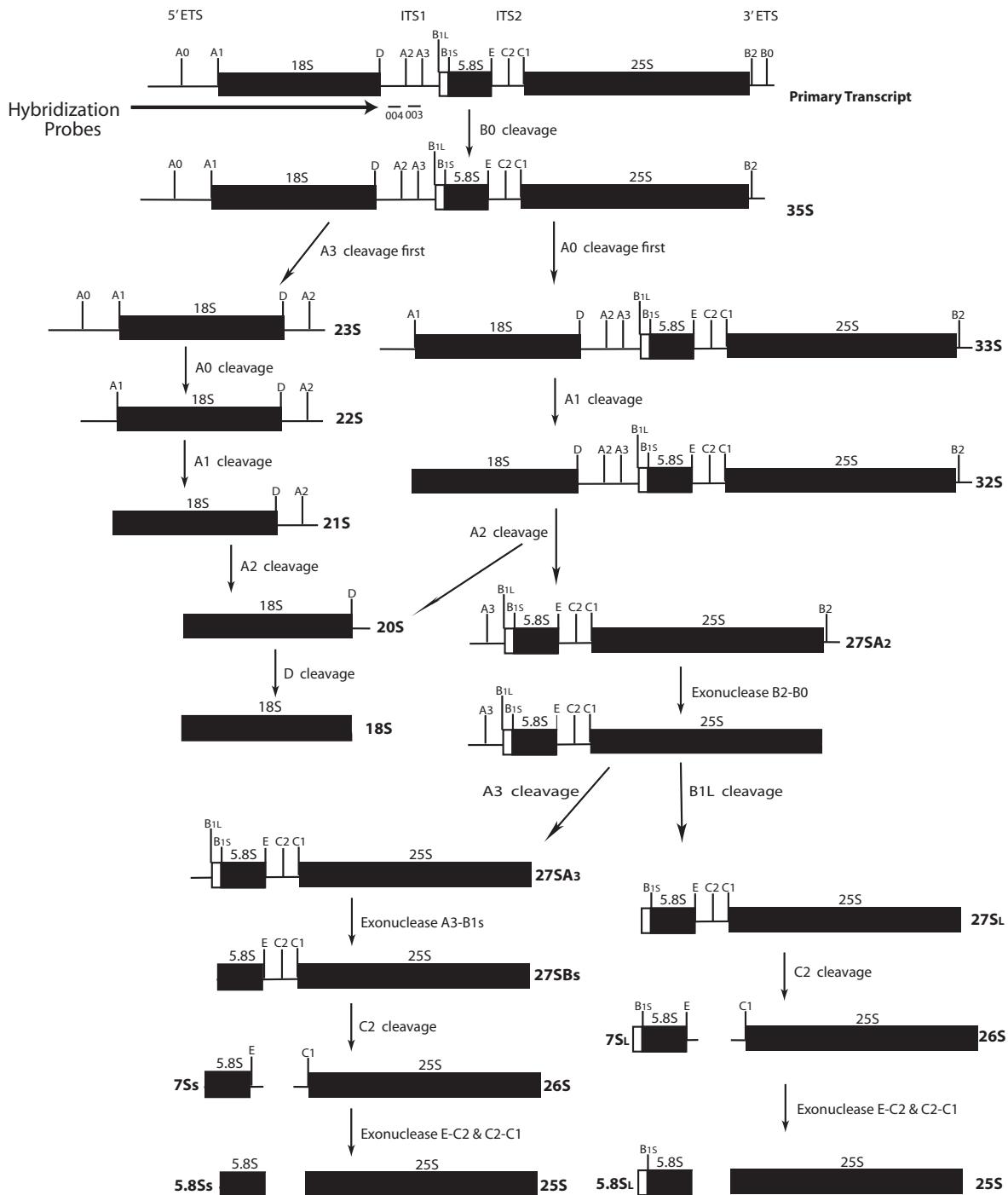
Open reading frames fused to the GAL1 promoter used in this study.

ORF	Gene Name	Alias
YBL026W	LSM2	"SMX5, SNP3"
YBL074C	AAR2	
YBR055C	PRP6	"RNA6, TSM7269"
YBR065C	ECM2	SLT11
YBR102C	EXO84	USA3
YBR119W	MUD1	"U1-A, U1A"
YBR152W	SPP381	
YBR188C	NTC20	
YBR237W	PRP5	RNA5
YCL018W	LEU2	
YCL037C	SRO9	
YCR046C	IMG1	
YDL030W	PRP9	
YDL043C	PRP11	RNA11
YDL084W	SUB2	
YDL087C	LUC7	"EXM2, EPE1"
YDL160C	DHH1	
YDL175C	AIR2	
YDL209C	CWC2	NTC40
YDR007W	TRP1	
YDR037W	KRS1	GCD5
YDR088C	SLU7	SLT17
YDR163W	CWC15	
YDR225W	HTA1	"H2A1, SPT11"
YDR230W	Dubious ORF	
YDR235W	PRP42	"MUD16, SNU65"
YDR240C	SNU56	MUD10
YDR243C	PRP28	
YDR283C	GCN2	AAS1
YDR364C	CDC40	"PRP17, SLT15, SLU4"
YDR381W	YRA1	SHE11
YDR416W	SYF1	NTC90
YDR432W	NPL3	"MTR13, MTS1, NOP3, NAB1"
YDR473C	PRP3	RNA3
YEL026W	SNU13	
YER006W	NUG1	
YER009W	NTF2	
YER013W	PRP22	
YER029C	SMB1	"Sm B, SmB"
YER107C	GLE2	RAE1
YER112W	LSM4	"SDB23, USS1"
YER146W	LSM5	
YFL003C	MSH4	
YFL017W-A	SMX2	"SNP2, YFL018W-A, SmG"
YFR005C	SAD1	
YGL030W	RPL30	"L30, L32, YL38, rp73"
YGL049C	TIF4632	eIF4G2
YGL120C	PRP43	
YGL122C	NAB2	
YGL128C	CWC23	
YGL147C	RPL9A	"L8A , L9A YL11, rp24"
YGL238W	CSE1	KAP109
YGR013W	SNU71	
YGR075C	PRP38	
YGR084C	MRP13	
YGR091W	PRP31	
YGR129W	SYF2	NTC31
YGR162W	TIF4631	eIF4G1
YGR204W	ADE3	
YGR278W	CWC22	
YHR041C	SRB2	"HRS2, MED20"
YHR086W	NAM8	"MRE2, MUD15"

YHR156C	LIN1	SNU40
YHR165C	PRP8	"DBF3, DNA39, RNA8, SLT21, USA2"
YIL061C-SNP1		"U1 70K, U1-70K"
YIR005W	IST3	SNU17
YIR009W	MSL1	YIB9
YJL080C-SCP160		
YJL124C-LSM1	SPB8	
YJL203W	PRP21	SPP91
YJR022W	LSM8	
YJR050W	ISY1	"UTR3, NTC30"
YKL012W	PRP40	
YKL074C	MUD2	
YKL095W	YJU2	CWC16
YKL173W	SNU114	GIN10
YKL214C	YRA2	
YKR022C	NTR2	
YKR086W	PRP16	
YLL036C	PRP19	PSO4
YLR016C	PML1	
YLR116W	MSL5	BBP
YLR117C	CLF1	"SYF3, NTC77"
YLR132C		Uncharacterized ORF
YLR147C	SMD3	"SLT16, Sm D3"
YLR183C	TOS4	
YLR226W	BUR2	CST4
YLR249W	YEF3	"TEF3, Sm D2"
YLR275W	SMD2	
YLR293C	GSP1	"CNR1, CST17"
YLR298C	YHC1	U1C
YLR347C	KAP95	RSL1
YLR409C	UTP21	
YLR424W	SPP382	"CCF8, NTR1"
YLR438C-A	LSM3	"SMX4, USS2"
YLR453C	RIF2	
YML025C	YML6	
YML029W	USA1	
YML046W	PRP39	
YML117W	NAB6	
YMR049C	ERB1	
YMR116C	ASC1	CPC2
YMR213W	CEF1	NTC85
YMR216C	SKY1	
YMR240C	CUS1	
YMR268C	PRP24	
YNL005C	MRP7	YmL23
YNL118C	DCP2	PSU1
YNL147W	LSM7	
YNL189W	SRP1	"KAP60, SCM1"
YNL210W	MER1	
YNL224C	SQS1	
YNL245C	CWC25	
YNL251C	NRD1	
YNL286W	CUS2	
YNR011C	PRP2	RNA2
YNR053C	NOG2	NUG2
YOL133W	HRT1	"RBX1, ROC1, HRT2"
YOL139C	CDC33	"TIF45, eIF4E"
YOR128C	ADE2	
YOR148C	SPP2	
YOR159C	SME1	Sm E
YOR167C	RPS28A	"S28A , S33A , YS27 "
YOR202W	HIS3	
YOR308C	SNU66	
YOR319W	HSH49	

YPL151C	PRP46	NTC50
YPL169C	MEX67	
YPL178W	CBC2	"MUD13, SAE1, CBP20"
YPL190C	NAB3	HMD1
YPL213W	LEA1	
YPL235W	RVB2	"TIP49B, TIH2, TIP49"
YPR057W	BRR1	
YPR082C	DIB1	SNU16
YPR094W	RDS3	
YPR101W	SNT309	NTC25
YPR161C	SGV1	BUR1
YPR176C	BET2	
YPR178W	PRP4	RNA4
YPR182W	SMX3	Sm F

FIGURE S1.—List of overexpression constructs used in this study. The galactose inducible *GAL1* promoter fusion constructs (GELPERIN *et al.* 2005) used here are listed as the relevant open reading frames (ORF) followed by the *Saccharomyces Genome Database* designated official gene name then any gene or protein aliases used in the literature.

Saccharomyces cerevisiae rRNA processing pathway

Adapted from: M. Kos, and D. Tollervey, 2005. Mol Cell 20:53-64;

S. Granneman *et. al.*, 2006. Mol Cell Biol 26:1183-94.

FIGURE S2.—A schematic of the yeast rRNA biogenesis. This image shows the parallel processing of the 35S precursor ribosomal RNA into the mature 25S, 18S and 5.8S rRNA products through two alternative paths based on initial cleave at the A3 (left) or A0 (right) site is shown (see (GRANNEMAN *et al.* 2006; KOS and TOLLERVEY 2005) for related discussion). The placement of the hybridization probes used for the detection of the 35 S precursor and the 23S and 20S (probe 004) and 27SA2 (probe 003) intermediates is indicated on the first line.

Saccharomyces cerevisiae G-patch domains (domain location/protein length)

Spp382p	(61-108/708)	..TYGIGAKL LSSMGYVAGK GL...GKDG. SGITTPPIETQ SRPMHNAGLG MFSN
Pxr1p	(25-72/271)	..TSRFGHQF LEKFGWPKPGM GL...GLSPM NSNTSHIKV. SIKDDNVGLG AKLK
Sqs1p	(720-767/767)	..NENIGRRM LEKLGWKSSE GL...GIQGN KGISEPIFA. KIKKNRSGLR HSES
Ylr271w	(41-87/274)	..IMPRGYKM MENMGYKEGE TL...G.SNE SALKEPIKV. EINTKRRGIR AEKP
Spp2p	(100-149/185)	VPVEEFGDAL LRGMGWESDS EQDSKGDKTQ SRNKDVSNVS QIHPDGLGIG
Consensus		..te.fG.kl LekmGwksGe gL...G.sg. sgikepIkv. sIk.dn.Glg a...

Spp382p	(61-108/708)	..TYGIGAKL LSSMGYVAGK GL...GKDG. SGITTPPIETQ SRPMHNAGLG MFSN
Pxr1p	(25-72/271)	..TSRFGHQF LEKFGWPKPGM GL...GLSPM NSNTSHIKV. SIKDDNVGLG AKLK
Sqs1p	(720-767/767)	..NENIGRRM LEKLGWKSSE GL...GIQGN KGISEPIFA. KIKKNRSGLR HSES
Ylr271w	(41-87/274)	..IMPRGYKM MENMGYKEGE TL...G.SNE SALKEPIKV. EINTKRRGIR AEKP
Spp2p	(100-149/185)	VPVEEFGDAL LRGMGWESDS EQDSKGDKTQ SRNKDVSNVS QIHPDGLGIG
Consensus		..te.fG.kl LekmGwksGe gL...G.sg. sgikepIkv. sIk.dn.Glg a...

FIGURE S3.—Primary sequence alignment of the G-patch domains. The *Saccharomyces* MIPS database ([\(<http://mips.gsf.de/genre/proj/yeast/>\)](http://mips.gsf.de/genre/proj/yeast/) (2009) was used to identify five annotated G-patch proteins in baker's yeast using InterProScan Version 7.1 (InterPro domain = IPR000467). The alignment of the G-patch was done using Multalin (CORPET 1988) with a consensus sequence included beneath the aligned sequences. In parentheses after each protein name is the amino acid domain used for alignment followed by the length of the entire protein.

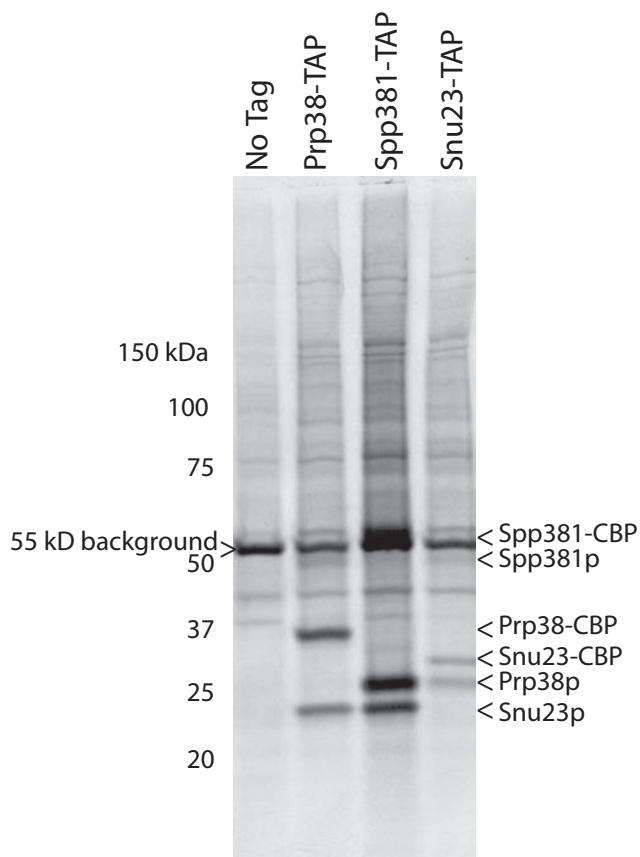


FIGURE S4.—Prp38p, Spp382p, and Snu23p form a heteromeric complex without stably bound Cwc23p or Sqslp. The indicated epitope tagged (TAP) yeast strains were metabolically labeled with Trans 35 S-label (ICN) for 4 hours at 30°C and then broken with glass beads in buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 200 mM NaCl, 10% glycerol, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 2 mM benzamide, 0.5% NP-40) and the lysate cleared by centrifugation at 14,000 X g. Sequential protein A agarose and calmodulin agarose affinity (TAP) selection was done as previously modified (WANG *et al.* 2003) except that the stringency of the wash steps was increased to 450 mM NaCl. The proteins were resolved on a SDS 10% polyacrylamide gel and imaged using a Typhoon 9600 phosphorimager (GE Healthcare). The positions of the molecular weight markers and the 55 kDa background band are noted at the left.

Three protein bands, Prp38p, Spp382p, and Spp381p co-purify independent of the protein used for selection. The band identities were confirmed by immune detection with anti-Prp38p antibody (Rymond, unpublished) and anti-CBP antibody (Santa Cruz Biotechnology) and by mass spectroscopy with a Deca Quadrupole ion trap mass spectrometer (ThermoElectron, Waltham, MA after extract scale-up using unlabeled sample prepared in an identical manner. CBP indicates the residual calmodulin binding peptide present in the TAP-tagged target protein after cleavage by TEV protease. Note: The Spp381 protein labels poorly with the 35 S met, cys mixture but resolves as a band that migrates just below the dark background band of approximately 55 kDa present in the untagged lane. We previously reported the anomalous electrophoretic migration of this 34 kDa protein detected using the small (9 amino acid) HA epitope (LYBARGER *et al.* 1999). Spp381-CBP migrates above the 55 kDa background band but is not well resolved on this gel system. Both Snu23p and Spp381p also interact with Prp38p in the two-hybrid assay (LYBARGER *et al.* 1999; UETZ *et al.* 2000).

- COPPET, F., 1988 Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res 16: 10881-10890.
 GELPERIN, D. M., M. A. WHITE, M. L. WILKINSON, Y. KON, L. A. KUNG *et al.*, 2005 Biochemical and genetic analysis of the yeast proteome with a movable ORF collection. Genes Dev 19: 2816-2826.
 GRANNEMAN, S., K. A. BERNSTEIN, F. BLEICHERT and S.J. BASERGA, 2006 Comprehensive mutational analysis of yeast DEXD/H box RNA helicases required for small ribosomal subunit synthesis. Mol Cell Biol 26: 1183-1194.
 KOS, M., and D. TOLLERVEY, 2005 The Putative RNA Helicase Dbp4p Is Required for Release of the U14 snoRNA from Preribosomes in *Saccharomyces cerevisiae*. Mol Cell 20: 53-64.
 LYBARGER, S., K. BEICKMAN, V. BROWN, N. DEMBLA-RAJPAI, K. MOREY *et al.*, 1999 Elevated Levels of a U4/U6.U5 snRNP-Associated Protein, Spp381p, Rescue a Mutant Defective in Spliceosome Maturation. Mol Cell Biol 19: 577-584.
 UETZ, P., L. GIOT, G. CAGNEY, T. A. MANSFIELD, R. S. JUDSON *et al.*, 2000 A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. Nature 403: 623-627.
 WANG, Q., K. HOBBS, B. LYNN and B. C. RYMOND, 2003 The Clf1p splicing factor promotes spliceosome assembly through N-terminal tetratricopeptide repeat contacts. J Biol Chem 278: 7875-7883.