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Supplemental Information

Exon Definition Complexes Contain the Tri-snRNP and Can Be Directly Converted into B-like Precatalytic Splicing Complexes

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Figure S1

A

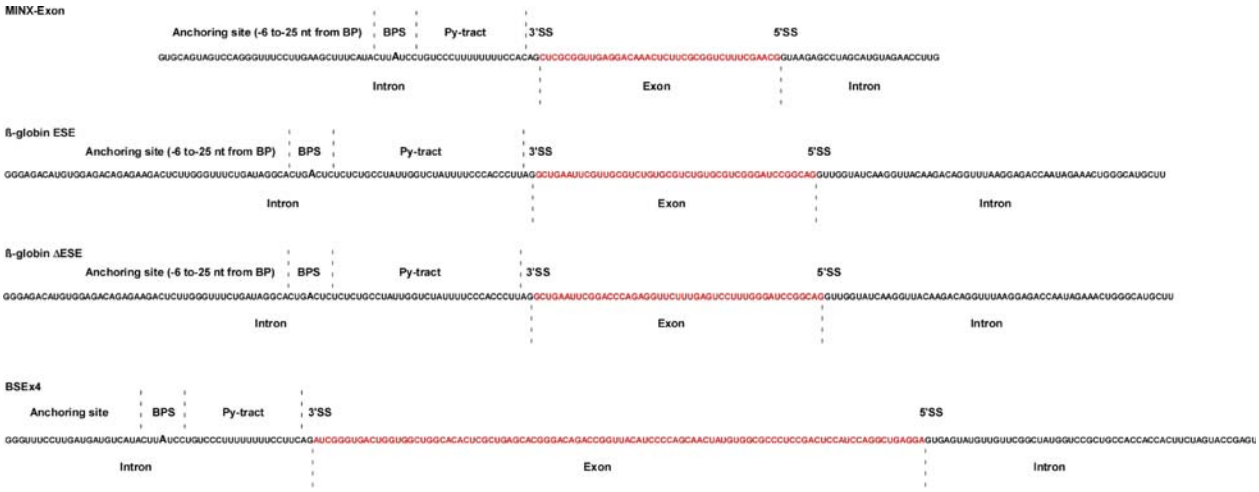

Figure S1. Data related to Figure 1

(A) Sequence of the constructs used in this study. Exon sequences are highlighted in red and the intron sequences in black. The anchoring site, branchpoint sequence (BPS), polypyrimidine tract (Py-tract), as well as the 3‘ and 5’ splice site (SS) are indicated above each sequence.

Figure S1

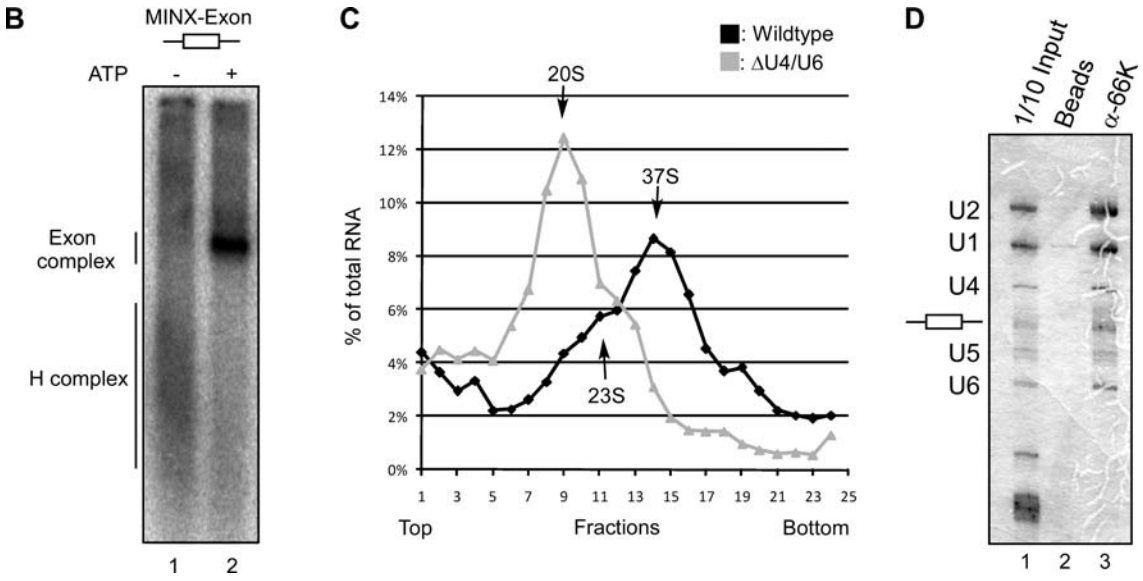

Figure S1 (cont). Data related to Figure 1

(B-D) Cross-exon complexes formed on a MINX exon RNA lacking MS2 aptamers contain U4/U6.U5 tri-snRNPs, in addition to U1 and U2 snRNPs. (B) Native gel analysis of splicing complexes formed after 10 min in HeLa nuclear extract on ^{32}P-labeled MINX exon RNA (lacking MS2 binding sites) in the presence (lane 2) or absence (lane 1) of ATP. The identity of the spliceosomal complexes is indicated on the left. **(C)** Splicing reactions were performed with ^{32}P-labeled MINX exon RNA lacking MS2 sites and untreated HeLa nuclear extract (black line) or extract depleted of U4/U6 snRNA (grey line), and then subjected to glycerol gradient centrifugation. The radioactivity in each fraction was determined by Cherenkov counting and expressed as the percent of the total ^{32}P-RNA loaded onto the gradient. S-values were determined by comparison with a reference gradient containing prokaryotic ribosomal subunits. **(D)** Gradient fractions containing 37S complexes formed in untreated nuclear extract (fractions 14-16) were pooled and immunoprecipitated with PAS beads lacking antibody (lane 1) or pre-bound with affinity-purified antibody against the U2-associated SF3a66 protein (Will et al., 2002) (lane 2). After extensively washing the beads with buffer containing 65 mM salt (G65 buffer), RNA was recovered and then separated by denaturing PAGE, and visualized by silver staining. The positions of the snRNAs and MINX exon RNA are indicated on the left.

Figure S1

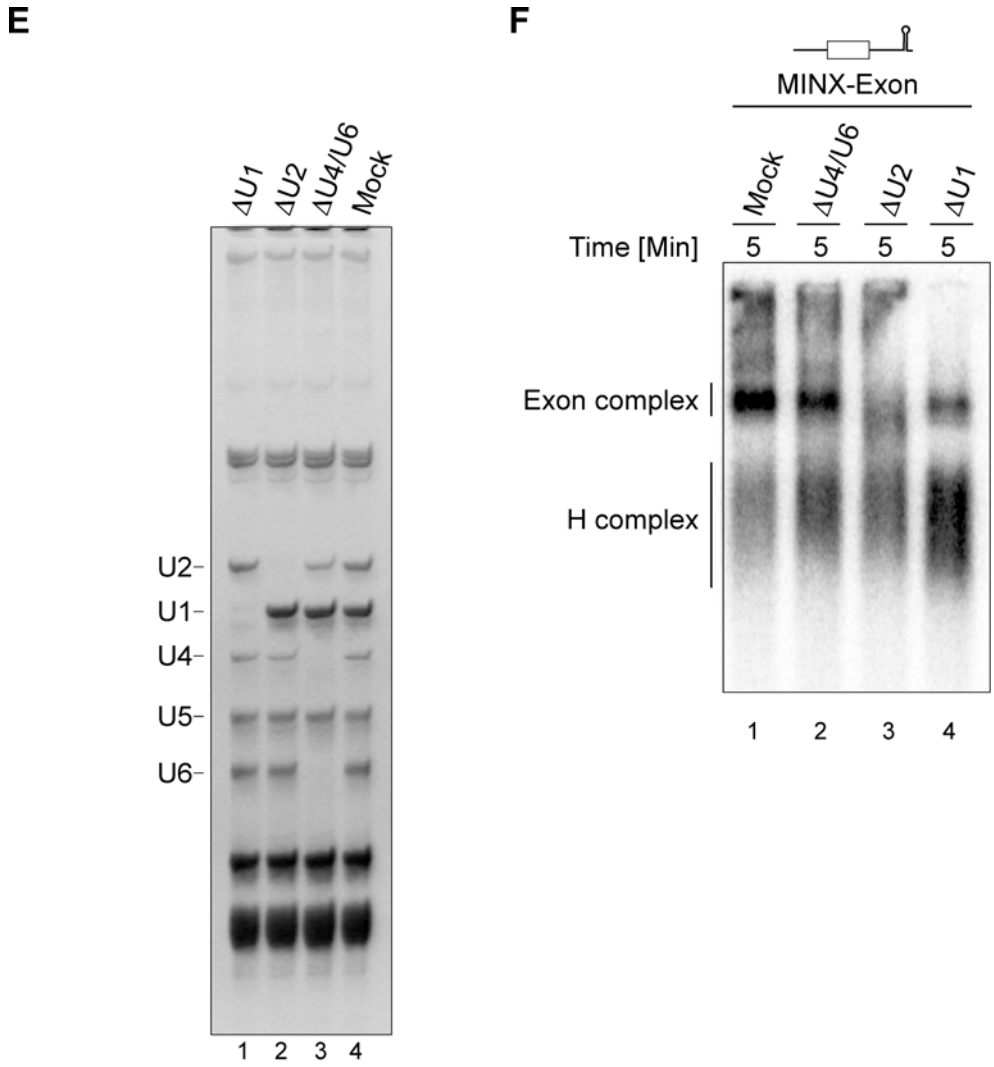

Figure S1 (cont). Data related to Figure 1

(E-F) Exon complex formation is dependent on the presence of the U2 snRNP.
(**E**) Nuclear extracts were depleted of U1 (lane 1), U2 (lane 2) or U4/U6 snRNPs (lane 3) with biotinylated 2'OMe oligonucleotides against U1 (nts 1-13), U2 (nts 1-20) or U6 (nts 87-101) snRNAs as described previously (Barabino et al. 1990; Blencowe et al. 1989; Segault et al. 1995). As a control, depletion was also carried out in the absence of oligonucleotide (Mock, lane 4). RNA from each extract was separated by denaturing PAGE and visualized by staining with ethidium bromide. The position of the snRNAs is indicated on the left. **(F)** ^{32}P-labeled MINX exon RNA was incubated under splicing conditions with the indicated nuclear extract for 5 min. Splicing complex formation was subsequently analysed on a 2% low melting agarose gel after addition of heparin (final concentration 0.1 µg/µl). The identity of the complexes is indicated on the left.

Figure S1

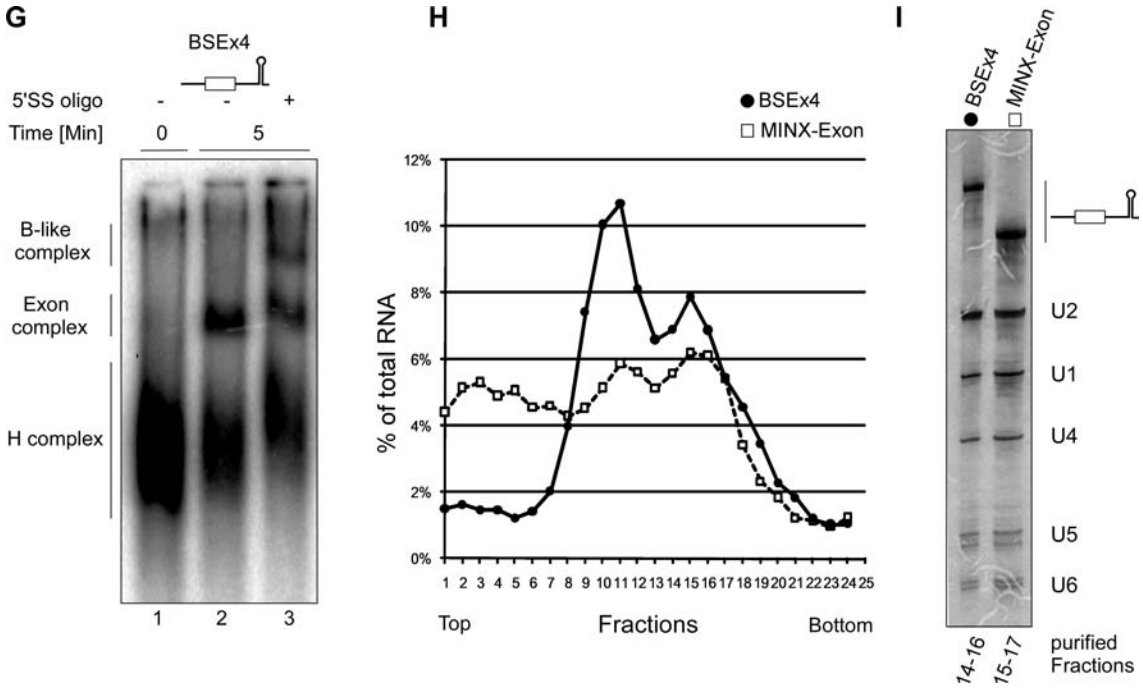

Figure S1 (cont). Data related to Figure 1

(G-I) Cross-exon complexes formed on BSEx4 exon RNA also contain U4/U6.U5 tri-snRNPs.

(G) Native gel analysis (in the presence of heparin) of splicing complexes formed at the indicated time points on ^{32}P-labeled BSEx4 RNA (Sharma et al., 2008) in the presence (lane 3) or absence (lanes 1-2) of a 100-fold excess of the 5'SS RNA oligo. The identity of the spliceosomal complexes is indicated on the left. **(H)** Splicing reactions with MINX exon RNA (line with open squares) or BSEx4 RNA (line with closed circles) were subjected to glycerol gradient centrifugation. The radioactivity in each fraction was determined by Cherenkov counting and expressed as the percent of the total ^{32}P-RNA loaded onto the gradient. **(I)** Gradient fractions containing 37S complexes (fractions 14-16) of MINX exon and BSEx4 RNA were pooled and subjected to MS2 affinity selection. RNA was recovered, separated by denaturing PAGE, and visualized by silver staining. The positions of the snRNAs and MINX exon RNA are indicated on the right.

Figure S2

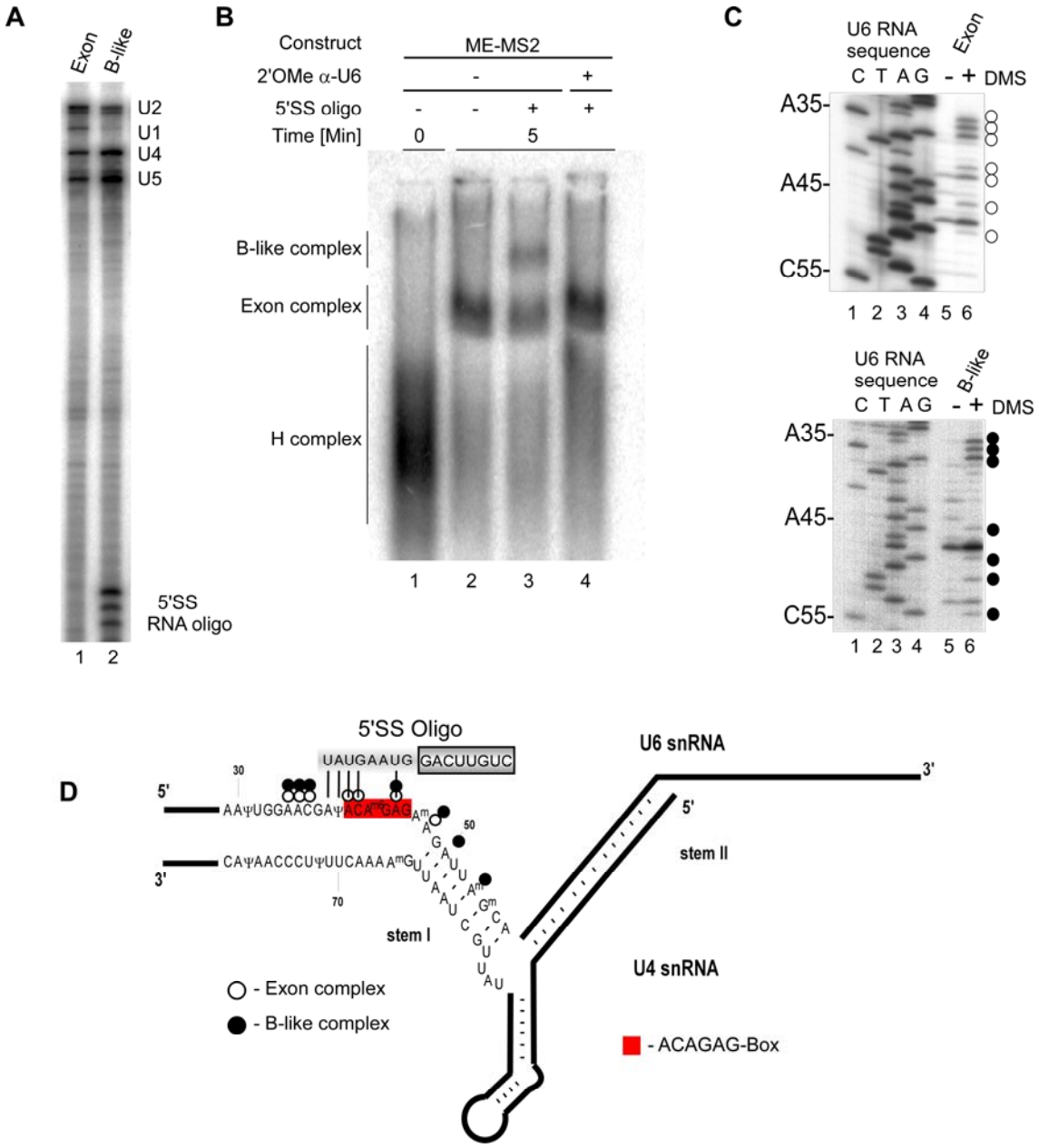
A
Exon
B-like
U2
U1
U4
U5
5'SS
RNA oligo
1 2
B
Construct
ME-MS2
2'OMe α-U6
-
+
5'SS oligo
- - + +
Time [Min]
0
5
B-like complex
Exon complex
H complex
1 2 3 4
C
U6 RNA
sequence
Exon
C T A G - + DMS
A35-
A45-
C55-
1 2 3 4 5 6
U6 RNA
sequence
B-like
C T A G - + DMS
A35-
A45-
C55-
1 2 3 4 5 6
D
5'SS Oligo
UAUGAAUG
GACUUGUC
5'
3'
30
50
70
stem I
stem II
U6 snRNA
U4 snRNA
- Exon complex
- B-like complex
- ACAGAG-Box

Figure S2. Data related to Figure 4

(A-D) The 5'SS oligo interacts with the ACAGAG box of U6 RNA in the B-like complex. (**A**) Exon and B-like complexes formed on MINX Exon RNA were MS2 affinity purified after glycerol gradient centrifugation. RNAs were recovered from the affinity purified complexes and then labeled at their 3' end with ^{32}P-pCp. Samples were analyzed on a 5% denaturing polyacrylamide gel. The identity of the bands is indicated on the right. Please note that U6 snRNA is generally labeled poorly at its 3'end. (**B**) Native gel analysis of splicing complexes formed at the indicated time points on ^{32}P-labeled MINX exon RNA (ME-MS2) in the presence (3-4) or absence (1-2) of a 100-fold excess of a 5'SS oligonucleotide. In lane 3, 5 µM (final concentration) of a 2'OMe oligo complementary to nucleotides 27-46 of U6 snRNA (including the ACAGAG box) were added prior to splicing. The identity of the spliceosomal complexes is indicated on the left. (**C**) Chemical footprinting of U6 snRNA in exon (upper panel) versus B-like complexes (lower panel). Experiments were essentially performed as described previously (Karaduman et al., 2006). In brief, 1 pmol of MS2 purified exon (left panel) or B-like complex (right panel) was incubated in the presence of DMS (lane 6) or its absence (lane 5). DMS modifications were carried out in 100 µl G65 buffer. The reaction was performed for 8 min at 20°C in the presence of 0.5 µl DMS and subsequently stopped by the addition of 100 µl of DMS stop buffer (400 mM Tris-HCl, pH 8.0, 400 mM ß-mercaptoethanol, 5 mM EDTA, 600 mM NaAcetate). After proteinase K digestion of the sample, the RNA was recovered and dissolved in 5µl H_2O. A primer extension reaction was performed with 1.25 µl of modified RNA as described (Karaduman et al., 2006). The region between U6 A35 and C55 is shown here. The sequence of U6 snRNA is also shown (lanes 1-4). White or black circles at the right of the gel indicate modifications in the exon and B-like complex, respectively. (**D**) A schematic comparison of modifications detected in the exon and B-like complex in the U6 region shown above.

Table S1

Protein	Mol mass (kDA)	A comp	Exon complex				B comp	B-like complex			GenBank accession no.
			#1	#2	#3	#4		#1	#2	#3	
A complex proteins											
BUB3	37.2	+	13	5	11	7		4	-	14	gi|4757880
MGC2803	18.4	+	4	1	1	-		-	-	1	gi|13128992
SF1	68.3	+	8	2	2	1		-	-	1	gi|42544123
tat SF1	85.7	+	8	1	2	1		-	-	-	gi|21361437
SF4	72.5	+	-	6	15	4		2	-	2	gi|33469964
TLS/FUS	53.4	+	2	2	5	4		1	-	1	gi|4826734
RES complex											
MGC13125	70.5		-	-	-	-	+	2	-	3	gi|14249338
CGI-79	39.7		-	-	-	-		1	-	4	gi|57471625
non-snRNP proteins											
DBPA	40.1		-	1	-	-		1	-	1	gi|16198465
DDX9	142.0	+	9	6	5	20		10	8	26	gi|4503297
FBP21	42.5		-	-	-	-	+	3	2	11	gi|6005948
hPRP4-Kinase	117.1		5	11	11	8	+	1	1	9	gi|20330554
Luc7-A	51.5		5	5	3	1		-	-	1	gi|19923485
p72/DDX17	80.5	+	-	3	6	3	+	6	-	11	gi|38201710
PPP1CA	38.6	+	-	-	-	1	+	1	-	1	gi|4506003
RBM10	104.0		-	-	-	1		3	-	1	gi|20127479
RBM7	30.5		-	2	3	1		3	-	2	gi|9994185
TFIP11	96.8		-	1	2	2	+	7	6	9	gi|8393259
ZNF265	37.4		-	1	1	1		1	-	6	gi|42741684
ZNF207	50.8	+	1	1	1	-		-	-	1	gi|4508017
SKIV2L2	117.8		2	6	4	5		3	1	1	gi|39930353

Cap binding complex											
CBP20	18.0	+	19	1	1	2	+	-	-	1	gi|19923387
CBP80	91.8	+	23	2	6	4	+	1	1	6	gi|4505343
General mRNP proteins											
ASR2B	100.0	+	31	3	4	-	+	2	-	1	gi|58331218
ELAV	36.1	+	1	1	3	3	+	5	2	7	gi|38201714
HCNGP	33.9	+	5	2	5	2		-	1	3	gi|9994179
LOC124245	104.0	+	17	5	11	14	+	2	2	12	gi|31377595
NF45	43.0	+	8	6	2	6	+	3	-	7	gi|24234747
NFAR	95.4	+	11	2	1	16	+	6	1	13	gi|24234750
YB-1	35.9	+	2	8	9	10	+	7	3	12	gi|34098946
EJC/mRNP											
RNPS1	34.2	+	1	1	2	3		1	-	2	gi|6857826
Acinus (fSAP152)	151.8	+	1	5	11	7		3	1	6	gi|7662238
Aly/REF	26.9		2	1	-	2		1	-	2	gi|55770864
UAP56	49.1		7	2	2	2		3	1	5	gi|21040371
DDX3	73.3		1	2	-	1		1	-	1	gi|87196351
TREX											
THOC1	75.6		-	1	2	5		2	-	10	gi|4826882
THOC2	169.6		-	-	-	3		1	1	3	gi|52486999
SR proteins											
SF2/ASF	27.8	+	39	15	31	18	+	6	-	11	gi|5902076
9G8	27.4	+	13	9	22	15	+	7	3	10	gi|72534660
SRp20	19.4	+	10	1	3	1		-	-	3	gi|4506901
SRp30c	25.5	+	-	5	3	6	+	1	-	7	gi|4506903
SRp40	31.3	+	4	3	6	6	+	5	-	9	gi|86991438
SRp55	39.6	+	13	10	20	16	+	12	4	8	gi|20127499
SRp75	56.8	+	2	2	2	1	+	1	-	3	gi|21361282
SC35	25.5	+	1	1	3	-		-	-	-	gi|47271443
hTra-2 alpha	32.7		1	1	2	1		-	-	2	gi|9558733
hTra-2 beta/SFRS10	33.7	+	3	-	2	-	+	1	-	1	gi|4759098
SFRS12	59.4		6	1	3	3		-	-	3	gi|21040255

SFRS11 (p54)	53.5		10	2	4	2		-	-	2	gi|4759100
SR related proteins											
FLJ10154	32.9	+	-	2	4	2	+	2	-	3	gi|48675817
SRm160	102.5	+	3	3	1	2	+	1	-	2	gi|33150824
SRm300	300.0	+	37	34	38	12		28	1	25	gi|19923466
hnRNP											
hnRNP A0	30.9		3	3	5	5		2	-	5	gi|5803036
hnRNP A1	38.7	+	28	22	60	43	+	25	11	56	gi|4504445
hnRNP A2/B1	37.4	+	9	8	29	19	+	12	4	23	gi|14043072
hnRNP A3	39.6	+	1	5	7	8	+	1	-	7	gi|34740329
hnRNP AB	36.0	+	-	3	3	5		3	-	7	gi|33112434
hnRNP C1/C2	33.3	+	2	-	4	3	+	3	-	7	gi|4758544
hnRNP D	38.4		-	2	5	2		1	1	3	gi|14110414
hnRNP G	42.4		1	-	2	-	+	2	-	3	gi|56699409
hnRNP H	49.2		-	2	1	-		2	-	1	gi|48145673
hnRNP M	77.5		5	3	7	9		1	1	12	gi|14141152
hnRNP Q	69.6	+	2	2	1	3		6	3	4	gi|23397427
hnRNP R	70.9		16	1	8	11		1	1	13	gi|5031755
PCBP1	37.5	+	8	6	16	7	+	9	4	9	gi|5453854
PCBP2	38.6		3	2	1	2		2	-	2	gi|14141166
RNPC2	59.4	+	26	9	25	16	+	13	7	19	gi|4757926
Transcription											
CDK9	42.8		-	1	3	4		2	-	2	gi|4502747
Cofactor for Sp1	72.9		3	-	3	5		1	-	1	gi|28558975
CRkRS	164.2		-	1	2	1		-	-	1	gi|7706549
cyclin K (CCNK)	40.8		-	2	1	1		-	-	1	gi|38176158
Cyclin L1	60.0		3	1	1	-		-	-	1	gi|9945320
Paf1	59.9		-	2	-	6		2	-	4	gi|42476169
Parafimbromin	60.6		-	2	-	9		2	-	3	gi|40018640
SON	263.8		-	1	3	2		5	-	9	gi|21040326
XRCC6	69.8		-	1	-	-		10	-	3	gi|4503841

RNA processing									
CPSF1	160.9	-	1	1	3	1	3	10	gi|56676371
FIP1L1	66.5	-	3	2	4	2	1	4	gi|40254978
RBM15	107.2	-	3	5	8	3	-	6	gi|47933339
RNA binding									
TIP-48	51.1	1	2	1	2	2	-	-	gi|5730023
TIP-49	50.2	2	-	2	2	-	-	-	gi|4506753
eIF2AK2	62.1	-	1	-	4	2	-	6	gi|4506103
ZC3HAV1	101.4	-	-	3	7	2	-	2	gi|27477136
Other cellular processes									
14-3-3 epsilon	29.2	5	-	1	1	1	-	1	gi|62131678
BAT2D1	308,6	-	-	-	1	6	1	19	gi|115298682
CG3173-PA	73.1	3	2	-	7	1	-	-	gi|113419429
GCFC	104.8	-	1	-	4	5	1	12	gi|22035565
MGC2655	37.5	-	2	-	1	2	-	7	gi|31543164
PDCD4	51.7	-	-	-	2	3	-	8	gi|21735596
PIB5PA	70.5	-	-	-	2	1	1	-	gi|13325070
PRKDC	469.1	40	3	9	5	42	5	47	gi|13654237
PRMT5	72.7	-	-	-	-	1	-	2	gi|2323410
RSRC1	38.7	-	-	-	2	1	-	4	gi|38488727
TRRAP	434.4	4	-	1	2	2	-	-	gi|4507691
Other cellular processes (thought to be non-nuclear)									
AHNAK	689.0	-	-	-	-	1	-	2	gi|61743954
BAT2	228.9	-	-	-	-	2	-	2	gi|15277263
FNBP4	110.2	-	-	-	-	3	1	4	gi|158534059
MYCBP2	510.2	-	-	-	-	6	-	2	gi|126116565
RACK1	34.9	4	4	6	7	11	5	19	gi|5174447
SEC31A	133.0	-	-	3	2	-	3	3	gi|41349439
SHCBP1	133.4	-	1	1	4	-	-	1	gi|7661950

Table S1. Proteins detected by mass spectrometry in MS2 affinity-purified cross-exon or B-like complexes. Proteins identified by LC-MSMS after separation by PAGE in at least two of three preparations are shown. Note that only a subset of proteins identified are shown here, with the remainder shown in Table 1. Proteins not reproducibly detected are summarized in Table S2. Proteins generally accepted to be common contaminants, such as ribosomal proteins, are not shown. The presence of a protein is indicated by a number which represents the absolute number of peptides sequenced for that protein in a particular preparation (i.e. #1, #2 , #3 or #4). A "+" represents the presence of a protein in purified human A (Behzadnia et al., 2007) or B (Deckert et al., 2006) complexes. Proteins are grouped in organisational and/or functional subgroups**.**

Table S2

Protein	Mol mass (kDa)	Exon complex				B-like complex			GenBank accession no.
		#1	#2	#3	#4	#1	#2	#3	
Splicing associated									
Abstrakt	69.8	-	-	-	-	-	-	1	gi|21071032
CDC2L2	91.0	9	-	-	-	-	-	-	gi|16357482
CHERP	100.0	-	-	1	4	2	-	-	gi|21359884
Cyp-E	33.4	-	-	-	-	-	-	1	gi|5174637
DDX35	78.9	-	-	-	-	-	-	2	gi|20544129
DHX36	114.8	1	-	-	-	-	-	-	gi|18497286
E1B-AP5	95.7	-	-	5	2	-	-	3	gi|3319956
eIF4A3	46.9	-	1	-	-	-	-	3	gi|7661920
hnRNP F	45.6	-	-	-	-	-	-	2	gi|16876910
hnRNP L	64.1	-	-	1	-	-	-	-	gi|52632383
hnRNP U	90.5	-	-	-	-	-	-	1	gi|14141161
hsp27	22.8	2	1	-	-	-	-	3	gi|4504517
KHDRBS1	48.2	-	-	1	-	-	-	1	gi|5730027
Luc7-like	43.8	2	-	-	2	-	-	-	gi|8922297
Luc7-like2	47.6	3	-	4	-	-	-	-	gi|7706310
matrin 3	94.6	-	-	-	-	1	-	-	gi|62750354
MGC23918	19.2	-	-	-	-	-	-	3	gi|21389497
NIPP1	38.5	-	-	-	-	-	-	1	gi|46255714
PPIG	88.7	-	-	-	1	-	-	-	gi|42560244
PTB	59.5	-	-	-	-	-	-	3	gi|4506243
RBM14	69.5	-	-	-	-	-	-	4	gi|5454064
RBM4	40.3	-	-	-	1	2	-	-	gi|12653083
RBM5/LUCA15	92.1	-	1	-	-	-	-	-	gi|5032031
SFRS16	77.2	-	-	-	-	-	-	25	gi|133922600
SNIP1	45.8	1	-	-	-	-	-	-	gi|21314720
SPF31	31.0	-	-	3	-	-	-	-	gi|7657611
SRp38	31.3	-	-	-	2	-	-	5	gi|5730079
SRp46	32.3	1	-	-	-	-	-	-	gi|15055543
SRPK1	74.3	-	1	-	-	-	-	1	gi|47419936
SRrp 35	30.5	-	1	1	-	-	-	-	gi|148612890
RBBP6	201.7	-	-	1	2	-	-	2	gi|33620716
Transcription associated									
BCLAF1	106.2	-	-	1	1	-	-	5	gi|7661958
CREBBP	265.4	-	-	-	1	-	-	1	gi|119943104
Cyclin T1	80.7	-	-	1	-	-	-	-	gi|17978466
DIDO1	243.8	-	-	-	-	-	-	1	gi|71044479
MED12	243.1	1	-	-	-	-	-	-	gi|110347429
MTA2	75.1	-	-	-	-	-	-	2	gi|14141170
NCOR1	270.2	-	-	-	-	1	-	8	gi|22538461
PHF3	229.5	-	-	-	-	-	-	1	gi|7662018
RP II H	17.1	2	-	-	-	-	-	-	gi|14589953
RPB 1	217.2	-	1	-	4	-	-	-	gi|4505939
RPB 5	24.6	4	-	1	-	-	-	-	gi|14589951
RPB 7	19.3	2	-	-	-	-	-	-	gi|4505947
SETD2	231.1	-	-	1	-	-	-	4	gi|30410779

TAF15	61.8	-	-	-	-	-	-	1	gi|4507353
TRAP25	20.3	2	-	-	-	-	-	-	gi|18087811
TRERF1	132.2	-	-	-	7	-	-	-	gi|15812222
TRFP	23.2	-	-	1	-	-	-	1	gi|30023851
RNA processing									
CPSF2	88.4	-	-	1	-	-	-	-	gi|34101288
CPSF3	77.5	-	-	-	-	-	-	3	gi|7706427
CPSF4	30.2	-	-	-	-	-	-	1	gi|5729939
CPSF6	68.0	-	-	-	-	1	-	-	gi|33300633
CSTF2	61.0	-	-	3	2	-	-	-	gi|4557493
ELG	38.9	1	-	-	-	-	-	-	gi|8923771
GEMIN3	92.2	-	-	-	2	-	-	2	gi|14251212
GEMIN1 (SMN1)	31.8	-	-	1	1	-	-	3	gi|4507091
GEMIN2	31.8	-	1	-	2	-	-	2	gi:10937869
GEMIN4	120.0	-	-	-	2	-	-	2	gi|122939157
GEMIN5	168.5	-	-	-	2	5	-	-	gi|22001417
GEMIN6	18.8	-	1	-	-	-	-	-	gi|41393577
MRNP 41	41.0	-	1	-	-	-	-	1	gi|4506399
STRAP	38.4	-	-	-	2	-	-	1	gi|148727341
THOC3	38.8	-	-	2	3	-	-	2	gi|14150171
RNA binding									
ADAR	136.0	-	-	-	3	-	-	-	gi|70166852
CIRP	18.7	-	1	-	-	-	-	-	gi|4502847
DDX 26	100.4	-	-	-	3	1	-	-	gi|11024694
EMG	26.8	-	-	-	-	-	-	2	gi|31652262
FB19	99.1	-	-	-	1	-	-	1	gi|25777671
IGF2BP3	63.7	-	-	3	-	-	-	3	gi|30795212
MGC10433	50.4	-	1	-	5	-	-	-	gi|21359951
PNO1	27.9	-	-	-	-	-	-	2	gi|10047140
PRPF40B	99.4	-	1	-	1	-	-	1	gi|29789008
RBM15B	97.2	-	-	-	-	-	-	1	gi|54607124
RBM4B	40.5	-	-	-	-	-	-	-	gi|13899354
RBMXL1	47.8	-	1	-	-	-	-	1	gi|21361809
SERBP1	45.0	-	-	-	-	-	-	1	gi|41349439
STRBP	73.7	3	-	-	-	-	-	-	gi|21361745
TPR	267.2	-	-	2	-	-	-	-	gi|114155142
WTAP	44.2	-	-	2	1	-	-	1	gi|21361159
Other cellular processes									
14-3-3 beta	28.1	1	-	-	-	-	-	-	gi|4507949
ABCA8	17.9	-	-	-	-	-	1	-	gi|6005701
AKAP8	76.1	-	-	1	-	-	-	2	gi|5031579
ANK2	433.6	-	-	-	-	-	-	1	gi|52426735
BAG2	23.6	-	1	-	-	-	-	1	gi|4757834
BCDIN3	74.4	2	2	-	-	-	-	4	gi|49114634
CARF	61.1	-	-	-	-	-	-	2	gi|8923040
CCAR1	132.8	-	-	2		-	-	3	gi|32441867
DNAJA1	44.9	-	-	1	1	-	-	2	gi|4504511
DNAJC1	63.9	-	-	-	3	-	5	-	gi|21361912
EAPP	32.7	-	-	-	-	-	-	5	gi|117938268
ERH	12.2	-	-	2	-	-	-	-	gi|4758302
FLJ10569	82.2	3	-	-	2	-	-	1	gi|83582809
FLJ10839		-	-	-	-	2	-	-	gi|46852388

FLJ12894	55.5	-	-	1	-	1	-	-	gi|19913371
GROS1	90.5	-	-	1	-	-	-	-	gi|21361918
GTL3	22.8	2	-	1	-	-	-	3	gi|8392875
H2A C	13.7	-	-	-	1	-	1	-	gi|4504239
H2B	13.8	2	-	-	-	-	-	1	gi|28173554
H2B J	13.8	-	1	-	-	-	-	-	gi|4504269
HsKin17	45.2	-		-	-	-	-	3	gi|13124883
HSPA2	70.0	-	1	-	-	-	-	-	gi|13676857
LARP1	123.5	-	-	-	-	-	-	1	gi|39725634
LOC115004	58.9	-	-	4	3	-	3	-	gi|115511030
LOC196441	226.2	-	-	-	-	-	-	1	gi|87116683
LOC23131	164.2	-	1	-	-	1	-	-	gi|50962882
LOC257169	52.1	1	-	-	-	-	-	-	gi|22749539
LOC25980	43.3	-	-	-	1	-	-	6	gi|18034690
LOC283987	88.6	-	2	-	2	-	-	-	gi|29825823
LOC284498	162.3	-	-	-	-	-	-	1	gi|113403400
LOC51574	66.8	-	1	-	-	-	-	-	gi|109809741
LOC55720	92.4	-	-	-	-	2	-	-	gi|39780588
LOC55726	80.1	-	-	-	4	-	-	3	gi|155030185
LOC79034	49.9	1	-	-	-	-	-	-	gi|21362070
LOC9703	253.6	-	-	2	-	-	1	-	gi|57242774
LTV1	54.8	-	-	-	-	-	-	3	gi|21361875
MEP50	36.6	-	-	-	-	-	1	-	gi|13129110
NCOR2	274.7	-	-	-	-	-	-	1	gi|116256453
NGEF	82.4	-	1	-	-	-	-	-	gi|166197688
NUMA1	238.1	-	1	-	2	-	-	2	gi|71361682
NYD-SP11	273.2	-	1	-	-	-	-	-	gi|89056816
p30 DBC	102.8	-	-	-	-	-	-	3	gi|24432106
PCNA	28.7	-	-	-	-	2	-	-	gi|4505641
PDCD6	21.9	9	-	-	-	-	-	-	gi|7019485
PKP2	97.3	-	-	-	-	2	-	-	gi|4758932
PLCB2	134.0	-	-	-	-	-	2	-	gi|95147333
PRMT1	42.4	-	-	2	-	-	-	-	gi|150456457
RBBP7	47.8	-	-	-	-	1	-	-	gi|4506439
RP III large subunit	155	2	-	-	-	-	-	-	gi|39725938
S100A8	10.7	-	-	1	-	-	-	-	gi|21614544
SMC1A	143.2	-	1	-	-	-	-	-	gi|30581135
SPEN	402.2	-	-	-	-	9	-	-	gi|14790190
SSRP1	81.0	-	-	-	-	2	-	-	gi|4507241
SUMO1	11.4	-	-	-	-	-	-	9	gi|4507801
SUMO3	11.5	-	-	-	-	-	-	4	gi|48928058
thioredoxin	11.7	-	1	-	-	-	-	-	gi|50592994
THOC5	78.5	-	-	3	2	-	-	7	gi|50959085
TOP2A	174.4	-	-	-	2	-	-	-	gi|19913406
VSIG8	43.9	-	-	1	-	-	-	-	gi|61966777
WDR33	145.9	-	-	-	1	-	-	1	gi|56243590
WDR61	33.6	-	2	-	2	-	-	-	gi|13376840
YTHDF2	62.3	-	-	1	-	-	-	-	gi|12803469
ZGPAT	57.3	-	-	1	-	-	-	2	gi|133925793
ZNF318	251.1	-	-	-	-	-	-	3	gi|120587019
ZNF598	98.7	-	-	-	-	-	-	1	gi|30039694
Other cellular processes (thought to be non-nuclear)									
14-3-3 zeta	27.6	-	1	-	-	2	-	-	gi|4507953
ADH7	41.5	-	-	4	5	-	-	-	gi|71743840

BYSL	49.6	-	-	-	-	-	-	1	gi|51173724
Desmoplakin	331.8	-	-	1	-	-	-	3	gi|58530840
DYNLL1	10.2	1	-	-	-	-	-	-	gi|4505813
ERC2	110.5	-	-	-	-	-	-	1	gi|34577114
filaggrin	435.2	-	-	-	-	-	1	-	gi|60097902
filaggrin 2	247.9	1	-	-	-	-		-	gi|62122917
FLJ23420	90.1	-	-	-	9	-	-	1	gi|40217803
FLJ23445	23.6	-	1	-	-	-	-	-	gi|156151381
GALNT3	72.6	-	-	-	-	-	-	1	gi|153266878
LOC57461	33.6	-	-	-	1	-	-	-	gi|29540545
PPIB	21.0	-	-	-	-	-	-	1	gi|4758950
PRDX1	22.1	-	-	-	-	-	1	-	gi|32455266
PRIC285	294.6	-	-	-	-	-	-	1	gi:156105693
PTCHD3	86.9	-	-	-	-	-	1	-	gi|77798175
Rtn4	129.8	-	-	1	-	-	-	-	gi|24431935
Signal Recognition P54	55.6	-	2	-	-	-	-	-	gi|4507215
TNFAIP8	22.9	-	1	-	-	-	-	-	gi|117676365
VG5Q	80.9	-	-	1	-	-	-	-	gi|39725952

Table S2. Proteins not reproducibly detected by mass spectrometry in MS2 affinity-purified cross-exon or B-like complexes. Proteins were identified by LC-MSMS after separation by PAGE. Proteins identified in maximally one-third of the preparations are shown. Proteins generally accepted to be common contaminants, such as ribosomal proteins or those involved in RNA degradation are not shown. The presence of a protein is indicated by a number which represents the absolute number of peptides sequenced for that protein in a particular preparation (i.e. #1, #2 , #3 or #4). The first column contains the name of the protein followed by its predicted molecular mass in kDa and the last column shows its GenBank acession number. Proteins are grouped in organisational and/or functional subgroups.

Supplemental References

Barabino, S.M., Blencowe, B.J., Ryder, U., Sproat, B.S., and Lamond, A.I. (1990). Targeted snRNP depletion reveals an additional role for mammalian U1 snRNP in spliceosome assembly. Cell 63, 293-302.

Blencowe, B.J., Sproat, B.S., Ryder, U., Barabino, S., and Lamond, A.I. (1989). Antisense probing of the human U4/U6 snRNP with biotinylated 2'-OMe RNA oligonucleotides. Cell 59, 531-539.

Karaduman, R., Fabrizio, P., Hartmuth, K., Urlaub, H., and Lührmann, R. (2006). RNA structure and RNA-protein interactions in purified yeast U6 snRNPs. J. Mol. Biol. 356, 1248-1262.

Segault, V., Will, C.L., Sproat, B.S., and Lührmann, R. (1995). In vitro reconstitution of mammalian U2 and U5 snRNPs active in splicing: Sm proteins are functionally interchangeable and are essential for the formation of functional U2 and U5 snRNPs. EMBO J. 14, 4010-4021.

Sharma, S., Kohlstaedt, L.A., Damianov, A., Rio, D.C., and Black, D.L. (2008). Polypyrimidine tract binding protein controls the transition from exon definition to an intron defined spliceosome. Nat. Struct. Mol. Biol. 15, 183-191.

Will, C.L., Urlaub, H., Achsel, T., Gentzel, M., Wilm, M., and Lührmann, R. (2002) Characterization of novel SF3b and 17S U2 snRNP proteins, including a human Prp5p homologue and an SF3b DEAD-box protein. EMBO J. 21, 4978-4988.