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Supplemental Data

The EF-G-like GTPase Snu114p Regulates Spliceosome Dynamics Mediated by Brr2p, a DExD/H Box ATPase

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Supplemental Experimental Procedures

Strains and Plasmids. (See Tables S1 and S2 below.) yJPS762 and yJPS1004 were made by TAP-tagging (Rigaut et al., 1999) *PRP43* or *PRP28*, respectively, through integrative transformation of BY4741 (Open Biosystems) with DNA amplified from pKG810 (Tasto et al., 2001) using primers Prp43-TAPF and Prp43-TAPR or Prp28-TAPF and Prp28-TAPR, respectively. The plasmid shuffle strains yJPS996, yJPS997 and yJPS998 were created by transforming yJPS762 with the wild-type, *URA3*-marked plasmids pSN123, pJPS1492 and pTB1, respectively, after which *BRR2* was replaced (Brachmann et al., 1998) with *LEU2* and *PRP22* and *SNU114* were replaced with *HIS3*, respectively. The plasmid shuffle strains yJPS1114 and yJPS1107 were created by transforming yJPS1004 with the wild-type, *URA3*-marked plasmid pSN123 and pTB1, respectively, after which *BRR2* was replaced with *LEU2* and *SNU114* was replaced with *HIS3*, respectively. The final, experimental strains were generated by first transforming yJPS996, yJPS997, yJPS998, yJPS1107, or yJPS1114 with plasmids encoding wild-type or mutant *BRR2*, *PRP22* or *SNU114* plasmids and then selecting against strains that maintained the corresponding *URA3*-marked plasmid by growth on 5-fluroorotic acid (Boeke et al., 1984). pJPS1492, pJPS1518, and pJPS1521 were made by subcloning the inserts in p358-PRP22 and p358-G810A (Schwer and Meszaros, 2000) into the appropriate vector (see Table S2) by restriction enzyme digest using SacI and SalI. pJPS1346 and pJPS1347 were made by subcloning the inserts in pJPS298 and pJPS643 (Leeds et al., 2006) into pRS313 by restriction enzyme digest using SacI and XhoI. pJPS654 was made by introducing the point mutation into pPR130 by QuickChange mutagenesis (Stratagene).

Oligonucleotides. Oligonucleotides for snRNA primer extensions were previously described (Staley and Guthrie, 1999; Leeds et al., 2006). For detection of psoralen-crosslinked species by Northern analysis, templates for random-primed probes were generated for U2, U5 and U6 by PCR of genomic DNA using the following primers.

U2F, 5′ACGAATCTCTTTGCCCTTTTGGCTTAGATCA

U2R, 5′CGCGAGAATAACAACTAAAGAAGCGAGCA

U5F, 5′AAGCAGCTTTACAGATCAATGGCGGAGGGA

U5R, 5′TTGAGAAAAAGGGCAGAAAAGTTCCAAAA

U6F, 5′GTTCGCGAAGTAACCCTTCGTGGACATTTGGTCAA

U6R, 5′AAAACGAAATAAATCTCTTTGTAAAACGGTTCATC

Oligonucleotides for Northern analysis for U3A precursors, intermediates and products were as follows.

U3A intron, 5′AGCTGCTGCAATGGTTG

U3A mRNA, 5′TTCCTATAGAAATGATCCTATGAAG

Oligonucleotides for Northern analysis of snRNAs were previously described (Noble and Guthrie, 1996).

Oligonucleotides for strain construction:

Prp43-TAPF,

5′TTGAAACAAGGTAAAAACAAAAAGAAGAGTAAGCACTCCAAGAAAATGAAGCGACGATGG

AAAAAGAATTTC

Prp43-TAPR,

5′AAATTTATATAAATCTATTTTTTTTTTTTTTTCGACACAAAATGTTACGACTCACTATAGGGA

Prp28-TAPF,

5′AACAAATATAACGTCGGCAAACAGCTCAGTAATGAGATTATATATATGAAGCGACGATGGA

AAAAGAATTTC

Prp28-TAPR,

5′TGAGATCTTGGGTCCATCAAAAAAAAGAAAGAAAATAATATAACTTACGACTCACTATAG

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Table S1. Yeast Strains Used in this Study

Strain	Relevant Gene(s)	Genotype	Source/Reference
yJPS797	*PRP43-TAP-KanMX*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ prp43::HIS3* pJPS1162	Leeds et al., 2006
yJPS773	*PRP22-TAP-KanMX*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ*	Leeds et al., 2006
yJPS798	*prp43-Q423N-TAP-KanMX*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ prp43::HIS3* pJPS1163	Leeds et al., 2006
yJPS762	*PRP43-TAP-KanMX*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ*	This study
yJPS997	*PRP43-TAP-KanMX PRP22*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ prp22::HIS3* pJPS1492	This study
yJPS1049	*PRP43-TAP-KanMX PRP22*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ prp22::HIS3* pJPS1518	This study
yJPS1052	*PRP43-TAP-KanMX prp22-G810A*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ prp22::HIS3* pJPS1521	This study
yJPS996	*PRP43-TAP-KanMX BRR2*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ brr2::LEU2* pSN123	This study
yJPS999	*PRP43-TAP-KanMX BRR2*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ brr2::LEU2* pPR130	This study
yJPS1000	*PRP43-TAP-KanMX brr2-1*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ brr2::LEU2* pPR133	This study
yJPS1001	*PRP43-TAP-KanMX brr2-R1107A*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ brr2::LEU2* pJPS654	This study
yTB135	*PRP43 SNU114*	*MAT***a** *his3Δ leu2Δ ura3Δ trp1Δ lys2Δ prp43::KanMX snu114::KanMX* pTB1 pTB123	Brenner et al., 2005
yJPS998	*PRP43-TAP-KanMX SNU114*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ snu114::HIS3* pTB1	This study
yJPS1053	*PRP43-TAP-KanMX SNU114*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ snu114::HIS3* pTB106	This study
yJPS1120	*PRP43-TAP-KanMX snu114-14*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ snu114::HIS3* pTB108	This study
yJPS1056	*PRP43-TAP-KanMX snu114-60*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ snu114::HIS3* pTB113	This study
yJPS1054	*PRP43-TAP-KanMX snu114-D271N*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ snu114::HIS3* pTB114	This study
yJPS1004	*PRP28-TAP-KanMX*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ*	This study
yJPS1107	*PRP28-TAP-KanMX SNU114*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ snu114::HIS3* pTB1	This study
yJPS1108	*PRP28-TAP-KanMX SNU114*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ snu114::HIS3* pTB106	This study
yJPS1111	*PRP28-TAP-KanMX snu114-D271N*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ snu114::HIS3* pTB114	This study
yJPS1114	*PRP28-TAP-KanMX BRR2*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ brr2::LEU2* pSN123	This study
yJPS1115	*PRP28-TAP-KanMX BRR2*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ brr2::LEU2* pPR130	This study
yJPS1117	*PRP28-TAP-KanMX brr2-R1107A*	*MAT***a** *his3Δ leu2Δ met15Δ ura3Δ brr2::LEU2* pJPS654	This study

Table S2. Plasmids Used in this Study

Plasmid	Genotype	Vector Backbone	Source or Reference
pJPS1162	*PRP43-TAP*	pRS316	(Leeds et al., 2006)
pJPS1163	*prp43-Q423N-TAP*	pRS316	(Leeds et al., 2006)
pJPS1492	*PRP22*	pRS316	This lab
pJPS1518	*PRP22*	pRS315	This lab
pJPS1521	*prp22-G810A*	pRS315	This lab
pSN123	*BRR2*	pRS426	(Raghunathan and Guthrie, 1998)
pPR130	*BRR2*	pSE362	(Raghunathan and Guthrie, 1998)
pPR133	*brr2-1*	pSE362	(Raghunathan and Guthrie, 1998)
pJPS654	*brr2-R1107A*	pSE362	This study
pJPS298	*PRP43*	pRS315	(Leeds et al., 2006)
pJPS643	*prp43-Q423N*	pRS315	(Leeds et al., 2006)
pJPS1346	*PRP43*	pRS313	This lab
pJPS1347	*prp43-Q423N*	pRS313	This lab
pTB123	*PRP43*	pSE360	(Brenner and Guthrie, 2005)
pTB1	*SNU114*	pRS316	(Brenner and Guthrie, 2005)
pTB106	*SNU114*	pRS315	C. Guthrie
pTB108	*snu114-14*	pRS315	C. Guthrie
pTB113	*snu114-60*	pRS315	C. Guthrie
pTB114	*snu114-D271N*	pRS315	C. Guthrie