SUPPLEMENTARY MATERIAL FOR:

Rps3/uS3 promotes mRNA binding at the 40S ribosome entry channel and stabilizes preinitiation complexes at start codons

Jinsheng Dong, Colin E. Aitken, Anil Thakur, Byung-Sik Shin, Jon R. Lorsch, and Alan G. Hinnebusch

Table S1. Summary of key phenotypes for Rps3 substitutions

Rps3 substitution	Relevant 48S PIC contacts (≤ 5Å)	Cell Growth (Slg$^-$)	Suppression of *SUI5* His$^+$ phenotype	Suppression of *SUI5* Slg$^-$ phenotype at 37°C	*HIS4-lacZ* UUG:AUG expression in *SUI5* cells
none (WT)	NA[1]	++++	none (WT)	-	0.151±0.019
K108D	none	++++	strong	strong	0.039±0.006
R116A	mRNA	-/+	NA[2]	NA[2]	ND[3]
R116D	mRNA	++++	moderate	strong	0.032±0.003
R117A	mRNA	++++	none	none	ND
R117D	mRNA	++++	strong	strong	0.026±0.004
K141A	latch	lethal	NA[2]	NA[2]	ND
K141D	latch	++	NA[2]	NA[2]	ND
R146A	latch/mRNA	lethal	NA[2]	NA[2]	ND
R146D	latch/mRNA	+++	slight	Moderate[4]	0.036±0.006
K148A	mRNA	++++	none	none	ND
K148D	mRNA	++++	moderate	slight	0.026±0.004

[1] NA, not applicable

[2] NA, as suppression of the effects of *SUI5* on growth in -His medium or in +His medium at 37°C could not be scored owing to lethality (*K141A* & *R146A*) or strongly impaired growth (*R116A*) conferred by the *rps3* mutation in the absence of *SUI5* (Fig. 2B).

[3] ND, not determined

[4] Although growth of the *R146D/SUI5* strain is only slightly better than that of the WT/*SUI5* control strain on +His medium at 37°C (cf. rows 1 and row 12 in Fig. 2B), taking into account the reduced growth of the *R146D/SUI5* strain relative to the WT/*SUI5* strain at 30°C (left panel) suggests a moderate versus slight degree of suppression.

ND, not determined

Table S2. Yeast strains employed in this work.

Strain	Genotype	Source
H2995	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)*	(Valasek et al. 2004)
HD2738	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX*	This study
HD2754	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH412 [lc[a] *LEU2* $RPS3^+$ in pRS315]	This study
HD2755	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pRS315 [lc *LEU2* vector]	This study
HD2765	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH424 [lc LEU2 *rps3-K148D* in pRS315]	This study
HD2764	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH431 [lc *LEU2 rps3-K148A* in pRS315]	This study
HD2767	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH425 [lc *LEU2 rps3-R116D* in pRS315]	This study
HD2766	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH432 [lc *LEU2 rps3-R116A* in pRS315]	This study
HD2769	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH482 [lc *LEU2 rps3-R117D* in pRS315]	This study
HD2768	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH433 [lc *LEU2 rps3-R117A* in pRS315]	This study
HD3120	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH13-37 [lc *LEU2 rps3K108D* in pRS315]	This study
HD2772	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH429 [lc *LEU2 rps3-R146D* in pRS315]	This study
HD2779	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH430 [lc *LEU2 rps3-K141D* in pRS315]	This study
HD2836	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH412 [lc *LEU2* $RPS3^+$ in pRS315] p4281 [sc[a] *TRP1 SUI5* in YCplac22]	This study

Strain	Genotype	Source
HD2911	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH412 [lc *LEU2* $RPS3^+$ in pRS315] YCplac22 [sc *TRP1* vector]	This study
HD2846	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH424 [lc *LEU2 rps3-K148D* in pRS315] p4281 [sc *TRP1 SUI5* in YCplac22]	This study
HD2868	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH431 [lc *LEU2 rps3-K148A* in pRS315] p4281 [sc *TRP1 SUI5* in YCplac22]	This study
HD2848	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH425 [lc *LEU2 rps3-R116D* in pRS315] p4281 [sc *TRP1 SUI5* in YCplac22]	This study
HD2861	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH432 [lc *LEU2 rps3-R116A* in pRS315] p4281 [sc *TRP1 SUI5* in YCplac22]	This study
HD2850	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH482 [lc *LEU2 rps3-R117D* in pRS315] p4281 [sc *TRP1 SUI5* in YCplac22]	This study
HD2849	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH433 [lc *LEU2 rps3-R117A* in pRS315] p4281 [sc *TRP1 SUI5* in YCplac22]	This study
HD3145	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH13-37 [lc *LEU2 rps3-K108D* in pRS315] p4281 [sc *TRP1 SUI5* in YCplac22]	This study
HD2841	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH429 [lc *LEU2 rps3-R146D* in pRS315] p4281 [sc *TRP1 SUI5* in YCplac22]	This study
HD2860	*MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)* P_{GAL}-*RPS3::kanMX* pDH430 [lc *LEU2 rps3-K141D* in pRS315] p4281 [sc *TRP1 SUI5* in YCplac22]	This study
HD2973	*MATa ura3Δ0 leu2Δ0 his3Δ0 met15Δ0 rps3Δ::kanMX* pDH459 [lc *URA3* $RPS3^+$ in pRS316]	This study
HD3240	*MATa ura3Δ0 leu2Δ0 his3Δ0 met15Δ0 rps3Δ::kanMX* pDH412 [lc *LEU2* $RPS3^+$ in pRS315]	This study

Strain	Genotype	Source
HD3241	*MATa ura3Δ0 leu2Δ0 his3Δ0 met15Δ0 rps3Δ::kanMX* pDH425[lc *LEU2 rps3-R116D* in pRS315]	This study
HD3242	*MATa ura3Δ0 leu2Δ0 his3Δ0 met15Δ0 rps3Δ::kanMX* pDH482 [lc *LEU2 rps3-R117D* in pRS315]	This study
HD3243	*MATa ura3Δ0 leu2Δ0 his3Δ0 met15Δ0 rps3Δ::kanMX* pDH13-37 [lc *LEU2 rps3-K108D* in pRS315]	This study
HD3244	*MATa ura3Δ0 leu2Δ0 his3Δ0 met15Δ0 rps3Δ::kanMX* pDH424 [lc *LEU2 rps3-K148D* in pRS315]	This study
HD3277	*MATa ura3Δ0 leu2Δ0 his3Δ0 met15Δ0 rps3Δ::kanMX* pDH429 [lc *LEU2 rps3-R146D* in pRS315]	This study

[a] lc, low copy number; sc, single copy

Table S3. Plasmids employed in this work.

Plasmid	Description	Source
pRS315	lc[a] *LEU2* vector	(Sikorski and Hieter 1989)
pRS316	lc *URA3* vector	(Sikorski and Hieter 1989)
p4281	sc *TRP1 TIF5-G31R* in YCplac22	(Valasek et al. 2004)
YCplac22	sc *TRP1* vector	(Gietz and Sugino 1988)
pDH412	lc *LEU2*, *RPS3*$^+$	This study
pDH459	lc *URA3*, *RPS3*$^+$	This study
pDH424	lc *LEU2*, *rps3-K148D*	This study
pDH431	lc *LEU2*, *rps3-K148A*	This study
pDH425	lc *LEU2*, *rps3-R116D*	This study
pDH432	lc *LEU2*, *rps3-R116A*	This study
pDH482	lc *LEU2*, *rps3-R117D*	This study
pDH433	lc *LEU2*, *rps3-R117A*	This study
pDH13-37	lc *LEU2*, *rps3-K108D*	This study
pDH429	lc *LEU2*, *rps3-R146D*	This study
pDH430	lc *LEU2*, *rps3-K141D*	This study
p3218	pFA6a-kanMX6-PGAL1	(Longtine et al. 1998)
p367	sc *URA3 HIS4(ATG)-lacZ*	(Donahue and Cigan 1988)

Plasmid	Description	Source
p391	sc *URA3 HIS4(TTG)-lacZ*	(Donahue and Cigan 1988)
pPMB24	sc *URA3 SUI1-lacZ*	(Martin-Marcos et al. 2011)
pPMB25	sc *URA3 SUI1-opt-lacZ*	(Martin-Marcos et al. 2011)

[a] lc, low copy number; sc, single copy.

Table S4. Primers used for mutagenesis (with mutated nucleotides underlined).

Primer	Sequence (5’ to 3’)	Mutation
RPS3-K148D	CTAAGAGCTGCCAGAGCTGATGCTATGAAATTTGCTGAC	*K148D*
RPS3-K148D-r	GTCAGCAAATTTCATAGCATCAGCTCTGGCAGCTCTTAG	*K148D*
RPS3-K148A	CTAAGAGCTGCCAGAGCTGCTGCTATGAAATTTGCTGAC	*K148A*
RPS3-K148A-r	GTCAGCAAATTTCATAGCAGCAGCTCTGGCAGCTCTTAG	*K148A*
RPS3-R116D	GAACGGTTTGGCTATCGATAGAGCTGCTTACGGTGTCG	*R116D*
RPS3-R116D-r	CGACACCGTAAGCAGCTCTATCGATAGCCAAACCGTTC	*R116D*
RPS3-R116A	GAACGGTTTGGCTATCGCTAGAGCTGCTTACGGTGTCG	*R116A*
RPS3-R116A-r	CGACACCGTAAGCAGCTCTAGCGATAGCCAAACCGTTC	*R116A*
RPS3-R117A	GGTTTGGCTATCAGAGCTGCTGCTTACGGTGTCGTCAG	*R117A*
RPS3-R117A-r	CTGACGACACCGTAAGCAGCAGCTCTGATAGCCAAACC	*R117A*
RPS3-R117D	GGTTTGGCTATCAGAGATGCTGCTTACGGTGTCGTCAG	*R117D*
RPS3-R117D-r	CTGACGACACCGTAAGCAGCATCTCTGATAGCCAAACC	*R117D*
RPS3-K108D	GCTGAATCTATGAAATTCGATTTGTTGAACGGTTTGGC	*K108D*
RPS3-K108D-r	GCCAAACCGTTCAACAAATCGAATTTCATAGATTCAGC	*K108D*
RPS3-R146D	GGTAAACTAAGAGCTGCCGATGCTAAGGCTATGAAATTT	*R146D*
RPS3-R146D-r	AAATTTCATAGCCTTAGCATCGGCAGCTCTTAGTTTACC	*R146D*
RPS3-K141D	GAAGTTGTTGTTTCCGGTGATCTAAGAGCTGCCAGAGCT	*K141D*
RPS3-K141D-r	AGCTCTGGCAGCTCTTAGATCACCGGAAACAACAACTTC	*K141D*

Figure S1. *RPS3* alleles do not significantly alter 40S:60S subunit ratios.

rps3Δ::kanMX deletion strains harboring plasmid-borne WT *RPS3*$^+$ (HD3240) or *rps3* alleles *R116D* (HD3241), *R117D* (HD3242), *K108D* (HD3243), *K148D* (HD3244) or *R146D* (HD3277) were grown in YPD medium to A_{600} ~1, and WCEs prepared in the absence of Mg^{+2} were resolved by velocity sedimentation through 5 to 30% sucrose gradients and scanned at 254 nm. Mean (± S.E.M.) 40S/60S subunit ratios calculated from three biological replicates are indicated. Asterisks indicate significant differences between mutant and WT as judged by a two-tailed, unpaired Student's t-test (*, $P<0.05$; **, $P<0.01$).

Dong *et* al_Fig. S1