Supplementary Data to

P1 AND P2 PROTEIN HETERODIMER BINDING TO THE P0 PROTEIN OF *Saccharomyces cerevisiae* IS RELATIVELY NON-SPECIFIC AND A SOURCE OF RIBOSOMAL HETEROGENEITY

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MATERIALS AND METHODS

Strains

Saccharomyces cerevisiae strains used are listed in Table S1. Conditional P0 null strains (DGP0 strains) derived from D46, D47, D56 and D57, were obtained by substituting the *RPP0* genomic copy by a copy under the control of the GAL1 promoter, as previously described for the parental W303 strain (33), although in this case the genetic marker used for selection was the kanamycin resistance gene Kan^r .

Table 1. Strains used in this work

Strain	Genotype	Reference
W303−1b	MATα, *leu2-3,112 trp1-1 his3-11,15 ura3-1, ade2-1, can1-100.*	(49)
D46	MATa, *RPP1B::TRP1, RPP2A::URA3, leu2-3,112, his3-11,15 can1-100*.	(30)
D47	MATa, *RPP1A::LEU2, RPP2A::URA3, his3-11,15, trp1-1, can1-100.*	(30)
D56	MATa, *RPP1B::TRP1, RPP2B::HIS3, ura3-1, leu2-3,112, can1-100*.	(30)
D57	MATa, *RPP1A::LEU2, RPP2B::HIS3, ura3-1, trp1-1, can1-100.*	(30)
W303dGP0	MATα, *leu2-3,112 trp1-1 his3-11,15 ura3-1, ade2-1, can1-100, RPP0::URA3-GAL1-RPP0*	(33)
D46dGP0	MATa, *RPP1B::TRP1, RPP2A::URA3, leu2-3,112, his3-11,15 can1-100, RPP0::KanMX4-GAL1-RPP0*	This work
D47dGP0	MATa, *RPP1A::LEU2, RPP2A::URA3, his3-11,15, trp1-1, can1-100 RPP0:: KanMX4-GAL1-RPP0*	This work
D56dGP0	MATa, *RPP1B::TRP1, RPP2B::HIS3, ura3-1, leu2-3,112, can1-100 RPP0:: KanMX4-GAL1-RPP0*	This work
D57dGP0	MATa, *RPP1A::LEU2, RPP2B::HIS3, ura3-1, trp1-1, can1-100 RPP0:: KanMX4-GAL1-RPP0*	This work

Table S2. Plasmids used in the work		
Plasmid	Characteristics	Reference
pFL36	LEU2, ARS-CEN6, pMB1 ori, *Amp*^{R}	(34)
pFL36-P0	pFL36 encoding protein P0	This work
pFL36-P0ΔA	pFL36 encoding P0ΔA	This work
pFL36-P0ΔB	pFL36 encoding protein P0ΔB	This work
pFL36-P0ΔAB	pFL36 encoding protein P0ΔAB	This work
pFL37	HIS3, ARS-CEN6, pMB1 ori, *Amp*^{R}	(50)
pFL37-P0	pFL37 encoding protein P0	This work
pFL37-P0ΔA	pFL37 encoding protein P0ΔA	This work
pFL37-P0ΔB	pFL37 encoding protein P0ΔB	This work
pFL37-P0ΔAB	pFL37 encoding protein P0ΔAB	This work
pFL38	URA3, ARS-CEN6, pMB1 ori, *Amp*^{R}	(34)
pFL38-P0	pFL38 encoding protein P0	This work
pFL38-P0ΔA	pFL38 encoding protein P0ΔA	This work
pFL38-P0ΔB	pFL38 encoding protein P0ΔB	This work
pFL38-P0ΔAB	pFL38 encoding protein P0ΔAB	This work
pFL39	TRP1, ARS-CEN6, pMB1 ori, *Amp*^{R}	(34)
pFL39-P0	pFL39 encoding protein P0	This work
pFL39-P0ΔA	pFL39 encoding protein P0ΔA	This work
pFL39-P0ΔB	vpFL39 encoding protein P0ΔB	This work
pFL39-P0ΔAB	pFL39 encoding protein P0ΔAB	This work

Table S3. Oligonucleotides used to construct the plasmids used in this work	
Deleted Site	Sequence
P0A3	5´-CGGTCAAGTGTTCACCTTGCCATCTGTCG-3´
P0A4	5´-CGACAGATGGCAAGGTGAACACTTGACCG-3´
P0B3	5´-CTCTTTGGCTATTGGTTACGAAATTGAAGATTTGGTTGA C-3’
P0B4	5´-GTCAACCAAATCTTCAATTTCGTAACCAATAGCCAAAGA G-3´
P0AB1	5´-CGACAACGGTCAAGTGTTCGAAATTGAAGATTTGGTTGA C-3´
P0A2	5´-GTCAACCAAATCTTCAATTTCGAACACTTGACCGTTGTCG-3´
P0N4	5´-ATT GAA ATT GTT TCT GAT GTC AAG-3´
P0taaXho	5´-GAC TCG AGA TCG AAT AAA CCG AAA CCC ATG-3´

Cell fractionation and ribosome preparation

Exponentially grown *S. cerevisiae* cells (A600 ~ 0,5) were resuspended in buffer 1 (10 mM Tris-HCl, pH 7.4, 20 mM KCl, 12.5 mM MgCl2, 1 mM DTT) supplemented with a mixture of protease inhibitors (aprotinine, antipain, leupeptine, chymostatin, pepstatine and PMSF at 10 μg/ml final concentration) and broken by shaking with glass beads in a FastPrep. The supernatant fraction S30 was obtained by centrifuging the extract at 30000 x g for 15 min at 4ºC. Ribosomes were prepared from the S30 by centrifugation at 100000 x g for 2 h at 4ºC, yielding a ribosomal pellet and the supernatant fraction S-100. The ribosomes were washed by centrifugation through a

discontinuous 20% and 40% sucrose gradient in buffer 2 (20 mM Tris-HCl, pH 7.4, 100 mM MgCl2, 0.5 M NH4Cl and 5 mM 2-mercaptoetanol) and resuspended in buffer 1. For activity tests, the ribosomes and fraction S-100 were obtained according to (51).

Analysis of polysomes and ribosomal subunits by sucrose gradients

S. cerevisiae cells were grown in YEPD medium then washed with buffer 3 (10 mM Tris-HCl pH 7.4, 80 mM KCl, 10 mM $MgCl_2$ and 100 μg/ml cycloheximide, 1% DTT). After breaking cells in Fastprep equipment, total extracts were obtained by centrifugation at 13,000 rpm for 30 min at 4ºC, and the supernatant loaded onto a linear 10 to 50% (w/v) sucrose gradient in buffer 2 and centrifuged in a SW40Ti rotor at 39.000 rpm for 3:45 h at 4ºC. Gradients were fractionated in a Teledyne ISCO 185 density gradient fractionator, and absorbance at 260 nm was monitored continuously by using an ISCO UA-5 UV monitor.

RESULTS AND DISCUSSION

Effect of truncated P0 expression on the accumulation of P1/P2 proteins

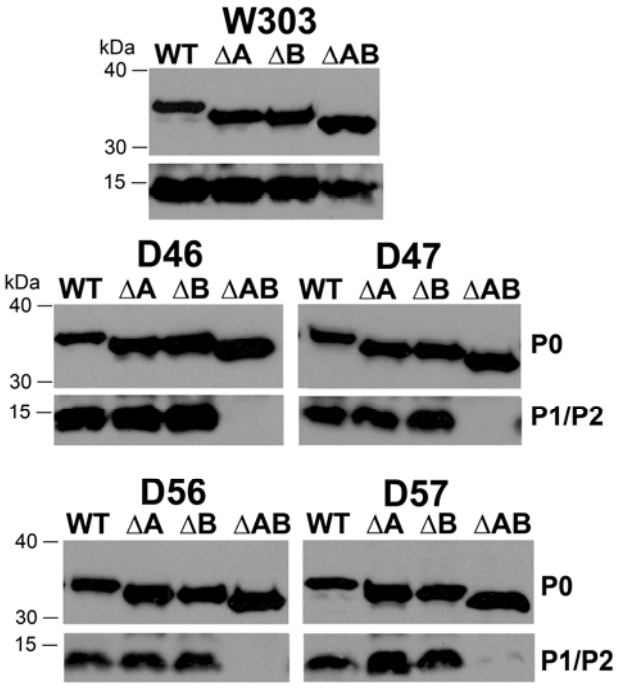

Fig S1.- Truncated P0 proteins expressed in the stalk mutant strains of *S. cerevisiae*. Strains W303dGP0, D46dGP0, D47dGP0, D56dGP0 and D57dGP0 were transformed with the corresponding plasmid to express wild-type P0, P0 lacking site A (ΔA), P0 lacking site B (ΔB) and P0 lacking both sites (ΔAB). The transformed cells were grown in glucose medium and the total cell extracts were resolved by SDS-PAGE. The P0 proteins were detected in immunoblots probed with antibody 3BH5(ppA-2), which cross-reacts with the C-terminal domain of all the stalk proteins (36).

Effect of P0 truncation on cell growth.

The failure to observe any alteration in the growth of strains D46 and D57 when a P0 acidic protein binding site is absent (Fig S2) indicates that P0 truncation does not affect protein function in a detectable way.

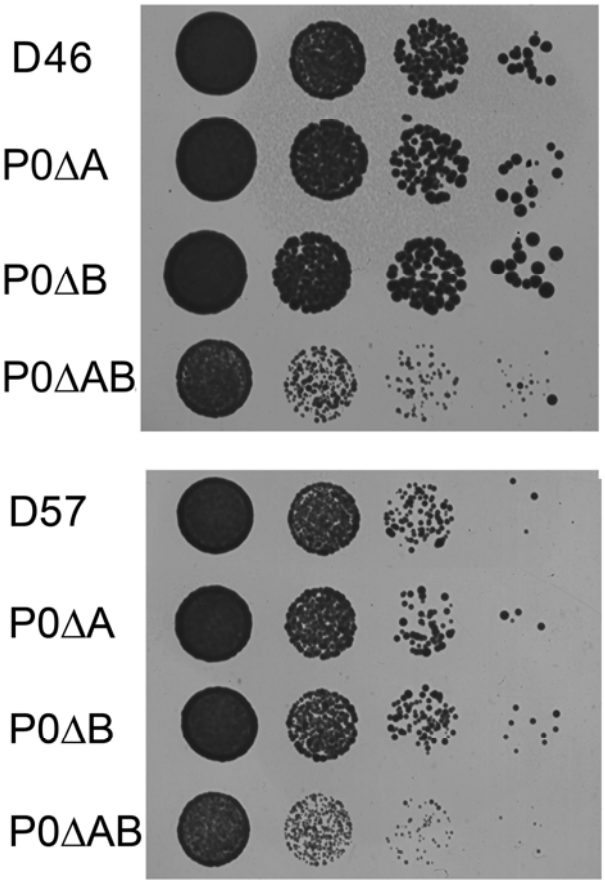

Fig S2.- Growth of *S. cerevisiae* strains expressing truncated P0 proteins lacking the P1/P2 binding sites. Serial dilutions of cells from the double D46 and D57 mutants, as well as that of the same strains expressing the different truncated P0 proteins were grown on YEPD agar plates at 30ºC for four days.

Proposed model for stalk pentamer assembly

The assembly of the P1/P2 protein into the stalk mainly yields a P1α/P2β-P0-P1β/P2α pentamer, as shown in Fig S3. However, since the two P0 binding sites A and B can bind all possible P1/P2 heterodimers (Fig 6), a small proportion of ribosomes could carry alternative combinations of acidic proteins (as illustrated in Fig S3 for a ribosome carrying two copies of the P1β/P2α). Indeed, since a minor fraction of P1α/P2β can bind to site B in the W303 strain (Fig 6A), the generation of ribosomes carrying two copies of this heterodimer is also possible, which is consistent with the cross-linking data showing the formation of P protein homodimers (29). Hence, there is some heterogeneity in the ribosome population that can be controlled by the cell regulating the amount of each acidic protein expressed in any given metabolic condition.

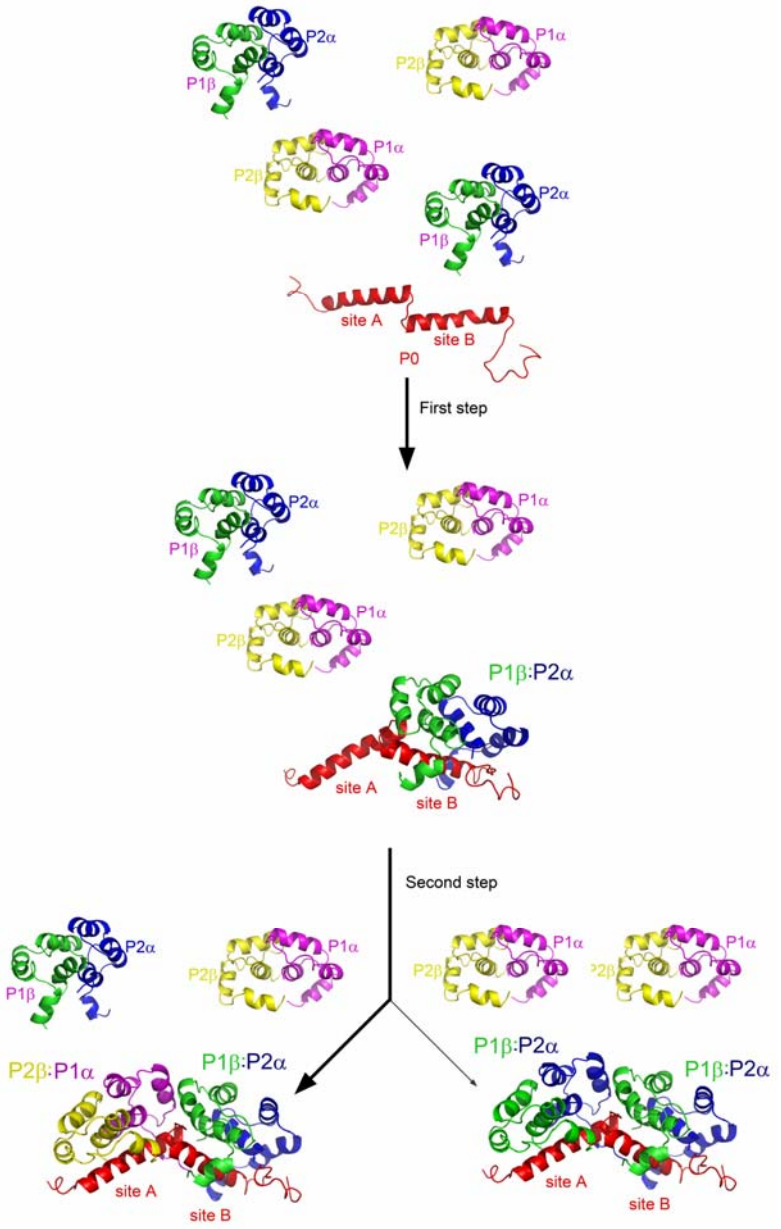

Fig S3. Proposed model for *S. cerevisiae* ribosomal stalk assembly. The width of the arrows in the second step suggests the relative probability of the two alternative pathways occurring. The structure of the protein complexes is based on previous models of the stalk structure (13).

References

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