Published in "Molecular Cell 46(1): 105–110 , 2012"
which should be cited to refer to this work.

Supplemental Information

Leucyl-tRNA Synthetase Controls TORC1 via the EGO Complex

Grégory Bonfils, Malika Jaquenoud, Séverine Bontron, Clemens Ostrowicz,
Christian Ungermann, and Claudio De Virgilio

Supplemental References

Arino, J., and Herrero, E. (2003). Use of tetracycline-regulatable promoters for functional analysis of protein phosphatases in yeast. Methods Enzymol *366*, 347-358.

Binda, M., Péli-Gulli, M.P., Bonfils, G., Panchaud, N., Urban, J., Sturgill, T.W., Loewith, R., and De Virgilio, C. (2009). The Vam6 GEF controls TORC1 by activating the EGO complex. Mol Cell *35*, 563-573.

Gietz, R.D., and Sugino, A. (1988). New yeast-*Escherichia coli* shuttle vectors constructed with *in vitro* mutagenized yeast genes lacking six-base pair restriction sites. Gene *74*, 527-534.

Hohmann, S., and Thevelein, J.M. (1992). The cell division cycle gene *CDC60* encodes cytosolic leucyl-tRNA synthetase in *Saccharomyces cerevisiae*. Gene *120*, 43-49.

Li, Z., Vizeacoumar, F.J., Bahr, S., Li, J., Warringer, J., Vizeacoumar, F.S., Min, R., Vandersluis, B., Bellay, J., Devit, M., *et al.* (2011). Systematic exploration of essential yeast gene function with temperature-sensitive mutants. Nature Biotech *29*, 361-367.

Urban, J., Soulard, A., Huber, A., Lippman, S., Mukhopadhyay, D., Deloche, O., Wanke, V., Anrather, D., Ammerer, G., Riezman, H., *et al.* (2007). Sch9 is a major target of TORC1 in *Saccharomyces cerevisiae*. Mol Cell *26*, 663-674.

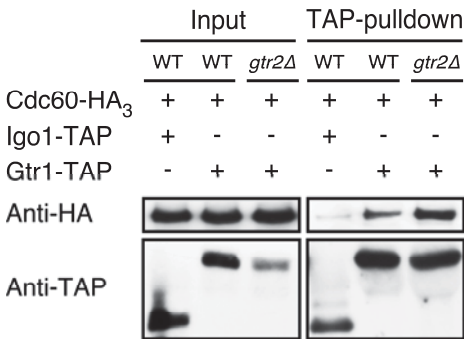

Figure S1. Cdc60-Gtr1 Interaction in the Absence of Gtr2, Related to Figure 1

Gtr1-TAP, or the control protein Igo1-TAP, was precipitated from extracts of Cdc60-HA_3-expressing wild-type (WT) or *gtr2*Δ cells that were grown to and harvested in exponential growth phase. Cell lysates (Input) and TAP pull-down fractions were subjected to SDS-PAGE and immunoblots were probed with anti-HA or anti-protein A (anti-TAP) antibodies as indicated.

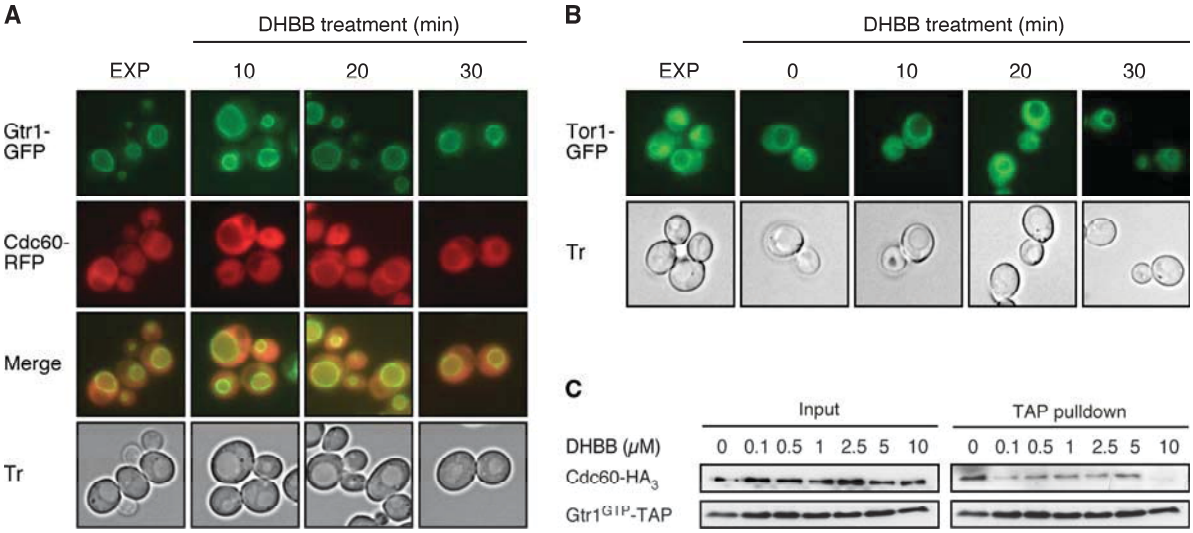

Figure S2. Additional Effects of DHBB Treatment, Related to Figure 2

(A and B) DHBB treatment does not affect the localization of Gtr1-GFP (A) or Tor1-GFP (B). Gtr1-GFP (A) and Tor1-GFP (B) mainly localize to the vacuolar membrane, while Cdc60-RFP (A) adjoins the limiting membrane of the vacuole, but mainly localizes to the cytoplasm in exponentially growing cells (EXP). DHBB (10 μM) treatment does not detectably alter the localization of Gtr1-GFP, Tor1-GFP, or Cdc60-RFP. Notably, given the high abundance of Cdc60-RFP within the cytoplasm, a potential DHBB-induced displacement of Cdc60-RFP from the vacuolar membrane may escape detection by standard fluorescence microscopic analyses. Tr, transmission.

(C) DHBB disrupts the Cdc60-Gtr1^{GTP} interaction in a concentration-dependent manner. Gtr1^{GTP}-TAP was precipitated from cells co-expressing Cdc60-HA_3. Cells were harvested in exponential growth phase either prior to (0) or following a 30-min period of treatment with the indicated DHBB concentrations. Cell lysates (Input) and TAP pull-down fractions were subjected to SDS-PAGE and immunoblots were probed with anti-HA or anti-protein A (anti-TAP) antibodies.

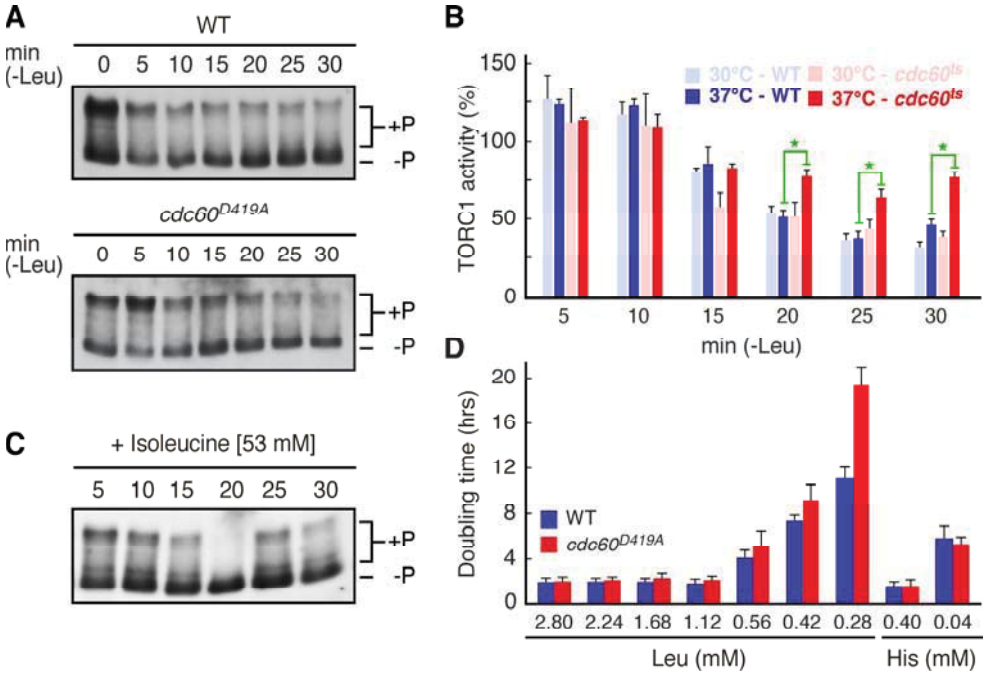

Figure S3. Physiological Relevance of LeuRS Editing, Related to Figure 4

(A) Leucine starvation causes TORC1 inactivation in both wild-type and *cdc60*^{D419A} mutant cells. Leucine (*leu2*Δ) auxotrophic wild-type (WT) and *cdc60*^{D419A} mutant cells were grown to exponential phase in medium containing leucine and then transferred to a medium lacking leucine (0 time point). Samples were taken at the times indicated following leucine starvation and TORC1 activity was assayed as in Figure 1D.

(B) Temperature-inactivation of Cdc60^{ts} protects TORC1 from leucine starvation-induced downregulation. Leucine (*leu2*Δ) auxotrophic wild-type and *cdc60*^{ts} mutant cells were grown to exponential phase in medium containing leucine, incubated for 1 hr at either 30°C or 37°C, and then transferred to a medium lacking leucine (0 time point). Samples were taken at the times indicated following leucine starvation (-Leu) and TORC1 activity was assayed as in Figure 1D. Data are expressed as relative values with respect to the 0 time point and reported as averages (n = 3), with standard deviations indicated by the lines above each bar. As assessed by two-way analysis of variance (ANOVA) followed by post-test analysis, the observed differences between wild-type and *cdc60*^{ts} cells at 37°C are statistically significant with p-values < 0.05 (indicated with one asterisk).

(C) Addition of isoleucine in disproportionate quantities causes transient TORC1 inactivation. Wild-type cells were grown (on SD medium) to exponential growth phase and treated with excessive amounts of isoleucine (*i.e.* final concentration of 53 mM). Samples were taken at the times indicated following isoleucine addition and TORC1 activity was assayed as in Figure 1D.

(D) LeuRS editing is specifically required for growth under leucine limiting conditions. Leucine (*leu2*Δ) and histidine (*his3*Δ) auxotrophic wild-type (blue bars) and LeuRS editing defective *cdc60*^{D419A} mutant (red bars) cells were grown in SD medium containing either 5 mM histidine and various levels of leucine (Leu [mM]), or 9 mM leucine and different levels of histidine (His [mM]) as indicated. Doubling times are reported as averages (n = 3), with standard deviations indicated by the lines above each bar.

Table S1. Proteins Identified in Gtr1-TAP Pull-Down Experiments, Related to Figure 1

Protein[1]	Function	No. of peptides (+ Leu)	No. of peptides (- Leu)
Rpl4A	Component of the large (60S) ribosomal subunit	9	0
Vas1	Mitochondrial and cytoplasmic valyl-tRNA synthetase	6	0
Ded1	DEAD-box helicase	6	0
Fas1	Fatty Acid synthetase	24	1
Cdc60	**Leucyl-tRNA synthetase**	11	1
Rpo21	DNA-directed RNA polymerase	9	1
Gnd1	6-Phosphogluconate dehydrogenase	8	1
Trr1	Thioredoxin reductase	6	1
Faa4	Fatty acyl-CoA synthetase	5	1
Acc1	Acetyl-CoA carboxylase	5	1
Pfk2	Phosphofructokinase	5	1
Rpn8	Regulatory subunit of the 26S proteasome	5	1
Fet5	Multicopper oxidase	5	1

[1]Proteins were identified by LC-MS-MS analysis of polypeptides in purified Gtr1-TAP preparations from exponentially growing (+Leu) or leucine-deprived (30 min; -Leu) cells. Only proteins for which at least one peptide was identified in the Gtr1-TAP preparations (confidence interval of 99.9%) and none in control preparations from non-tagged wild-type cells were retained for further analysis. Proteins for which at least 5 peptides were identified in the +Leu samples and none in the corresponding –Leu samples, or proteins for which the ratio of the number of peptides in the +Leu versus the -Leu samples was > than 5, were retained for this table.

Table S2. Strains Used in This Study

Strain	Genotype	Source	Figure
BY4741	*MAT***a**; *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	Euroscarf	S3B
BY4742	*MATα*; *his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0*	Euroscarf	
Y08149	[BY4741] *MAT***a**; *cdc60*^{ts}	Li et al., 2011	S3B
YL515	[BY4741/2] *MATα*; *his3Δ1, leu2Δ0, ura3Δ0*	Binda et al., 2009	
YL516	[BY4741/2] *MAT***a**; *his3Δ1, leu2Δ0, ura3Δ0*	Binda et al., 2009	2A-C, 3A-D, 4C
GB2381	[YL516] *MAT***a**; *CDC60-HA*$_3$*::HIS3*	This study	1A/B, 2D/E, S1, S2C
GB2382	[YL516] *MAT***a**; *IGO1-TAP-kanMX4, CDC60-HA*$_3$*-HIS3*	This study	1A, S1
GB2549	[YL516] *MAT***a**; *CDC60-HA*$_3$*-HIS3, gtr2Δ::kanMX4*	This study	S1
GB2523	[YL516] *MAT***a**; *KOG1-HA::kanMX4*	This study	2F
MPG1630	[YL516] *MAT***a**; *gtr1Δ::kanMX4, gtr2Δ::kanMX4*	This study	2A-C, 3B/C
GB2378	[YL516] *MAT***a**; *cdc60*^{D418R}	This study	2A-C
GB2379	[YL516] *MAT***a**; *cdc60*^{D418R}*- HA*$_3$*::HIS3*	This study	2E
MB32	[YL516] *MAT***a**; *gtr1Δ::kanMX4*	Binda et al., 2009	4A
MJA2638	[YL515] *MATα; cdc60Δ::KanMX4* [YCplac111-*CDC60*]	This study	4A, S3A, S3D
MJA2786	[YL515] *MATα; cdc60Δ::KanMX4* [YCplac111-*CDC60*^{D419A}]	This study	S3A, S3D, S3C
MJA2784	[YL515] *MATα; cdc60Δ::KanMX4* [*CEN, HIS3, CDC60*]	This study	S3D
MJA2785	[YL515] *MATα; cdc60Δ::KanMX4* [*CEN, HIS3, CDC60*^{D419A}]	This study	S3D
MJA2604	[YL515] *MATα; cdc60Δ::KanMX4* [YCplac111-*cdc60*^{S414F}]	This study	4A
8003	*MATα*; *leu2Δ0, ura3, trp1, his3, ade8, cdc60*^{ts}	Hohmann and Thevelein, 1992	1C/D
MPG2389	*MAT***a**; *HIS3::GTR1-GFP, gtr1Δ::natMX4, ura3-52, leu2, trp1*	This study	S2A
MP52-2A	[YL516] *MAT***a**; *TOR1-D330-3xGFP*	Binda et al., 2009	S2B
NMY51	*MAT***a**; *his3Δ200, trp1-901, leu2-3,112, ade2, LYS::(lexAop)4-HIS3, ura3::(lexAop)8- lacZ, ade2::(lexAop)8-ADE2 GAL4*	Dualsystems	4B

Table S3. Plasmids Used in This Study

Plasmid	Description	Source	Figure
YCplac33	*CEN, URA3*	Gietz and Sugino, 1988	2A-C, 3A-D, 4A-D
YCplac111	*CEN, LEU2*	Gietz and Sugino, 1988	2A-C, 3A-C, 4A
pMB1344	YCplac33-*GTR1-TAP*	Binda et al., 2009	1A-B, 2D-F, 4C, S1
pCM264	*CEN, URA3,* Tet_{OFF}-HIS_6-HA_3	Arino and Herrero, 2003	1C/D, 4A
pGB1957	pCM264-Tet_{OFF}-HIS_6-*CDC60*	This study	1C/D
pJU1462	pRS413-$SCH9^{T570A}$-HA_5	Urban et al., 2007	1D, 2A-C, 3A-D, 4D, S3C
pMB1394	YCplac33-Tet_{ON}-$GTR1^{Q65L}$	Binda et al., 2009	2A-C, 3B/C, 4A
pPM1623	YCplac111-Tet_{ON}-$GTR2^{S23L}$	This study	2A-C, 3B/C, 4A
pMJ1974	YCplac111-*CDC60*	This study	4A, S3D
pMJ2113	YCplac111-$CDC60^{S414F}$	This study	4A
pDL2-Alg5	2µ, *ADH1-HA-NubG, TRP1*	Dualsystems	4B
pAI-Alg5	2µ, *ADH1-HA-NubI,TRP1*	Dualsystems	4B
pPR3-N	2µ, *CYC1-NubG-HA, TRP1*	Dualsystems	4B
pNP1689	pPR3-N-*CYC1-NubG-HA-GTR1*	Binda et al., 2009	4B
pNP1692	pPR3-N-*CYC1-NubG-HA-GTR2*	Binda et al., 2009	4B
pMJ1868[1]	pCabWT-*CYC1-Cub-LexA-*$Cdc60^{CP1}$	This study	4B
pMJ2115[1]	pCabWT-*CYC1-Cub-LexA-*$Cdc60^{CP1\text{-}S414F}$	This study	4B
pMPG1574	2µ, Tet_{ON}-HIS_6-HA_3, *URA3*	Binda et al., 2009	4C/D
pMJ2059[1]	pMPG1574-Tet_{ON}-HIS_6-HA_3-$CDC60^{CP1}$	This study	4C/D
pMJ2116[1]	pMPG1574-Tet_{ON}-HIS_6-HA_3-$CDC60^{CP1\text{-}S414F}$	This study	4C/D
pMB1372	*YCplac33-* $GTR1^{Q65L}$ *-TAP*	Binda et al., 2009	S2C
pMJA2192	pRS416-*CYC1-CDC60-RFP, URA3*	This study	S2A
pMJA2069	YCplac111-$CDC60^{D419A}$	This study	S3A, S3D
pJU1436	pRS416-$SCH9^{T570A}$-HA_5	Urban et al., 2007	S3C
pMJA2168	*CEN, HIS3, CDC60*	This study	S3C, S3D
pMJA2167	*CEN, HIS3,* $CDC60^{D419A}$	This study	S3D

[1]Plasmids pMJ1868/pMJ2115 and pMJ2059/pMJ2116 express the Cdc60 CP1 editing domain encompassing amino acids 263-548 and 263-530 of Cdc60, respectively.