Supplementary Information

Table S1: yeast strains used for this study

Name	Genotype	reference
NSY1471	wild type	(Scrivens et al., 2009)
NSY1472	*trs20ts*	(Scrivens et al., 2009)
NSY1517	Bet3-GFP	this study
NSY1518	BET3-GFP/trs20ts	this study
NSY1515	TRS130-GFP	this study
NSY1516	TRS130-GFP/*trs20ts*	this study
NSY1513	TRS120-GFP	this study
NSY1514	TRS120-GFP/*trs20ts*	this study
NSY1519	TRS120-myc	this study
NSY1520	TRS120-myc/*trs20ts*	this study
NSY1521	TRS130-HA	this study
NSY1522	TRS130-HA/*trs20ts*	this study
NSY752	PJ469-4α	(James et al., 1996)
NSY468	PJ469-4a	(James et al., 1996)
NSY1523	TRS20::HygMX + pNS1397	this study
NSY1524	TRS20::HygMX + pNS1398	this study
NSY1525	TRS20::HygMX + pNS1399	this study
NSY128	wild type	D. Botstein, DBY4975
NSY 862	COP1-RFP	(Huh et al., 2003)
NSY 863	CHC1-RFP	(Huh et al., 2003)
NSY1547	TRS130-GFP/CHC1-RFP	this study
NSY1548	TRS130-GFP/CHC1-RFP/*trs20ts*	this study
NSY1549	TRS120-GFP/CHC1-RFP	this study
NSY1550	TRS120-GFP/CHC1-RFP/*trs20ts*	this study
NSY1551	BET3-GFP/CHC1-RFP	this study
NSY1552	BET3-GFP/CHC1-RFP/*trs20ts*	this study
NSY1553	BET3-GFP/COP1-RFP	this study
NSY1554	BET3-GFP/COP1-RFP/*trs20ts*	this study
NSY1555	*trs20ts*	this study
NSY1556	*trs20D46Y*	this study
NSY1557	wild type	this study

Table S2: Plasmids used for this study

Name	backbone	insert(s)	reference
pNS981	pCDFDuet-1	His_6-TRS120	(Tokarev et al., 2009)
pNS1000	pETDuet-1	GST-BET5 + TRS23-S	this study
pNS1043	pRSFDuet-1	BET3-MBP + TRS31-myc	this study
pNS1436	pCDFDuet-1	His_6-TRS33	this study
pNS1437	pCDFDuet-1	His_6-TRS33 + TRS20-HA	this study
pNS1385	pCDFDuet-1	TRS20-HA	this study
pNS1386	pCDFDuet-1	*trs20ts*-HA	this study
pNS1387	pCDFDuet-1	*trs20D46Y*-HA	this study
pNS422	pYEX4T-1	GST	(Martzen et al., 1999)
pNS424	pYEX4T-1	GST-BET5	(Martzen et al., 1999)
pNS206	pGBDU-C2	none	(James et al., 1996)
pNS1115	pGBDU-C2	TRS120	this study
pNS1106	pGBDU-C2	BET3	this study
pNS196	pACT2	none	Clontech Laboratories, Inc.
pNS1152	pACT2	TRS20	this study
pNS1395	pACT2	*trs20D46Y*	this study
pNS1394	pACT2	*trs20ts*	this study
pNS1096	pACT2	BET3	this study
pNS1396	pRS316	TRS20	this study
pNS1397	pRS315	TRS20	this study
pNS1398	pRS315	*trs20D46Y*	this study
pNS1399	pRS315	*trs20ts*	this study
pNS1432	pRS416	CFP-N-BET3	this study
pNS1428	pRS413	CFP-C-TRS20	this study
pNS1429	pRS413	CFP-C-*trs20D46Y*	this study
pNS1435	pRS413	YFP-C-TRS120	this study
pNS1433	pRS416	YFP-N-TRS20	this study
pNS1434	pRS416	YFP-N- *trs20D46Y*	this study
pNS661	pRS315	GFP-YPT31	R. Collins, pRC647
pNS1430	pRS315	yEVenus-YPT1	this study

Supplemental Methods

Construction of Yeast Strains

The *trs20ts* and its cognate wild type strain (NSY1471 and 1472) were a generous gift from M. Sacher. Strains expressing Trs120-myc and Trs130-HA were constructed by mating *trs20ts* with NSY1176 (Trs130-HA::*HIS3*/Trs120-myc::*TRP1*), followed by sporulation and tetrad dissection. Strains expressing Chc1-RFP in wild type and *trs20ts* were created by PCR and homologous recombination of RFP::KanMX into NSY1471 and 1472. Trs130, Trs120, and Bet3 were then tagged with yEGFP in these strains by PCR and homologous recombination of yEGFP::HygMX. Similarly, wild type and *trs20ts* strains expressing COPI-mCherry were created by PCR and homologous recombination of mCherry::HygMX into NSY1471 and 1472. Bet3 was then tagged with GFP in these strains using yEGFP::KanMX. Strains expressing Trs20 wild type, Trs20-D46Y, or Trs20ts on plasmids covering *trs20Δ* were created by transforming *TRS20* in pRS316 into NSY1471, deleting *TRS20* from the chromosome via homologous recombination using Hygromycin B resistance, transforming the resulting strain with *TRS20* wild type, *trs20*-D46Y, or *trs20ts* expressed from pRS315, and *selecting* the final strains from 5-FOA plates. Wild type, *trs20ts, and trs20D46Y* strains used for Ypt1 and Ypt31 localization were created by PCR and homologous recombination of a TRS20, *trs20V92A/F133S,* or *trs20D46Y*-HygMX cassette into the TRS20 locus.

Construction of Plasmids

Plasmids pNS1015, 981, 1000, 1043, 1385, and 1387 were used for bacterial expression. The remaining plasmids were transformed into yeast. All plasmids constructed for this study were created using PCR, restriction digestion, and ligation into the destination vector, unless indicated otherwise. For pNS1015 and 1000, the GST tag was first subcloned from pET41a (Novagen) into pETDuet-1 (Novagen) before adding the TRAPP subunits. For pNS1043, MBP was subcloned from pMAL-C2X (NEB) into pRSFDuet-1 (Novagen). The myc and HA epitopes were added to Trs31 and Trs20, respectively, by engineering the sequence into the reverse primer during cloning. For cloning wild type or mutant *TRS20* into pRS vectors, *TRS20*, along with 400 base pairs upstream and 200 downstream, was sub-cloned from *TRS20* in pRS425, a generous gift from Michael Sacher. To create *trs20*-D46Y and *trs20ts* mutants in each vector, PCR-based mutagenesis was utilized to mutate T275C and T398C (for the V92A and F133S amino acid substitutions in the trs20ts constructs) or G136T (for D46Y) from the wild type DNA sequence. To construct the BiFC plasmids, *TRS120* was cloned in frame with YFP-C into pNS1426 (YFP-C in pRS413). *TRS20* or *trs20-D46Y* were cloned in frame with YFP-C into pNS1425 (YFP-N in pRS416). *BET3* was cloned in frame with CFP-N into pNS1431 (CFP-N in pRS415) (Lipatova et al., 2012).

Cell Fractionation

To calculate membrane attachment of Trs120 and Trs130, cells were grown to mid-log phase at 26° in synthetic dextrose medium, and 50 OD_{600} units were harvested. 37° samples were shifted to 37° for 90 minutes before harvesting cells. Cells were lysed using glass beads in the same way as for the yeast pull-down experiments, and lysates pre-cleared at 1000 g for 5 minutes. Lysates were then centrifuged at 100,000 g for 60 minutes; membrane (P100) and supernatant (S100) fractions were collected. Membrane fractions were resuspended in lysis buffer, TCA was added to added to both fractions, to a final concentration of 10%. Proteins were washed twice with 400µl acetone, resuspended in 50µl laemmli buffer, and run on 9% acrylamide gels.

Supplemental References

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Supplemental Figure Legends

FigureS1: GST-pull-down of bacterial TRAPP subunits. A. Shown is the full anti-His_6 immuno-blot of lysate (left) and pull-downs used in Figure 1A. Cleared bacterial lysate (S100) expressing His_6-Trs120 was used for GST-pull-down, as in Figure 1A. In the pull-down lanes, the His_6-tagged Trs120 and Trs33 can be seen. **B.** Tca17-S does not attach to GST-Bet5 complexes. This experiment was done as described for Figure 1A, except that the effect of expressing S-tagged Tca17 together with TRAPP I core + Trs33 was determined. Tca17 was expressed (lysates, left), but it did not co-precipitate with GST-Bet5 complexes and did not affect the association of Trs120 with TRAPP I (pull-downs, right). Trs20 is shown as a positive control for the pull-down.

Figure S2. The effect of the *trs20ts* mutation on membrane attachment of Trs120 and Trs130. Cells expressing GFP-tagged Trs120 (top) or Trs130 (bottom) were grown at 26°C and shifted to 37°C for 90 minutes. Cell lysates were subjected to 100,000 g spin, and the level of Trs120 or Trs130 in the total (T), pellet (P) and sup (S), was determined using immuno-blot analysis and anti-GFP antibodies. The bands were quantified and the percent of Trs120 and Trs130 in the P100 (P) fraction is shown under the blots; +/- represents SD of results from two independent experiments. The levels of Trs120 and Trs130 in the P100 fraction are lower in *trs20ts* mutant cells when compared to wild type cells.