Figure EV1. Expression analysis and growth characteristics of mutants used in this study. All strains are listed in Appendix Table S1.

A. Mutations in the Dsl1p lasso domain and the deletion of the lasso domain do not affect the stability of the protein. Since Dsl1p antibodies preferentially recognize the Dsl1 protein carrying an intact lasso domain, we analyzed myc-tagged proteins. The sequences encoding two myc-tags were introduced 3’ of the DSL1 alleles as described in Materials and Methods. Extracts of two parallel samples were analyzed using the 9B11 antibody (Cell Signaling Technologies, Danvers, MA). α-COP was used as a loading control. As an additional loading control, a Ponceau S-stained protein band at about 120 kDa is shown.

B. C. Immunoblot analysis of strains expressing either VN or VC tags alone. For the upper panels, COP1-specific antibodies were used and untagged COP1 subunits were employed as a loading control. For the blots shown in the second row, GFP-specific antibodies were used. An additional loading control, a Ponceau S-stained protein band at about 120 kDa is shown. The results indicate that expression of α-COP and β-COP is not affected by the tags, while the tagged versions of genes encoding δ-COP and ε-COP seem to be less well expressed. We do not know why the expression varies so much since both tagged genes except RER1 are under the control of their own promoter, and all 3’-tagged genes are followed by the ADH1 3’-UTR. Our results with VC-RER1 indicate that the codon usage of the tag may be important at least when the tag is at the 5’ end (Lipatova et al, 2015). We obtained very similar results when we used a chromosomally inserted VC-RER1 version under the control of the strong RPL7B promoter or a single-copy vector-encoded version under the endogenous promoter where the codon usage of the VC tag was adapted to yeast.

D. Expression analysis of strains expressing different BiFC combinations. The cell density of exponentially growing cells was adjusted to 1 OD600. These suspensions as well as three serial dilutions were spotted on agar plates. Images were taken after 48 h incubation at the indicated temperature. Rer1p marked with one asterisk indicates that the expression of tagged genes was examined under the control of the RPL7B promoter from the RER1 locus. The plasmid-encoded version with codon-adapted VC tag under the control of the endogenous promoter is marked by two asterisks. The growth test shown was performed on selective minimal medium to prevent the loss of plasmids.

I. Extracellular secretion was determined using Mabs to VC-RER1 VN from both RER1p (third lane) and Dsl1p/VC-RER1 VN expressing cells. We observed a reduction in the Emp47p level of VN-RER1 VN expressing cells. We observed a reduction in the Emp47p level which is not due to the BiFC formation. It can already be observed in cells expressing the tagged protein. The tagged protein is produced. Tandem affinity-purified complexes were analyzed on SDS PAGE. Gels were dried and analyzed using a phosphorimager (FLA7000, Fujifilm).

K. The presence of untagged proteins in addition to the VC-tagged Dsl proteins reduces the BiFC signal, while it does not change the polarized appearance of BiFC spots. For the micrographs shown, diploid strains were imaged that express VN-tagged β-COP and Sec20p from both SEC27 loci. At the DLS3 loci, they express a wild-type and a VC-tagged version (upper panel) or two DSL3 alleles (lower panel). The same result was obtained with strains that are homozygous or homozygous for the equivalent DSL1 versions. Moreover, a β-COP/VN–β-COPVN, DSL1 ort/DLS1, DSL3 ort/DLS3 strain could not be distinguished from a homozygous β-COP/VN–β-COPVN DSL1 ort/DLS1 strain (H. Schmitt, unpublished results). Thus, the BiFC spot formation is not due to a deletion of Dsl proteins. Scale bar, 5 μm.

L. PLA detection of COPI/Dsl and COPI/ER-SNARE interactions. Superimposed representative images of fixed yeast cells carrying Dsl3pCFP; Sec20pYFP, or no tag (wild type). Cells were fixed with PFA and immunodecorated with primary antibodies against endogenous COP1, as well as GFP. Subsequently, the PLA reaction was carried out according to the manufacturer’s instructions, using Duolink® In Situ red PLA reagents. Proximity of COPI to Dsl3pCFP or Sec20pYFP produced to a fluorescent spot in situ. Gray channel: DIC; blue: DAPI; red: PLA signal. Scale bar, 10 μm.
Figure EV1.
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K The dsl

L A collection of tags other than the BiFC tags was also analyzed for synthetic lethality with the dsl

D No synthetic lethality was observed when the 1 G Dsl

J All COPI

I The cargo receptor Rer

H The main conclusion that one can draw from the results shown in the first two rows of (H) as well as panels (I) and (J) is that VC-tagged ER proteins other than the Dsl proteins cannot suppress the COPI\(^{\text{VC}}\)/dsl\(^{\text{1-5xWA}}\) combination. The lack of suppressor activity is not due to weak BiFC interactions since these combinations gave BiFC signals with an intensity as high as that of the combinations used in (E) (see Fig 1C). The VN tag fused to SEC16 slightly improved the growth of dsl\(^{1-5xWA}\) cells producing \(\alpha\)-COPI\(^{\text{VC}}\), while the equivalent \(\varepsilon\)-COPI\(^{\text{VC}}\)-producing cells grew less well (compare with C). However, more samples would be necessary to prove that these minor differences are significant. The strain used in row 6 carries a BiFC combination that gave a very low fluorescence signal (Fig 1H). Both tags alone had no effect in the presence of the dsl\(^{1-5xWA}\) mutation and accordingly their combination resulted in cells that grew normally at all temperatures. Row four and five of (H)

F As shown in (C), cells with VC-tagged \(\delta\)-COPI stopped growing when the dsl\(^{1-5xWA}\) mutant allele was the only Dsl1 version expressed in the cells. The growth of these cells was improved by the VN tag fused to Dsl1p (compare the last rows of C and F). This effect was limited to 30°C since the cells were temperature-sensitive.

G Dsl1p carrying the VC tag rescued two of the four COPI\(^{\text{VC}}\)/dsl\(^{1-5xWA}\) combinations (\(\varepsilon\)-COPI\(^{\text{VC}}\) and \(\beta\)-COPI\(^{\beta}\)). In contrast to Dsl1\(^{379}\), Dsl1\(^{379}\) could not suppress the equivalent combinations with \(\alpha\)-COPI\(^{\text{VC}}\) and \(\delta\)-COPI\(^{\text{VC}}\). For these tests, a plasmid-encoded VC-tagged version of dsl\(^{1-5xWA}\) was used. The failure of this plasmid to support growth of \(\alpha\)-COPI\(^{\text{VC}}\) and \(\delta\)-COPI\(^{\text{VC}}\)-producing cells may indicate that Dsl1\(^{379}\) is less efficient as suppressor than Dsl1\(^{379}\). However, one must keep in mind that the BiFC tags fused to the dsl\(^{1-5xWA}\) protein alone caused a growth defect (D). Therefore, the fact that cells with a \(\varepsilon\)-COPI\(^{\text{VC}}\)/dsl\(^{1-5xWA}\) VC/BIFC pair grew well at all temperatures, and \(\beta\)-COPI\(^{\beta}\)-dsl\(^{1-5xWA}\)VC were viable at 30°C, shows that the BiFC formation is able suppress the negative effects of two harmful tags in the same cell.

E The lethal COPI/dsl\(^{1-5xWA}\) combinations shown in (B) were viable when Dsl1\(^{375}\) was expressed as well, suggesting that the BiFC interaction can reconstitute the normal function of the coat/other interaction. Note that the \(\beta\)-COPI\(^{\beta}\)/\(\delta\)st3\(^{\text{VC}}\) combination, which includes the most efficiently expressed BiFC-tagged COPII subunit, grew well even at 37°C (see also Fig 1F), while the combination that includes the \(\beta\)-COPI\(^{\beta}\) version could not grow at 37°C. As discussed below, this observation indicates that for suppression to occur, it is optimal if the BiFC formation is very efficient.

A Plasmid-encoded wild-type and mutant versions of Dsl1 support growth of Dsl1 shuffl cells that carry no other modification.

B The presence of unpaired VN tags at the C-term of \(\alpha\), \(\beta\), \(\delta\), and \(\varepsilon\)-COPI were lethal when combined with the dsl\(^{1-5xWA}\) mutation. Cells producing \(\alpha\)-COPI\(^{\text{VC}}\) were temperature-sensitive no matter what Dsl1 version was present in the cells. \(\delta\)-COPI\(^{\text{VC}}\)-producing cells were also temperature-sensitive, but a surplus of Dsl1p suppressed the temperature sensitivity.

C Compared to the VN tag, the VC fragment at the C-terminus of COPII subunits had a less dramatic effect on growth when combined with the dsl\(^{1-5xWA}\) mutation. Cells producing \(\delta\)-COPI\(^{\text{VC}}\) were an exception. The \(\delta\)-COPI\(^{\text{VC}}\) cells were temperature-sensitive no matter what Dsl1 version was expressed. It has been observed previously that \(\delta\)-COPI\(^{\text{VC}}\) cells are also temperature-sensitive (Zink et al, 2009; see also F and G).

D No synthetic lethality was observed when the dsl\(^{1-5xWA}\) mutation was expressed in VN or VC tags at the C-terminus of Dsl proteins or COPII subunits Sec12p and Sec16p. However, cells expressing a VN or VC-tagged dsl\(^{1-5xWA}\) protein grew slower than the corresponding wild-type version even at room temperature.

The Authors

Polarization of ER arrival sites

Saskia Schröter et al

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Figure EV2.
Figure EV3.
Figure EV3. Kymographs of BiFC polarization patterns and organelle association.

Time-lapse micrographs of agarose-embedded cells were taken at RT with fresh PM Glc + ura medium supply throughout one budding cycle. Single-cell kymographs were generated by concatenating the central section of the cell (sliced along the pole axis, frames at the width of the bud neck) of one time-lapse dataset. Images were acquired at 5-min intervals, except for the datasets Dsl1pGFP+VCRer1p (10-min intervals) and β'-COPmRFP+Dsl3pVCRer (2-min intervals). In all combinations, fluorescent foci localized to areas of membrane expansion in the bud. Frequently, another prominent fluorescent spot appeared in the mother cell on the side of the bud neck. Schematic representations of a yeast cell depict the observed fluorescence pattern in the respective strains. Scale bars, 10 μm.

A Kymographs of strains depicted in Fig 3. Cells expressing complementary BiFC-tagged proteins between subunits of the Dsl complex, the COPI complex, the COPII complex, and the COPII adaptor Sec16p were analyzed. The combination β'-COPmRFP+Dsl3pVC A206K contains a mutated VC tag whose alteration is equivalent to the A206K mutation in GFP, which prevents the formation of dimers (Zacharias et al, 2002). The COPmRFP dataset depicts β'-COPmRFP fluorescence foci in Dsl1p-depleted GAL-DSL1 cells after incubation in glucose-containing medium (YEPD) for 4 h at 30°C.

B Kymographs of strains depicted in Fig 4. In all samples, the COPI+Dsl combination β'-COP+Dsl3pVC was analyzed. The cells additionally expressed organelle markers from plasmids: the ER marker HDEL, the cis- and trans-Golgi markers Sed5p, Cos1p, and Sec7pCFP, the COPI cargo Rer1p, or the secretory vesicle marker Sec4p. The ERES marker Sec13pmRFP was expressed from its normal locus. BiFC signals are pseudocolored green; organelle markers are pseudocolored red. BiFC signals showed a spatial and temporal association with shown organelle markers.
Figure EV4.
Figure EV4. ER and ERES association with ER arrival sites.

A COPI+Dsl foci association with the ER. Time-lapse fluorescence micrographs of cells carrying ER and COPI+Dsl BiFC markers. Agarose-embedded cells were grown at RT with fresh PM Glc + ura medium supply and imaged in 10-min intervals. Tunicamycin was added to the medium to a final concentration of 125 ng/ml. tubER and COPI+Dsl signals retracted simultaneously from the bud upon tunicamycin treatment. Arrows mark ER and BiFC signals in the bud, arrowheads mark loss of signals from the bud. Scale bar, 5 μm.

B ERES and ERAS coalescence. Fluorescence micrographs of cells carrying the COPI+Dsl BiFC pair β-COP96•Dsl33p10C, as well as the ERES marker Sec13pRFP in a sec12 wild-type or sec12-4 background. BiFC signals and ERES distribution were normal at 25°C (left panels). At 37°C (right panels), ERES aggregated in the sec12-4 mutant, while BiFC foci lost their polarized distribution. Importantly, COPI+Dsl BiFC foci did not colocalize with coalescing ERES in the sec12-4 mutant. Scale bar, 10 μm.

C Association of ERES and ERAS during growth and quiescence. Fluorescence micrographs of cells carrying COPI+Dsl BiFC pairs β-COP96•Dsl33p10C or ε-COP96•Dsl13p10C, as well as ERES marker Sec13pRFP. In fast-growing cells (panels 1-3), ERAS were polarized, and each ERAS was located in the immediate vicinity of an ERES. In non-growing cells (panel 4), ERAS were more numerous, appeared evenly distributed, and each ERAS appeared to have a corresponding ERES in immediate vicinity (arrowheads). Scale bar, 10 μm.

D Characteristic positioning of ERES toward ERAS in individual cells (D) or during time-lapse imaging of a single cell (E). Fluorescence micrographs of cells carrying COPI+Dsl BiFC pair β-COP96•Dsl33p10C, as well as ERES marker Sec13pRFP. For (E), agarose-embedded cells were grown at RT with fresh PM Glc + ura medium. Scale bar, 5 μm.

Figure EV5. Summary of findings and model for coupled material transport at the bud tip.

A Spatial arrangement of the ERAS through self-organization. BiFC results of protein complex interactions and their subcellular localization are displayed graphically. The presented data support the hypothesis that ERAS form depending on organelle localization, namely Golgi placement. ERAS localization is a logical consequence of placement of Golgi, ER, and ERES, with Golgi potentially being the critical factor.

B Schematic overview of observed subcellular localizations of organelles and interaction sites between COPI vesicles and Dsl complex (ER).

C Model of a hotspot at the bud tip. Trans-Golgi is transported into the bud tip via Myo2p, where it supplies the newly inherited ER with material that may be utilized for the generation of cis-Golgi.

D Proposed ER-Golgi interaction hub, which facilitates efficient spatially compact material exchange in anterograde and retrograde direction.