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SPOTLIGHT

p24 family Tango(1) at the endoplasmic reticulum exit site to organize cargo exit

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The p24 family of proteins have been regarded as cargo receptors for endoplasmic reticulum (ER) to Golgi transport; however, their precise functions have yet to be revealed. In this issue, Pastor-Pareja and colleagues (https://doi.org/10.1083/jcb.202309045) show that the interaction of these proteins with Tango1 is critical for their localization at the ER exit site (ERES) and efficient transport of secretory proteins in *Drosophila*.

Proteins synthesized in the ER enter the secretory pathway through the ER exit site (ERES) and are ultimately transported to the plasma membrane or outside the cell (1). At ERES, some nascent proteins are recognized by cargo receptors, which usually traffic the cargoes to the Golgi. The p24 family of proteins are thought to be cargo receptors involved in the trafficking of many cargoes, including lipid-binding proteins and membrane proteins, and are conserved across yeast, plants, and mammals. Based on sequence homology, p24 proteins are classified into four subfamilies (α, β, γ, and δ) (2).

In this study, Yang et al. (3) started by systematically depleting each subfamily of p24 proteins in flies and observed the resultant phenotypes (with two types suppressed for the β subfamily). It became evident that suppressed expression of one member of each subfamily was associated with a general impairment of protein secretion. These findings necessitate consideration of the hierarchical localization of p24 subfamilies, as demonstrated later in this paper. Specifically, the localization to ERES of the γ subfamily requires all other subfamilies, whereas β subfamily requires α and δ, and δ subfamily requires α and β for ERES localization. Interestingly, localization to ERES of α does not require any other family members. Given the importance of correct localization related to proper functioning of the p24 proteins, it can be inferred that the γ family is at least involved in general secretion. The impact on secretion by other families may be due to the action of p24 subfamilies, including γ, which localize ectopically. From these results, it is now evident that the p24 protein family has broader functions beyond working merely as cargo receptors for specific molecules, and that a coordinated and sequential recruitment process of the p24 protein family to the ERES is crucial for efficient transport of secreted proteins.

In addition to the interaction between p24 proteins, interaction with Tango1 has been demonstrated to be crucial for the localization of p24 proteins to the ERES (Fig. 1). Tango1 was initially identified as a factor involved in secretion through genome-wide siRNA screening in *Drosophila* S2 cells (4). Subsequent analysis in mammalian cells demonstrated that TANGO1L, a mammalian ortholog of Tango1, specifically participates in collagen secretion (5). Further analysis revealed that *Drosophila* Tango1 and human TANGO1 family (TANGO1L and TANGO1S) are involved in the organization of ERES, as demonstrated by Pastor-Pareja and our group, respectively (6, 7).

Interestingly, all p24 families exhibited binding affinity for Tango1 via their Golgi dynamics (GOLD) domains, a cargo recognition domain of the p24 family, with the γ subunit showing a robust interaction. The strong interaction of the γ subunit, which is presumed to be positioned downstream in the interaction among p24 proteins, with Tango1 is unexpected and intriguing. Therefore, it would be interesting to discover the mechanisms by which each subfamily physiologically forms a multimer and how these multimers adapt their composition when each subfamily is knocked down.

ERES localization of Tango1 was also demonstrated to be regulated by interaction between SH3 domain of Tango1 and p24 GOLD domain. Upon p24 knockdown, Tango1 relocates ectopically to the plasma membrane. However, the mechanism by which Tango1 is transported to the plasma membrane upon suppression of p24 remains to be elucidated. The authors had previously shown that deletion mutants of only the cytoplasmic domain of Tango1 could rescue the phenotype of Tango1 knockdown in flies (6). Interestingly, in mammalian cells, the TANGO1 isoform, TANGO1S, which lacks the SH3 domain but retains the membrane-binding domain, could solely rescue the inhibition of collagen secretion caused by TANGO1L knockdown (8). Since TANGO1S is not expected to interact with p24, direct involvement of p24 in the ERES localization of TANGO1S is unlikely. We have previously shown the necessity for an interaction between Sec16 and

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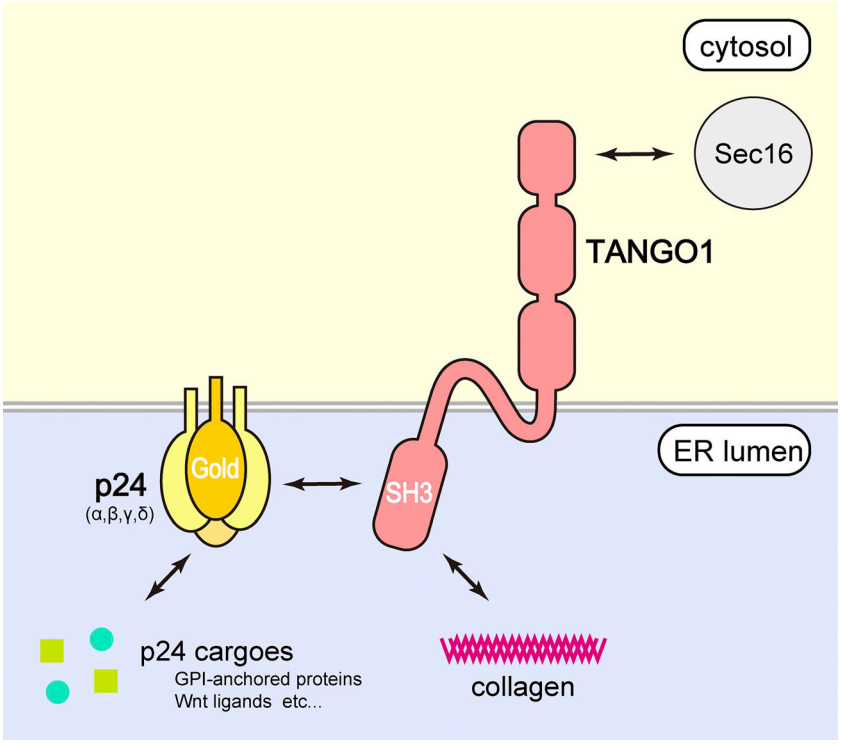

Figure 1. **The SH3 domain of Tango1, which is involved in collagen binding, interacts with p24 GOLD domain.** This interaction is essential for both proteins to be correctly localized at the ERES.

TANGO1L/TANGO1S in mammalian cells for localization to ERES (7). Although interaction between Tango1 and Sec16 is yet to be reported in flies, understanding how the mechanism of ERES localization of Tango1 is regulated across species is of significant interest.

Yang et al. (3) found that the coat protein complex 2 (COPII)–coated area, stained by COPII outer coat factor Sec13 and the small GTPase Sar1, expands upon suppression of p24 expression. Analysis using focused ion beam–scanning electron microscopy revealed an increase in the number of vesicles between the ERES and the Golgi with p24 depletion. By analyzing the size of these vesicles, they show that the number of vesicles corresponding to COPII between the ER and Golgi likely increases due to p24 suppression. From these findings, the authors speculate that the p24 family may act to restrain the budding of incomplete vesicles with improperly sorted cargoes. The mechanism through which p24 can exert this function remains to be determined. The authors previously showed that loss of Tango1 reduced the size of the ERES (6), whereas Tango1 overexpression induced enlargement of ERES in *Drosophila* (9). Here they propose that Tango1 and p24 collaborate to bind various cargoes via their SH3 and GOLD domains, functioning as concentration receptors or ERES stabilization factors at ERES, preventing them from being exported indiscriminately (Fig. 1). Notably, domains involved in the interaction between Tango1 and p24 are SH3 and GOLD domains, respectively. In this model, Tango1's multimerization and complex formation of the p24 family are both necessary to recruit cargoes to the ERES.

Although the current study has revealed that p24 plays a broader role beyond being merely a cargo receptor, many studies have shown that p24 functions as a cargo receptor cycling between the ER and the Golgi interacting with COPI and COPII components (2). Conversely, as the authors also mentioned in the discussion section, it is believed that under normal conditions, Tango1 is unlikely to be transported from the ERES toward the Golgi (5). Considering the function of mammalian TANGO1L as a collagen exporter and its collaboration with TANGO1S in ERES organization, it is conceivable that both *Drosophila* Tango1 and mammalian TANGO1L function as cargo exporters, detecting the folding status of cargoes, including p24 complex and collagens, using their SH3 domain and/or luminal side intrinsically disordered region (Fig. 1). Thus, p24 identified in this study might represent one of the cargoes of Tango1 as a cargo exporter, ensuring its proper folding and complex formation, similar to collagens. This idea is also supported by the authors' observation that under p24 depletion, most of Tango1 seems localized correctly at ERES with a fraction of Tango1 mislocalized to the plasma membrane, whereas Tango1 depletion led to the severe mislocalization of the p24 family with ER diffusion and plasma membrane. These possibilities await further analysis to elucidate the impact of the interaction between p24 and Tango1 on their respective functions.

In conclusion, this study by Yang et al. (3) is highly significant as it sheds light on the influence of p24 on ERES composition, the hierarchical nature of p24's function, and the role of p24–Tango1 interaction in ERES.

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References

1. Robinson, C., et al. 2024. *Front. Mol. Biosci.* https://doi.org/10.1007/s00709-015-0858-6
2. Pastor-Cantizano, N., et al. 2016. *Protoplasma.* https://doi.org/10.1007/s00709-015-0858-6
3. Yang, K., et al. 2024. *J. Cell Biol.* https://doi.org/10.1083/jcb.202309045
4. Bard, F., et al. 2006. *Nature.* https://doi.org/10.1038/nature04377
5. Saito, K., et al. 2009. *Cell.* https://doi.org/10.1016/j.cell.2008.12.025
6. Liu, M., et al. 2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201611088
7. Maeda, M., et al. 2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201703084
8. Maeda, M., et al. 2016. *Mol. Biol. Cell.* https://doi.org/10.1091/mbc.e16-03-0196
9. Yang, K., et al. 2021. *Cell Rep.* https://doi.org/10.1016/j.celrep.2021.109707