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Localization of arabidopsis SYP125 syntaxin in the plasma membrane sub-apical and distal zones of growing pollen tubes

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Key words: $[Ca^{2+}]_c$, endocytosis, exocytosis, secretion, syntaxins, tip growth

Abbreviations: $[Ca^{2+}]_c$, cytosolic free calcium; CLSM, confocal laser scanning microscopy; *PIP2*, phosphatidylinositol-4,5-bisphosphate; *PIP5K4*, phosphatidylinositol-4-monophosphate-5-kinase; *PM*, plasma membrane; *SYP*, syntaxin in plant (referring to all Q-SNAREs with a trans-membrane domain)

Tip growth in pollen tubes occurs by continuous vesicle secretion and delivery of new wall material, but the exact subcellular location of endocytic and exocytic domains remains unclear. Here we studied the localization of the *Arabidopsis thaliana* pollen specific syntaxin SYP125 using GFP-fusion constructs expressed in *Nicotiana tobaccum* pollen tubes. In agreement with the predicted role for syntaxins, SYP125 was found to be associated with the plasma membrane and apical vesicles in growing cells. At the plasma membrane, SYP125 was asymmetrically localized with a higher labeling 20–35 μm behind the apex, a distribution which is distinct from SYP124, another pollen-specific syntaxin. Competition with a related dominant negative mutant affected the specific distribution of SYP125 but not tip growth. Co-expression of the phosphatidylinositol-4-monophosphate-5-kinase 4 (PIP5K4) or of the small GTPase Rab11 perturbed polarity and the normal distribution of GFP-SYP but did not inhibit the accumulation in vesicles or at the plasma membrane.

Taken together, our results corroborates previous observations that in normal growing pollen tubes, the asymmetric distribution of syntaxins helps to define exocytic sub-domains but requires the involvement of additional signaling and functional mechanisms, namely phosphoinositides and small GTPases. The localization of syntaxins at different membrane domains likely depends on the interaction with specific partners not yet identified.

Introduction

Pollen tube growth and reorientation occur at the extreme apex of the tube due to polarized transport, docking and fusion of secretory vesicles.[1,2] It was generally assumed that exocytosis events occur mostly at the extreme apex while membrane recycling (endocytosis) would take place further back from the tip, at the flanks of the apex and/or at sub-apical regions.[3,4] Recent data questioned this paradigm suggesting that the preferential location for fusion is on a limited membrane domain at the sub-apical flanks and not at the extreme apex.[5,6] The hypothesis that two endocytic modes could co-exist according to the growth conditions as also been raised[7-9] and requires further testing.

We recently showed that a pollen specific syntaxin, SYP124 (Microarray Database Genevestigator, GeneChip; reviewed in ref. 10), accumulates at the membrane of apical vesicles and at the plasma membrane flanks (10–25 μm behind the apex) of growing pollen tubes.[11] SYP124 belongs to a highly conserved family of Qa-SNAREs (for soluble N-ethylmaleimide sensitive factor attachment protein receptor) which interact on the target membrane with two other partners classified as Qb- and Qc-SNAREs.[12] The resulted target complex known as t-SNARE binds with R-SNARE on transport vesicle and assures docking and thus driving membrane fusion.[13] Therefore, our reported localization suggests a role for SYP124 in the regulation of localized exocytosis and a net flow of fusion events in the flanks of the growing pollen tube apex. According to theoretical predictions,[14] SYP124 has functional redundancy with SYP125, the other pollen-specific syntaxin reported to have asymmetric localization.[15] So in this work we further investigated the distribution of SYP125 during pollen tube growth and upon modulation by regulatory proteins (PIP5K4 and Rab11). Using GFP-fusion proteins we found that SYP125 also accumulates at apical vesicles and at the plasma membrane but, in contrast to SYP124, the highest fluorescent labeling was observed in more distal plasma membrane regions, 20–35 μm behind the apex of growing pollen tubes.

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Results and Discussion

The location, dynamics and regulatory mechanisms of exocytic events in pollen tubes remains unclear.[2,4,5,21] Recent data has suggested that the preferential location for fusion is on a limited membrane domain at the sub-apical flanks and not at the extreme apex, in agreement with the hypothesis of an annulus shaped release zone.[5,6,22] In a previous work we found that SYP124, a pollen-specific Qa-SNARE, showed a specific distribution with a higher labeling at the plasma membrane flanks of growing pollen tubes, 10–25 μm behind the apex.[11] This distribution was affected by regulators of membrane secretion (Ca^{2+}, PIP5K4 and Rab11),[11] indicating that syntaxins alone do not provide the level of specificity required for apical growth and that additional signaling and functional mechanisms are required.

To further study this topic, here we analyzed the distribution of SYP125, another pollen-specific Qa-SNARE predicted to have functional redundancy with SYP124.[14] For such purpose, pollen of *Nicotiana tabacum* was transformed with a GFP-SYP125 fusion construct controlled by the Lat52 promoter and actively growing pollen tubes (growth rate of at least 3 um·min^{-1}) imaged by confocal laser scanning microscopy 5–6 h after transformation. Control experiments involved transformation of cells with free GFP which exhibits cytosolic distribution.[11]

SYP125 accumulates in apical vesicles and at the membrane flanks of the growing pollen tube. Similarly to SYP124,[11] in growing cells expressing GFP-SYP125 (n = 10), the fluorescence mainly localized in the characteristic inverted cone of apical vesicles and at the plasma membrane with a higher signal at the flanks of the pollen tube (**Fig. 1A and B**). However, the highest labeling at the plasma membrane was observed 20–35 μm behind the apex, a localization that overlaps but not superimposes with SYP124 (10–25 μm behind the extreme tip) (**Fig. 1C**). In the non-growing pollen tubes (n = 5), there was dissipation in SYP125 apical vesicle labeling and localization in the plasma membrane was still visible but with no accumulation pattern (**Fig. 1D**). Overexpression of GFP-SYP125 (observations made 7–9 h after transformation; n= 10) leads to a significantly higher localization associated with the plasma membrane (**Fig. 1E**) and perturbations in apical morphology (changes in tip diameter-n= 3; oscillatory or wavy pattern-n = 4) but not to a significant disturbance of polarity.

Co-expression with a truncated version consisting only in the N-terminal portion of SYP (SYP125T),[19] led to no visible change in pollen tube morphology or growth rate (n = 5). However, as in non-growing cells, localization in the plasma membrane was still visible but with no specific accumulation pattern (**Fig. 1F**) suggesting a perturbation in the interaction of SYP125 with its targets. We hypothesize this was due to the dominant negative potential of the truncated form that competes with the native protein for the SNARE partner but has minimal impact on the formation of functional complexes.[23] Consequently, the "pool" of interactors with the non-mutated SYP is reduced and the protein will flow on the membrane differently.

Localization of SYP125 is modulated by signaling pathways. Previously we found that the localization of SYP124 was affected by the addition of 20 μM gadolinium chloride.[11] Gadolinium (Gd^{3+}) is an inhibitor of plasma membrane cationic channels[24] known to reduce intracellular $[Ca^{2+}]_c$ levels in pollen tubes[1,4,7] and at this concentration transiently reduces growth rates allowing fast recovery upon wash-out.[11] Along with a reduction in growth rate, the addition of this ion to the extracellular medium caused a transient but significant (≥5%) reduction in the association of the fluorescent signal with the apical and sub-apical plasma membrane (**Fig. 2**; n = 5). This is in agreement with the observation that SNARE fusion is inherently Ca^{2+}-insensitive but an increase in Ca^{2+} enhances SNARE-mediated liposome fusion[25] and consistent with a reduction in fusion rates but continued membrane recycling. In contrast, the signal associated with both apical and sub-apical cytoplasmic regions exhibited no significant changes (<5%; **Fig. 2**) suggesting that, similarly to SYP124, influx of extracellular Ca^{2+} is important for specific SYP125 localization (and accumulation) at the plasma membrane but not to its trafficking through secretory vesicles.

The localization of SYP125 was also found to be modulated by the *Arabidopsis thaliana* phospholipid kinase PIP5K4 and by the small GTPase RAB11. To directly compare SYP125 results with our previous report in reference 11, here we co-expressed GFP-SYP125 with either PIP5K4 or with a dominant-negative GDP-bound version of Rab11 (Rab11DN). Overexpression of these two proteins was shown to induce depolarization of the growth axis and to disturb membrane trafficking.[20,26,27] In all analyzed cells (n = 10) PIP5K4 expression (~7–9 h after transformation) caused changes in GFP-SYP125 localization, the association of GFP fluorescence with the apical plasma membrane and dissipation of the typical higher labeling of the apical cytoplasm (**Fig. 3A–C**). Eight of these cells also exhibited significant disturbance of polarity as depicted in **Figure 3A and B**. Rab11DN overexpression (**Fig. 4A–C**; n = 10) led to similar observations with altered SYP125 localization, dissipation of apical vesicle labeling (n = 10) and depolarization of the growth axis (n = 9).

These results stress the similarity between SYP124 and SYP125 in agreement with a functionally redundant role for the two syntaxins. They also support our previous suggestion that syntaxins play mostly a structural role in pollen tube growth rather than specifying the rate of vesicle delivery. Their turnover and localization most likely follows the changes triggered by the multiple signaling pathways acting on pollen tube secretion.

Material and Methods

Plasmids and vectors. Lat52 Pollen Promoter [pLat52,[16]] was amplified from pGreen pLat52-GFP vector[17] as previously described in reference 18 and 19. AtSYP125 cDNA (AT1G11250, The Arabidopsis Information Resource) was amplified with forward (5'-GTC GAC CAT GAA CGA TTT ATT CTC TAA TTC ATT C-3') and reverse (5'-CTG CAG TTC ACT TCA ACA TGA GCA TTA TGT GAG G-3') primers carrying *Sal*I and *Pst*I restriction sites (underlined) respectively. The resulting DNA fragment was used to replace the *Sal*I-*Pst*I fragment in plasmid pSGFP5K.[18] After digestion with *Nde*I and *Eco*RI the resulting DNA fragment was used to replace the corresponding

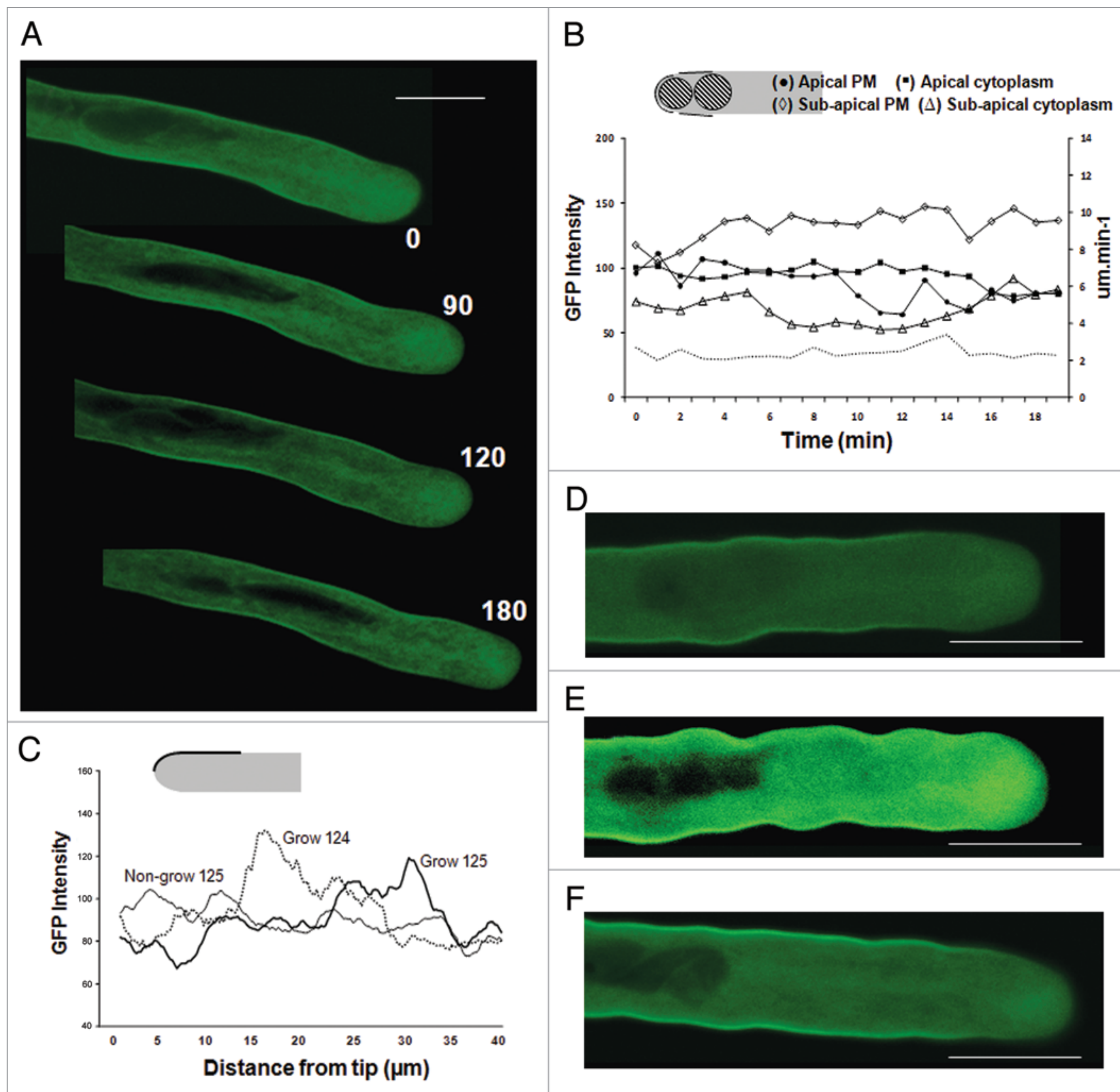

Figure 1. Confocal imaging of *N. tabacum* pollen tubes expressing GFP-SYP125. Scale bars = 20 μm. (A) Time-course series of a growing tobacco pollen tube showing the subcellular localization of SYP125 (observation made ~6 h after transformation). The protein seemed to accumulate mostly in apical vesicles and at the flanks of the plasma membrane. Numbers refer to the time interval (in seconds). Images are representative of ten independent experiments. (B) Time course analysis of GFP fluorescence intensity in the growing pollen tube shown in (B). Each line is representative of measurements of the fluorescent signal made in the lines/circles depicted in the diagram for 12 different pollen tubes: apical plasma membrane (black circle); apical cytoplasm (black square); sub-apical plasma membrane (lozenge); sub-apical cytoplasm (triangle). Growth rate measurements ($\mu m \cdot min^{-1}$) are displayed by the dashed line. See Supplemental file for Z-stack series of SYP125 localization. (C) Distribution of average fluorescence intensity along the first 40 μm of the pollen tubes plasma membrane as depicted in the diagram for growing (Grow-thick solid line; n = 10) and non-growing (Non-grow-thin solid line; n = 5) pollen tubes. Dotted line represents equivalent measurements for growing pollen tubes expressing GFP-SYP124 (n = 12); differences to the localization pattern of SYP125 are statistically significant ($p < 5E^{-8}$). Error bars not displayed for the sake of clarity. See Supplemental file for co-expression image of GFP-125 and RFP-SYP124. (D) Optical section of a non-growing pollen tube transformed with GFP-SYP125. (E) Pollen tube expressing high levels of GFP-SYP125 exhibited disturbed growth and perturbations in apical morphology. The protein accumulates at the flanks but also at the apical plasma membrane. (F) Pollen tube co-expressing SYP125T (truncated protein lacking the transmembrane domain) with full GFP-SYP125. The cell exhibited no perturbations in apical morphology but protein localization exhibits a higher labeling in the plasma membrane further away from the sub-apical region.

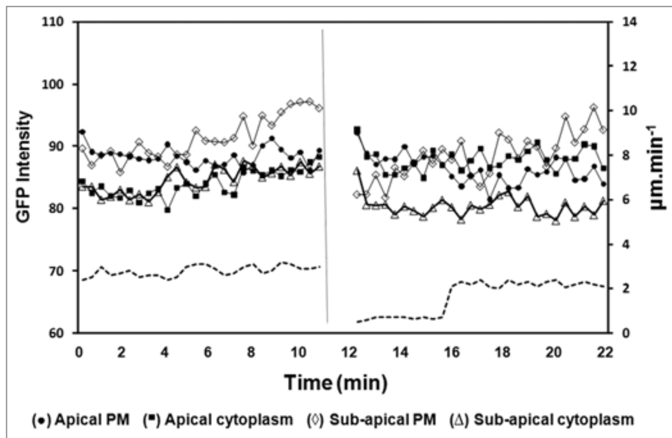

Figure 2. Time course analysis of GFP fluorescence intensity in *N. tabacum* growing pollen tubes transformed with GFP-SYP125 and exposed to 20 µM of extracellular Gd^{3+}. Imaging and measurements were performed under the same conditions and settings as in **Figure 1A**. Growth rate measurements ($\mu m \cdot min^{-1}$) are displayed by the dashed lines. Addition of the inhibitor was performed at the time indicated by the vertical dotted line and collection of data resumed after inhibitor wash out. The graph is representative of five independent pollen tubes. Symbols indicate measurements performed in the apical plasma membrane (black circle), apical cytoplasm (black square), sub-apical plasma membrane (lozenge) and sub-apical cytoplasm (triangle) according to the diagram in **Figure 1B**.

fragment in plasmid GFP-AtSYP122F, resulting in GFP-AtSYP125F fusion coding sequence controlled by the pLat52.

Construct 125T was similarly obtained but amplifying GFP-AtSYP125F with forward (5'-GCC CAT GGC CCC CCA TGA AGA CTA ATC TTT TTC TCT T-3') and reverse (5'-ACT GCA GAT CAA GTC CAT TTC CTC GAG CTC TT-3') primers carrying *Nco*I and *Pst*I restriction sites (underlined) respectively, resulting in AtSYP125ΔC257-K283 coding sequence controlled by the pLat52.

Constructs pLat52:GFP-AtSYP124F, pLat52:AtRab11DN and pLat52-AtPIP5K4 were generated as previously in reference 11, 19 and 20.

For co-expression experiments a construct RFP-AtSYP124 was generated in two steps. RFP from the vector pHD334 was amplified with forward (5'-ATC ATC GGT ACC ATG GCC TCC TCC GAG GAC GTC-3') and reverse (5'-AAT AAT GCG GCC GCT TAG GCG CCG GTG GAG TG-3') primers carrying KpnI and NotI restriction sites (underlined) respectively and cloned into pHD32. AtSYP124F was amplified with forward (5'-GGA GCC GTC GAC AAT GAA TGA TTT ATT CTC-3') and reverse (5'-CAT GAT CCA TGG TCT TCA ACA TGA GCA TGA-3') primers carrying SalI and NcoI restriction sites (underlined) respectively. The resulted fragment was cloned into pHD32 giving rise to RFP-SYP124 controlled by the pLat 52.

Plant material. *Nicotiana tabacum* (cv. Petit Havana SR1) plants were grown in a greenhouse under standard conditions. Mature pollen was collected and germinated in culture as previously described in reference 20.

Transient transformation and GFP imaging in tobacco pollen tubes. A helium-driven PDS-1,000/He particle delivery system (BioRad) was used for the biolistic transformation of tobacco pollen as described by reference 20. The methods for plasmid expression, pollen germination, confocal imaging and drug treatments were as previously described in reference 11.

Data analysis. Numerical data extraction was performed using Image-Pro Plus 6.0 software (Media Cybernetics). Fluorescence measurements of the pollen tube cytoplasm and/or plasma membrane correspond to medium fluorescence intensity in the areas depicted in the figure diagrams. Fluorescence was quantified in terms of average pixel intensity and statistically analyzed with a t-student test (2-tailed distribution). Variations of >5% were considered to be meaningful. Except where mentioned, numerical data in figures correspond to single cell analysis of typical experiments and not to summary statistics.[8,20]

Conclusions

The data presented here indicates that pollen-specific syntaxins SYP125 and SYP124,[11] have a complementary distribution and corroborates previous suggestions of a functionally redundant role for these two Qa-SNAREs.[14] The observed differences in protein accumulation at the plasma membrane might reflect specific interactions with unidentified targets (e.g., PIP2 and phosphatidic acid; ref. 28) which, under normal growth conditions of wild-type pollen tubes, could translate into discrete asymmetric secretory events and relate to the two endocytic modes suggested to co-exist in these cells.[9,22] However, we cannot rule out the hypothesis that this overlapping but not superimposed distribution is due to the use of a heterologous system (expression of Arabidopsis genes in tobacco).

Similarly to SYP124,[11] the changes in SYP125 distribution observed upon growth modulation are likely to reflect membrane dynamics and a repositioning of the vesicle's docking machinery upon intra- and extracellular stimuli. Although these findings highlight the importance of syntaxins in secretion and tip growth, they must be carefully interpreted because the localization reported reflects protein accumulation and not necessarily the point of vesicle fusion.[11,21] Further studies on the complex machinery required for vesicle fusion are thus required.

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Authors' Contributions

R.M. conceived the study and prepared the manuscript. RUR carried out the experiments and contributed to the manuscript. RUR and PAS made the molecular constructs. All authors discussed the results, read and approved the final manuscript.

Note

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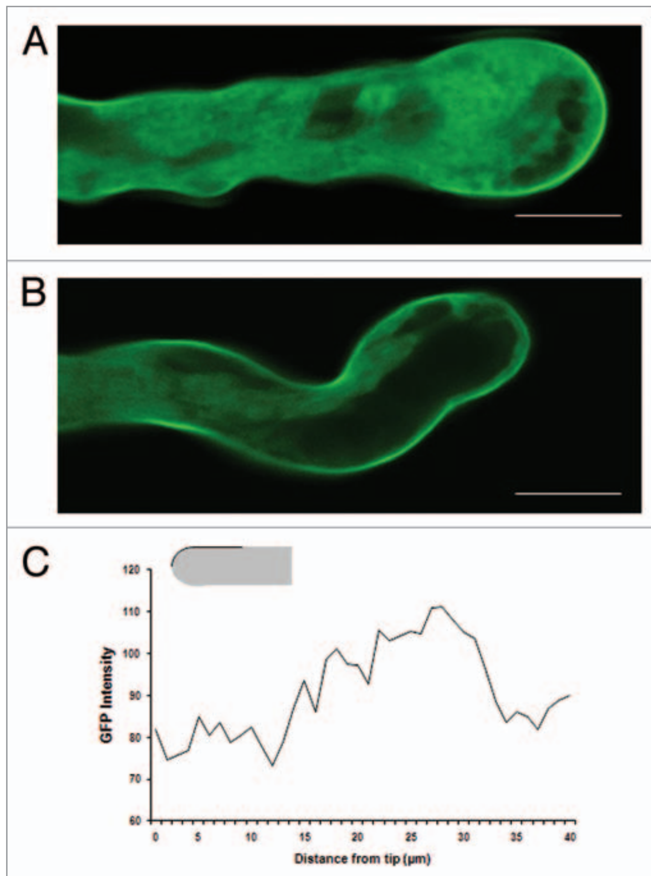

Figure 3. Confocal imaging of *N. tabacum* pollen tubes co-expressing PIP5K4 with GFP-SYP125. Scale bars = 20 μm. (A and B) Optical section of pollen tubes overexpressing PIP5K. Along with high protein localization in the flanks and apical plasma membrane, labeling of large endomembrane-like structures is visible. (C) Distribution of average fluorescence intensity along the first 40 μm of the pollen tubes plasma membrane (n = 10) as depicted in the diagram. The distribution is statistically different ($p = 2.6E^{-24}$) from GFP-SYP125 alone (compare with **Fig. 1B**). Error bars not displayed for the sake of clarity.

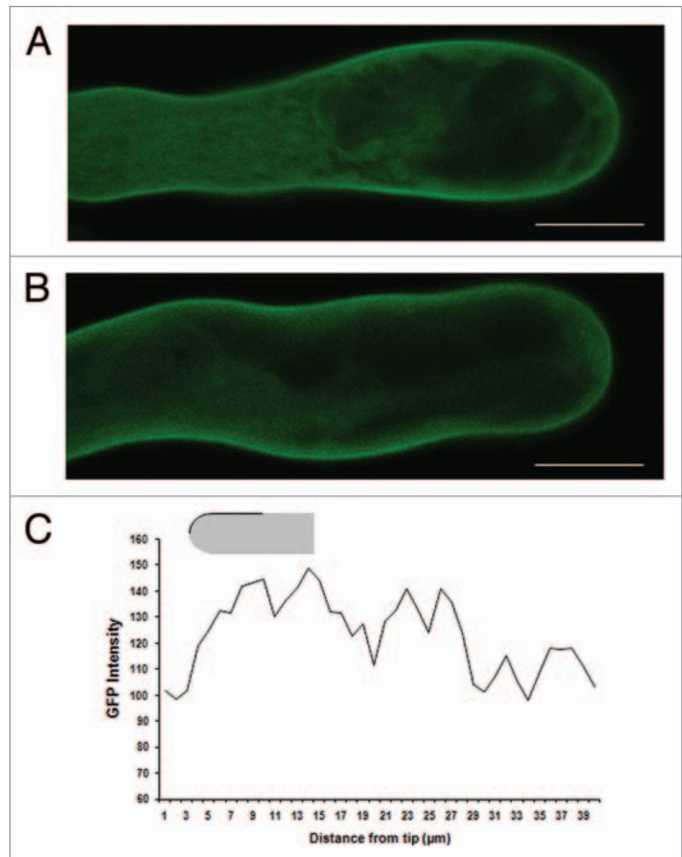

Figure 4. Confocal imaging of *N. tabacum* pollen tubes co-expressing Rab11DN with GFP-SYP125. Scale bars = 20 μm. (A and B) Optical section of pollen tubes overexpressing Rab11DN. The cells exhibit partial loss of polarity, high protein localization in the flanks and apical plasma membrane and almost complete dissipation of the apical vesicle labeling. (C) Distribution of average fluorescence intensity along the first 40 μm of the pollen tubes plasma membrane (n = 10) as depicted in the diagram. The distribution is statistically different ($p = 1.4E^{-12}$) from GFP-SYP125 alone (compare with **Fig. 1B**). Error bars not displayed for the sake of clarity.

References

1. Holdaway-Clarke TL, Hepler PK. Control of pollen tube growth: Role of ion gradients and fluxes. New Phytol 2003; 159:539-63. DOI:10.1046/j.1469-8137.2003.00847.x.
2. Malhó R, Castanho Coelho P, Pierson E, Derksen J. Endocytosis and membrane recycling in pollen tubes. In: Samaj J, Baluska F, Menzel D. *The Plant Endocytosis*. Germany: Springer-Verlag 2005; 277-91.
3. Picton JM, Steer MW. Determination of secretory vesicle production rates by dictyossomes in pollen tubes of Tradescantia using Cytochalasin D. J Cell Sci 1981; 49:261-72. PMID: 7309807.
4. Castanho Coelho P, Malhó R. Correlative analysis of apical secretion and $[Ca^{2+}]_C$ in pollen tube growth and reorientation. Plant Signal Behav 2006; 1:152-7.
5. Zonia L, Munnik T. Vesicle trafficking dynamics and visualization of zones of exocytosis and endocytosis in tobacco pollen tubes. J Exptl Bot 2008; 59:861-73. PMID: 18304978.
6. Bove J, Vaillancourt B, Kroeger J, Hepler PK, Wiseman PW, Geitmann A. Magnitude and direction of vesicle dynamics in growing pollen tubes using spatiotemporal image correlation spectroscopy and fluorescence recovery after photobleaching. Plant Physiol 2008; 147:1646-58. PMID: 18508956.
7. Camacho L, Malhó R. Endo-exocytosis in the pollen tube apex is differentially regulated by Ca^{2+} and GTPases. J Exptl Bot 2003; 54:83-92. PMID: 12456758
8. Monteiro D, Liu Q, Lisboa S, Scherer GEF, Quader H, Malhó R. Phosphoinositides and phosphatidic acid regulate pollen tube growth and reorientation through modulation of $[Ca^{2+}]_c$ and membrane secretion. J Exptl Bot 2005; 56:1665-74. PMID: 15837704
9. Moscatelli A, Ciampolini F, Rodighiero S, Onelli E, Cresti M, Santo N, et al. Distinct endocytic pathways identified in tobacco pollen tubes using charged nanogold. Journal of Cell Science 2007; 120:3804-19. PMID:17940063.
10. Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W. GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. Plant Physiol 2004; 136:2621-32. PMID: 15375207.
11. Ângelo Silva P, Rehman R, Rato C, Di Sansebastiano GP, Malhó R. Asymmetric localization of Arabidopsis syntaxins at the pollen tube apical and sub-apical zones is involved in tip growth. BMC Plant Biol 2010; 10:179. PMID: 20718953.
12. Di Sansebastiano GP, Faraco M, Zouhar J, Dalessandro G. The study of plant SNAREs specificity in vivo. Plant Biosystems 2009; 143:621-9.
13. Lipka V, Kwon C, Panstruga R. SNARE-ware: The role of SNARE-domain proteins in plant biology. Annu Rev Cell Dev Biol 2007; 23:147-74; PMID:17506694.
14. Kato N, He H, Steger AP. A systems model of vesicle trafficking in Arabidopsis pollen tubes. Plant Physiol 2010; 152:590-601. PMID:19933386.
15. Enami k, Ichikawa M, Uemura T, Kutsuna N, Hasezawa S, Nakagawa T, et al. Differential expression control and polarized distribution of plasma membrane-resident SYP1 SNAREs in *Arabidopsis thaliana*. Plant Cell Physiol 2009; 50:280-9. PMID: 19098073.
16. Twell D, Yamaguchi J, McCormick S. Pollen-specific expression in transgenic plants: Coordinate regulation of two different promoters during microsporogenesis. Development 1990; 109:705-13. PMID:2401221.
17. Hellens RP, Edwards EA, Leyland NR, Bean S, Mullineaux PM. pGreen: A versatile and flexible binary Ti vector for Agrobacterium-mediated plant transformation. Plant Mol Biol 2000; 42:819-32. PMID: 10890530.
18. Di Sansebastiano GP, Paris N, Marc-Martin S, Neuhaus JM. Specific accumulation of GFP in a non-acidic vacuolar compartment via a C-terminal propeptide-mediated sorting pathway. Plant J 1998; 15:449-57. PMID: 9753772.

19. Rehman RU, Stigliano E, Lycett GW, Sticher L, Sbano F, Faraco M, et al. Tomato rab11a characterization evidenced a difference between SYP121-dependent and SYP122-dependent exocytosis. Plant Cell Physiol 2008; 49:751-66. PMID: 18385165.
20. Sousa E, Kost B, Malhó R. Arabidopsis Phosphatidylinositol-4-Monophosphate-5-Kinase 4 regulates pollen tube growth and polarity by modulating membrane recycling. Plant Cell 2008; 20:3050-64. PMID: 19033528.
21. Zonia L, Munnik T. Uncovering hidden treasures in pollen tube growth mechanics. Trends Plant Sci 2009; 14:318-27. PMID: 19446491.
22. McKenna ST, Kunkel JG, Bosch M, Rounds CM, Vidali L, Winship LJ, et al. Exocytosis precedes and predicts the increase in growth in oscillating pollen tubes. Plant Cell 2009; 21:3026-40. PMID: 19861555.
23. Geelen D, Leyman B, Batoko H, Di Sansabastiano GP, Moore I, Blatt MR. The abscisic acid-related SNARE homolog NtSyr1 contributes to secretion and growth: Evidence from competition with its cytosolic domain. Plant Cell 2002; 14:387-406. PMID: 11884682.
24. Schumaker KS, Sze H. Inositol-1,4,5-trisphosphate releases Ca^{2+} from vacuolar membrane vesicles of oat roots. J Biol Chem 1987; 262:3944-6. PMID: 2881929.
25. Mahal LK, Sequeira SM, Gureasko JM, Sollner TH. Calcium-independent stimulation of membrane fusion and SNAREpin formation by synaptotagmin I. Journal of Cell Biology 2002; 158:273-82. PMID: 12119360.
26. Ischebeck T, Stenzel I, Heilmann I. Type B Phosphatidylinositol-4-Phosphate 5-Kinases mediate Arabidopsis and *Nicotiana tabacum* pollen tube growth by regulating apical pectin secretion. Plant Cell 2008; 20:3312-30. PMID: 19060112.
27. De Graaf BHJ, Cheung AY, Andreyeva T, Levasseur K, Kieliszewski M, et al. Rab11 GTPase-regulated membrane trafficking is crucial for tip-focused pollen tube growth in Tobacco. Plant Cell 2005; 17:2564-79. PMID: 16100336.
28. Vicogne J, Vollenweider D, Smith JR, Huang P, Frohman MA, Pessin JE. Asymmetric phospholipid distribution drives in vitro reconstituted SNARE-dependent membrane fusion. Proc Natl Acad Sci USA 2006; 103:14761-6. PMID:1595425.