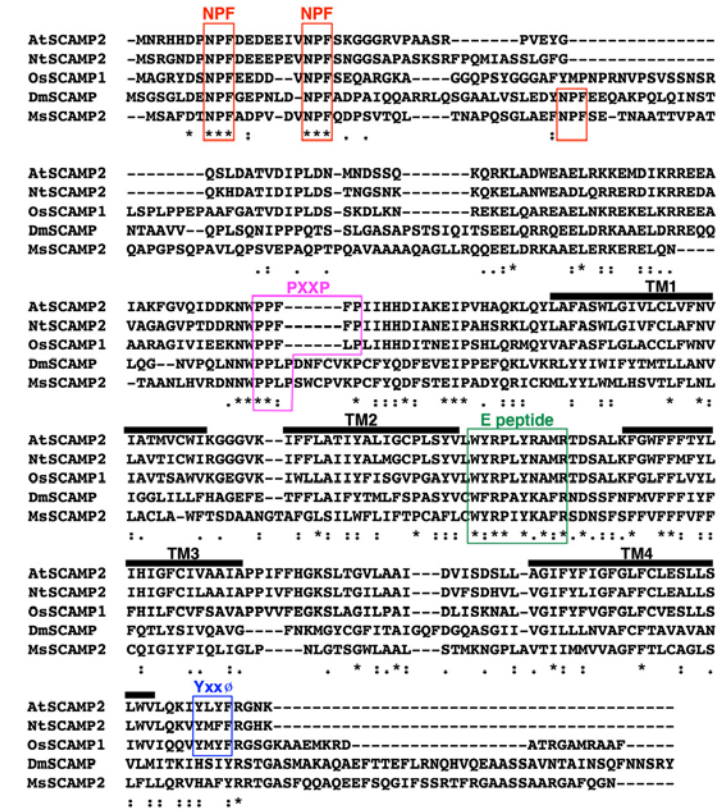

Figure 1. Alignment of SCAMP2 amino acid sequences. Sequences of tobacco SCAMP2 (Nt SCAMP2, accession No. AB295617), *Arabidopsis* (At, At1g32050), rice (Os, Os07g0564600), *Drosophila* (Dm, CG9195-RA) and mouse (Ms, mouse SCAMP2) were aligned for maximal homology using *T-Coffee* (http://tcoffee.vital-it.ch/cgi-bin/Tcoffee/tcoffee_cgi/index.cgi). Horizontal bars indicate transmembrane domains (TM). Frames indicate NPF sequence (NPF), proline-rich domain (PXXP), E peptide and tyrosine motif (Yxx{Phi}).

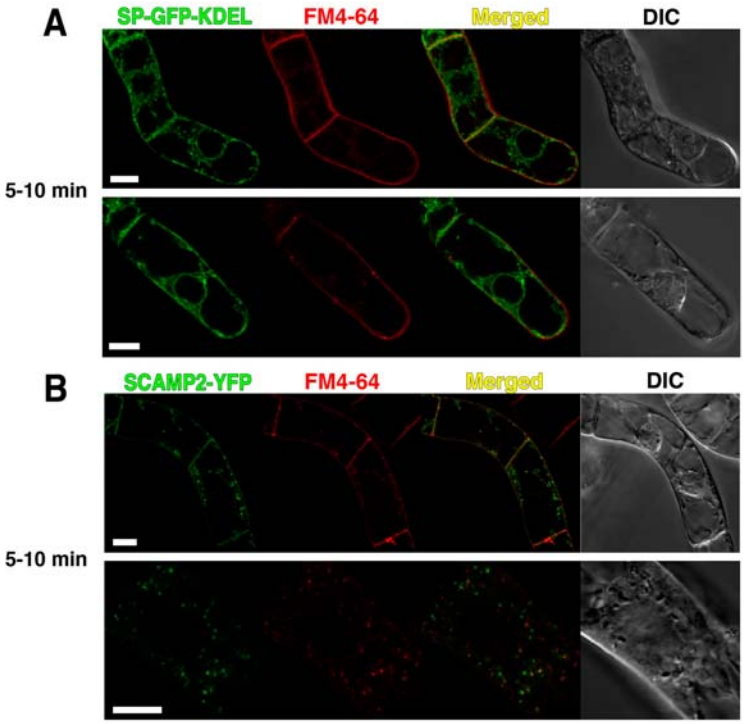

Figure 2. Subcellular localization of FM4-64. (A) Distribution of FM4-64 in BY-2 cells expressing ER marker, signal peptide attached to GFP with a KDEL-tail (Yuasa et al., Plant J **41,** 81-94). Cells were incubated with medium containing 33 μM FM4-64 on ice for 10 min and then washed with cold medium. Samples were then incubated at room temperature for 5–10 min before collecting

FM4-64 in BY-2 cells expressing tobacco SCAMP2-YFP. Cells were incubated with medium containing 17 μM FM4-64 on ice for 10 min and then washed with cold medium. Samples were then incubated at room temperature for 5–10 min before collecting fluorescent images.

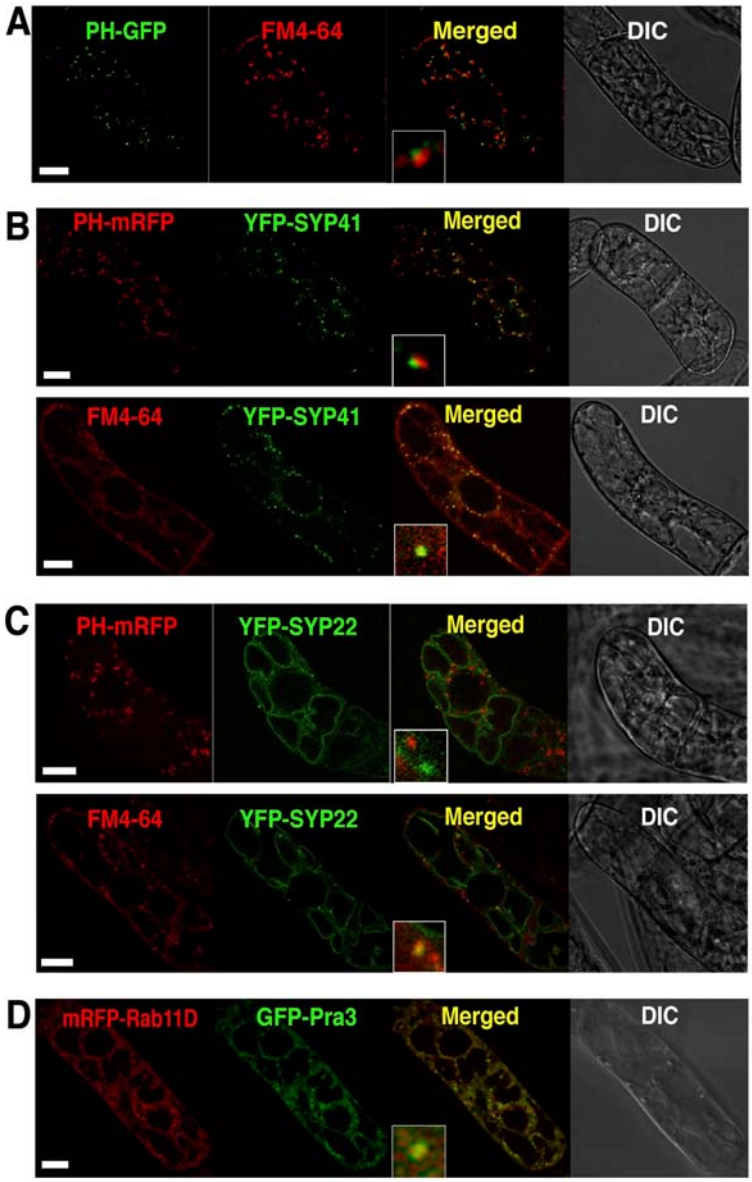

Figure 3. Subcellular localization of FM4-64 with organelle markers. (A) Localization of PH-GFP and FM4-64 incubated for 30-40 min. Many of the FM4-64 signals located by the side of a cis-Golgi marker PH-GFP indicate the TGN localization of this dye. This observation is consistent with the previous observations (Dhonukshe et al., 2006; Lam et al., 2007). Other FM4-64 dots represent the endosome. (B) Localization of tobacco SYP41 with either PH-mRFP or FM4-64 incubated for 30–40 min. Insert in the merged image show 4-fold enlarged image of a randomly-chosen fluorescence dot. Some YFP-SYP41 localized at the side of cis-Golgi marker PH-mRFP and colocalized with FM4-64 signal. This localization pattern of YFP-SYP41 is consistent with the localization pattern of SYP41 in *Arabidopsis*. (C) Localization of tobacco SYP22 with either PH-mRFP or FM4-64 incubated for 30–40 min. Insert in the merged image show 4-fold enlarged image of a randomly-chosen fluorescence dot. Clear localization of YFP-SYP22 at the vacuolar membrane is apparent. Some YFP-SYP22 show localization of intracellular bright dots. No PH-mRFP and YFP-SYP22 dots were associated. Some YFP-SYP22 dots colocalized with FM4-64 signal. This localization pattern of YFP-SYP22 is consistent with the localization pattern of SYP22 in *Arabidopsis*. (D) Localization of mRFP-Rab11D with GFP-PsPra3 (Inaba et al., 2002). >85% of the fluorescent dots showed both green and red signals. Bars in A-D, 20 μm

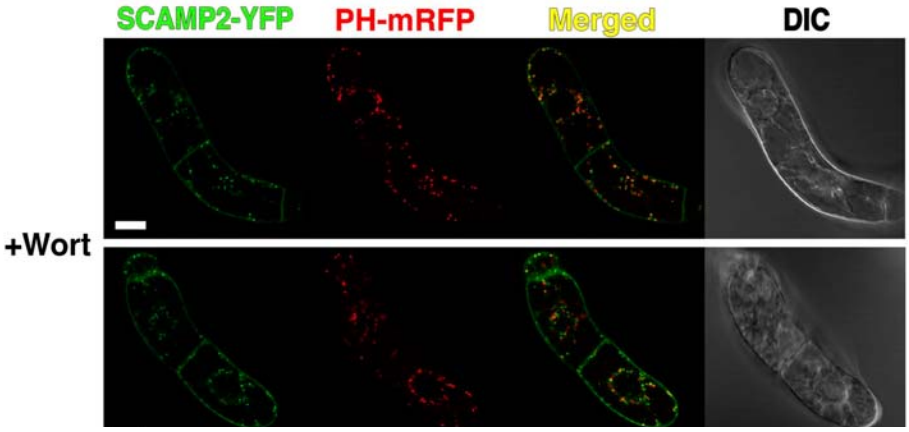

Figure 4. Subcellular Localization of SCAMP2 in the presence of wortmannin. BY-2 cells expressing PH-mRFP and SCAMP2-YFP were treated with 16.5 μM of wortmannin for 1 h.

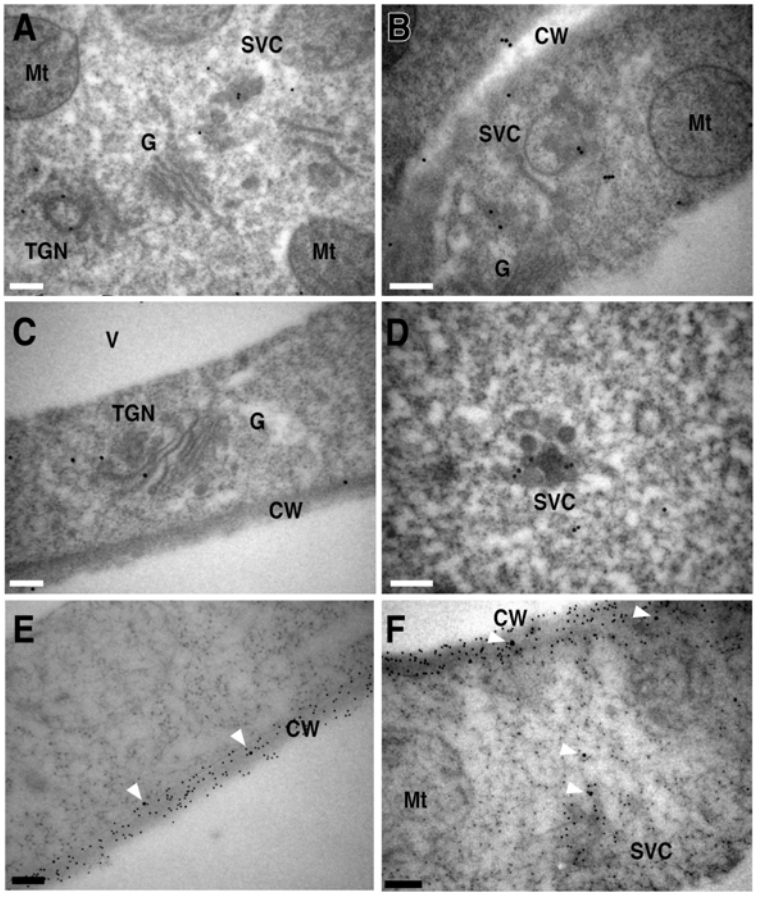

Figure 5. Immunogold labeling using anti-SCAMP2 antibody. (A-D) The ultrathin sections of BY-2 cells were stained using anti-SCAMP2. (E and F) The ultrathin sections of BY-2 cells were stained using anti-SCAMP2 (18 nm) and JIM7 (12 nm) antibodies. Arrowheads show gold particles of SCAMP2. Key: G, Golgi apparatus; CW, cell wall; V, vacuole; Mt, mitochondrion. Bars = 200 nm.

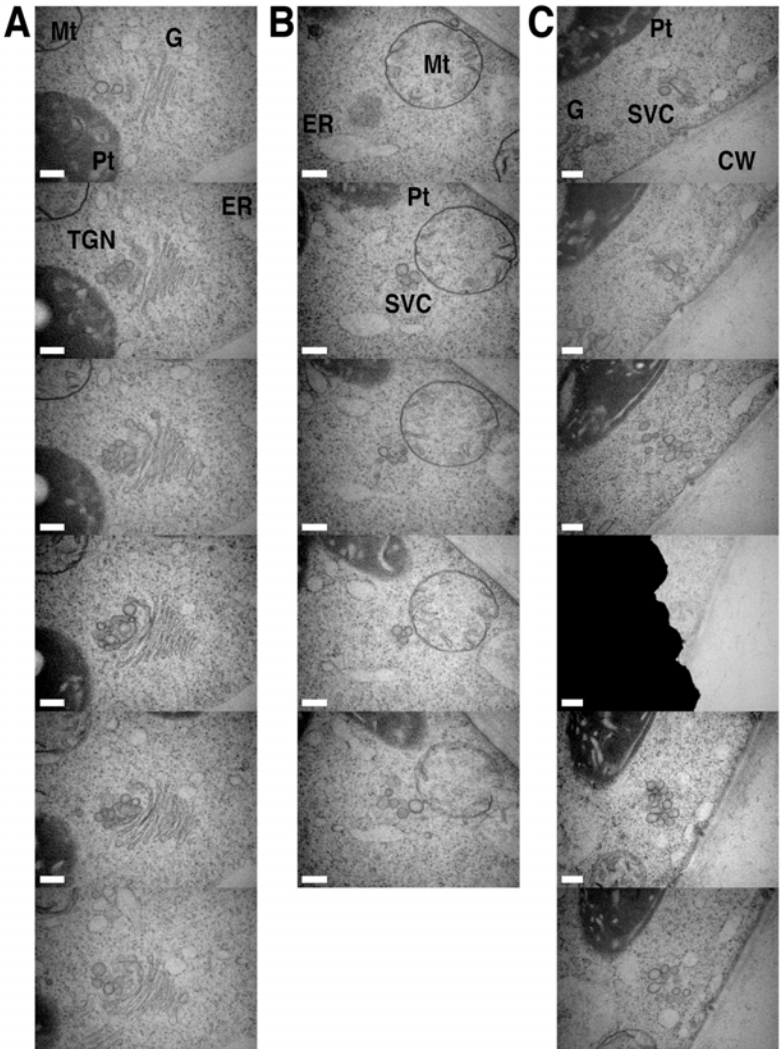

Figure 6. Electron micrographs of ultrathin serial sections of BY-2 cells for the comparison of TGN and SVC. Blocks of BY-2 cells prepared by HPF/FS were stained with 1% tannic acid in acetone and the sections were observed. (A) Ultrathin serial sections of the Golgi apparatus and TGN. (B and C) Ultrathin serial sections of a SVC. Key: G, Golgi apparatus; Pt, plastid; CW, cell wall; V, vacuole; Mt, mitochondrion. Bars = 200 nm.

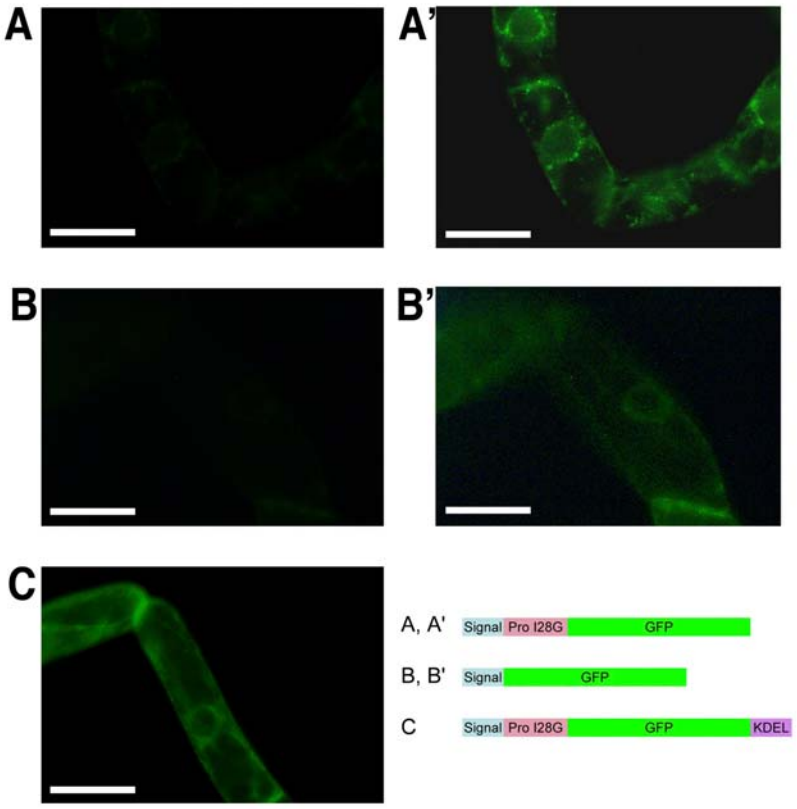

Figure 7. Localization of SPO41(I28G)-GFP, sporamin signal peptide-GFP and SPO41(I28G)-GFP-KDEL in tobacco BY-2 cells. Epifluorescence images of 3-day old tobacco cells expressing constructs indicated in lower right panel. A, B, and C show representative transformants with the same data acquisition and presentation conditions, whereas A' and B' show the same images with contrast adjusted to optimal intensity for presentation. Note that the pattern of fluorescence of SPO41(I28G)-GFP and sporamin signal peptide-GFP differs significantly although cell clumps expressing both constructs showed brighter fluorescence between two attached cells. This suggested that these proteins were secreted to the extracellular space. The absence of secreted GFP fluorescence signals in the culture medium region as well as cell walls that face the medium is due to the volume of culture being much larger than the volume of cells.

The intracellular fluorescence pattern of sporamin signal peptide-GFP resembles to that of SPO41(I28G)-GFP-KDEL (Yuasa et al., Plant J **41,** 81-94). as observed in the case of secretory GFP with signal peptide from of pumpkin 2S albumin (Mitsuhashi et al., Plant Cell Physiol. 41, 993-1001, 2000) whereas SPO41(I28G)-GFP showed punctate pattern in the cell. This difference might be the result of the rate of transport in the cell as a point mutation of the propeptide of sporamin, the 27th proline to asparagine mutation, decreased the speed of intracellular transport (Matsuoka and Nakamura, Plant Mol. Biol. 41, 825-835, 1999). Bars = 50 μm.

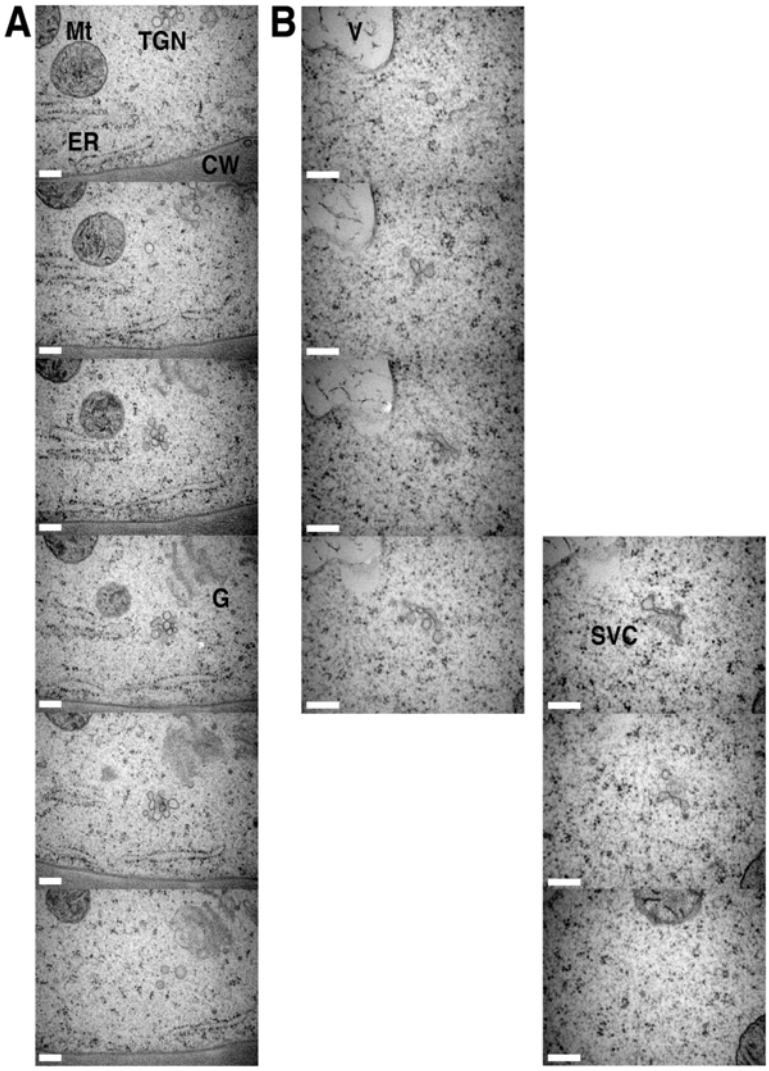

Figure 8. Electron micrographs of ultrathin serial sections of rice culture cells for the comparison of TGN and SVC. Blocks of rice culture cells prepared by HPF/FS were stained with 1% tannic acid in acetone and the sections were observed. (A and B) Ultrathin serial sections of a SVC. Key: G, Golgi apparatus; CW, cell wall; V, vacuole; Mt, mitochondrion. Bars = 200 nm.

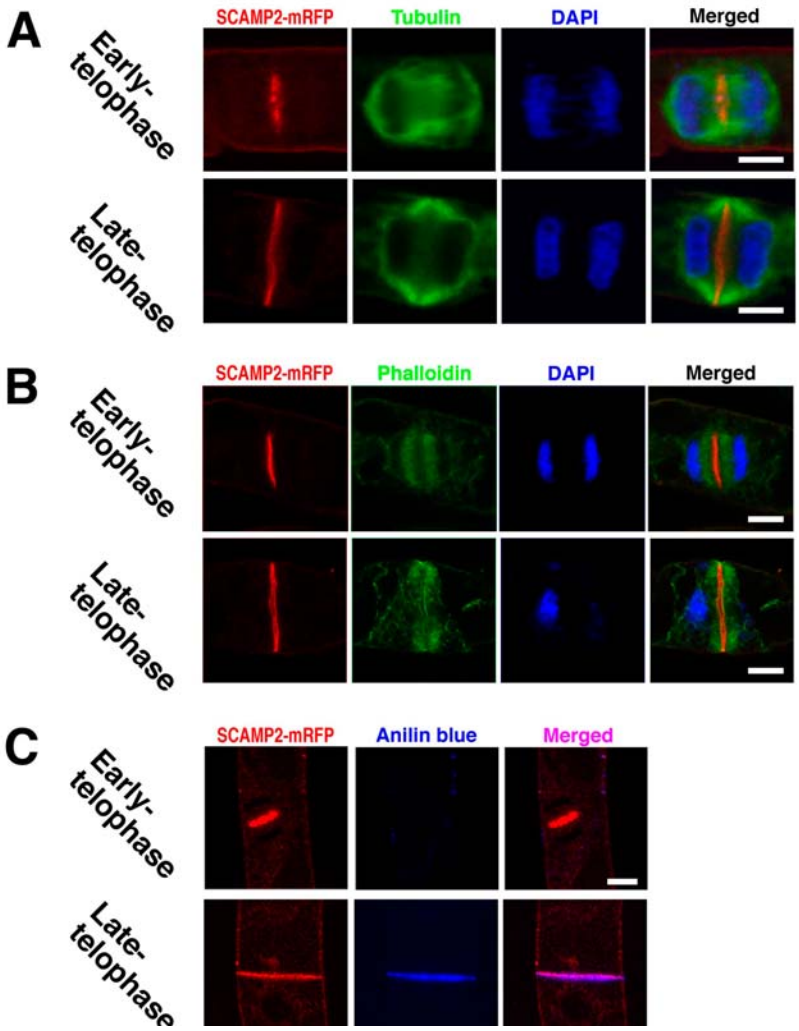

Figure 9. Distribution of SCAMP2 and cytoskeletones in dividing cells. BY-2 cells expressing SCAMP2-mRFP at lag phase of growth were stained with anti-tubulin for microtubles (A), phalloidin for actin (B) and aniline blue for callose or DAPI for DNA. Upper panels show early-telophase cells and lower panels show late-telophase cells. Bars = 20 μm.

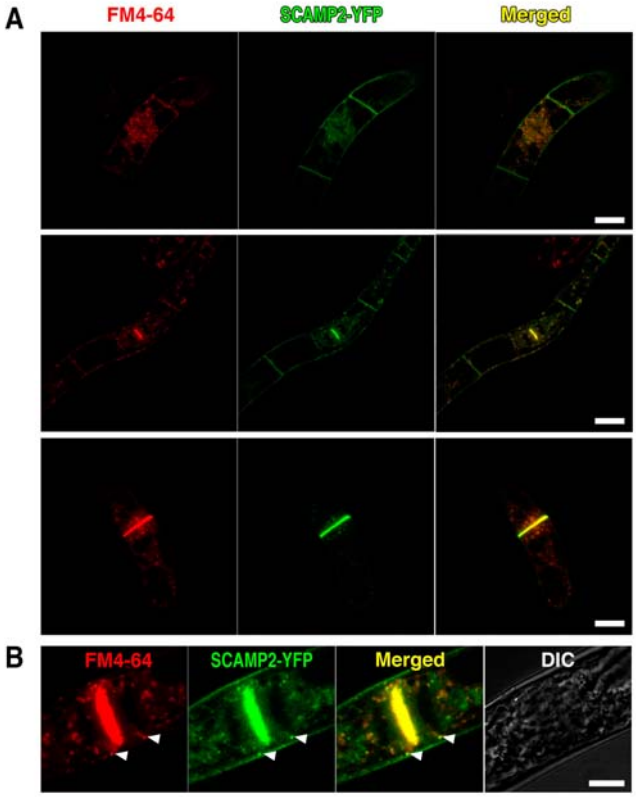

Figure 10. Both SCAMP2 and FM4-64 accumulate in the cell plate. BY-2 cells expressing SCAMP2-YFP at lag phase of growth were labeled with FM4-64 and observed using confocal laser scanning microscope. (A) Subcellular localization of SCAMP2 and FM4-64 during cell division. Top, metaphase; middle, early telophase; bottom, late telophase. Bar = 20 μm. (B) Movement of an SCAMP2 dot containing FM4-64 during cytokinesis. Arrowheads indicate the position of FM4-64 positive SVC. Bar = 10 μm.

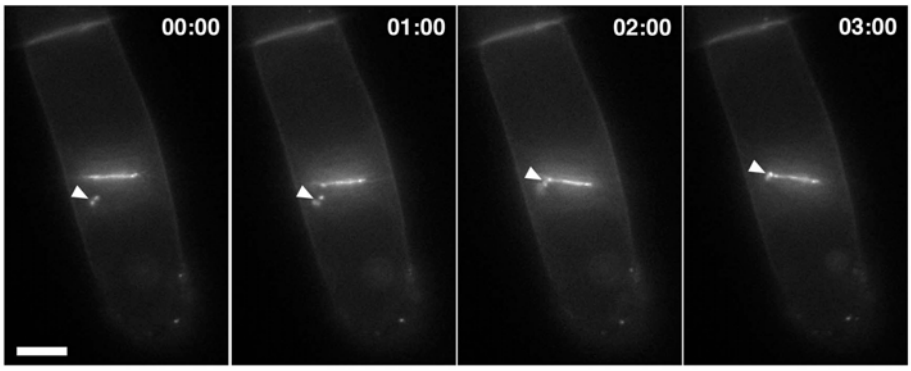

Figure 11. Accumulation of SCAMP2-YFP and transport of SVCs to the cell plate in the presence of BFA. BFA (5 μg/ml, final concentration) was added to culture of BY-2 cells expressing SCAMP2-YFP at lag phase and incubated for 2 h. Image of mitotic cell was collected at indicated time using a fluorescence microscope (Toyooka et al., Autophagy **2,** 96-106). Bar = 20 μm

Supplemental table

Primers used for this study.

Gene	DDBJ Accession No	5' Primer	3' Primer
tobacco SCAMP2	AB295617	CACCATGAGTAGAGGAAACGATCCGAATCCGTTTGAC	CTTGTGCCCTCGAAAAAACATGTACACTTTCTGCAGC
tobacco SYP41	AB295618	CACCATGGCGTCGAGAAATAGAACGATATTGT	TCAGAATAATATCTCCTTCAAAATTAGGAGGACC
tobacco SYP22	AB295619	CACCATGAGTTTTCAAGATCTTGAAGCGGG	TCAGGCTGCAAGTACTATGATCACAATGAG
tobacco RAB11D	AB470307	GGGTCTAGAGGTACCATGGCGAGTGGATATGGGGA	GGGAAGCTTGAATTCACGTCGAATCCTGATATC