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Atsyp51 and Atsyp52 Differently Affect Sorting of Gfpchi and Aleugfp Vacuolar Markers

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Introduction

Many targeting signals in the plant secretory pathway have been identified but mechanisms of protein storage and of tissue- and species-specific accumulation strategies are not yet fully understood [1-3]. Two vacuolar reporters derived by the GFP fusion in frame with the C-terminal Vacuolar Sorting Determinant (VSD) of tobacco chitinase (GFPChi) and the N-terminal fusion in frame with the sequence-specific VSD of the cysteine protease barley Aleurain (AleuGFP) have been used in several occasions to characterize vacuolar sorting and were recently used them to distinguish between the specific function of the two closely related Qc-SNARE SYP51 and SYP52 [4-6]. SYP51 appeared more essential to GFPChi sorting to the central vacuole while SYP52 appeared important for the transport of both markers [6]. We present here a more detailed analysis of SYP5 effect on fluorescent markers than that reported by De Benedictis [6] and further discuss the SYP5s effect on the last step of vacuolar sorting.

Experimental

Gene constructs

The constructs used were previously described: AtSYP51 based 51F and 51T; AtSYP52 based 52F and 52T; GFPChi and AleuGFP [4,6].

Protoplasts transformation

Arabidopsis thaliana (ecotype Colombia) protoplasts were prepared from leaves of 3 weeks old rosettes and transformed as previously described for tobacco protoplasts [4].

Confocal microscopy

Protoplasts were examined with a confocal laser-microscope (LSM 710 Zeiss, ZEN software, GmbH, Germany). GFP were detected in the lambda range 505-530 nm, assigning the green color. Chlorophyll epifluorescence was detected above 650 nm assigning the red color.

Results

After 24 hours of transient expression, both GFPChi and AleuGFP labeled the central vacuole of most of protoplasts but at least four patterns were distinguishable. De Benedictis et al. [6] discussed two main populations of cells, whichever marker was expressed: cells with a labeled central vacuole and cells with a non-fluorescent central vacuole. But the additional structures are very interesting.

When AleuGFP was expressed we could recognize: an empty central vacuole with labeling restricted to bright dots/PVC (Figure 1A); abnormal aggregates of dots and membranous structures (Figure 1B); a homogeneously labeled central vacuole (Figure 1C); homogeneously labeled central vacuole with several peripheral bright dots/PVC (Figure 1D). We counted about 200 cells per population, in 3 independent experiments. The distribution of these four patterns among the control population expressing AleuGFP alone was compared with the distributions of the same patterns in four different populations co-expressing 51F, 51T, 52F or 52T respectively (Figure 1E). The "F" forms correspond to the native (coded by the cDNA) forms of SYP51 and SYP52 and clearly affected the marker distribution. 52F induced the most significant decrease of labeled central vacuoles as those observed in figures 1C and 1D. Also 51F induced a significant alteration, increasing the visibility of PVCs in cells with a labeled central vacuole as seen in figure 1D. The forms 51T and 52T, obtained by the deletion of the trans-membrane C-terminal domain, had no significant effect on patterns distribution.

Also GFPChi was distributed in four recognizable patterns: an empty central vacuole with labeling restricted to ER (Figure 1F); a homogeneously labeled central vacuole (Figure 1G); a homogeneously labeled central vacuole with visible ER and small vacuoles (Figure 1H); fluorescence restricted to small vacuoles and abnormal membranous

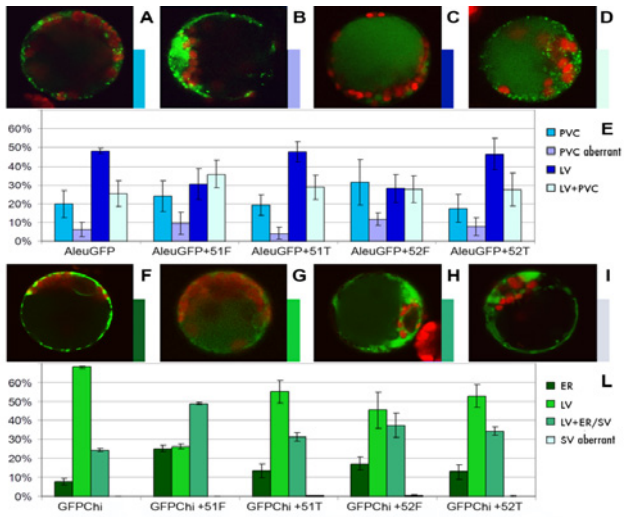

Figure 1: Different fluorescent patterns of AleuGFP (A-D) and GFPChi (F-I) in control conditiona as well as when co-expressed with SYP5 F and T forms. Percentage of patterns observed (E and L) was calculated counting about 200 transformed cells per population in four independent experiments (n=4).

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structures (Figure 1I). In this case 51F was the only co-expressed construct inducing a significant change in patterns distribution (Figure 1L).

Discussion

With our study we support the hypothesis that *At*SYP51 and *At*SYP52, despite their high homology sequence and similar localization on the tonoplast and endosome/TGN [6], do not share the same function in vacuolar sorting. SYP51 is mainly involved in GFPChi sorting to the vacuole; on the contrary, AleuGFP was more affected by SYP52. De Benedictis et al. already reported that the effect appeared to be restricted to the last step of fusion between pre-vacuolar compartments (AleuGFP) or small vacuoles (GFPChi) with the central one [6], similarly to the effect already described by other authors about the Qa-SNARE SYP21 [7].

In this analysis of the data we evidence that GFPChi suffer a very specific effect due to 51F overexpression. This marker can accumulate in small vacuoles, not yet fully characterized, before to reach the central vacuole by heterotypic fusion so the abnormal persistence of small vacuoles and other unusual membranous structures, correlate perfectly with the i-SNARE effect on the very last event of fusion with the tonoplast [4].

We previously speculated that SYP51 reach the tonoplast by default (probably when the SNARE complexes on pre-vacuoles are saturated). AtSYP52 seems to work preventing a different fusion event, equally required for the transport of GFPChi and AleuGFP. It was hypothesized that it was an event of homotypic fusion but observing the four distribution patterns we can also conclude that, if 51F drastically impair a fusion event, 52F simply reduce the efficiency of a different fusion event, possibly with a different mechanism. In fact other data previously reported evidence possible differences in the interference mechanisms due to the two SYP5s [6]. Further investigation is needed to shed light on this sophisticated regulation of membrane traffic.

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