A NOVEL ADAPTOR PROTEIN COMPLEX DRIVES CLATHRIN MEDIATED ENDOCYTOSIS IN PLANTS

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Clathrin-mediated endocytosis (CME) at the plasma membrane functions to recycle membrane and transmembrane proteins thereby altering nutrient uptake, cell polarity and modulation of various signalling pathways. TPLATE is a plant-specific adaptor-like protein hypothesized to play a role in CME based on its interaction with clathrin and its sensitivity to drugs affecting this process. A proteomic and cell biological approach now provides further insight into the function of TPLATE in plant endocytosis at the PM. Tandem Affinity Purifications (TAP) using TPLATE as bait identified TPLATE as part of an eight-subunit complex with seven novel interactors which all localize at the PM in Arabidopsis root cells. These interactors have putative functions in endocytosis based on the presence of specific domains and/or structural homology with known adaptor proteins. Reverse TAP and co-immunoprecipitation experiments using the identified interactors as baits confirmed the initial interactions and furthermore connected the TPLATE complex with several members of the dynamin protein family, the clathrin scaffolding proteins, ENTH/ANTH domain containing proteins and the AP-2 endocytic adaptor complex. Induced silencing of the TPLATE complex subunit AtTFL severely inhibited the uptake of the styryl dye FM4-64 in Arabidopsis root cells and affected the internalization of several cargo proteins, suggesting a general effect on PM endocytosis. TPLATE, AtTFL and an AP-2 complex subunit localize in dynamic foci at the PM with comparable lifetimes and AtTFL recruitment to the PM precedes dynamin and clathrin recruitment. Simultaneous imaging of TPLATE and AP-2 complex subunit markers in Arabidopsis roots showed that the amount of dynamic TPLATE and AtTFL foci exceeded the amount of AP-2 foci at the PM. Furthermore, the majority of AP-2 foci co-localized with the TPLATE complex markers. This observation strongly suggests a common function for both complexes in endocytosis and explains the strong reduction in FM uptake upon inhibiting TPLATE complex function in the presence of endogenous levels of AP-2. The joint function of the TPLATE complex and AP-2 is underlined by the fact that the TPLATE complex subunit AtTFL is distantly related to the muniscin proteins which act as endocytic hubs and are recruited prior to AP-2 and clathrin.

In conclusion, we have identified a highly novel, multi-subunit plant-specific complex which links AP-2, dynamins and the clathrin scaffolding proteins and which plays a major role in plant plasma membrane vesicle internalization.