Functional characterisation of the yeast tumour suppressor homologue Sro7p

ABSTRACT

Correct targeting of newly synthesized proteins to appropriate domains of the cell membrane is crucial to cellular architecture, polarity and function, making it no surprise that many proteins of the secretory machinery are conserved throughout evolution. This work presents a functional characterisation of the *Saccharomyces cerevisiae* cell polarity protein and tumour suppressor homologue, Sro7p. This protein, and its paralogue Sro77p, belong to the Lgl-family of WD-40 repeat proteins that are conserved from yeast to human. Deletion of *Lgl* genes produces different phenotypes that all seem to share the common denominator of defective targeting of critical cell surface proteins. Yeast cells lacking *SRO7* become sensitive to NaCl and we here show that this defect is due to mis-targeting of the sodium transporter Ena1p. In *sro7* mutants Ena1p becomes routed to the vacuole for degradation via the multi-vesicular body (MVB) pathway, instead of being properly expressed at the cell surface. Isolation and analysis of post-Golgi secretory vesicles showed a defective sorting of Ena1p into these vesicles from *sro7* mutants, implying mis-sorting in late Golgi or early endosomes. The diversion of Ena1p into the MVB pathway further required ubiquitylation by the ubiquitin ligase Rsp5p. Isolation of suppressors of the *sro7* salt sensitivity identified two genes of unknown function, *RSN1* encoding a trans-membrane protein, and *ART5* (*RSN2*), encoding an arrestin-like protein. Deletion of either gene in *sro7* mutants re-establishes salt tolerance and retargets Ena1p to the cell surface. Previous proteomic studies have shown that Art5p interacts with Rsp5p and we showed that deletion of *ART5* in *sro7* mutants inhibits ubiquitylation of Ena1p. Our data are consistent with Art5p being a selective adaptor protein that helps Rsp5p recruiting Ena1p for ubiquitylation. To identify further candidate proteins for mis-sorting in salt stressed *sro7* mutants we performed the first proteomic analysis of purified yeast post-Golgi vesicles (PGVs), using quantitative proteomics techniques. By this analysis we could identify 107 genuine vesicle residents in control yeast cells, including a number of cargo proteins not previously identified in PGVs. Vesicles derived from *sro7* mutants contained essentially the same list of proteins but were depleted of a subset of proteins, thus being candidates for mis-routing. The present study finally analysed possible Lgl conservation in plants by characterising two *Arabidopsis thaliana* Lgl homologues. Sequence based modelling showed that both proteins can fold into the twin β-propellers shown by the published Sro7p crystal structure. However, only one of the proteins, AtLGL1, could partially substitute for the yeast Sro7/77 proteins. The other, AtLGL2 showed structural similarities with tomosyn that is known to regulate vesicle fusion in mammals. Homozygous T-DNA insertion mutants in *A. thaliana* exhibited defects in lateral root formation, a phenotype associated with changed cell- and tissue polarity.

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