Functional studies of the Sro proteins, yeast homologues of the *Drosophila lethal(2) giant larvae* tumour suppressor

Ingrid Wadskog

Department of Cell and Molecular Biology, Microbiology, Göteborg University
Medicinaregatan 9, Box 462, SE-405 30 Göteborg, Sweden

ABSTRACT

The *SRO7* encoded tumour suppressor homologue of *Saccharomyces cerevisiae* belongs to a family of WD40 repeat proteins with homologues in species as diverse as yeast, *Drosophila*, mouse and man. The founding member of this family of proteins is the *Drosophila lethal(2) giant larvae* (*L(2)GL*) tumour suppressor.

In yeast, deletion of the *SRO7* gene leads to increased NaCl sensitivity and accumulation of sodium to toxic intracellular levels. Unlike many other salt sensitive mutants, the *sro7* mutant displays no reduction in tolerance to high osmolarity in general, but specifically only to NaCl. Immunolocalization of Sro7p revealed a cytoplasmic distribution and cell fractionation studies showed that a significant portion of Sro7p was loosely associated with a sedimentable, membrane rich fraction. Expression of a *Drosophila l(2)gl* cDNA in strain lacking *SRO7* and its homologue *SRO77*, partially restored the Na^+ tolerance of the cells, indicating a functional relationship between the Sro proteins and the tumor suppressor protein. The NaCl sensitivity of the *sro7* mutant is due to defective sorting of Ena1p, the main sodium pump in yeast. On exposure of *sro7* mutants to NaCl stress the *ENA1* gene is induced as in wild type cells but the produced protein fails to be targeted to the cell surface. Instead, this Na^+ extruding ATPase is transported to and degraded in the vacuole. The *SRO7* gene also exhibits genetic interaction with temperature sensitive *sec* mutants defective for components of the post-Golgi exocytic machinery, strengthening observations from other researchers on a role for Sro7p in late exocytosis. Search for suppressors of Sro7p deficiency identified *RSN1*, a novel gene encoding a membrane protein. Over-expression of *RSN1* stabilizes Ena1p and restores NaCl tolerance consistent with correct re-targeting of Ena1p to the cell surface. The phenotype of *sro7* mutants is also suppressed by external addition of Ca^{2+}, whereas deletion of genes encoding plasma membrane Ca^{2+} channels aggravates the NaCl sensitivity of *sro7* mutants.

The *sro77* single mutant exhibits no observable growth phenotype, but deletion of *SRO77* in an *sro7* mutant background results in hypersensitivity to salt in addition to more general sensitivity to osmotic stress. Sro77p is localized to internal membranes but is redistributed from inner membranes to the cell periphery following exposure of cells to salt stress. Sro77p shows two hybrid interactions with Yca1p, a yeast metacaspase which induces apoptotic-like cell death in yeast cells during stressed conditions. The Yca1p dependent loss of viability disappears in *sro77* mutants, even though there are signs of typical apoptotic morphological changes, which might indicate the existence of an alternative Yca1p independent apoptotic-like cell death response in yeast.

Key words: Ion homeostasis, salt stress, exocytosis, protein targeting, apoptosis, *l(2)gl* tumour suppressor, *S. cerevisiae*, *SOP1, SRO7, SOP2, SRO77, ENA1*

Göteborg 2003 ISBN 91-628-5737-1