THE ROLE OF DYNAMIN-RELATED PROTEINS IN VACUOLE BIOGENESIS IN FISSION YEAST (*Schizosaccharomyces pombe*)

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ABSTRACT

Dynamins are GTPases concerned with membrane tubulation and scission (Praefcke and McMahon, 2004). In the fission yeast, *Schizosaccharomyces pombe,* the dynamin-related proteins (DRPs) Vps1 and Dnm1 act redundantly in peroxisome biogenesis (Jourdain *et al.*, 2008) but nothing is known about their other cellular roles. Fission yeast cells contain ~20 small, spherical vacuoles that undergo fission or fusion in response to environmental signals (Bone *et al.*, 1998). *S. pombe* cells lacking Vps1 had smaller vacuoles with reduced capacity for fusion in response to hypotonic stress but enhanced fission in response to hypertonic conditions. Unlike wild type, *vps1*Δ vacuoles showed no change in diameter in response to temperature stress. Vps1-Cgfp localised to the vacuolar membrane both in living cells and in isolated vacuoles. *vps1*Δ cells showed close to wild type levels of vacuole protein processing and normal actin organisation and endocytosis. Overexpression of Vps1 caused a global transformation of vacuoles from spherical to tubular. Spherical vacuoles were restored by repression of *vps1* expression or by induction of vacuole fusion. Tubulation was blocked in the presence of GTPγS and in a *vps1* mutant that lacked the entire GTPase domain. Vacuole tubulation was more extensive in the absence of a second DRP, Dnm1. The absence of Dnm1 abolished the hyper fission phenotype of *vps1*Δ*,* whereas overexpression of Dnm1 induced vacuole fission. These results are consistent with a model of vacuole fission in which Vps1 creates a tubule of an appropriate diameter for subsequent scission by another DRP. Preliminary evidence suggests that Dnm1 serves the latter role.

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ABBREVIATIONS

CDCFDA: 5[6]-Carboxy-2',7'-Dichlorofluorescein Diacetate

CPY: Carboxypeptidase Y

DAPI: 4', 6-diamidino-2-phenylindole

DIC: Differential interference contrast

DMSO: Dimethylsulfoxide

DNA: Deoxyribonucleic acid

dNTP: Deoxyribonucleotide triphosphate

DRP: Dynamin-related protein

EDTA: Ethylenediaminetetraacetic acid

EMM: Edinburgh minimal medium

GED: GTPase effector domain

GFP: Green fluorescent protein

GTP: Guanosine triphosphate

Lat-A: Latrunculin A

LB: Luria-Bertani medium

MSA: Minimal supporting agar

MT: Microtubule

nmt1: No message in thiamine

OD: Optical density

PBS: Phosphate buffered saline

PCR: Polymerase chain reaction

PEG: Polyethylene glycol

PH: Pleckstrin homology domain

PRD: Proline-rich domain

SH3: Src 3 homology

TBZ: Thiabendazole

Vps: Vacuolar protein sorting

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