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Imp2, the PSTPIP homolog in fission yeast, affects sensitivity to the immunosuppressant FK506 and membrane trafficking in fission yeast

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ABSTRACT

Cytokinesis is a highly ordered process that divides one cell into two cells, which is functionally linked to the dynamic remodeling of the plasma membrane coordinately with various events such as membrane trafficking. Calcineurin is a highly conserved serine/threonine protein phosphatase, which regulates multiple biological functions, such as membrane trafficking and cytokinesis. Here, we isolated *imp2-c3*, a mutant allele of the *imp2*$^+$ gene, encoding a homolog of the mouse PSTPIP1 (proline-serine-threonine phosphatase interacting protein 1), using a genetic screen for mutations that are synthetically lethal with calcineurin deletion in fission yeast. The *imp2-c3* mutants showed a defect in cytokinesis with multi-septated phenotypes, which was further enhanced upon treatment with the calcineurin inhibitor FK506. Notably, electron micrographs revealed that the *imp2-c3* mutant cells accumulated aberrant multi-lamella Golgi structures and putative post-Golgi secretory vesicles, and exhibited fragmented vacuoles in addition to thickened septa. Consistently, *imp2-c3* mutants showed a reduced secretion of acid phosphatase and defects in vacuole fusion. The *imp2-c3* mutant cells exhibited a weakened cell wall, similar to the membrane trafficking mutants identified in the same genetic screen such as *ypt3-i5*. These findings implicate the PSTPIP1 homolog Imp2 in Golgi/vacuole function, thereby affecting various cellular processes, including cytokinesis and cell integrity.

1. Introduction

Cytokinesis is the final step in the cell cycle and is the process that physically separates a mother cell into two daughter cells [1,2]. In order to successfully complete this process, cells have to dynamically organize a multitude of proteins, such as components of the actomyosin contractile ring and of a physical membrane barrier. Recently, the functional link between cytokinesis and other cellular events regulating membrane dynamics, especially membrane trafficking has received much attention [3,4].

We have been using the fission yeast *Schizosaccharomyces pombe* (*S. pombe*) as a model system for studying the cellular functions and the regulatory mechanism of calcineurin, which is an evolutionarily conserved Ca^{2+}/calmodulin-dependent serine/threonine protein phosphatase and an important regulator of Ca^{2+} signaling. Ppb1, the *S. pombe* calcineurin, regulates multiple biological processes, such as cytokinesis, membrane trafficking, ion homeostasis and cell wall integrity [5–7]. The immunosuppressive drug FK506 blocks the activation of calcineurin through the formation of complexes with immunophilins, and this drug has been frequently used to delineate various cellular functions mediated by calcineurin [8,9]. In order to identify genes that share an essential function for growth with calcineurin, we performed a chemical genetic screen to isolate mutants that exhibit sensitivity to FK506 [10,11], and identified various membrane trafficking genes, including *ypt3*$^+$ (encoding a Rab 11 homolog) [12], *ryh1*$^+$ (encoding a Rab 6 homolog) [13], *gdi1*$^+$ (encoding a Rab GDI) [14], *apm1*$^+$ (μ1 subunit of the Adaptor–Protein complex 1) [15], and *sip1*$^+$ (AP-1 accessory protein) [16]. Notably, these membrane trafficking mutants displayed defects in cytokinesis besides those in membrane trafficking. Here, we isolated the *imp2-c3*, a mutant allele of the

Abbreviations: PSTPIP1, proline-serine-threonine phosphatase interacting protein 1; YPD, yeast extract-peptone-dextrose; EMM, Edinburgh minimal medium; YES, yeast extract with supplements; GFP, green fluorescent protein; YFP, yellow fluorescent protein; ORF, open reading frame.

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Table 1
S. pombe strains used in this study.

Strain	Genotype	Reference
HM123	*h⁻ leu1-32*	Our stock
HM528	*h⁺ his2*	Our stock
KP201	*h⁻ leu1-32 cis3-1/imp2-c3*	This study
KP630	*h⁻ leu1-32 ura4-D18 apm1::ura4⁺*	[15]
KP208	*h⁻ leu1-32 ura4-D18 pmk1::ura4⁺*	[20]

imp2$^+$ gene, encoding a homolog of the mouse PSTPIP1 [17], that is structurally similar to the Pombe Cdc15 homology (PCH) Proteins, as a new addition to the genes which affect sensitivity to FK506 upon mutation. Imp2 was previously isolated as a component of the actin contractile ring and is structurally similar to the *S. pombe* Cdc15, a founding member of the F-BAR (Fes/Cip4 homology-Bin/Amphisin/Rvsp) domain protein, involved in actin ring organization [18]. In this study, we have characterized a novel role for Imp2 in membrane trafficking and showed that Imp2 was involved in Golgi/vacuole function, suggesting a functional interplay between actomyosin ring-mediated cytokinesis and membrane trafficking events.

2. Materials and methods

2.1. Strains, media, and genetic and molecular biology methods

S. pombe (*S. pombe*) strains used in this study are listed in Table 1. The complete medium (yeast extract-peptone-dextrose; YPD) and

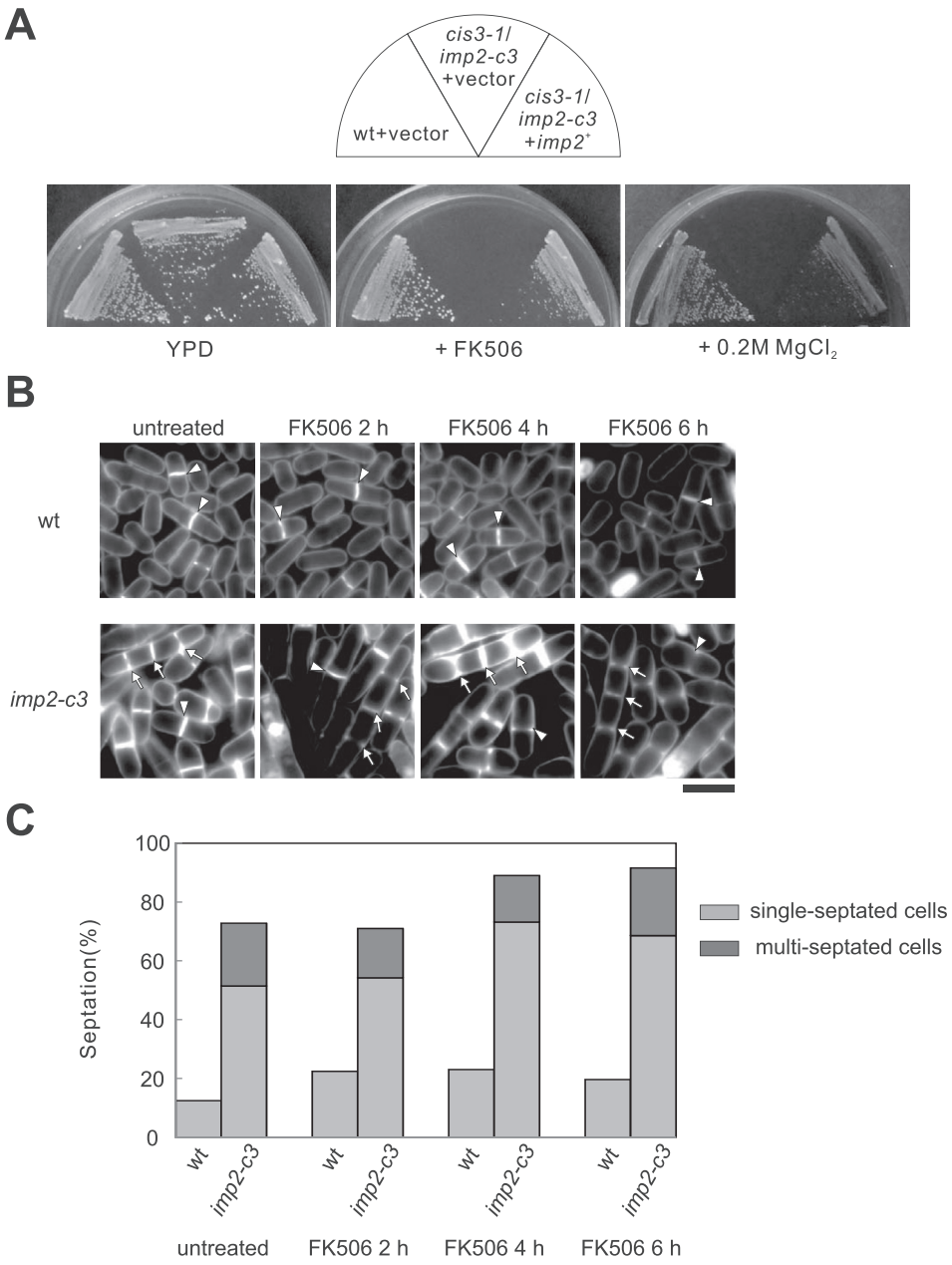

Fig. 1. Mutation in the *cis3*$^+$/*imp2*$^+$ gene causes immunosuppressant- and chloride-sensitive phenotypes and defects in cytokinesis. (A) The immunosuppressant and chloride sensitivities of the *cis3-1/imp2-c3* mutant cells are shown. Cells transformed with the multi-copy vector pDB248 or the vector containing the *cis3*$^+$ gene were streaked on to the plates as indicated, and then incubated for 3 days at 27°C. (B) Fluorescence micrographs of wild-type (wt) and *imp2-c3* mutant cells stained with Calcofluor. Cells were incubated in YPD or YPD plus FK506 at 27°C and then stained with Calcofluor to visualize cell wall and septum. Bar, 10 μm. (C) Percentage of cells forming a division septum at each time point in wild-type (wt) and *imp2* mutant cells after the addition of FK506 at 27 °C. Values are the average of 5 times with 100 cells counted for each time point.