

CHARACTERIZATION OF THE ROLES OF PHOSPHOLIPASE B1 AND THE CYCLIC-AMP (cAMP)-PROTEIN KINASE A (PKA) PATHWAY IN NUTRITIONAL AND OSMOTIC STRESS RESPONSE IN *SCHIZOSACCHAROMYCES POMBE*

Dissertation Defense

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Physiological stress is a reality faced by all cells. Even though these stresses are often disruptive of normal cellular function, cells must adapt to the stressor or perish. This study utilizes the model organism *Schizosaccharomyces pombe*, commonly known as fission yeast, to study a novel stress response pathway for adaptation to nutrient deprivation and hyperosmotic stress involving phospholipase B1 (Plb1) and components of the cyclic-AMP (cAMP)-protein kinase A (PKA) pathway. A previous study showed that deletion of *plb1* confers sensitivity to hyperosmotic stress in the form of potassium chloride (KCl). This phenotype could be rescued by over-expression of components of the cAMP-PKA pathway, and likewise, deletion of these components also lead to KCl-sensitivity phenotypes. This study shows that deletion of these genes also correlates with increased fragmentation of mitochondria under conditions of hyperosmotic stress. Interestingly, addition of rotenone or loss of mitochondrial PE synthesis – conditions that have previously been associated with increased mitochondrial fragmentation – exacerbated phenotypes of the *plb1*Δ mutant. Mitochondrial fragmentation was also accompanied by increased mitophagy in KCl-treated *plb1*Δ cells. Cell cycle arrest in G2/M and cytokinesis was observed in KCl-treated cells in which the gene encoding the PKA catalytic subunit *pka1* had been deleted. These phenotypes – mitochondrial fragmentation and failure to complete cytokinesis – may be due to dysregulation of PS and PE synthesis and cellular distribution. In addition to participating in hyperosmotic stress response, Plb1 and the cAMP-PKA pathway cooperate to respond to nutrient deprivation. The function of the cAMP-PKA pathway in glucose sensing is well established in yeast. Previous studies have suggested a role for phospholipases B (PLBs) in nutrient scavenging, though a limited number of studies have examined how nutrient content affects secretion of these PLBs. In this study, we have found that the secretion of Plb1 is increased in nutrient-poor media. In particular, glucose content greatly affects Plb1 secretion, since incubation in low-glucose media highly increases secretion whereas addition of glucose reduces secretion. Since the cAMP-PKA pathway is responsible for detecting glucose in the media, we predicted that deletion of *pka1* would lead to increased Plb1 secretion. However, Plb1 secretion was not appreciably altered in a *pka1*Δ mutant. Interestingly, *pka1*Δ cells did have increased levels of Plb1 protein, and the localization of Plb1 in these cells resembled that seen in glucose-starved cells, suggesting that Plb1 and the cAMP-PKA pathway may interact with regards to glucose sensing.