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Elucidation of Mechanism of Action of Osmotin, a Plant Antifungal Protein, Using Yeast Genetic System

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Among families of antimicrobial proteins associated with plant defense are the Pathogenesis Related proteins of family 5 (PR-5) that are structurally related to the sweet tasting protein thaumatin (Veronese et al., 2003). Osmotin is an antifungal tobacco PR-5 protein. It induces programmed cell death in *Saccharomyces cerevisiae* by signaling suppression of cellular stress responses via RAS2/cAMP (Narasimhan et al., 2001).

Most of the PR proteins, including osmotin, have specific wide-spectrum antifungal activities suggesting that target recognition maybe determined by their interaction with pathogen cell surface components. Specific fungal cell wall components enhance or suppress osmotin antifungal activity (Narasimhan et al., 2003; Veronese et al., 2003). In *S. cerevisiae*, the PIR family of cell wall glycoproteins are osmotin-resistance determinants (Yun et al., 1997), whereas phosphomannans of cell wall glycoproteins have been shown to increase osmotin toxicity, probably by serving as docking structures to facilitate osmotin diffusion across the cell wall (Ibeas et al., 2000). Genetic analyses have revealed that SSD1, a protein that affects cell wall morphogenesis and deposition of PIR proteins, is a critical determinant of resistance to osmotin (Ibeas et al., 2001). Unidentified changes in the yeast cell wall that enhance toxicity are induced by osmotin via activation of a mitogen-activated protein kinase cascade (Yun et al., 1998).

Specific interactions at the plasma membrane are also required for osmotin antifungal activity, because yeast spheroplasts that are susceptible to tobacco osmotin can be resistant to PR-5 proteins from other plant species (Yun et al , 1997). We report here the isolation of a plasma membrane determinant of osmotin sensitivity that was identified by its ability to confer an osmotin supersensitive phenotype to yeast cells upon gene overexpression. The gene, *ORE20/PHO36*, encodes a seven transmembrane domain receptor-like protein, homologous to mammalian adiponectin receptors, and is involved in yeast lipid and phosphate metabolism (Karpichev et al., 2002; Yamauchi et al., 2003a). Adiponectin is an antidiabetic and antiatherosclerotic protein hormone in mammals that conditions sensing of energy status, fatty acid oxidation and glucose transport upon interaction with adiponectin

receptors (Diez and Iglesias, 2003; Yamauchi et al., 2003a,b). The nature of the PHO36 ligand is unknown. We report that ORE20/PHO36 specifically binds osmotin at the plasma membrane and controls osmotin-induced cell death via a *RAS2* signaling pathway. We also show that osmotin can induce AMP kinase phosphorylation in mammalian C2C12 myocytes via adiponectin receptors.

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