Neurofibromin Homologs Ira1 and Ira2 Affect Glycerophosphoinositol Production and Transport in Saccharomyces cerevisiae

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Saccharomyces cerevisiae produces extracellular glycerophosphoinositol through phospholipase-mediated turnover of phosphatidylinositol and transports glycerophosphoinositol into the cell upon nutrient limitation. A screening identified the RAS GTPase-activating proteins Ira1 and Ira2 as required for utilization of glycerophosphoinositol as the sole phosphate source, but the RAS/cyclic AMP pathway does not appear to be involved in the growth phenotype. Ira1 and Ira2 affect both the production and transport of glycerophosphoinositol.

Membrane phospholipids are continually synthesized and degraded as cells grow and respond to environmental conditions. A major pathway of phosphatidylinositol (PI) turnover in Saccharomyces cerevisiae is its decylation to produce extracellular glycerophosphoinositol (GroPIns) (3). Plb3, an enzyme with phospholipase B (PLB)/lysophospholipase activity, is thought to be primarily responsible for the production of extracellular GroPIns, with Plb1 playing a lesser role (11, 12, 13). GroPIns is transported into the cell by the Git1 permease (17). GIT1 expression is upregulated by phosphate limitation and inositol limitation. In fact, GroPIns can act as the cell’s sole source of both inositol (17) and phosphate (1).

A screening for gene products involved in the process by which GroPIns enters the cellular metabolism identified Ira1 and Ira2, yeast homologs of the mammalian protein neurofibromin. Alterations in NF1, the gene encoding neurofibromin, are associated with the pathogenesis of neurofibromatosis type 1, an autosomal dominant genetic disease (4, 5, 25). Ira1 and Ira2 affect both the production and transport of glycerophosphoinositol.

Identification of IRA4 genes as affecting GroPIns metabolism. To identify genes involved in the metabolic process by which GroPIns is used as a phosphate source, we screened the MATα viable yeast knockout collection (Research Genetics) for strains displaying compromised growth when GroPIns rather than low Pi was supplied as the phosphate source. Synthetic complete medium, high-Pi (10 mM KH2PO4) medium, low-Pi (0.2 mM KH2PO4) medium, and GroPIns+ (70 μM) medium were made as described previously (2). Mutants were transferred by hand pinner from a master plate to a 96-well plate containing low-Pi medium and allowed to grow at 30°C for 3 days. From low-Pi medium, cell inocula were transferred to plates containing GroPIns+ medium. Growth was monitored at 395 nm after 4 days of incubation at 30°C. The ratio of absorbance in low-Pi to absorbance in GroPIns+ medium was determined. This screening was performed twice. Mutants with a value of 3 or greater for the ratio of absorbance in low-Pi medium to absorbance in GroPIns+ medium were subjected to a second screening by the monitoring of their growth in test tubes. As expected, the GIT1 gene was required for growth, and no other mutant displayed a complete growth abatement phenotype (Fig. 1). In particular, we did not identify a gene likely to encode a glycerophosphodiesterase responsible for GroPIns catabolism. A likely explanation for this result is that multiple gene products are involved in the mechanism(s) by which GroPIns enters cellular metabolism. However, a number of strains exhibited a slow-growth phenotype, one of which was the ira1Δ mutant (Fig. 1A). To confirm the validity of this result, we analyzed growth on GroPIns in strains of the unrelated Σ1278b background (gifts from M. Cardenas, Duke University), and found that not only the ira1Delta mutant but also the ira1Δ ira2Δ double mutant (Table 1) exhibited greatly reduced growth on GroPIns (Fig. 1B). The ira1Δ mutant was not present in the deletion set screened initially.

To probe the role of the RAS/cAMP pathway in GroPIns metabolism, we analyzed other strains bearing alterations in the pathway. Interestingly, a pde2Δ mutant that, like the ira1Δ mutant, exhibits increased cAMP levels (15) was not defective for growth on GroPIns. Similarly, a strain bearing a plasmid-borne hyperactive allele of RAS2, RAS2-V19 (a gift from P. Herman, Ohio State University) (9, 10, 22), was not defective for growth on GroPIns (Fig. 1A). Thus, upregulation of the RAS/cAMP pathway may not be the primary reason for the growth defect...
Cells were transferred to low-P$_i$ or GroPIns/H$_9018$

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V19 plasmid pPHY453, containing the hyperactive Wild-type cells transformed with empty vector pRS415 (vector) or 0.005). Absorbance was recorded after 48 h of growth at 30°C.

Using GroPIns as a phosphate source. Strains in the BY4742 (A) or experiments.

Standard errors of the means of results of at least two independent strains in the BY4742 background 1278b background were grown overnight in low-P$_i$ medium. A

ras2 a MAT MLY187 ras1 MAT MLY41 WT, wild type.

ras1 MAT MLY186 ira1 MAT THY336 ira2 MAT THY337 ira1 MAT JPV469 git1 MAT JPV597 pde2 MAT JPV574 ira2 MAT

| Strain     | Relevant genotype | Genotype                  | Source or reference |
|------------|------------------|---------------------------|---------------------|
| BY4742 background |                 |                           |                     |
| JPV203     | WT               | MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 | Research Genetics   |
| JPV597     | pde2Δ            | MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pde2::kanMX | Research Genetics |
| JPV212     | git1Δ            | MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 git1::kanMX | Research Genetics |
| JPV574     | ira2Δ            | MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ira2::kanMX | Research Genetics |

* WT, wild type.
involves the RAS/cAMP pathway or other, less defined functions involved in the cell’s ability to utilize GroPIns as a phosphate source. Ira1 and Ira2 are large proteins, each over 3,000 amino acids. The RAS GAP-related domain of each protein is contained within a few hundred amino acids, and C-terminal regions of approximately 200 amino acids bind to Gpb1 or Gpb2 and are important for protein stability (8). In addition, yeast Ira1 and Ira2 proteins and the human neurofibromin type 1 protein all contain bipartite phospholipid binding modules consisting of a Sec14 homologous segment and a pleckstrin homology-like domain (6). Both in vitro phospholipid binding and lipid exchange activity have been documented for neurofibromin (24), suggesting that Ira1 and Ira2 may have similar activities. Indeed, Ira1 appears to play a membrane-anchoring role for adenylate cyclase in addition to its role as a RAS GAP (14). It is tempting to speculate that Ira1 or Ira2 might also play a role in the membrane association or activation of an enzyme or enzymes responsible for the metabolism of internalized GroPIns. A test of that hypothesis awaits the identification of the gene products involved in the process by which GroPIns enters cellular metabolism.

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