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Negative Regulation of Phosphatidylinositol 4,5-Bisphosphate Levels by the INP51-associated Proteins TAX4 and IRS4*

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Phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) is an important second messenger in signaling pathways in organisms ranging from yeast to mammals, but the regulation of $PI(4,5)P_2$ levels remains unclear. Here we present evidence that $PI(4,5)P_2$ levels in *Saccharomyces cerevisiae* are down-regulated by the homologous and functionally redundant proteins TAX4 and IRS4. The EPS15 homology domain-containing proteins TAX4 and IRS4 bind and activate the $PI(4,5)P_2$ 5-phosphatase INP51 via an Asn-Pro-Phe motif in INP51. Furthermore, the INP51-TAX4/IRS4 complex negatively regulates the cell integrity pathway. Thus, TAX4 and IRS4 are novel regulators of $PI(4,5)P_2$ and $PI(4,5)P_2$-dependent signaling. The interaction between TAX4/IRS4 and INP51 is analogous to the association of EPS15 with the 5-phosphatase synaptojanin 1 in mammalian cells, suggesting that EPS15 is an activator of synaptojanin 1.

Phosphoinositides are conserved from yeast to mammals as second messengers. They mediate the signal transduction involved in many different cellular processes and thereby comprise a complex signaling system (1). One of the more thoroughly studied phosphoinositides is phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$).[1] $PI(4,5)P_2$ was originally shown to be cleaved by phospholipase C to generate the two second messengers inositol-1,4,5-phosphate and diacylglycerol (2). More recently, uncleaved $PI(4,5)P_2$ has also been shown to act as a second messenger (3). Uncleaved $PI(4,5)P_2$ acts by binding conserved domains in target proteins, such as the pleckstrin homology domain (4–6). In mammalian cells, $PI(4,5)P_2$ signaling is important in regulating vesicular transport, the organization of the actin cytoskeleton, and the regulation of ion channels (7–9). In the model organism *Saccharomyces cerevisiae*, $PI(4,5)P_2$ is also essential and is produced by MSS4, the sole phosphatidylinositol 4-phosphate 5-kinase in yeast (10, 11).

One target of $PI(4,5)P_2$ signaling in *S. cerevisiae* is the GDP/GTP exchange factor ROM2. ROM2 contains a pleckstrin homology domain that binds $PI(4,5)P_2$ in the plasma membrane, thus mediating the localization of ROM2 (12, 13). ROM2 has two homologs, ROM1 and TUS1, which also harbor putative pleckstrin homology domains (12, 14). ROM2 and its homologs are components of the cell integrity pathway necessary for the cellular response to cell wall damage induced by stress such as heat shock (14, 15). The response to cell wall damage includes a reorganization of the actin cytoskeleton and an up-regulation of cell wall synthesis (16–18). Activation of the cell integrity pathway is mediated by its most upstream component, the cell wall sensor WSC1, which signals to ROM2 (19, 20). Subsequently, ROM2 stimulates the exchange of GDP to GTP in the Rho GTPase RHO1, thereby activating RHO1 (12). The GTPase-activating protein (GAP) SAC7 converts RHO1-GTP into the inactive GDP-bound form (21). RHO1 in its active (GTP-bound) form has several effectors such as the glucan synthase FKS1 and the PKC1-MAP kinase cascade (22–25). Activation of the MAP kinase MPK1 induces transcription of genes involved in cell wall biosynthesis such as *CHS3* encoding chitin synthase III, which is important for cell wall repair (16, 18, 26).

The ROM2-MAP kinase-signaling pathway is also an effector branch of the TOR signaling network. The TOR signaling network contains two structurally and functionally distinct complexes, TOR complex 1 (TORC1) and 2 (TORC2) (27). TORC2 regulates the polarization of the actin cytoskeleton, and this regulation is via the ROM2-MAP kinase pathway (21, 27–29). Interestingly, the sole phosphatidylinositol 4-phosphate 5-kinase MSS4 is required for the polarization of the actin cytoskeleton, and overexpression of MSS4 restores growth in conditional TORC2 mutants (10, 11, 27, 30). However, the link between $PI(4,5)P_2$ and TORC2 in the regulation of ROM2 and, ultimately, the actin cytoskeleton is not understood.

The phosphoinositide 5-phosphatases INP51, INP52, and INP53 (also known as SJL1–3) mediate the turnover of $PI(4,5)P_2$ and are implicated in several cellular processes such as cell wall biosynthesis and the organization of the actin cytoskeleton (31, 32). Heat shock has been observed to induce an increase in $PI(4,5)P_2$ levels, suggesting the existence of a mechanism regulating the levels of this phosphoinositide (10). However, the nature of this regulatory mechanism and, more specifically, the possible regulation of the phosphoinositide 5-phosphatases are not well understood. In mammalian cells, the INP family orthologue synaptojanin 1 interacts with several proteins involved in endocytosis in nerve terminals (33–35). To investigate the regulation of $PI(4,5)P_2$ turnover in *S. cerevisiae*, we focused on INP51, INP52, and INP53 and their role in $PI(4,5)P_2$ signaling linked to the cell integrity pathway and the TORC2 pathway.

Here we present evidence for two novel positive regulators of the $PI(4,5)P_2$ 5-phosphatase INP51, the redundant EPS15 homology (EH) domain-containing proteins TAX4 and IRS4. INP51 associates with TAX4 or IRS4 to form a complex impor-

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[1] The abbreviations used are: $PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; EH, EPS115 homology; GAP, GTPase-activating protein; HA, hemagglutinin A; MAP, mitogen-activated protein; TOR, target of rapamycin; TORC, TOR complex; TRITC, tetramethylrhodamine isothiocyanate; ts, temperature sensitive.

TABLE I
Strains used in this study

Strain	Genotype
TB50**a**	*MAT***a** *leu2–3,112 ura3–52 trp1 his3 rme1 HML***a**
PA66-2A	TB50**a** *wsc1*::*HIS3MX6*
TS124-2A	TB50**a** *rom2*::*kanMX4*
SF8-2D	TB50**a** *sac7*::*kanMX4*
SF36-2B	TB50**a** *tor2*::*kanMX4*/*YCplac33*::*tor2–21*^{ts}
HM44-1C	TB50α *inp51*::*HIS3*MX6
HM47-1C	TB50**a** *INP51-HA3-kan*MX4
HM52-2D	TB50α *inp51*::*HIS tor2*::*kan*MX4/YCplac33::*tor2–21*^{ts}
HM59-2C	TB50**a** *inp51*::*HIS rom2*::*kan*MX4
HM64-1B	TB50**a** *inp51*::*HIS wsc1*::*HIS3*MX6
HM77-1B	TB50**a** *inp51*::*HIS sac7*::*kan*MX4
HM90-1A	TB50**a** *irs4*::*kan*MX4
HM92-1A	TB50α *TAX4-MYC13-HIS3*MX6
HM93-1A	TB50α *tax4*::*HIS3*MX6
HM96-1B	TB50**a** *INP51-HA3-kan*MX4 *TAX4-MYC13-HIS3*MX6
HM102-3B	TB50**a** *INP51*Δ*NPF-HA3-HIS3*MX6
HM104-2D	TB50α *IRS4-MYC13-HIS3*MX6
HM112-2C	TB50α *tax4*::*HIS3*MX6 *irs4*::*kan*MX4
HM119-2B	TB50**a** *INP51-HA3-kan*MX4 *IRS4-MYC13-HIS3*MX6
HM122-9B	TB50**a** *tax4*::*HIS3*MX6 *irs4*::*kan*MX4 *sac7*::*kan*MX4
HM125-1A	TB50α *tax4*::*HIS3*MX6 *irs4*::*kan*MX4 *tor2*::*kan*MX4/YCplac33::*tor2–21*^{ts}
HM127-3B	TB50**a** *INP51*Δ*NPF-HA3-HIS3*MX6 *TAX4-MYC13-HIS3*MX6
HM132-2D	TB50**a** *INP51*Δ*NPF-HA3-HIS3*MX6 *IRS4-MYC13-HIS3*MX6

tant for the turnover of PI(4,5)P_2 linked to the cell integrity pathway.

EXPERIMENTAL PROCEDURES

Strains and Media—The *S. cerevisiae* strains used in this study are listed in Table I. All strains were isogenic derivatives of TB50. Rich medium (yeast extract, peptone, and dextrose (YPD)) and minimal medium (synthetic dextrose (SD)) were as described previously (36). PCR cassettes were used to generate gene deletions and for tagging with HA, Myc, or the tandem affinity purification tag, as described (37–39). Yeast transformation was performed by the lithium acetate procedure (40). Correct integration was verified by PCR, and tagged proteins were tested for their functionality.

Spot Assay—Logarithmically growing cells were harvested and resuspended in 10 mM Tris-HCl (pH 7.4). The resuspended cells were diluted in a 10-fold dilution series. 3 μl of each dilution (10×, 100×, 1,000× and 10,000× diluted) were spotted on a YPD plate. Growth was scored after 2 days at 30 or 37 °C.

INP51 Purification—Cells expressing a tagged or untagged (mock purification) version of INP51 were grown in 5 liters of YPD to an A_{600} of 0.8 at 30 °C, harvested by centrifugation, and washed with cold water before resuspension in lysis buffer. The lysis buffer used to prepare cell extracts contained phosphate-buffered saline, 5% glycerol. 0.5% Tween 20, phosphatase inhibitors (10 mM NaF, 10 mM NaN_3, 10 mM *p*-nitrophenylphosphate, 10 mM sodium pyrophosphate, 10 mM β-glycerophosphate, and 1 mM phenylmethylsulfonyl fluoride), and protease inhibitor mixture tablets (Roche Applied Science). Cell lysate was obtained by glass bead lysis. Cell lysates containing ~700 mg of protein were cleared with a 5-min, 500 × *g* spin, diluted with lysis buffer to 10 mg/ml, and subsequently passed over an ion exchange resin (SP-Sepharose). The resin was washed twice with lysis buffer and twice with lysis buffer containing 50 mM potassium acetate. Bound proteins were eluted with lysis buffer containing 600 mM potassium acetate. The eluate was precleared over a protein A-Sepharose (Amersham Biosciences) column prior to the addition of anti-HA (12CA5) cross-linked to protein A-Sepharose beads. After 5 times washing with lysis buffer, immunoprecipitated proteins were visualized by silver staining (41). Analysis of protein bands by mass spectrometry was performed as described (27).

Immunoprecipitation—Yeast extracts from cells (500-ml culture in YPD grown to A_{600} of 0.8 at 30 °C) expressing tagged proteins of interest were prepared as described above. An aliquot of extract containing 10 mg of protein was adjusted to 1 ml with lysis buffer plus inhibitors. For immunoprecipitations, 20 μl of anti-HA (12CA5) or anti-Myc (9E10) cross-linked to protein A-Sepharose beads were added and mixed for 4 h at 4 °C. Beads were collected by centrifugation, washed five times with 1 ml of lysis buffer, and resuspended in 5× SDS-polyacrylamide gele sample buffer for electrophoresis. After SDS-PAGE, the proteins were blotted onto nitrocellulose membranes, blocked in 5% dry milk powder in 1× phosphate-buffered saline and 0.1% Nonidet P-40, and incubated with primary antibody anti-HA (clone 12CA5) or anti-Myc (clone 9E10) (1:10000 in blocking solution). Subsequently tagged proteins were detected using horseradish peroxidase-conjugated goat anti-mouse secondary antibodies and ECL reagents (Amersham Biosciences).

Phosphoinositide Analysis—Yeast cells were diluted 5 × 10^4/ml in SD medium lacking inositol containing 10 μC/ml [^{3}H]inositol and grown to a density of 2–4 × 10^6/ml (12–16 h). 2-ml aliquots were taken and mixed with 2 ml of MeOH. Cells were pelleted by centrifugation and processed as described previously (42).

Differential Centrifugation—Differential centrifugation was performed as described previously (43).

Indirect Immunofluorescence—Logarithmically growing cells containing INP51-HA, TAX4-Myc, or IRS4-Myc were fixed for 2 h in the growth medium supplemented with formaldehyde (3.7% final) and potassium phosphate buffer (100 mM final, pH 6.5). Cells were washed and resuspended in sorbitol buffer (1.2 M sorbitol and 100 mM potassium phosphate, pH 6.5). Cell walls were digested for 45 min at 37 °C in sorbitol buffer supplemented with β-mercaptoethanol (20 mM final) and zymolyase 20T (12.5 mg/ml; Seigagaku Corporation). Spheroblasts were fixed on poly-L-lysine-coated glass slides and permeabilized with PBT (53 mM Na_2HPO_4, 13 mM NaH_2PO_4, 75 mM NaCl, 1% bovine serum albumin, and 0.1% Triton X-100). Immunofluorescence directed against the HA epitope was performed by application of a primary antibody anti-HA (clone 12CA5) or anti-Myc (clone 9E10) at a dilution of 1:1,000 in PBT for 2 h and, subsequently, the application of a Cy3-conjugated rabbit anti-mouse IgG (Molecular Probes) diluted 1:1,000 in PBT for 90 min. Washed cells were examined with a Zeiss Axiophot microscope (100× objective). Immunofluorescent detection of FKS1 was performed as described previously (17).

MAP Kinase Activation Assay—YPD cultures of logarithmically growing cells at 24 or 39 °C were harvested, and cell extracts were prepared as described previously (44). Protein concentrations of extracts were determined by using a Bradford assay (Bio-Rad). Samples were denatured by the addition of 5× SDS-PAGE sample loading buffer and heating at 95 °C for 5 min. A total of 25 μg of protein (for MPK1 protein detection) or 40 μg of protein (for phosphorylated MAP kinase detection) was loaded for standard SDS-PAGE (10% acrylamide) and Western blot. For immunodetection, a goat anti-MPK1 antibody (clone yN-19, 1:1000 dilution; Santa Cruz Biotechnology) and a rabbit anti-phospho-p44/42 MAP kinase (Thr202/Tyr204) antibody (1:1000; Cell Signaling) were used. The anti-MPK1 and anti-phospho-MAP kinase were verified to specifically recognize activated MPK1 under heat stress conditions (data not shown). Secondary antibodies were horseradish peroxidase-conjugated anti-goat (anti-MPK1) or anti-rabbit (anti-phospho-MAP kinase) secondary antibody and detection by ECL reagents (Amersham Biosciences). The nitrocellulose membrane used for the Western blot was stained with Coomassie Blue for the visualization of total protein.

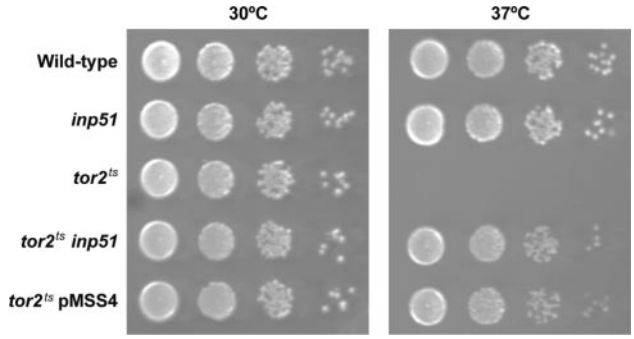

FIG. 1. **Disruption of *INP51* restores growth of *a tor2* temperature-sensitive mutant (*tor2*^{ts}).** Wild-type (TB50**a**), *inp51*, *tor2*^{ts}, and *tor2*^{ts} *inp51* cells and *tor2*^{ts} cells transformed with plasmid overexpressing MSS4 were spotted on to YPD medium and incubated at 30 or 37 °C. *tor2*^{ts} cells are not viable at 37 °C.

Actin Staining—Logarithmically growing cells were fixed in formaldehyde (3.7%) and potassium phosphate buffer (100 mM, pH 6.5) and stained with TRITC-phalloidin (Sigma) to visualize actin, as described previously (45).

Chitin Staining—1 ml of cells at an A_{600} of 0.5 were collected by centrifugation and washed with water. Cells were then incubated in a solution with 0.5 mg/ml calcofluor white (Sigma) for 5 min to visualize chitin. To remove residual calcofluor white, cells were washed twice with water. Cells were examined with a Zeiss Axiophot microscope (100× objective).

RESULTS

Disruption of INP51 Restores Growth of a tor2 Mutant—To understand further how the turnover of PI(4,5)P_2 is important for PI(4,5)P_2 signaling, we tested whether the phosphoinositide 5-phosphatase INP51, INP52, or INP53 antagonizes MSS4 signaling. We asked if the lack of any *INP* gene mimics MSS4 overexpression in suppressing a TOR2 signaling defect. A deletion of each *INP* gene was introduced into a temperature-sensitive *tor2* (*tor2*^{ts}) mutant defective in the organization of the actin cytoskeleton. The double mutants were grown at permissive (30 °C) and restrictive (37 °C) temperatures to determine whether an *INP* mutation can suppress the *tor2*^{ts} mutation. Deletion of only *INP51*, *INP52*, or *INP53* conferred no growth defect at any temperature (data not shown). Interestingly, the deletion of *INP51*, but not the deletion of *INP52* or *INP53*, suppressed the growth defect of *tor2*^{ts} cells (data not shown). The *inp51 tor2*^{ts} cells grew almost as well as wild-type cells and as well as *tor2*^{ts} cells overexpressing MSS4 (Fig. 1). This result suggests that INP51, but not INP52 or INP53, antagonizes the role of MSS4 in the TORC2 signaling pathway. Curiously, although *inp51* suppressed the growth defect of *tor2*^{ts}, it did not appear to suppress the actin defect of the *tor2*^{ts} mutant (data not shown).

INP51 Synthetically Interacts with Mutations Affecting the Cell Integrity Pathway—The finding that an *inp51* deletion suppresses the growth defect of a *tor2*^{ts} mutant is similar to the previous observation that activation of the cell integrity pathway suppresses the growth defect of a *tor2*^{ts} mutant (15) and suggests that INP51 may antagonize the cell integrity pathway. Thus, we investigated whether an *inp51* mutation interacts with mutations affecting components of the cell integrity pathway. Specifically, we asked if an *inp51* deletion suppresses or enhances the effect of mutations in *WSC1, ROM2, SAC7*, or *MPK1*. We observed that the deletion of *INP51* suppresses the growth defects of *wsc1* and *rom2* cells at 37 and 30 °C, the restrictive temperatures of these mutants, respectively (Fig. 2, *A* and *B*). Conversely, the combination of *inp51* with *sac7* conferred a synthetic growth defect (Fig. 2*C*). Finally, the growth defect of an *mpk1* mutant grown at restrictive temperature (38 °C) was not suppressed by *INP51* deletion (data not shown). The above results show that *INP51* genetically interacts with at least some components of the cell integrity pathway. Deletion of *INP51* suppresses mutations in positive components of the pathway (*WSC1* and *ROM2*) and causes a synthetic defect when combined with a mutation in a negative element of the pathway (*SAC7*). The nature of these interactions suggests that the phosphoinositide phosphatase INP51 negatively regulates signaling through the cell integrity pathway. This conclusion is consistent with the previous conclusions (13, 27, 30, 46) that the phosphoinositide kinase MSS4 and PI(4,5)P_2 act positively on the TORC2 and cell integrity pathways.

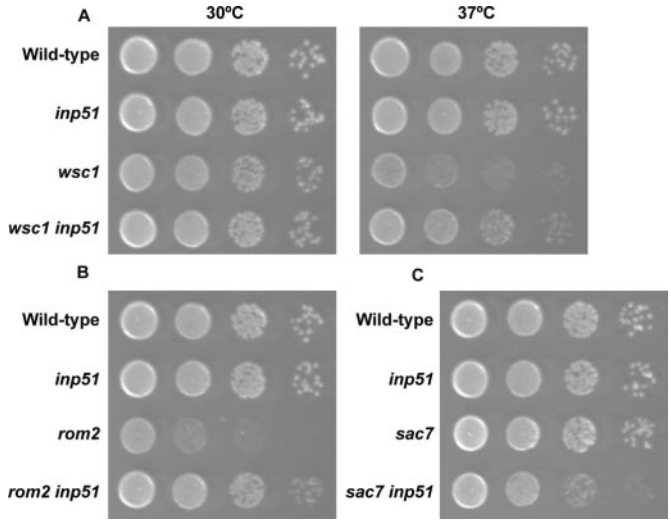

FIG. 2. **INP51 synthetically interacts with mutations affecting the cell integrity pathway.** *A*, wild-type, *inp51*, *wsc1*, and *wsc1 inp51* cells were spotted on to YPD medium and incubated at 30 or 37 °C. *wsc1* has a growth defect at 37 °C. *B*, wild-type, *inp51*, *rom2*, and *rom2 inp51* cells were spotted onto YPD medium and incubated at 30 °C. The *rom2* mutant has a growth defect at 30 °C. *C*, wild-type, *inp51*, *sac7*, and *sac7 inp51* cells were spotted onto YPD medium and incubated at 30 °C.

INP51 Associates with the EH Domain-containing Proteins TAX4 and IRS4—In mammalian cells, the kinases and phosphatases that determine PI(4,5)P_2 levels are regulated by interacting proteins (33, 47–51). An indication that the level of PI(4,5)P_2 is regulated in *S. cerevisiae* has been suggested previously by the observation that PI(4,5)P_2 levels increase upon heat shock (10). However, proteins regulating such kinases and phosphatases in *S. cerevisiae* remain to be identified. To identify potential INP51 regulatory proteins, we opted to isolate INP51-interacting proteins by using a biochemical approach described previously (27). A functional, epitope-tagged version of INP51 (INP51-HA) was constructed and purified from yeast cell extracts as described under "Experimental Procedures." Two homologous proteins were co-purified with INP51, IRS4, and an uncharacterized protein encoded by the open reading frameYJL083w that we named TAX4 (Fig. 3). The interaction between INP51 and TAX4 or IRS4 was confirmed by coimmunoprecipitation using epitope-tagged versions of TAX4 (TAX4-Myc) and IRS4 (IRS4-Myc), as described under "Experimental Procedures" (Fig. 4). We did not observe interaction between TAX4 and IRS4 by coimmunoprecipitation of the heterologously tagged versions (TAX4-TAP and IRS4-Myc) of these proteins (data not shown). IRS4 was identified previously in a screen for mutants defective in rDNA silencing (52). Untagged TAX4 has an apparent molecular mass of 77 kDa and a predicted size of 68.7 kDa. Untagged IRS4 is observed as two bands between 70 and 75 kDa, slightly larger than the predicted size of 68.8 kDa. TAX4 and IRS4 have an overall identity of 31% and contain a C-terminal EH domain. The EH domains

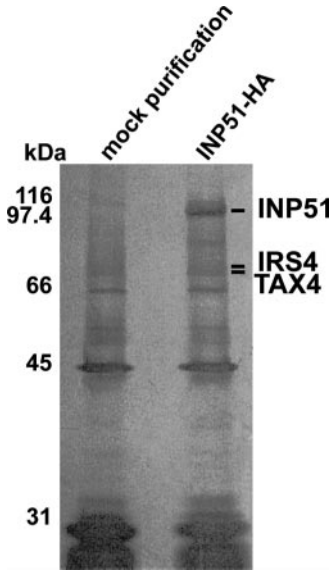

FIG. 3. **INP51 co-purifies with TAX4 and IRS4.** A silver-stained gel of INP51 purification (see "Experimental Procedures") from HM47-1C cells (INP51-HA) and TB50 cells (*mock purification*) is shown. Protein bands unique to the INP51 purification are indicated. These bands were excised and identified by mass spectrometry (see "Experimental Procedures"). Equivalent regions of the mock purification were also excised to confirm the co-purification with INP51.

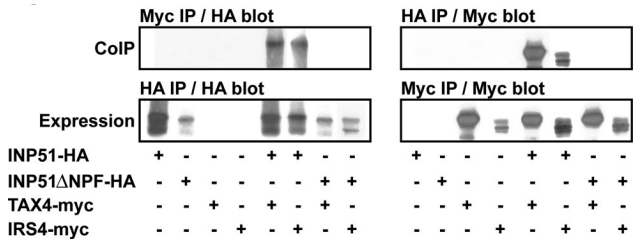

FIG. 4. **INP51 coimmunoprecipitates with TAX4 and IRS4.** Lysates from cells expressing INP51-HA (HM47-1C), TAX4-Myc (HM92-1A), IRS4-Myc (HM104-2D), or INP51ΔNPF-HA (HM102-3B) or co-expressing INP51-HA and TAX4-Myc (HM96-1B), INP51-HA and IRS4-Myc (HM119-2B), INP51ΔNPF-HA and TAX4-Myc (HM127-3B), or INP51ΔNPF-HA and IRS4-Myc (HM132-2D) were used to analyze the interaction between INP51 and TAX4 and IRS4. *IP*, immunoprecipitation.

of TAX4 and IRS4 are 64% identical. The EH domain, conserved from yeast to human, is a protein-protein interaction domain of ~100 amino acids that interacts specifically with short motifs containing an asparagine-proline-phenylalanine (NPF) core (53, 54). INP51 has a C-terminal NPF motif (55), amino acids 932–934, which is important for the interaction between INP51 and TAX4 or IRS4. The absence of the NPF motif and amino acids C-terminal to NPF in INP51 (*i.e.* the deletion of amino acids 932–946) abolishes the interaction between INP51 and TAX4 or IRS4, as assayed by coimmunoprecipitation (Fig. 4). Our results show that INP51, via its NPF-motif, interacts separately with the two EH domain-containing proteins TAX4 and IRS4, suggesting that INP51 forms separate INP51-TAX4 and INP51-IRS4 complexes.

There are additional EH-containing proteins in *S. cerevisiae*, including PAN1, END3, and EDE1 (45, 56, 57). Interestingly, a conditional *pan1* mutation shows synthetic interaction with an *inp51* mutation (58). Because PAN1 and END3 belong to the same protein complex, we tested whether PAN1 and/or END3 interact with INP51. We could not detect, by coimmunoprecipitation, an interaction between INP51 and PAN1 or END3, suggesting that INP51 interacts specifically with TAX4 and IRS4.

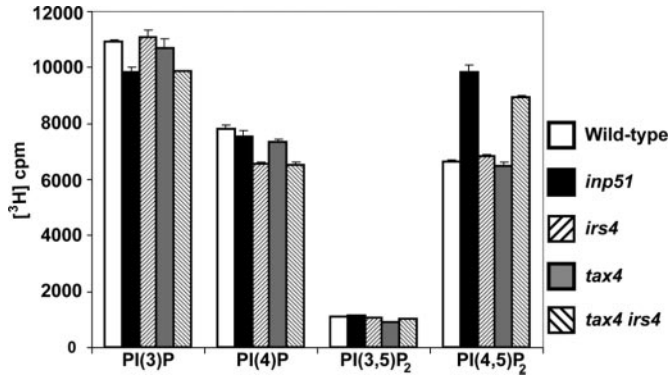

FIG. 5. **PI(4,5)P2 is increased in *inp51* and *tax4 irs4* cells.** Wild-type, *inp51*, *tax4*, *irs4*, and *tax4 irs4* cells were labeled at 30 °C with [^{3}H]inositol, and [^{3}H]inositol-containing lipids from the cell extracts were deacylated, separated by high pressure liquid chromatography, and quantified. *PI(3)P*, phosphatidylinositol 3-phosphate; *PI(4)P*, phosphatidylinositol 4-phosphate; *PI(3,5)P$_2$*, phosphatidylinositol 3,5-bisphosphate.

TAX4 and IRS4 Positively Regulate INP51—The interaction of INP51 with TAX4 and IRS4 led us to examine a possible involvement of TAX4 and IRS4 in the function of INP51. *TAX4* and *IRS4* are non-essential genes, as shown previously (59). Furthermore, cells lacking both *TAX4* and *IRS4* (a *tax4 irs4* double mutant) did not display a detectable growth defect on standard rich or minimal media at various temperatures (Fig. 6 and data not shown).

To determine the function of TAX4 and IRS4, we measured the levels of all detectable *S. cerevisiae* phosphoinositides, namely phosphatidylinositol 3-phosphate, phosphatidylinositol 4-phosphate, phosphatidylinositol 3,5-bisphosphate, and PI(4,5)P_2, in *inp51*, *tax4*, *irs4*, and *tax4 irs4* mutant cells. The double mutant *tax4 irs4* was examined in anticipation of the likelihood that the structurally homologous TAX4 and IRS4 proteins are also functionally homologous. We observed that an *inp51* mutant has higher levels of PI(4,5)P_2 than does wild-type, as observed previously (60), but is not altered in the levels of other phosphoinositides (Fig. 5). The deletion of *TAX4* or *IRS4* did not affect the level of any phosphoinositide. However, the double deletion mutant *tax4 irs4*, like the *inp51* mutant, exhibited a significant and specific increase in the level of PI(4,5)P_2. Our data suggest that TAX4 and IRS4 indeed act redundantly as positive regulators of INP51 activity and, thereby, PI(4,5)P_2 turnover.

Because TAX4 or IRS4 appears to be required for INP51 activity, we asked if the *tax4 irs4* double mutation, like *inp51*, genetically interacts with *TOR2* and components of the cell integrity pathway. We combined the *tax4 irs4* double mutation with *tor2^{ts}*, *rom2*, or *sac7*. In agreement with the positive role of TAX4 and IRS4 on INP51 activity, *tax4 irs4* suppressed the growth defect of a *tor2^{ts}* mutant at a non-permissive temperature to the same extent as did the deletion of *INP51* or the overexpression of MSS4 (Fig. 6). The growth defect of a *rom2* mutant was also suppressed by the *tax4 irs4* mutation (data not shown). Finally, the combination of *tax4 irs4* with a *sac7* mutation produced a synthetic growth defect similar to the combination of *inp51* and *sac7* (Fig. 7). Deletion of *TAX4* or *IRS4* alone showed no synthetic interaction with components of the cell integrity pathway (data not shown). Our results suggest that TAX4 or IRS4, like INP51, is important for PI(4,5)P_2 turnover linked to TORC2 signaling and the cell integrity pathway.

To investigate the mechanism by which TAX4 and IRS4 regulate INP51, we examined the cellular localization of TAX4, IRS4, and INP51 by indirect immunofluorescence on strains expressing epitope-tagged versions of these proteins. We observed an intracellular punctate staining pattern for both TAX4 and IRS4 (data not shown). However, for unknown rea-

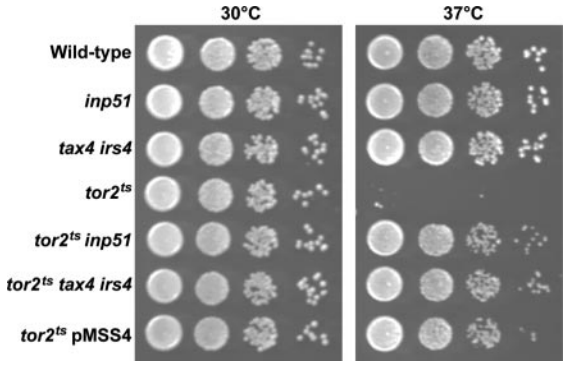

FIG. 6. **Double deletion of *TAX4* and *IRS4* restores growth of *tor2*^{ts}.** Wild-type, *inp51*, *tax4 irs4*, *tor2*^{ts}, *tor2*^{ts} *inp51*, and *tor2*^{ts} *tax4 irs4* cells and *tor2*^{ts} cells transformed with plasmid overexpressing MSS4 were spotted onto YPD medium and incubated at 30 or 37 °C. *tor2*^{ts} cells are not viable at 37 °C.

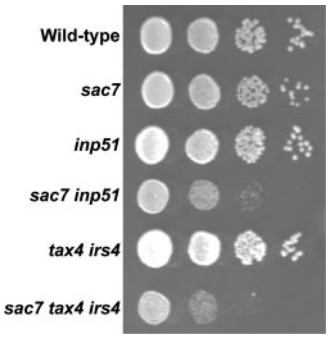

FIG. 7. ***tax4 irs4* double mutation interact with mutations in the cell integrity pathway.** Wild-type, *sac7, inp51*, *sac7 inp51, tax4 irs4*, and *sac7 tax4 irs4* cells were spotted onto YPD medium and incubated at 30 °C.

sons, we were unable to detect a signal for INP51 and were thus unable to determine by this assay if TAX4 or IRS4 is required for the correct localization of INP51. INP51 has previously been shown to be membrane-associated (31). However, by differential centrifugation (see "Experimental Procedures") we observed that INP51 is a cytosolic protein and that this localization did not change in a *tax4 irs4* double mutant (data not shown).

INP51, TAX4, and IRS4 Negatively Regulate MPK1—The genetic interactions described above suggest that INP51, TAX4, and IRS4 negatively regulate signaling through the cell integrity pathway. However, the above experiments (and data not shown for TAX4 and IRS4) did not reveal a genetic interaction between MPK1 and the INP51 complexes. To investigate further a possible functional interaction between the MPK1 and the INP51 complexes, we examined MPK1 phosphorylation in wild-type cells and mutant cells containing *rom2*, *sac7*, *inp51*, *tax4*, *irs4*, *tax4 irs4,* or any possible combination of *rom2* or *sac7* with *inp51*, *tax4*, *irs4*, or *tax4 irs4*. Upon stimulation of the cell integrity pathway, MPK1 is phosphorylated and thereby activated (61). MPK1 phosphorylation was analyzed with an anti-phospho-p44/p42 MAP kinase antibody that also specifically recognizes the phosphorylated form of MPK1. The amount of total MPK1 was assayed with a separate antibody that recognizes all forms of MPK1. As shown previously, MPK1 phosphorylation is almost undetectable in wild-type cells grown at 23 °C (non-inducing condition) but increases upon shift to 39 °C (61). We examined the phosphorylation state of MPK1 in wild-type cells and mutant cells grown at low temperature (24 °C) and at high temperature (39 °C). The level of MPK1 phosphorylation in heat-shocked cells (shift to 39 °C for 30 and 60 min) was not altered in any of the mutants examined (data not shown). However, the *sac7*, *sac7 inp51*, and *sac7 tax4 irs4* mutations affected the basal level (24 °C) of MPK1, as follows. A *sac7* mutation resulted in a slight increase in MPK1 phosphorylation but also a similar increase in the amount of the MPK1 protein as compared with wild-type (Fig. 8). The *sac7 inp51* and *sac7 tax4 irs4* cells exhibited a 10-fold increase in MPK1 phosphorylation and a 2.5-fold increase in MPK1 protein level compared with the *sac7* cells. Thus, *inp51* and *tax4 irs4* mutations cause constitutive MPK1 activity, although this effect is detectable only when *inp51* and *tax4 irs4* mutations are combined with a *sac7* mutation. Furthermore, as suggested by some of the genetic interactions described above, these findings confirm that INP51 complexes indeed negatively regulate signaling through MPK1 and the cell integrity pathway.

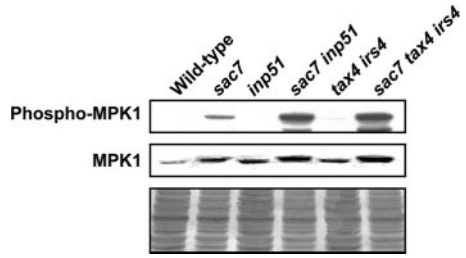

FIG. 8. **MPK1 activity increases in *sac7 inp51* and *sac7 tax4 irs4* cells.** MPK1 activation and protein levels were analyzed in wild-type, *sac7*, *inp51*, *sac7 inp51*, *tax4 irs4*, and *sac7 tax4 irs4* cells at 24 °C. Analysis of activated MPK1 with antibodies recognizing specific Thr/Tyr phosphorylation (*top section*) and MPK1 protein level with antibodies recognizing MPK1 (*middle section*) was performed. The *bottom section* (total extract) shows a Coomassie-stained version of the gel from the middle section. Equal amounts of protein were loaded in each lane, *i.e.* 40 μg for the gel of top section and 25 μg for the gel of the middle and bottom sections.

The Distribution of Chitin Is Negatively Regulated by INP51, TAX4, and IRS4—To further investigate the regulation of MPK1 signaling by the INP51 complexes, we examined MPK1 readouts in *sac7 inp51* and *sac7 tax4 irs4* cells. In particular, we examined the actin cytoskeleton, glucan synthase (FKS1) and chitin (see the Introduction) in *sac7*, *inp51*, *tax4 irs4*, *sac7 inp51*, and *sac7 tax4 irs4* cells grown at low (24 °C) and high temperatures (39 °C). Heat stress transiently depolarizes both the actin cytoskeleton and glucan synthase (17). The above mutant cells behaved like wild-type cells with regard to the actin cytoskeleton and FKS1 (data not shown), suggesting that INP51, TAX4, and IRS4 do not regulate signaling of the cell integrity pathway to these targets. However, the amount and distribution of chitin was changed in some mutants. In wild-type cells, chitin is most visible at the neck and at a future bud site (Fig. 9). In the *sac7* and *tax4 irs4* cells the chitin signal was slightly depolarized, whereas in the *sac7 inp51* and *sac7 tax4 irs4* cells the chitin signal and depolarization were strikingly increased. In *sac7 inp51* and *sac7 tax4 irs4* cells, the chitin was clearly distributed over the entire cell surface in unbudded cells, the mother cell surface in small budded cells, and both mother and daughter cell surfaces in large budded cells (Fig. 9). The changes in chitin amounts and distribution suggest that INP51, TAX4, and IRS4 regulate a specific branch of the cell integrity pathway affecting chitin.

To investigate if the affect on chitin is the cause of the observed growth defect of *sac7 inp51* and *sac7 tax4 irs4* cells (Fig. 7), *CHS3* was deleted in these mutants. *CHS3* encodes chitin synthase III that is responsible for 90% of the chitin produced in the cell, although deletion of *CHS3* does not cause a growth defect (62–64). A deletion of *CHS3* in *sac7 inp51* and *sac7 tax4 irs4* mutants reduced the amount of chitin to wild-type levels but did not suppress the growth defect (data not shown). This suggests that targets of the cell integrity pathway other than chitin could be affected (hyperactivated), thus caus-

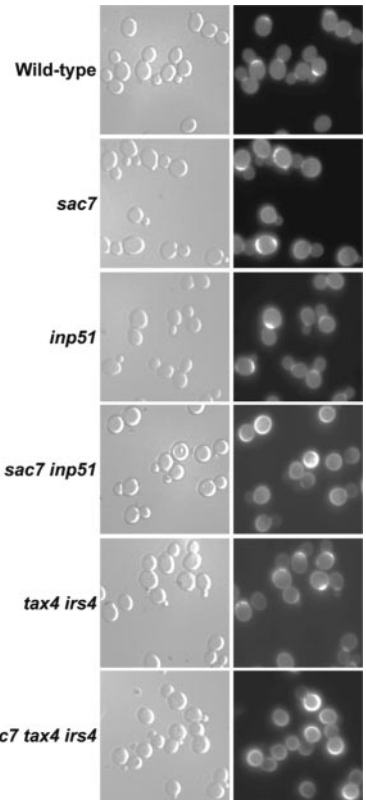

FIG. 9. **Chitin distribution is delocalized in *sac7 inp51* and *sac7 tax4 irs4* cells.** Cells from logarithmically grown cultures were fixed with formaldehyde and stained with calcofluor white. The cells were examined with Nomarski optics and fluorescence to visualize the chitin.

ing the growth defect observed in *sac7 inp51* and *sac7 tax4 irs4* cells.

DISCUSSION

Here we present two proteins, TAX4 and IRS4, that are novel partners of the phosphoinositide 5-phosphatase INP51. The structurally homologous and functionally redundant TAX4 and IRS4 proteins positively regulate INP51 activity and thereby negatively regulate PI(4,5)P_2 levels (Fig. 10). Furthermore, the INP51 complexes (INP51-TAX4 and INP51-IRS4) negatively regulate signaling through the cell integrity pathway, including the MAP kinase MPK1.

TAX4 and IRS4 contain an EH domain. EH domains are found mainly in proteins involved in endocytosis and the organization of the actin cytoskeleton, and they interact specifically with short motifs containing an NPF core (54, 56, 57, 65, 66). INP51 contains an NPF motif (55) that is important for the interaction with TAX4 or IRS4 and is not found in any other phosphoinositide phosphatase in *S. cerevisiae*. Thus, the interaction between INP51 and TAX4 or IRS4 is similar to the interaction between the mammalian phosphoinositide 5-phosphatase synaptojanin 1 (NPF-containing) and EPS15, suggesting a conserved regulatory mechanism (33). The functional consequence of EPS15 binding to synaptojanin 1 is not known, but our findings suggest that this binding may result in the activation of synaptojanin 1 phosphatase activity.

How does TAX4 or IRS4 activate INP51? The EH domain has been suggested to play an important role in recruiting proteins to a specific cellular location (67). Our studies did not reveal a change in INP51 localization in the absence of TAX4 and IRS4; however, TAX4 and IRS4 might control a small pool of INP51 whose change in localization cannot be detected in the fractionation experiment. Furthermore, we did not detect a change in

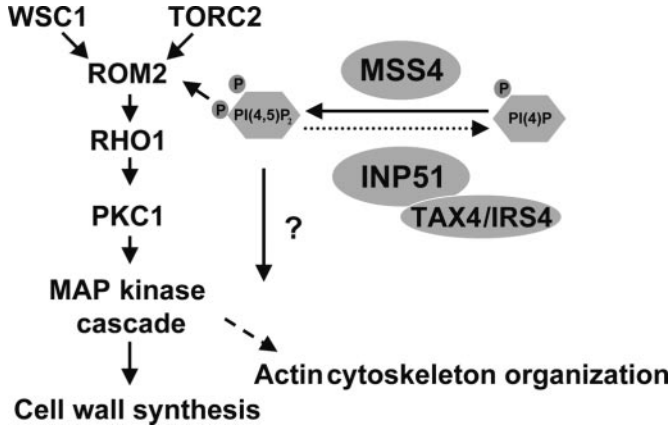

FIG. 10. **Model showing PI(4,5)P2 regulation linked to the cell integrity pathway.** PI(4,5)P2 synthesis and turnover is regulated by MSS4 and INP51, respectively. INP51 activity is controlled by TAX4 and IRS4. PI(4,5)P2 signaling impinges on a PKC1 pathway controlled by two independent pathways, the TORC2 signaling pathway and the cell integrity pathway. PI(4,5)P2 could signal to ROM2 or through an unknown parallel pathway.

the binding of TAX4 or IRS4 to INP51 in response to several different types of cell wall stress, including heat shock and detergent treatment (data not shown). Future studies will be required to determine the mechanism by which TAX4 or IRS4 activates INP51.

We also present evidence that INP51, TAX4, and IRS4 genetically interact with two signaling pathways, the TORC2 signaling pathway and the cell integrity pathway (Fig. 10). The mechanism by which *inp51* and *tax4 irs4* mutations suppress the growth defect of a *tor2* mutant remains unclear but could be through the activation of the cell integrity pathway. The *inp51* or *tax4 irs4* mutations activate MPK1, and the cell integrity pathway is a downstream effector pathway of TORC2. The activation of MPK1, which is detected only when *inp51* or *tax4 irs4* is combined with a *sac7* mutation, could be a result of increased RHO1 activity (balance toward the active, GTP-bound form of RHO1). The *sac7* mutation eliminates a RHO1 GAP and thereby prevents down-regulation of RHO1, whereas *inp51* or *tax4 irs4* causes an increase in the level of PI(4,5)P_2 that could then activate RHO1 via the pleckstrin homology domain in the RHO1 GDP/GTP exchange factors.

The combined action of a GAP (SAC7) and a phosphoinositide phosphatase (INP51) could be a conserved mechanism for achieving proper spatial and temporal regulation of a signaling pathway, in this particular case signaling through the cell integrity pathway. The GAP and phosphoinositide phosphatase combination has been observed in other organisms such as *Dictyostelium discoideum*, where the phosphoinositide phosphatase Dd5P4 contains both a PI(4,5)P_2 5-phosphatase catalytic domain and a GAP catalytic domain (68). This domain combination is also present in the human PI(4,5)P_2 5-phosphatase OCRL1 that, when mutated, causes mental retardation and kidney and eye failure (oculocerebrorenal Lowe syndrome) (69–71).

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REFERENCES

1. Martin, T. F. (1998) *Annu. Rev. Cell Dev. Biol.* **14,** 231–264
2. Berridge, M. J., and Irvine, R. F. (1989) *Nature* **341,** 197–205
3. Liscovitch, M., Chalifa, V., Pertile, P., Chen, C. S., and Cantley, L. C. (1994) *J. Biol. Chem.* **269,** 21403–21406
4. Gascard, P., Sauvage, M., Sulpice, J. C., and Giraud, F. (1993) *Biochemistry* **32,** 5941–5948
5. Harlan, J. E., Hajduk, P. J., Yoon, H. S., and Fesik, S. W. (1994) *Nature* **371,** 168–170
6. Itoh, T., Koshiba, S., Kigawa, T., Kikuchi, A., Yokoyama, S., and Takenawa,T.

(2001) *Science* **291,** 1047–1051
7. Cremona, O., and De Camilli, P. (2001) *J. Cell Sci.* **114,** 1041–1052
8. Yin, H. L., and Janmey, P. A. (2003) *Annu. Rev. Physiol.* **65,** 761–789
9. Hilgemann, D. W., Feng, S., and Nasuhoglu, C. (2001) *Science's STKE* http://stke.sciencemag.org/cgi/content/full/sigtrans;2001/111/re19
10. Desrivieres, S., Cooke, F. T., Parker, P. J., and Hall, M. N. (1998) *J. Biol. Chem.* **273,** 15787–15793
11. Homma, K., Terui, S., Minemura, M., Qadota, H., Anraku, Y., Kanaho, Y., and Ohya, Y. (1998) *J. Biol. Chem.* **273,** 15779–15786
12. Ozaki, K., Tanaka, K., Imamura, H., Hihara, T., Kameyama, T., Nonaka, H., Hirano, H., Matsuura, Y., and Takai, Y. (1996) *EMBO J.* **15,** 2196–2207
13. Audhya, A., and Emr, S. D. (2002) *Dev. Cell* **2,** 593–605
14. Schmelzle, T., Helliwell, S. B., and Hall, M. N. (2002) *Mol. Cell. Biol.* **22,** 1329–1339
15. Bickle, M., Delley, P. A., Schmidt, A., and Hall, M. N. (1998) *EMBO J.* **17,** 2235–2245
16. Igual, J. C., Johnson, A. L., and Johnston, L. H. (1996) *EMBO J.* **15,** 5001–5013
17. Delley, P. A., and Hall, M. N. (1999) *J. Cell Biol.* **147,** 163–174
18. Jung, U. S., and Levin, D. E. (1999) *Mol. Microbiol.* **34,** 1049–1057
19. Verna, J., Lodder, A., Lee, K., Vagts, A., and Ballester, R. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 13804–13809
20. Philip, B., and Levin, D. E. (2001) *Mol. Cell. Biol.* **21,** 271–280
21. Schmidt, A., Bickle, M., Beck, T., and Hall, M. N. (1997) *Cell* **88,** 531–542
22. Qadota, H., Python, C. P., Inoue, S. B., Arisawa, M., Anraku, Y., Zheng, Y., Watanabe, T., Levin, D. E., and Ohya, Y. (1996) *Science* **272,** 279–281
23. Drgonova, J., Drgon, T., Tanaka, K., Kollar, R., Chen, G. C., Ford, R. A., Chan, C. S., Takai, Y., and Cabib, E. (1996) *Science* **272,** 277–279
24. Kamada, Y., Qadota, H., Python, C. P., Anraku, Y., Ohya, Y., and Levin, D. E. (1996) *J. Biol. Chem.* **271,** 9193–9196
25. Nonaka, H., Tanaka, K., Hirano, H., Fujiwara, T., Kohno, H., Umikawa, M., Mino, A., and Takai, Y. (1995) *EMBO J.* **14,** 5931–5938
26. Garcia-Rodriguez, L. J., Trilla, J. A., Castro, C., Valdivieso, M. H., Duran, A., and Roncero, C. (2000) *FEBS Lett.* **478,** 84–88
27. Loewith, R., Jacinto, E., Wullschleger, S., Lorberg, A., Crespo, J. L., Bonenfant, D., Oppliger, W., Jenoe, P., and Hall, M. N. (2002) *Mol. Cell* **10,** 457–468
28. Helliwell, S. B., Schmidt, A., Ohya, Y., and Hall, M. N. (1998) *Curr. Biol.* **8,** 1211–1214
29. Schmidt, A., Kunz, J., and Hall, M. N. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 13780–13785
30. Helliwell, S. B., Howald, I., Barbet, N., and Hall, M. N. (1998) *Genetics* **148,** 99–112
31. Stolz, L. E., Huynh, C. V., Thorner, J., and York, J. D. (1998) *Genetics* **148,** 1715–1729
32. Stefan, C. J., Audhya, A., and Emr, S. D. (2002) *Mol. Biol. Cell* **13,** 542–557
33. Haffner, C., Takei, K., Chen, H., Ringstad, N., Hudson, A., Butler, M. H., Salcini, A. E., Di Fiore, P. P., and De Camilli, P. (1997) *FEBS Lett.* **419,** 175–180
34. McPherson, P. S., Garcia, E. P., Slepnev, V. I., David, C., Zhang, X., Grabs, D., Sossin, W. S., Bauerfeind, R., Nemoto, Y., and De Camilli, P. (1996) *Nature* **379,** 353–357
35. Haffner, C., Di Paolo, G., Rosenthal, J. A., and de Camilli, P. (2000) *Curr. Biol.* **10,** 471–474
36. Guthrie, C., and Fink, G. R. (eds) (1991) *Methods in Enzymology* Vol. 194, Academic Press, New York
37. Wach, A., Brachat, A., Pohlmann, R., and Philippsen, P. (1994) *Yeast* **10,** 1793–1808
38. Longtine, M. S., McKenzie, A., III, Demarini, D. J., Shah, N. G., Wach, A., Brachat, A., Philippsen, P., and Pringle, J. R. (1998) *Yeast* **14,** 953–961
39. Puig, O., Caspary, F., Rigaut, G., Rutz, B., Bouveret, E., Bragado-Nilsson, E., Wilm, M., and Seraphin, B. (2001) *Methods* **24,** 218–229
40. Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) *J. Bacteriol.* **153,** 163–168
41. Shevchenko, A., Wilm, M., Vorm, O., and Mann, M. (1996) *Anal. Chem.* **68,** 850–858
42. Dove, S. K., Cooke, F. T., Douglas, M. R., Sayers, L. G., Parker, P. J., and Michell, R. H. (1997) *Nature* **390,** 187–192
43. Kaiser, C. A., Chen, E. J., and Losko, S. (2002) *Methods Enzymol.* **351,** 325–338
44. Martin, H., Rodriguez-Pachon, J. M., Ruiz, C., Nombela, C., and Molina, M. (2000) *J. Biol. Chem.* **275,** 1511–1519
45. Benedetti, H., Raths, S., Crausaz, F., and Riezman, H. (1994) *Mol. Biol. Cell* **5,** 1023–1037
46. Yoshida, S., Ohya, Y., Goebl, M., Nakano, A., and Anraku, Y. (1994) *J. Biol. Chem.* **269,** 1166–1172
47. Malecz, N., McCabe, P. C., Spaargaren, C., Qiu, R., Chuang, Y., and Symons, M. (2000) *Curr. Biol.* **10,** 1383–1386
48. Song, W., and Zinsmaier, K. E. (2003) *Neuron* **40,** 665–667
49. Honda, A., Nogami, M., Yokozeki, T., Yamazaki, M., Nakamura, H., Watanabe, H., Kawamoto, K., Nakayama, K., Morris, A. J., Frohman, M. A., and Kanaho, Y. (1999) *Cell* **99,** 521–532
50. Weernink, P. A., Meletiadis, K., Hommeltenberg, S., Hinz, M., Ishihara, H., Schmidt, M., and Jakobs, K. H. (2004) *J. Biol. Chem.* **279,** 7840–7849
51. Chong, L. D., Traynor-Kaplan, A., Bokoch, G. M., and Schwartz, M. A. (1994) *Cell* **79,** 507–513
52. Smith, J. S., Caputo, E., and Boeke, J. D. (1999) *Mol. Cell. Biol.* **19,** 3184–3197
53. Wong, W. T., Schumacher, C., Salcini, A. E., Romano, A., Castagnino, P., Pelicci, P. G., and Di Fiore, P. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 9530–9534
54. Salcini, A. E., Confalonieri, S., Doria, M., Santolini, E., Tassi, E., Minenkova, O., Cesareni, G., Pelicci, P. G., and Di Fiore, P. P. (1997) *Genes Dev.* **11,** 2239–2249
55. Wendland, B., Emr, S. D., and Riezman, H. (1998) *Curr. Opin. Cell Biol.* **10,** 513–522
56. Tang, H. Y., and Cai, M. (1996) *Mol. Cell. Biol.* **16,** 4897–4914
57. Gagny, B., Wiederkehr, A., Dumoulin, P., Winsor, B., Riezman, H., and Haguenauer-Tsapis, R. (2000) *J. Cell Sci.* **113,** 3309–3319
58. Wendland, B., and Emr, S. D. (1998) *J. Cell Biol.* **141,** 71–84
59. Giaever, G., Chu, A. M., Ni, L., Connelly, C., Riles, L., Veronneau, S., Dow, S., Lucau-Danila, A., Anderson, K., Andre, B., Arkin, A. P., Astromoff, A., El-Bakkoury, M., Bangham, R., Benito, R., Brachat, S., Campanaro, S., Curtiss, M., Davis, K., Deutschbauer, A., Entian, K. D., Flaherty, P., Foury, F., Garfinkel, D. J., Gerstein, M., Gotte, D., Guldener, U., Hegemann, J. H., Hempel, S., Herman, Z., Jaramillo, D. F., Kelly, D. E., Kelly, S. L., Kotter, P., LaBonte, D., Lamb, D. C., Lan, N., Liang, H., Liao, H., Liu, L., Luo, C., Lussier, M., Mao, R., Menard, P., Ooi, S. L., Revuelta, J. L., Roberts, C. J., Rose, M., Ross-Macdonald, P., Scherens, B., Schimmack, G., Shafer, B., Shoemaker, D. D., Sookhai-Mahadeo, S., Storms, R. K., Strathern, J. N., Valle, G., Voet, M., Volckaert, G., Wang, C. Y., Ward, T. R., Wilhelmy, J., Winzeler, E. A., Yang, Y., Yen, G., Youngman, E., Yu, K., Bussey, H., Boeke, J. D., Snyder, M., Philippsen, P., Davis, R. W., and Johnston, M. (2002) *Nature* **418,** 387–391
60. Stolz, L. E., Kuo, W. J., Longchamps, J., Sekhon, M. K., and York, J. D. (1998) *J. Biol. Chem.* **273,** 11852–11861
61. Kamada, Y., Jung, U. S., Piotrowski, J., and Levin, D. E. (1995) *Genes Dev.* **9,** 1559–1571
62. Bulawa, C. E. (1992) *Mol. Cell. Biol.* **12,** 1764–1776
63. Shaw, J. A., Mol, P. C., Bowers, B., Silverman, S. J., Valdivieso, M. H., Duran, A., and Cabib, E. (1991) *J. Cell Biol.* **114,** 111–123
64. De Nobel, J. G., Klis, F. M., Ram, A., Van Unen, H., Priem, J., Munnik, T., and Van Den Ende, H. (1991) *Yeast* **7,** 589–598
65. Morinaka, K., Koyama, S., Nakashima, S., Hinoi, T., Okawa, K., Iwamatsu, A., and Kikuchi, A. (1999) *Oncogene* **18,** 5915–5922
66. Benmerah, A., Gagnon, J., Begue, B., Megarbane, B., Dautry-Varsat, A., and Cerf-Bensussan, N. (1995) *J. Cell Biol.* **131,** 1831–1838
67. Santolini, E., Salcini, A. E., Kay, B. K., Yamabhai, M., and Di Fiore, P. P. (1999) *Exp. Cell Res.* **253,** 186–209
68. Loovers, H. M., Veenstra, K., Snippe, H., Pesesse, X., Erneux, C., and van Haastert, P. J. (2003) *J. Biol. Chem.* **278,** 5652–5658
69. Attree, O., Olivos, I. M., Okabe, I., Bailey, L. C., Nelson, D. L., Lewis, R. A., McInnes, R. R., and Nussbaum, R. L. (1992) *Nature* **358,** 239–242
70. Suchy, S. F., Olivos-Glander, I. M., and Nussabaum, R. L. (1995) *Hum. Mol. Genet.* **4,** 2245–2250
71. Faucherre, A., Desbois, P., Satre, V., Lunardi, J., Dorseuil, O., and Gacon, G. (2003) *Hum. Mol. Genet.* **12,** 2449–2456

Negative Regulation of Phosphatidylinositol 4,5-Bisphosphate Levels by the INP51-associated Proteins TAX4 and IRS4

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