The interactions of protein kinases and phosphatases with their regulatory subunits and substrates underpin cellular regulation. We identified a kinase and phosphatase interaction (KPI) network of 1844 interactions in budding yeast by mass spectrometric analysis of protein complexes. The KPI network contained many dense local regions of interactions that suggested new confidence interactions were recovered for 120 protein kinases (fig. S5; see fig. S6 and table S6). High-confidence interactions were recovered for 120 protein kinases (fig. S5; see fig. S6 and table S6 for validation). For a number of kinases, we demonstrated that associated proteins were substrates in vitro (figs. S7 and S8 and table S7). Our dataset doubled the number of KPIs obtained in previous low-throughput (LTP) studies and performed as well as LTP data against an unbiased HTP high-confidence (HTP-HC) benchmark dataset (fig. S1 (2)). Clustering of all kinases and phosphatases by their interaction profiles revealed locally dense regions in the KPI network (Fig. 1A and fig. S9). The Cdc14 phosphatase formed one of the largest single hubs in the network with 53 interaction partners, including 23 kinases and 5 phosphatases (Fig. 1B, fig. S6, and table S6). Cdc14 antagonizes mitotic cyclin-dependent kinase (CDK) activity and is activated by the mitotic exit network (MEN) upon completion of anaphase (1). New connections between Cdc14 and other mitotic regulators included the CDK-inhibitory kinase Swe1, the nuclear RENT complex (3), and the DNA damage checkpoint kinases Chk1 and Dun1. Cdc14, Net1, and Sir2 each interacted with the acetylase Sir2 that together with Cdc14 form the nucleolar RENT complex (4). New connections between Cdc14 and other mitotic regulators included the CDK-inhibitory kinase Swe1, the cytokinesis checkpoint protein Boi1 (5), and two activators of cytokinesis, Cbk1 and Ace2 (6). Cdc14, Net1, and Sir2 each interacted with the DNA damage checkpoint kinases Chk1 and Dun1. In support of a role for Cdc14 in the DNA damage response, we found that ectopic expression of Cdc14 caused sensitivity to the DNA-damaging agent methylmethane sulfonate (MMS), while a strain defective for Cdc14 function was sensitive to the ribonucleotide reductase inhibitor hydroxyurea (Fig. 1C). Interactions between the kinase and phosphatase interaction (KPI) network upon pH upshift (fig. S7). The sensor might be the needle subunit itself, which has been implicated in signaling the translocator to effector switch in Shigella (11) and Yop secretion by Yersinia (12). Another possibility is that translocon pore assembly changes the pH gradient within the needle channel and that the sensor is located toward the base of the secretion apparatus. Changes in pH from mildly acidic to neutral can have dramatic effects on protein folding; for example, some bacterial toxins re-fold after their translocation from acidic endosomes to the host-cell cytosol in a partially unfolded state (13). The SPI-2 T3SS pH sensor might thus undergo a conformational change on exposure to neutral pH and transduce a dissociation signal to the Ssa1/SsaM/SpiC complex.

The interaction of protein phosphorylation mediates cellular responses to growth factors, environmental signals, and internal processes by the regulation of protein interactions, enzyme activity, or protein localization (1). However, the protein interactions of kinases, phosphatases, and their regulatory subunits and substrates remain sparse.
RENT and the nutrient-sensing TOR complex 1 (TORC1) were supported by the finding that increased Cdc14 activity caused rapamycin sensitivity, whereas reduced Cdc14 function caused rapamycin resistance (Fig. 1D), suggesting that Cdc14 may antagonize TOR signaling. Cdc14 also interacted with the energy-sensing adenine 5’-monophosphate (AMP)-activated kinase (AMPK) Snf1 and its upstream kinase Sak1; AMPK activates glucose-repressed genes in yeast and is an upstream inhibitor of TOR activity in metazoans (7). Derepression of Cdc14 caused a severe defect in growth on glycerol medium and sensitivity to 2-deoxyglucose (Fig. 1D).

Cdc14 exhibited connections with three different mitogen-activated protein kinase (MAPK) modules. Interaction of the pheromone MAPK pathway kinases Fus3 and Ste7 with Cdc14 was supported by the finding that constitutive expression of Cdc14 caused partial pheromone resistance (fig. S10). Cdc14 interacted with the high osmolarity glycerol (HOG) pathway MAPK kinase Pbs2; consistently, constitutive expression of Cdc14 caused sensitivity to osmotic stress (Fig. 1E). The HOG pathway is also known to stimulate mitotic exit (8). The upstream cell wall integrity (CWI) MAPK kinase Bck1 interacted with Cdc14; a cdc14-3 strain was sensitive to the cell wall stress agent calcofluor white (Fig. 1E). These CWI interactions extended along each pathway because the conditional MEN alleles mob1-77 and cdc15-2 exhibited specific synthetic lethal interactions with either slt2Δ or bck1Δ mutations; this lethality was alleviated by growth on iso-osmotic medium but not by a catalytically inactive mutant of Slt2 (Fig. 1F and fig. S10). These data reveal Cdc14 as a nexus for cell cycle, checkpoint, metabolic, and stress signals (fig. S10).

The TORC1 and TORC2 kinase complexes are conserved from yeast to human and control macromolecular synthesis and polarized morphogenesis, respectively; TORC1 is sensitive to the macrolide rapamycin, whereas TORC2 is not (9). In the KPI dataset, TORC1 and TORC2 formed a highly connected subnetwork of 28 interaction partners, including 13 kinases and 4 phosphatases (figs. S6 and S11 and table S6). These connections established new links between TORC1 and the mitochondrial retrograde (RTG) signaling pathway (10), which induces genes required for glutamate production (fig. S12). Multiple TORC1 subunits exhibited previously undocumented interactions with the kinases Fmp48, Nnk1, Npr1, and Ksp1 (Fig. 2A and fig. S11).

Fmp48 is a kinase of unknown function that is associated with mitochondrial subcellular fractions (11). Consistent with interactions among

Fig. 1. Cdc14 phosphatase network. (A) Hierarchical two-dimensional clustering of bait interaction profiles in the KPI dataset. See fig. S9 for full clustergram. Networks for indicated clusters and other kinases are shown in fig. S19. (B) Cdc14-Net1-Sir2 (RENT) interaction network. Kinases are in orange, phosphatases in blue, kinase-associated proteins in yellow, and other proteins in gray. Red connecting lines indicate KPI interactions, gray lines LTP interactions, and gray dashed lines HTP-HC interactions. Line thickness indicates peptide count of interaction; node size is proportional to total number of interactions in the KPI dataset. Bold dashed circle indicates RENT complex and known associated proteins. RAM, regulation of Ace2p activity and cellular morphogenesis. (C) Sensitivity of a GAL1-CDC14 strain to 0.03% methyl methanesulfonate (MMS) when induced by 0.02% galactose (see fig. S50 for expression titration) and a cdc14-3 strain to 200 mM hydroxyurea (HU) at 33°C. (D) Sensitivity of a GAL1-CDC14 strain to either rapamycin (5 ng/ml) or glycerol medium when induced by 0.05% galactose. Resistance of a cdc14-3 strain to rapamycin (20 ng/ml) and sensitivity to 2-deoxyglucose (DG, 100 μg/ml) at 33°C. (E) Sensitivity of a GAL1-CDC14 strain to 1 M sorbitol when induced by 0.05% galactose. Sensitivity of a cdc14-3 strain to calcofluor white (CFW, 18 μg/ml) at 33°C. (F) Representative tetrads bearing combinations of slt2Δ, bck1Δ, cdc15-2, and mob1-77 alleles. Double-mutant spore clones are circled in yellow; pbs2Δ and tor1Δ served as negative controls.
Fmp48, TORC1, and the RTG inhibitor Mks1, elevated expression of FMP48 caused a growth defect on nonfermentable glycerol medium and rapamycin resistance on a fermentable carbon source (Fig. 2B). Overexpression of FMP48 caused abnormal mitochondrial morphology (Fig. 2C) and repression of genes encoding tricarboxylic acid cycle enzymes, electron transport chain components, and subunits of the adenosine 5′-triophosphate (ATP) synthase (Fig. 2D). Fmp48-associated kinase activity was specifically increased by rapamycin treatment (Fig. 2E), suggesting that Fmp48 relays TORC1 signals to the RTG pathway and mitochondrial function.

The uncharacterized kinase Ykl171w, renamed Nnk1 for nitrogen kinase, associated with all TORC1 subunits (fig. S11) and with Gdh2, the NAD+-dependent glutamate dehydrogenase that catalyzes deamination of glutamate to α-ketoglutarate and ammonia (12). Gdh2 was phosphorylated by Nnk1 complexes in vitro (Fig. 2F), and a gdh2Δ strain was resistant to rapamycin when grown on glutamate as the sole nitrogen source (Fig. 2G), whereas overexpression of NNK1 conferred hypersensitivity to rapamycin (Fig. 2H). Nnk1 also interacted with the TORC1 effector Ure2, which regulates the nitrogen catabolite response by sequestering the transcription factor Gln3 in the cytoplasm (12). Overexpression of NNK1 induced rapid nuclear accumulation of Gln3 (Fig. 3F) and increased transcription of Gln3 target genes (Fig. 2J), suggesting that Nnk1 activity antagonizes the Ure2-Gln3 inter-

Fig. 2. TORC1 kinase network. (A) Partial network of new TORC1-associated kinases. (B) Overexpression of GAL1-FMP48 inhibits growth on glycerol and confers rapamycin resistance. (C) Overexpression of GAL1-FMP48 causes abnormal mitochondrial morphology as visualized with an Ilv3GFP mitochondrial matrix fusion protein (GFP, green fluorescent protein). DIC, differential interference contrast. (D) Genome-wide expression profiles of GAL1-FMP48 and GAL1-MKS1 strains induced with 0.2% galactose. RTG-responsive (orange), mitochondrial (red), stress-responsive (green), and Gln3/Gcn4-responsive (blue) genes are marked. (E) Fmp48FLAG or Sch9FLAG complexes were immunopurified from cells grown in the presence or absence of rapamycin (200 ng/ml) for 30 min, then incubated with [33P]-γ-ATP, and radiolabeled species were resolved by SDS–polyacrylamide gel electrophoresis. Nonregulated Sch9-associated activity served as a negative control. (F) Immunopurified Nnk1FLAG complexes were incubated with [33P]-γ-ATP, then denatured, and radiolabeled Gdh2 species were repurified with antibody to hemagglutinin (HA). (G) A gdh2Δ strain is rapamycin resistant when glutamate is the sole nitrogen source. (H) Expression of GAL1-NNK1 in 2% galactose confers sensitivity to rapamycin (5 ng/ml). (I) Expression of GAL1-NNK1 in 2% galactose for 1.5 hours specifically induces Gln3 target genes. Color bar indicates fold increase (red) or decrease (green) relative to empty vector control.
action. The expansive TORC1 network also included other nutrient-sensing kinases (Npr1, Snf1, Gen2, and Ksp1; fig. S11) (13), transcription-associated kinases (Tra1 and Tpk2), MAPK module components (Bck1 and Kdx1), cell cycle kinases (Ime2, Mih1, and Cib2-Cdc28), an mRNA splicing kinase (Sky1), and a ribosome biogenesis kinase (Rio2). These findings underscore the central role of TOR in cell growth.

In a global protein interaction network constructed from the KPI, LTP, and HTP-HC datasets (2), kinase-kinase (K-K) interactions were significantly enriched compared to all other kinase interaction partners \((P < 3 \times 10^{-6})\) and collectively formed a highly interconnected K-K network (Fig. 3A, figs. S13 and S14, and table S8). Consistent with a trans-kinase phosphorylation network (14), we assigned 607 phosphorylation sites on 98 kinases (fig. S15 and table S9). This K-K network was extremely robust to fragmentation by hub deletion (Fig. 3B) and was far less modular than previous less-complete K-K networks (Fig. 3A, figs. S13 and S14, and table S8). The expansive TORC1 network also interacted with kinase interaction partners (Fig. 3C and fig. S18). The multifunctionality of kinases, as defined by associated GO terms, was markedly increased by the KPI dataset (Fig. 3D).

Cellular processes are controlled by a multitude of low-affinity interactions, as often mediated by short linear motifs embedded in disordered protein regions (15, 16). The KPI network is highly enriched for disordered regions as compared to the entire proteome \((P < 10^{-16})\). This physical organization may allow the cell to overcome stochastic limitations in signal propagation, integration, and downstream responses (16). In human cells, kinase-mediated signaling can readily propagate across pathways (17) and may dictate complex decisions through a broadly distributed network of effectors (18, 19). Moreover, phosphorylation-based feedback loops often enable cooperative responses, tuning of network outputs, and entrained states (20–22). The densely connected and nonmodular architecture of the KPI network suggests that the interaction of many such circuits will underpin cellular information flow (23).

**References and Notes**

2. Supporting material is available on Science Online.  
24. We thank B. Rought, A. Amon, L. Harrington, J. Bader, M. Costanzo, B. Andrews, C. Boone, R. Aebersold, B. Bodenmiller, I. Sadowski, and F. Sichert for discussions; J. P. Zhang and D. Fermin for technical support; and M. Snyder, Y. Ohsumi, S. Piatti, S. Hahn, H. Reinman, S. Biggins, T. Peters, M. Longhese, and D. Mao for reagents. Supported by grants from the Canadian Institutes of Health Research to A.C.G. (MOP-84314), T.P. (MOP-57793), and M.T. (MOP-12246); the Ontario Research Fund to T.P. and A.C.G. (REO#-044); the National Institutes of Health to M.T. (RO1R0020431 from the National Center for Research Resources) and A.L.N. (CA-126239); a Terry Fox Foundation Research Studentship from the National Cancer Institute of Canada to J.R.S.; Federation of European Biochemical Societies and Marie Curie Fellowships to V.N.; Canada Research Chairs in Functional Genomics and Bioinformatics (to M.T.) and in Functional Proteomics (to A.C.G.); the Lea Reichmann Chair in Cancer Proteomics to A.C.G.; and a Scottish Universities Life Sciences Alliance Research Professorship and a Royal Society Wolfson Research Merit Award to M.T.

**Supporting Online Material**

[www.sciencemag.org/cgi/content/full/328/5981/1043/DC1](www.sciencemag.org/cgi/content/full/328/5981/1043/DC1)

Materials and Methods  
Figs. S1 to S27  
Tables S1 to S15  
References  
19 May 2009; accepted 7 April 2010  
10.1126/science.1176495