almost at the same positions on the two complementary helices: in the yeast ARS1 origin, the start sites of the two leading strands are displaced by 2 bp (*1*) and in the human lamin B2 origin they overlap by 3 bp. As in yeast, the initiation event occurs within an A+T-rich area where specific nucleoprotein complexes assemble in G_1 and S phase. In both systems initiation occurs within the extended protected region detectable in G_1. From our data it is also apparent that, on the retrograde arms, in both directions, the first two Okazaki fragments are of nearly identical length, close to 140 nucleotides, whereas the following ones (up to the fifth or sixth) are shorter.

We are currently addressing identification of the proteins belonging to these complexes and we intend to check the possibility that some of them might be the human counterparts of the yeast ORC and MCM proteins (*13*).

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A Role for Nuclear Inositol 1,4,5-Trisphosphate Kinase in Transcriptional Control

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Phospholipase C and two inositol polyphosphate (IP) kinases constitute a signaling pathway that regulates nuclear messenger RNA export through production of inositol hexakisphosphate (IP_6). The inositol 1,4,5-trisphosphate kinase of this pathway in *Saccharomyces cerevisiae*, designated Ipk2, was found to be identical to Arg82, a regulator of the transcriptional complex ArgR-Mcm1. Synthesis of inositol 1,4,5,6-tetrakisphosphate, but not IP_6, was required for gene regulation through ArgR-Mcm1. Thus, the phospholipase C pathway produces multiple IP messengers that modulate distinct nuclear processes. The results reveal a direct mechanism by which activation of IP signaling may control gene expression.

How diverse extracellular stimuli elicit selective cellular responses through the activation of IP signaling pathways remains an important question in eukaryotic biology. Central to this process is phosphatidylinositol-specific phospholipase C, activation of which produces second messenger molecules such as inositol 1,4,5-triphosphate (IP_3), a regulator of calcium release (*1, 2*). Modification of IP_3 to form higher phosphorylated inositols such as IP_4, IP_5, and IP_6, the functions of which are less well understood, indicates that the regulatory effects of IP messengers may not be limited to calcium signaling (*2, 3*). Further diversity may be achieved through the spatial localization of IP pathways to cellular organelles (*4*). For example, a phospholipase C–dependent IP kinase signaling pathway contributes to IP_6-mediated mRNA export from the nucleus. In this pathway, phosphatidylinositol 4,5-bisphosphate is hydrolyzed to IP_3, which is then sequentially phosphorylated to IP_6 by two IP kinases: a putative kinase with dual specificity for IP_3 and IP_4, whose locus has not been mapped, and an IP_5 2-kinase (Ipk1) that is localized to the nuclear envelope–pore complex (*5*).

Many agonists that activate IP signaling also initiate specific changes in gene expression. Tight control of transcription is facilitated through the assembly of site-specific protein complexes on DNA promoter elements that regulate the activity of the general transcriptional machinery. One such multiprotein assembly is the arginine-responsive ArgR-Mcm1 complex, which comprises four proteins—Arg80, Arg81, Arg82, and Mcm1—each of which is required for proper transcriptional control (*6, 7*). Arg80 and Arg81 function as arginine-specific transcription factors, whereas Arg82 and Mcm1 are pleiotropic regulators. Mutants of *arg82* have defects in responses to nutrients, sporulation, mating, and stress (*8*). Mcm1 is a versatile transcription factor required for regulation of diverse gene sets including those involved in cell-cycle control, cell-type specificity, and pheromone and nutrient responses (*6, 9*). As a canonical member of the MADS (*MCM1*, agamous, deficiens, serum response factor) box transcription factor family, Mcm1 typically acts in concert with factors that have more limited cellular roles to achieve specificity in transcriptional control (*10*). Thus, combinatorial interplay between the dedicated arginine-specific transcription factors Arg80 and Arg81 and the more general factors Mcm1 and Arg82 allows interpretation of changes in extracellular arginine levels into transcriptional control of genes that participate in biosynthesis and catabolism of arginine. We find that Arg82 is a dual-specificity IP_3-IP_4 kinase and thus establish a potential link between phospholipase C–activated IP signaling and transcriptional control.

The phospholipase C–dependent IP signaling pathway apparently uses a single gene product to convert IP_3 to IP_5. IP_3 and IP_4 kinase activities copurify from both budding and fission yeast extracts through multiple chromatography steps (*11*). Also, a mutation of a single locus in *Saccharomyces cerevisiae*, *GSL3*, results in the loss of IP_4 and IP_5 production in vivo (*5*). To identify the IP_3-IP_4 kinase, we speculated that an evolutionary relation might exist among IP_3 kinases. In metazoans, a family of IP_3 3-kinases has been identified (*12*). Multisequence alignment of this family was used to identify several conserved elements. These elements were then used to search the yeast database and revealed Arg82 as a candidate IP kinase with 30% identity and 55% similarity throughout two 30–amino acid conserved elements (Fig. 1). Arg82 has a predicted molecular mass similar to that of the purified yeast IP_3-IP_4 kinase activity (*11*).

To test directly whether *ARG82* encodes an intrinsic IP_3 kinase, we analyzed purified, recombinant Arg82. Incubation of recombinant protein with [^{3}H]inositol 1,4,5-trisphosphate and adenosine triphosphate (ATP) resulted in the formation of inositol 1,3,4,5,6-pentakisphosphate, indicating that Arg82 performs two phosphorylation steps (Fig. 2A). Recombinant Arg82 in the presence of pure inositol 1,4,5-

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trisphosphate and trace mass amounts of [γ-^{32}P]ATP generated a single ^{32}P-labeled IP peak, which coeluted with inositol 1,4,5,6-tetrakisphosphate and was resistant to hydrolysis by type I inositol polyphosphate 5-phosphatase (Fig. 2B). With pure D-inositol 1,4,5,6-tetrakisphosphate, a single radiolabeled product peak of inositol 1,3,4,5,6-pentakisphosphate was observed (Fig. 2B). Seven other pure IP isomers were also tested as possible substrates, and under these conditions, kinase activity was not detected (*13*). Furthermore, the entire pathway from IP_3 to IP_6 could be recapitulated in vitro by incubation of IP_3, ATP, and recombinant Ipk2 and Ipk1. Thus, we conclude that Arg82 is a dual-specificity IP_3 6-kinase or IP_4 3-kinase, which we designate Ipk2 (for inositol polyphosphate kinase).

The functional role of Ipk2 in vivo was determined by analysis of *ipk2* mutant strains. One copy of *IPK2* (*ARG82*) was disrupted in diploid yeast with the *HIS3* gene by homologous recombination. Haploid *ipk2* null (Δ) mutants were recovered by sporulation and dissection of the heterozygous *IPK2/ipk2*Δ strain. All four progeny were viable when grown at 30°C; however, *ipk2*Δ mutants grew slowly at 30°C and were not viable at 37°C. Soluble inositol phosphates were measured in mutant and wild-type cells by steady-state [^{3}H]inositol labeling and high-performance liquid chromatography (HPLC). The predominant IP isomer in wild-type yeast under these conditions is inositol hexakisphosphate (IP_6), the end product of a Plc1–dependent inositol kinase pathway (*5*). Cells lacking Ipk2 showed a loss of IP_4, IP_5, and IP_6, and had increased amounts of IP_3 and an unidentified isomer of IP_2 (IP_2x) (Fig. 3). This is consistent with a blockade of the major IP_3 kinase activity in *ipk2*Δ cells. Production of IP_6 was restored to a state similar to that in wild-type cells by reintroduction of *IPK2* into mutant cells (Fig. 3).

To further examine this IP pathway, we analyzed the relation between Ipk2 and Plc1, the sole phosphatidylinositol-specific phospholipase C from yeast (*14*). In wild-type cells, overproduction of Plc1 resulted in large increases in the levels of IP_3, IP_4, IP_5, and IP_6 (*5*). Overexpression of Plc1 in *ipk2*Δ cells resulted in 20-fold increases of IP_3 and IP_2x above those observed in *ipk2*Δ strains; however, IP_4, IP_5, and IP_6 remained essentially undetectable (*15*). To test whether IP_2x and IP_3 arise solely from Plc1 activation, we analyzed IP levels in an *ipk2*Δ *plc1*Δ double mutant. As was the case in *plc1*Δ cells, IPs were not detected in the double mutant, indicating that Plc1 activity produces the relevant IP_3 substrate for Ipk2 in vivo.

Given the kinase selectivity of Ipk2, and the similar IP profiles of the *ipk2*Δ strain and previously described *gsl3* mutants, we speculated that the *gsl3* mutants had mutations in *IPK2*. A plasmid harboring *IPK2* was found to complement the *gsl3-3 gle1-2* synthetic lethal phenotype and the *gsl3-3* temperature-sensitive phenotype and resulted in a strain that produced IP_4, IP_5, and IP_6 (*16*). When the *gsl3-3* and the *ipk2*Δ strains were mated, the resulting diploid was temperature-sensitive, and the alleles did not cosegregate in tetrad dissection analysis (*16*). These results confirm that *gsl3-3* is an allele of *IPK2*.

Previous studies of Arg82 (Ipk2) indicate that it regulates cellular processes at the level of gene transcription (*8*). An in vitro assay for ArgR-Mcm1–mediated control is the ability of cellular extracts to form multiprotein assemblies on site-specific DNA promoter elements (*17*). Inability to form such complexes has been correlated with a loss of transcriptional control (*18*). For this reason, we characterized the role of Ipk2-mediated IP production in formation of ArgR-Mcm1 protein–DNA complexes. Extracts prepared from wild-type cells were incubated with a ^{32}P-radiolabeled fragment of the *ARG5,6* promoter element that can be bound by the ArgR-Mcm1 complex. The binding mixture was resolved on nondenaturing polyacrylamide gels, such that a band of

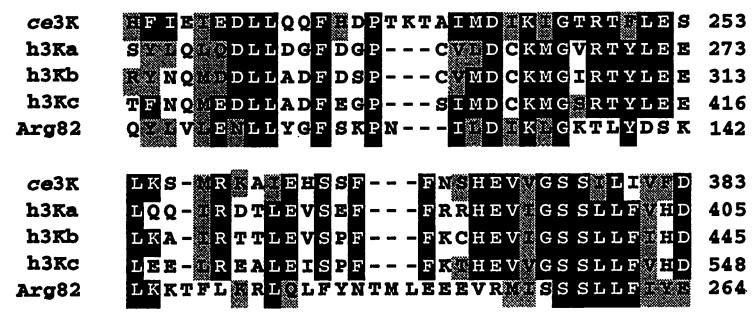
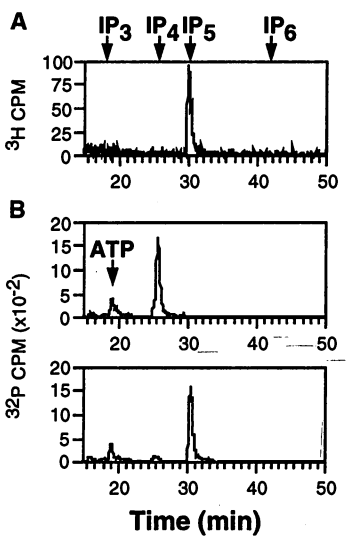


Fig. 1. A yeast gene product, Arg82, with similarity to conserved elements in an inositol phosphate kinase gene family. Amino acid numbering is provided to the left of the sequences. Identical residues, black; residues in which the nature of the side chain is preserved, gray. The sequences are as follows: ce3K, *Caenorhabditis elegans*, accession number (acc) AAC38962; h3Ka, *Homo sapiens*, acc Swissprot P23677; h3Kb, *H. sapiens*, acc Swissprot P27987; h3Kc, *H. sapiens*, acc Swissprot D38169; Arg82, *S. cerevisiae*, acc Swissprot P07250.

Fig. 2. Arg82, renamed Ipk2, an intrinsic IP_3-IP_4 kinase. The coding sequence of *IPK2* was fused in frame to glutathione *S*-transferase (GST), expressed in bacteria and purified. (**A**) GST-Ipk2 (10 ng) was incubated in 50 mM Hepes (pH 7.3), 50 mM NaCl, and 10 mM $MgCl_2$ with 10 μM [^{3}H]I(1,4,5)P_3 (DuPont Biotechnology Systems, Boston, Massachusetts) and 2 mM adenosine triphosphate (ATP) at 37°C for 30 min, and reaction products were resolved by HPLC. Conversion was time- and dose-dependent with approximate activity of 1 μmol/min per milligram. (**B**) GST-Ipk2 (10 ng) was incubated as above except with trace amounts of [γ-^{32}P]ATP and either 10 μM pure I(1,4,5)P_3 (top) or I(1,4,5,6)P_4 (bottom) (Matreya, Pleasant Gap, Pennsylvania). Incubation of GST (10 μg) alone with any IP isomer yielded no detectable radiolabeled IP product. All reaction products were analyzed by Partisphere (Whatman) strong anion exchange (SAX) (4.6 mm by 125 mm) HPLC using a linear gradient from 10 mM to 1.7 M ammonium phosphate (pH 3.5) over 25 min, followed by further elution with 1.7 M ammonium phosphate for 25 min. Individual IP isomers were assigned on the basis of coelution with known IP standards and sensitivity or resistance to various inositol phosphate phosphatase enzymes.



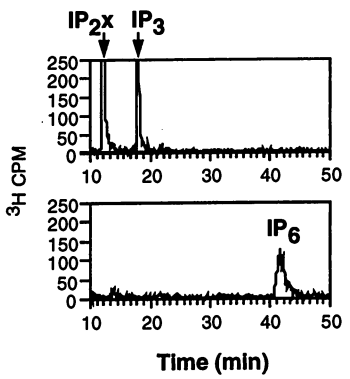


Fig. 3. Ipk2, the primary I(1,4,5)P_3 kinase in yeast. HPLC analysis of soluble extracts prepared after steady-state ^{3}H-labeling of *ipk2*Δ (top) or *ipk2*Δ + pIpK2 (bottom) in which an *ipk2*Δ strain expressed a fluorescent GFP-Ipk2 fusion protein (*26*). Cells were incubated in complete minimal medium containing [^{3}H]inositol (20 μCi/ml) and harvested during logarithmic growth as described (*27*). Soluble fractions from approximately 1×10^7 cells were resolved by HPLC as described above.

large apparent molecular size was observed in wild-type extracts that corresponded to the multiprotein complex bound to DNA (Fig. 4A). When we used extracts of mutant cells in which *IPK2* was deleted, no such complex was detected (*17*). Reintroduction of *IPK2* in an *ipk2*Δ strain restored complex formation. Because mutations in *IPK2* impair production of IP_6, we examined whether failure to form the ArgR-Mcm1 transcriptional complex reflected a defect in IP_6-regulated mRNA export. ArgR-Mcm1 complexes did form in extracts from the *ipk1*Δ strain, indicating that defects in cellular IP_6 production and mRNA export do not hinder ArgR-Mcm1 complex formation.

Phosphatidylinositol-specific phospholipase C (Plc1) activity produces the IP_3 substrate for Ipk2, and *plc1*Δ cells do not produce detectable IPs (*5*). Therefore, if any products of the Ipk2 reactions were required for complex formation, binding should be abolished in the *plc1*Δ strain as well. In extracts from a *plc1*Δ strain, complexes were formed (Fig. 4A). Additionally, in extracts prepared from *ipk2*Δ strains expressing a kinase-inactive Ipk2 mutant (*ipk2kin*$^-$), complexes were also able to form (*19*) (Fig. 4A). Thus, cellular production of IPs through phospholipase C and Ipk2 kinase activity are not necessary to maintain components of the ArgR-Mcm1 transcriptional complex, whereas the cellular presence of the Ipk2 protein is required.

Because the DNA mobility shift experiments do not distinguish between active and inactive transcriptional complexes, we also used a phenotypic assay for transcriptional control. In wild-type yeast strains provided with arginine (or its biosynthetic precursor, ornithine) as the sole nitrogen source, activation of the ArgR-Mcm1 complex results in induction of two arginine catabolic genes and repression of six arginine anabolic genes (*7, 20*). Therefore, strains that do not form or activate the ArgR-Mcm1 transcriptional complex are inviable on medium with arginine or ornithine as the sole nitrogen source. Wild-type, *ipk2*Δ, *ipk2*Δ + pIPK2, *ipk1*Δ, and *plc1*Δ strains were plated at 30°C on rich medium or medium containing ornithine as the sole nitrogen source (*21*) (Fig. 4B). Wild-type, *ipk1*Δ, and *ipk2*Δ + pIPK2 strains grew normally, but *ipk2*Δ and *plc1*Δ strains failed to grow. We also examined the *ipk2*Δ + pIpk2kin$^-$ strain and found that, despite their ability to form ArgR-Mcm1 complexes, these cells were unable to grow on ornithine medium (Fig. 4C). We conclude that production of inositol polyphosphates may be dispensable for ArgR-Mcm1 complex assembly, but that phospholipase C-induced, Ipk2-mediated production of IP_4-IP_5, but not IP_6, is required in vivo for the function of the ArgR-Mcm1 transcriptional complex.

Because Plc1 and Ipk2 but not Ipk1 appear to be required for transcriptional activity, the biologically relevant IP molecule may be IP_4 or IP_5. To further define the isomer or isomers required for regulation of the ArgR-Mcm1 complex, we examined an allele of *IPK2*, *ipk2-3*, which produces IP_4 but displays a significant reduction in the levels of IP_5 and IP_6 in cells grown at 30°C. In extracts from *ipk2-3* mutants, complex formation with the *ARG5,6* promoter was similar to that in wild-type (Fig. 4A), and the cells grew normally on ornithine (Fig. 4B). Thus, it appears that compromising the production of IP_5 and IP_6 does not significantly influence ArgR-Mcm1 function.

Several enzymes in IP signaling pathways are found in the nucleus, including Ipk1 and phospholipase C (*4, 5, 22*). Moreover, an Ipk2-lacZ fusion protein fractionates with the nucleus (*23*). To visualize the subcellular localization of Ipk2, a green fluorescent protein (GFP)-tagged Ipk2 was expressed in *ipk2*Δ cells and analyzed by direct fluorescence microscopy. Living cells expressing GFP-Ipk2 exhibited concentrated fluorescence in the nucleus and weak cytoplasmic staining (*24*). Thus, localized production of IPs in the nucleus may influence transcriptional control.

Our results emphasize that Ipk2 influences transcriptional responses by two mechanisms. First, Ipk2 protein but not IP synthesis is needed to enable formation of ArgR-Mcm1 complexes on DNA promoter elements. Second, production of I(1,4,5,6)P_4 and possibly I(1,3,4,5,6)P_5 through both phospholipase C and Ipk2 kinase activity is required to properly execute transcriptional control as measured by growth on ornithine as the sole nitrogen source. Together, this pathway produces distinct messengers involved in regulating nuclear processes [see Web fig. 1 (*25*)]. The coordination of gene expression through Ipk2 and the regulation of mRNA export through Ipk1 may contribute to the mechanisms by which diverse cellular agonists induce selective responses.

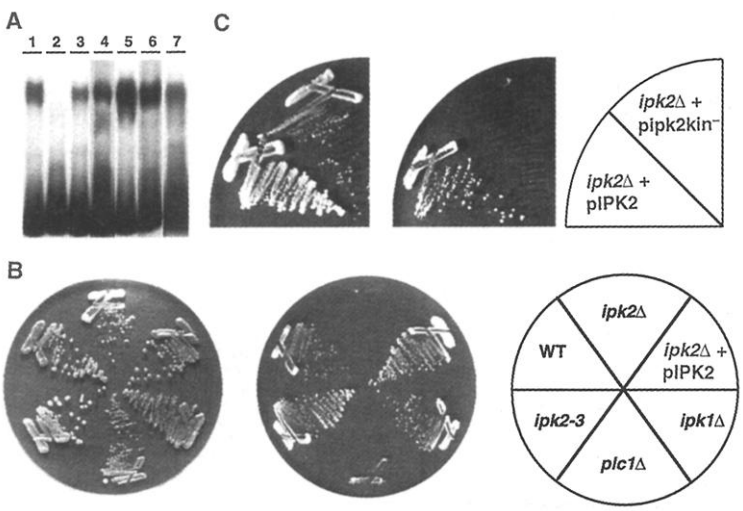


Fig. 4. Requirement of inositol polyphosphate production for function, but not formation, of the ArgR-Mcm1 transcriptional complex. (**A**) ArgR-Mcm1 complex formation using DNA mobility shift assay. Cells (5×10^8) of (1) wild-type, (2) *ipk2*Δ, (3) *ipk2*Δ + pIPK2, (4) *ipk1*Δ, (5) *plc1*Δ, (6) *ipk2-3*, and (7) *ipk2*Δ + pIpk2kin$^-$ strains were grown to early logarithmic growth phase in rich medium or complete minimal medium lacking tryptophan (pIPK2 and pIpk2kin$^-$ strains). Crude extracts were obtained by mechanical lysis of yeast cells with glass beads (*28*). The relevant promoter region of the *ARG5,6* gene was amplified by the polymerase chain reaction, gel-purified, and end-labeled with [γ-^{32}P]ATP. Soluble protein (50 μg) from each strain was incubated with ^{32}P-labeled probe; complexes were resolved by electrophoresis and visualized by autoradiography (*28*). (**B**) Phenotypic assay of ArgR-Mcm1 transcriptional activity. Freshly grown single colonies of appropriate strains (as labeled) were streaked onto rich medium (left) versus plates containing ornithine as the sole nitrogen source supplemented with limiting amounts (20 μg/ml) of nitrogen compounds for which our strain is auxotrophic (histidine, adenine, tryptophan, uracil, and leucine) (*7*). Plates were incubated at 30°C for 3 days. (**C**) Ipk2-kinase activity is required for ArgR-Mcm1 function. Freshly grown single colonies of *ipk2*Δ + pIPK2 or *ipk2*Δ + pIpk2kin$^-$ were streaked onto either complete minimal (left) or ornithine medium (both of which lacked tryptophan) and incubated as above.

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13. The following D-IP isomers were tested: I(1,4)P_2, I(1,3,4)P_3, I(1,2,6)P_3, I(1,3,4,5)P_4, I(3,4,5,6)P_4, I(1,3,4,5,6)P_5, and IP_6. For each, <1% of total radioactivity was detected as radiolabeled IP product. During revision of this manuscript, it was reported that GST-Arg82 (referred to as ArgRIII) expressed in human cells possessed IP_3 kinase activity [A. Saiardi, H. Erdjument-Bromage, A. M. Snowman, P. Tempst, S. H. Snyder, *Curr. Biol.* **9**, 1323 (1999)].
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15. Of note, a small peak of IP_4x (similar to that reported in *gsl3-3* strains after shift to 37°C) and a small peak of a novel IP_5x were detected in these strains, indicating that other IP kinases may be present in yeast cells.
16. Genomic sequence encoding *ARG82* was isolated using *Taq* polymerase with yeast genomic DNA as template and oligonucleotide primers containing Bam HI restriction sites. Primers were designed based on yeast genomic DNA sequence (DDBJ/EMBL/GenBank). The resulting fragment was cloned into the Bam HI site of plasmids pRS315 and pRS316. These were transformed into strain SWY1852 (*MATα gle3-3 gle1-2 ade2 ade3 ura3 his3 leu2 trp1* pGLE1/URA3/ADE3/CEN). Rescue of synthetic lethality was tested by monitoring for red and white sectoring of a *gsl3-3 gle1-2* pGLE1/URA3/ADE3/CEN pIPK2/LEU2/CEN strain on yeast extract, peptone, and dextrose (YPD) medium. The same strain was tested for temperature-sensitive growth at 37°C on medium lacking uracil (–Ura). Whereas a pLEU2/CEN plasmid does not confer sectoring on YPD or growth at 37°C on –Ura, the pIPK2/LEU2/CEN plasmid resulted in sectoring and growth at 37°C. Soluble IP levels were analyzed as described above for the *ipk2*Δ strains. Because the haploid *ipk2*Δ strains are defective for mating (*8*), the *ipk2*Δ strain was transformed with pIPK2/URA3/CEN. The resulting haploid was mated with SWY1852 (plus pRS315), and the diploid was assayed for temperature sensitivity after growth on 5-fluoroorotic acid.
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19. A mutant *ipk2* lacking IP_3-IP_4 kinase activity (*ipk2kin*$^-$) was constructed using a PCR-based mutagenesis strategy that resulted in a single amino acid substitution (replacement of aspartate 131 with alanine). The coding sequence of *ipk2kin*$^-$ was fused in frame to GST, expressed in bacteria, and purified. GST-ipk2kin$^-$ (100 ng) did not exhibit detectable kinase activity toward IP_3 and IP_4. The coding sequence of *ipk2kin*$^-$ was cloned into pUNI-10 and recombined with GFP-acceptor plasmid (*26*) to generate pGFP-ipk2kin$^-$. Cells lacking *ipk2*Δ were transformed with pGFP-ipk2kin$^-$ (*ipk2*Δ + pIpk2kin$^-$) and analyzed. Expression of GFP-tagged ipk2kin$^-$ restored ArgR-Mcm1 complex formation in *ipk2*Δ strains similar to GFP-tagged Ipk2; however, in contrast to GFP-Ipk2, expression of the GFP-ipk2kin$^-$ mutant was unable to rescue the slow growth at 30°C, inviability at 37°C, inviability on ornithine medium, and IP_4 or IP_5 production (as measured by steady-state inositol labeling) observed in *ipk2*Δ strains.
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21. Single-cell plating efficiencies of the relevant strains were determined to confirm the results obtained by streaking cells on ornithine solid medium. Sonicated cultures of known cell number were plated in parallel at multiple dilutions to rich medium versus medium containing ornithine as a sole nitrogen source. Plates were grown at 30°C for 3 to 4 days, and colony-forming units (CFU) were determined by visualization. Percent plating efficiency was defined as (CFU on ornithine medium/CFU on rich medium) × 100. Percent plating efficiency of wild-type cells on ornithine medium was 100%; for *ipk2*Δ, 0%; for *ipk1*Δ, 87%; for *plc1*Δ, <2%; and for *ipk2-3*, 85%. Results are averages obtained from two independent trials. These strains were also plated onto solid medium containing glutamate as the sole nitrogen source, and all strains were able to grow as single colonies having plating efficiencies of greater than 85%. Cells lacking *plc1* or *ipk2* did not exhibit measurable changes in PI(4,5)P_2 levels as compared with wild-type using a steady-state radiolabeling analysis. Additionally, *inp51*Δ strains, which exhibit greater than twofold increases in cellular PI(4,5)P_2 (*27*), grew normally on ornithine medium.
22. N. Liu, K. Fukami, H. Yu, T. Takenawa, *J. Biol. Chem.* **271**, 355 (1996); M. Yamaga, M. Fujii, H. Kamata, H. Hirata, H. Yagisawa, *J. Biol. Chem.* **274**, 28537 (1999).
23. J. Bercy, E. Dubois, F. Messenguy, *Gene* **55**, 277 (1987).
24. The amino-terminal end of *IPK2* was fused in frame to GFP and expressed from a centromeric *TRP1* plasmid under control of the *CDC42* promoter in an *ipk2*Δ strain (*26*). Cells were grown to logarithmic growth phase in complete minimal medium minus tryptophan in the presence of 1 μg/ml DAPI. Cells were transferred to slides and viewed directly in growth medium using a Zeiss Axioskop microscope (Zeiss, Thornwood, NY) equipped with epiflorescent and differential interference contrast (DIC) optics. The tagged protein was functionally similarly to native Ipk2. Expression of GFP-tagged Ipk2 on a centromeric plasmid (with the *CDC42* promoter) rescues the temperature sensitivity at 37°C, growth on ornithine, and defects in inositol phosphate production (as determined by steady-state labeling and HPLC analysis) of the *ipk2*Δ strain.
25. Supplementary material is available to *Science* Online subscribers at www.sciencemag.org/feature/data/1045815.shl.
26. The *ipk2*Δ + pIPK2 strains were constructed using modifications to the univector system [Q. Liu, M. Z. Li, D. Leibham, D. Cortez, S. J. Elledge, *Curr. Biol.* **8**, 1300 (1998)]. The *IPK2* gene was amplified from wild-type genomic DNA by using the polymerase chain reaction and cloned in frame with a Lox recombination site in pUNI-10. In vitro site-specific recombination with recombinant GST-Cre enzyme joined this resulting vector and yeast host vector pLOX/GFP/TRP1/CEN to create a centromeric *TRP1* plasmid with an amino-terminal GFP-tagged *IPK2*, under transcriptional control of the *CDC42* promoter. pUNI-10 and pLOX/GFP/TRP1/CEN were kindly provided by D. Lew and J. Moskow, Duke University.
27. L. E. Stolz, W. J. Kuo, J. Longchamps, M. K. Sekhon, J. D. York, *J. Biol. Chem.* **273**, 11852 (1998).
28. Crude extracts were obtained by mechanical lysis of yeast cells with glass beads, in the presence of 200 mM Tris (pH 8.0), 10 mM $MgCl_2$, 1 mM EDTA (pH 8.0), 10% glycerol, 10 mM dithiothreitol, and multiple protease inhibitors (500 μM phenylmethylslfonyl fluoride, 5 μg/ml pepstatin A, 1 μg/ml chymostatin, 100 mM *p*-aminobenzamidine, 100 mM ε-aminocaproic acid, 5 μg/ml aprotinin). The relevant promoter region of the *ARG5,6* gene (bases –142 to +15) was amplified by the polymerase chain reaction, gel-purified, and end-labeled with [γ-^{32}P]ATP by the polynucleotide kinase reaction according to manufacturer's instructions. Soluble protein (50 μg) from each strain was incubated with 10,000 cpm of ^{32}P-labeled probe for 15 min in 4 mM tris (pH 8.0), 30 mM NaCl, 4 mM $MgCl_2$, 5 μM L-arginine, 0.5 μg/ml herring sperm DNA, and 5% glycerol; complexes were resolved by electrophoresis on 5% nondenaturing polyacrylamide gels and visualized by autoradiography.

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Rapid Extragranular Plasticity in the Absence of Thalamocortical Plasticity in the Developing Primary Visual Cortex

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Monocular deprivation during early postnatal development remodels the circuitry of the primary visual cortex so that most neurons respond poorly to stimuli presented to the deprived eye. This rapid physiological change is ultimately accompanied by a matching anatomical loss of input from the deprived eye. This remodeling is thought to be initiated at the thalamocortical synapse. Ocular dominance plasticity after brief (24 hours) monocular deprivation was analyzed by intrinsic signal optical imaging and by targeted extracellular unit recordings. Deprived-eye responsiveness was lost in the extragranular layers, whereas normal binocularity in layer IV was preserved. This finding supports the hypothesis that thalamocortical organization is guided by earlier changes at higher stages.

The establishment of precise neural circuits is believed to result from activity-dependent rearrangements of neural connections during normal development (*1*). A conventional assumption present in models of visual cortical development and reorganization is that synaptic connections are remodeled serially in the order in which information is processed