Inositol Diphosphate Signaling Regulates Telomere Length*

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Activation of phospholipase C-dependent inositol polyphosphate signaling pathways generates distinct messengers derived from inositol 1,4,5-trisphosphate that control gene expression and mRNA export. Here we report the regulation of telomere length by production of a diphosphorylinositol tetraakisphosphate, PP-IP₆, synthesized by the KCS1 gene product. Loss of PP-IP₆ production results in lengthening of telomeres, whereas overproduction leads to their shortening. This effect requires the presence of Tel1, the yeast homologue of ATM, the protein mutated in the human disease ataxia telangiectasia. Our data provide in vivo evidence of a regulatory link between inositol polyphosphate signaling and the checkpoint kinase family and describe a third nuclear process modulated by phospholipase C activation.

Appropriate cellular responses to environmental changes involve intracellular second messenger systems that transduce the signals from cytoplasm to nucleus, thereby initiating adaptive genetic programs. One well described intracellular messenger system works through receptor-coupled activation of phospholipase C (PLC),¹ which hydrolyzes phosphatidylinositol 4,5-bisphosphate to yield inositol 1,4,5-trisphosphate (IP₃) (reviewed in Refs. 1 and 2). In metazoans, cellular production of IP₃ functions as a signal for calcium release from intracellular stores through allosteric activation of an IP₃ receptor channel. Recent studies indicate that IP₃ also plays an important role as precursor to multiple inositol polyphosphates (IP₆), each with potentially unique signaling capability (reviewed in Ref. 3). This signaling potential is not restricted to the cytoplasm, because IP₆ are known to function in nuclear processes in budding yeast (reviewed in Ref. 4). Activation of yeast phospholipase C (Plc1) produces IP₃, which is rapidly phosphorylated by the dual specificity 6-3 kinase Ipk2 (initially cloned as ArgRIII/Arg82 (5)) to yield IP₄ and IP₅ (6–8). This phosphorylation step is coupled to transcriptional regulation, possibly through chromatin remodeling (7, 9–11). IP₄ is then phosphorylated by the 2-kinase Ipk1, generating IP₅, which is required for efficient mRNA export from the nucleus (12–14). Both IP₅ and IP₆ can be converted to the diphosphoryl inositol phosphates PP-IP₄ and PP-IP₅ through the action of Kcs1, a kinase required for normal vacuolar morphology (15–18). PP-IP₅ has recently been suggested to play a role in chemotaxis in Dictyostelium (19). A nuclear role for Kcs1 is implied by its initial cloning as a regulator of mitotic DNA recombination, and inositol kinase activity is required for this regulation, but further understanding of its nuclear activity is lacking (20, 21).

Recent work has provided further linking of IP production and nuclear function through an important family of protein serine/threonine kinases known as phosphatidylinositol 3-kinase related kinases (PIKKs). IP₅ stimulates DNA repair by non-homologous end joining with mammalian proteins in vitro (22). Non-homologous end joining can be reconstituted in vitro with a limited number of purified proteins, including the Ku heterodimer, which binds DNA ends and IP₅ (23, 24), and the PIKK, DNA-dependent protein kinase (DNA-PKcs) (reviewed in Refs. 25 and 26). Other PIKKs, namely ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) gene products in mammalian cells and Tel1 and Mec1 in yeast, have been shown to have important activities in DNA damage checkpoint and telomere maintenance (27–29).

Telomeres are specialized protein-nucleic acid structures at the ends of linear, eukaryotic chromosomes that preserve genetic information during DNA replication, promote genomic stability, and are important in both cellular senescence and oncogenic transformation (reviewed in Refs. 30–32). Telomeric DNA is maintained through the action of the ribonucleoprotein telomerase, which acts in late S-phase to add template-independent, species-specific TG-rich repeats to the lagging strand, ensuring that chromosome length and coding sequences are maintained. Telomere length is heterogeneous among chromosomes and cells, with the average length established by a dynamic equilibrium between forces that shorten (exonuclease access, low telomerase activity) and those that lengthen telomeric sequences (capping proteins, high telomerase activity). Here we describe a role for the phospholipase C-dependent IP signaling pathway in the regulation of telomere length through production of an inositol diphosphate, PP-IP₄, synthesized by Kcs1. This modulation is dependent on Tel1 providing a functional connection between PIKKs and inositol phosphates in vivo.

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1 The abbreviations used are: PLC, phospholipase C; IP, inositol phosphate; IP₃, inositol hexakisphosphate; IP₄, inositol 1,4,5-trisphosphate; IP₅, inositol tetraakisphosphate; IP₆, inositol pentakisphosphate; PP-IP₄, diphosphorylinositol tetraakisphosphate; PP-IP₅, bis(diphosphoryl)inositol triakisphosphate; PP-IP₆, diphosphorylinositol penta-kisphosphate; HPLC, high performance liquid chromatography; kin, catalytically inactive kinase; PIKK, phosphatidylinositol 3-kinase-related kinase.

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**EXPERIMENTAL PROCEDURES**

**Strains, Media, and Genetic Methods—** Yeast strains used in this study are isogenic with W303a (leu2–3,112 his3–11,15 ura3–1 ade2–1 trp1–1 rad5–535 can1–100) except for changes introduced by transformation as indicated. Yeast were grown either in standard rich (yeast-peptone-dextrose) medium or in complete minimal medium containing 2% glucose or galactose and lacking appropriate nutrients to verify and maintain plasmids and markers. Yeast genetic manipulations were performed using standard procedures. Deletion strains pcl1::KANMX, ipk2::KANMX, ipk1::KANMX, tel1::ura3, and yku70::ura3 ura3::HIS3 were generated as described previously (7, 12, 33, 34). Double and triple deletion strains were constructed by mating to isogenic strains of the opposite mating type followed by sporulation of the resulting diploid and dissection of tetrads. Nutrient requirements, inositol labeling, and PCR were used to screen spores for double mutations.

The entire KCS1 and DDP1 open reading frames were deleted from the diploid Saccharomyces cerevisiae strain W303 and replaced with the selectable marker HIS3 as described (35). Tetrads were dissected using a Zeiss micromanipulator following sporulation of the heterozygous diploid. 0.3% potassium acetate plates. The kcs1::HIS3 and ddp1::HIS3 spores shared segregated 2:2 (not shown) and were identified by growth patterns on medium lacking histidine as well as by PCR analysis.

**Gene Identification and Plasmid Construction—** To identify candidate mammalian inositol diphosphoryl synthases, human expressed sequence tag data bases were searched with BLASTP, blasted BLASTP, or TBLASTN with appropriate query sequences. The full-length human cDNA of each candidate (designated as hIPk, an orthologue of yeast Kcs1) was obtained from Research Genetics, Inc. as an insert in pBluescript SK (−)

The entire open reading frame minus the codons for the first six amino-terminal amino acids was inserted in-frame into the NcoI and XhoI sites of pGEX-KG, derived from pGEX-2T (Amersham Biosciences) and kindly obtained from Research Genetics, Inc. as an insert in pBluescript SK(−). Note that the Southern blot strategies are the same as published previously (37). Genomic DNA was prepared from 5-ml cultures grown to saturation and digested with PstI before electrophoresis on a 1% Tris borate-EDTA-agarose gel. The fragments were transferred to Hybond N+ nylon membranes (Amersham Biosciences) and hybridized at 65 °C to a probe derived from pVT14 containing telomeric DNA and a portion of the Y′ subtelomeric repeat. The probe was labeled with [α-32P]dCTP using Ready-To-Go labeling beads (Amersham Biosciences). Note that the Southern blot strategies are the same as the accepted methods in the field; however, the effect appears small because the Pest1 site used for cleaving is about 800 bp from the chromosome end. Thus less than half of the fragment is composed of telomeric sequences. The bottom smear represents the terminal fragment resulting from cutting at the last Y′ elements (one of the subtelomeric repeats) on the chromosome. The bands near the top of the gel reflect hybridization to fragments generated by cutting tandem Y′ elements of different sizes. These bands vary in intensity, even in wild-type strains, probably as a consequence of a high rate of unequal recombination. Although we cannot exclude the possibility that there is a somewhat higher rate of this type of recombination in one or more of the mutants, none of them has the dramatic phenotype seen in yeast strains undergoing alternative lengthening of telomere recombination, where the upper bands become extremely prominent. For example, see Fig. 3 in Ritchie et al. (37).

**RESULTS**

**Genetics of Inositol Polyphosphate Synthesis in S. cerevisiae—** A graphic summary of the metabolism of soluble inositol phosphates in budding yeast is depicted in Fig. 1. A single phospholipase C (Plc1) hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to release IP3, from the membrane. IP3 serves as substrate for the inositol kinase Ipk2, which phosphorylates both the D-6 and D-3 position hydroxy groups to produce IP6 and IP5. IP6 can be further phosphorylated to PP-IP6 by one of two inositol diphosphoryl synthases in this strain, Kcs1, or a second kinase, Ids1. Kcs1 also phosphorylates IP5 to make PP-IP5. The kinase(s) responsible for synthesis of PP-IP6 is currently not defined, although it is likely that Kcs1 or Ids1 functions at this step. The inositol diphosphates PP-IP4, PP2-IP3, and PP-IP5 are dephosphorylated to PP6 and IP6 by an inositol diphosphophosphatase Ddp1. Note that the ring position phosphates of Kcs1 and Ids1 phosphate are undetected; it is possible that the PP-IP6 products of each kinase are distinct isomers (which are omitted from the diagram only for the sake of simplicity).

**Fig. 1. Schema of phospholipase C-dependent inositol polyphosphate metabolism in S. cerevisiae.** A single phospholipase C (Plc1) hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to release IP3, from the membrane. IP3 serves as substrate for the inositol kinase Ipk2, which phosphorylates both the D-6 and D-3 position hydroxy groups to produce IP6 and IP5. IP6 can be further phosphorylated to PP-IP6 by one of two inositol diphosphoryl synthases in this strain, Kcs1, or a second kinase, Ids1. Kcs1 also phosphorylates IP5 to make PP-IP5. The kinase(s) responsible for synthesis of PP-IP6 is currently not defined, although it is likely that Kcs1 or Ids1 functions at this step. The inositol diphosphates PP-IP4, PP2-IP3, and PP-IP5 are dephosphorylated to PP6 and IP6 by an inositol diphosphophosphatase Ddp1. Note that the ring position phosphates of Kcs1 and Ids1 phosphate are undetected; it is possible that the PP-IP6 products of each kinase are distinct isomers (which are omitted from the diagram only for the sake of simplicity).

**DISCUSSION**

To further study the role of these kinases and phosphatases in inositol metabolism we analyzed a series of yeast mutant strains undergoing alternative lengthening of telomere recombination, where the upper bands become extremely prominent. For example, see Fig. 3 in Ritchie et al. (37).

**RESULTS**

**Genetics of Inositol Polyphosphate Synthesis in S. cerevisiae—** A graphic summary of the metabolism of soluble inositol phosphates in budding yeast is depicted in Fig. 1. A single phospholipase C (Plc1) and three inositol polyphosphate kinases, Ipk2, Ipk1, and Kcs1, function to generate over seven species of water-soluble IP messengers. Plc1 hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to release IP3, from the membrane (6). IP3 serves as substrate for the inositol kinase Ipk2 (also known as Arg82), which phosphorylates both the D-6 and D-3 position hydroxy groups to produce IP6 and IP5 (7, 38). IP6 is phosphorylated at the 2-hydroxyl by Ipk1 to generate IP6(12). Kcs1 has been reported to function as an inositol diphosphophosphatase to produce PP-IP4 and PP-IP5, from IP6 and IP6 substrates, respectively (17). Dephosphorylation of inositol diphosphates occurs through a phosphatase Ddp1 (36).

To further study the role of these kinases and phosphatases in inositol metabolism we analyzed a series of yeast mutant strains undergoing alternative lengthening of telomere recombination, where the upper bands become extremely prominent. For example, see Fig. 3 in Ritchie et al. (37).
but are not observed under these labeling conditions (therefore HPLC traces only show relevant times of elution). A significant branch point in the IP pathway is exposed in metabolically labeled cells upon deletion of the IP₆ 2-kinase (ipkΔ) cells, which accumulate IP₆ and a diphosphoryl inositol, PP-IP₄ (Fig. 2A) (12). Deletion of KCS1 in the ipkΔ strain abrogates the peak of PP-IP₄, which can be complemented with active Kcs1; thus production of PP-IP₄ is primarily dependent on Kcs1 in vivo (Fig. 2A) (17). Disruption of KCS1 alone does not alter IP₆ levels, demonstrating that PP-IP₄ is not required for synthesis of IP₆ (Fig. 2A). Disruption of the DDP1 gene exposes a peak of PP-IP₄, which is not seen at appreciable levels in wild-type cells (Fig. 2B) (36). Elimination of KCS1 in the ddp1Δ strain (kcs1Δ ddp1Δ) leads to an increase rather than an ablation of PP-IP₅ production (Fig. 2B), demonstrating the existence of a second inositol diphosphoryl synthase (designated Ids1), which phosphorylates IP₆ to a PP-IP₅ isomer (see Fig. 1 schematic). This is surprising in light of the published work of others that Kcs1 is the major IP₆ kinase activity in yeast (17). Cellular extracts prepared from kcs1Δ ddp1Δ mutants have significant IP₆ kinase activity, further indicating the presence of a second kinase (data not shown). Additionally, it does not appear that Ids1 is encoded by IPK2, as extracts from ipk2Δ kcs1Δ mutant cells harbor IP₆ kinase activity, and we were not able to detect this activity using recombinant Ipk2 under a range of conditions (data not shown).

It is noteworthy that the chemical nature of the PP-IP₅ isomers generated by Kcs1 and Ids1 are not known; thus it is plausible that these kinases phosphorylate different positions, thereby generating distinct PP-IP₅ products that are not resolved by our HPLC analysis. The observed elevation of PP-IP₅ in the kcs1Δ ddp1Δ strain suggests either that: 1) Ids1 activity is negatively regulated by Kcs1 and/or one of its products; or 2) Kcs1 converts the product of Ids1 to PP-IP₅, thus when Kcs1 is deleted, PP-IP₅ accumulates. Ids1 activity may also function as a minor kinase relative to Kcs1 in the synthesis of PP-IP₅ from Ins₁,3,4,5,6P₅, as analysis of ipkΔ kcs1Δ ddp1Δ mutant cells reveals a small but significant peak of PP-IP₅ (not shown). Furthermore, PP-IP₃ derivatives are observed when DDP1 is eliminated in ipkΔ strains; however, it is not known whether Kcs1 or Ids1 activity is responsible for generating this product(s). Collectively, our new results along with previously published data lead to the schematic pathway depicted in Fig. 1.

**Inositol Phosphates and Telomere Length in Vivo—Evidence implicating IPs in the regulation of nuclear function and DNA repair processes in mammalian and yeast systems provided the impetus to examine these pathways in budding yeast. Examination of non-homologous end joining repair in plc1, ipk2, ipk1, or kcs1 mutant yeast revealed that loss of inositol signaling pathways does not result in detectable disruption of non-homologous end joining in living yeast (data not shown). It is of interest that yeast possess two PIKKs, Tel1 and Mec1, which are orthologues of ATM and ATR that play an important evolutionarily conserved role in the regulation of telomere length.**

*Signaling-dependent Telomere Maintenance*

**FIG. 2.** Steady state analysis of inositol polyphosphates in mutant strains. Haploid yeast strains were labeled with [³²P]inositol to steady state in overnight cultures, and then soluble inositols were isolated and analyzed by HPLC. IP standards are indicated as arrows. Strains in A are, top to bottom, wild type (wt), kcs1Δ, ipkΔ, ipkΔ kcs1Δ, ipkΔ kcs1Δ transformed with pRS-KCS1 (pKCS1), and ipkΔ kcs1Δ transformed with pRS-cks1kin ‘(pks1kin’). Strains in B are, top to bottom, wild type, ddp1Δ, kcs1Δ, and ddp1Δ kcs1Δ.
have significantly elevated levels of PP-IP4 (not shown) and ipk1/H9004, wild type, negative regulator of telomere length. In contrast, all effects of PP-IP4 at the telomere are abolished in the absence of Tel1. Both the double (ipk1Δ tel1Δ and kcs1Δ tel1Δ) and triple (ipk1Δ kcs1Δ tel1Δ) mutant strains have stable, shortened telomeres indistinguishable from tel1Δ cells (Fig. 5B). This result indicates that the IPs function in the same pathway of telomere regulation as this ATM homologue. Because loss of Tel1 function leads to short telomeres, the data are consistent with Kcs1 production of PP-IP4 acting as a negative regulator of Tel1 function.

**DISCUSSION**

Here we report a novel role of a phospholipase C-dependent signaling pathway in the control of telomere length. Individual deletion of each of the three enzymes in the synthetic pathway to PP-IP4 causes an identical lengthening of telomeres. The long telomere phenotype is seen in yeast lacking all soluble inositol phosphates (plc1-null) as well as in those lacking only expression does not (Fig. 4), demonstrating that kinase activity is required. The ability of Kcs1 to complement in dextrose indicated that the GAL promoter was not “off” and may be leaky in these strains. We therefore performed complementation analysis in the ipk1Δ kcs1Δ strains, which enable detection and quantification of PP-IP4. Indeed, the Kcs1 strains produced significant levels of PP-IP4 even in dextrose, confirming the leaky expression. Furthermore, growth of cells in galactose, which is a strong inducer of GAL promoters, only modestly elevated PP-IP4 levels as compared with dextrose (Fig. 4).

Analysis of ipk1Δ kcs1Δ complemented strains confirm that expression of KCS1 restores telomere length equivalent to the ipk1Δ strain alone (Fig. 4). Intriguingly, expression of inactive kinase in this strain not only fails to complement telomere length but also further lengthens telomeres, suggestive of a direct kinase-effector protein interaction at the telomere (Fig. 4). Consistent with this idea, hIP6K only partially restores telomere length to the length observed in ipk1Δ cells in the ipk1Δ kcs1Δ mutant cells, despite PP-IP4 synthesis equivalent to exogenous Kcs1 (HPLC data not shown). These results indicate a requirement for kinase activity and PP-IP4 production, as well as a possible role for a non-catalytic protein component, such as a determinant that mediates proper localization or regulation.

**Kcs1 Regulation of Telomeres Requires Tel1**—With this evidence for PP-IP4 as a regulator of telomere length, we used genetics to identify other components of the novel PP-IP4 pathway. Because of the initial report that IP6 interacts with DNA-dependent protein kinase, we considered Tel1 and Ku70/80 to be reasonable effector candidates, because both have important yet independent roles in maintenance of the yeast telomere (34). We predicted that the effect of PP-IP4 would be absent in cells missing a critical component of the IP effector pathway.

Analysis of yku70 mutant cells indicates an average telomere length of −150 bp (Fig. 5A), consistent with published reports. Double (ipk1Δ yku70Δ and kcs1Δ yku70Δ) and triple (ipk1Δ kcs1Δ yku70Δ) mutant strains were constructed and tested for telomere length (Fig. 5A). In a yku70Δ background, the loss of Kcs1 function again lengthens telomeres about 50 bp (average length of 200 bp) consistent with Ku and Kcs1 functioning in independent pathways. To probe the gain of the PP-IP4 effect on shortening telomeres in the absence of Ku, we examined telomere length in both yku70Δ ipk1Δ (Fig. 5A) and yku70Δ ipk1Δ overexpressing Plc1 (not shown). Neither condition resulted in further shortening beyond the 150-bp length. Furthermore, we did not find evidence of recombination or senescence to suggest telomeres have reached a critical length, indicating either the gain of function effect cannot be reproduced in the setting of short telomeres or is dependent on the Ku pathway.

In contrast, all effects of PP-IP4 at the telomere are abolished in the absence of Tel1. Both the double (ipk1Δ tel1Δ and kcs1Δ tel1Δ) and triple (ipk1Δ kcs1Δ tel1Δ) mutant strains have stable, shortened telomeres indistinguishable from tel1Δ cells (Fig. 5B). This result indicates that the IPs function in the same pathway of telomere regulation as this ATM homologue. Because loss of Tel1 function leads to short telomeres, the data are consistent with Kcs1 production of PP-IP4 acting as a negative regulator of Tel1 function.

Collectively, these results implicate phospholipase C-dependent PP-IP4 production through Kcs1 activity as a negative regulator of telomere length.

To test whether increases in PP-IP4 production would lead to further telomere shortening, we overexpressed PLC1 in several mutant strains, which (based on our previous studies) significantly elevates IP production in cells (12). Wild-type strains overexpressing PLC1 have increased IP6 levels (20-fold), no detectable increase in PP-IP4 levels, and normal telomere length (Fig. 3B). In contrast, ipk1Δ cells overexpressing PLC1 have significantly elevated levels of PP-IP4 (not shown) and undergo further shortening of telomeres to an average length of 265 bp from the 325 bp observed in wild type (Fig. 3B). When Kcs1 is eliminated from this strain, the cells again have a long telomere phenotype (Fig. 3B). These data are consistent with a gain of function effect mediated by PP-IP4. Comparing the telomere lengthening in the absence of inositol diphosphates and the shortening seen with increases in P1c1- and Kcs1-mediated production of PP-IP4, we see a range of modulation of telomere length of over 120 bp, accounting for roughly one-third of the total wild-type telomere length (Fig. 3B).

To confirm that inositol kinase activity is required for normal telomere length, we examined telomeres in kcs1Δ strains complemented with either wild-type Kcs1, kinase-inactivated Kcs1 (kcs1kin−), or a human inositol diphosphoryl synthase (hIP6K) (Fig. 4). Both Kcs1 and hIP6K expression complement telomere length in the kcs1Δ strain, whereas Kcs1 kinase-inactive ex-vivo.
Kcs1 kinase activity, which do synthesize PP-IP₅ by way of Ids1 activity. Because ipk1Δ-null cells with no PP-IP₅ have the opposite phenotype, loss of PP-IP₅ cannot explain the longer telomeres observed. Therefore the kinase activity of Kcs1 must be producing something else, proximal to IP₆ on the IP pathway, which is needed for normal telomere length. Because IP₅ is a known substrate for Kcs1, the simplest interpretation is that PP-IP₄ loss leads to longer telomeres. We cannot formally exclude PP₂-IP₃ and other molecules downstream of PP-IP₄ or Kcs1 phosphorylation of another inositol substrate such as IP₄ as the molecules acting at the telomere. Importantly, we also report that further increases in PP-IP₄ synthesis lead to further shortening of telomeres. The opposing effects of gain and loss of PP-IP₄ demonstrate that this signaling molecule regulates the equilibrium of telomere length. These dual criteria also distinguish Kcs1 and its products from a number of reported genes involved in the maintenance of telomeres in yeast, most of which have only been reported to either lengthen or shorten telomeres but not both. Such regulation by a signaling-activated intracellular messenger pathway puts telomere maintenance under the potential control of extracellular stimuli, providing a mechanism to alter the average telomere length based on growth conditions or other environmental inputs.

Because neither the loss nor gain of PP-IP₄ has any impact on telomere length in the absence of Tel1, our work also provides a novel functional interaction between IPs and the activity of a PIKK family member, linking two important nuclear signaling pathways. Given that loss of Tel1 and increase in PP-IP₄ both lead to shortened telomeres, the simplest explanation is that PP-IP₄ inhibits Tel1 kinase function, either through direct or indirect kinase inhibition, consistent with Tel1 being a downstream component of this IP pathway. Assigning Kcs1 a

FIG. 4. Complementation of telomere phenotype requires Kcs1 kinase activity. ipk1Δ or ipk1Δ kcs1Δ cells were transformed with plasmids encoding various inositol diphosphoryl synthases behind a GAL10 promoter. Cultures were grown in complete minimal medium-Ura with either dextrose (D) or galactose (G) as carbon source, and telomeric sequences were probed as described under “Experimental Procedures.” The plasmids, from left to right, are pKCS1 (encoding wild-type Kcs1), pVEC (empty vector control), pkcs1kinΔ (encoding an active site point mutant of Kcs1), and pIP6K (encoding a human inositol diphosphoryl synthase). The length of wild type (wt) telomeres is indicated by the oval and arrow on right. nd, not detected.

FIG. 5. Regulation of telomere length by Kcs1 is independent of Ku but dependent on Tel1. A, epistasis analysis of KCS1, IPK1, and YKU70 at the telomere. B, epistasis analysis of KCS1, IPK1, and TEL1 at the telomere. For both panels, single, double, and triple mutant strains were generated from spores grown for at least 100 doublings prior to the Southern analysis shown. Genotypes are indicated below each lane. The length of wild-type (wt) telomeres is indicated by the oval and arrow on right.
role as a negative regulator of Tel1 activity is consistent with earlier reports of the initial cloning of KCS1 as a second site suppressor of a mitotic recombination phenotype (20) because loss of Tel1 function increases recombination rates (33). Tel1 is not the only potential target of IP regulation as other candidate effectors in this pathway of telomere regulation include the Mre11-Rad50-Xrs2 protein complex components, but their precise biochemical roles at the telomere remain elusive. Because the Mre11-Rad50-Xrs2 complex as well as Tel1 favors lengthening telomeres, they must somehow favor telomerase access to the telomere end, and it is likely that the activity of Kcs1 opposes that access, thus changing the equilibrium to favor average loss of telomere sequence. Given the reported roles for Kcs1 and inositol diphosphates in cellular signaling events, we cannot rule out at this point that other processes are involved. Other important regulators may be gleaned from recent studies describing a genome-wide screen for deletion mutants with a telomere phenotype that yielded many mutants with length alterations similar to the plc1 and kcs1 phenotypes (39). The components of the PP-IP₄ pathway were not identified in this laborious screen; however, the authors concede they failed to identify one-third of the described previously deletion strains for strain construction.

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