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Inositol pyrophosphates modulate cell cycle independently of alteration in telomere length

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ABSTRACT

Synthesis of inositol pyrophosphates through activation of Kcs1 plays an important role in the signalling response required for cell cycle progression after mating pheromone arrest. Overexpression of Kcs1 doubled the level of inositol pyrophosphates when compared to wild type cells and 30 min following the release from α-factor block further increase in inositol pyrophosphates was observed, which resulted that cells overexpressing Kcs1 reached G_2/M phase earlier than wild type cells. Similar effect was observed in *ipk1Δ* cells, which are unable to synthesize IP_6-derived inositol pyrophosphates (IP_7 and IP_8) but will synthesize IP_5-derived inositol pyrophosphates (PP-IP_4 and $(PP)_2$-IP_3). Although *ipk1Δ* cells have shorter telomeres than wild type cells, overexpression of Kcs1 in both strains have similar effect on cell cycle progression. As it is known that PP-IP_4 regulates telomere length through Tel1, inositol polyphosphates, cell cycle and telomere length were determined in *tel1Δ* cells. The release of the cells from α-factor block and overexpression of Kcs1 in *tel1Δ* cells produced similar effects on inositol pyrophosphates level and cell cycle progression when compared to wild type cells, although *tel1Δ* cells possesses shorter telomeres than wild type cells. It can be concluded that telomere length does not affect cell cycle progression, since cells with short telomeres (*ipk1Δ* and *tel1Δ*) progress through cell cycle in a similar manner as wild type cells and that overexpression of Kcs1 in cells with either short or normal telomeres will increase S phase progression without affecting telomere length. Furthermore, IP_5-derived inositol pyrophosphates can compensate for the loss of IP_6-derived inositol pyrophosphates, in modulating S phase progression of the cell cycle.

1. Introduction

In mammalian cells, the activation of several isoforms of phosphoinositide-specific phospholipase C (Plc) has been demonstrated in nuclei during proliferation and synchronous progression through the cell cycle. However, downstream

Abbreviations: Plc, phospholipase C; IP_3, inositol trisphosphate; IP_4, inositol tetrakisphosphate; IP_5, inositol pentakisphosphate; IP_6, inositol hexakisphosphate; IP_7 (also referred as PP-IP_5), diphosphoinositol pentakisphosphate; IP_8 [also referred as $(PP)_2$-IP_4], bisdiphosphoinositol tetrakisphosphate; PP-IP_4, diphosphoinositol tetrakisphosphate; $(PP)_2$-IP_3, bisdiphosphoinositol trisphosphate; HPLC, high performance liquid chromatography.

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targets of Plc-generated second messengers in nuclei have remained poorly described (reviewed in Visnjic and Banfic, 2007; Follo et al., 2014). Divecha et al. (1993) first observed that there are many similarities between nuclear phospholipid signalling in mammals and phospholipid signalling in the budding yeasts *Saccharomyces cerevisiae* (Irvine, 2003). Budding yeasts have a sole Plc gene, PLC1, that can generate diacylglycerol and inositol 1,4,5-trisphosphate from phosphatidyl-inositol 4,5-bisphosphate, but they do not utilize diacylglycerol to activate protein kinase C and have no I(1,4,5)P_3 receptor in their genome to mobilize calcium. Instead, yeast use I(1,4,5)P_3 as a precursor for the synthesis of higher inositol phosphates, and these products have been proved to regulate important nuclear events in several genetic and biochemical studies of Plc-mediated signalling (Tsui and York, 2010). Therefore, the focus of interest has progressively shifted toward understanding of pathways responsible for generation of higher phosphates and pyrophosphates and their possible roles in eukaryotic cells.

In *S. cerevisiae*, four different kinases participate in generation of inositol phosphates and pyrophosphates. Ipk2 phosphorylates I(1,4,5)P_3 into I(1,4,5,6)P_4 (IP_4) and I(1,3,4,5,6)P_5 (IP_5). A major role of Ipk2 and its products, IP_4 and IP_5, is transcriptional regulation by modulation of the chromatin remodelling in response to nutrients (Odom et al., 2000; Steger et al., 2003). The yeast Ipk1 and its product, IP_6, which is generated by phosphorylation of IP_5, regulate mRNA export through conjunction with Gle1 which regulates the activity of the DEAD-box protein Dbp5 at the nuclear pore complexes cytoplasmic face (York et al., 1999; Folkman et al., 2014) and nonhomologous end joining (Hanakahi et al., 2000). The synthesis of diphosphoinositol phosphates or pyrophosphates in yeasts is mediated by two kinases: Kcs1 phosphorylates IP_5 into 5-PP-IP_4, and IP_6 into 5-PP-IP_5 (IP_7); Vip1 phosphorylates IP_6 into IP_7 isomer 1-PP-IP_5. $(PP)_2$-IP_4 (IP_8) is generated by either Kcs1-mediated phosphorylation of 1-PP-IP_5 or Vip1-mediated phosphorylation of 5-PP-IP_5 (Tsui and York, 2010). Kcs1 has been reported to modulate telomere length by generation of 5-PP-IP_4 (Saiardi et al., 2005; York et al., 2005), and Vip1-generated IP_7 binds to the cyclin-CDK-CDK inhibitor complex to regulate transcription of the yeast phosphate (Pi)-responsive (PHO) genes (Lee et al., 2007).

Our recent study demonstrated changes in inositol phosphate levels in α-factor-treated *S. cerevisiae,* which allows cells to progress synchronously through the cell cycle after release from a G_1 block. The results of the study showed an increase in the activity of Plc1 early after release from the block with a concomitant increase in the level of IP_7 and IP_8. The enzymatic activity of Kcs1 *in vitro* and HPLC analysis of ^{3}[H]inositol-labelled *kcs1*Δ cells confirmed that Kcs1 is the principal kinase responsible for generation of pyrophosphates in synchronously progressing cells. Furthermore, disruption of the *DDP1* gene, which encodes a phosphatase that dephosphorylates both IP_8 and IP_7 down to IP_6, increased the level of pyrophosphates following release from α-factor block. While *ddp1*Δ cells reached G_2/M phase earlier than wild type cells, FACS analysis of α-factor synchronized *plc1*Δ and *kcs1*Δ yeast mutants revealed a block in S phase, suggesting that progression through the S phase may be regulated by inositol pyrophosphates (Banfic et al., 2013).

In this study we have investigated how overexpression of Kcs1 affects level of inositol pyrophosphates and progression of cells through the cell cycle. Furthermore, as *ipk1Δ* cells are unable to synthesize IP_6, IP_7 or IP_8, but will accumulate alternative inositol pyrophosphates, PP-IP_4 and $(PP)_2$-IP_3 and have telomeres slightly shorter than wild type (Saiardi et al., 2005; York et al., 2005), therefore herein we have investigated how overexpression of Kcs1 affects the level of alternative inositol pyrophosphates, telomere length and progression of cells through the cell cycle.

2. Materials and methods

2.1. Materials

2.1.1. Reagents were obtained from the following sources

All media and supplements for yeast growth from Formedium; α-factor mating pheromone from Zymo Research; glass beads (diameter 425–600 μm), RNase A and proteinase K from Sigma; [^{3}H]inositol (30 Ci/mmol) from Perkin Elmer or American Radiolabeled Chemicals and Sytox from Invitrogen. All other chemicals were of analytical grade.

2.2. Yeast strains and growth condition

S. cerevisiae strains used in this study are isogenic with W303 MAT**a** (*leu2-3,112*, *his3-11,15*, *ura3-1*, *ade2-1*, *trp1-1*, *rad5-535*, *can1-100*). Deletion strains *ipk1*:*KANMX*, *tel1*:*ura3*, as well as strains in which Kcs1 was overexpressed were generated as described previously (York et al., 2005). For labelling experiments, cells were inoculated in synthetic medium without inositol with addition of 5.7 mg/ml ammonium sulphate, 0.82 mg/ml amino acids, 2% glucose, and 5 μCi/ml ^{3}[H]inositol (labelling medium) and grown to achieve isotopic equilibrium (8–9 divisions) in a shaker incubator at 30 °C and 200 r.p.m. (Banfic et al., 2013). At the end of incubation, cells were collected, counted, resuspended in fresh labelling medium containing 2% galactose at the concentration of 2.5×10^6/ml, grown into mid-logarithmic phase, and then incubated in the presence of 5 μM α-factor mating pheromone for 6 h. The G_1-arrested cells were washed twice with synthetic medium lacking inositol, released into fresh labelling medium containing 2% raffinose, and allowed to progress synchronously through the cell cycle. At the indicated time points, cells were harvested by centrifugation (2000 × g, 4 °C, 4 min) For flow cytometric analysis and telomere length determination, cells were grown as described above, except that cold inositol was added into the medium at the same concentration as ^{3}[H]inositol.