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Inositol phosphate multikinase dependent transcriptional control

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ABSTRACT

Production of lipid-derived inositol phosphates including IP_4 and IP_5 is an evolutionarily conserved process essential for cellular adaptive responses that is dependent on both phospholipase C and the inositol phosphate multikinase Ipk2 (also known as Arg82 and IPMK). Studies of Ipk2, along with Arg82 prior to demonstrating its IP kinase activity, have provided an important link between control of gene expression and IP metabolism as both kinase dependent and independent functions are required for proper transcriptional complex function that enables cellular adaptation in response to extracellular queues such as nutrient availability. Here we define a promoter sequence cis-element, 5′-CCCTAAAAGG-3′, that mediates both kinase-dependent and independent functions of Ipk2. Using a synthetic biological strategy, we show that proper gene expression in cells lacking Ipk2 may be restored through add-back of two components: IP_4/IP_5 production and overproduction of the MADS box DNA binding protein, Mcm1. Our results are consistent with a mechanism by which Ipk2 harbors a dual functionality that stabilizes transcription factor levels and enzymatically produces a small molecule code, which together coordinate control of biological processes and gene expression.

1. Introduction

Inositol phosphates (IPs) are conserved eukaryotic signaling molecules implicated in the regulation of many biological processes, including the export of mRNA from the nucleus, embryonic development, signaling, and responses to environmental stress (Wilson et al., 2013; Lee et al., 2012; Folkmann et al., 2011; Chakraborty et al., 2011; Hatch and York, 2010; Michell, 2008; Bhandari et al., 2007; Irvine and Schell, 2001; Majerus, 1992). In the yeast *Saccharomyces cerevisiae*, the generation of IPs begins with a single phospholipase C enzyme (Plc1) that hydrolyzes the lipid phosphatidylinositol 4,5-bisphosphate (PIP_2) to release the soluble head group inositol 1,4,5-trisphosphate (IP_3) from diacylglycerol (DAG) (Flick and Thorner, 1993; York et al., 1999). Inositol phosphate multikinase, Ipk2 (also known as IPMK and Arg82), converts IP_3 to inositol 1,4,5,6-tetrakisphosphate (IP_4) and inositol 1,3,4,5,6-pentakisphosphate (IP_5) by sequential phosphorylation at the 6- and 3-positions, respectively (York et al., 1999; Odom et al., 2000; Saiardi et al., 1999). IP_5 is converted to inositol 1,2,3,4,5,6-hexakisphosphate (IP_6), the most abundant species in wild-type *S. cerevisiae*, by the activity of Ipk1 (York et al., 1999; Ives et al., 2000). The abridged yeast lipid-dependent inositol phosphate pathway (Fig. 1A) is evolutionarily conserved (Hatch

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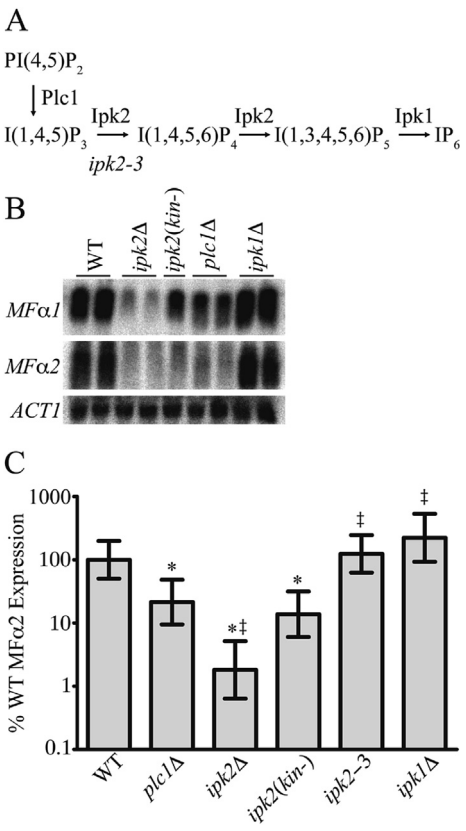

Fig. 1. Ipk2 and IPs are required for efficient MFα2 expression. A) Diagram outlining the IP production pathway in *S. cerevisiae*. Diphosphate species and phosphatases have been omitted for simplicity. B) Northern blot analysis of MFα1 and MFα2 expression in WT and IP pathway mutant strains. *ACT1* is shown as a loading control. C) Real-time PCR quantification of MFα2 expression levels in WT and IP pathway mutant strains. Values are the mean ± SD of at least three independent cultures, * indicates significant difference from WT expression ($p < 0.05$), and ‡ indicates significant difference from *plc1*Δ ($p < 0.05$).

and York, 2010); however in some eukaryotes Ipk2 exhibits distinct "multikinase" activities that lead to more than one metabolic pathway leading to formation of IP_6 (Hatch and York, 2010; Otto et al., 2007; Verbsky et al., 2005; Stevenson-Paulik et al., 2005; Seeds et al., 2004).

The discovery that Ipk2 encodes an inositol phosphate kinase isogenic to the transcriptional regulator Arg82 (ArgRIII complementation group) provided an unanticipated link between inositol phosphate signaling and the control of gene expression (Odom et al., 2000). Prior to defining its enzymatic function, studies of Arg82 defined its role as a transcriptional regulator in response to nutrient conditions of arginine as the sole nitrogen source (El Bakkoury et al., 2000; Dubois and Messenguy, 1994; Messenguy and Dubois, 1993; Messenguy et al., 1991; Qiu et al., 1990; Dubois et al., 1987; Bercy et al., 1987; Delforge et al., 1975; Bechet et al., 1970). Arg82 is a member of the ArgR-Mcm1 transcription complex that regulates the transcription of genes in the arginine metabolic pathway. The ArgR-Mcm1 complex consists of four members, Mcm1, Arg80, Arg81 and Ipk2 (Messenguy and Dubois, 1993). Mcm1 is a founding member of the family of MADS (Mcm1, Agamous, Deficients, Serum Response Factor) box transcription factors, and it is essential for cell viability (Messenguy and Dubois, 2003; Treisman and Ammerer, 1992). Arg80 is also a MADS box transcription factor, but its function appears to be limited to the regulation of arginine metabolic genes (Messenguy and Dubois, 2003). Together, Mcm1 and Arg80 are able to bind elements in the promoters of arginine-responsive genes, but when excess arginine is present in the medium, Arg81, the arginine sensor, and Arg82 also assemble on DNA to form the complete complex (Yoon et al., 2004). In addition, studies of metazoan Ipk2/IPMK protein suggest an evolutionarily conserved function as a partner with a variety of nuclear proteins to regulate nutrient responses and gene expression (Kim et al., 2016; Xu and Snyder, 2013; Xu et al., 2013a,b; Kim et al., 2013; Wu et al., 2011; Kim and Snyder, 2011; Kim et al., 2011).

In the past decade studies have documented Ipk2's role in regulating gene expression occurs through both kinase-dependent and independent effects. Kinase-dependence and production of IP products has been shown to be required for a number of transcriptional responses to nutrient changes, including amino acids, phosphate and nitrogen (Odom et al., 2000; Guzinska et al., 2009; Steger et al., 2003; El Alami et al., 2003). Additionally, conditional-phenotypes observed in *ipk2* null yeast, including temperature sensitivity and growth on arginine as the sole nitrogen source, are complemented by heterologous expression of either plant, fly or metazoan Ipk2 in a kinase-dependent fashion, consistent with an essential role for its

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