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Maize protein kinase CK2: regulation and functionality of three β regulatory subunits

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Summary

Biochemical and crystallographic data suggest that, in contrast with other organisms, the active maize protein kinase CK2 might be composed simply of a catalytic polypeptide (CK2α), thus lacking CK2β regulatory subunits. To investigate the existence and functionality of CK2β regulatory subunits in *Zea mays*, we have screened a maize cDNA library using different approaches and have isolated three full-length cDNAs encoding CK2β regulatory subunits (CK2β-1, CK2β-2 and CK2β-3) and a cDNA coding for a novel CK2α catalytic subunit, CK2α-3. The pattern of expression of all these α/β subunits has been studied in different organs and developmental stages using specific probes for each isoform, and indicates that while CK2α subunits are constitutive, CK2β subunits are expressed differentially during embryo development. The yeast two-hybrid system and pull-down assays have been used to study specific interactions between the different subunits. While CK2α subunits are unable to self-associate, preferential interactions between α/β isoforms and β/β isoforms can be predicted. Furthermore, we show that maize CK2α/β subunits assemble into a structural tetrameric complex which has very similar properties to those described in other organisms, and that expression of maize CK2β subunits in yeast allows the rescue of the phenotypic defects associated to the lack of CK2 function, thus demonstrating the functionality of maize CK2β regulatory subunits.

Keywords: protein kinase CK2, CK2β regulatory subunits, two-hybrid system, pull-down assays, autophosphorylation, salt tolerance.

Introduction

Protein kinase CK2 is a ubiquitous and highly conserved Ser/Thr kinase present in the nucleus and cytoplasm of all eukaryotic cells examined to date (Guerra and Issinger, 1999; Guerra *et al.*, 1999). It is well known that CK2 can use both ATP and GTP as phosphate donors, shows a preference for acidic protein substrates, is not responsive to known second-messenger molecules, and is able to phosphorylate more than 160 substrates; however its precise biological functions remain unknown. The CK2 enzyme has been widely studied in several organisms, demonstrating that is involved in different processes such as cell proliferation (Seldin and Leder, 1995); cell-cycle progression (Espunya *et al.*, 1999; Hanna *et al.*, 1995); signal transduction (Chen *et al.*, 1997); or transcriptional control (Lüscher *et al.*, 1989). In plants, CK2 appears to be involved in light-regulated gene expression and plant growth (Lee *et al.*, 1999), and the enzyme is able to phosphorylate a number of transcription factors such as GBF1 (Klimczak *et al.*, 1992), the transactivation factor AT-1 (Roux, 1993), or CCA1 (Sugano *et al.*, 1998; Sugano *et al.*, 1999). In maize, the abscisic acid- and water stress-responsive phosphoprotein Rab17 has been shown to be phosphorylated *in vitro* by CK2 (Goday *et al.*, 1994; Plana *et al.*, 1991). Therefore CK2 activity is likely to be involved in a large number of relevant cellular processes in plants.

In mammals and yeast, the enzyme is a heterotetramer composed of two types of subunit, giving rise to different