forms: $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, $\alpha'_2\beta_2$. The $\alpha$ (42–44 kDa) and $\alpha'$ (38 kDa) subunits are catalytically active by themselves, and are structurally related, although they are encoded by different genes (Litchfield *et al.*, 1990). The CK2$\alpha$ subunits are highly conserved among different species and are closely related to the cdc2 group of protein kinases. In plants, two cDNA clones have been identified in *Arabidopsis thaliana* that encode proteins 72% identical to the human CK2$\alpha$ catalytic subunit (Mizoguchi *et al.*, 1993), whereas in maize two clones have been described to date (Dobrowolska *et al.*, 1991; Peracchia *et al.*, 1999) that are also highly similar to the human CK2$\alpha'$ subunit.

The CK2$\beta$ regulatory subunits (26–40 kDa) present no homology to regulatory subunits or domains of other protein kinases, except to the *Drosophila melanogaster Stellate* gene product (Livak, 1990). This subunit presents three main properties: it is inactive by itself but can stimulate CK2$\alpha$ catalytic activity (Grankowski *et al.*, 1991); it confers stability to the enzyme (Meggio *et al.*, 1992); and it provides specificity for the interaction with substrates and inhibitors (Bidwai *et al.*, 1993). Whereas in most organisms only one gene for CK2$\beta$ has been described, two genes (*CKB1* and *CKB2*) have been identified in *Saccharomyces cerevisiae* (Bidwai *et al.*, 1994). At least two genes exist in *D. melanogaster* (Bidwai *et al.*, 1999; Saxena *et al.*, 1987), plus a $\beta$-like protein encoded by the *Stellate* locus (Livak, 1990). In plants, three cDNA clones encoding CK2$\beta$ regulatory subunits have been identified in *A. thaliana* (Collinge and Walker, 1994; Sugano *et al.*, 1999).

Analysis of CK2 function has been carried out in the yeast *S. cerevisiae* by constructing mutants in the different subunits of the kinase. In this organism, simultaneous disruption of the *CKA1* and *CKA2* genes encoding $\alpha$ and $\alpha'$ catalytic subunits is lethal for the cell (Padmanabha *et al.*, 1990). Deletion of *CKB1* and/or *CKB2* genes coding for regulatory subunits does not affect yeast growth under normal conditions, but results in a phenotype of hypersensitivity to $Na^+$ and $Li^+$ cations (Bidwai *et al.*, 1995).

The level of identity between plant, yeast and human CK2$\beta$ regulatory subunits is not as high as in the case of CK2$\alpha$ subunits, making difficult to assess the presence of this type of protein in a given organism. In this regard there is controversy about the existence of CK2$\beta$ subunits in maize. For instance, antibodies raised against chicken CK2$\beta$ failed to recognize the presence of this protein in maize extracts (Dobrowolska *et al.*, 1992). Furthermore, the resolution of the crystal structure of *Zea mays* CK2$\alpha$ (Niefind *et al.*, 1998) showed that the enzyme is more stable than recombinant human CK2$\alpha$. This stability, and the high specific activity of the maize catalytic subunit, allow us to speculate that it can exist without the presence of CK2$\beta$. On the other hand, two forms of the maize enzyme were originally purified: CK2A, which appears to correspond to the typical heterotetramer; and CK2B, which is a monomeric form related to the catalytic subunit CK2$\alpha$ (Dobrowolska *et al.*, 1992). However, the properties of the monomeric form CK2B are different from those of the recombinant maize CK2$\alpha$ subunit, because CK2B is unable to assemble with human CK2$\beta$, whereas recombinant maize CK2$\alpha$ does (Battisttuta *et al.*, 2000; Boldyreff *et al.*, 1993).

In this paper we show the existence of CK2$\beta$ proteins in maize through the isolation of three cDNA clones corresponding to CK2$\beta$ subunits. In addition, a novel clone that encodes a third CK2$\alpha$ subunit is reported. The expression of the diverse maize $\alpha/\beta$ subunits has been studied during embryo development and in different plant organs. Using the two-hybrid system and pull-down assays, we have analysed the maize CK2 structure by testing the specific interactions between its subunits. We found that maize CK2 is active in the heterotetrameric form, and CK2$\beta$ is able to stimulate CK2 activity. Furthermore, maize CK2$\beta$ can function in yeast by complementation of the phenotypic defects associated to lack of CK2 function.

## Results

### *Isolation of a cDNA encoding a maize CK2β subunit*

The BLAST program (Altschul *et al.*, 1990) was used to screen the NCBI dbest database using the *A. thaliana* CK2B1 cDNA sequence (accession number L22563). One candidate maize EST clone (AA979779) of 585 bp was identified, obtained and used as a probe to screen a maize cDNA library. One clone of 1330 bp, named CK2$\beta$-1, was isolated and sequenced. The first ATG in position 208 is proposed as the translation start site. The ORF of 830 bp encodes a predicted protein of 276 amino acids, 30.5 kDa and pI = 5.16.

The amino acid sequence of CK2$\beta$-1 has been aligned with the sequences of CK2$\beta$ from *Homo sapiens, D. melanogaster, A. thaliana* and *S. cerevisiae* (Figure 1). *Arabidopsis thaliana* CK2$\beta$ subunits present an $NH_2$-terminal extension of ≈90 amino acids that shares no homology to other known proteins and is not present in the other CK2$\beta$ sequences. This region is also present in maize CK2$\beta$-1 and retains a significant level of amino acid identity (55%). Within the rest of the molecule, maize CK2$\beta$-1 is highly similar to other CK2$\beta$ proteins: it presents 84% identity with *A. thaliana* CK2B1 and 56% identity with human CK2$\beta$ in the central and COOH-terminal regions. However, at the COOH terminus, 22 residues that are conserved in *Drosophila, Xenopus* and mammals are absent in maize and *Arabidopsis* CK2$\beta$ subunits. The consensus site present in the COOH-terminal region in human CK2$\beta$, which is phosphorylated by $p34^{cdc2}$ (Litchfield *et al.*, 1991), is not present in maize CK2$\beta$-1.