originally purified (Dobrowolska *et al.*, 1992): CK2A, which seems to correspond to the typical heterotetrameric structure; and CK2B, a monomeric form related to the catalytic subunit CK2α.

Recent studies demonstrate the functional specialization of CK2 isoforms in mammalian cells (Vilk *et al.*, 1999). The existence of several forms of CK2α/β in maize raises the possibility that differential expression and/or functional differences could be responsible for the regulation of enzyme activity through specific interaction with substrates or assembly of the holoenzyme. We have analysed possible differences in expression during embryo development of all CK2 subunits. Our data indicate that CK2β is present during all stages of development, but CK2β-1 shows higher levels of expression during late embryogenesis, whereas CK2β-2 and CK2β-3 are more prominent in the earlier and intermediates stages of development. The correlation observed between CK2β-1 and *rab*17 expression during late embryogenesis raised the possibility that Rab17 might be phosphorylated *in vivo* by a CK2 enzyme containing the CK2β-1 subunit. However, while this possibility may still hold true, no correlation between water stress and CK2β-1 expression was observed in vegetative tissues.

The results obtained for the CK2α isoforms indicate preferential expression of the three isoforms in the earlier stages of development, and suggest that CK2 may play an important role in plant embryogenesis. A similar pattern of expression was found in animals; it has been reported that expression and activity of CK2 are high during early embryogenesis and decrease in the latter stages (Hu and Rubin, 1990; Maridor *et al.*, 1991).

Data obtained using yeast two-hybrid system and pull-down assays indicate that all three CK2β subunits can interact with other CK2α and CK2β subunits, and are therefore potentially able to compose the typical heterotetrameric structure previously described for other CK2 enzymes. The three maize CK2α subunits present a 96% of identity at the amino acid level; for this reason differences obtained in CK2α/β interactions were surprising, especially in the case of CK2α-1/CK2β-2. However, the results obtained were confirmed by both methods. To better understand these variations, further work is needed to determine which amino acids are involved in specific interactions between CK2 subunits. Our results indicate that CK2β-2 is the only isoform that is fully unable to interact with itself. Interestingly, in CK2β-2 the Val^{212} present in the other maize CK2β subunits is changed to Ala^{212}. As this change affects the cysteine-rich motif, a region determining the stable homodimerization of the protein (Chantalat *et al.*, 1999), it is tempting to speculate that this conserved amino acid change might be responsible for the failure to form CK2β-2/CK2β-2 homodimers. We also observed that the maize CK2α subunits are unable to self-associate, a circumstance that has been described for human CK2 (Gietz *et al.*, 1995).

Functional expression experiments in budding yeast demonstrate that maize CK2β regulatory subunits can replace the yeast CKB1 protein, encoding a yeast CK2 regulatory subunit. This has been proved by testing two different, and apparently independent, phenotypes: the rescue of the saline hypersensitivity characteristic of yeast strains lacking CK2 regulatory subunits; and the compensation of the temperature–sensitive growth defect due to presence of the *cka2-8* allele as the sole source of CK2 catalytic activity. The latter phenotype can be rescued, not only by expression of CK2α subunits from different species (Bidwai *et al.*, 1992), but also by overexpression of the yeast *CKB1* subunit (Hanna *et al.*, 1995). The mechanisms for this compensation are currently unknown, although it has been proposed that the CK2β subunit may interact with the defective protein to restore its function. In any case, such a functional conservation is remarkable considering that the level of identity between maize CK2β-1 and yeast CKB1 is barely above 40% at the amino acid level.

We have also demonstrated that when maize CK2α/β subunits are mixed in equimolar proportions, the enzyme can be reconstituted in the heterotetrameric form, as assessed by CK2β autophosphorylation. The functional significance of autophosphorylation is not well understood, but it is suspected to be involved in tuning of the kinase activity (Lin *et al.*, 1994). Maize CK2β subunits contain more putative phosphorylation sites (Figure 2a) than the human counterpart; therefore maize CK2 appears to be suitable for studying the relevance of CK2β autophosphorylation. The reconstitution of maize holoenzyme results in a stimulation of the catalytic activity of CK2α towards the Rab17 and β-casein substrates, confirming that maize CK2β subunits are not only structural but also functional homologues of the other CK2β previously described.

Experimental procedures

Plant material

Embryos of maize (*Zea mays*) pure inbred line W64A were collected before pollination (BP) and at 1,4,7,10,20, 30, and 40 days after pollination (DAP). Maize seedlings grown for 3 days were used to obtain leaf and root tissues. Water-stress treatments of maize seedlings were performed as described previously (Gómez *et al.*, 1988).

Northern analysis

For Northern blot analysis, total RNA was prepared from roots, leaves and wild-type embryos at different stages of development by phenol extraction, as previously described (Busk and Pagès, 1997). RNA (25 μg per lane) was separated in 1.5% agarose-