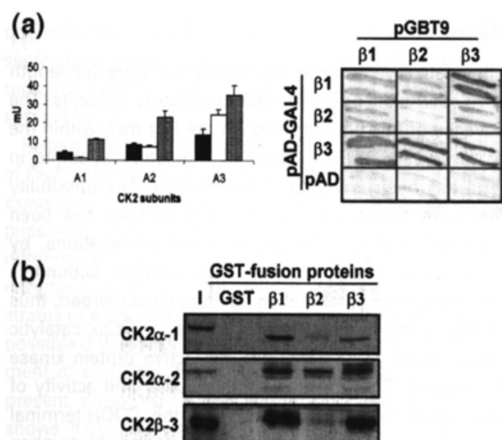

Figure 4. Interactions between CK2 subunits.
(a) Interactions between CK2 subunits in yeast. Left, graphic representation of β-galactosidase activity measurement in liquid assay. Values are expressed as Miller units and are the mean standard deviation from three independent assays: A1, pAD-GAL4-CK2-α1; A2, pAD-GAL4-CK2-α2; and A3, pAD-GAL4-CK2-α3 were tested for interaction with maize pGBT9-CK2β1 (solid bars); pGBT9-CK2β2 (open bars) or pGBT9-CK2β3 (shaded bars). Right, β-galactosidase assay performed on a filter. Different CK2β subunits were expressed with binding domain, pGBT9 vector (left) or activation domain, pGAD424 vector (top). Each panel shows duplicate patches of yeast expressing two CK2β subunits. Dark colour indicates β-galactosidase activity after 4 h incubation with substrate.
(b) *In vitro* interactions with CK2 subunits. Autoradiography shows *in vitro* interactions among translated [^{35}S]methionine-labelled CK2α-1, CK2α-2, CK2β-3, and GST alone, GST-CK2β subunit fusion proteins. Input (I) represented 10% of the [^{35}S]methionine-labeled proteins.

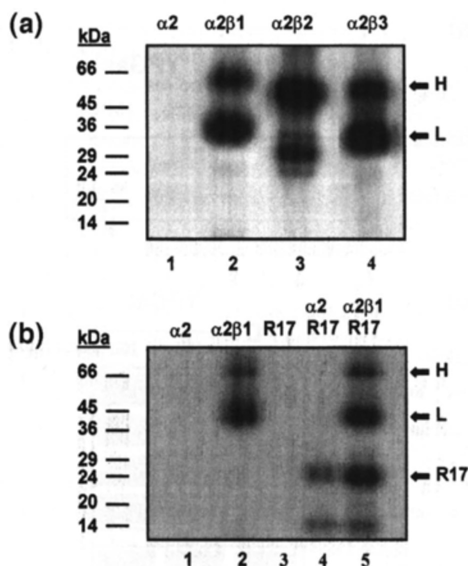

Figure 5. Analysis of maize CK2 activity.
(a) Autophosphorylation of maize CK2β subunits *in vitro*. Autoradiography of phosphorylated CK2β subunits. Lane 1, CK2α-2; lane 2, CK2α-2 and CK2β-1; lane 3, CK2α-2 and CK2β-2; lane 4, CK2α-2 and CK2β-3. H, High molecular weight proteins correspond to recombinant proteins CK2β subunits (MW ≈30 kDa) fused to GST proteins (29 kDa); L, low molecular weight proteins, intermediate products of GST fusion proteins.
(b) *In vitro* phosphorylation of Rab17 by CK2. Lane 1, CK2α-2 alone; lane 2, CK2α-2 and CK2β-1; lane 3, Rab17 alone; lane 4, CK2α-2, CK2β-1 and Rab 17; lane 5 CK2α-2, CK2β-1, and Rab17.

subunits are unable to interact with each other (data not shown).

To confirm the *in vitro* specific binding among the CK2 subunits, we employed pull-down assays with GST-CK2β subunit fusion proteins and *in vitro*-translated [^{35}S]methionine-labelled CK2α/β subunits (Figure 4b). The results obtained for CK2α/β interactions were consistent with the yeast two-hybrid system experiments. Strong CK2β/β interactions previously detected were confirmed using pull-down assays, whereas results corresponding to CK2β-2/CK2β-3 indicated a weak interaction.

Functionality of maize CK2

To determine if maize CK2 enzyme is able to autophosphorylate its own CK2β subunits, we expressed all three CK2β subunits as GST-fusion proteins, and CK2α-2 using the His-tagged system. The enzyme was reconstituted assembling CK2α-2 with the three CK2β subunits and, as shown in Figure 5(a), all CK2β subunits are strongly phosphorylated. The high molecular weight proteins of ≈60 kDa (H) correspond to the CK2β subunits fused to GST proteins; there are also proteins of lower molecular weight, ≈30–40 kDa (L), which are also phosphorylated and may correspond to intermediate products of these fusion proteins. All phosphorylated proteins contain CK2β subunits because GST alone is not phosphorylated by CK2 (data not shown). Figure 5(b) shows the stimulatory effect of CK2β addition on CK2 activity of CK2α, using Rab 17 as substrate. It has been demonstrated previously that CK2α alone is able to phosphorylate Rab 17 *in vitro* (Goday *et al.*, 1994; Plana *et al.*, 1991); however, addition of the maize CK2β subunit enhances kinase activity towards the substrate, indicating the functionality of the heterotetrameric form. A similar effect has also been found using β-casein as a substrate (data not shown).

Furthermore, complementation experiments using yeast mutants were performed. As mentioned above, deletion of both *CKA1* and *CKA2* genes encoding the catalytic subunits of CK2 in *S. cerevisiae* results in a lethal phenotype. The presence of the *cka2-8* allele prevents lethality at the permissive temperature (30°C), although it does not allow growth at 37°C. Overexpression of yeast *CKB1*, however, allows growth at the restrictive temperature (Hanna *et al.*, 1995). As shown in Figure 6(a), whereas strain YDH8 is able to grow at 30°C both in glucose and galactose plates, only YDH8 cells transformed with pYES-CK2β1 could grow at