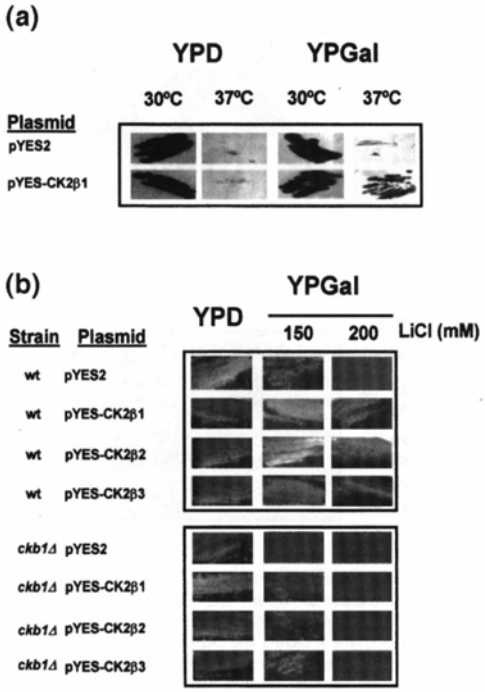

Figure 6. Functional expression of the maize CK2β regulatory subunit CK2β-1 in yeast.
(a) Compensation of the cka2-8 temperature-sensitive mutation by maize CK2β-1. Strain YDH8 was transformed with pYES2 vector and pYES-CK2β1, and incubated at 30 or 37°C in rich plates containing either glucose or galactose as carbon source. Growth was monitored after 5 days.
(b) Maize CK2β subunits are able to complement the LiCl sensitivity of a ckb1Δ mutant. The wild-type strain YPH499 and its isogenic strain MAR1 (*ckb1Δ*) were transformed with plasmids pYES2, pYES-CK2β1, pYES-CK2β2 and pYES-CK2β3, and grown in rich medium containing glucose (YPD) or galactose (YPGal) at the indicated concentrations of LiCl. Growth was monitored after 4 days.

37°C on galactose plates (which induces transcription of the *GAL1* promoter).

Yeast cells lacking one of the CK2β isoforms display a characteristic phenotype of increased sensitivity to sodium and lithium ions. Wild-type cells, as well as the hypersensitive strain MAR1 (*ckb1Δ*), were transformed with plasmids pYES-CK2β1, pYES-CK2β2 and pYES-CK2β3. Positive clones were grown on rich medium containing either glucose or galactose (to induce the *GAL1* promoter), in the presence of different concentrations of LiCl. As shown in Figure 6(b), expression of CK2β-1, CK2β-2 and CK2β-3 on a wild-type background increases tolerance to lithium ions, as has been observed on high-copy expression of yeast *CKB1* (data not shown). Overexpression of the three CK2β subunits also improved the tolerance of the hypersensitive *ckb1Δ* mutant. Taken together, these results suggest the functionality of all three maize CK2β subunits.

Discussion

In this paper we describe the isolation of three full-length maize cDNAs encoding for CK2β regulatory subunits. The relevance of this finding relies on the fact that, within the last few years, the existence of regulatory subunits in maize has been a source of controversy. The possibility that maize might not contain CK2β proteins has been supported, among other experimental observations, by the discovery that the maize CK2α catalytic subunit is significantly more stable than its human counterpart, thus leading to the notion that, in maize, a CK2α catalytic subunit alone could constitute the active protein kinase CK2 enzyme. The unusually high stability and activity of maize CK2α relies on the fact that the COOH-terminal region of the maize enzyme is 60 amino acids shorter than that of human CK2α (Boldyreff *et al.*, 1993). This might explain why maize CK2α is the only CK2 catalytic subunit crystallized to date (Niefind *et al.*, 1998).

Our results clearly demonstrate that multiple CK2β regulatory subunits do exist, and are expressed in maize. These subunits are able to interact with other CK2α and/or CK2β subunits, allowing the formation of the typical heterotetrameric structure described in all the organisms examined to date. The available data in maize (this work) and *A. thaliana* (Collinge and Walker, 1994; Sugano *et al.*, 1999) suggest that a high level of heterogeneity for the CK2β isoforms exists in plants. Moreover, a novel form of CK2α is described here in addition to the two previously reported isoforms. Because previous data suggested the possibility that a third CK2α subunit exists in *A. thaliana* (Mizoguchi *et al.*, 1993), it will be interesting to assess whether the existence of three CK2α subunits is a general characteristic for plants. In any case, our data demonstrate that the structure of CK2 in maize can be rather complex. In humans, the α′ subunit is 40 amino acids shorter than the α subunit in the carboxy-terminal region. It is interesting that this difference does not exist between maize CK2α subunits (CK2α-2 is simply one residue shorter than CK2α-1 and CK2α-3). Indeed, the sequences of plant CK2α subunits reported to date are more similar to human α′ than to the α subunit. This suggests that in plants, the CK2α family may be composed only by one type of subunit.

It has been reported (Chantalat *et al.*, 1999) that the last 33 residues of the human CK2β regulatory subunit were relevant for oligomerization of the tetramer, as deletion of this region reduced the intensity of its interaction with the CK2α catalytic subunit. It is noteworthy that all the three maize CK2β reported here lack 20 of the mentioned 33 residues. This might imply that, in maize, the interaction between CK2α/β subunits is weaker than in the case of the human CK2 holoenzyme, thus making possible the existence of the two forms of the CK2 enzyme that were