filters were washed twice at 65°C in 1 × SSC, 1% SDS and 3 times at 65°C in 0.1 × SSC, 0.1% SDS; as a result, the first CK2β subunit (CK2β-1) was isolated. Moreover, a low stringency screening was performed as described, except that filters were washed twice at 65°C in 1 × SCC, 1% SDS and 3 times at 0.5 × SSC, 0.1% SDS and a second CK2β subunit (CK2β-2) was cloned. Plasmids were excised from a homogeneous population of hybridizing phages into *E. coli* according to the manufacturer (Stratagene). The largest inserts were completely sequenced using the Automated Laser Fluorescent (ALF) system of Pharmacia.

Yeast two-hybrid screening

The maize CK2β-1 (pGBT9-CK2β1) was used as a bait for the two-hybrid screening of the HybriZap two-hybrid vector system library.

For screening, the Saccharomyces cerevisiae strain HF7c (MAT**a**, ura3-52, his3-200, ade2-101, lys2-801, trp1-901, leu2-3,112 gal4-542, gal80-538, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3,URA3::GAL4$_{17MERS(3X)}$-CYC1$_{TATA}$-LacZ) was transformed with the pGBT9-CK2β1 plasmid according to the manufacturer (Clontech matchmaker™). Positive clones were selected in plates lacking triptophan, and transformed with the HybriZap two-hybrid library. Transformants were selected in Leu$^-$, Trp$^-$, His$^-$ plates containing 1 mM 3-amino-1,2,4,-triazole. Purified colonies were tested for β-galactosidase activity using filter assays according to the manufacturer (Clontech matchmaker™). Plasmids from His$^+$ LacZ$^+$ colonies were isolated and electrophorated into *E. coli*, and the DNA sequence of the inserts was determined. Using this method, new maize CK2α subunits (CK2α-3) and CK2β subunits (CK2β-3) were isolated.

Results

CK2α subunits

In mammals and yeast the CK2 enzyme is a heterotetramer composed of two types of catalytic subunits, (α and α′) and regulatory subunits, (β) giving rise to different forms: $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, $\alpha'_2\beta_2$. The α (42–44 kDa) and α′ (38 kDa) subunits are catalytically active by themselves, and structurally related, although they are encoded by different genes [16]. The CK2α subunits are highly conserved among different species and are closely related to the cdc2 group of protein kinases. This high degree of conservation suggests that some of the main CK2 functions have to be conserved between the different species. CK2 function has been analyzed in *Saccharomyces cerevisiae* by constructing mutants for the different kinase subunits. In this organism, simultaneous disruption of the *CKA1* and *CKA2* genes encoding α and α′ catalytic subunits is lethal for the cell [17].

In plants, the composition of the CK2α family seems to be quite different. In *Arabidopsis thaliana* two cDNA clones have been identified that encode proteins 72% identical to the human CK2α′ catalytic subunit [9], and the same authors, based on the Southern analysis, suggest the existence of a third CK2α subunit. Furthermore, computational analysis of Arabidopsis genome, which has been completely sequenced, indicates the existence of at least four different genes encoding for CK2α subunits. In maize three clones have been described to date [8, 13, 18] that are also highly similar to the human CK2α′ subunit. In Fig. 1, plant CK2α subunits have been aligned with human CK2α and α′ sequences. The different plant CK2α subunits are very similar to each other; they present more than 90% of identity at amino acid level and almost the same length. Most of the different residues are located in the C-terminal region but the structural determinants defined for CK2α catalytic subunits are conserved in all the cases. Both Arabidopsis and maize present no significant homology between their CK2α genes in the 3′ noncoding region at the nucleotide level. According to the data, we can postulate that in plants the CK2α subunits belong to a multigenic family composed at least by three members but only by one type of subunit. The human CK2α′ is 40 amino acids shorter than the α subunit in the carboxyterminal region. It is interesting that the sequences of plant CK2α subunits, due to their length, are more similar to human α′ than α subunit. The C-terminal region of the maize enzyme is 60 amino acids shorter than human CK2α; this fact should explain the unusual high stability and activity of maize CK2α and the reason why maize CK2α is the only CK2 catalytic subunit crystallized to date [12]. Recently, several reports about the maize CK2α structure, have been published [19–21].

The maize CK2α gene corresponding to CK2α-1 is the only CK2 genomic sequence described in plants [18]. The maize genomic clone is 7.5 Kb long and contains 10 exons separated by 9 introns of different sizes. In the promoter region we can find typical elements of eukaryotic promoter motifs, such as TATA boxes, CAAT boxes or GC-boxes. However, further data on plant CK2 genomic sequences will be required to determine if the organization of the maize gene is shared in other plant species.

CK2β subunits

The CK2β 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 [22]. This CK2β subunit presents three main properties: it is inactive by itself but can stimulate CK2α catalytic