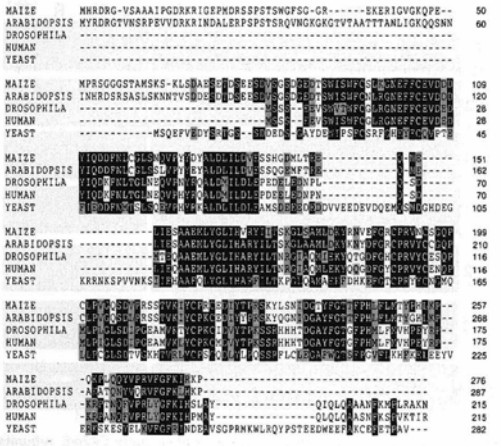

Figure 1. Alignment of the maize CK2β-1 protein with CK2β regulatory subunits from various species.
The amino acid sequence of maize CK2β-1 (accession number AF239816) is shown aligned with CK2β regulatory subunits of *Arabidopsis thaliana* (CK2B1, accession number L22563), *Drosophila melanogaster* (CK2β-1, M16532), *Homo sapiens* (CK2β, X16312) and *Saccharomyces cerevisiae* (CKB1, U21283). Alignment was created by using the CLUSTALW program. Invariant residues are indicated in reverse type, similar residues in shaded boxes, and dashes indicate gaps introduced to maximize alignment.

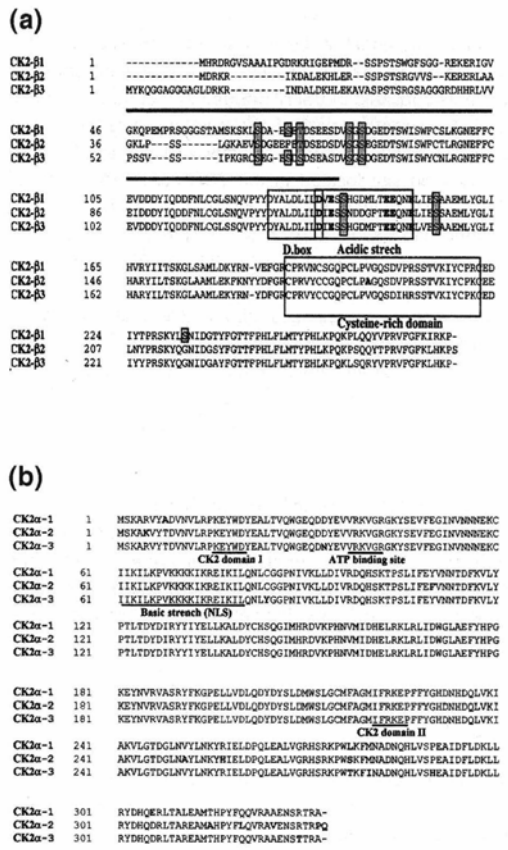

Figure 2. Multiplicity of maize CK2 subunits.
(a) Comparison of the deduced amino acid sequences of maize CK2β-1, CK2β-2 and CK2β-3. Underlined regions correspond to the NH_2-terminal extension characteristic of plant CK2β regulatory subunits. The destruction box consensus, acidic stretch and cysteine-rich motif are boxed. Serine and threonine residues that are putative autophosphorylation sites are indicated as shaded boxes. Functionally essential acidic residues (see main text) are indicated by bold letters.
(b) Comparison of the amino acid sequences of maize CK2α-1, CK2α-2 and CK2α-3. Characteristic domains of CK2α catalytic subunits are underlined. Residues that vary in one of the three sequences are indicated in bold and boxed type.

Isolation of CK2β-2, CK2β-3 and CK2α-3

Southern hybridization of total genomic DNA with the complete CK2β-1 cDNA showed multiple bands, even under high-stringency conditions (data not shown), suggesting that CK2β-1 belongs to a multigenic family.

Using the full-length CK2β-1 maize cDNA as a probe, we screened the maize library under low-stringency conditions and isolated a new clone (1088 bp), named CK2β-2. CK2β-2 encodes a predicted protein of 260 amino acids (molecular mass 29.57 kDa, pI = 4.89) (Figure 2a). CK2β-2 also contains a characteristic NH_2-terminal extension, although it is somewhat shorter than in CK2β-1.

As an alternative approach, the CK2β-1 cDNA was cloned into pGBT9 vector and used as a bait in the two-hybrid system. We screened the maize cDNA library and isolated multiple clones His^+, βGAL^+. Twenty per cent of the clones that gave the strongest signal corresponded to a new cDNA encoding a third CK2β regulatory subunit, which we named CK2β-3. The CK2β-3 clone is 1382 bp long and encodes a predicted protein of 273 amino acids (30.52 kDa, pI = 5.48).

The alignment of the three clones encoding CK2β regulatory subunits is represented in Figure 2(a). The main differences between the three sequences are located in the NH_2-terminal region. All three proteins present every major conserved feature found in CK2β subunits from other organisms (Reed *et al.*, 1994). They contain the CK2 regulatory subunit signature (C-P-X_3-C-X_{22}–CPXC), a cysteine-rich motif involved in the zinc-finger structure. The characteristic acidic stretch is also present in maize CK2β and, even though its amino acid sequence is not identical to the other CK2β subunits, the acidic residues that have been shown to be essentials by site-directed mutagenesis (Krehan *et al.*, 1996) are conserved. Adjacent to the acidic domain is located a region of 9 amino acids, previously reported in other CK2β regulatory subunits (Allende and Allende, 1995), that is described as a potential 'destruction box'. In addition to the conserved autophos-