formaldehyde gels, then transferred to nylon membranes (Hybond-N, Amersham Pharmacia Biotech, Uppsala, Sweden). RNA loading was visualized by ethidium bromide staining. Filters were prehybridized at 65°C in 250 mM sodium phosphate buffer containing 7% SDS, 1 mM EDTA, 100 μg ml^{-1} salmon sperm DNA. Hybridization was performed overnight at 65°C using the same solution supplemented with the ^{32}P-labelled probe (2×10^6 cpm ml^{-1}) and final washes were done at 65°C in 0.1 × SSC, 1% SDS. Filters were washed twice at 65°C in 20 mM sodium phosphate buffer plus 1% SDS and 1 mM EDTA.

Yeast two-hybrid library screening

A HybriZap two-hybrid vector system library was constructed from poly(A)+ RNA maize stressed leaf of the inbred line W64A, according to the manufacturer (Matchmaker, Clontech Laboratories Inc., Palo Alto, CA, USA). For the cDNA library screening, hybridization with the maize EST clone (accession number AA979779) was carried out at 65°C in a 250 mM sodium phosphate buffer containing 7% SDS and 1 mM EDTA. For high-stringency screening, filters were washed twice at 65°C in 1 × SSC, 1% SDS and three times at 65°C in 0.1 × SSC, 0.1% SDS. A low-stringency screening was performed as described, except that filters were washed twice at 65°C in 1 × SCC, 1% SDS and three times at 0.5 × SSC, 0.1% SDS. Plasmids were excised from a homogeneous population of hybridizing phages into *E. coli* according to the manufacturer (Stratagene, La Jolla, CA, USA). The largest inserts were completely sequenced using the Automated Laser Fluorescent (ALF) system of (Amersham Pharmacia Biotech, Uppsala, Sweden).

To generate the GAL4 binding domain/CK2β-1 fusion (pGBT9-CK2β1) to be used as a bait for the two-hybrid screening (Fields and Song, 1989), the pAD-GAL4-CK2 β1 clone isolated in the cDNA screening was cloned into *Eco*RI/*Sma*I sites of pGBT9 vector.

For screening, the *S. cerevisiae* strain HF7c (MATa, ura3–52, his3–200, ade2–101, lys2–801, trp1–901, leu2–3112 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 (Matchmaker, Clontech). Positive clones were selected in plates lacking tryptophan, 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 (Matchmaker, Clontech). Plasmids from His^+ $LacZ^+$ colonies were isolated and electroporated into *E. coli*, and the DNA sequence of the inserts was determined.

Yeast two-hybrid interaction testing

To generate the GAL4 binding domain (BD) fusion proteins, the cDNAs encoding CK2β-2, CK2β-3 and CK2α-2 were cloned into pGBT9 vectors. pGBT9-CK2β2 was constructed using primers 5′-CCC GGG GAT AAC TCA ACC TG-3′ and 5′-CAG CTG GCG AAT TCG TAC AT-3′ to amplify the insert and cloning the PCR product into the *Sma*I/*Sal*I sites of pGBT9. To obtain pGBT9-CK2β3, the pAD-GAL4-CK2β3 clone isolated in the cDNA screening was digested with restriction enzymes *Eco*RI and *Bgl*II, and the insert cloned into pGBT9 *Eco*RI/*Bam*HI sites.

Some of the fusion proteins with GAL4-activation domain (AD), such as pAD-GAL4-CK2β1, pAD-GAL4-CK2β3, pAD-GAL4-CK2α1 and pAD-GAL4-CK2α3, were isolated in the two-hybrid library screening using CK2β1 as a bait. pGBT9-CK2α2 and pGAD424-CK2α2 were obtained by digesting the full-length cDNA of pBS-CK2α2 (Peracchia *et al.*, 1999) with *Sma*I/*Xho*I and cloning into the *Sma*I/*Sal*I sites. For interaction studies, both plasmids containing chimeric proteins fused to AD and BD domains were co-transformed into *S. cerevisiae* Y187 strain and selected into Leu–Trp plates. Transformants were tested for β-galactosidase activity using filter and liquid assays as described by the manufacturer.

In vitro *pull-down assays*

To obtain the GST-CK2β fusion proteins, the cDNAs encoding CK2β-1, CK2β-2 and CK2β-3 were cloned in-frame into pGEX-2T vector (Pharmacia Biotech). Expression and purification of GST-CK2β fusion proteins were as described (Frangioni and Neel, 1993). CK2α-1, CK2α-2 and CK2β-3 were translated in a 25 μl reaction in the presence of [^{35}S]methionine (>1000 μCi $mmol^{-1}$, Amersham) using the T7-TNT Quick coupled Transcription/Translation System (Promega Corporation, Madison, WI, USA). For *in vitro* binding assays, 2–3 μg of each GST-fusion protein was attached to 30 μl glutathione–agarose beads and incubated with 20 μl ^{35}S-labelled protein in 180 μl binding buffer (20 mM Hepes pH 7.9, 50 mM KCl, 2.5 mM MgCl, 10% glycerol, 0.2% NP-40, 1 mM DTT, 3 μl rabbit non-immune serum). After incubation overnight at 4°C with shaking, the beads were washed four times with wash buffer (10 mM Tris–HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.2% NP-40) resuspended in 20 μl electrophoresis sample buffer, boiled for 5 min and separated by 12% SDS–PAGE. The radiolabelled proteins were visualized by autoradiography.

In vitro *phosphorylation assays*

To obtain CK2α recombinant protein, CK2 α-2 cDNA was cloned in-frame into pET28c vector, expressed and purified according to the pET system manual (Novagen, Madison, WI, USA).

For the phosphorylation assay, 2 pmol CK2α-2 and 2 pmol of each GST-CK2β fusion protein were added in a total volume of 30 μl CK2 buffer (8.9 mM $MgCl_2$, 0.5 mM EGTA, 27 mM β-glycerol phosphate, 0.5 mM EDTA, 1 mM DTT, 0.08 mM ATP, 3 μCi [γ-^{32}P]ATP). In some experiments, 0.4 μg Rab 17 purified protein was added. Samples were incubated for 20 min at 30°C. Reactions were stopped by addition of electrophoresis sample buffer, and the phosphorylated proteins were separated by 12% SDS–PAGE and visualized by autoradiography.

Functional expression of maize CK2β-1 in yeast

The cDNAs of CK2 β-1 and CK2β-3 were excised from pAD-GAL4 vectors by digestion with *Eco*RI/*Xba*I and ligated into the same sites of plasmid pYES2 (Invitrogen, San Diego, CA, USA); for pYES-CK2β2 construction *Hind*III/*Sac*I sites were used. The pYES2 plasmid allows strong transcription from the *GAL1* promoter when cells are grown in galactose. These constructs were used to transform the wild-type strain YPH499 (*MATa CKB1 ura 3–52 leu2-Δ1 ade2–101 lys2–801 hisΔ 3–200 trp1Δ-63*), as well as its isogenic derivative strain MAR1 (*ckb1::TRP1*). Strain MAR1 was constructed as described previously (de Nadal *et al.*, 1999). Positive clones were selected in Ura^- plates. Tolerance to lithium ions was tested by streaking cultures in yeast extract–peptone–dextrose (YPD) or yeast extract peptone Galactose (YPGal) plates containing different concentrations of LiCl.