phorylation site described in other organisms, maize CK2β contain additional putative CK2 phosphorylation sites (according to the S/T-XX-D/E CK2 consensus), most of them also located in the NH_2-terminal region. CK2β-2 and CK2β-3 present several of the putative phosphorylation sites identified in CK2β-1, but they lack the single phosphorylation site located in the COOH-terminal region of CK2β-1. Moreover, CK2β-2 encodes a protein slightly shorter than CK2β-1 and CK2β-3. At the amino-acid level, the identities between CK2β-1/CK2β-2, CK2β-1/CK2β-3 and CK2β-2/CK2β-3 are 77, 75 and 80%, respectively.

Previous studies using Southern blot analysis demonstrated that maize CK2α catalytic subunit genes also belong to a multigenic family (Peracchia *et al.*, 1999). Two cDNA clones (CK2α-1 and CK2α-2) have been described previously (Dobrowolska *et al.*, 1991; Peracchia *et al.*, 1999). Several of the remaining CK2β-1 interacting clones corresponded to a new cDNA highly related to the reported CK2α catalytic subunits (termed CK2α-3). As shown in Figure 2(b), the degree of identity between the different maize CK2α subunits is much higher than in the case of CK2β subunits, and CK2α-3 shares every structural determinant previously defined for CK2α catalytic subunits.

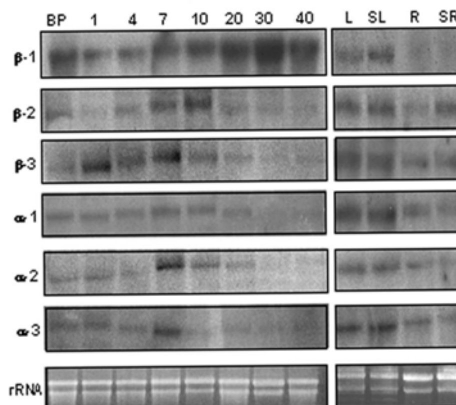

Figure 3. Expression pattern of genes encoding maize CK2α/β subunits in different organs and during embryo development using specific probes.
25 μg total RNA was loaded per lane. BP, before pollination; 1–40, days after pollination (DAP); L, leaves; SL, stressed leaves; R, roots; SR, stressed roots. β-1, CK2β-1; β-2, CK2β-2; β-3, CK2β-3; α-1, CK2α-1; α-2, CK2α-2; α-3, CK2α-3. rRNA, ribosomal RNAs.

Differential expression of the maize α/β CK2 multigenic family

The pattern of expression of all known maize α/β subunits identified has been analysed in various organs and developmental stages using Northern blot analysis (Figure 3). For CK2β subunits the differential NH_2-terminal regions were used as probes. The expression of CK2β-1 appears to be higher in embryos of 20, 30 and 40 days after pollination (DAP), whereas CK2β-2 show the highest expression in intermediate stages of embryo development (7 and 10 DAP), and CK2β-3 is predominantly expressed at early stages (1, 4 and 7 DAP). In maize, the Rab17 protein is strongly induced and phosphorylated during late embryogenesis and in vegetative tissues under drought conditions. CK2 has been shown to phosphorylate *in vitro* Rab17 protein (Goday *et al.*, 1994; Plana *et al.*, 1991). The high level of expression of CK2β-1 during late embryogenesis, coinciding with the expression of the *rab*17, prompted us to analyse if a similar situation occurred in vegetative tissues under water-stress conditions. Northern analysis of RNA obtained from leaves and roots indicated that mRNA for all CK2β subunits is expressed in these organs. However expression of the CK2β-1 was not increased after water stress.

Previous data based on Northern analysis using the full-length CK2α-1 as probe suggested a constitutive expression of CK2α subunits during embryo development (Peracchia *et al.*, 1999). Here the 3′ non-coding regions of the three CK2α subunits were used as gene-specific probes to test for differential expression of the isoforms. The results obtained indicate that the expression pattern is roughly the same for the three CK2α isoforms The levels of mRNA decreased at the latest stages of embryo development, particularly in the case of CK2α-2. Northern analysis of RNA obtained from leaves and roots indicates that mRNAs for all CK2α subunits are expressed in these organs, the level being higher in leaves than in roots. No mRNA changes were detected for any subunit under water-stress conditions.

Interactions between CK2α and CK2β subunits

As a first approach, the yeast two-hybrid system was used to investigate if specific interactions between the different CK2 subunits exist. As shown in Figure 4(a), all the CK2α subunits are able to interact with all the CK2β subunits, although with different levels of intensity. β-galactosidase assay performed on filter indicates that a very weak interaction is detected between CK2β-1/CK2β-1 and CK2β-1/CK2β-2, and no detectable interaction is observed between CK2β-2/CK2β-2, whereas CK2β-1/CK2β-3 and CK2β-3/CK2β-3 present the strongest interaction. The CK2β-2/CK2β-3 interaction differs depending on which cDNA is cloned in the binding or in the activation domain. Using a construct carrying CK2α-2 fused to the binding domain, we analysed the interaction between this clone and the other CK2α subunits joined to the activation domain. The results obtained indicated that maize CK2α