Supplementary data

Purification of B. oleracea telomeric DNA-binding proteins.

The purification scheme is shown in Figure 6A. Most of the telomeric DNA activity from *B. oleracea* nuclear extract precipitated at 60% ammonium sulfate saturation (Supplementary Figure 2A). The protein sample from this fraction was dialyzed and fractionated by preparative isoelectric focusing in the Rotofor system (Bio-Rad). As shown in Supplementary Figure 2B, most DNA-binding complexes appeared in fractions 14 and 15 (pH 4.4 - 4.5). A second peak of complex B appeared in fractions 17 and 18 (pH 4.7 – 4.8). The intensities of complexes C and D were significantly reduced, perhaps due to dissociation of the protein components of these complexes.

To further purify complexes A and B, samples from fractions 14 and 15 were combined and subjected to size-exclusion chromatography on a Superose 12 column (Supplementary Figure 3). The peak of complex A eluted in fractions 25-26 (50-70 kDa) and the peak of complex B eluted in fractions 22-23 (150-170 kDa). To estimate the apparent molecular weight of the DNA-binding component of complex A, fractions 20-28 from Superose 12 column were used in a cross-linking assay (see Figure 6B).

Supplementary methods

Protein purification

Ammonium sulfate was added to *B. oleracea* nuclear extract in 20% increments to 80% saturation at 4^{o}C with stirring. The precipitate was collected by centrifugation, resuspended in TMG-50 buffer (50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 10% glycerol) and dialyzed against dialysis buffer. The sample was then mixed with ampholytes (3-5 pH range) and subjected to isoelectric focusing on the Rotofor system (Bio-Rad) according to the manufacturer's instructions. Fractions with telomere binding activity were pooled and size-fractionated on a Superose 12 column (Pharmacia) in 20 mM Tris-HCl pH 8.0, 10% glycerol, 150 mM NaCl, 1 mM $MgCl_2$. The eluted sample was DNA-affinity purified as described below. Activity was monitored at all stages by EMSA.