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A recombinant C-terminal fragment of maize Initiator Binding Protein 2 (IBP2) binds to telomere-repeat DNA in vitro.

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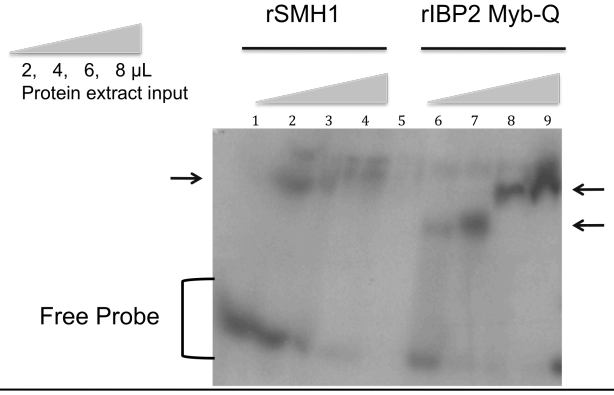
The maize (*Zea mays* L.) *initiator-binding protein2* (*IBP2*) gene is known to encode a protein, IBP2, that binds to the *initiator* (*inr*) element in the *Shrunken1* promoter (Lugert and Werr 1994; Plant Mol Biol, 25, 493–506). The duplicate genes *IBP1* and *IBP2* were initially identified in ligand-binding screens using cDNA expression libraries. The binding of IBP to the transcription start site *inr* element was confirmed by footprint and band-shift assays. We and others have observed that IBP1 and IBP2 also resemble telomere DNA-binding proteins from other plant species. We investigated therefore whether the IBP2 protein exhibits telomere DNA-binding activity.

Using Electrophoretic Mobility Shift Assay (EMSA) we found that a C-terminal sublcone of the IBP2 protein (rIBP2 Myb-Q, AA 579-667, GenBank GU080214), binds telomeric repeat DNA *in vitro* (Fig. 1, arrows indicate band shifts). We also found that rIBP2 Myb-Q binds strongly to double stranded telomere repeat sequences with three or four tandem repeats, but only weakly to sequences with two (Fig. 2, arrows indicate band shifts). Furthermore, we found no evidence of binding with only one telomere repeat (5' TTTAGGG 3') sequence. Single point mutations (T1:A and G6:T) in the three-repeat sequence diminished, but did not entirely abolish, binding activity (via competition EMSA assays, data not shown).

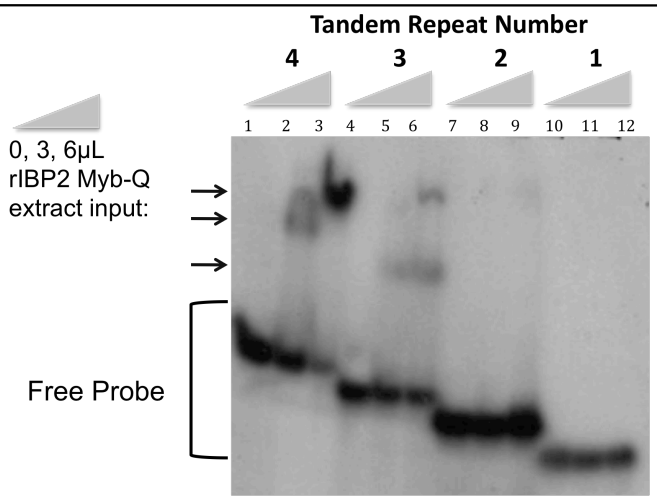
In order to compare the binding of rIBP2 Myb-Q to telomere vs. initiator DNA, we carried out filter binding assays. In this experiment, total protein extract from E coli cultures expressing rIBP2 Myb-Q (IPTG+) were incubated with nitrocellulose filters. These filters were then incubated with radiolabeled oligonucleotide probes corresponding to telomere DNA or *Sh1 initiator* DNA (Fig. 3). From these experiments, we found that the rIBP2 Myb-Q showed very weak binding to the *initiator* sequence of the *Shrunken*1 promoter. These findings confirm the prediction that the maize IBP protein (IBP1 or IBP2) has telomere-DNA-binding activity. Consequently, maize IBP1/2 may reside at maize telomeres, raising the intriguing possibility of dual functions for IBP, telomeric and transcriptional.

Fig. 1. Telomere DNA binding Activity of C-terminal Fragment of IBP2



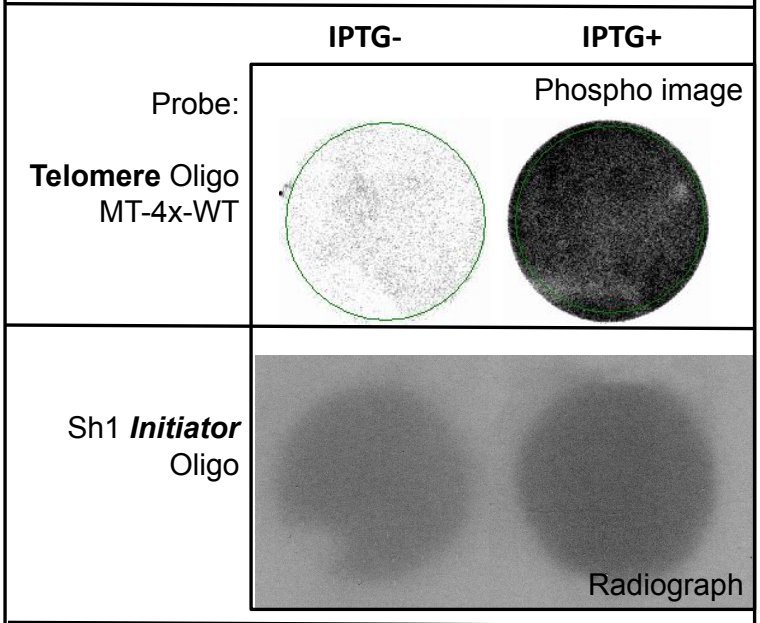
Triangles represent increasing amounts of dialyzed crude protein extract added to otherwise identical binding reactions. The ^{32}P-radiolabled ds 3-telomere-repeat probe, MT-3x-WTF-809, migrates as a bandshift product (arrows) or as free probe (lower band). Bandshifts with 5 nmol radiolabled telomere probe were incubated with increasing amounts of rSMH1-containing extract (lanes 1-4) as a positive control or with increasing amounts of rIBP2 Myb-Q-containing extracts (lanes 6-9). Lane 5 has no sample. Amounts of extract added are indicated at the bottom, and the position of detected bandshift products are denoted by arrows.

Fig. 2. Telomere Repeat Number Required for rIBP2 MybQ binding



rIBP2 Myb-Q-containing protein extract was incubated with 0.8 pmol ^{32}P-radiolabeled telomere probe (TTTAGGG) per lane. Lanes 1, 2, and 3 contain 0, 3, and 6µL rIBP2 Myb-Q incubated with radiolabled 4 repeat probe (MT-4x-WTF-809). Lanes 4-6 represent the 3 repeat probe (MT-3x-WTF-809) in otherwise identical reactions. Lanes 7-9 and lanes 10-12 correspond to binding reactions using the 2 repeat probe (MT-2x-WTF-809) and the single repeat probe (MT-1x-WTF 809), respectively. The positions of the most conspicuous bandhsift products are indicated by arrows. Free probe is evident as the bottom band in each lane.

Fig. 3. Nitrocellulose Filter Binding Assay



Nitrocellulose filters probed with radioactive Initiator probe. Phosphor-image or autogradiographs of filters incubated with uninduced (IPTG-) or induced (IPTG+) extracts are shown and radiolabeled probes. Probes used are indicated at the left. Relatively weak binding is seen for the Sh1 initiator, compared to telomeric DNA. This relative affinity of rIBP2 MybQ for telomere vs. initiator DNA was also observed using EMSAs.