DNA binding properties of a bHLH transcription factor fused with green fluorescent protein

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Basic-helix-loop-helix (bHLH) transcription factors play roles in cell fate determination and regulate transcription of tissue-specific genes. They bind to a specific DNA sequence, E-box (CANNTG), as a dimer. Crystal structure of a bHLH protein, E47 suggested that a glutamic acid Eb9 located at 9th position in basic region is important for recognition of the nucleotide sequence (Longo et al., 2008). However, the role of Eb9 in recognition of E-box sequence has not completely been elucidated.

We made a fusion protein (HEB-GFP), consisting of a bHLH domain of HEB (*Hela* E-box binding protein) and GFP. It was indicated HEB-GFP selectively binds to E-box sequence from our gel shift analyses. We also expressed HEB-GFP mutants in which Eb9 was substituted to other amino acids. When Eb9 was replaced to V, L, I, T, F, Y, W and D, HEB-GFP lost the affinity to DNA, probably lacking the ability of conformational change required for binding to DNA. Mutants bearing K, R, Q, N and S at b9 position bound to E-box sequence although sequence selectivity was weak compared to that of wild type. It was suggested that bHLH domain prefer to bind to E-box sequence and Eb9 enhance the sequence selectivity.