TATA-box Binding Protein interacts with Antp, Scr, Ubx and AbdB through their N-terminal domains

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Background

Hox proteins are transcriptional factors (TFs) that define segment identity during embryonic development regulating specific target genes. These TFs interact with cofactors for DNA specificity and other TFs to regulate gene expression, which include basal transcriptional machinery members like BIP2, Med19, TFIIEβ, M1BP and TBP. Since TBP glutamine homopeptide (PolyQ) act as an interaction domain involved transcriptional regulation, we analyzed if TBP interact with Antp, Scr, Ubx and AbdB through its PolyQ region.

Methods

We used Bimolecular Fluorescent Complementation (BiFC) to determine TBP interaction with Antp, Scr, Ubx and AbdB as well as the implication of their homeodomain (HD) and the TBP polyQ region. We used expression vectors carrying the sequences of TBP and its PolyQ lacking version (TBPΔQ) fused to the N-terminal half of Venus (VN) and Antp, Scr, Ubx and AbdB as well as their HDs fused to the C-terminal (VC). All VN and VC constructions were co-transfected with pCAGmCherry in HEK293 cells. Fluorescent cells were quantified and BiFC percentage was calculated as green cells per each 100 red cells. We also used UAS-GAL4 system to direct the expression of VNTBP and VCAntp or AntpHD in *D. melanogaster* embryos using a *Ptc*-GAL4 driver. BiFC fluorescent embryos were acquired by confocal microscopy.

Results

The results showed that TBP interact with Antp (78%), Scr (72%), Ubx (70%) and AbdB (95%) in cell culture. The interaction with HD decreased interaction percentage as follows: AntpHD(50%), ScrHD(44%), UbxHD(41%) and AbdBHD(61%) indicating the implication of the N-terminal in these interactions. Also, the PolyQ deletion in TBP decreased the signal to 41%, 25%, 34% and 49%, respectively, confirming the PolyQ importance in these interactions. The combination of deletions both in TBP and homeoproteins showed an additive effect. Additionally, we corroborrated the TBP-Antp interaction in *D. melanogaster* embryos by BiFC and the Antp N-terminal involvement in this interaction as well.

Conclusions

TBP interaction with Antp, Scr, Ubx and AbdB is mediated by Hox N-terminal regions and the PolyQ domain of TBP as well. Furthermore, TBP-Antp interaction also occurs *in vivo,* suggesting that they could have similar transcriptional regulation during development of *Drosophila melanogaster*.