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The Role of Proximal Promoter Binding Transcription Factors(GAGA, NTF-1 and *Zeste*) in Regulating *Ultrabithorax* in the Anterior 50% of the Embryo.

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Drosophila development is controlled by a temporally ordered cascade of regulatory genes. Among the latter acting members of this hierachy are the homeotic genes. These genes act to instruct groups of cells which body segment to develop into.

We are investigating the transcriptional regulation of the homeotic gene *Ultrabithorax*(*Ubx*). Earlier biochemical experiments have identified and purified three trans-acting factors(GAGA, NTF-1, and *Zeste*) that bind to the *Ubx* proximal promoter and activate its transcription in vitro. The role of each of these transcription factors in regulating the pattern of *Ubx* expression is being investigated. Transgenes containing 22kb upstream sequence from the transcription start site are expressed similarly to the endogenous *Ubx* gene. We have utilized this 22kb *Ubx*-LacZ fusion gene system to study the function of GAGA, NTF-1, *Zeste* binding sites in the proximal promoter. A series of differently mutated *Ubx* promoter constructs containing binding sites for none, one, or all three of these transcription factors have been introduced into *Drosophila* by P-element transformation. Interestingly, transgenes containing no proximal factor binding sites are expressed very weakly only in the nerve cord of the embryo. Also, binding sites for each transcription factor were found to strongly activate different pattern of transcription in the embryo, suggesting a specific role for each factor in regulating the spatial pattern of *Ubx*. In GAGA and *Zeste* transgenic lines, strong repression of *Ubx* expression in CNS, labial portion and some of thoracic segments is maintained.

Encouraged by these findings, we made additional constructs which have binding sites both for GAGA and NTF, and also prepared constructs with *Zeste* and NTF binding sites in various spatial arrangements. Surprisingly, in the transgenic lines, GAGA was able to suppress the ubiquitous expression contributed by NTF binding sites. Also *Zeste* was able to suppress the activity of NTF but rather weakly compared to GAGA. GAGA and *Zeste* seem to mask the transcriptional activation of NTF in the anterior and terminal portion of embryos during early embryogenesis. Thus, these factors may affect the silencing activity of distant regulatory elements, which is initiated by *Hunchback* and maintained by *Polycomb* genes. Currently, we are investigating which cis-element residing within the 22kb upstream element is communicating with the GAGA and/or *Zeste* which bind to the proximal promoter of *Ubx*. Eventually, we will define the factors which interact with these two transcription factors.