

Functional analysis of heterochromatin-associated Boundary Elements and their genes

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Chromatin insulators are thought to participate in the segregation of the genome into active and inactive domains. In *Drosophila melanogaster*, Boundary Elements (BEs) interact with the BEAF protein (Boundary Element-Associated Factor) and we have characterized a BEAF binding-signature (the BEAF *Dual-Core*) that incorporates two clusters of CGATA BEAF-binding sites separated by a ~ 200-bp AT-rich region that contains DNase I-hypersensitive and protected regions and corresponds to presumptive nucleosome-positioning sequences. Of hundreds of BEAF dual-cores found in the *Drosophila* genome, a subset has been associated with epigenetic regulation of a specific group of euchromatin genes, where they serve to control the deposition of histone modifications. Others map to heterochromatin and we have also focused on BEAF dual-cores found at or near hetero-/euchromatin borders of the X chromosome. Using a BEAF siRNA knockdown approach, we have analyzed the effects of BEAF depletion on the expression of heterochromatin-proximal genes and of genes within heterochromatin. This approach has also been combined with an analysis of the effects of the depletion of heterochromatin-associated proteins, including HP1 and the non-histone D1 architectural protein that is preferentially associated with the 1.688 g/cm³ satellite III 359-bp repeats (SAT III). Because the 11-Mbp SAT III array is located next to the rDNA locus, we have also investigated the possible role of SAT III and its associated proteins with the regulated expression of the 18S and 28S rDNA precursor. Taken together, our results suggest a regulatory role for BEAF for the expression of genes located within heterochromatin, including pericentromeric rDNA repeats. Because the centromere-proximal region of the *Drosophila* X chromosome remains poor in sequence information, the regulatory role for BEAF at hetero-/euchromatin borders requires further characterization and might be implicated in processes more complex than forming a simple barrier against heterochromatin spreading.

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