

Insulators: linking genome architecture to gene regulation

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Abstract

Insulator elements have long been associated with a proposed domain boundary function, ensuring appropriate associations between regulatory elements and transcription units through the physical organisation of the genome into looped domains. Recent experiments in *Drosophila* have, however, highlighted a more direct involvement of insulators in transcriptional regulation.

Introduction and context

A major goal of genomic studies is to understand the regulatory architecture of the genome; how, for example, powerful regulatory elements, such as enhancers and silencers, which have the ability to act over large genomic distances, are constrained to act only on appropriate transcription units. There is considerable evidence for a domain model of genomic regulatory architecture where the actions of long-range regulatory elements are confined within chromosomal domains [1-3]. The promiscuity of enhancers is restricted to regulation of transcription units within the same domain. In this model of genome organisation, insulator elements are proposed to play a key role as domain gate-keepers, shielding genes within the domain from the influence of outside regulatory elements. How insulators function is still unclear but they have been proposed to associate together to form chromosomal loops providing a physical basis for chromosomal domains. Insulator elements have been shown to have appropriate activities for such a role; they are defined by their ability to block enhancer-promoter interaction and/or to provide boundaries between different chromatin states and have been demonstrated to be involved in chromosome looping. But does the segregation of the genome into domains through the formation of chromosomal loops really represent the endogenous function of insulator elements? Although this question has been asked in the past [4,5], recent results in *Drosophila* emphasising links between insulators and the regulation of transcription have once again brought it into focus.

Major recent advances

A number of proteins have been associated with insulator function in *Drosophila*, including the Suppressor of Hairy-wing [Su(Hw)] protein [6], CCCTC-binding factor (CTCF) [7], Centrosomal protein 190 (CP190) [8], Boundary element-associated factor (BEAF) [9], GAGA factor (GAF) [10] and Zeste-white-5 (Zw5) [11], and over the past few years, genomic binding sites for several insulator components have been mapped [12-18]. In support of the domain model, these studies reveal associations between particular insulator components and boundaries of chromatin state domains; for example, Bartkuhn *et al.* [14] document that CP190 and CTCF binding correlates strikingly with the boundaries of repressive chromatin domains marked by the H3K27me3 modification. This accords with genomic studies in vertebrates demonstrating that CTCF binding sites mark chromatin state boundaries [19,20]. However, the *Drosophila* genomic mapping experiments also reveal a strong association between particular insulator components and transcription start sites. This is especially true for some components, CP190, GAF and BEAF, but not for Su(Hw), suggesting that there may be distinct types of insulator complexes [15]. BEAF 32A and B were initially identified as factors binding to an insulator element (scs') associated with the 87A hsp70 heat shock locus [9]. Insulator activity is dependent on BEAF binding sites and BEAF is involved in the formation of a chromosomal loop through interaction with Zw5 bound to a second element (scs) [21]. However, despite its strong 'domain boundary'

credentials, more than 85% of the 1820 BEAF binding sites in the genome are less than 300 bp from transcription start sites [16]. Many (50%) of these BEAF peaks are associated with head-to-head gene pairs, although these BEAF sites do not appear to function to insulate these adjacent promoter regions from each other. Rather, BEAF appears to be generally required to promote transcription. Sharing of components between insulators and promoters may fit with shared structural features of these elements (for example, both CTCF-binding sites and active transcription start sites are marked by the presence of specific histone variants and are associated with nucleosomes containing both the H3.3 and H2A.z variants [22]).

While this may be interpreted as indicating a link between insulators and transcriptional regulation, it might also suggest that 'insulator' components may serve a variety of functions and not all their binding sites are necessarily associated with insulator function. However, other experimental approaches provide support for a fundamental role for insulator-promoter interaction. It has been known for some time that some promoters can behave as insulators in enhancer-blocking assays [10]. However, it was never clear whether the mechanism of enhancer-block was the same in both cases. Recently, Chopra *et al.* [23] have uncovered a connection between promoters with enhancer-blocking activity and the presence of stalled RNA polymerase II (Pol II). Testing the ability of several Hox gene promoters to exhibit enhancer-blocking, promoters associated with stalled Pol II showed blocking function whereas several unstalled promoters did not. The enhancer-blocking activity was dependent on the function of the elongation factors Negative elongation factor (NELF) and DRB sensitivity-inducing factor (DSIF), which are thought to stabilise Pol II at the pause site. However, the key observation that draws together insulators and promoters is that the enhancer-blocking activity of two bithorax-complex insulators, *Fab7* and *Fab8*, is also dependent on the elongation factors NELF and DSIF. As *Fab7* and *Fab8* do not seem to contain stalled polymerase, the implication is that the insulators interact with promoters to inhibit elongation. This fits with observations, using DamID (DNA adenine methyltransferase identification) or chromosomal conformation capture, that the *Fab7* and *Fab8* insulators can form loops that bring them into contact with promoter regions of the *Abd-B* Hox gene and that this configuration is associated with repression of *Abd-B* transcription [24,25].

In support of the generality of interaction between insulators and the regulation of transcription elongation, Jiang *et al.* [16] found considerable overlap between BEAF and NELF binding sites. In addition, vertebrate studies using chromatin topology assays have identified

insulator-promoter associations at several loci, including *Igf2-H19* [26,27] and *CFTR* [28].

Future directions

Overall, the above combination of genomic binding site location and functional analysis currently indicates a more intimate relationship between insulators and the regulation of gene transcription than is suggested by the domain boundary/chromosome loop model. Insulators may serve a diverse range of functions; as classical boundaries, as boundaries of regulatory regions [13,29], and as regulators of transcription. Even the regulatory effects of insulators can apparently be diverse; BEAF appears to be predominantly required to facilitate transcription whereas the above interactions between the *Fab* insulators and *Abd-B* promoters occur in a repressive context. Is there a common thread in this diversity? It is feasible that insulator elements in general are involved in the specification of chromosomal topology; this could provide a common function but, depending on the context, could have diverse consequences for chromatin state and transcriptional regulation.

Abbreviations

BEAF, Boundary element-associated factor; CP190, Centrosomal protein 190; CTCF, CCCTC-binding factor; DamID, DNA adenine methyltransferase identification; DRB, 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole; DSIF, DRB sensitivity-inducing factor; GAF, GAGA factor; NELF, Negative elongation factor; Su(Hw), Suppressor of Hairy-wing; Pol II, RNA polymerase II; Zw5, Zeste-white-5.

Competing interests

The author declares that he has no competing interests.

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