Chromatin boundaries: **Punctuating the genome**

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Transcriptional enhancers are constrained to act within domains defined by boundary elements. How these elements work is a mystery. A recent study emphasizes their autonomous activity; another emphasizes their dependence on nuclear organization. Both effects need to be accounted for by any successful model.

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One of the major structural puzzles concerning the organization of the eukaryotic nucleus is the means for subdividing the chromatin fiber into domains of enhancer function. Precise control over the expression of a gene can be exerted through interactions between the basic transcriptional machinery at the gene's promoter and specific protein complexes at enhancer elements. Enhancers are capable of acting over long distances, in an orientation-independent manner, from either the 5′ or 3′ end of the gene, or sometimes from within an intron. Enhancer activities are generally not promoter-specific; how, then, is an enhancer prevented from inappropriately activating the promoters of neighboring genes as well as its own?

An important step towards a resolution of this problem came with the discovery of a class of boundary or insulator elements that block communication between enhancer and promoter. This class includes the scs and scs′ elements from the *Drosophila* heat shock locus [1,2]; several similar elements from vertebrate genomes, identified in the sequences flanking genes or gene clusters (reviewed in [3]); and a subset of the Fab-7 sequences at the bithorax complex that are responsible for delimiting the paraseg-ment-specific regulatory regions of these homeotic genes [4,5]. The cluster of binding sites for the protein Suppressor of Hairy wing (Su(Hw)) in the *Drosophila* transposable element *gypsy* also exhibits boundary activity [6]. The best studied are the scs/scs′ elements and the Su(Hw) binding sites that form the *gypsy* insulator, and we shall limit our discussion to these.

The activity of a boundary element is dependent on its position relative to the enhancer and promoter; it only interferes with communication between the latter two elements when interposed between them. Importantly, the boundary activity does not normally inactivate either the promoter or the enhancer, which can interact with other regulatory elements on the same side of the boundary. The activity of boundary elements is not restricted to blocking positive regulatory interactions; these elements protect transgenes from the full spectrum of positive and negative euchromatic chromosomal position effects. The *gypsy* insulator also protects the origin of DNA replication required for chorion gene amplification from such effects [7]. In addition, these boundary elements protect a test *mini-white* gene from the silencing activity of a Polycomb response element [8,9], and the *gypsy* insulator protects a test gene against the silencing induced by heterochromatin [3], suggesting an ability to interfere with folding or assembly of an extensive chromatin complex [10].

How do boundary elements work? The various models proposed fall into two general categories: those involving a local interaction between the proteins of the boundary element and the proteins of the enhancer (or other regulatory element), and those in which the boundary activity is coupled to a structural role in higher-order nuclear organization (see Figure 1) [1]. These models need not be considered mutually exclusive — both local and long-range effects may be important. In a simple model of the first category, the insulator is suggested to act as a decoy that short-circuits enhancer–promoter interactions (Figure 1a) [3]. In this model, the boundary acts as a competitive inhibitor of enhancer–promoter interactions. A related model postulates that the interaction between the boundary and enhancer proteins does not have a negative impact on enhancer activity, but rather imposes a positive directionality on the action of the enhancer (Figure 1b) [11].

The second group of models includes those in which the boundary elements delimit higher-order chromatin structures. For example, boundary elements might form the base of a chromatin loop, physically separating or constraining adjacent domains to prevent interaction between regulatory elements; such structures might limit the type of chromatin assembly dictated by particular regulatory elements (Figure 1c) [1]. Alternatively, a boundary element might direct localization into a nuclear domain with chromatin-assembly characteristics that differ from other subnuclear domains; a shift in the availability of certain chromosomal proteins might limit the interactions of regulatory elements by eliminating assembly of chromatin complexes that act over a distance (Figure 1d).

Two recent papers have helped to focus our thinking about these models, but in very different ways [11,12]. Krebs and Dunaway [11] tested the effect of scs and scs′ elements on

Figure 1
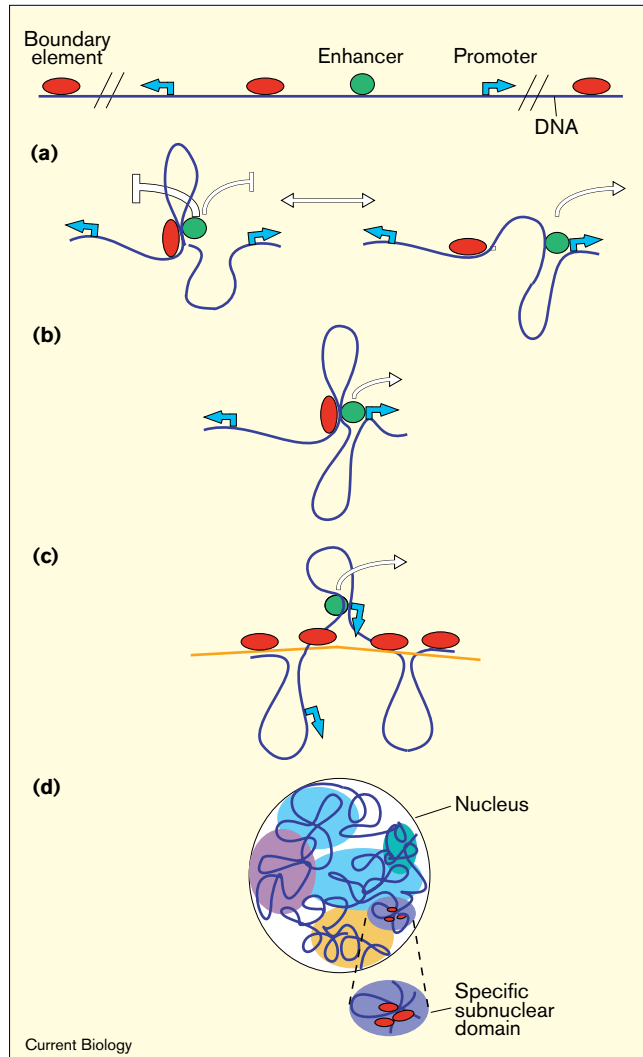
Models for boundary function. The cartoons illustrate local interaction models based on enhancer decoy **(a)** or enhancer orientation **(b)** activities; structural models are based on organization of chromatin loops **(c)** or on positioning in different nuclear subdomains **(d)**. In each case, boundary elements are represented by red ovals; enhancers by green circles; promoters by blue arrows; and a nuclear matrix element by a yellow line. The large colored areas in (d) represent nuclear domains, each with a unique local concentration of various chromosomal proteins. White arrows above enhancers indicate positive or negative effects on promoter activation.

enhancer activation of alternative rRNA promoters, distinguished by the length of their transcripts, in plasmid constructs. The regulatory elements were either on the same plasmid (in *cis*), or on opposite rings of dimeric catenanes (in *trans*). The template was assayed by injection of the DNA into a *Xenopus* oocyte nucleus; control experiments showed that a stable transcription complex formed rapidly, well before chromatin assembly was complete. In the absence of scs/scs′ elements, enhancer-activated transcription apparently occurred by random collision of enhancer

and promoter from either configuration. With all regulatory elements in *cis*, one observes a two-fold loss of enhancer activity in the presence of one copy of scs/scs′; a full block of enhancer activity requires the presence of two scs/scs′ elements, surrounding either the promoter or the enhancer.

This result lends support to the idea that a boundary element imposes directionality on enhancer action; in an intact circular DNA molecule, both directions must be closed off completely to block the enhancer. In this assay system, the enhancer can function in a *trans* configuration, activating a promoter on a separate, but linked plasmid; once again, complete blockade of the enhancer requires two scs/scs′ elements bracketing one of the regulatory sequences. The experiments indicate that this boundary activity can be observed in the absence of complete chromatin assembly. The specific DNA fragments required for full activity in this assay [11] are, however, smaller than the minimal scs/scs′ sequence required for full activity in transgenic flies [13], suggesting that additional factors may contribute to full boundary activity within chromosomes.

The recent work of Gerasimova and Corces [12] indeed focuses our attention on the role of *in vivo* nuclear architecture. This study analyzes the *gypsy* insulator, making extensive use of *Drosophila* genetics to manipulate the chromosomal proteins present. The experiments concern Su(Hw), a zinc-finger DNA binding protein which, as mentioned above is essential for boundary function of the *gypsy* insulator. To function normally, Su(Hw) requires a second protein, Mod(mdg4). Loss of Mod(mdg4) results in either loss of insulator function or conversion of the Su(Hw) binding sites into a repressor element, the specific effect depending on the promoter under study [14,15]. Mod(mdg4) does not bind directly to DNA itself, but is thought to do so by interacting with other proteins, presumably through its 'BTB' domains.

Gerasimova and Corces [12] have now shown, by immunofluorescent localization on polytene chromosomes, that Su(Hw) and Mod(mdg4) are both apparently associated with known *gypsy* element insertions at the y^2 and sc^1 loci. Mod(mdg4) is associated with about 500 sites on polytene chromosomes of strains that lack gypsy insertions; Su(Hw) protein is associated with about 200 sites, all a subset of the Mod(mdg4) sites. Su(Hw) is required for localization of Mod(mdg4) to *gypsy* elements in polytene chromosomes; conversely, a mutant *mod(mdg4)* allele appears to result in reduced Su(Hw) binding. Functional and cytological assays, as well as earlier biochemical studies, thus indicate that these two proteins work together, in physical association, to create a boundary [12,14].

Further observations show that Su(Hw) and Mod(mdg4) colocalize in the diploid nucleus in 10–20 spots. These spots are thought to be associated with the nuclear periphery, but

Figure 2
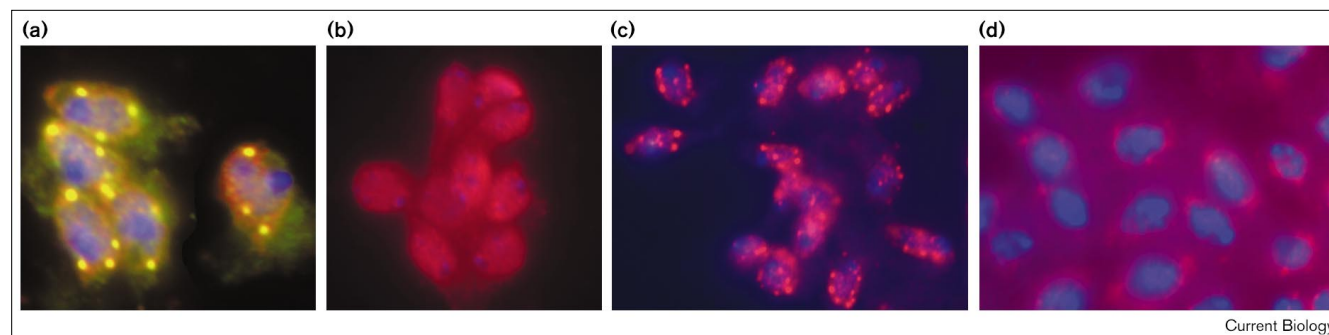
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The distribution of Mod(mdg4) protein in diploid nuclei of *Drosophila* imaginal dics cells is affected by mutations in chromosomal proteins. In each panel DNA is stained with DAPI (blue). **(a)** Simultaneous localization of Mod(mdg4) (red) and Su(Hw) (green); sites where the two proteins colocalize are labeled yellow. **(b)** Localization of Mod(mdg4) in nuclei from a strain carrying a *su(Hw)* null allele. **(c)** Localization of Mod(mdg4) in nuclei from a strain carrying a *mod(mdg4)* mutation. **(d)** Localization of Mod(mdg4) in nuclei of imaginal disc cells from a strain carrying both a *mod(mdg4)* mutation and mutations of trithorax-group genes. (Reproduced with permission from [12].)

this has not yet been definitively established by a three-dimensional analysis. Interestingly, a deficiency of one protein affects the distribution of the other. The subnuclear localization of Mod(mdg4) appears diffuse in flies carrying one null allele of *Su(Hw)*, although some small spots can still be seen; conversely, the intensity of Su(Hw) spots is decreased by a deficiency of Mod(mdg4) [12] (Figure 2a,b). Thus, not only do these two proteins work together, localizing to a discrete set of subdomains in the nucleus, but changes in the presence of one perturbs the distribution of the other, altering the nuclear architecture.

Less is known about proteins that interact with the scs/scs′ elements. Laemmli and co-workers [16] have purified a protein, BEAF-32, that binds to a repeated palindromic sequence from scs′, and have shown that a multimer of the binding site has partial boundary activity. This protein is also located at hundreds of sites on polytene chromosomes, and is restricted to discrete subnuclear regions. It is not known whether this localization coincides with that of Su(Hw) and Mod(mdg4), nor is the degree of structural and mechanistic similarity between scs/scs′ and *gypsy* insulators yet known.

These are not the only recent findings that call our attention to the importance of nuclear architecture in the regulation of gene activity. It has long been noted that heterochromatin — the condensed, inactive form of chromatin that can turn off nearby genes in a stochastic manner, resulting in 'position-effect variegation'— occupies distinct regions of the nucleus, and the intranuclear position of a test gene has been correlated in a number of studies with the effectiveness with which it is silenced [17]. For example, the telomeres of yeast chromosomes are assembled into discrete protein complexes located at

the periphery of the nucleus, and mutations in the associated proteins that result in a loss of localization also result in a loss of silencing of telomere-associated test genes [18]. A recent study of mouse B-cell nuclei has suggested that the regulatory Ikaros protein inactivates genes by recruiting them to a position adjacent to heterochromatin [19]. Whether these changes in gene activity are dependent on an altered protein composition in that area of the nucleus, an altered structural state of the chromatin, or both, remains to be seen.

Although the present picture is very incomplete, there are tantalizing clues that relate other gene regulatory systems, more subtle than the establishment of euchromatin and heterochromatin domains, to nuclear organization. Although *mod(mdg4)* is allelic to an enhancer of position-effect variegation, *En(var)3-93D*, it also shows genetic characteristics that place it in the *trithorax* group [12]. The products of the *trithorax* group of genes in *Drosophila* help maintain appropriate homeotic genes in an active state, while those of the *Polycomb* group maintain the inactive state. Not only do *mod(mdg4)* alleles affect this regulatory system, but mutations in some trithorax-group and Polycomb-group genes can affect *gypsy* insulator function.

Despite this evidence for functional interaction, there is relatively little overlap in the distribution patterns of Mod(mdg4) and trithorax-group or Polycomb-group proteins on polytene chromosomes. It appears, therefore, that the interactions between these proteins may be indirect. In combination with a mutant *mod(mdg4)* allele, some mutations in *trithorax* group or *Polycomb* group genes cause complete loss of normal Mod(mdg4) nuclear localization — the remaining Mod(mdg4) protein is distributed to the cytoplasm or diffusely throughout the nucleus, rather than

in spots (Figure 2c,d) [12]. The correlation between changes in nuclear organization and changes in boundary function suggests that the establishment of distinct functional domains within the nucleus, and association of boundary elements with these domains, is of critical importance for boundary function. One is reminded of the changes in nuclear distribution of the transcription-silencing Sir proteins that occur on mutation of one member of the complex, with profound effects on gene expression and chromosome maintenance [20].

While the mechanism of boundary action remains obscure, the accumulating data suggest some possible components of a model. As discussed above, boundary elements limit the *cis* activity of regulatory elements to within the bounded area; proteins involved in boundary function are part of a general system that governs nuclear organization; and mutations that disrupt nuclear organization can disrupt boundary function. As suggested by Pirrotta and colleagues [8,10], from the results of studies with the Polycomb response element, boundaries might act by blocking the assembly of *cis*-organized multiprotein complexes, whether those required to bring together an enhancer with a promoter, or those required to assemble a silenced domain around a Polycomb response element. Where the assembly of such complexes is dependent on weak DNA–protein interactions, the system might be sensitive to changes in nuclear localization that change the effective concentrations of chromosomal proteins.

The recent findings might thus point to some combination of the models illustrated in Figure 1b and 1d. While speculative, ideas along these lines can clearly be tested, both by examining the patterns of protein–DNA interaction in chromatin by cross-linking assays [21] and by examining changes in nuclear localization of a given gene in relation to both regulatory events and genetic manipulation [19]. The next few years should see an explosion of new findings in this fascinating area, bringing together studies of gene regulation, chromatin assembly and nuclear architecture.

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