Insulators help in organizing the eukaryotic genomes into physically and functionally autonomous regions through the formation of chromatin loops. Recent findings in Drosophila and vertebrates suggest that insulators anchor multiple loci through long-distance interactions which may be mechanistically linked to insulator function. Important to such processes in Drosophila is CP190, a common co-factor of insulator complexes. CP190 is also known to associate with the nuclear matrix, components of the RNAi machinery, active promoters and borders of the repressive chromatin domains. Although CP190 plays a pivotal role in insulator function in Drosophila, vertebrates lack a probable functional equivalent of CP190 and employ CTCF as the major factor to carry out insulator function/chromatin looping. In this review, we discuss the emerging role of CP190 in tethering genome, specifically in the perspective of insulator function in Drosophila. Future studies aiming genome-wide role of CP190 in chromatin looping is likely to give important insights into the mechanism of genome organization.

Introduction

One of the fundamental questions in nuclear biology is how eukaryotes package their relatively large genome in the tiny confines of the nucleus. Studies over the past many years have strongly supported the idea that eukaryotic genomes are organized into a series of structurally and functionally independent domains of chromatin through inter and intra-chromosomal interactions among various regulatory regions. The establishment as well as the maintenance of such domains involve the action of cis-regulatory elements referred to as “insulators” or “boundaries.” Chromatin insulators were first discovered in Drosophila melanogaster and subsequently in a variety of organisms including yeast, mosquito, Xenopus, chicken, mice and humans suggesting their widespread importance in genome organization. These elements exert their effects by preventing inappropriate cross-talk between genomic regions such as enhancers and promoters and active and silent states of the chromatin (Fig. 1). Insulators have been identified using transgenic assays in which they block enhancer-promoter interactions when present between the two (hence, referred to as enhancer-blockers) and/or prevent the spreading of the silencing effects of the heterochromatin (referred to as barriers). While some of the characterized insulators have been shown to act primarily as barriers to heterochromatin, others may possess both enhancer-blocking and barrier activity. For their function, insulators depend on specific proteins that associate with these elements. Although the presence of GAGA factor (GAF) has been reported recently, CTCF remains the major protein that mediates the insulator function in vertebrates. However, in Drosophila, multiple insulator elements have been characterized that vary widely in their DNA sequences and in the proteins that bind to them. Among the best studied elements are the so and scs from the bdp70 heat shock locus, gypsy from the gypsy retrotransposon, SFI from the Antennapedia complex and Fab-7 and Fab-8 from the bithorax complex. While so and scs’ ‘use Zest-white-5 (Zw5) and Boundary Element Associated Factor (BEAF), respectively, as their main DNA binding proteins, the gypsy insulator binds to Suppressor of Hairy-wing [Su(Hw)], Fab-7 and SFI bind to GAGA factor (GAF) and Fab-8 binds to the Drosophila homolog of the vertebrate CTCF (dCTCF). The function of these insulators often depends on the insulator co-factor, CP190 through protein-protein interactions. Recent studies have suggested that insulator proteins such as Su(Hw), BEAF, and dCTCF bind specific DNA sequences and recruit CP190 and Mod(mdg4), which through homotypic and heterotypic protein-protein interactions bridge contacts between distant genomic region. In this review, we will largely focus our discussion on the role of CP190 as a key player in insulator function and genome organization.

The Centrosomal Protein 190 (CP190)

CP190 (for centrometal protein of 190 kDa) is a protein of 1,096 amino acids with a predicted molecular weight of 121 kDa and an apparent molecular weight of about 190 kDa. The protein contains an N-terminal BTB/POZ (broad-complex, tramtrack and bric-a-brac/poxvirus and zinc finger) domain, an aspartic-acid-rich D-domain, three C2H2 zinc finger motifs, and a C-terminal E-rich domain (Fig. 2). Apart from these motifs, CP190 also contains a centrosomal targeting domain (CENT) for its localization to centrosomes during mitosis. The BTB/POZ, the aspartic-acid rich
Earlier studies on the localization of CP190 have shown it to be associated with centrosomes throughout the nuclear division cycle in syncytial Drosophila embryos. However, after the cellularization of the embryo, CP190 is exclusively found in nucleus during the interphase. CP190 was found at a number of sites along the entire length of the chromosomes localizing to band and interband boundaries. These early observations indicated the function of CP190 beyond centrosomes. A variety of non-histone proteins have been shown to have a structural or regulatory role in chromatin. For example, the Polycomb group (PcG) proteins maintain the compact and transcriptionally repressive state of the homeotic genes during development. In contrast, the proteins of the trithorax group, maintain an open state of chromatin. GAF, a product of trithorax-like (Trl) gene is a zinc-finger protein that associates with a large number of chromosome loci and its mutation leads to enhancement of position effect variegation, suggesting its role in organizing chromatin structure. Similarly, Su(Hw) and dCTCF are also zinc-finger proteins which have been found to localize to a number of sites in the genome especially at the boundaries between bands and interbands and are important for insulator function and organization of chromatin.

Genomic Distribution of CP190

CP190 was originally identified in Drosophila melanogaster using a monoclonal anti-centrosomal antibody and was subsequently used to select the CP190 gene from a Agt11 expression library. Like other Drosophila insulator proteins (dCTCF and GAF being the exception) CP190 appears to be restricted to insects. Although CP190 was initially identified and characterized as a result of its association with centrosomes and microtubules, later studies showed it to be localized in the nucleus and bind to specific sites on polytene chromosomes, suggesting its role in the interphase nuclei. Early biochemical studies also suggest that CP190 is a component of nuclear matrix. Depleting CP190 in culture cells does not significantly interfere with centrosomes and microtubule organization, cell division or with cell viability. However, flies that are homozygous mutant for CP190 die at the late pupal stages, suggesting that it is essential for fly development. CP190 does not bind directly to DNA; however, it is crucial for the insulator function of Su(Hw) dependent gypsy and dCTCF dependent Fab-8 insulators. CP190 has also been shown to associate with other subclasses of insulator complexes such as a BEAF-32.
that there is no distinction between individual CP190 sites and sites co-bound by Su(Hw)+CP190 and dCTCF+CP190 in S2 and BG3 cells as well as in whole embryos suggesting that co-binding of CP190 is not a product of tissue specific regulation. Moreover, around 80% robust CP190 binding sites overlap with dCTCF, Su(Hw) or BEAF-32. Interestingly, they also found a number of sites in the genome where CP190 binds independently of Su(Hw), dCTCF and BEAF-32. It was also found that recruitment of CP190 and BEAF to co-bound sites is independent of each other as knockdown of either BEAF or CP190 do not effect each other’s recruitment. This study also found that majority of
tempt us to think that CP190, which has a global genomic distribution like other insulator factors, may be another protein with a larger role in organization of chromatin structure. Early studies using a genetic screen for dominant enhancers of mod(mdg4) identified CP190 as a third component of the gypsy insulator-complex. CP190 was found to co-localize extensively with Mod(mdg4)2.2 and the Su(Hw) proteins at endogenous insulator sites and at the borders of bands/interbands on the polytene chromosomes. Subsequently, CP190 was also shown to occupy a number of dCTCF target sites, when analyzed on polytene chromosomes. These results indicated that, although Su(Hw) and dCTCF have diverse binding specificity, they share co-factors. Recent genome-wide ChIP-chip data revealed an extensive overlap of CP190 with the target sites of four major insulator factors; dCTCF, Su(Hw), BEAF and GAF. Although dCTCF and Su(Hw) target sites do not overlap, CP190 largely overlaps with both these insulator proteins and with around 80% of the GAF sites. Additionally, around 80% of the Stromalin (vertebrate cohesin counterpart in Drosophila) sites and most of the binding sites of dCTCF also overlap with CP190. This analysis found that although many sites of the insulator subclasses overlap, a subset show cell-type specific localization. Similar results were obtained when dCTCF target sites were compared in S2 and Kc cells, where again a subset of dCTCF target sites showed cell-type specificity. These observations indicate that different subclasses of insulators organize genome in a cell-type specific manner which may be responsible for differential gene expression. However, More recently, using quantitative genome-wide analysis of insulator protein binding, Schwartz et al., found that there is no distinction between individual CP190 sites and sites co-bound by Su(Hw)+CP190 and dCTCF+CP190 in S2 and BG3 cells as well as in whole embryos suggesting that co-binding of CP190 is not a product of tissue specific regulation. Moreover, around 80% robust CP190 binding sites overlap with dCTCF, Su(Hw) or BEAF-32. Interestingly, they also found a number of sites in the genome where CP190 binds independently of Su(Hw), dCTCF and BEAF-32. It was also found that recruitment of CP190 and BEAF to co-bound sites is independent of each other as knockdown of either BEAF or CP190 do not effect each other’s recruitment. This study also found that majority of
the insulator binding sites that showed enhancer-blocking activity in transgenic assays belong to those bound exclusively by CP190 or co-bound by BEAF suggesting that these sites may represent the major group of robust insulator elements in *Drosophila*.

### CP190 Affects the Structure of Chromatin

The organization of nucleosomes and their chemical and compositional modifications play a key role in regulation of gene expression. Recent studies have focused on nucleosome organization and the factors that determine this organization. Underlying DNA sequences, the binding of a transcription factor or chromatin remodelling machinery can influence position of nucleosomes. Repositioning or partial or complete disruption of nucleosomes may allow DNA binding proteins to access their regulatory elements. Given the role of insulators in genome organization, it becomes interesting to look if they have any effects on the organization or re-organization of the nucleosomes. Previous work has shown that mammalian CTCF binding sites are associated with positioned nucleosomes. In *Drosophila*, boundary regions have also been associated with reduced nucleosome occupancy across the bithorax complex. These results are intriguing and point out a key role of insulator elements and their associated proteins in nucleosome organization. CP190 has been shown to bind promoters of active genes and such promoters are depleted of nucleosomes. Target sites which are occupied by both dCTCF and CP190 show lack of H3 and therefore, loss of nucleosomes. In contrast, sites exclusively bound by dCTCF do not show any changes in the nucleosome occupancy. When CP190 is depleted, H3 levels are increased at these sites, suggesting that the loss of nucleosomes is due to CP190. The inverse-correlation is depleted, H3 levels are increased at these sites, suggesting that the loss of H3 and decrease in H3K27me3 at the borders of repressed promoters can be meditated by CP190. This report raises the possibility that, in some cases, CP190 may be sufficient to mediate the insulator function. This is supported by a recent study in which many individual CP190 sites were found across the *Drosophila* genome that act as strong enhancer blockers in transgenic assay. Alternatively, CP190 may be recruited to such sites by yet to be identified DNA binding factors.

### Immunofluorescence

Immunofluorescence studies have shown that Su(Hw), Mod(mdg4)2, dCTCF, and CP190 colocalize and form nuclear speckles termed insulator bodies. It has been suggested that these structures are formed as a result of interaction among individual insulator-containing complexes located at distant genomic locations. However, other studies have argued against the clustering of distinct insulator DNA sequences within an insulator body and instead shown that these structures are aggregates of insulator proteins much like the promyelocytic leukemia nuclear bodies (PML-NB). Whether insulator bodies are functional insulator complexes or aggregates of insulator proteins is debatable, however, it is now established that CP190 is crucial to the formation of insulator bodies. It is now established that CP190 is a key player in the formation of insulator bodies when CP190 and dCTCF mutants, the loss of H3 and decrease in H3K27me3 at the borders of repressive domains was found to be dependent on CP190 and not on dCTCF. Similar results were obtained by Negre et al. when they analyzed binding sites of insulator associated proteins including CP190 and found that these regions have low nucleosome density and high histone replacement or displacement. Taken together, these results strongly suggest a possible role of CP190 in chromatin/nucleosome organization and raise the possibility of barrier-like function for CP190 bound regions.

### CP190 is a Common Component of Multiple Insulator Complexes

Unlike vertebrates, which appear to employ only a single factor CTCF for insulator activity, *Drosophila* possesses at least five classes of insulators defined by their DNA binding proteins. These include the Su(Hw), dCTCF, Z5w5, BEAF and GAF. Genetic studies and genome-wide analysis have shown that, at least four of the insulator factors, Su(Hw), dCTCF, BEAF and GAF share a common component, the CP190 protein, although, in case of GAF it has not been conclusively proven (Fig. 3). CP190 physically interacts with Su(Hw) and Mod(mdg4)2. Furthermore, it was shown that CP190 is required for gypsy insulator function. Similarly, using a combination of co-immunoprecipitation, DNA FISH and affinity chromatography, two individual studies showed that CP190 interacts with dCTCF and is required for the formation of Zeste-22 insulator. Using a transgenic enhancer-blocking assay, CP190 was also shown to be required for the boundary activity of HS1 insulator in the *Drosophila* bithorax complex. Genome-wide ChIP-chip analysis has also revealed extensive overlap of CP190 with BEAF sites. These results suggest that most of the insulator factors recruit CP190 as a co-factor and, therefore, may utilize a common mechanism for their insulator function. However, a recently identified insulator, Wari at the 3’ end of the white gene lacks binding sites for any of the known insulator factors but is bound by CP190. This report raises the possibility that, in some cases, CP190 may be sufficient to mediate the insulator function.
two insulators play an important role in mediating their interaction. Similarly, using reporter assay, Fab-7 and Fab-8 have been shown to interact with a CTCF site in the Abd-B promoter.

Several studies have shown that Polycomb target sites interact over long distances, and in certain cases, such interactions are mediated by insulators. For example, a single copy of gypsy insulator restricts a PRE (polycomb response element) from interacting with a distal promoter, while two copies of gypsy bring a PRE to a downstream target gene to mediate its repression. Similarly, the interaction of Mcp and Fab-7 elements, which contain both PRE and insulator activity, has been shown to depend on the underlying insulator activity of these elements.

These studies suggest insulators to be the chief factors mediating long-range interactions among diverse regulatory elements in Drosophila.

While the involvement of major insulator factors in long-range interactions has been shown previously, recent observations point to an important role of CP190 in mediating such interaction. It is proposed that CP190 is recruited to different insulator sites either by itself or by insulator binding proteins which then act as a common adaptor that mediates interaction among other proteins with which CP190 interacts to carry out insulator function.

“Loops” of the Genome: An Emerging View of Insulator Function

Recent data emerged from studies employing high resolution techniques such as 3D-FISH and chromosome conformation capture assay (3C) and its variants, support the idea that insulators directly and physically interact with each other or with other regulatory elements such as promoters, enhancers and silencers, to form chromatin loops. Depending upon the nature and context in which such interactions take place, the target loci could either be relocated to a transcription factory, resulting in transcriptional activation or to a Polycomb group (PcG) body, causing transcriptional repression (Fig. 4). One of the earliest evidence of the existence of insulator-mediated long-range interaction in Drosophila came from studies on the ssc and ssc‘ insulators of the heat shock locus. 3C assay showed that Zex5 bound ssc and BEAF bound ss‘ physically interact with each other. Similar interactions have been observed between two insertions of the gypsy in a Su(Hw) dependent manner. GAF binding insulators such as Fab-7 or Mcp and dCTCF/CP190 binding Fab-8 have been shown to mediate long-range interaction in the bithorax complex. It has been found that Abd-B (Abd-B) and Antennapedia (Antp) genes, which are located far away (~10 Mb) in chromosome 3R, colocalize in nuclei of cells in which both genes are repressed. However, when Fab-7 or Mcp is deleted their colocalization is reduced suggesting that these two insulators play an important role in mediating their interaction.

Similarly, using reporter assay, Fab-7 and Fab-8 have been shown to interact with a CTCF site in the Abd-B promoter. Several studies have shown that Polycomb target sites interact over long distances, and in certain cases, such interactions are mediated by insulators. For example, a single copy of gypsy insulator restricts a PRE (polycomb response element) from interacting with a distal promoter, while two copies of gypsy bring a PRE to a downstream target gene to mediate its repression. Similarly, the interaction of Mcp and Fab-7 elements, which contain both PRE and insulator activity, has been shown to depend on the underlying insulator activity of these elements.

These studies suggest insulators to be the chief factors mediating long-range interactions among diverse regulatory elements in Drosophila. While the involvement of major insulator factors in long-range interactions has been shown previously, recent observations point to an important role of CP190 in mediating such interaction.
different insulator sites (Fig. 4). For example, Moskvich and coworkers showed that chromosomal looping in the Abd-B locus is dependent on CP190.44 When CP190 is depleted in S2 cells, long-range interactions and loop formation among regulatory elements in the Abd-B locus is impaired. Further evidence for the role of CP190 in promoting chromatin looping has come from a recent study involving a CTCF dependent insulator at the Eip57B locus.45 CP190 was shown to stabilize loop formation necessary for restricting transcriptional activation to edysome regulated genes. More recently, Hou et al. found that domain boundaries which are enriched for insulator factors dCTCF, BEAF and CP190 interact more frequently which may facilitate clustering of active and silent genes to transcription factories and Polycomb (Pc) bodies respectively.7

It is interesting to note that CP190, which is critical for insulator function and chromatin looping in Drosophila, has not been conserved in vertebrates. Instead vertebrate CTCF co-operates with cohesin which creates or stabilizes chromatin loops by physically linking different CTCF-binding sites on the same or different chromosomes.28,79 Thus, it is tempting to propose that cohesin might play a role equivalent to that of CP190 in Drosophila. It has been observed that upon heat shock CP190 disassociates from the chromatin or insulator complexes while the localization of DNA binding proteins is not affected.45,72 Similarly, depletion of cohesin results in disruption of chromatin looping and changes in expression of genes under CTCF control, without affecting the expression or binding of CTCF.23,28 These results suggest that recruitment of CP190 and cohesin may function as a regulatory mechanism of controlling insulator activity in Drosophila and vertebrates, respectively. Another interesting connection between CP190 and cohesin is their interaction with DEAD-box RNA helicases and the regulation of insulator activity. CP190 physically interacts with RNA helicase Rm62 in an RNA dependent manner to negatively regulate insulator function of the gypsy insulator.64 Accordingly, reduction in the levels of Rm62 restores the insulator activity of the gypsy insulator. Similarly, RNA dependent interactions have also been observed between RNA helicase p68 (the vertebrate counterpart of Rm62) and cohesin.114 p68 interacts with cohesin to positively regulate insulator activity of IgH/Ig19 ICR which upon depletion of p68 is compromised.45 Although the interaction between CP190 and Rm62 in Drosophila and that of p68 and cohesin in vertebrates have opposing effects, the conservation of this interaction is remarkable. Overall, the view emerging from these studies suggest that the central function of insulators may be to create or stabilize chromosome interactions and that CP190 plays a key role in mediating such interactions in Drosophila. However, in vertebrates, it appears that cohesin may have replaced CP190 to mediate long-range interactions.

Concluding Remarks

It is clear that CP190 acts as a common anchor protein of different insulator classes and plays a wide role in genome organization through the formation of chromatin loops. Several genome wide studies have shown that CP190 co-localizes with all the major insulator binding partners.45,73,80 It was observed that a number of sites in the Drosophila genome where CP190 does not co-localize with any of the known insulator proteins. Whether these represent CP190 target sites to which it binds on its own, as suggested in a recent study by Schwartz et al.,46 or sites where CP190 is recruited by DNA binding factors that are yet to be identified. In either case, it is clear that CP190 has broad range of genomic loci under its regulatory influence. Another feature of CP190 is its correlation with nucleosome occupancy. At active promoters and borders of repressive chromatin domains, CP190 negatively correlates with nucleosome occupancy suggesting that it may directly disrupt the nucleosome assembly or recruit histone modifiers and/or chromatin remodelers.86 Insulators mediate long-range interactions between distant genomic regions and, interestingly, such regions also include Polycomb target sites.64,73 It is tempting to speculate that CP190, which is involved in insulator mediated looping, may have a role in targeting such sites to polycomb bodies.64,73 Furthermore, given its global genomic distribution and the availability of high resolution 3C technique, it will also be important to study the role of CP190 in long-distance interactions on a genome-wide scale. Finally, while a global regulator of chromatin structure is expected to be conserved across the species, vertebrates appear to lack a CP190 counterpart. It remains to be seen if CTCF acquired different partners in vertebrates such as cohesin or a functional homolog of CP190 exists that remains to be identified.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 2012; 497:356-60. PMID:22495300; http://dx.doi.org/10.1038/nature12107.
2. Heo C, Li Z, Qin ZS, Corces VG. Gene density, transcription, and insulators contribute to the partition of the Drosophila genome into physical domains. Mol Cell 2012; 48:873-84; PMID:22841285; http://dx.doi.org/10.1016/j.molcel.2012.08.031.
3. Lubben-Ansen A, van Belkum NL, Williams L, Braaker M, Regoes R, Tilling A, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science 2014; 345:209-35; PMID:25197076; http://dx.doi.org/10.1126/science.1251403.
4. Saunders T, Sibley E, Kristjanson J, Bavro A, Leblanc B, Harcheian M, et al. Three-dimensional folding and associated organization principles of the Drosophila genome. Cell 2012; 148:956-72; PMID:22265598; http://dx.doi.org/10.1016/j.cell.2012.01.030.
5. Alahverdi SH, Saunaghi A, Vanetti D, Shouche YS, Mehra BK. Conserved boundary elements from the Hox complex of mosquitoes. Anopheles gambiae, Aedes albopictus, and Aedes aegypti. Insect Mol Biol 2003; 12:543-58; PMID:12861783; http://dx.doi.org/10.1046/j.1365-2583.2003.00011.x.
6. Chung H, Whibley M, Felsenfeld G. A novel element of the chicken beta-globin domain serves as an insulator in human erythroid cells and protects against position effect in Drosophila. Cell 1999; 74:505-16; PMID:10348617; http://dx.doi.org/10.1016/S0092-8674(99)00832-2.
1. Schlenke S, Hagstrom K, Laemmli UK. The scs' bound-
gary element: characterization of boundary element-
and nuclear structures: Drosophila CP60 and CP190. J
2. Schoborg TA, Oegema K, Parry H, Whitfield KG, Laem-
mation marks the boundaries of cis-regulatory domains.
3. Schwartz YB, Linder-Brooks A, Tolstorukov MY, Kim M,
4. Schwartz YB. Histone replacement and insulator func-
tions of position-orientation variation. Genes Dev 1993, 7:1999-1995. PMID:808125.
5. Franke A, DeCamillis M, Zick D, Chong N, Brock PN, Henikoff JG. Histone replacement and insulator func-
tions of position-orientation variation. Genes Dev 1993, 7:1999-1995. PMID:808125.
6. Schwartz YB, Linder-Brooks A, Tolstorukov MY, Kim M, Li HB, et al. Nature and
7. Vyas S, Liu LP, Song X, Liu X, Baugher A, Paro R. Polycomb and polyhomeotic
8. Butcher RD, Chodagam S, Basto R, Tolkhorov MY, Kim M, Li HB, et al. Nature and
9. Messmer SF, Karch F, Paro R. Polycomb and polyhomeotic
10. Li Q, Sumantyaprasit G. Hypersensitive sites 5
11. Mandel LK, Kasten SA, Zinn KE. The ti boundary element may facilitate independent gene
12. West AG, Gehrke S, Oegema K, Corces VG. DNA position-specific repres-
sor proteins CTCF and CP190 link enhancer block-
13. Schoborg TA, Oegema K, Parry H, Whitfield KG, Laemmli UK. The scs' bound-
gary element: characterization of boundary element-
and nuclear structures: Drosophila CP60 and CP190. J
14. Schoborg TA, Oegema K, Parry H, Whitfield KG, Laemmli UK. The scs' bound-
gary element: characterization of boundary element-
and nuclear structures: Drosophila CP60 and CP190. J
15. Li Q, Sumantyaprasit G. Hypersensitive sites 5
16. West AG, Gehrke S, Oegema K, Corces VG. DNA position-specific repres-
sor proteins CTCF and CP190 link enhancer block-
17. Oegema K, Parry H, Whitfield KG, Laemmli UK. The scs' bound-
gary element: characterization of boundary element-
and nuclear structures: Drosophila CP60 and CP190. J
18. Oegema K, Parry H, Whitfield KG, Laemmli UK. The scs' bound-
gary element: characterization of boundary element-
and nuclear structures: Drosophila CP60 and CP190. J
19. Henderson DS, Raff JW, et al. The Drosophila centro-
dependent localization of the CP190 centrosomal protein is determined by the coordinate action of
two separable domains. J Cell Biol 1995; 131:1261-
20. Yang J, Ceson VG. Insulators, lineage interfaces,
and genome functions. Cont. Opin. Genet. Dev. 2012, 22:86-92. PMID:22225527, http://dx.doi.
21. Olson D, Shmueli B, South H, Alkabi O, Pa VC. The chromosomal insulator element. Curr. Op. Genet. Dev. 2012, 22:86-92. PMID:22225527, http://dx.doi.
22. Butcher RD, Chodagam S, Basto R, Tolkhorov MY, Kim M, Li HB, et al. Nature and
23. Schwartz YB. Histone replacement and insulator functions of position-orientation variation. Genes Dev 1993, 7:1999-1995. PMID:808125.
24. Schlenke S, Hagstrom K, Laemmli UK. The scs' bound-
gary element: characterization of boundary element-
and nuclear structures: Drosophila CP60 and CP190. J
25. Chodagam S, Basto R, Tolkhorov MY, Kim M, Li HB, et al. Nature and
26. Butcher RD, Chodagam S, Basto R, Tolkhorov MY, Kim M, Li HB, et al. Nature and
27. Yang J, Ceson VG. Insulators, lineage interfaces,
and genome functions. Cont. Opin. Genet. Dev. 2012, 22:86-92. PMID:22225527, http://dx.doi.
28. Oegema K, Parry H, Whitfield KG, Laemmli UK. The scs' bound-
gary element: characterization of boundary element-
and nuclear structures: Drosophila CP60 and CP190. J
29. Oegema K, Parry H, Whitfield KG, Laemmli UK. The scs' bound-
gary element: characterization of boundary element-
and nuclear structures: Drosophila CP60 and CP190. J
30. Olson D, Shmueli B, South H, Alkabi O, Pa VC. The chromosomal insulator element. Curr. Op. Genet. Dev. 2012, 22:86-92. PMID:22225527, http://dx.doi.
31. Olson D, Shmueli B, South H, Alkabi O, Pa VC. The chromosomal insulator element. Curr. Op. Genet. Dev. 2012, 22:86-92. PMID:22225527, http://dx.doi.
32. Butcher RD, Chodagam S, Basto R, Tolkhorov MY, Kim M, Li HB, et al. Nature and
33. Schwartz YB. Histone replacement and insulator functions of position-orientation variation. Genes Dev 1993, 7:1999-1995. PMID:808125.
34. Schwartz YB. Histone replacement and insulator functions of position-orientation variation. Genes Dev 1993, 7:1999-1995. PMID:808125.
35. Schlenke S, Hagstrom K, Laemmli UK. The scs' bound-
gary element: characterization of boundary element-
and nuclear structures: Drosophila CP60 and CP190. J
36. Schlenke S, Hagstrom K, Laemmli UK. The scs' bound-
gary element: characterization of boundary element-
and nuclear structures: Drosophila CP60 and CP190. J
37. Chodagam S, Basto R, Tolkhorov MY, Kim M, Li HB, et al. Nature and
38. Butcher RD, Chodagam S, Basto R, Tolkhorov MY, Kim M, Li HB, et al. Nature and
39. Henderson DS, Raff JW, et al. The Drosophila centro-
dependent localization of the CP190 centrosomal protein is determined by the coordinate action of
two separable domains. J Cell Biol 1995; 131:1261-
40. Yang J, Ceson VG. Insulators, lineage interfaces,
and genome functions. Cont. Opin. Genet. Dev. 2012, 22:86-92. PMID:22225527, http://dx.doi.
41. Olson D, Shmueli B, South H, Alkabi O, Pa VC. The chromosomal insulator element. Curr. Op. Genet. Dev. 2012, 22:86-92. PMID:22225527, http://dx.doi.
42. Butcher RD, Chodagam S, Basto R, Tolkhorov MY, Kim M, Li HB, et al. Nature and
43. Schwartz YB. Histone replacement and insulator functions of position-orientation variation. Genes Dev 1993, 7:1999-1995. PMID:808125.
44. Schwartz YB. Histone replacement and insulator functions of position-orientation variation. Genes Dev 1993, 7:1999-1995. PMID:808125.
45. Schlenke S, Hagstrom K, Laemmli UK. The scs' bound-
gary element: characterization of boundary element-
and nuclear structures: Drosophila CP60 and CP190. J
46. Schlenke S, Hagstrom K, Laemmli UK. The scs' bound-
gary element: characterization of boundary element-
and nuclear structures: Drosophila CP60 and CP190. J
47. Chodagam S, Basto R, Tolkhorov MY, Kim M, Li HB, et al. Nature and
48. Butcher RD, Chodagam S, Basto R, Tolkhorov MY, Kim M, Li HB, et al. Nature and
49. Henderson DS, Raff JW, et al. The Drosophila centro-
dependent localization of the CP190 centrosomal protein is determined by the coordinate action of
two separable domains. J Cell Biol 1995; 131:1261-
50. Yang J, Ceson VG. Insulators, lineage interfaces,
and genome functions. Cont. Opin. Genet. Dev. 2012, 22:86-92. PMID:22225527, http://dx.doi.
51. Olson D, Shmueli B, South H, Alkabi O, Pa VC. The chromosomal insulator element. Curr. Op. Genet. Dev. 2012, 22:86-92. PMID:22225527, http://dx.doi.
52. Butcher RD, Chodagam S, Basto R, Tolkhorov MY, Kim M, Li HB, et al. Nature and
53. Schwartz YB. Histone replacement and insulator functions of position-orientation variation. Genes Dev 1993, 7:1999-1995. PMID:808125.
54. Schwartz YB. Histone replacement and insulator functions of position-orientation variation. Genes Dev 1993, 7:1999-1995. PMID:808125.
55. Niles-Llach S, Czarnowiski S, Ascoli P, Episkopou V. Characterization of a new regulatory element within the Drosophila forkhead complex. Nucleus. 2008; 1(3):46-53. PMID:19798017; http://dx.doi.org/10.1007/s12077-008-0008-8.

56. Erokhin M, Parshikov A, Georgiev P. Chromatin insulators determine the nuclear localization of DNA. Mol Cell. 2000; 4:1025-35. PMID:11067672; http://dx.doi.org/10.1016/S1097-2765(00)00101-5.

57. Gerasimov A, Paredes O, Toshchakov S, Potapova A, Georgiev P. SUMO conjugation of proteins but not of insulators. EMBO Rep. 2008; 9(9-10):1380-6; PMID:18830600; http://dx.doi.org/10.1038/embobio.2008.32.

58. Gerasimov A, Volkov I, Georgiev P. U2AF35 arginine is required for the assembly of Drosophila Su(Hw) and HUA43 insulator bodies. Curr Opin Genet Dev. 2012; 22:101-9; PMID:22178420; http://dx.doi.org/10.1016/j.ogc.2011.12.001.

59. Pirrotta V. The chromatin insulator determines the nuclear localization of DNA. Mol Cell. 2000; 6:1025-35; PMID:11106742; http://dx.doi.org/10.1016/S1097-2765(00)00101-5.

60. Kohchi T, Towle A, Corces VG. The ubiquitin ligase dTAPORS mediates the association of Polycomb response element (PRE) contacts with chromatin. Mol Cell. 2006; 23:215-26; PMID:16549355; http://dx.doi.org/10.1016/j.molcel.2006.06.016.

61. Kyrchanova O, Toshchakov S, Potapova A, Georgiev P. Functional interaction between the Fab-7 and Fab-8 boundaries and the upstream promoter region in the Drosophila Abd-B gene. Mol Cell Biol. 2008; 28:4188-95; PMID:18426914; http://dx.doi.org/10.1128/MCB.00229-08.

62. Muller M, Hagegeon K, Guzyatskaya H, Pirrotta V, Schell P. The cyp element from the Drosophila melanogaster leukemia complex mediates long-distance regulatory interactions. Genome Res. 1999; 9:1333-56; PMID:9945463.

63. Neveu J, Muller M, Pirrotta V, Sadie PE. The cnp domain elements mediate long-range chromosome interactions in Drosophila. Mol Cell Biol. 2006; 26:1721-30; PMID:16495353; http://dx.doi.org/10.1128/MCB.00849-10.

64. Corset I, Scherberich B, Seaton T, Cardall G. A chromatin insulator driving three-dimensional Polycomb response element (PME) contacts and Polycomb association with the chromatin. Proc Natl Acad Sci U S A. 2011; 108:2094-9; PMID:21813466; http://dx.doi.org/10.1073/pnas.1012180108.

65. van Straelen B, Delker D. Genomatix tools for inferring chromatin architecture. Nat Biotechnol. 2010; 28:1089-95; PMID:20944601; http://dx.doi.org/10.1038/nbt.1680.

66. Bartkuhn M, Bredlow L, Kyrchanova O, Toshchakov S, Podstreshnaya Y, Parshikov A, Georgiev P. Functional interaction between the Drosophila insulator and Polycomb response element (PRE) contacts with chromatin. Mol Cell. 2011; 44:29-38; PMID:21981916; http://dx.doi.org/10.1016/j.molcel.2011.01.013.

67. Chu H, Buck K, Zhang J, Xue S, Camerini-Otero RD, Felsenfeld G. Mediation of CTCF transcriptional insulator by DEAD-box RNA-binding protein p68 and neural receptor RNA activator SRA. Genes Dev. 2010; 24:2543-55; PMID:20984584; http://dx.doi.org/10.1101/gad.1967810.

68. Kyrchanova O, Toshchakov S, Potapova A, Georgiev P. Chromatin insulators form gene loops by interacting with promoters in Drosophila. Development. 2011; 138:497-506; PMID:21842594; http://dx.doi.org/10.1242/dev.057070.

69. Pirrotta V, Li HR. A view of nuclear Polycomb bodies. Gene. 2008; 413:101-6; PMID:18179422; http://dx.doi.org/10.1016/j.gene.2008.11.004.

70. Bhatia J, Ganote M, Shull P. Protein:protein interactions and the pairing of boundary elements in vivo. Genes Dev. 2010; 17:664-75; PMID:20281908; http://dx.doi.org/10.1101/gad.185303.

71. Gavrilova E, Kruse K, Li J, Loo B, Gerasimov A, Schuhroth H, Bonnet J, et al. Polycomb-dependent regulatory contacts between distant chromosomal loci in Drosophila. Cell. 2011; 144:216-26; PMID:21262819; http://dx.doi.org/10.1016/j.cell.2011.02.026.

72. Galkin AV, Georgiev P. Functional interaction between the Fab-7 and Fab-8 boundaries and the upstream promoter region in the Drosophila Abd-B gene. Mol Cell Biol. 2008; 28:4188-95; PMID:18426914; http://dx.doi.org/10.1128/MCB.00229-08.

73. Tomishige K, Li HB, Muller M, Bahechar IA, Kyrchanova O, Toshchakov S, Podstreshnaya Y, Parshikov A, Georgiev P. Functional interaction between the Drosophila insulator and Polycomb response element (PRE) contacts with chromatin. Mol Cell. 2011; 44:29-38; PMID:21981916; http://dx.doi.org/10.1016/j.molcel.2011.01.013.

74. Rohrb C, Rosen DJ, Welsh PL, Detrich CM, Krippes GW, Budge NS, et al. CTFC physically links cohesin to chromatin. Proc Natl Acad Sci U S A. 2008; 105:8309-14; PMID:18550811; http://dx.doi.org/10.1073/pnas.0806468105.

75. Schedl P, Wang F, Liu S, Kind J, Bartmann MS, Lehmann PH. Cohesin interactions with CTFC at the KHSRP locus control region and at cellular c-myc and H19/IGF2 insulators. EMBO J. 2009; 28:4196-60; PMID:19235772; http://dx.doi.org/10.1038/sj.emboj.2008.118.

76. Wondi KS, Yoshida K, Koh T, Badele M, Kori B, Schlaghofer E, et al. Cohesin mediates transcriptional silencing by CCCTC-binding factor. Nature. 2008; 451:76-81; PMID:18219272; http://dx.doi.org/10.1038/nature06634.

77. Nascimento R, Wondi KS, Sr X, Hodgkinson J, Hutt- Lewis S, Woodall K, et al. Cohesin is required for higher-order chromatin configuration at the imprinted IGf2-H19 locus. PLoS Genet. 2009; 5:e1000739; PMID:19956766; http://dx.doi.org/10.1371/journal. pgc.1000739.

78. Le PP, Corset I, Scherberich B, Seaton T, Cardall G. A chromatin insulator driving three-dimensional Polycomb response element (PME) contacts and Polycomb association with the chromatin. Proc Natl Acad Sci U S A. 2011; 108:2094-9; PMID:21813466; http://dx.doi.org/10.1073/pnas.1012180108.

79. Barcelo J, Garcia-Manero L, Mora A, Butera G. Cohesin functionally associates with CTFC on mammalian chromatin. Cell. 2008; 132:42-5; PMID:18327772; http://dx.doi.org/10.1016/j.cell.2008.03.011.

80. Bantignies F, Reguero V, Comet I, Leblanc B, Lachaud G, Pierre V, et al. Cohesins possess an H2A.Z-specific binding activity. EMBO J. 2010; 29:1296-307; PMID:20241211; http://dx.doi.org/10.1038/emboj.2010.5.

81. Bantignies F, Reguero V, Comet I, Leblanc B, Lachaud G, Pierre V, et al. Cohesins possess an H2A.Z-specific binding activity. EMBO J. 2010; 29:1296-307; PMID:20241211; http://dx.doi.org/10.1038/emboj.2010.5.

82. Bantignies F, Reguero V, Comet I, Leblanc B, Lachaud G, Pierre V, et al. Cohesins possess an H2A.Z-specific binding activity. EMBO J. 2010; 29:1296-307; PMID:20241211; http://dx.doi.org/10.1038/emboj.2010.5.