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REVIEW: GENE REGULATION

Insulators and Boundaries: Versatile Regulatory Elements in the Eukaryotic Genome

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Insulators mark the boundaries of chromatin domains by limiting the range of action of enhancers and silencers. Although the properties of insulators have been well studied, their role in vivo has largely been a subject of speculation. Recent results make it possible to ascribe specific and essential functions to the insulators of *Drosophila*, yeast, and vertebrates. In some cases, insulator activity can be modulated by nearby regulatory elements, bound cofactors, or covalent modification of the DNA. Not simply passive barriers, insulators are active participants in eukaryotic gene regulation.

Within the eukaryotic nucleus, each gene is embedded within a chromosomal environment of other DNA sequences that have the potential to affect its expression. In some cases, regulatory elements—enhancers or silencers—associated with nearby genes could be close enough to disrupt normal expression patterns. In other cases, a transcriptionally active gene is surrounded by regions of condensed chromatin that could overflow their borders and silence the gene.

How does a gene with its own programmed pattern of expression defend itself against its neighbors? Work over the last several years suggests that specialized DNA sequence elements called insulators, which flank some genes, may be responsible for providing a barrier against incursions from surrounding domains. Although the insulator elements vary greatly in their DNA sequences and the specific proteins that bind to them, they have at least one of two properties related to barrier formation. First, insulators have the ability to act as a "positional enhancer blocker": If the insulator lies between a promoter and an enhancer, then enhancer-mediated activation of the promoter is impaired, but if the insulator lies outside the region between enhancer and promoter, little or no effect is observed. Insulators are neutral barriers to enhancer action; they do not inactivate either the enhancer or the promoter.

Second, insulators have the ability to protect against position effects. When genes are moved from their native context, as in transgenic animals, the dominant effect of the new chromosomal environment becomes apparent. Expression levels at the new location often bear no resemblance to that of the gene in its native position. This variability can result from the proximity of an endogenous enhancer or silencer. It can also reflect the location of the reporter gene near a region of condensed, inactive chromatin. Flanking a transgene with insulators can suppress this variability. Having the ability to protect against position effects and/or to block distal enhancer activity has come to form the operational definition of an insulator.

Most of the properties of insulators in *Drosophila* and vertebrates have been determined using artificial constructs, but there has been little direct evidence as to the role of these elements at their natural genomic sites. Here, we place special emphasis on recent findings that illustrate the role of insulators and boundaries in their native context. Clear functional significance has now been demonstrated for insulators in organisms from yeast to humans. Furthermore, recent results show that some insulators do not behave simply as static barriers; these insulators act as a kind of modulatable switch, allowing them to function as sophisticated regulatory elements.

Enhancer Blocking

Early evidence for the existence of the enhancer-blocking activity of insulators came from two *Drosophila* mutations. The first involved an insertion of a transposable element, *gypsy*, near the *yellow* gene (*1*). Expression of this gene is controlled by multiple tissue-specific enhancers located both 5′ and 3′ of its promoter. The presence of a *gypsy* element upstream of the *yellow* promoter prevents the enhancers located 5′ of this insertion from activating *yellow*, but has no adverse effect on the downstream enhancers. Similar results were obtained at another locus (*2*). The second mutation occurred near the *Abd-B* gene within the *Drosophila* Bithorax complex. This gene, involved in specification of parasegmental identity, is controlled by a series of parasegment-specific enhancers (see below). Mutations in regions (*Fab-7* and *Fab-8*) that lie between the enhancers result in transformation of one parasegment into another, attributable to the merging of enhancer domains (*3*–*6*). Detailed analysis reveals that these mutations have destroyed enhancer-blocking elements within *Fab-7* and *Fab-8*, which are normally responsible for maintaining the separate identities of the enhancers.

The connection between enhancer-blocking activity and chromatin boundary function was made after identification of two elements, scs and scs′, that appeared to mark the ends of a chromatin domain at the *Drosophila hsp-70* locus (*7*). As part of their investigation (*8*) of the boundary properties of these elements (see below), Kellum and Schedl showed that scs and scs′ function as positional enhancer blockers (*9*). They devised an assay that measured the effect of placing an element between an enhancer and promoter in transgenic fruit flies. This enhancer-blocking assay became a defining test for insulator activity.

Proteins have been identified that bind to *gypsy*, scs, and scs′ and that are implicated in their enhancer-blocking activity. Surprisingly, no significant similarity is evident among any of the insulator proteins of fruit flies, yeast, or vertebrates. In the case of *gypsy*, the protein suppressor of hairy wing [Su(Hw)] (*1*) is essential to enhancer-blocking properties. Other proteins, zest-white-5 (Zw5) and BEAF-32, have been shown to bind to scs and scs′, respectively (*10, 11*).

Recently, it was proposed that deletion of a short sequence element with demonstrated enhancer-blocking activity is responsible for the altered expression pattern of the *facet-strawberry* allele of *Notch* (*12*). This mutation suggests a link between insulators and chromosome architecture because the ~880–base pair (bp) deletion eliminates an interband and fuses 3C7 and 3C6 bands in polytene chromosomes.

A number of insulators have now been identified both in other invertebrate species and in vertebrates (*13*). These elements are found in loci with quite different cell-type specificity and function, and they include sites in the sea urchin histone H3 genes (*14*), the ribosomal RNA genes of *Xenopus* (*15*), the human T cell receptor (TCR)-α/δ locus

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(*16*), and the chicken β-globin genes (*17*). All of these insulators are found between genes with independent profiles of expression (Fig. 1), consistent with the idea that they serve to prevent inappropriate interaction between the regulatory elements of the neighboring gene loci.

The first vertebrate insulator to be described, cHS4, is located near the 5′ end of the chicken β-globin locus (*17, 18, 23*). It shares a number of properties with a related insulator element located at the 3′ end of the same locus (*19*). These insulators are associated with deoxyribonuclease I–hypersensitive sites that mark the positions of binding sites for the ubiquitous DNA binding protein CTCF (*20*). Fragments of DNA containing these hypersensitive sites have the enhancer-blocking properties of insulators, and the CTCF sites are both necessary and sufficient for this activity.

Protection Against Position Effects

A second defining activity of insulators is the ability to protect a gene against the encroachment of neighboring silencing or activating signals. Such properties might be expected of elements with the putative role of maintaining chromatin domain boundaries and shielding a locus against outside influences. Some sequences are specialized only to block against silencing from adjacent condensed chromatin, as in the case of the yeast elements at telomeres and mating-type loci mentioned below. There are many elements, however, that can both prevent encroachment by condensed chromatin and block the action of external enhancers and promoters.

The *Drosophila* scs and scs′ elements were first defined as sequences able to protect against position effects (*8*). Fruit flies transformed with a transposable element carrying an eye-color gene have variable eye color depending on the site of integration, a manifestation of the position effect. Surrounding this transgene with scs and scs′ results in suppression of the variability in eye color. These early experiments established the existence of elements able to protect against both activating and inactivating effects that derive from the chromosomal environment. Subsequent analyses demonstrated similar properties for the *gypsy* insulator, which can protect a transgene or a DNA replication origin from position effects (*21*, *22*).

In addition to harboring positional enhancer-blocking activity, the vertebrate insulator element cHS4 can protect a transgene from position effects in *Drosophila* and in early-erythroid chicken cell lines (*17*, *24*). The element was shown also to protect against gradual extinction of expression in culture (*24*). Two copies of a 250-bp HS4 "core" work as well in this assay as two copies of the full 1.2-kb HS4 element, although the CTCF site appears unnecessary to protect against position effects (*25*), suggesting that two overlapping insulator activities coexist at the 5′ end of the chicken β-globin locus. A role in vivo for both activities is suggested by the presence of a nearby gene encoding a folate receptor, separated from the 5′ boundary of the β-globin domain by 16 kb of condensed chromatin (*26*) (Fig. 1A). In this location, the HS4 insulator element might serve both as a barrier to the spread of the condensed chromatin and as an inhibitor of cross talk between the regulatory elements of the two loci. This model of HS4 action remains to be tested in vivo. However, HS4 has proven useful in generating stably expressing transgenic mice (*27*), rabbits (*28*), and cell lines (*24*, *29–31*). The use of insulators results in protection against chromosomal position effects in all tissue types, and in each case, confers uniform reporter expression at most integration sites.

Barriers to the encroachment of silencing signals have also been identified recently in the neighborhood of telomeres and within the mating-type loci of the yeast *Saccharomyces cerevisiae* [reviewed in (*32*)]. The protein components responsible for heterochromatin formation at these loci are well known, and fairly detailed models have been proposed for the condensed chromatin structures. The role of these boundary elements appears to be confined to interference in the condensation process. The binding sites and proteins involved in boundary function at these loci are different from each other and they share no obvious relationship with the fruit fly or vertebrate proteins. A contribution by SIR proteins is common to the silencing mechanisms at the telomeres and mating-type loci of yeast. Because both the SIR proteins of yeast and the polycomb proteins of *Drosophila* are fairly well conserved even in vertebrates, perhaps some commonality among silencing mechanisms and their blockage will ultimately be found.

Modulation of Insulator Activity

Insulator elements have previously been thought of as marking fixed boundaries. A number of recent results, however, show that this is too simple a view: the boundary function of some insulators can be modified or even abrogated in a potentially regulated fashion. Modifying factors may bind to adjacent regulatory sites or to the insulator proteins themselves. More directly, methylation of the DNA binding site for the insulator protein can block binding and insulator activity completely. *Fab-7* and *Fab-8* serve to prevent interaction between adjacent enhancer elements (*iab-6*, *iab-7*, and *iab-8*; see Fig. 2). Although the central regions of *Fab-7* and *Fab-8* are able to block enhancer action over considerable distances in heterologous assays, these long-range disruptions do not appear to occur at their natural chromosomal location. This apparent conflict may be resolved by the identification of a new element (*4*), the promoter-targeting sequence (PTS), which permits the upstream enhancer *iab-7* to bypass *Fab-8* insulation and to activate *Abd-B* (see Fig. 2B legend). The presence of the PTS does not interfere with the local insulating activity of the *Fab* element. It remains to be shown that a PTS is present near other *iab* elements, but the discovery of the first of these suggests how each parasegment-specific enhancer can operate independently of its neighbors, while still allowing each to have

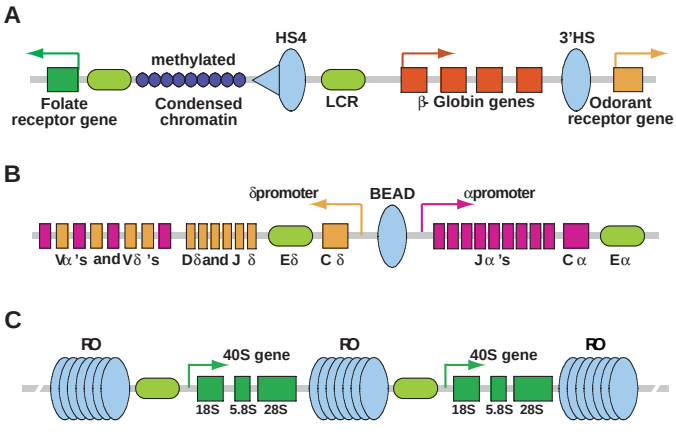

Fig. 1. Vertebrate insulators (blue ovals) are found between genes and enhancers (light green) with distinct profiles of expression. The protein CTCF is required for the enhancer-blocking activity in reporter assays of all the elements shown here (*19*, *20*). (**A**) Chicken β-globin gene locus. The HS4 enhancer-blocking element separates the regulatory elements of the late erythroid-specific globin genes (red) from those of an independently regulated early erythroid-specific folate receptor gene (dark green) (*17*, *23*, *26*). The 3′HS element separates a downstream odorant receptor gene (orange) from the globin locus control elements (*19*). HS4 may also block (triangle) the spread of condensed chromatin (dark blue) that separates these two loci (*25*, *26*). (**B**) Human TCRα/δ locus. The BEAD (blocking element alpha/delta) element separates the differentially regulated TCRα (magenta) and TCRδ (orange) genes from each other's enhancers. The BEAD element displays position-dependent enhancer-blocking activity in reporter assays (*16*, *20*). (**C**) *Xenopus* ribosomal RNA repeats. Tandem arrays of the 40S ribosomal RNA (rRNA) genes are separated into independent transcriptional units by sequence repeats termed the repeat organizer (RO). RO confers polarity of action on the 60/ 81-bp enhancer (*15*), and a single RO repeat unit displays position-dependent enhancer-blocking activity in reporter assays (*20*).

access to the promoter when appropriate.

Modulation of the kind directed by the PTS could involve direct interactions between insulator- and PTS-bound proteins. Some precedent for this kind of modulation of insulator activity is suggested by the observation that mutations in the gene *Mod*(*mdg4*) affect the properties of the Su(Hw) binding sites in *gypsy* (*33*) (Fig. 2A). A large splice variant of this gene (*34*) codes for a protein that binds to the Su(Hw) protein, but not to DNA. Mutations in *Mod*(*mdg4*) can convert the insulator into a partially bidirectional silencer of enhancer action (*33*). This mutation reveals a pathway through which protein-protein interactions could modulate insulator activity.

A more direct way of nullifying the action of an insulator is to prevent binding of the protein responsible for its activity. Recent results show that the imprinting mechanism at the *Igf2/H19* locus uses this strategy (*35*, *36*). Imprinting results in expression of *H19* only from the maternally transmitted allele and of *Igf2* only from the paternal allele (Fig. 2C). Furthermore, the paternal allele is methylated differentially in a region between the two genes (now called the imprinted control region or ICR) even in the gametes. A number of earlier experimental results led to the proposal (*37*) that *Igf2* expression was inhibited on the maternal allele because of a putative insulator, located between *Igf2* and *H19*, that could block the action of a downstream enhancer on the *Igf2* promoter (Fig. 2C). It was suggested that the observed methylation of the ICR could somehow abolish insulator activity, allowing the downstream enhancer to activate *Igf2* expression on the paternal allele.

Direct evidence for the presence of enhancer-blocking activity within the ICR has now come from experiments in which sequences from the mouse or human ICR were inserted between test promoters and enhancers, either in cell lines or in transgenic mice (*35, 36*, *38–40*). Further analysis revealed that mouse, rat, and human ICRs share multiple copies of a single sequence motif with very strong homology to the central region of the binding site for CTCF within the chicken β-globin 5′ insulator (see above) (*35*, *36*, *41*). Seven such sites are present in humans, and four in mouse; in vivo footprinting shows that these sites are occupied in mouse primary embryo fibroblasts (*41*). Gel-shift assays confirm that CTCF binds to these sites; mutations of the sites abolish both binding and insulating activity.

With this insulator in place between the *H19* enhancer and the *Igf2* promoter, it is not surprising that *Igf2* is inactive on the maternal allele, but why is it active on the paternal allele? The answer lies in the observation that methylation of the sites within the ICR abolishes CTCF binding both in vitro and in vivo (*35*, *36*, *41*). Because the activity of this insulator depends on the presence of functional CTCF binding sites, there is no insulation on the methylated paternal allele, and *Igf2* expression is consequently activated. Consistent with the suggestion that methylation allows *Igf2* expression, mouse embryos in which methylation has been eliminated do not express *Igf2* from either allele, because the insulator is now active on both (*42*). In contrast, when the ICR is deleted in mice, the normally silent maternal allele of *Igf2* is expressed (*43*). Thus, the ICR is an insulator through which imprinted expression is directed. Interestingly, conditional deletion of the ICR in mice at different stages of tissue development reveals that removal of this insulator at any stage is sufficient to allow activation of the maternal *Igf2* allele (*39*). In this locus, the insulator is required for establishment and maintenance of an allele-specific silent state, but silencing is directional (having no effect on neighboring *H19*). Furthermore, insulation is not achieved through permanent inactivation of the *Igf2* promoter or the *H19* enhancer; once the insulator is removed, the block is relieved.

Mechanisms

Since the discovery of the first insulators, conflicting models have been proposed to account for their properties. In the case of positional enhancer blocking, it is necessary to explain why placement of the insulator between enhancer and promoter is critical. Position effect protection implies not only this ability to block external enhancers and silencers, but the further capacity to block heterochromatinization. Explanations of enhancer-blocking depend on our uncertain understanding of how enhancers work in higher eukaryotes. Models to explain enhancer blocking include derailment of tracking enhancers and decoys that interfere with looping enhancers; these have been amply reviewed (*13*, *32*, *44*). Provocative new observations have led to other more complex models that propose topological or ultrastructural restrictions on how enhancers and insulators function within the nucleus. A growing body of evidence points to an important role for nuclear compartmentalization in the orchestration of nuclear events and a number of seemingly disparate observations about enhancers, insulators, and silencing can be tied together by such models.

Gerasimova and Corces (*45*) have shown that the location of Su(Hw) completely overlaps that of a subset of mod(mdg4) proteins within the nucleus and, furthermore, that the proteins tend to be arranged in clusters near the nuclear periphery of interphase diploid cells. They have suggested that the Su/mod complex may be tethered to nuclear lamina or

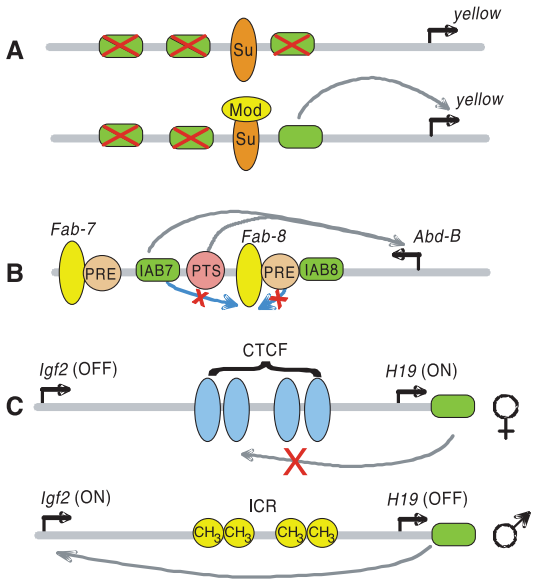

Fig. 2. Modulation of insulator activity. In (A), (B), and (C), the insulators are all shown as ellipses, but each insulator involves a different binding site and protein. (**A**) When Su(Hw) binding sites (orange) in the *gypsy* retrotransposon are inserted between enhancers (green) at the *Drosophila yellow* locus, the effect in the absence of the protein product of *mod*(*mdg4*) is to silence expression from all enhancers (top). Positional enhancer blocking at this locus requires the modifying interaction of mod(mdg4) with Su(Hw) at this site (*33*). With mod(mdg4) present, only enhancers upstream of the insulator are blocked. Enhancers downstream of the promoter are omitted from this diagram. (**B**) The regulatory region for the *Drosophila Abd-B* gene contains parasegment-specific developmentally regulated enhancers including *iab-7* and *iab-8*. *Fab-8* contains an insulator (yellow) that prevents *iab-7* from interfering with the *iab-8* program and also blocks the silencing action of a polycomb response element (PRE) (blocking shown by blue arrows). Although the presence of the *Fab-8* enhancer-blocking activity might be expected to prevent *iab-7* from activating the *Abd-B* promoter in parasegment 12, the recently discovered PTS element modifies this behavior (gray arrows) to allow such long-range interactions (*4*). (**C**) The mouse *Igf2/H19* locus contains four binding sites (blue) for the protein CTCF (seven in humans). CTCF binding is associated with known insulator elements (*20*). On the maternally inherited allele, the CTCF sites in the *Igf2/H19* locus serve to block the action of a downstream enhancer (green) on the *Igf2* promoter. Consequently, *Igf2* is not expressed from the maternal allele. On the paternal allele, the CTCF sites are methylated, and CTCF does not bind, inactivating the insulator. The enhancer is now free to activate *Igf2* expression from the paternal allele (*35*, *36*). [Inactivation of *H19* expression on the paternal allele is controlled by a separate mechanism (*50*).]

other components of the nuclear architecture, creating a series of separate loop domains (a more general version of this is shown in Fig. 3). Strong additional evidence for such a model has been presented in a new paper (*46*) that confirms the location of Su(Hw) protein at the nuclear periphery and shows: (i) The intranuclear location of a DNA sequence near a gypsy element largely overlaps the sites of mod(mdg4) protein clusters ("insulator bodies"). In strains lacking that gypsy insertion, there is much less overlap. (ii) When the gypsy insulator element insert is present, nearby DNA sequences normally distributed throughout the nucleus move to the nuclear periphery. (iii) When copies of the element are inserted near two different sequences normally found at separate nuclear locations, the sequences colocalize. (iv) The effects in (ii) and (iii) are dependent on expression of Su(Hw) protein.

If we suppose in the simplest model that an enhancer and a promoter can only interact when they are within the same loop and that the bound Su/mod proteins create two separate loops, then the positional enhancer-blocking effect is accounted for. If the presence of such a loop also interferes with the extension of an adjacent condensed chromatin structure, general protection against position effects can also be explained. The creation of a looped domain might not require attachment to some fixed site in the nucleus, but only interaction between proteins at the base of the loop.

There is a growing list of proteins that may be involved in establishing the higher-order organization of chromatin. Dorsett and his colleagues [see reviews (*47*, *48*)] have identified two genes, *Chip* and *Nipped-B*, that could serve as "facilitators" of enhancer-promoter interaction (*47*). Chip can promote dimerization of homeodomain (HD) proteins (*49*). HD binding sites are widely distributed in the *Drosophila* genome [see (*48*)], suggesting that Chip could serve as a bridge to gather together the region between enhancer and promoter, bringing them close to each other. *Nipped-B* may also play an architectural role, perhaps in stabilizing the chromatin loop domains. *Chip* mutations enhance the insulator phenotype of *gypsy*, and Chip interacts directly with the Su(Hw) protein. Thus, this insulator could work by interfering with the formation of the gathered structure (*47*, *48*). The effect might be to create two separate such structures, perhaps with Su(Hw) bound to the nuclear lamina, leaving enhancers upstream of the *gypsy* element isolated.

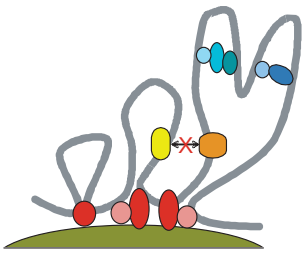

Fig. 3. Generalized diagram of loop domain models. Chromatin fibers are attached to structural components (green) within the nucleus by specific DNA binding proteins (red symbols). These create separate loop domains. It is assumed (without implying a specific mechanism) that an enhancer (yellow symbol) in one loop domain cannot interact with a promoter (orange) in another. Further subdivisions of loops might be created by other classes of proteins (blue), which could either attach elsewhere within the nucleus or perhaps simply interact with each other to cordon off a region. [See (*45*) and (*46*) for more detailed models of this kind.]

Much of the data on other insulators is consistent with a loop-domain model, although some experiments, particularly with plasmids, may be more difficult to accommodate. [See (*13*) for further discussion.] In every case, it will be useful to try to determine whether specific sites of localization for these elements play a role in their function within the nucleus.

Conclusion

The fact that insulator activity can be modulated adds greatly to the range of regulatory possibilities for such elements. The PTS element of fruit flies may have a functional equivalent in vertebrates, and selective methylation similar to that seen at the *Igf2/H19* locus may well be a widely used stratagem.

Drawing on the example of the *Abd-B* locus in *Drosophila*, we suggest that insulators might be found wherever a cluster of enhancers with distinct developmental patterns of control acts on a single gene. Insulators might also be found within clusters of genes whose associated regulatory elements direct different programs of expression, like the arrangement in the chicken folate receptor/β-globin/odorant receptor loci. It also will be worth looking for insulators at the naturally occurring borders between genes and extended domains of condensed chromatin. Given the great diversity of insulator elements and their associated binding proteins in *Drosophila*, it is a good guess that many kinds of insulators remain to be discovered in vertebrates as well, and that they will play important roles in regulating patterns of gene expression.

Note added in proof: Two papers in this issue of *Science* (*51*, *52*) provide important information about insulator action and its relation to loop domain models. They show that in contrast to the known insulating properties of single Su(Hw) elements, insertion of a pair of Su(Hw) elements between enhancer and promoter does not lead to insulation. The data provide strong evidence for interaction between nearby Su(Hw) binding arrays, consistent with the idea that these sites are involved in loop formation. Some of the results suggest, however, that the mode of action of this insulator may involve rather more complicated mechanisms than those of the simplest loop domain models.

References and Notes

1. P. K. Geyer, V. G. Corces, *Genes Dev.* **6**, 1865 (1992).
2. D. Dorsett, *Genetics* **134**, 1135 (1993).
3. J. Mihaly *et al.*, *Development* **124**, 1809 (1997).
4. J. Zhou, M. Levine, *Cell* **99**, 567 (1999).
5. S. Barges *et al.*, *Development* **127**, 779 (2000).
6. K. Hagstrom, M. Muller, P. Schedl, *Genes Dev.* **10**, 3202 (1996).
7. A. Udvardy, E. Maine, P. Schedl, *J. Mol. Biol.* **185**, 341 (1985).
8. R. Kellum, P. Schedl, *Cell* **64**, 941 (1991).
9. _____, *Mol. Cell Biol.* **12**, 2424 (1992).
10. M. Gaszner, J. Vazquez, P. Schedl, *Genes Dev.* **13**, 2098 (1999).
11. C. M. Hart, K. Zhao, U. K. Laemmli, *Mol. Cell Biol.* **17**, 999 (1997).
12. J. Vazquez, P. Schedl, *Genetics* **155**, 1297 (2000).
13. A. C. Bell, G. Felsenfeld, *Curr. Opin. Genet. Dev.* **9**, 191 (1999).
14. F. Palla *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 2272 (1997).
15. C. C. Robinett, A. O'Connor, M. Dunaway, *Mol. Cell Biol.* **17**, 2866 (1997).
16. X. P. Zhong, M. S. Krangel, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 5219 (1997).
17. J. H. Chung, M. Whiteley, G. Felsenfeld, *Cell* **74**, 505 (1993).
18. T. R. Hebbes *et al.*, *EMBO J.* **13**, 1823 (1994).
19. N. Saitoh *et al.*, *EMBO J.* **19**, 2315 (2000).
20. A. C. Bell, A. G. West, G. Felsenfeld, *Cell* **98**, 387 (1999).
21. R. R. Roseman, V. Pirrotta, P. K. Geyer, *EMBO J.* **12**, 435 (1993).
22. L. Lu, J. Tower, *Mol. Cell Biol.* **17**, 2202 (1997).
23. M. Reitman, G. Felsenfeld, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 6267 (1988).
24. M. J. Pikaart, F. Recillas-Targa, G. Felsenfeld, *Genes Dev.* **12**, 2852 (1998).
25. F. Recillas-Targa *et al.*, unpublished observations.
26. M. N. Prioleau *et al.*, *EMBO J.* **18**, 4035 (1999).
27. Y. Wang *et al.*, *Nature Biotechnol.* **15**, 239 (1997).
28. F. Taboit-Dameron *et al.*, *Transgen. Res.* **8**, 223 (1999).
29. T. Inoue *et al.*, *J. Hum. Genet.* **44**, 152 (1999).
30. S. Rivella *et al.*, *J. Virol.* **74**, 4679 (2000).
31. D. W. Emery *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 9150 (2000).
32. F. L. Sun, S. C. Elgin, *Cell* **99**, 459 (1999).
33. T. I. Gerasimova *et al.*, *Cell* **82**, 587 (1995).
34. K. Buchner *et al.*, *Genetics* **155**, 141 (2000).
35. A. C. Bell, G. Felsenfeld, *Nature* **405**, 482 (2000).
36. A. T. Hark *et al.*, *Nature* **405**, 486 (2000).
37. A. L. Webber *et al.*, *Nature* **391**, 711 (1998).
38. C. Kanduri *et al.*, *Curr. Biol.* **10**, 449 (2000).
39. M. Srivastava *et al.*, *Genes Dev.* **14**, 1186 (2000).
40. C. Kanduri *et al.*, *Curr. Biol.* **10**, 853 (2000).
41. P. Szabo *et al.*, *Curr. Biol.* **10**, 607 (2000).
42. E. Li, C. Beard, R. Jaenisch, *Nature* **366**, 362 (1993).
43. J. L. Thorvaldsen, K. L. Duran, M. S. Bartolomei, *Genes Dev.* **12**, 3693 (1998).
44. A. Udvardy, *EMBO J.* **18**, 1 (1999).
45. T. I. Gerasimova, V. G. Corces, *Cell* **92**, 511 (1998).
46. T. I. Gerasimova, K. Byrd, V. G. Corces, *Mol. Cell* **6**, 1025 (2000).
47. D. Dorsett, *Curr. Opin. Genet. Dev.* **9**, 505 (1999).
48. M. Bulger, M. Groudine, *Genes Dev.* **13**, 2465 (1999).
49. E. Torigoi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 2686 (2000).
50. R. A. Drewell *et al.*, *Development* **127**, 3419 (2000).
51. H. N. Cai, P. Shen, *Science* **291**, 493 (2001).
52. E. Murayova *et al.*, *Science* **291**, 495 (2001).

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