Nuclear location of a chromatin insulator in \textit{Drosophila melanogaster}

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Introduction

Chromatin-related activities are organized at different anatomic levels in the nucleus (Belmont, 2001; Chubb and Bickmore, 2003; Chubb et al., 2002; Cremer and Cremer, 2001; Cremer et al., 2000; Dillon and Festenstein, 2002; Gasser, 2002; Heun et al., 2001; Marshall et al., 2001; Parada and Misteli, 2002; Spector and Gasser, 2003; Vazquez et al., 2001). For example, constitutively silent centromeric and telomeric heterochromatin and gene-poor chromosomes are frequently found near the nuclear periphery. By contrast, regulated transcription might be organized at a more local scale. In human interphase nuclei, inactive genes are often found in the interior of chromosomal territories whereas active genes are often positioned near the periphery or in the interchromatin space (Chubb et al., 2002; Cremer and Cremer, 2001; Croft et al., 1999; Feuerbach et al., 2002; Galy et al., 2000; Mahy et al., 2002a; Mahy et al., 2002b).

Chromatin boundaries (insulators) delimit chromatin domains of distinct structures and transcriptional activities (Gerasimova and Corces, 2001; Geyer and Clark, 2002; Labrador and Corces, 2002). They play important roles in regulating gene activity in complex genetic loci in diverse organisms from yeast to humans (Bi and Broach, 2001; Ishii and Laemmli, 2003; Mihaly et al., 1998; Noma et al., 2001; West et al., 2002). Insulators are characterized by two distinct assays for their ability to block enhancer-promoter interactions and to protect transgenes from influences of neighboring chromatin. Despite extensive studies, molecular mechanisms underlying insulator activity remain controversial (Cai and Shen, 2001; Dorsett, 1999; Gerasimova et al., 2000; Geyer, 1997).

The \textit{Drosophila} gypsy retrotransposon insertion disrupts communications between distal enhancers and a downstream gene promoter (Geyer and Corces, 1987). A 340 bp stretch near the 5’ long terminal repeat (LTR) of the retrotransposon, which binds to Suppressor of Hairy Wing (SUHW) and interacts with Modifier of Midget 4 [MOD(MDG4)] indirectly, is necessary and sufficient for the insulator activity (Gerasimova et al., 1995; Spana et al., 1988). We have previously shown that the cis-arrangement of suHw insulator crucially affects its activity: a single intervening suHw blocks enhancer-promoter interactions, whereas two tandem suHw elements abolish their insulator activity (Cai and Levine, 1997; Muravyova et al., 2001). These observations suggest that suHw might interact with another insulator and/or unidentified nuclear anchor sites, thereby separating enhancers and promoters into different chromatin loops. The interaction between the tandemly paired suHw insulators neutralizes their ability to mediate formation of such chromatin loops, which leads to the loss of insulator activity.

Consistent with this observation, it was recently reported that the gypsy retrotransposon is associated with the nuclear periphery (Gerasimova et al., 2000; Gerasimova and Corces, 1998). Furthermore, SUHW and MOD proteins also localize near the nuclear periphery in large foci. Genetic mutations in \textit{suHw} and \textit{mod(mdg4)} that abolish the insulator function also disrupt these protein foci and the association of gypsy with the nuclear periphery. Based on these observations it was proposed that the enhancer-blocking activity of the suHw DNA depends on its interaction with an insulator complex near the nuclear periphery (Gerasimova et al., 2000).

To test whether association to the nuclear periphery is
required for the suHw insulator function, we examined the nuclear localization of suHw in multiple transgenic lines containing either a single or tandemly paired suHw insulators. Using fluorescent in situ hybridization (FISH) analysis, we show that genomic loci containing the full-length gypsy retrotransposon were present at a higher proportion near the nuclear periphery than loci without the gypsy retrotransposon. However, transgenes containing the functional 340 bp suHw insulator did not exhibit such biased distribution towards the nuclear periphery. Antibody stain showed that the SUHW protein foci are present both at the periphery and the nuclear interior, similar to the distribution of the transgenes containing the suHw DNA. A subset of interior FISH foci also colocalized with the SUHW protein foci. The enhancer-blocking activity of suHw remained intact under the heat shock conditions previously shown to disrupt the association of gypsy with the nuclear periphery. Our results suggest that the insulator activity of suHw is independent of its proximity to the nuclear periphery.

Materials and Methods

Fly strains

All flies were maintained in standard culture media at 23°C. Strains y1w67c2 and w1118 were used as nontransgenic controls. All transgenic fly strains used in this study have been previously described (Belozerov et al., 2003; Cai and Levine, 1995; Cai and Levine, 1997; Cai and Shen, 2001; Cai et al., 2001). Briefly, the majority of the transgenes are pCaSpeR derivatives that contain the suHw insulators in various arrangements and embryonic or larval enhancers (Pirrotta et al., 1987). Embryonic enhancers are inserted upstream of the eve basal promoter (−42 to +200) fused in frame with lacZ coding region (Cai and Levine, 1997; Small et al., 1992). Larval enhancers such as the glass GMR enhancer and the bristle specific enhancers of the yellow gene were cloned, along with suHw, into the first intron of transgenic mini-yellow genes (Belozerov et al., 2003). The following gypsy strains were provided by the Bloomington stock center: #189 y2, w1118, In(3L)R208/TM3, Sb1; #171 y1 ct K; bw1 and #1502 zw564 cf6. The following gypsy strains were generously provided by T. Gerasimova and V. Corches (Johns Hopkins University, Baltimore, MD): y2 w1118 cf6 snv+/Y X SXY (TGI) and y2 w1118 cf6 snv+/Y X X/Y v y2/Y (TG2).

In situ hybridizations

Probe preparation: LacZ and genomic DNA from the cut locus were used to prepare FISH probes were cloned by PCR into the pTOPO vector (Invitrogen, CA) with the following primers:

- LacZ/W: GGGCGTTACCAACTTATCG, LacZ/Cl: GACA- CGAGGACCACTGTT, LacZ/CC: GCCGGAGCGGTGTCAA- TC, C3/34:62; GTTGGCTCTCTACTGTGCTG, C5/154: ACACT- CAAAAGGACGCTAC, C6+6k5: GCAAACAGACTGCTA- ATCGACCAC, C5+6k3: GCCTGGATTACCTGTGGTGGT- GC.

FISH analysis was performed following a published protocol (Dernburg, 2000). The fluorescence probes were prepared as follows. Plasmids or purified fragments were digested with six 4-base-recognizing restriction enzymes to an average 100-150 bp. TdT (terminal transferase) labeling of probe with Cy3-dUTP was according to manufacturer’s instruction (TdT labeling Kit, Roche). Probe was resuspended in 0.8x prehybridization buffer (1x prehybridization buffer: 10% dextran sulfate, 50% formamide, 5x SSC). Imaginal discs from third instar larvae were dissected in 1x buffer B [10 mM KH2PO4 (pH 6.8), 15 mM NaCl, 45 mM KCl, 2 mM MgCl2]. Several discs were then transferred into 2% formaldehyde in 1x PBS and fixed for 25 minutes. The discs were then treated with 45% acetic acid for 6 minutes on Denhardt-gelatin treated slides and covered with siliconized cover slips without pressure. Slides were stored in liquid N2 or at −80°C until frozen solid. The cover slip of the frozen slide was quickly flipped off and the slide was dipped in 100% methanol and was dehydrated for 2 hours at room temperature or overnight at −20°C. Slides were then washed in 2x SSCT [2x SSC (0.3 M NaCl, 0.03 M citric acid), 0.1% Tween-80], followed by a series of washes that change to 50% formamide in 4x SSCT. Prehybridization was in 50% formamide in 4x SSCT for 1 hour at 37°C. After the probe was applied to the disc tissues, the slide was covered with a cover slip and denatured for 2 minutes on a heat block at 94°C in a humidifier chamber and hybridized overnight at 37°C in a humidifier chamber. After hybridization, samples were washed for 1 hour at 37 °C in 50% formamide in 4x SSCT. Slides were blocked with 10% BSA in 1x PBS for 30 minutes and incubated with primary antibody (anti-lamin B-Dm, rabbit 836, Paul Fisher, SUNY Stony Brook, NY) in PB/Tx [1x PBS (0.126 NaCl, 10 mM NaH2PO4, 0.5 M NaCl, 1% BSA, 0.1% Triton X-100] at room temperature for 3 hours, washed and incubated with secondary antibody (such as Alexa488 anti-rabbit) for 3 hours or overnight at 4°C. The optional fixation condition for the SUHW protein requires longer fixation time (25-40 minutes) (for details see Gerasimova et. al, 2001). Samples were counter-stained with DAPI and mounted in the antifading mounting medium Citifluor (Vector Lab).

Polytene chromosomes in situ hybridization was done as described previously (Cai et al., 1994) with the following modifications. After adding the probe, squashed chromosomes were heat-denatured by incubating the slides on a heat block at 94°C for 2 minutes.

Whole-mount embryo RNA in situ hybridization was performed with digoxigenin-UTP labeled antisense RNA probes. Expression patterns were visualized by colorimetric reaction following an incubation with anti-digoxigenin antibody conjugated to alkaline phosphotase (Genius Kit, Boehringer) (Cai et al., 2001; Tautz and Pfeifle, 1989).

Data analysis and statistical modeling.

For confocal microscopy a Leica TCS SP2 spectral multiphoton confocal microscope was used. Images were collected from 0.3 μm serial optical sections at a 2x zoom. The distance between hybridization foci and nuclear lamin staining was measured either with the Leica software package or manually on projected images, and expressed as a percentage of the nuclear radius (longest radius when ovoid shaped nuclei were used). The shortest distance between transgene foci and the lamin staining from three viewing angles of a single nucleus were selected and used for 3-dimensional distance measurements. Data for transgene groups (such as NS, SS, DS or gypsy) were averaged using multiple fly lines. Statistical analyses of data from paired groups were done by Student’s t-test and a Chi square analysis (Kruskal-Wallis test). A significance level of 0.05 was used for all tests. The random sample model is: U=−(3/2)·t·SQRT(1-t²)·(1−(1−t²)·SQRT(1−(1−t²))·t), where U describes the shortest distance between a point inside an ideal spherical space and the exterior of the sphere within a plane that is perpendicular to a fixed axis of the sphere (light path). A probability density function is a function defined on a continuous interval so that the area under the curve (and above the x-axis) described by the function is equal to 1.

Results and Discussion

We decided to investigate the correlation between nuclear location and insulator function of the suHw element. For this purpose, we determined the nuclear localization of reporter genes linked to the suHw DNA in diploid larval cells from a collection of fly lines that each harbor an independently
inserted suHw transgene. This collection offers several advantages. First, the transgenes contain the suHw DNA, either as a functional single element or as a tandem pair, allowing us to directly assess the correlation between enhancer-blocking activity and nuclear localization of the insulator. Second, each of the transgenes is located in a distinct chromosomal position, providing an important control for the possible interference by different chromosomal environments. Finally, the 340 bp suHw insulator was used instead of the full-length gypsy retrotransposon, thus minimizing potential influences from the non-insulator sequence of the gypsy element.

Sub-nuclear localization of transgenes
Chromosomes assume a stereotypical spatial organization in interphase nuclei in human cells (Chubb et al., 2002; Mahy et al., 2002b; Tanabe et al., 2002; Walter et al., 2003). Genes were also found to localize non-randomly along the apical-basal axis that corresponds to their relative chromosomal positions in Drosophila embryonic nuclei (Wilkie and Davis, 2001; Wilkie et al., 1999). To evaluate how the presence of the suHw insulator might impact the nuclear compartmentalization of its neighboring DNA, we first examined the nuclear distribution of transgenes that harbor no suHw insulator. Since all of the transgenes used here contain a lacZ reporter gene, driven by either embryonic or larval enhancers, we used a lacZ-specific DNA probe for FISH analysis.

Larval imaginal disc cells from different fly lines containing non-suHw (NS) transgenes were fixed with 2% paraformaldehyde, hybridized to a Cy-3 labeled lacZ probe (red) and counter stained with a polyclonal antibody against nuclear lamin (green). Using confocal microscopy we detected fluorescent foci at various positions along the radial axes of these nuclei (NS-Cy3) (Fig. 1A). The control experiment using imaginal disc nuclei from non-transgenic yw-Cy3, Fig. 1B; see Materials and Methods).

We collected confocal images of imaginal disc tissues from four NS transgenic lines. The shortest distance between the FISH foci and the ring of the lamin staining was measured (n=61-90 for each line) and expressed as a function of the radius (% Radius). The histogram of foci distribution indicates that these NS transgenes are present throughout the nuclear volume (Fig. 1D).

To assess the distribution of NS transgenes in a three-dimensional space, a mathematical model was used to simulate spherical space with randomly distributed foci (Fig. 1F,G) (see Materials and Methods). The model has been corrected for the under-representation of peripheral foci when viewed from a fixed angle (for an example, see arrowheads in Fig.1A,C). The distribution pattern of a total of 325 Cy3-labeled foci from four independent lines of transgenes was plotted against the random distribution model (Fig. 1F,G). The median distance between the NS transgene foci and the nuclear lamin is 31% of radius (Fig. 1F, blue bar), which is significantly different from that for the random model of 25% of the radius (Fig. 1F, gray bar, P<10^{-8}). Comparison of the NS-Cy3 foci histograms with the random distribution model (Fig. 1G, blue and gray profiles) shows that this difference is mainly the result of a reduced presence of transgenes in the most peripheral 0-15% of the radius (perinuclear zone, blue shaded area, Fig. 1G).

Interestingly, transgene foci were found at a higher frequency than expected in the nuclear volume immediately interior to the perinuclear zone, in a region that spans the next 25% of the radius and which we termed sub-perinuclear zone (orange shaded area, Fig. 1G). Such non-random distribution is also seen with the histograms of the individual NS transgenes (Fig. 1D, colored lines). In addition to projected distance measured from top view panels (2D measurements), we further calculated the real foci-lamin distance using measurements from all three views (Fig. 1A, top and two side view panels, 3D measurements). The 3D measurements from 27 NS nuclei also showed a reduced presence of transgenes in the perinuclear region (Fig. 1D, black line). To investigate the reason for the under-representation of the transgenes, we counter-stained these nuclei with DAPI and quantified fluorescent intensity across the nucleus by using high-resolution confocal microscopy. Our results indicated that DNA concentration is uneven throughout the nuclear interior and that it appears to be reduced near the periphery (data not shown). Therefore, the low perinuclear concentration of transgenes might reflect the property of the bulk DNA. Taken together, our results indicate that the NS transgenes are not preferentially associated with the nuclear periphery.

The effect of full-length gypsy insertion on the sub-nuclear localization of neighboring DNA
It was previously reported that the gypsy retrotransposon preferentially localizes near the nuclear periphery (Gerasimova et al., 2000) and that this peripheral association was abolished by mutations in the SUHW and MOD(MDG4) genes that abolished the insulator function (Gerasimova et al., 2000). We examined the distribution of the gypsy retrotransposon in three independent insertion lines using FISH with Cy3-labeled probes against the yellow and cut sequences near the insertion sites. We found a higher proportion of gypsy foci near the nuclear periphery compared with NS transgenes (Fig. 1C). The median distance between the gypsy foci and the lamin staining from 254 measurements was 26% of the radius, significantly less than the 33% radius for the NS transgenes (Fig. 1F, P<10^{-7}). Although the gypsy foci were still under-represented in the perinuclear zone when compared with the random model (Fig. 1F,G), they were, at the expense of the nuclear interior, more narrowly concentrated in the sub-perinuclear region: 75% of the foci were within 33% of the radius from the lamin staining, compared with 45% of the radius for the random model and an average of 41% of the radius for the NS transgenes (Fig. 1F,G). Every gypsy line showed a single narrow peak of foci distribution that centered around 26% of radius, whereas the majority of NS lines exhibited broader foci distribution and were centered around 35-40% of radius (Fig. 1D,E). We also measured the 3D distance between lamin and gypsy foci in a small number of nuclei. The result confirmed the closer association between gypsy and the nuclear periphery (black line, Fig. 1E).

The above results showed that the gypsy and surrounding DNA assume a more peripheral distribution than the NS transgenes. However, the average distance between the gypsy DNA and the lamin staining in this study appears to be greater than the one reported previously (Gerasimova et al., 2000). One possible explanation for this apparent discrepancy might be the...
different genomic DNA used as probes. We used probes against a 6-8 kb region, whereas a much larger region was used in the previous study. A larger probe could increase the size of the fluorescent foci, which may change the apparent nuclear localization. To verify this, we tested the same gypsy lines used in the previous study (courtesy of T. Gerasimova and V. Corces), using the shorter probes. We did not observe closer periphery association in these gypsy lines (data not shown).

Transgenes containing the suHw insulator do not associate with the nuclear periphery

We next examined the subnuclear localization of transgenes containing the 340 bp suHw insulator. We first tested transgenes containing a single functional suHw (SS) by using a Cy3-labeled lacZ probe. The transgene foci do not appear to preferentially associate with the nuclear periphery (Fig. 2A). The median distance between the lamin and SS transgene foci

![Fig. 1. The effect of the gypsy sequence on the nuclear localization of neighboring chromosomal DNA.](image)
from 333 measurements is 34% of radius (Fig. 2E, red bar), comparable to that of the NS transgenes (32.5% of radius, Fig. 1F), and significantly different from that of the gypsy transposon (26% radius, $P < 10^{-9}$, Fig. 1F). A combined foci histogram of the SS transgenes is also more similar to that of NS transgenes than that of gypsy lines (compare Fig. 2F with Fig. 1G). As seen before, the 2D foci histograms from individual lines (Fig. 2C) are in good agreement with the 3D histogram and the combined 2D histogram for the group (black line, Fig. 2C,F).

To test further whether changes in the suHw insulator function are accompanied by changes in its nuclear localization, we investigated the nuclear localization of transgenes containing tandemly paired suHw (DS), which neutralized their enhancer-blocking activity. The DS transgene foci labeled with the Cy3-lacZ probe were found along the nuclear radius, within the same range than the single insulator transgenes (Fig. 2B,D). The median distance between transgene and nuclear lamin from 307 measurements is 35% of the radius. This is similar to the SS transgenes (35.5% of radius, Fig. 2E) and different to that of gypsy (26% radius, $P < 10^{-9}$) (Fig. 1F, Fig. 2E). Again, the foci distribution histograms of individual DS transgenes, the combined histogram of the DS group, and the 3D foci distribution histogram of the group were in good agreement and showed little difference from those of the NS and the SS groups (Fig. 2D,F). These results indicate that, unlike the full-length gypsy retrotransposon, the suHw insulator does not appear to be closely associated with the nuclear periphery.

Fig. 2. The nuclear localization of transgenes containing the suHw insulator in functional or non-functional arrangements. (A) Confocal image of imaginal disc nuclei from a single-suHw (SS) transgenic larva, hybridized with Cy3-labeled lacZ DNA probes (red) and an anti-lamin antibody (green) (SS-Cy3 group). (B) Confocal image of a transgenic line with paired-suHw (DS) (DS-Cy3 group). (C-D) Histogram of foci distribution along the radius of the SS-Cy3 (C) and the DS-Cy3 group (D) in independent transgene lines (colored lines). 3D foci histograms of pooled samples are represented by the black lines. (E) Summary of foci distribution in a quartile diagram. The distance between FISH foci and lamin staining as a fraction of the nuclear radius for each sample group (colored bars) is compared with a model of randomly distributed samples (gray bar). (F) Foci histograms along the nuclear radius of each sample groups (colored lines) and the probability density function along the nuclear radius for the random distribution model (gray dashed line). (G) Diagram of cytological locations of insertion sites in selected transgenes. Small vertical lines represent transgene insertions at various locations in the three major chromosomes (black, gypsy; blue, NS; red, SS; green, DS). Insertion sites for individual lines are listed below.
We were surprised by this difference between the suHw insulator and the full-length gypsy retrotransposon. To exclude the possibility that clustering of a transgene group in unusual chromosomal locations influenced their nuclear localization, we mapped the chromosomal sites of selected lines from all groups by in situ hybridization. Our results showed that the insertion sites for all groups appear to be random in the euchromatic regions, and no clustering was seen (Fig. 2G).

Also, unlike the full-length gypsy retrotransposon, the functional suHw insulator does not tether DNA to the nuclear periphery. These results suggest that the suHw insulator is functional in the nuclear interior. Gypsy sequences unrelated to the suHw insulator might be responsible for the periphery localization of the transposon. Consistent with our findings, it was recently reported that the boundary activity of suHw does not require its association with the nuclear periphery (Calvi and Spradling, 2001).

Enhancer-blocking activity of the suHw insulator is permitted at the nuclear interior

Previous studies showed that the gypsy retrotransposon and two insulator proteins, SUHW and MOD(MDG4) colocalized to the nuclear periphery in larval imaginal disc cells (Gerasimova et al., 2000). Mutations in suHw and mod(mdg4) that disrupt insulator function abolished the gypsy association with the nuclear periphery (Gerasimova et al., 2000; Gerasimova and Corces, 1998). Based on these observations,
it has been suggested that the insulator activity of the gypsy element is mediated by the interaction between these two proteins and anchor sites at or near the nuclear envelope (Gerasimova et al., 2000). Our finding that suHw insulators are localized both in the sub-perinuclear and more interior region of cell nuclei (Fig. 2) raised the possibility that insulator proteins might be localized in the nuclear interior as well.

To test this, we analyzed the nuclear localization of the SUHW protein by immunofluorescence staining using an anti-SUHW antibody (courtesy of T. Gerasimova and V. Corces) (Gerasimova et al., 2000; Gerasimova and Corces, 1998). Confocal images showed a punctuated pattern of SUHW distribution, with large foci at the periphery and within the nuclei (Fig. 3A-D). In addition, smaller foci and more diffused staining was observed throughout the nuclei. The 2D distance between 68 SUHW protein foci and the nuclear periphery showed that the SUHW foci distribution resembles those of the transgene DNAs, with a substantial portion within the sub-perinuclear zone and further interior (Fig. 3E). This result indicates that the SUHW protein, which is essential for suHw insulator function, could colocalize with the suHw DNA at both the periphery and the interior. We also conducted triple staining of suHw DNA, SUHW protein and lamin. The triple staining efficiency was lower owing to the difference in optimal fixation conditions for SUHW and for FISH (with the short probe). However, these stainings showed that at least 50% (n>25) of the suHw DNA foci at internal sites colocalize with the SUHW protein (Fig. 3F-H). In summary, our results support the insulator function of SUHW in the interior regions of the nucleus.

Heat shock of larvae at 37°C for 20 minutes is sufficient to disrupt association between gypsy and the nuclear periphery (Gerasimova et al., 2000). If anchoring of suHw insulator to the nuclear periphery is necessary for its enhancer-blocking activity, we reasoned that heat-induced disassociation of SUHW and insulator DNA might disrupt its insulator activity. To test this, we examined the enhancer-blocking activity of the suHw insulator in heat-shocked transgenic embryos. In transgenic embryos containing the evenskipped (eve) enhancers, Stripe 2 (E2) and Stripe 3 (E3), which are separated by a neutral spacer (T) (3T2 embryos), both stripe enhancers activated the downstream eve-lacZ reporter gene, as shown by the lacZ expression (Fig. 3I). By contrast, in embryos in which the T was replaced by the suHw insulator DNA (3S2 embryos), the distal E3 enhancer was blocked from the ev-lacZ promoter and the E3 stripe expression was lost (Fig. 3J). The function of the proximal E2 enhancer was unaffected as seen by the single E2 stripe (Fig. 3I). The nuclear location of the transgenes containing the neutral spacer (transgene 3T2, line C196) and suHw (transgene 3S2, line C36) are similar to other transgenes in the same group (Fig. 1D, blue line; Fig. 2C, red line). We next tested the enhancer-blocking function of suHw in 3S2 embryos that had been heat shocked for 30 minutes at 37°C. The suHw-mediated block of the distal E3 enhancer remained intact (Fig. 3K) indicating that the peripheral association of the SUHW protein is not essential for enhancer-blocking activity of the suHw insulator.

In conclusion, we showed that, unlike the full length gypsy retrotransposon, the functional suHw insulator DNA is not preferentially associated with the nuclear periphery. Rather, it is positioned throughout the nucleus. We showed that SUHW protein is present both at the nuclear periphery and in the nuclear interior, in distributions similar to that of suHw DNA. We showed that the 340 bp suHw insulator DNA and the SUHW protein are colocalized at some of the interior sites. We further showed that heat shock conditions, which resulted in the disassociation of SUHW and MOD(MDG4) from the nuclear periphery, do not disrupt the enhancer-blocking function of the suHw insulator in transgenic embryos. These results provided structural and functional evidence that the suHw insulator activity does not require association with the nuclear periphery and that components in the nuclear interior can support the insulator function of suHw, such as providing anchor sites for insulator interactions. Identification of these components should provide insights into the mechanisms of insulator activity. A recent study linking the chromatin barrier activity in the yeast HML silent loci with nuclear-pore components at the nuclear periphery (Ishii et al., 2002) suggests that diverse mechanisms might underlie the function of different insulators or chromatin boundaries from different organisms.

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