

# Diverse transcription influences can be insulated by the *Drosophila* SF1 chromatin boundary

Parimal Majumder, Sharmila Roy, Vladimir E. Belozherov, Dimple Bosu, Meena Puppali and Haini N. Cai\*

Department of Cellular Biology, University of Georgia, Athens, GA 30602, USA

Received November 13, 2008; Revised April 22, 2009; Accepted April 23, 2009

## ABSTRACT

**Chromatin boundaries regulate gene expression by modulating enhancer–promoter interactions and insulating transcriptional influences from organized chromatin. However, mechanistic distinctions between these two aspects of boundary function are not well understood. Here we show that SF1, a chromatin boundary located in the *Drosophila* Antennapedia complex (ANT-C), can insulate the transgenic *miniwhite* reporter from both enhancing and silencing effects of surrounding genome, a phenomenon known as chromosomal position effect or CPE. We found that the CPE-blocking activity associates with different SF1 sub-regions from a previously characterized insulator that blocks enhancers in transgenic embryos, and is independent of GAF-binding sites essential for the embryonic insulator activity. We further provide evidence that the CPE-blocking activity cannot be attributed to an enhancer-blocking activity in the developing eye. Our results suggest that SF1 contains multiple non-overlapping activities that block diverse transcriptional influences from embryonic or adult enhancers, and from positive and negative chromatin structure. Such diverse insulating capabilities are consistent with the proposed roles of SF1 to functionally separate fushi tarazu (*ftz*), a non-Hox gene, from the enhancers and the organized chromatin of the neighboring Hox genes.**

## INTRODUCTION

Chromatin organization has long been known to affect gene activity [for reviews see (1–4)]. Expression of integrated transgenes is also influenced by chromatin organization of the surrounding genome. For example, *Drosophila* carrying the transgene *miniwhite* marker

display a wide variety of eye colors depending on the transgene insertion site, a phenomenon referred to as chromosomal position effect (CPE) (5,6). In vertebrates, integrated transgenes are often progressively silenced by the neighboring chromatin in an insertion-site-dependent fashion (7,8). Chromatin boundaries are specialized DNAs located between genomic domains of distinct chromatin structure and function. They can block communication between gene promoter and regulatory enhancers, and protect integrated reporter genes from positive or negative effects of the surrounding chromatin (9–21) [see (22–28) for reviews]. Indeed, some of the best-characterized boundary elements were initially identified by their ability to protect reporter transgenes. For example, the *Drosophila* *scs* and *scs'* elements were shown to protect *miniwhite* against CPE, resulting in more consistent and lighter eye colors (11). The vertebrate  $\beta$ -globin *cHS4* boundary can also protect reporter genes from the silencing effect of the genome (10).

Recent studies indicated that the activity that impedes the spread of silent chromatin within the  $\beta$ -globin boundary, called a barrier, depends on different *cis*- and *trans*-factors from the activity that block enhancers. The barrier recruits histone-modification enzymes to establish centers of active chromatin (12,29). These results indicate that the two aspects of boundary function are mediated by distinct mechanisms (13,30). Related mechanisms have also been proposed for certain boundaries in the yeast telomeres or silent mating loci (15,31–36). Although parallels have been drawn between the fly CPE-blocking activity and vertebrate barriers, separation of CPE-blocking and enhancer-blocking activities has not been reported in *Drosophila*. In particular, the *Drosophila* Gypsy suHw boundary appears to support both of its enhancer-blocking and CPE-blocking activities through the same DNA sequence and the same zinc finger protein SUHW (37). These observations are inconsistent with a common mechanism underlying all ‘position effects’ in different organisms, and consequently, a common mechanism for all ‘barrier-like’ activities.

\*To whom correspondence should be addressed. Tel: +1 706 542 3329; Fax: +1 706 542 4271; Email: hc.ai@uga.edu

The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

© 2009 The Author(s)

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/2.0/uk/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

To address these questions we have probed the CPE-blocking activity associated with SF1, a 2.4-kb boundary element located in the intergenic region between the non-Hox gene *ftz* and the homeotic gene *Sex comb reduced (Scr)* in the *Drosophila* ANT-C homeotic complex. We have previously shown that SF1 contains a potent embryonic enhancer-blocking activity (38). In this study, we report that SF1 can protect the *miniwhite* reporter from the influences of organized chromatin surrounding the transgene insertion site. We show that the DNA regions within SF1 that support CPE block is different from the element that mediates enhancer block; and that GAF sites, critical for the latter, is dispensable for the former. Importantly, we provide evidence that the CPE-blocking activity cannot be attributed to a potential enhancer-blocking activity in the developing eye. Our findings suggest that the *Drosophila* SF1 boundary contains multiple non-overlapping activities that block enhancers or chromatin-mediated effects. These functional properties of SF1 may be important for the insulation of the non-homeotic *ftz* gene from neighboring enhancers and repressive chromatin associated with homeotic genes. Our results also suggest the diverse mechanisms may underlie 'chromosomal position effect', as well as the activities that impede them.

## METHODS

### Construction of CPE-blocking transgenes

The full-length SF1 and its sub-fragments SF1a–SF1c were generated by PCR using primers containing Not I site and cloned into pCRII/TOPO vector (Invitrogen). The resulting constructs were digested with Not I or Nsi I and the DNA inserts were gel extracted, purified and ligated into the respective sites flanking the *miniwhite* reporter in the pCaSpeR transformation vector. Site-directed mutagenesis of the two GAF sites in SF1c was performed using the single-stranded DNA method as described previously (38). The base substitution in the GAF sites was done using the following oligonucleotides: 5' ACAATGAACAGGATCCTGATGAATTA 3' and 5' GTTGTGATGCAGATCTGCTTACTTAG 3'.

### Construction of enhancer-blocking transgenes

The G5 enhancers (provided by Jumin Zhou) were digested with Bam HI, purified, and ligated into the unique Bam HI site of the CaSpeR vector, resulting in the CA-G5 plasmids. SF1 or suHw insulators were inserted into the unique Not I site (converted from the original Eco RI site) in the CA-G5 plasmids. Details of the embryonic enhancer-blocking assay in Figure 2 are described previously (38).

### P-element-mediated germline transformation

P-element mediated transformation was carried out as described previously (43,44) (Rainbow Transgenic Inc, California). The  $y^1w^{67c23}$  and  $w^{1118}$  *Drosophila* strains were used to generate transgenic lines. Eighteen or more independent lines were generated for all CPE-blocking

tests. Five or more independent transgenic lines were obtained and characterized for each enhancer-blocking construct.

### Eye color assessment and pigment measurement

The eye color of 5–7 days old heterozygous females was assigned and color level by visual assessment according to a 12-point scale of progressively darker color shown in Figure 1A, under 10× objective and intermediate illumination with NCL150 cold light source. Eye pigment was extracted from 20 7-day old flies of indicated eye color as described previously (45): flies were homogenized in 100  $\mu$ l AEA buffer (30% EtOH, 0.1% concentrated HCl) and brought to 1 ml by adding 900  $\mu$ l AEA. The samples were then vortexed for 30 min and spun for 10 min in a microcentrifuge. Twenty microliters of 0.5% hydrogen peroxide was added to the supernatant to oxidize the extracted pigment. The samples were mixed, spun and measured for absorbance at OD<sub>480</sub> using Genova Life Analyzer spectrophotometer. Each OD<sub>480</sub> reading was repeated three times and the mean value was used to generate the chart in Figure 1B.

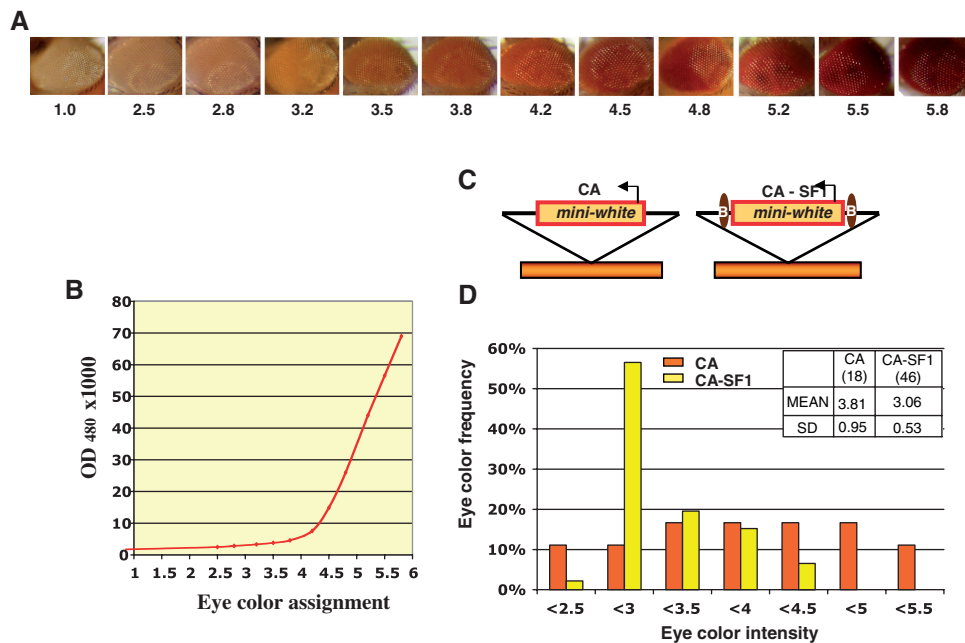
### Statistical analysis

The P value in Figure 2A is calculated by Chi-square test using an on-line calculator from QuickCalcs (GraphPad Software, Inc., La Jolla, CA), where number of lines in each eye color category was compared to that of the control (CA). Using color distribution expected from CA transgenic lines, the probability (*P*) of observing eye color distribution as seen in CA-SF1 lines is <0.0001 ( $\chi^2 = 16.767$  with 1 degree of freedom). Similar calculation was done for CA-SF1a (*P* < 0.0630); CA-SF1b (*P* < 0.9307); and CA-SF1c (*P* < 0.0190). For eye enhancer-blocking assays in Figure 4 with no insulator, suHw or SF1, eye color of transgenic lines was scored according to the color standard in Figure 1A. Data compilation and statistical analyses, except otherwise indicated, were done using the Microsoft excel software.

## RESULTS

### SF1 contains a CPE-blocking activity

Transgenic flies carrying the *miniwhite* reporter exhibit wide range of eye colors depending on the site of transgene insertion (Figure 1A). Such variation, known as CPE, is attributed to the influences of the surrounding chromatin on an otherwise weak *miniwhite* promoter (5,6,46). Since the effect of CPE on *miniwhite* expression has been evaluated by visual assessment of eye color in previous studies, we categorized the eye color of transgenic flies carrying *miniwhite* into 12 intensity levels, and use them to evaluate the CPE-blocking results reported below (Figure 1A). However, we also defined the eye color standard by measuring the absorbance (OD<sub>480</sub>) of the eye pigment extracted from these flies (Figure 1B, see 'Methods' section). Our measurements indicate that the CPE-caused variation in the *miniwhite* expression, as measured by



**Figure 1.** The CPE-blocking activity of SF1. (A) Eye color intensity standard shown by eyes of  $w^{1118}$  and transgenic flies showing increasing eye color. The number below each eye indicates the color designation, with 1 being the parental strain  $w^{1118}$  and 5.8 being the darkest eye color observed. (B)  $OD_{480}$  absorbance of eye pigment extracted from flies in each eye color category (see Methods section). (C) Schematic representation of unprotected (CaSpeR, or CA) or SF1-flanked (CaSpeR-SF1, or CA-SF1) *miniwhite* randomly integrated into genome (brown bars). Arrows represent *miniwhite* promoter and ovals represent the SF1 boundary. (D) Bar graph showing eye color distribution of CA and CA-SF1 transgenic lines. Each independent line was assigned an eye color score according to chart in B. The Y-axis indicates the percentage of lines displaying eye color within the indicated range (shown in X-axis). The inset table provides sample number ( $N$ , in parentheses), eye color mean (MEAN) and SD for CA and CA-SF1 transgenes.

$OD_{480}$ , can range up to 70-fold, and much of it is above the sensitive range of the human eye.

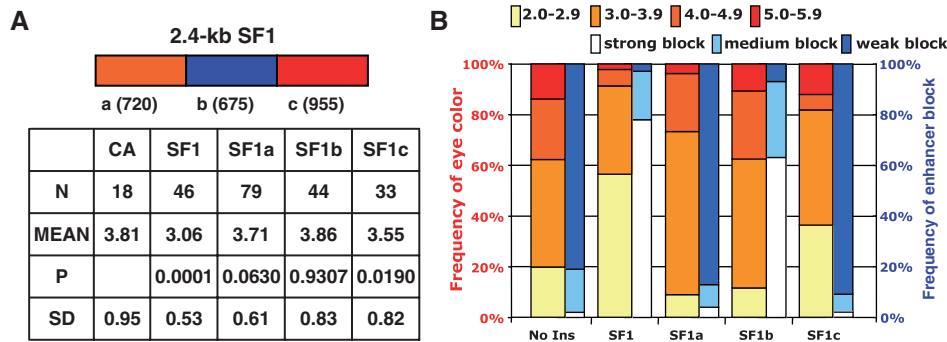
To test the ability of SF1 to protect *miniwhite* from CPE, transgenic flies carrying the *miniwhite* reporter with or without the protection of flanking SF1 were assigned an eye color intensity level according to the color standard (Figure 1C). We found that fly lines carrying unprotected *miniwhite* in the pCaSpeR vector (CA) exhibit a wide range of eye colors with a comparable number of lines in each color category from 2.5 to 5.5 (Figure 1C and D). This is indicative of a strong CPE. In contrast, fly lines carrying *miniwhite* protected by the full-length SF1 (brown ovals, CA-SF1) display predominantly yellow to light orange eye colors (Figure 1C and D). Among 46 CA-SF1 lines, 78% exhibited eye colors between 3.0 and 3.5 (compared to 27% among unprotected CA lines) with few lines exhibiting extremely light or dark colors. In addition to decreasing eye color variation, shown by lower standard deviation (SD, Figure 1D), flanking SF1 also appeared to reduce the average eye color (MEAN, Figure 1D). Both such effects have been previously reported for the *scs* and *suHw* insulators and have been attributed to the insulation of primarily positive influences of the surrounding chromatin (11,37). The average eye color in SF1 protected lines appears to be lighter than those in *suHw* and *scs* protected lines. This could be due to the slight variances in the assay parameters, such as the color standard, the inclusion of a yellow marker in the P-element in the previous studies, or to potential

repressive effects of SF1 (37). Taken together, our results indicate that SF1 contains a potent CPE-blocking activity.

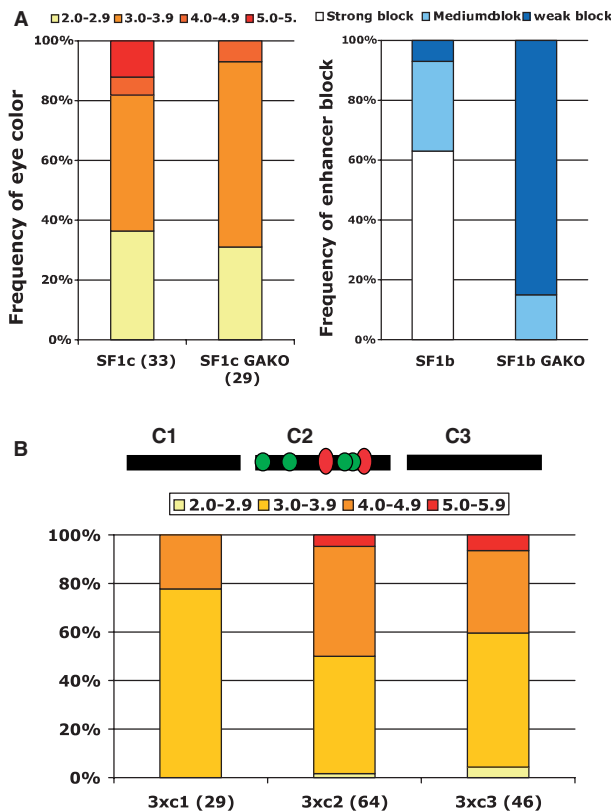
### Molecular dissection of the SF1 CPE-blocking activity

To identify and characterize the CPE-blocking activity within SF1, we dissected the 2.4-kb full-length boundary into three fragments of comparable sizes (SF1a, SF1b and SF1c, Figure 2A), and tested them individually for CPE-blocking activity. For each SF1 sub-fragment, a large number of independent lines were scored for eye colors ( $N$ , Figure 2A and B). Compared to the no insulator controls (No ins, CA in Figure 1C and D), SF1a- and SF1c-containing flies showed more lines with yellow and light-orange eyes, and/or fewer lines with dark-red eyes (Figure 2B). This is indicative of CPE-blocking activity associated with these two elements, although both appeared significantly weaker than that of the full-length SF1. In contrast, the eye color variation among SF1b lines was similar to those of the CaSpeR control (Figure 2B). Statistical analysis of sample groups indicates that SF1, SF1a and SF1c, but not SF1b, showed significant difference in the eye color distribution from the CaSpeR control (see  $P$ -value in Figure 2A).

The lack of CPE-blocking activity in the SF1b region is somewhat unexpected because this element was previously found to exhibit about 80% of the enhancer-blocking activity of full-length SF1 in a transgenic embryo enhancer-blocking assay [for comparison see blue bars in Figure 2B (38)]. In contrast, the SF1a and SF1c showed



**Figure 2.** SF1 contains separate enhancer-blocking and CPE-blocking activities. (A) Analysis of CPE-blocking activity in SF1 sub-fragments. Top: A schematic of the three sub-fragments within the SF1 boundary, with size of each fragment in base pairs shown in parentheses. Bottom: A table summarizing the total numbers of lines (*N*), mean eye color (MEAN), SD and the probability distribution (*P*) for each group against CA was calculated by chi-square test. (B) Comparison of CPE- and enhancer-blocking activities in SF1 and its sub-fragments. The yellow-orange bar graph summarizes the CPE-blocking activity of no insulator (No ins, CA), SF1 and three SF1 sub-fragments using the assay outlined in Figure 1C. The percentage of lines displaying eye colors within the designated range, as shown on top of bar graph, is indicated on the left y-axis. The white-blue bar graph summarizes the embryonic enhancer-blocking activity in the corresponding DNA elements (38). The percentage of embryos showing strong (70–100%), medium (30–70%) or weak (0–30%) block, as shown on top of bar graph, is indicated on the right y-axis.



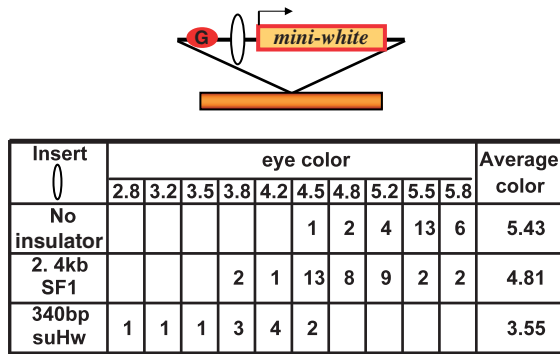
**Figure 3.** CPE-blocking activity is independent of GAF sites and TATA-like motifs in SF1c. (A) Effect of GAF site mutations on CPE-blocking activity of SF1c and enhancer-blocking activity of SF1b. Left panel: bar graph summarizing CPE-blocking activity of SF1c (left) and SF1cGAKO (right). The percentage of lines displaying eye colors within the designated range, as shown on top of bar graph, is indicated on the left y-axis. The positions of the GAF sites (red ovals) within SF1c are indicated in diagram on top in (B). Right panel: bar graph summarizing enhancer-blocking activity of SF1b (left) and SF1bGAKO [right, (38)]. (B) CPE-blocking activity of SF1c sub-fragments. Top: diagram of three SF1c sub-regions (SF1c1-c3), with positions of GAF sites (red ovals) and TATA-like motifs (green circles) indicated. Bottom: bar graph summarizing CPE-blocking activity of three tandem copies of SF1c1-3. The percentage of lines displaying eye colors within the designated range, as shown on top of bar graph, is indicated on the left y-axis.

little insulator activity in the enhancer-blocking assay. This result suggests that the SF1 boundary may contain two potent and non-overlapping activities: one that blocks embryonic enhancers and the other insulates against positive and negative CPE in the developing eye.

#### CPE-blocking activity is independent of GAF sites and TATA-like motifs

To further analyze the distinctions between the enhancer- and CPE-blocking activities within SF1, we tested whether they require different sequence motifs, including binding sites for insulator protein GAF, a BTB/zinc finger protein. Our previous study showed that mutations in GAF binding sites in the SF1b element abolished its enhancer-blocking activity (Figure 3A, right panel) (38,47). To test whether GAF sites are also required for the CPE-blocking activity, we replaced the two GAF sites in SF1c with unrelated sequences. As shown in Figure 3A, mutations in the GAF sites in SF1c (SF1cGAKO) did not compromise the CPE-blocking activity. In fact, the proportion of SF1cGAKO lines with medium to light eye colors is slightly higher. This could be due to the loss of binding by GAF, which is also known to mediate transcription activation. Our results suggest that the CPE-blocking activity in SF1c and the insulator activity in SF1b depend on distinct *cis*- and *trans*-components (Figure 3A).

In addition to GAF sites, SF1c also contains multiple TATA-like motifs in its central region (green circles, Figure 3B). Similar AT-rich motifs and other promoter-like sequences are found in several other boundary elements, prompting the hypothesis that these *cis*-elements could serve as a sink of regulatory influences (48). To further define the *cis*-requirement of CPE-blocking activity in SF1c and to test whether TATA-motifs contribute to the CPE-blocking activity, we dissected the SF1c region further into three sub-fragments and tested each in three tandem copies in *miniwhite*-protection assay (Figure 3B). Our results indicate that the central region of SF1c (3c2) does not contain higher level of CPE-blocking activity than the neighboring regions, suggesting that



**Figure 4.** The SF1 contains little enhancer-blocking activity in the developing eye. Top: diagram of eye enhancer-blocking transgenes. The transgene containing G5 enhancer (G, red circle) and the *miniwhite* transgene (arrow), separated by insert DNA (open oval), is randomly integrated in the chromosome (orange bar). Table: rows from top to bottom, number of lines in each eye color category for G5-*miniwhite* transgene containing no insert, 2.4-kb SF1 or 340-bp suHw insulators, respectively.

GAF and TATA-like motifs do not contribute significantly to the CPE-blocking function of SF1c.

#### The CPE-blocking activity is distinct from a late eye enhancer-blocking activity in SF1

The *Drosophila* CPE has often been compared to the vertebrate position effect, which is the gradual silencing of integrated reporters by the genome or chromatin surrounding the transgene. However, key differences exist between the two phenomena. First, the *Drosophila* CPE appears to be both negative and positive in nature, as shown by the decrease of both extreme light and extreme dark eye colors in boundary-protected transgenic lines. Second, the *Drosophila* CPE is more dramatically manifested in the behavior of the *miniwhite* reporter, which is also used in most boundary protection studies (11,37). Other features, including a lack of time-dependence in the *Drosophila* CPE, also suggest mechanistic differences between the two effects. An alternative explanation for the *Drosophila* CPE suggests the action of eye-specific enhancers or silencers around transgene insertion sites. If this were true, the two non-overlapping activities in SF1 would represent two enhancer-blocking activities, one that functions in the embryo and the other in the developing eye.

To distinguish between these two mechanisms, we tested SF1 for its ability to block enhancers in the same tissue and developmental stage as in the CPE-blocking assay. We used the eye-specific glass multiple repeat (G5) and the *miniwhite* reporter to perform the blocking tests (Figure 4) (49,50). As controls, we also made G5-*miniwhite* transgene with no insulator, or with the suHw insulator, which has been shown to contain enhancer-blocking activity in the adult eye. We found that the G5 enhancer without intervening insulator can strongly activate *miniwhite* expression in majority of transgenic lines, resulting an average eye color of 5.43, which corresponds to OD<sub>480</sub> level of 53 (Figure 4, no insulator). The 340-bp suHw placed between G5 and

*miniwhite* strongly reduced the average eye color and shifted the peak of eye color distribution to the lighter range, with an average eye color of 3.55 and OD<sub>480</sub> level of 4 (Figure 4, suHw insulator). The 93% reduction in the OD<sub>480</sub> level is consistent with the enhancer-blocking activity of suHw in the eye tissue. However, the 2.4-kb SF1 only weakly reduced average eye color, with an average eye color of 4.81 and OD<sub>480</sub> level of 30 (Figure 4, SF1 insulator). This result suggests the SF1 contains much weaker or little enhancer-blocking activity in the adult eye, especially considering the 2.4-kb linear distance that separates the G5 enhancer from *miniwhite* due to the insertion of SF1. This is in strong contrast to the strong CPE-blocking activity SF1 exhibited in the same tissue. It is also in strong contrast to its potent activity in blocking diverse embryonic enhancers (Figure 2B) (38). Taken together, our results do not support the hypothesis that an eye-specific enhancer-blocking activity is responsible for the CPE-blocking behavior of SF1. Our results also indicate that the ability of SF1 to block enhancers in the eye is weak, suggesting that the boundary element may be regulated in a stage-specific and/or tissue-specific fashion.

#### DISCUSSION

In this study we have characterized the CPE-blocking activity associated with the *Drosophila* SF1 boundary. Our results suggest that SF1 contains at least two non-overlapping boundary activities, a strong embryonic enhancer-blocking activity associated with SF1b element, and strong CPE-blocking activities associated with SF1a and SF1c elements. Mutagenesis and dissection studies indicate that the CPE-blocking activity depends on different *cis* and *trans* components from the embryonic enhancer-blocking activity. We further showed that the CPE-blocking activity is unlikely to be attributed to a late stage enhancer-blocking activity in the developing eye.

*Drosophila* CPE, manifested predominantly by the enhancement or suppression of *miniwhite*, was thought to result from the active or repressive chromatin around the transgene insertion sites.

CPE-blocking activity, therefore, has been compared to the vertebrate barrier activity and long used as a defining feature for chromatin boundaries in *Drosophila* (11,48). However, the ability of *Drosophila* boundaries to block both positive and negative CPE argues against a shared mechanism between these elements and the vertebrate barriers such as the  $\beta$ -globin barrier, which counter the progression of silent chromatin by establishing centers of active chromatin (13,29).

An alternative explanation for the *Drosophila* CPE invokes the action of enhancers or silencers near the integrated transgenes. This model is consistent with the ability of boundaries to block both positive and negative effects. It also accommodates the fact that for some *Drosophila* boundaries the CPE-blocking activity depends on the same *cis*- and *trans*- components as the enhancer-blocking activity (14,37,39,40,51). However, this hypothesis would predict widespread presence of eye-specific enhancers

and silencers in the genome to account for the prevalence of the CPE effect.

Our analysis of the SF1 boundary provides the first evidence that the CPE-blocking activity can be separated from the enhancer-blocking activity, suggesting that these two insulating functions may be mediated through distinct mechanisms in *Drosophila*. It is possible that the CPE-blocking activities in *Drosophila* form structures that are transcriptionally 'neutral', and able to insulate the weak *miniwhite* promoter from the effect of local chromatin. It is unclear, however, whether such local chromatin effect can compare, in range or strength, to that of constitutive heterochromatin, or whether such effect influences *Drosophila* gene promoters in general. A previous study showed that human MAR sequence could facilitate CPE blocking either arranged to flank the reporter or placed upstream in tandem copies (41). This is distinct from the CPE-blocking behavior of *Drosophila* boundaries such as suHw and scs, further demonstrating the diverse mechanisms that could influence the regulation of the *miniwhite* reporter.

The SF1 boundary is located in the *Scr-ftz* genomic interval in the *Drosophila* ANT-C, which differs from other Hox clusters in that it contains both homeotic and non-homeotic genes. Proper regulation of these genes requires modulation of enhancer traffic as well as insulation of chromatin-mediated effects. The SF1 compound boundary fulfills both requirements: the SF1b element can restrict long-range enhancers from interfering with the *ftz* and *Scr* promoter (38); and the SF1a and SF1c elements may protect the non-Hox *ftz* gene from chromatin-mediated regulation, such as the PRE/TRE maintenance of the neighboring Hox genes. Separation and selective association of different types of boundary activities could determine the regulatory role of compound boundaries and provide flexibility in their function.

## FUNDING

Funding for open access charge: National Institutes of Health GM058458.

*Conflict of interest statement.* None declared.

## REFERENCES

- Bell, A., Boyes, J., Chung, J., Pikaart, M., Prioleau, M.N., Recillas, F., Saitoh, N. and Felsenfeld, G. (1998) The establishment of active chromatin domains. *Cold Spring Harb. Symp. Quant. Biol.*, **63**, 509–514.
- Richards, E.J. and Elgin, S.C. (2002) Epigenetic codes for heterochromatin formation and silencing: rounding up the usual suspects. *Cell*, **108**, 489–500.
- Grewal, S.I. and Moazed, D. (2003) Heterochromatin and epigenetic control of gene expression. *Science*, **301**, 798–802.
- Ahmad, K. and Henikoff, S. (2002) Epigenetic consequences of nucleosome dynamics. *Cell*, **111**, 281–284.
- Levis, R., Hazelrigg, T. and Rubin, G.M. (1985) Effects of genomic position on the expression of transduced copies of the white gene of *Drosophila*. *Science*, **229**, 558–561.
- Pirrotta, V., Steller, H. and Bozzetti, M.P. (1985) Multiple upstream regulatory elements control the expression of the *Drosophila* white gene. *EMBO J.*, **4**, 3501–3508.
- Festenstein, R., Sharghi-Namini, S., Fox, M., Roderick, K., Tolaini, M., Norton, T., Saveliev, A., Kioussis, D. and Singh, P. (1999) Heterochromatin protein 1 modifies mammalian PEV in a dose- and chromosomal-context-dependent manner. *Nat. Genet.*, **23**, 457–461.
- Ayyanathan, K., Lechner, M.S., Bell, P., Maul, G.G., Schultz, D.C., Yamada, Y., Tanaka, K., Torigoe, K. and Rauscher, F.J. 3rd. (2003) Regulated recruitment of HP1 to a euchromatic gene induces mitotically heritable, epigenetic gene silencing: a mammalian cell culture model of gene variegation. *Genes Dev.*, **17**, 1855–1869.
- Bell, A.C., West, A.G. and Felsenfeld, G. (1999) The protein CTCF is required for the enhancer blocking activity of vertebrate insulators. *Cell*, **98**, 387–396.
- Chung, J.H., Whiteley, M. and Felsenfeld, G. (1993) A 5' element of the chicken beta-globin domain serves as an insulator in human erythroid cells and protects against position effect in *Drosophila*. *Cell*, **74**, 505–514.
- Kellum, R. and Schedl, P. (1991) A position-effect assay for boundaries of higher order chromosomal domains. *Cell*, **64**, 941–950.
- Recillas-Targa, F., Pikaart, M.J., Burgess-Beusse, B., Bell, A.C., Litt, M.D., West, A.G., Gaszner, M. and Felsenfeld, G. (2002) Position-effect protection and enhancer blocking by the chicken beta-globin insulator are separable activities. *Proc. Natl. Acad. Sci. USA*, **99**, 6883–6888.
- West, A.G., Huang, S., Gaszner, M., Litt, M.D. and Felsenfeld, G. (2004) Recruitment of histone modifications by USF proteins at a vertebrate barrier element. *Mol. Cell*, **16**, 453–463.
- Geyer, P.K. and Corces, V.G. (1992) DNA position-specific repression of transcription by a *Drosophila* zinc finger protein. *Genes Dev.*, **6**, 1865–1873.
- Donze, D., Adams, C.R., Rine, J. and Kamakaka, R.T. (1999) The boundaries of the silenced HMR domain in *Saccharomyces cerevisiae*. *Genes Dev.*, **13**, 698–708.
- Cai, H. and Levine, M. (1995) Modulation of enhancer-promoter interactions by insulators in the *Drosophila* embryo. *Nature*, **376**, 533–536.
- Morcillo, P., Rosen, C. and Dorsett, D. (1996) Genes regulating the remote wing margin enhancer in the *Drosophila* cut locus. *Genetics*, **144**, 1143–1154.
- Yao, S., Osborne, C.S., Bharadwaj, R.R., Pasceri, P., Sukonnik, T., Pannell, D., Recillas-Targa, F., West, A.G. and Ellis, J. (2003) Retrovirus silencer blocking by the cHS4 insulator is CTCF independent. *Nucleic Acids Res.*, **31**, 5317–5323.
- Zhou, J., Ashe, H., Burks, C. and Levine, M. (1999) Characterization of the transvection mediating region of the abdominal- B locus in *Drosophila*. *Development*, **126**, 3057–3065.
- Hagstrom, K., Muller, M. and Schedl, P. (1996) Fab-7 functions as a chromatin domain boundary to ensure proper segment specification by the *Drosophila* bithorax complex. *Genes Dev.*, **10**, 3202–3215.
- Golovnin, A., Birukova, I., Romanova, O., Silicheva, M., Parshikov, A., Savitskaya, E., Pirrotta, V. and Georgiev, P. (2003) An endogenous Su(Hw) insulator separates the yellow gene from the Achaete-scute gene complex in *Drosophila*. *Development*, **130**, 3249–3258.
- Celniker, S.E. and Drewell, R.A. (2007) Chromatin looping mediates boundary element promoter interactions. *Bioessays*, **29**, 7–10.
- Wallace, J.A. and Felsenfeld, G. (2007) We gather together: insulators and genome organization. *Curr. Opin. Genet. Dev.*, **17**, 400–407.
- Wei, G.H., Liu de, P. and Liang, C.C. (2005) Chromatin domain boundaries: insulators and beyond. *Cell Res.*, **15**, 292–300.
- Cai, H.N. (2006) In Ma, J. (ed.), *Gene Expression and Regulation*. Higher Education Press, Beijing.
- Sipos, L. and Gyurkovics, H. (2005) Long-distance interactions between enhancers and promoters. *FEBS J.*, **272**, 3253–3259.
- Valenzuela, L. and Kamakaka, R.T. (2006) Chromatin insulators. *Annu. Rev. Genet.*, **40**, 107–138.
- Gaszner, M. and Felsenfeld, G. (2006) Insulators: exploiting transcriptional and epigenetic mechanisms. *Nat. Rev. Genet.*, **7**, 703–713.
- Mutskov, V.J., Farrell, C.M., Wade, P.A., Wolffe, A.P. and Felsenfeld, G. (2002) The barrier function of an insulator couples

- high histone acetylation levels with specific protection of promoter DNA from methylation. *Genes Dev.*, **16**, 1540–1554.
30. Huang,S., Li,X., Yusufzai,T.M., Qiu,Y. and Felsenfeld,G. (2007) USF1 recruits histone modification complexes and is critical for maintenance of a chromatin barrier. *Mol. Cell Biol.*, **27**, 7991–8002.
  31. Ferrari,S., Simmen,K.C., Dusserre,Y., Muller,K., Fourel,G., Gilson,E. and Mermod,N. (2004) Chromatin domain boundaries delimited by a histone-binding protein in yeast. *J. Biol. Chem.*, **279**, 55520–55530.
  32. Bi,X. and Broach,J.R. (2001) Chromosomal boundaries in *S. cerevisiae*. *Curr. Opin. Genet. Dev.*, **11**, 199–204.
  33. Oki,M. and Kamakaka,R.T. (2002) Blockers and barriers to transcription: competing activities? *Curr. Opin. Cell Biol.*, **14**, 299–304.
  34. Donze,D. and Kamakaka,R.T. (2001) RNA polymerase III and RNA polymerase II promoter complexes are heterochromatin barriers in *Saccharomyces cerevisiae*. *EMBO J.*, **20**, 520–531.
  35. Bi,X., Braunstein,M., Shei,G.J. and Broach,J.R. (1999) The yeast HML I silencer defines a heterochromatin domain boundary by directional establishment of silencing. *Proc. Natl Acad. Sci. USA*, **96**, 11934–11939.
  36. Bi,X. and Broach,J.R. (1999) UASrpg can function as a heterochromatin boundary element in yeast. *Genes Dev.*, **13**, 1089–1101.
  37. Roseman,R.R., Pirrotta,V. and Geyer,P.K. (1993) The *su(Hw)* protein insulates expression of the *Drosophila melanogaster* white gene from chromosomal position-effects. *EMBO J.*, **12**, 435–442.
  38. BelozeroV,V.E., Majumder,P., Shen,P. and Cai,H.N. (2003) A novel boundary element may facilitate independent gene regulation in the Antennapedia Complex of *Drosophila*. *EMBO J.*, **22**, 3113–3121.
  39. Ip,Y.T., Levine,M. and Small,S.J. (1992) The bicoid and dorsal morphogens use a similar strategy to make stripes in the *Drosophila* embryo. *J. Cell Sci. Suppl.*, **16**, 33–38.
  40. Rubin,G.M. and Spradling,A.C. (1982) Genetic transformation of *Drosophila* with transposable element vectors. *Science*, **218**, 348–353.
  41. Cai,H.N., Zhang,Z., Adams,J.R. and Shen,P. (2001) Genomic context modulates insulator activity through promoter competition. *Development*, **128**, 4339–4347.
  42. Gindhart,J.G. Jr. and Kaufman,T.C. (1995) Identification of Polycomb and trithorax group responsive elements in the regulatory region of the *Drosophila* homeotic gene *Sex combs reduced*. *Genetics*, **139**, 797–814.
  43. Levis,R., O'Hare,K. and Rubin,G.M. (1984) Effects of transposable element insertions on RNA encoded by the white gene of *Drosophila*. *Cell*, **38**, 471–481.
  44. Farkas,G., Gausz,J., Galloni,M., Reuter,G., Gyurkovics,H. and Karch,F. (1994) The Trithorax-like gene encodes the *Drosophila* GAGA factor. *Nature*, **371**, 806–808.
  45. Kuhn,E.J. and Geyer,P.K. (2003) Genomic insulators: connecting properties to mechanism. *Curr. Opin. Cell Biol.*, **15**, 259–265.
  46. Moses,K. and Rubin,G.M. (1991) Glass encodes a site-specific DNA-binding protein that is regulated in response to positional signals in the developing *Drosophila* eye. *Genes Dev.*, **5**, 583–593.
  47. Ellis,M.C., O'Neill,E.M. and Rubin,G.M. (1993) Expression of *Drosophila* glass protein and evidence for negative regulation of its activity in non-neuronal cells by another DNA-binding protein. *Development*, **119**, 855–865.
  48. Patton,J.S., Gomes,X.V. and Geyer,P.K. (1992) Position-independent germline transformation in *Drosophila* using a cuticle pigmentation gene as a selectable marker. *Nucleic Acids Res.*, **20**, 5859–5860.
  49. Mallin,D.R., Myung,J.S., Patton,J.S. and Geyer,P.K. (1998) Polycomb group repression is blocked by the *Drosophila* suppressor of Hairy-wing [*su(Hw)*] insulator. *Genetics*, **148**, 331–339.
  50. Gerasimova,T.I., Gdula,D.A., Gerasimov,D.V., Simonova,O. and Corces,V.G. (1995) A *Drosophila* protein that imparts directionality on a chromatin insulator is an enhancer of position-effect variegation. *Cell*, **82**, 587–597.
  51. Namciu,S.J. and Fournier,R.E. (2004) Human matrix attachment regions are necessary for the establishment but not the maintenance of transgene insulation in *Drosophila melanogaster*. *Mol. Cell Biol.*, **24**, 10236–10245.