Transvection Is Common Throughout the *Drosophila* Genome

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ABSTRACT Higher-order genome organization plays an important role in transcriptional regulation. In *Drosophila*, somatic pairing of homologous chromosomes can lead to transvection, by which the regulatory region of a gene can influence transcription in *trans*. We observe transvection between transgenes inserted at commonly used phiC31 integration sites in the *Drosophila* genome. When two transgenes that carry endogenous regulatory elements driving the expression of either LexA or GAL4 are inserted at the same integration site and paired, the enhancer of one transgene can drive or repress expression of the paired transgene. These transvection effects depend on compatibility between regulatory elements and are often restricted to a subset of cell types within a given expression pattern. We further show that activated UAS transgenes can also drive transcription in *trans*. We discuss the implication of these findings for (1) understanding the molecular mechanisms that underlie transvection and (2) the design of experiments that utilize site-specific integration.

THOUGH much of transcriptional regulation is due to regulatory elements that act in *cis*, relatively near the transcriptional start site of a gene, long-range and *trans* interactions can also affect gene regulation (reviewed in Henikoff and Comai 1998; Dekker 2008). To understand the prevalence and importance of these interactions, work has focused on higher-order genome organization, identifying which DNA sequence elements physically interact, and determining how these interactions affect transcription at the molecular level. One area where much progress has been made in understanding *trans* interactions is the study of transvection effects in *Drosophila* (reviewed in Duncan 2002; Kennison and Southworth 2002). Chromosomes are somatically paired in Diptera, so both copies of a gene are usually in proximity, even during interphase (Stevens 1908; Metz 1916; Csink and Henikoff 1998; McKee 2004). In some cases, transvection occurs when the enhancer of one copy of a gene regulates expression of the paired copy of the gene in *trans*. Most examples of transvection were discovered as cases of intragenic complementation, in which two hypomorphic or loss-of-function alleles of a gene exhibit pairing-dependent complementation. Because of the low frequency of finding such complementary mutations, work on understanding transvection has focused on test cases including, but not limited to, the *yellow* (Geyer *et al.* 1990), *Ultrabithorax* (Lewis 1954), and *white* loci (Jack and Judd 1979; Gelbart and Wu 1982).

Despite the limited number of gene loci amenable to transvection studies, several important features of its underlying mechanism have been elucidated. For example, it has been established that enhancers of a gene more strongly activate transcription of the paired copy in *trans* if the *cis* core promoter is weakened or removed (Martínez-Laborda *et al.* 1992; Morris *et al.* 1999, 2004; Lee and Wu 2006; Gohl *et al.* 2008). Transvection may also be modified by *zeste* (Jack and Judd 1979), which has been found to be required for some examples of transvection (Kaufman *et al.* 1973; Gelbart and Wu 1982; Leiserson *et al.* 1994), and may facilitate physical interactions between alleles at some loci (Benson and Pirrotta 1988; Bickel and Pirrotta 1990). Finally, the genome appears to be generally permissive for transvection (Chen *et al.* 2002), so it is likely that many more genes undergo transvection than those that have been identified based on intragenic complementation.

The possible widespread nature of transvection presents a potential problem for transgene usage in *Drosophila*, specifically with regard to site-specific integration using the

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phiC31 integration system (Groth *et al.* 2004). Site-specific integration of transgenes is becoming increasingly common, as it provides the opportunity to control for and minimize genomic position effects (Markstein *et al.* 2008; Pfeiffer *et al.* 2010). Given the use of multiple transgenes in many crossing schemes, it is common to use two transgenes that have been independently inserted into the same integration site (*attP* site). This raises the question of whether it is possible to elicit transvection between two transgenes by simply inserting them into the same genomic location and pairing them. Indeed, we have found that transvection occurs between transgenes at *attP* sites, which imposes significant restrictions on experimental design.

Our analysis of transvection focuses on the ventral nerve cord (VNC) of the *Drosophila* larva, which contains a segmentally repeating, stereotyped array of stem cells (neuroblasts). Each neuroblast generates a stereotyped lineage that is easily identifiable during late larval life based on the characteristic morphologies of its constituent neurons (Truman *et al.* 2004). These lineages are further subdivided into hemilineages based on Notch activity (Truman *et al.* 2010), and these hemilineages can also be recognized by their neuron position and morphology. To have a genetic handle on these populations of neurons, we have been taking advantage of a recently developed toolset composed of transgenes in which GAL4 is driven by *cis*-regulatory modules (CRMs) taken from various gene loci (Pfeiffer *et al.* 2008). These transgenes have been inserted into the commonly used *attP2* integration site. We have identified GAL4 lines that are expressed in select neuroblast lineages and hemilineages and are using these as an entry point for investigations of VNC development, structure, and function. One strategy has been to use dual binary transcription systems (GAL4/UAS and LexA/LexAop) to independently label or manipulate multiple neuroblast lineages and study their physical and functional relationships (Brand and Perrimon 1993; Lai and Lee 2006). While examining multiple combinations of GAL4 and LexA lines inserted into *attP2*, we noted several cases in which part of the expression pattern driven by the regulatory element of one transgene was found where we had expected to see only the expression pattern of the paired transgene. Further analyses showed that this crosstalk was due to transvection.

We further investigated the transvection phenomena we encountered for two reasons. First, many experimental strategies, including the refinement of expression patterns using intersectional genetic approaches, require independence between the transcriptional control of multiple genetic components (Luan and White 2007; Simpson 2009). We therefore were motivated to document the observed transvection effects found at integration sites to prevent or minimize potential problems when using these tools. Second, because our genetic toolset essentially provides a simple and robust system for studying transvection, we took the opportunity to address questions regarding the nature and prevalence of transvection. Most importantly we find that (1) the ability of a *cis*-regulatory module to support transvection is common, and (2) the only requirement for a regulatory element to drive expression in *trans* is its ability to bind transcriptional activators. These results provide strong evidence that transvection is common throughout the *Drosophila* genome and must be considered when designing experiments that utilize transgenes inserted into the genome by site-specific integration.

Materials and Methods

Fly stocks

All crosses were carried out at 25° under standard conditions on cornmeal/molasses food. All GAL4 lines were created as described by Pfeiffer *et al.* (2008). CRM lines driving a transactivator composed of the LexA fused to the full-length GAL4 activation domain (LexA::GADfl) were created as described by Pfeiffer *et al.* (2010) using the pBPLexA::GADflUw vector. The sequences of the primers used to clone the CRMs used are available as Supporting Information, Table S1.

PhiC31 integrated pJFRC2-10XUAS-IVS-mCD8::GFP and pJFRC21-10XUAS-IVS-mCD8::RFP lines (Pfeiffer *et al.* 2010) were a gift from B. Pfeiffer and G. Rubin. Fly lines containing the *P{LexAop-rCD2::GFP}* (Lai and Lee 2006) and *P{LexAop-rCD2::RFP}* reporters were gifts from T. Lee. A fly line containing a second chromosome insert of *P{UAS-mCD8::mRFP.LG}* was a gift of E. Gavis and is available from the Bloomington Stock Center (no. 27398).

Preparation and examination of tissues

Tissues were dissected in PBS and fixed in 4% formaldehyde in PBS (pH 7.0) for 45–60 min, then rinsed in PBS-TX [phosphate buffered saline (pH 7.2) with 1% Triton X-100]. Antibody staining was performed as described by Truman *et al.* (2004) using the following antibodies: 1:1000 dilution of rabbit anti-GFP (Invitrogen), 1:500 rat antimouse CD8a (Invitrogen), 1:500 Alexa Fluor 488 conjugated goat anti-rabbit (Invitrogen), 1:500 Texas Red conjugated Donkey antirat IgG (Jackson ImmunoResearch Laboratories).

Imaging was performed on a Zeiss LSM 510 confocal microscope and *Z* stacks were analyzed using ImageJ (http://rsbweb.nih.gov/nih-image) and Vaa3D (Peng *et al.* 2010). ImageJ was used for all postprocessing of images.

Construction of R24B02-Ptrans-GAL4, R58G08-Ptrans-GAL4, and 24B02-ΔP-LexA

To create integration vectors for R24B02-Ptrans-GAL4 and R58G08-Ptrans-GAL4, Gateway clones for the R24B02 and R58G08 CRMs were transferred into a vector derived from pBPGw (Pfeiffer *et al.* 2008) that contained the P-transposase promoter (pBPGPtw; a gift from B. Pfeiffer and G. Rubin). To create the integration construct for 24B02-ΔP-LexA, the *Drosophila* synthetic core promoter (DSCP) was excised with *Fse*I and *Kpn*I from an integration vector for 24B02-LexA::p65, a gift from B. Pfeiffer (Pfeiffer *et al.* 2010). The resulting

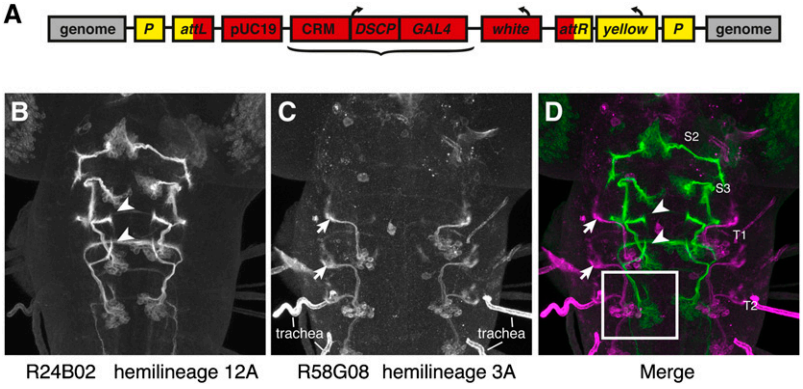

Figure 1 Independent labeling of two hemilineages in the larval ventral nervous system. (A) Schematic of a CRM-GAL4 transgene inserted into an *attP* site. Red-shaded boxes indicate sequences that originate from the *attB*-containing CRM-GAL4 vector. Yellow-shaded boxes indicate sequences from the *attP*-containing *P*-element. Gray boxes indicate the surrounding genomic region. *attR* and *attL* are the hybrid sites that result from phiC31-mediated recombination between *attP* and *attB*. Arrows indicate direction of transcription. The area above the brace indicates the sequences that vary in this study. For simplicity, we show only these elements in subsequent figures. Abbreviations: P, *P*-element sequence; CRM, *cis*-regulatory module; DSCP, *Drosophila* synthetic core promoter; *white*, mini-*white* used to score for integrations; pUC19, plasmid backbone from the CRM-GAL4 vector containing bacterial origin of replication and ampicillin resistance gene. (B–D) Maximum confocal *z* projection of the thoracic region of a wandering third instar larval central nervous system of the same genotype as in Figure 2A [w; *P{UAS-mCD8::mRFP.LG}*/R24B02-LexA (*attP40*); R58G08-GAL4(*attP2*)/*P{LexAop-CD2::GFP}*]. R24B02-LexA expression is shown in B and in green in D. It is expressed in hemilineage 12A in segments S2 to T2, and in A1. Hemilineage 12A's cell bodies are located relatively medially, and its neurite bundle projects dorsally. In T1 and T2, the 12A bundle splits into a medial and dorsal branch (arrowheads). In T2, the dorsal branch of 12A projects across the midline. R58G08-GAL4 is shown in C and in magenta in D. It is expressed in hemilineage 3A in segments T1–T3. The cell bodies of 3A are located more laterally than those of 12A. The 3A bundle initially projects dorsally, but then turns laterally and projects to the dorsal region of the leg neuropil (arrows). The white box indicates the area shown in Figure 2A. Background staining of trachea is indicated in C.

linearized plasmid was then blunted, and the plasmid was recircularized by blunt-end ligation. Transgenic fly lines were generated by Genetic Services (Cambridge, MA) via phiC31-integrase–mediated insertion into *attP40* or *attP2*.

Results

Transvection can occur between two transgenes inserted at the same attP site

The development of the CRM-GAL4 collection that we screened for ventral nerve cord neuronal lineage expression has been previously described (Pfeiffer *et al.* 2008). Briefly, each CRM-GAL4 contains: (1) a fragment taken from the *cis*-regulatory region of a preselected set of neuronally expressed genes (the CRM), (2) a synthetic core promoter that was designed to be both highly active and promiscuous (the DSCP) and (3) the yeast transcription factor GAL4, which is used to drive expression of one or more UAS transgenes that are inserted elsewhere in the genome. The CRM-GAL4 transgenes have been inserted into the genome at defined target sites via phiC31-mediated recombination (Pfeiffer *et al.* 2008). The target sites (*attP* sites) were themselves introduced into the genome via *P*-element–mediated transformation. In this study, we used *attP2* (Groth *et al.* 2004), which is located on the left arm of the third chromosome and *attP40* (Markstein *et al.* 2008), which is located on the left arm of the second chromsome. When a CRM-GAL4 is inserted into an *attP* site, the final result is a locus that is flanked by 5′ and 3′ *P*-element sequences and contains: (1) a copy of the *yellow* gene (the positive marker for *P*-element transformants), (2) one each of *attL* and *attR* sites (the outcome of recombination between *attP* and *attB*), (3) mini-*white* (a positive marker for phiC31 transformants), (4) the CRM-GAL4, and (5) the pUC19-derived backbone (Invitrogen) of the vector used to introduce the CRM-GAL4. A schematic of an integrated CRM-GAL4 is shown in Figure 1A.

While screening through CRM-GAL4s that had been integrated into *attP2*, we identified two transgenes, R24B02-GAL4 and R58G08-GAL4, that drove expression in neuroblast hemilineages 12A and 3A (Truman *et al.* 2010), respectively. When examined individually, the expression patterns of R24B02- and R58G08-driven transgenes (Figure 1, B–D) are easily distinguishable from one another due to the morphological characteristics of the hemilineages that they label. To independently label both hemilineage 12A and hemilineage 3A in the same fly, the CRMs R24B02 and R58G08 were used to create R24B02-LexA::GADfl and R58G08-LexA::GADfl (hereafter referred to as R24B02-LexA and R58G08-LexA; GADfl, full-length GAL4 activation domain). This allowed us to examine two different transgene pairs: R24B02-LexA/R58G08-GAL4 and R24B02-GAL4/R58G08-LexA.

When R58G08-GAL4 and R24B02-LexA were used to drive pJFRC21-10XUAS-IVS-mCD8::RFP (hereafter referred to UAS-mCD8::RFP) and LexAop-rCD2::GFP, we expected to see hemilineages 12A and 3A labeled with their respective fluorescent protein (Figures 1, B–D and 2A). Surprisingly, when we examined larvae that had both CRM drivers inserted into *attP2*, neurons from both hemilineages were found labeled by both fluorescent proteins (Figure 2B). In sibling larvae that lacked UAS-mCD8::RFP, neurons of the R58G08-GAL4 pattern still expressed the LexAop-rCD2::GFP reporter (Figure 2C). Because R58G08-GAL4 does not activate LexAop-rCD2::GFP on its own (our unpublished observations; Figure 2A), we conclude that there is an interaction between the R24B02-LexA and R58G08-GAL4 transgenes themselves when they are paired in *attP2*. Examining the complementary pair of transgenes in which the GAL4 and LexA were swapped (R24B02-GAL4 and R58G08-LexA) resulted in the same pattern of crosstalk (Figure 2D),

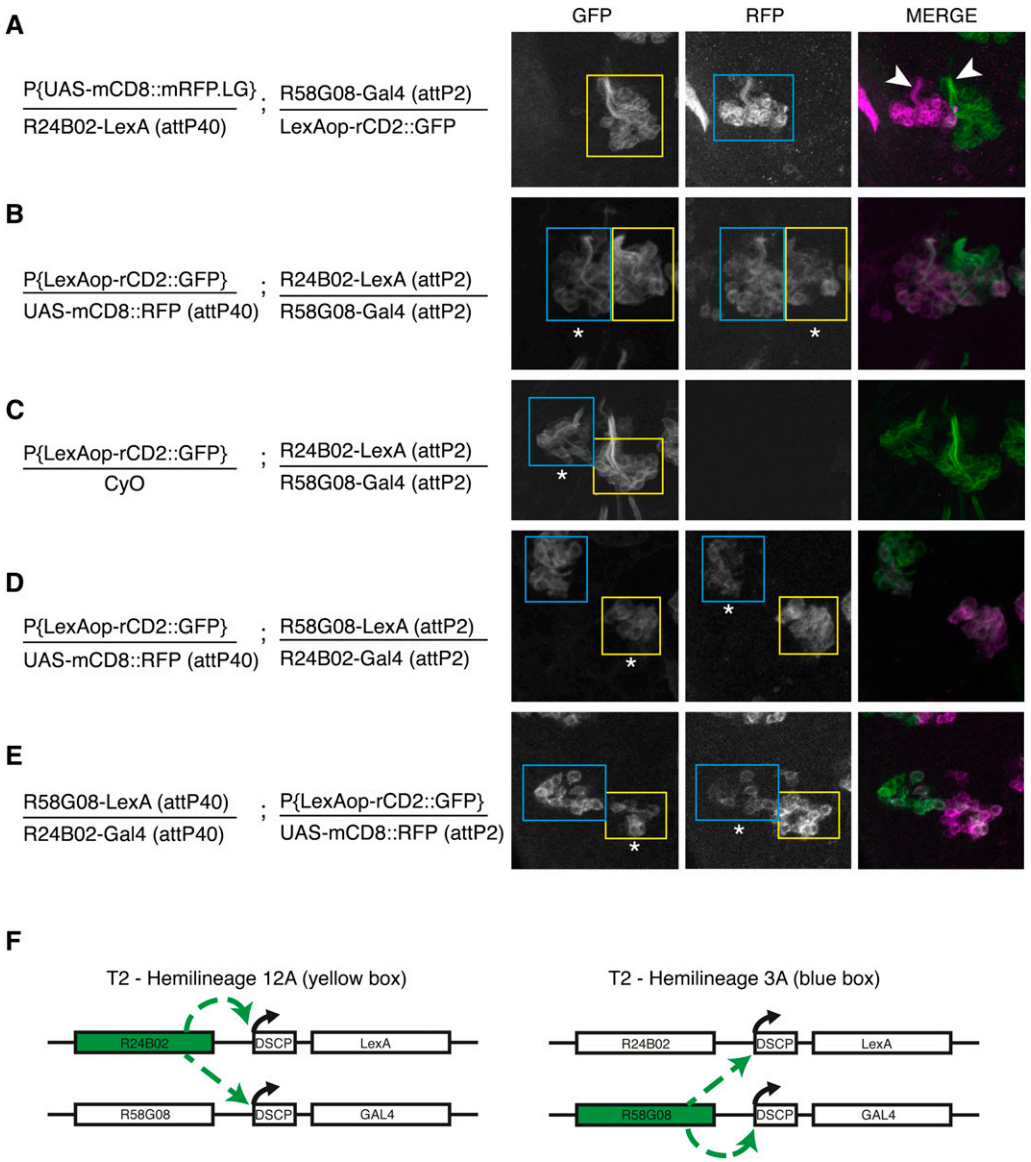

Figure 2 Transvection between transgenes inserted into attP2. (A–E) Partial *z* projections through the left T2 hemisegment in the ventral nerve cord of the indicated genotypes. Only the second and third chromosome genotypes are shown, all X chromosome genotypes are w^{1118}. Neurons belonging to hemilineage 12A are surrounded by a yellow box, whereas neurons belonging to hemilineage 3A are surrounded by a blue box. Asterisks indicate ectopic expression. (A) When no transgenes are paired, transvection does not occur. Shown is a partial stack through the cell body layer of the same CNS as shown in Figure 1. Hemilineages 12A and 3A are independently labeled with rCD2::GFP and mCD8::RFP, respectively, without crosstalk. In addition to being labeled by different fluorescent proteins, the cell bodies can be assigned to their respective hemilineage based on their position and all of the neurites of a hemilineage form a fasciculated bundle (arrowheads). (B) An example of transvection between R24B02-LexA and R58G08-GAL4 when both transgenes are inserted in *attP2*. In contrast to the example in A, neurons from both hemilineages are labeled by both fluorescent proteins. (C) An example of a sibling from the genotype in B that carries CyO instead of UAS-mCD8::RFP. Neurons from both hemilineages express LexAop-rCD2::GFP. (D) Swapping CRMs between R24B02-LexA and R58G08-GAL4 to make R58G08-LexA and R24B02-GAL4 does not affect transvection when both are inserted in *attP2*. (E) Moving both R58G08-LexA and R24B02-GAL4 to *attP40* does not eliminate transvection. (F) Schematic summarizing transvection that occurs in B and C. Green-shaded enhancers are active in the indicated cell population. Green arrows indicate activation. Solid arrows from promoter indicate transcription. When R24B02-LexA and R58G08-GAL4 are inserted into the same integration site and paired, each CRM can drive transcription off of both promoters in some cell types. In T2 hemilineage 12A (yellow box in image panels; left schematic), the R24B02 CRM activates both promoters, leading to the transcription of both GAL4 and LexA. In T2 hemilineage 3A (blue box in image panels; right schematic), the R58G08 CRM activates both promoters.

suggesting the interaction occurs between the CRMs/promoters of the two transgenes.

We reasoned that transvection might be the cause of the interaction between R58G08-GAL4 and R24B02-LexA at *attP2*. If so, the effect should be pairing dependent, and inserting either transgene into a distant site should eliminate the interaction. To address this, we examined larvae in which no two transgenes were inserted into the same *attP* site. To generate these animals, we first integrated R24B02-LexA into *attP40*. We also used UAS/LexAop reporters that were inserted into the genome by *P*-element–mediated transformation. We were then able to examine flies that carried four transgenes at four different locations: R24B02-LexA (*attP40*), a third chromosome insertion of *P{LexAop-CD2::GFP}* (Lai and Lee 2006), R58G08-GAL4(*attP2*), and a second chromosome insertion of *P{UAS-mCD8::mRFP.LG}*. In this genotype, we observed RFP and GFP patterns in the larval VNC that were distinct from each other and labeled only the specific neuronal hemilineages expected for independent expression of each CRM (Figure 2A). Thus, the interaction we had previously observed between the CRM transgenes was completely disrupted when they were moved to distant genomic locations, as would be expected if transvection were the basis for the effect.

We next examined a genotype in which both R58G08-GAL4 and R24B02-LexA were integrated into *attP40*, restoring the ability of the two transgenes to pair at a location distant from *attP2*. This genotype exhibited the same pattern of transvection that we had previously observed with the *attP2* insertions (Figure 2E). Because the strength and pattern of transvection for the paired CRM transgenes was qualitatively similar between *attP2* and *attP40*, we also conclude that there is no significant position effect on transvection, at least with respect to these two sites. We did not examine other *attP* sites, so it is possible that there may be regions in the genome that prevent transvection and that some *attP* sites may lie within those regions. However, previous work from others indicates that the genome is generally permissive for transvection (Chen *et al.* 2002), and our results with *attP40* and *attP2* are consistent with that view.

Promoter competition influences transvection directionality between transgenes

We next asked whether the type of promoter used in the CRM transgenes influences transvection. To test whether the DSCP (Pfeiffer *et al.* 2008) used in the construction of the initial CRM-GAL4 library is required for transvection effects, we replaced it with the *P*-element transposase gene promoter (O'Hare and Rubin 1983) in two CRM transgenes to create R58G08-Ptrans-GAL4 and R24B02-Ptrans-GAL4. When examined individually, both P-transposase promoter transgenes expressed in the neuronal hemilineages in which they expressed when their CRMs were combined with the DSCP, but at reduced levels (data not shown), consistent with previous observations that the P-transposase promoter is a weaker promoter than the DSCP for some CRM transgenes (Pfeiffer *et al.* 2008; B. Pfeiffer, personal communication). Both P-transposase promoter transgenes were also expressed in cells that are not found in the expression patterns of the DSCP transgenes. Thus the pattern and strength of expression driven by the R58G08 and R24B02 CRMs are partially dependent on the identity of the core promoter.

Having established that the CRM-Ptrans-GAL4 transgenes are each expressed in the same hemilineage as the original CRM-DSCP-GAL4 transgenes, we next tested for transvection between R58G08-Ptrans-GAL4 and R24B02-LexA when both were inserted in *attP2*. We also tested the complementary pairing between R24B02-Ptrans-GAL4 and R58G08-LexA in *attP2*. In both cases, the CRM of the transgene utilizing the P-transposase promoter activated transcription in *trans*, whereas the CRM of the transgene utilizing the DSCP did not (Figure 3, A and B). This unidirectional transvection could be due to one of two reasons. First, it may be that the DSCP can be regulated by the *trans* CRM, but the P-transposase promoter cannot. The second possibility is that transvection may tend to operate in one direction when there is an imbalance in the strength of the promoters. The DSCP may outcompete the *trans* P-transposase promoter for transcriptional activation by the CRM in *cis* to the DSCP, leading to the apparent unidirectionality of the transvection effect that we observed. Previous studies have shown that the strength or presence of the *cis* promoter can influence whether an enhancer will act in *trans* (Geyer *et al.* 1990; Martínez-Laborda *et al.* 1992; Morris *et al.* 1999, 2004; Lee and Wu 2006), supporting the latter hypothesis.

To test whether the DSCP promoter was responsible for the unidirectional transvection we observed between R58G08-Ptrans-GAL4 and R24B02-LexA, we generated the promoterless construct R24B02-ΔP-LexA and inserted it into *attP2*. R24B02-ΔP-LexA did not drive specific expression in hemilineage 12A on its own, as expected, but instead produced a low level of nonspecific neuronal expression (Figure 3E). However, when we paired *attP2* insertions of R24B02-ΔP-LexA and R58G08-Ptrans-GAL4, we found that the R24B02 CRM drove GAL4 expression specifically in hemilineage 12A in *trans* (Figure 3, E–G). Thus, the P-transposase promoter can be regulated by a *trans*-acting enhancer. This result excludes the possibility that the unidirectional transvection we observed between CRM-Ptrans-GAL4 and CRM-DSCP-LexA transgenes was due to the insensitivity of the P-transposase promoter to transvection. We conclude that the DSCP can outcompete a weak *trans* promoter for activation by the CRM in *cis* to the DSCP, which can affect the apparent directionality of transvection between two transgenes.

Transvection between R58G08-LexA and R24B02-GAL4 does not require zeste

Some examples of transvection depend on the function of the *zeste (z)* gene (Kaufman *et al.* 1973; Gelbart and Wu 1982), although it is not required in all cases (Pattatucci and Kaufman 1991; Hendrickson and Sakonju 1995). To test whether *z* function is required for transvection between paired transgenes inserted at *attP2*, we examined transvection between *attP2* insertions of R24B02-GAL4 and R58G08-LexA in larvae that were homozygous or hemizygous for z^a, a putative loss-of-function or strongly hypomorphic allele (Jack and Judd 1979). The pattern of transvection in these *z* mutant larvae was no different from what was found in larvae wild type for *z*, indicating that *z* is not required to mediate transvection between R24B02-GAL4 and R58G08-LexA (Figure 3C). We cannot rule out that the neomorphic mutation z^1 might modify the transvection phenotype, as it was not tested. This is important to note because z^1 can cause phenotypes that are pairing dependent even when there is no obvious *z*-null phenotype. The *zeste-white* interaction is one such example (Jack and Judd 1979; Gelbart and Wu 1982). Nonetheless, we conclude that *z* function is not necessary for pairing and transvection between transgenes inserted at *attP2*.

A large fraction of regulatory DNA can support transvection

The example of transvection between transgenes built with the R58G08 and R24B02 CRMs led us to ask whether this effect is specific to this particular combination of CRMs. To test whether transvection is common between transgenes

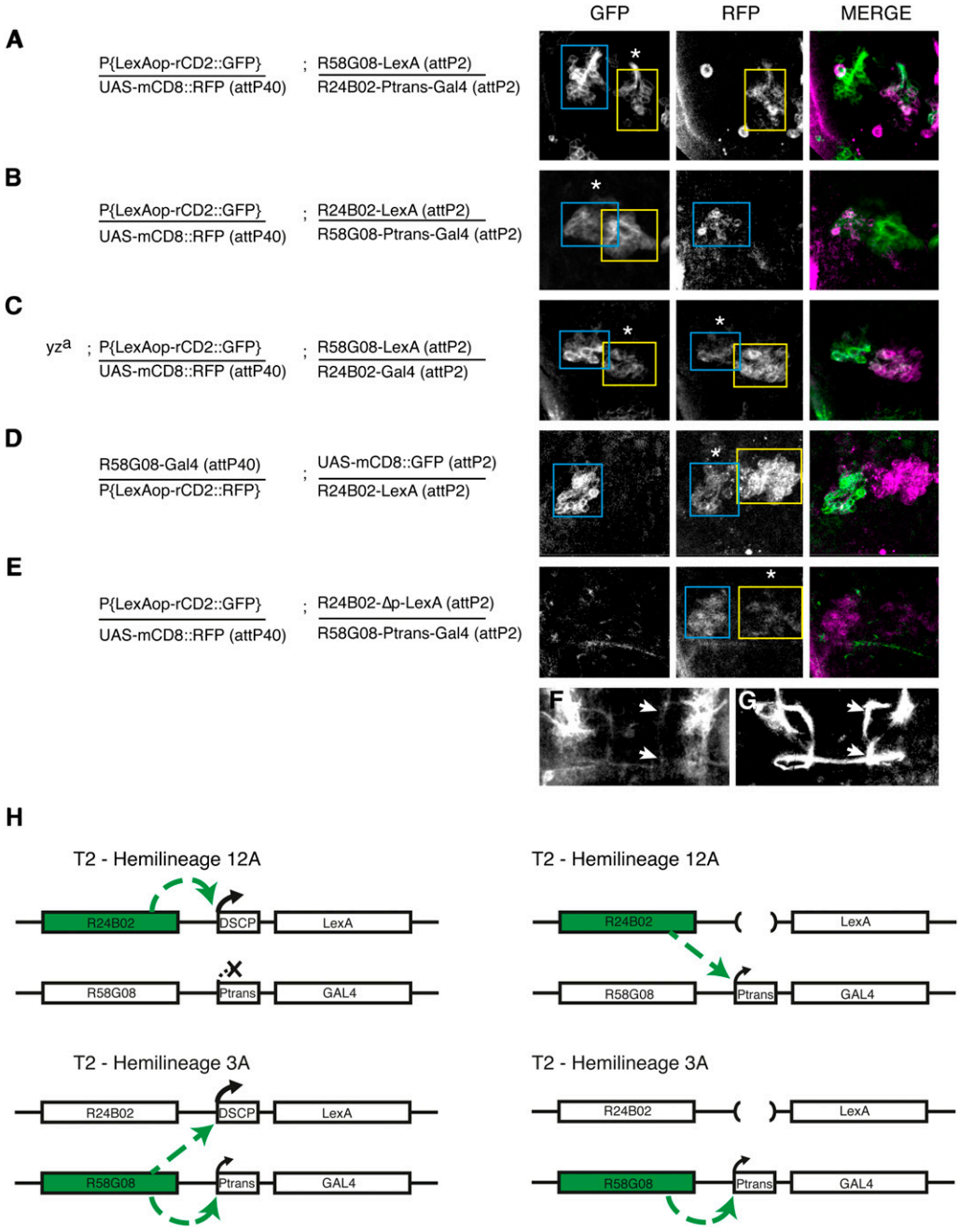

Figure 3 Transvection between integrated transgenes is influenced by promoter type, but not zeste. (A–D) Images and genotypes presented as in Figure 2. (A) Transvection between R24B02-Ptrans-GAL4 and R58G08-LexA is unidirectional. R24B02 drives inappropriate expression of LexA in hemilineage 12A, but R58G08 does not drive expression of GAL4 in 3A. (B) Transvection between R58G08-Ptrans-GAL4 and R24B02-LexA is also unidirectional. R58G08 drives inappropriate expression of LexA in hemilineage 3A, but R24B02 does not drive expression of GAL4 in 12A. (C) Same genotype as in Figure 2D, but in a yz^a background. Transvection still occurs in the absense of *zeste* function. (D) Example of unidirectional transvection between UAS-mCD8::GFP and R24B02-LexA. In this case, UAS-mCD8::GFP is activated by GAL4 in hemilineage 3A, and this causes LexA to misexpress in hemilineage 3A. R24B02 does not activate transcription in UAS-mCD8::GFP. (E) The R24B02 CRM acts in *trans* to drive expression of GAL4 from the R58G08-Ptrans-GAL4 transgene if the R24B02 transgene lacks an intact core promoter. (F) Inappropriate expression of GAL4 in the R24B02 pattern due to transvection from R24B02-ΔP-LexA to R58G08-Ptrans-GAL4 is also evident from the appearance of 12A projections in the dorsal neuropil of T1 and T2 (arrowheads). (G) An optical slice through the 24B02-GAL4 pattern at the same dorsoventral coordinates as in F. (H) Schematic summarizing transvection between transgenes utilizing promoters of unequal strength (data shown in B and E). Green-shaded enhancers are active in the indicated cell population. Green arrows indicate activation. Solid arrows from promoter indicate transcription, with the size of the arrow indicating the strength of the promoter. Broken arrows with an X indicate no transcription. (Left) When R24B02-LexA, which uses the strong DSCP, is paired with R58G08-Ptrans-GAL4, which uses the weaker P-transposase promoter, transvection only operates in one direction. In T2 hemilineage 12A, R24B02 acts only on its *cis* promoter, whereas in T2 hemilineage 3A, R58G08 acts on both promoters. (Right) When R24B02 does not have a functional core promoter, it is free to activate the *trans* P-transposase promoter in T2 hemilineage 12A. In T2 hemilineage 3A, R58G08 acts only on its own P-transposase promoter because there is no *trans* core promoter.

inserted at the same *attP* site, we assembled a panel of 21 CRM-GAL4s whose larval CNS expression patterns we had previously characterized, including R58G08-GAL4 and R24B02-GAL4. The CRM for R23G07-GAL4, also included in the panel, was used to make 23G07-LexA::GADfl (Pfeiffer *et al.* 2010) (hereafter referred to as R23G07-LexA) and, together with R58G08-LexA and R24B02-LexA, crossed to the GAL4 panel to test for transvection between 60 different pairs of transgenes at *attP2*. We were conservative in scoring for transvection in that we disregarded any ambiguous cases where two patterns might have true overlap in their patterns of expression. Moreover, we only focused on the ventral nerve cord of third-instar larvae, as that was the only tissue for which we had previously characterized the expression pattern of the CRMs in our panel. Our assessment of transvection frequency should then be considered conservative.

Table 1 Summary of transvection occurring in test crosses to three CRM-LexA strains

GAL4 used	R24B02	R23G07	R58G08
R24B02	NA[a]	↑[b]	←↑
R23G07	←[c]	NA	←↑
R58G08	←↑	←↑	NA
R54E05	←↑	←	←↑
R11G08	←	R[d]	←
R36B03	↑	↑	—
R54G12	↑	↑	—
R64F08	←	—	←
R29B04	←	—	—
R16H05	—	↑	—
R64E01	—	—	↑
R19D12	—	—	—
R33G12	—	—	—
R49D02	—	—	—
R23H08	—	—	—
R26B05	—	—	—
R32G10	—	—	—
R41G09	—	—	—
R44E04	—	—	—
R66B07	—	—	—
R29D02	—	—	—

[a] Crosses between a GAL4 and LexA using the same CRM are not scorable for transvection because they express in the same pattern.
[b] Up arrow indicates LexA is ectopically expressed in elements of the CRM-GAL4 pattern.
[c] Left arrow indicates GAL4 is ectopically expressed in elements of the CRM-LexA pattern.
[d] *Trans*-repression was seen in this cross (see Figure 5).

The results of the transvection test crosses are summarized in Table 1. Most importantly, transvection was frequently observed. Excluding the three crosses that were GAL4/LexA swaps between CRMs, we found that the transvection frequency among unique pairs of CRMs was 17/57 (30%). The fraction of CRM-GAL4s that participated in transvection with any of the three CRM-LexAs was 11/21 (52%). Assuming this collection of CRMs is representative of enhancer fragments found throughout the genome, we conclude that a large fraction of regulatory DNA is able to support transvection if removed from its endogenous locus and used to drive the expression of a transgene.

Transvection and its directionality requires compatibility between CRMs

For the 10/21 CRM-GAL4s that showed no evidence of transvection, it might be that sequence elements within those CRMs prevent transvection from occurring. However, for the 11 CRM-GAL4s that did show transvection, six of them showed transvection with at least one, but not all of the CRM-LexAs that we tested. This indicates that there are differences in transvection compatibility between different pairs of CRMs and suggests that there are sequences within the CRMs that encode these compatibilities. Thus it may be that those CRM-GAL4s that showed no transvection are simply incompatible with the three CRM-LexAs that we used for testing.

One interesting finding from the 60 test crosses is that transvection can be unidirectional between transgenes that use the same core promoter (DSCP), but different CRMs. Thirteen of the 57 unique pairs of CRMs showed unidirectional transvection. This directionality is also dependent on compatibility between CRM pairs. For example, transvection with R24B02-GAL4 was bidirectional when it was paired with R58G08-LexA, but unidirectional when it was paired with R23G07-LexA, as features of the R24B02 pattern showed up with the LexAop reporter but no element of the R23G07 pattern showed up with the UAS reporter. This directionality was not due to differences between the GAL4/LexA systems, because swapping GAL4/LexA between R24B02 and R23G07 had no effect on the directionality between the CRMs (Table 1).

Transvection does not always occur in all cells

For many of the combinations of the CRM transgenes we examined, transvection only occurred in a subset of cell types in which the transgenes were expressed. One example of this comes from the R24B02/R58G08 pair, which exhibited bidirectional transvection. Though R24B02 is able to drive expression in *trans* in hemilineage 12A in segments S2 to T2 (Figure 4, A and B), there is very little transvection in 12A in segment A1 (Figure 4, A and C). R24B02 also drives extensive expression in neurons in the optic lobes, but also fails to participate in transvection in these cells (Figure 4D). This pattern of cell type specificity is consistent between individuals and does not differ between integration sites, because the pattern of cells showing transvection between R24B02/R58G08 is the same regardless of whether the transgenes are inserted in *attP40* or *attP2* (data not shown). The consistency of the pattern of cell type specificity suggests that it is due to sequences within the CRMs. We also saw several cases among our 60 test crosses where transvection was sparse and/or stochastic (data not shown). We conclude from these observations that the factors affecting transvection must differ between cell types.

GAL4 bound to UAS can activate transcription in trans

The observation that transvection occurs only between compatible pairs of CRMs led us to consider the possibility that there are sequences in the CRMs that regulate transvection. Compatibility between CRMs could then be viewed as compatibility between binding sites for one or more transvection-mediating factors. To ask whether there are sequences that confer the ability of an enhancer to activate transcription in *trans*, we examined the effect of pairing R24B02-LexA with pJFRC2-10XUAS-IVS-mCD8::GFP (hereafter referred to as UAS-mCD8::GFP). The UAS-mCD8::GFP transgene contains no regulatory DNA other than GAL4 binding (UAS) sites and the *hsp70* basal promoter (Brand and Perrimon 1993; Pfeiffer *et al.* 2010); thus it is a minimal construct that should not contain any of the sequences that would be present in endogenous *Drosophila* enhancers that may uniquely confer the ability to act in *trans*.

When we examined larvae that had UAS-mCD8::GFP paired with R24B02-LexA in *attP2*, we found that driving

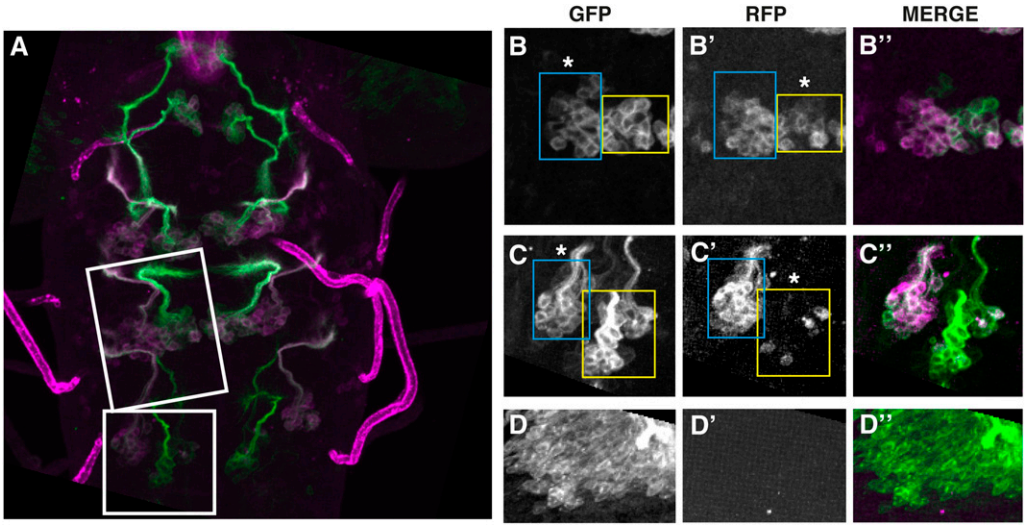

Figure 4 Cell specificity of transvection. (A) Maximum confocal *z* projection of a wandering third instar larval ventral nerve cord from a w; LexAop-rCD2::GFP/pJFRC21-10XUAS-IVS-mCD8::RFP(*attP40*); R24B02-LexA(*attP2*)/R58G08-Gal4 (*attP2*) animal. White boxes indicate the regions from which the partial stacks in Figure 3, B and C were taken. (B) Transvection occurs between R24B02-LexA and R58G08-GAL4 in hemilineages 12A and 3A in T2. (C) Transvection occurs between R24B02-LexA and R58G08-GAL4 in hemilineage 3A in segment T3, but only weakly in hemilineage 12A in segment A1 (a few cells of the 12A hemilineage are present in T3 in some animals, and these also show limited transvection). (D) No transvection is seen in cells in the optic lobe that express R24B02-LexA.

UAS-mCD8::GFP (*attP2*) with R58G08-GAL4 (*attP40*) resulted in expression of LexAop-mCD8::RFP in much of the R58G08 pattern (Figure 3D). This is particularly notable in that there is no sequence homology between the core elements of the R24B02-LexA and UAS-mCD8::GFP transgenes (although there is flanking homology from the integration construct and integration site; see Figure 1A). That the GAL4 protein produced by R58G08-GAL4(*attP40*) was sufficient to activate transcription in *trans* from its target UAS sites suggests that transvection can occur wherever a transcriptional activator is targeted to sequences present in *trans* to a functional core promoter. This result also suggests that there is no additional requirement for a transvection-mediating protein to bind a CRM for it to function in *trans*. The inability of some CRMs to drive transcription in *trans* must then be due to either (1) active repression by proteins recruited by the *trans* CRMs or (2) the inability of the *trans* promoter to initiate transcription (see *Discussion*).

Trans-repression can occur between transgenes integrated at attP sites

We observed *trans*-repression in at least 1 of our 60 transvection test crosses, in the case where 23G07-LexA was paired with R11G08-GAL4 in *attP2*. R11G08-GAL4 normally drives robust expression in hemilineages 12A and 11A (Figure 5B). R23G07 drives expression in lineage 11 and in other neuron types, but is what we call a "stochastic" CRM (Figure 5A). The expression patterns of stochastic CRMs vary between individuals in that a subset of lineages may or may not be labeled in a given individual. The on/off decision appears to be independent for each lineage, because we typically see bilateral asymmetry. The on/off expression decision also appears to occur early in the life of the neuroblast and then is epigenetically inherited by its daughters, so that either all of the cells in a lineage or hemilineage express the transgene or they all do not.

Although R11G08-GAL4 consistently drives expression in all 11A hemilineages when present alone, after pairing with R23G07-LexA, R11G08-GAL4 expression was very weak or absent in some 11A hemilineages. Interestingly, in six of the seven samples of the R23G07-LexA/R11G08-GAL4 pairing, hemilineage 11A expression of R11G08-GAL4 was suppressed in the same lineages that were not expressed in the stochastic R23G07-LexA pattern (Figure 5, C and D). In contrast, all five of the siblings in which R11G08-GAL4 was present alone (R11G08-GAL4/TM6B) showed typical, nonstochastic expression of R11G08-GAL4 in all 11A hemilineages. We can draw two conclusions from these data. First, the stochasticity seen with the R23G07 CRM is probably due to stochastic repression, rather than activation. Second, the mechanism for repressing R23G07-LexA in some lineages can act in *trans* to similarly repress R11G08-GAL4. We do not know whether this *trans* interaction occurs early in the life of the neuroblast, coincident with the R23G07-LexA silencing event or whether the repression persists through development. Finally, we observed that pairing R11G08-GAL4 with R23G07-LexA also reduced expression of R11G08-GAL4 in hemilineage 12A, although this repression was not as severe as in hemilineage 11A. R23G07 is never expressed in 12A, thus transgenes may affect the expression of a paired transgene in cell types that fall outside of their own typical expression pattern.

Discussion

Insights into transvection from using the phiC31 integrase system

One issue that complicates the study of transvection is the difficulty in assessing the effect at the single cell level. Most

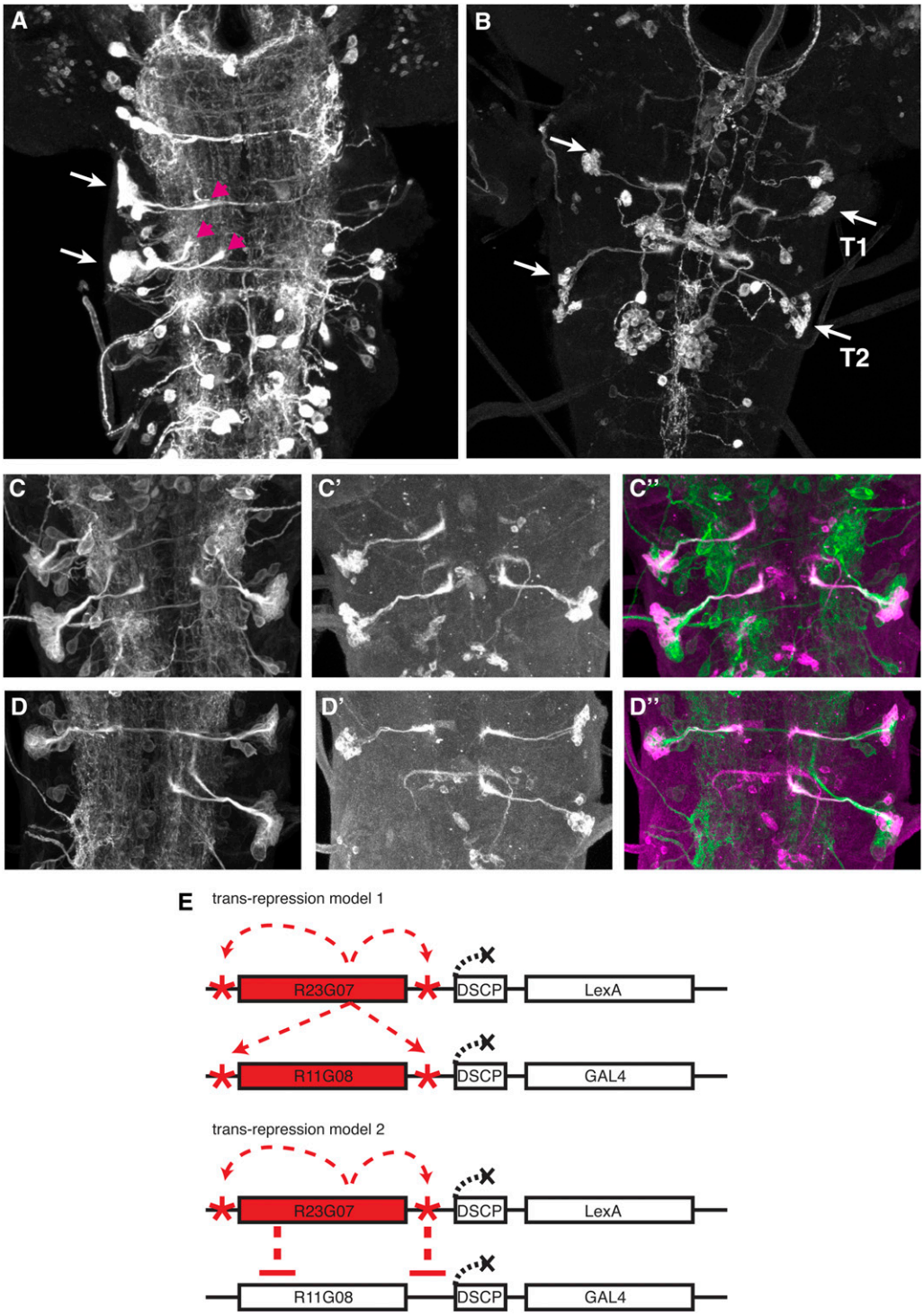

Figure 5 *Trans*-repression between integrated transgenes. (A) Confocal *z* projection of a wandering third instar larval VNC from an animal driving UAS-mCD8::RFP with R23G07-GAL4. Expression is found in many neuronal cell types, including lineage 11 (cell bodies indicated with white arrows; projections with magenta arrowheads). Note the stochastic expression in this individual: no expression is found in lineage 11 on the right side of the VNC. (B) Confocal *z* projection of a wandering third instar larval VNC from an animal driving UAS-mCD8::RFP with R11G08-GAL4. Expression in the VNC is largely limited to hemilineages 11A (cell bodies indicated with arrows) and 12A. Robust expression is found in every 11A/12A hemilineage in segments T1 and T2. (C–D'') Two examples of VNCs from w; LexAop-rCD2::GFP/UAS-mCD8::RFP (attP40); R23G07-LexA (attP2)/R11G08-GAL4(attP2) animals. Expression of R23G07-LexA (C and D) is stochastic in lineage 11, as expected. Expression of R11G08-GAL4 (C' and D') in hemilineage 11A is also stochastic, but in the same pattern as R23G07-LexA. Merged images are shown in C'' and D''. (E) Two possible models for *trans*-repression. Red shading and asterisks represent repressive epigenetic modifications and red arrows indicate recruitment of chromatin silencing proteins. In model 1, R23G07 recruits chromatin silencers that modify both paired transgenes. In model 2, only R23G07 is epigenetically modified, but these modifications directly or indirectly lead to the repression of both transgenes.

studies of transvection depend on intragenic complementation as the readout of transvection efficiency. To further investigate the molecular mechanisms of transvection, it will be useful to have a robust, controlled genetic system that enables both the molecular dissection of key sequence elements as well as a highly detailed and quantitative analysis of the transvection effect itself. One attractive approach is to use the phiC31 integrase system to insert transgenes into defined integration sites (*attP* sites) and test for their ability to support transvection. This system (1) allows for the use of transgenes that contain the minimal number of sequence elements sufficient to support transvection, (2) inserts transgenes into a common chromosomal location to allow for testing with multiple pairwise combinations while minimizing position effects, and (3) allows for distinct readouts for the transcriptional activity of both alleles (*e.g.*, two fluorescent reporters) to minimize the ambiguity that is often present when attempting to quantify the

rescue of mutant phenotypes when testing for intragenic complementation. This allows for an assessment of transvection at essentially the single molecule level, because each transgene pair is present as a single copy in each cell.

Although the system we have described here should be very valuable for studying transvection, there is one significant drawback. First, using the phiC31 integrase system along with engineered transgenes requires that all tested sequences be removed from their endogenous locus and combined with other foreign sequence elements. Thus, CRMs may behave very differently in this system than they do in their endogenous locus. It is therefore encouraging that changing the promoters in our transgenes did not eliminate transvection effects, and the patterns of transvection were qualitatively similar between two distant *attP* sites for the pairs that were tested at both sites (Figure 2, D and E; D. J. Mellert, unpublished observations). These observations suggest that transvection is robust to the surrounding genomic environment.

A second limitation of our study, but not the transgenic system we propose *per se*, is that it did not include the use of chromosomal rearrangements that disrupt pairing, which is usually the test for the pairing dependence of transvection effects. This raises the question as to whether the interactions we see are actually pairing dependent. That both transgenes require insertion into the same genomic location is strong evidence for this possibility. The prevailing view of transvection is that proximity of the two participating alleles is required. Although it is formally possible that two transgenes that substantially differ in sequence could interact even in the presence of chromosomal rearrangements (for an example of such a strong transvection effect, see Hopmann *et al.* 1995), it is not clear how transvection would be position dependent in such a case, because it would imply both transgenes would have the ability to associate independent of flanking homology. It has been shown that it is possible for two alleles to participate in transvection across large chromosomal distances or between chromosomes, but only if there is sufficient contiguous flanking homology (Ou *et al.* 2009). These ambiguities could be resolved in future studies. The phiC31 system for studying transvection is amenable to more classic techniques using chromosomal rearrangements, as it would allow for many different transgenes to be inserted into the same location on a rearranged chromosome. This could be used to test differences between CRMs in the robustness of transvection to various chromosomal rearrangements.

Despite the preceding caveats, we have provided some insight into the mechanism of transvection using the phiC31-integration strategy. One particularly surprising feature of transvection is that it can occur reciprocally between two fully functional alleles. This has been a difficult question to address because it requires the ability to assess transcription of each allele independently, and most studies of transvection depend on intragenic complementation between one allele with a disrupted regulatory region and one allele with a disrupted coding sequence (however, see Goldsborough and Kornberg 1996). We have shown that enhancers can act in *trans*, even if they are in *cis* to a fully functioning and strong core promoter and an intact coding sequence. This is consistent with previous reports that have shown that enhancers may work on *trans* promoters in the presence of a functional *cis* promoter (Goldsborough and Kornberg 1996; Casares *et al.* 1997; Sipos *et al.* 1998). Thus enhancer preference for the *cis* promoter does not necessarily prevent transvection between wild-type alleles, supporting the possibility that transvection is common throughout the wild-type *Drosophila* genome. Further supporting this view, we found that ~50% of CRMs we tested could undergo transvection when paired with at least one of three different transgenes. Given that most genes contain multiple CRMs in their regulatory regions and that the genome is generally permissible for transvection, it is probably the case that most genes contain CRMs that are competent to undergo some degree of transvection.

Our ability to observe the activity of enhancers at single cell resolution also reveals something about the dynamics of enhancer action. In cases where both promoter strengths are equal, some CRMs drive expression of both GAL4 and LexA in the same cell. This means that in some circumstances, a single enhancer can activate both paired promoters. Thus, when transvection is directional due to differences in promoter strength, it is not the case that the stronger promoter locks its *cis* enhancer into a configuration that physically prevents it from interacting with the weaker *trans* promoter. Rather, the stronger promoter probably outcompetes the weaker promoter for either enhancer input or transcription initiation factors that are present at a limiting local concentration (Morris *et al.* 1999). On the other hand, our data show that an enhancer can still act on its own weak *cis* promoter even in the presence of a strong *trans* promoter, demonstrating that enhancers exhibit some degree of *cis* preference even when there is competition between paired promoters.

The finding that UAS sequences can drive transcription in *trans* in the presence of GAL4 protein also shows that transvection does not necessarily require sequence homology between genes/transgenes, because somatic pairing and flanking homology will still ensure that they are in close proximity in the nucleus. Moreover, it is possible that there is no sequence that uniquely confers the ability of an enhancer to act in *trans*. The ability to bind a transcriptional activator is sufficient. This suggests to us a model for transvection in which any transcriptional activator can act in *trans* provided (1) the *trans* promoter is competent to initiate transcription in a given cell type (*i.e.*, not epigenetically silenced, can recruit and bind RNA polymerase II, *etc.*), and (2) there is not active repression by proteins bound to the *trans* regulatory region. We stress some caution with this interpretation, however. All of our transgenes were inserted into the genome along with copies of *mini-white* and *yellow* (Figure 1A). Because pairing-dependent *trans* effects have

been observed at both of these gene loci (Jack and Judd 1979; Gelbart and Wu 1982; Geyer *et al.* 1990), it remains a possibility that there are sequences carried in the transgenic constructs used to insert the *attP* sites and CRM transgenes that promote or are necessary for transvection. One solution to this problem would be to use recombinase-mediated cassette exchange (Bateman *et al.* 2006) to cleanly integrate transgenic constructs without inserting dominant markers.

We also find that transvection does not always occur throughout the entire expression pattern, suggesting some element of cell-type specificity. This observation may reflect a difference in the degree of somatic pairing between cell types. We favor a second possibility: that distinct elements of an expression pattern depend on unique combinations of transcription factors, and these transcription factor combinations differ in their ability to activate the *trans* promoter. Thus, the cell specificity of transvection and compatibility differences between CRMs may be due to the same underlying mechanism.

Trans-repression and a possible role for Polycomb group proteins

Surprisingly, we find evidence that the epigenetic state of a transgene can be transmitted in *trans* in *Drosophila*. *Trans*-repression and transmission of an epigenetic state has been observed not only in *Drosophila* (Henikoff and Dreesen 1989), but also plants (reviewed in Chandler 2007) and mammals (Rassoulzadegan *et al.* 2002), so epigenetic *trans*-repression may be a common feature of genome function. The phiC31 system could be used with defined CRM transgenes to work out mechanisms of *trans*-repression in the same way it can be used to study *trans*-activation, providing insight into this feature of gene regulation that may be applicable across diverse species.

One explanation for the *trans*-repression that we describe here is that there are sequences within R23G07 that recruit chromatin-silencing proteins, and these chromatin silencers produce chromatin modifications on both transgenes that are inherited and maintained by the neuroblast progeny (Figure 5E; model 1). Alternatively, the *trans*-repression we observe may not require both CRMs to be epigenetically modified. Instead, the transgene that is modified may persistently repress its paired, unmodified transgene (Figure 5E; model 2). We do not know how common *trans*-repression may be, because it is much more difficult to detect than positive *trans* interactions using the transgene crossing scheme that we used. However we expect that the frequency we observed (1 out of 57 crosses) is a low estimate.

Though we do not know the mechanism of silencing that occurs with R23G07, one attractive possibility is that it is regulated by Polycomb group proteins (reviewed in Mateos-Langerak and Cavalli 2008; Simon and Kingston 2009). In support of this, the region of the genome containing the R23G07 fragment has been found to bind Polycomb group (PcG) proteins Pho, Pcl, Pc, Psc, and dSfmbt in embryos and derived cell lines (Oktaba *et al.* 2008; Celniker *et al.* 2009). Moreover, R23G07 contains at least one sequence, CCGGCCATTCG, that is a reasonable match (8/11 nucleotides) for a position sequence-specific matrix reconstructed from ChiP-chip analysis of Pho and dSfmbt binding (G[C/A][C/G]GCCAT[T/C]TT; Oktaba *et al.* 2008). Given that PcG proteins are involved in pairing-dependent silencing (Kassis *et al.* 1991; Brown *et al.* 1998), it would be interesting to determine whether they are involved in the *trans*-repression we have reported here. If so, the phiC31 system could also be a powerful tool for dissecting the mechanism that underlies the *trans* effects of PcG-dependent gene silencing.

Implications for transgene usage in Drosophila

We first encountered transvection while attempting to independently label and manipulate multiple classes of neurons (R. Harris, personal communication). This goal requires transgenes to function independently. Independence in the transcriptional regulation of transgenes is particularly important for intersectional strategies, whereby the expression patterns of two or more transgenes are subdivided or combined. For example, the split-GAL4 system (Luan *et al.* 2006) is often used to target cells that are in common between two expression patterns. The DNA binding domain of GAL4 is expressed in one pattern (CRM1-GAL4DBD), the activation domain of GAL4 is expressed in a second pattern (CRM2-GAL4AD), and only in the intersection of the two patterns does the reconstituted GAL4 protein drive expression of UAS transgenes. The lack of independence in the transcriptional regulation of each split GAL4 component could result in an unexpected pattern. For example, transvection between CRM1-GAL4AD and CRM2-GAL4DBD could broaden the expression domain of CRM2-GAL4DBD to include more CRM1-GAL4AD expressing cells than expected. This could lead to an experimental result being misinterpreted.

Because of the apparent widespread nature of transvection between transgenes inserted at integration sites, caution should be exercised when using these genetic tools. To minimize the confounding effects of transvection, we propose the following guidelines:

(1) Wherever possible, the use of integrations sites should be limited to one insertion per site, per genotype. We have seen no case of transvection between transgenes inserted into the genome randomly by transposon-based methods or transvection between two transgenes inserted at distant sites.

(2) If the same site must be used for two different transgenes, those two transgenes must not require independence. For example, there should be no problem with using a CRM-GAL4 and UAS-GFP in the same site, because there is an intended activation interaction between those two transgenes.

(3) If two transgenes must be independent in their transcriptional control and also inserted into the same genomic site, controls must be in place to test for possible

transvection effects. These would include an indirect readout of the expression patterns of the two transgenes (*i.e.* UAS- or LexAop-GFP) or a direct readout via antibody staining or *in situ* hybridizations.

Finally, our observation that UAS sequences can drive transcription in *trans* is also of interest given the widespread use of the GAL4/UAS system in *Drosophila*. There may be cases where activating a UAS-driven effector drives expression not only of the effector, but also of a paired gene in *trans*. The chances of this phenomenon substantially affecting the outcome or interpretation of an experiment are probably low, but worth considering.

Acknowledgments

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Note added in proof: In this issue of *GENETICS*, Bateman and colleagues (pp. 1143–1155) report an independently developed, targeted transgene integration strategy for investigating transvection. Their results agree with our own in several important respects. First, they describe a minimal enhancer construct that can act in *trans*, supporting the conclusion that there is no special sequence element that gives an enhancer that ability. Second, they show that a single enhancer can act on both a *cis* and *trans* promoter simultaneously (or by rapid switching). Finally, they nicely quantify competition between *cis* and *trans* promoters for enhancer activity and show that this competition is skewed in favor of the *cis* promoter.

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Transvection Is Common Throughout the *Drosophila* Genome

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Table S1

CRM ID	Nearest Gene	L Primer	R Primer
R24B02	CG7807(AP-2)	GAGTCTACCAGTGCAACTGCTACTT	CTCCAAGTGTACCCAGCATCGCTTT
R23G07	CG7807(AP-2)	CGCCCTGCCACAAATTCGGACTTAA	CCATTTATATTTCGGAAGCCACGCC
R58G08	CG17907 (Ace, Acetylcholine esterase)	ACTTGCCAAAAGTCAGGAGTTGGGC	TGGAAGCACTTCCCGAATCGCCATC
R54E05	CG3967	GTTTTGCTGTCCCGCCCCATCAAAG	CTTAATGGAGCGTTGCGAAAGCGGT
R11G08	CG14029 (vri, vrille)	CACCGCCACAGTGGTCGTCGATCGAGCTT	GAGAGGTTTTGTGCACCCTTATCAG
R36B03	CG42234 (Dbx)	CCACCAGCTACGGCGTTGAATAATG	GTTGTTCCTCGATGAGCTGGTAGCG
R54G12	CG14994 (Gad1, Glutamic acid decarboxylase 1)	GTGCTCCACGATCCATTTCAAGGAG	GATACTCCGCCGACATCTGATTGCA
R64F08	CG8985 (DmsR-1, Dromyosuppressin receptor 1)	CACCGCCAAGTCAACACCACTAAGAACCT	CTTACGTTTGTGGCTCACCCCTTTT
R29B04	CG42636 (Gyc76C, Guanylyl cyclase at 76C)	GCATCCGCCATACCGACATTCGCCC	GAAGGGCAAGCAGCGACTTAACGAC
R16H05	CG4889 (wg, wingless)	CTTCTCGAGGGGCAAAAATCTATTC	GATTGCGTAAATGAAGCTCGTCTCT
R64E01	CG8985 (DmsR-1, Dromyosuppressin receptor 1)	CACCAACGCCTCCGCGAATTTGTTCGTGC	GCTCCATTTGAATTGCTTGGCTGGG
R19D12	CG17941 (dachsous, ds)	GCAGGGGGAGGGACTGACTCCTAGA	GGGAGGCAGGGGACTGATACCATAA
R33G12	CG1072 (Arrowhead, Awh)	CCCCAAACCCCCTTTTAGCCGAGAT	CACAAATGCCCCTCAACCACCCGAT
R49D02	CG12660 (rdgA, retinal degeneration A)	GTCACATCCAACAAGTGCGACAGAG	CAAGTGGAAACTAACACGGTACCAC
R23H08	CG4971 (Wnt10)	GCCACCATCTGAGCTCTGTTTACAC	CCTTTCCCTCCACTTGGCCACAATG
R26B05	CG42301 (CCKLR-17D1, CCK-like receptor at 17D1)	GAGCCATGAACTTTGGCAGCAGTGG	CATGTCACTGTTGCACACGCCTCGA
R32G10	CG2212 (swiss cheese, sws)	GATGGCTGCTACACGAACAACGTGC	CACATCGATGGCGATTATGTGAGCA
R41G09	CG8524 (NK7.1)	GTTCCATCCGAATTCACCGACAGTT	GGCTTCGCATTCGAGGATTTGTGCT
R44E04	CG1765 (CG8347, EcR, Ecdysone receptor)	CAGCAACTGCGTCAAGATTCGTGCA	CATCGCACTGACGCTTTAGTCTGGA
R66B07	CG6515 (Tachykinin-like receptor at 86C, Takr86C)	CACCGTCAGCCTCTTGTCGAGCATTATCG	ATGGGTAGGAAACCGCGACCACTTC
R29D02	CG42636 (Gyc76C, Guanylyl cyclase at 76C)	GCAGCAGAACCGAAAGTAAACCAAG	GTGTAGTCGCAGCCACAGATCCAGG