Transvection in 2012: Site-Specific Transgenes Reveal a Plethora of *Trans*-Regulatory Effects

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In this commentary, Judith Kassis discusses Bateman et al., "Comparing Enhancer Action in cis and in trans" and Mellert and Truman "Transvection is Common Throughout the Drosophila Genome", which are published in this issue of GENETICS.

IN *Drosophila*, homologous chromosomes are paired in somatic cells (reviewed in McKee 2004), leading to the opportunity for regulatory DNA on one chromosome to influence the expression of a promoter located on the homologous chromosome (reviewed in Duncan 2002; Kennison and Southworth 2002). Such *trans*-regulatory interactions were first reported by Ed Lewis (Lewis 1954) who found that allelic complementation between particular mutations within the bithorax complex did not occur when the pairing of homologous chromosomes was disrupted. He called this pairing-dependent interaction "transvection." Since that time, other examples of *trans*-regulation, both negative and positive, have been observed, and transvection at a few genes has been extensively characterized leading to the following findings: (1) enhancers can act either in *cis* or in *trans*, but they prefer to work in *cis*, and the presence of a promoter in *cis* can inhibit the *trans*-interactions (Geyer *et al.* 1990; Morris *et al.* 1999; Lee and Wu 2006 and references within); (2) many regions of the genome are permissive for transvection (Kassis *et al.* 1991; Kassis 1994; Chen *et al.* 2002); and (3) there are specific DNA fragments that facilitate some transvection interactions (*e.g.*, Hopmann *et al.* 1995). It is not known whether these findings are gene specific or if they can be generalized. The development of site-specific transgenes in *Drosophila* (Groth *et al.* 2004) has made the study of transvection much more tractable, and two articles in this issue of *GENETICS* suggest that *trans*-regulation is widespread in the *Drosophila* genome (Bateman *et al.* 2012; Mellert and Truman 2012).

Both groups of researchers used the phi-C31 system to integrate transgenes into specific genomic locations to look at the ability of one transgene to activate the expression of another, greatly increasing our knowledge of *trans*-interactions and suggesting many experiments for the future. However, beyond that, their approaches to studying transvection and the questions they addressed differ. Bateman *et al.* (2012) used recombination-mediated cassette exchange (Bateman *et al.* 2006) to insert a simple, defined enhancer, the GMR (which consists of five binding sites for the eye transcriptional activator Glass) and a defined promoter driving the expression of either GFP or mCherry into three different chromosomal insertion sites to address the question of whether a defined, simple test gene could undergo transvection. The answer was "yes!" This shows that no special transvection-mediating sequences are required for transvection. Mellert and Truman (2012) studied transvection using phi-C31 constructs developed as tools for study of the larval central nervous system (Pfeifer *et al.* 2008). In this system, the integrated transgenes are flanked by the mini-*white* gene, the *yellow* gene, and *pUC* DNA at the chromosomal insertion site. The *yellow* gene has been used extensively for studies of transvection and could have regions of DNA that facilitate transvection; thus, sequences within this integration platform could have contributed to the *trans*-regulatory interactions observed. Nevertheless, using this platform, Mellert and Truman show that many different enhancers can act in *trans* to either activate or repress transcription.

There are several interesting findings from these studies. First, activation in *trans* is qualitatively different from activation in *cis*. Both groups found that activation in *trans* occurred stochastically (Figure 1). In the case of the GMR transgene, activation in *trans* occurred in very few cells, but in those cells where *trans*-activation occurred, it was at a high level. This leads to the unanswered question of whether transcription in *cis* and in *trans* occur by the same mechanism.

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doi: 10.1534/genetics.112.142893
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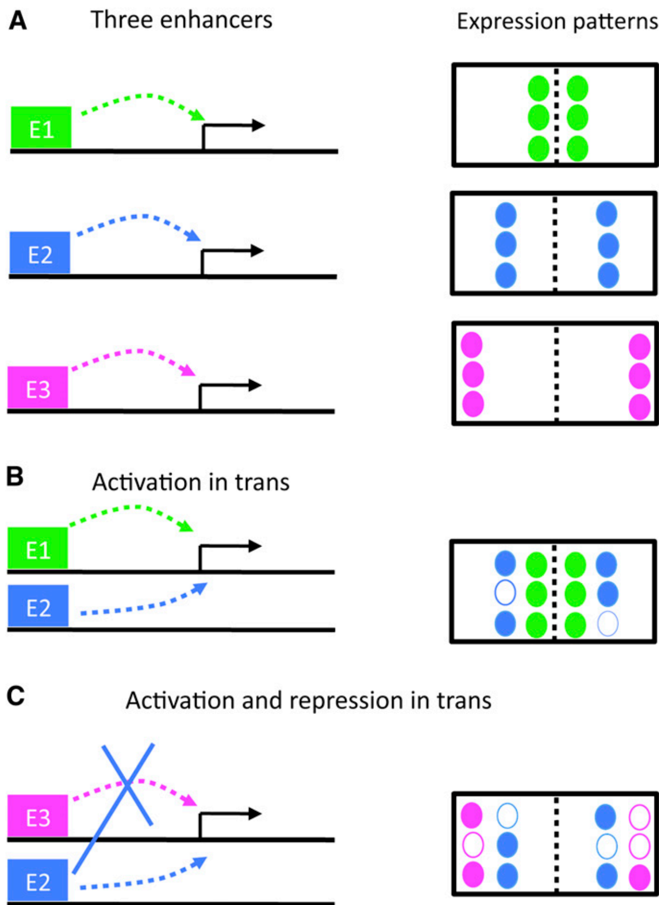

Figure 1 *Trans*-regulatory interactions can either activate or repress transcription. (A) (Left) Three hypothetical transgenes with three different fragments of regulatory DNA (colored boxes E1, E2, and E3) that stimulate transcription (dotted arrows) of the promoter (black arrow) in the patterns shown on the right. (Right) The box represents one segment; the dashed line is the axis of symmetry; each oval is one cell. Each enhancer fragment stimulates transcription in a different row of cells. (B) Each transgene is inserted in the same chromosomal location. In this case, E2 stimulates transcription from the promoter on the other chromosome. Note that transcription does not occur in all blue cells. (C) In addition to enhancer activity, the E2 DNA fragment interferes with the enhancer activity of the E3 fragment.

Both groups also showed that a single enhancer could activate both the *cis*- and *trans*-promoters in the same cell. The current experiments could not distinguish whether the enhancer worked on both promoters at the same time or cycled between the promoters.

Mellert and Truman (2012) tested the ability of 21 different enhancers in 60 different combinations and found that 11/21 enhancers could *trans*-regulate in some combination. In addition to *trans*-activation, Mellert and Truman found some enhancers that could repress transcription in *trans* (Figure 1C). Like *trans*-activation, *trans*-repression was found to be stochastic. In addition, they showed that GAL4, when bound to a UAS-driven transgene, could activate expression of a promoter in *trans*, again showing that a simple enhancer could support *trans*-activation.

Practical Implications for Experimental Design in *Drosophila*

Both of these studies emphasize the promiscuity of the *Drosophila* genome for *trans*-regulatory interactions. This has important practical implications for experimental design in *Drosophila*. One should always assume that two transgenes inserted into the same integration site might *trans*-regulate. The precautions that one should use when designing experiments using site-specific transgenes are well addressed in Mellert and Truman (2012).

What Can Transvection Tell Us About the Mechanism of Enhancer Activity?

One very interesting finding made by Mellert and Truman (2012) is that only a subset of enhancer combinations could mediate transvection in their transgenes. What can this tell us about how enhancers activate transcription? Distal enhancers can use many mechanisms to activate transcription (Bulger and Groudine 2011). It is possible that some mechanisms of transcriptional activation are incompatible with each other or that only some mechanisms support *trans*-activation. Furthermore, some enhancers may contain sequences that mediate repression in other cell types. It is possible that one could use the transvection system to look for such repression effects hidden within enhancers by setting up a system with a ubiquitous reporter construct to assay for *trans*-repression effects. Also, one could explore whether the chromatin modifications often associated with transcriptional activation and repression occur on both alleles of a *trans*-activated gene. Let the experiments begin.

Acknowledgment

J.A.K. is supported by the intramural research program of the National Institutes of Health, National Institute of Child Health and Human Development.

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