which fluctuations last. In theory, a blip in the signal (that is, some fluctuation about the mean steady state value) will continue longer with positive feedback than without, leading to a more slowly decaying autocorrelation function. The authors demonstrated this effect experimentally by weakening the positive feedback. Using either overexpression of a human inhibitor of Tat or introduction of a specific mutation in the *Tat* gene, they reduced the feedback strength and subsequently measured a decrease in the autocorrelation function, suggesting that the expression transients were shorter lived. They also found that reducing the viral feedback resulted in a higher latency rate by using a GFP reporter to measure promoter activity in cells. The human inhibitor that the authors overexpressed is a protein called SirT1 (a histone deacetylase and homolog of the yeast Sir2 protein), which de-acetylates and de-activates Tat, but which is also required for Tat transactivation[9]. SirT1 belongs to the sirtuin family of proteins implicated in a range of functions, from metabolism and aging to differentiation. Therefore, it is interesting to speculate whether and how those other functions might modulate Tat feedback.

Implications for HIV lifecycle

Weinberger *et al.* describe the Tat circuit as a generator of variable-length expression transients and propose that these variable transients serve as a probabilistic switch between latency and lysis[2]. It is not yet clear, however, to what extent this positive feedback circuit plays such a role *in vivo*. In addition to such feedback in the viral gene circuits, the state of the host cell is likely to have an important role in regulating this post–HIV infection fate decision[10]. For example, HIV preferentially infects active T cells, whereas quiescent T cells are not prone to infection, and, if infected, they do not promote viral replication. A 'resting' T cell, in which weak signals promote entry into G1 but not into subsequent stages of the cell cycle, can provide the conditions needed for infection that is not followed by replication, leading to latency[10]. In this manner, it is possible that the decision between latency or active replication and lysis could be determined solely by changes in external signals and the host cell state and that the Tat feedback characterized here might have a secondary role in executing this fate decision. In future studies, it will be important to consider the relevance of feedback within the HIV viral components in the context of the state of the host cell.

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Delivery codes for fly transgenics

Thomas C Tubon Jr & Jerry C-P Yin

Chromosomal position effects influence the transcription of exogenously introduced transgenes. A new study identifies molecular tools that exploit these properties to fine-tune transgenic gene expression through the use of site-specific integration and the gypsy insulator element.

As in real estate, location is (almost) everything in transgenic biology. The local chromatin structure that surrounds a randomly inserted transgene considerably affects the level and spatial pattern of expression. In *Drosophila*, the traditional solution to this inherent variability has been to generate and sort through many independent insertions in order to find ones that match the experimental need, as this can be done quickly and cheaply. However, unlike in real estate, there is now a systematic solution for this problem in *Drosophila*. On page 476 of this issue, Michele Markstein and colleagues[1] use information from two unrelated lines of work—site-specific recombination and position-effect variegation—to provide an elegant solution to transgenic biology's 'real estate' problem.

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Transgenesis in *Drosophila*

Reverse genetic approaches rely heavily on inducible gene expression systems to establish the relationship between a gene and its function. The current arsenal of transgenic techniques has facilitated the study of the effects of single gene manipulations in the context of a living organism. These techniques have paved the way for new discoveries in diverse disciplines ranging from learning and memory formation to disease pathogenesis. In *Drosophila*, several widely used approaches for transgenic gene manipulation are currently available. The first type involves placing the gene of interest under the control of the heat shock 70 (Hsp70) promoter, enabling a defined window of expression[2]. Hsp70-regulated transgenes are capable of rapid and robust induction, but they lack the ability to spatially restrict expression to specific tissues. A second approach, which uses the bipartite Gal4–upstream activating sequences (UAS) system[3], is the most widely used system in flies for achieving spatially restricted gene expression (**Fig. 1**). The primary limitation of this system is its inability to temporally control gene expression, a substantial problem whenever the function of a gene is being assayed late in development, usually after earlier usage. In light of this, variations of the Gal4-UAS system have been developed to introduce temporal control, including the use of the temperature-sensitive Gal80^{ts} protein[4,5], hormone ligands such as RU486 (GeneSwitch)[6–8] or the drug-based tetON/tetOFF system[9,10]. These developments allow temporal control to be added to the spatially restricted Gal4 system.

Although elegant, these approaches all rely on the use of one or more transgenes that are inserted randomly and that are therefore susceptible to the effects of local chromatin structure, whether repressive or activating, or acting with cell type–specific constraints. This randomness necessitates that multiple inserts be assayed, and even this is often not done thoroughly. For

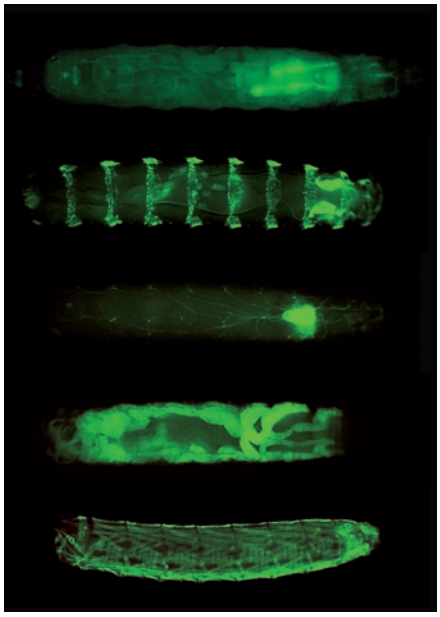

Figure 1 *Drosophila* larvae carrying Gal4 drivers that promote the expression of UAS::eGFP transgenes in specific tissues. The study by Markstein *et al.* shows that position effects complicating the use of transgenes can be quantified and overcome through the use of defined integration sites and the gypsy retrovirus insulator. Images courtesy of Michele Markstein.

example, it is common practice to use UAS-reporter transgenes (UAS-GFP or UAS-lacZ, for example) to indicate where a particular Gal4 driver line is expressed. The UAS–gene of interest transgene is then crossed with the driver, and the expression pattern is inferred to be similar, if not identical, to what is seen with the reporter transgene. Unfortunately, the difference in insertion sites between the UAS-reporter and the UAS–gene of interest transgenes can change the actual expression pattern. Rarely is the expression pattern of the gene of interest shown with immunocytochemistry to be the same as, or very similar to, the pattern of the reporter.

Toward precise regulation

Markstein *et al.*[1] adopted the phiC31 site-specific integration system[11] to circumvent these issues. Previous pioneering work used this bacteriophage-based system to produce large numbers of transgenic flies with 'attP' sites randomly inserted in various fly chromosomes[12]. In their new study, Markstein *et al.* integrated a UAS-luciferase reporter construct at 20 independent sites, and characterized expression in the absence ('basal') or presence ('induced') of a common Gal4 transgene. They also examined the tissue specificity of expression in the larval brain, muscle and fat body. Perhaps not surprisingly, each insertion showed unique tissue-type effects on basal and induced expression. Furthermore, the well-characterized gypsy insulators maximized induced but not basal expression. This latter result suggests that specific mechanistic interactions exist between the Gal4 acidic activation domain and proteins that interact with the gypsy insulator, such as suppressor of Hairy wing[13]. Whatever the details of this interaction, it apparently leads to increased expression only in the presence of the Gal4 protein and can maximize transgene expression in all tissues examined. This maximally induced level of expression was higher than that seen at any of the 20 sites in the absence of the gypsy insulators.

The availability of flies with these insertion sites means that new transgenes can be integrated into any given site with confidence that a certain pattern and level of expression will result. This will decrease the number of lines that experimenters need to make for a given transgene and can predictably lead to maximal expression, at least using Gal4. It will also facilitate structure/function studies where a series of changes are made to a transgene. All of the variants can be integrated in parallel into the same chromosomal location, and the effects of the alterations can be measured without expression differences between flies. Conversely, in an elegant proof-of-principle experiment, Markstein *et al.* generated an allelic series using the *Notch* gene. Specifically, they integrated an RNAi knockdown construct into different sites, producing a series of flies with a gradient of *Notch* expression. These flies showed a corresponding series of wing morphology phenotypes, consistent with existing information from mutant analysis.

All of this heavy lifting effectively ushers in a new generation of transgenic tools that permit precise regulation of exogenously inserted genes. In the near future, these advances can be integrated with the temporal 'switches' using the Gal80^{ts} TARGET, hormone receptor or tetracycline systems. This further refinement will provide high-precision temporal and spatial regulation of gene expression. These advances strengthen the position of *Drosophila* as a premier reverse genetic system, complementing its established place of honor in the pantheon of organisms used for forward genetics.

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