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The GAL4 System

A Versatile System for the Expression of Genes

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Summary

Over the past decade the adoption and refinement of the GAL4 system by the *Drosophila* field has resulted in a wide array of tools with which the researcher can drive transgene expression in a precise spatiotemporal pattern. The GAL4 system relies on two components: (1) GAL4, a transcriptional activator from yeast, which is expressed in a tissue-specific manner and (2) a transgene under the control of the upstream activation sequence that is bound by GAL4 (UAS_G). The two components are brought together in a simple genetic cross. In the progeny of the cross, the transgene is only transcribed in those cells or tissues expressing the GAL4 protein. Recent modifications of the GAL4 system have improved the control of both the initiation and the spatial restriction of transgene expression. Here we describe the GAL4 system highlighting the properties that make it a powerful tool for the analysis of gene function in *Drosophila* and higher organisms.

Key Words: Conditional gene expression; *Drosophila*; transcriptional activation; transgenesis; UAS; GAL4.

1. Introduction

Ectopic expression has proved an excellent technique for analyzing gene function in *Drosophila* and other model organisms. By altering the gene expression profile of a given cell, one can: (1) induce a cell fate change ***(1)***; (2) induce altered cell fates in neighboring cells ***(2)***; and (3) alter the cell's physiology ***(3)***. Therefore, ectopic expression of a gene can test whether it is sufficient for cell identity and whether its mode of action is autonomous or nonautonomous. Furthermore, ectopic expression is useful in the determination of signaling pathways and the response of a given cell or tissue to these pathways ***(4,5)***. As it has been estimated that up to 60% of *Drosophila* genes will have no loss-of-function phenotype ***(6)***, ectopic gene expression will be important in obtaining functional data on a large

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number of genes. In addition, with the advent of RNA interference (RNAi) technology it is now possible to use ectopic expression to perform gene knockdown experiments to determine if a gene is necessary for a given process ***(7–9)***.

The GAL4 system is a genetic tool allowing the ectopic expression of any given sequence, be it protein coding or a noncoding RNA (e.g., RNAi) ***(10)***. A key strength of the GAL4 system is its remarkable flexibility, with modifications and improvements of the original system giving the *Drosophila* geneticist a large selection of tools with which to drive expression in a restricted spatiotemporal pattern. This flexibility has facilitated the development of many ingenious procedures including labeling mutant cells within chimeric tissue, performing tissue-specific gain-of-function or loss-of-function screens, analyzing the developmental and/or functional role of defined cell populations, inhibition of neuronal function, and genetic rescue experiments as well as functional characterization of a given gene.

The GAL4 system was built on the characterization of transcriptional regulation in yeast. GAL4 is an archetypal eukaryotic transcription factor isolated as an activator of the genes responsible for galactose metabolism in *Saccharomyces cerevisiae* ***(11)***. Analysis of the GAL4 protein revealed that it binds DNA as a dimer through a Zn(2)-Cys(6) zinc finger and has two transactivation domains ***(12,13)***. Furthermore, the activity of GAL4 is repressed by a physical interaction with the GAL80 protein, which is alleviated when galactose is the only carbon source ***(14,15)***. The target sequence of GAL4 was defined as a 17-mer, four copies of which are found in the upstream activation sequence (UAS_G, hereafter UAS) of the galactose metabolism genes, *GAL10* and *GAL1* ***(16–18)***. The high level of conservation in the eukaryotic transcriptional machinery means that GAL4 can activate transcription in other species, as distantly related as humans and plants ***(18–20)***. Crucially, GAL4 is able to regulate transcription from the UAS in *Drosophila* ***(21)*** enabling the GAL4 system to be developed.

2. Key Features of the GAL4 System

The GAL4 system is a bipartite system in which one transgenic line, the driver, expresses GAL4 in a known temporal or spatial pattern and a second transgenic line, the responder, contains a UAS-dependent transgene (**Fig. 1A**) ***(10)***. When using the GAL4 system the most important requirement is an appropriate expression pattern of GAL4. From the outset the GAL4 system was designed to generate a range of driver lines in which a diverse array of regulatory elements controlled GAL4 expression ***(10)***. By adapting the enhancer-detection technique ***(22,23)*** a vector, pGawB ***(10)***, was constructed in which GAL4 is under the control of a weak P-transposase promoter. GAL4 expression depends on the regulatory elements surrounding the integration site of the vector. Thus, the spatial and temporal control of GAL4 expression is based on endogenous

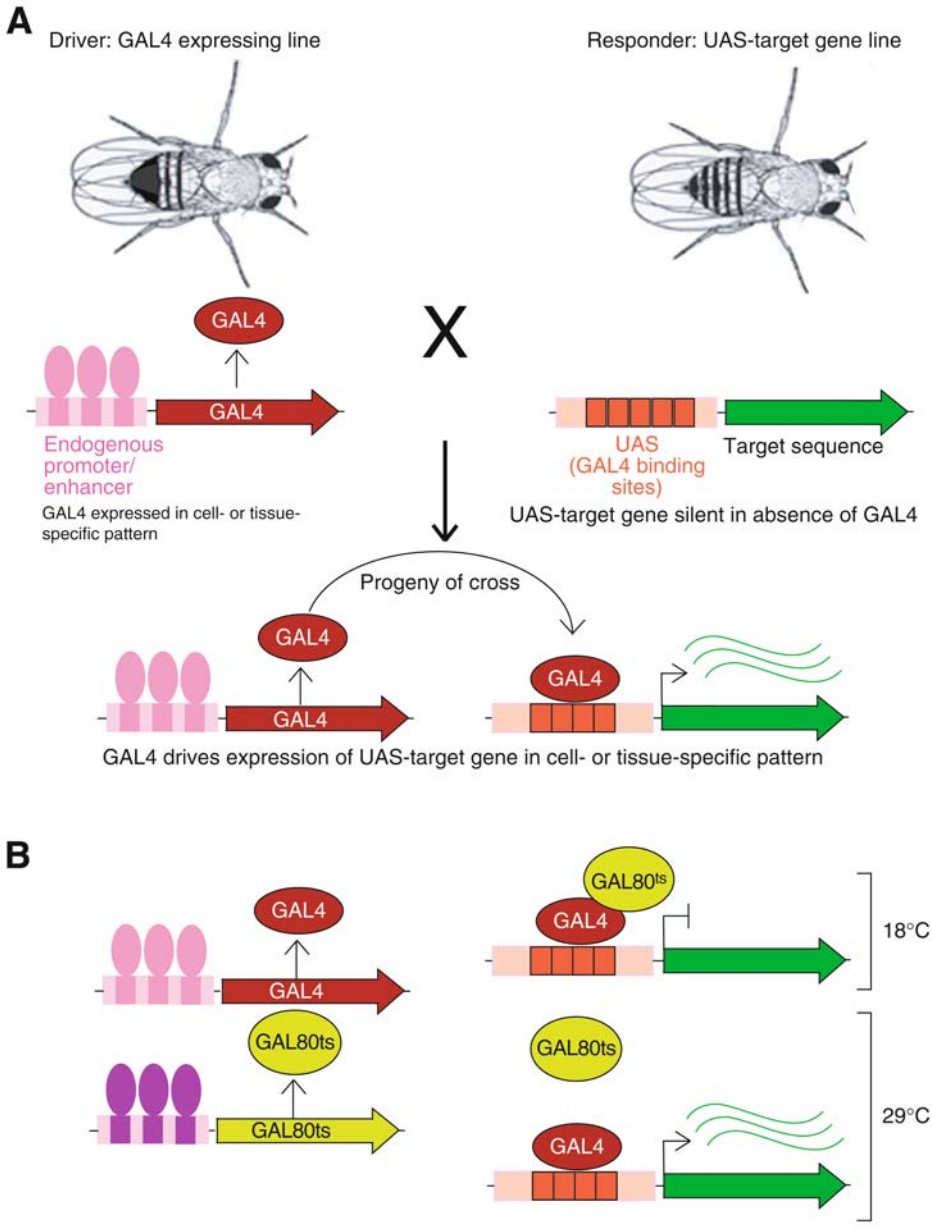

Fig. 1. *(Continued)*

enhancers. A large number of lines can be generated and screened for GAL4-expression pattern to obtain drivers in the tissue of interest. Almost 7000 GAL4 drivers are available and documented online at the GAL4 Enhancer Trap Data Base (*see* **ref. *24***).

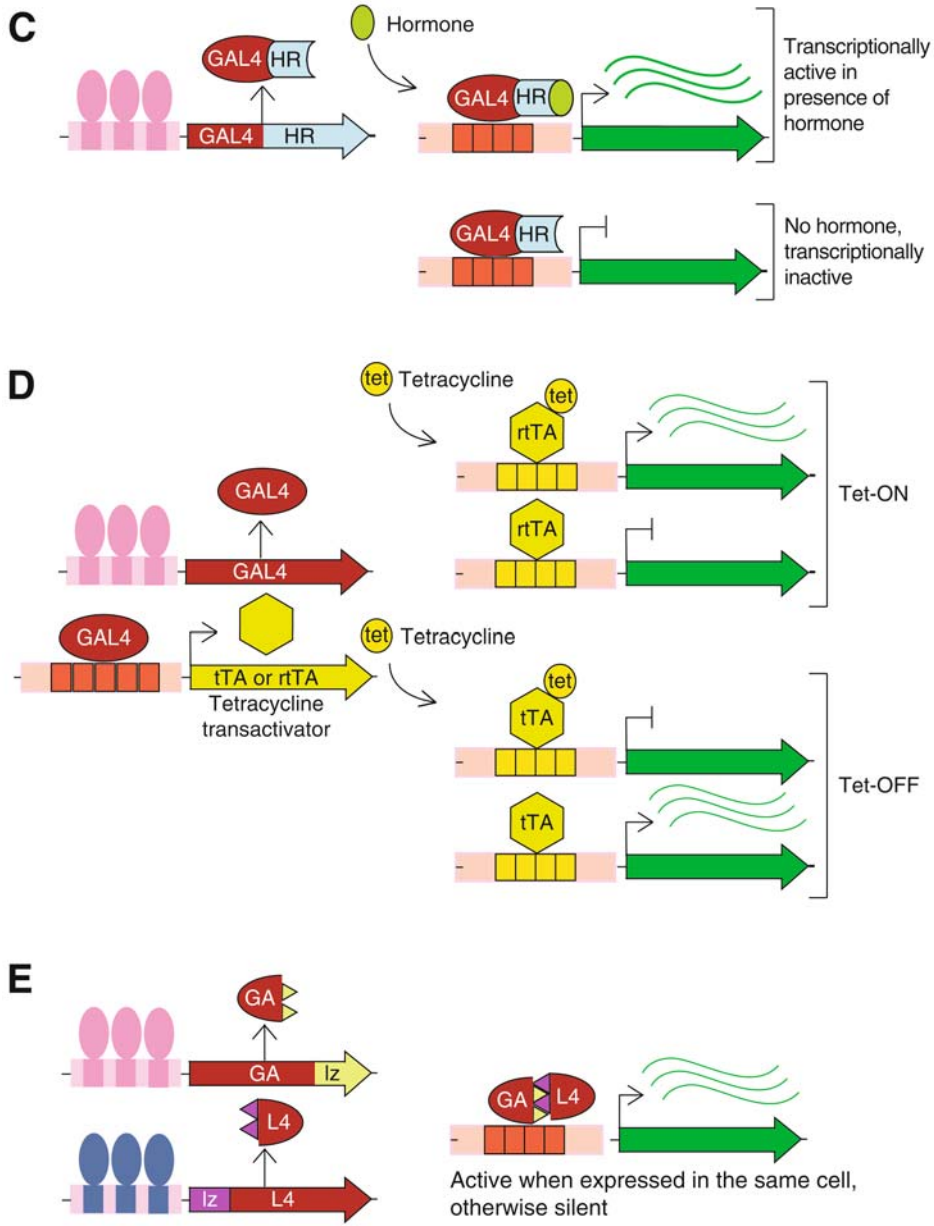

Fig. 1. *(Continued)*

Separation of the GAL4 driver and the UAS responder offers several key advantages. First, a single UAS-dependent transgene can be analyzed in multiple tissues and/or time-points through the use of different drivers. Second, as the UAS construct is effectively silent in the absence of GAL4, transgenics encoding

toxic proteins such as ricin, or apoptotic proteins such as reaper or head involution defective (hid), can be generated ***(25,26)***. Furthermore, rescue experiments can be performed by recapitulating endogenous gene expression with the appropriate drivers in a mutant background (e.g., **ref.** ***27***).

Temporal control, without cell or tissue specificity, of GAL4 activity can be achieved by driving GAL4 expression from a heat-shock promoter ***(28)***. Although the heat-shock procedure does offer good temporal control of the onset of expression a disadvantage is ubiquitous transgene expression. An important advance of the GAL4 system has been the development of several methods offering improved temporal control of the GAL4 system.

3. Temporal Control of the GAL4 System

3.1. Antagonizing GAL4 With the GAL80 Repressor

One approach to regulate temporal expression of a UAS-responder is to make use of the yeast protein GAL80, which binds the transactivation domain of GAL4 and prevents GAL4 from activating transcription in yeast ***(14,15)***. GAL80 can also repress GAL4 in *Drosophila* ***(29–31)*****,** and when expressed ubiquitously under the control of the *tubulin 1*α promoter, represses GAL4 activity in all tissues (**Fig. 1B**) ***(29)***. This feature paved the way for Lee and Luo to develop the mosaic analysis with a repressible cell marker (MARCM) technique to generate marked mutant clones (*see* **refs.** ***29*** and ***30***).

Recently, a series of balancer chromosomes bearing the *tub-GAL80* transgene have been generated ***(32)***. Vef et al. present a strategy in which *tub-GAL80* containing balancer chromosomes can be used in mutagenesis screens to rapidly identify homozygous mutants based on expression of green fluorescent protein (GFP). Briefly, lines containing a chromosome bearing a GAL4 driver and *UAS-GFP*

Fig. 1. *(Opposite page)* Schematic representation of the GAL4-based systems for transgene expression. The original scheme for the GAL4 system is shown in **(A)** GAL4 activity can be antagonized by GAL80 and forms the basis of the TARGET method of transgene regulation **(B)**. Use of a temperature sensitive GAL80 ($GAL80^{ts}$) allows the greater temporal control over the onset of expression. Panel **C** illustrates the hormone induction of transgene expression using a GAL4-hormone receptor (HR) fusion, hormone receptors used include estrogen and progesterone receptor. The Tet-ON and Tet-Off systems have been combined with GAL4 **(D)** affording inducible control of transgene expression by the feeding or withdrawal of tetracycline. The Split-GAL4 system is shown in panel **E**, the GAL4 DNA-binding domain is fused to a heterodimerizing leucine zipper (lz) motif as is the activation domain. The different fusion proteins are expressed in overlapping expression patterns using endogenous promoters/enhancers. When the fusion proteins are expressed in the same cell the leucine zippers direct heterodimerization, resulting in the formation of a functional activator. Either the GAD or a heterologous activation domain (e.g., VP16) can be used. B–E show only the progeny of the cross.

responder (i.e., GFP expressing) are mutagenized, and subsequently, balanced over the *tub-GAL80* balancer. Homozygous mutants are easily distinguished by GFP expression (from the GAL4/*UAS-GFP* on the mutagenized chromosome), which is lacking in heterozygotes because of GAL80 repression of GAL4 activity.

Perhaps the most promising and flexible development of temporal control of the GAL4 system is the development of the temporal and regional gene expression targeting (TARGET) technique ***(33)***. In the TARGET technique a temperature-sensitive version of the GAL80 protein ($GAL80^{ts}$) ***(34)*** is expressed ubiquitously under the control of the *tubulin 1α* promoter. GAL80 repression of GAL4 is alleviated by a simple temperature shift, giving the researcher exact control of the onset of expression. Crucially, the TARGET system is fully compatible with the vast array of GAL4 lines already established. The strength of the TARGET system is illustrated by the dissection of *rutabaga*, a type I adenylyl cyclase, function in short-term memory in *Drosophila* ***(33)***. Using TARGET to activate UAS-*rutabaga* expression in the mushroom bodies of adult flies rescued the memory defect of *rutabaga* mutants. Thus, the olfactory memory phenotype of *rutabaga* mutants is not owing to abnormal mushroom body development but abnormal neural physiology in adult neurons lacking the *rutabaga*-encoded type I adenylyl cyclase.

3.2. Inducible GAL4

Another approach to regulate temporal expression are hormone inducible variants of GAL4 ***(35–37)***. Two GAL4-hormone receptor chimeras are available: GAL4-estrogen receptor ***(35)*** and a second called GeneSwitch, which is a fusion of GAL4-progesterone receptor, and the activation domain of p65 ***(36,37)***. Transcriptional activation in both systems requires the addition of the appropriate ligand as both are transcriptionally silent until bound by the ligand (**Fig. 1C**). The onset of expression is controlled by feeding the fly or larva the ligand at an appropriate time, thereby circumventing possible detrimental effects of early transgene expression. Expression levels in the GeneSwitch system are ligand dose-dependant ***(36–38)***. Furthermore, the system is reversible by removal of the fly or larva from feed containing the ligand. However, reversal is restricted by the slow off-rate kinetics of GeneSwitch ***(36)*** and the perdurance of the transgene product.

The major limitation of this approach is that drug treatment requires feeding, so the embryonic and pupal stages of the lifecycle are not amenable to study. In addition, new driver lines must be generated expressing either GeneSwitch or GAL4-estrogen receptor. Nevertheless, elegant experiments can be performed using these drug inducible GAL4 variants. For example, in a recent demonstration, signaling from the heterotrimeric G(o) protein is required for associative learning ***(39)***; GeneSwitch, expressed from a mushroom body-specific promoter,

was used to drive expression of an inhibitor of G(o) signaling, *pertussis toxin* (PTX) (UAS-PTX), in the mushroom body. PTX expression was induced by feeding adult flies the GeneSwitch ligand, RU486, ensuring that the phenotypes were not the result of developmental defects. In this way, inhibition of G(o) signaling in the mushroom body was shown to impair olfactory associative learning and memory. In another study ***(40)***, GeneSwitch was used to express dFOXO, the downstream transcription factor of the insulin/insulin-like growth factor signaling pathway, in the adult fat body. Adult fat body-specific activation of the transcriptional response to insulin signaling resulted in an increased life span.

3.3. FLP-Out GAL4

One approach to refining temporal regulation of the GAL4 system is to combine it with the FLP-out technique ***(41–44)***. For example, a terminator cassette flanked by FLP recognition target (FRT) sites can be placed between the UAS promoter and the gene to be expressed, rendering the transgene silent ***(42)***. To activate the transgene requires the expression of the FLP recombinase to remove the terminator cassette. Use of a heat shock inducible *FLP recombinase (hs-FLP)* affords temporal control to the onset of transgene expression. A similar result can be achieved by placing a FRT-flanked terminator cassette in front of the GAL4-coding sequences ***(41,43)***. In this case, *GAL4* expression is reliant on the removal of the terminator cassette by FLP recombinase, which can be conveniently supplied using *hs-FLP* to control the timing of expression. In addition to the commonly used *hs-FLP* it is possible to use other characterized promoters to drive FLP expression in a spatiotemporal fashion (e.g., the eye-specific driver *ey-FLP* ***[45]***).

Recent work combined the FLP-out technique ***(44)*** with GeneSwitch to investigate the role of the decapentaplegic (DPP) morphogen gradient in controlling growth in the wing imaginal disc ***(46)***. A FRT-flanked terminator cassette was placed between the actin promoter and the GeneSwitch-coding sequence. The cassette was removed by induction of *hs-FLP* during larval stages, resulting in a mosaic animal in which GeneSwitch expression was clonally restricted. The GeneSwitch ligand RU486 was fed to transgenic larvae promoting the activation of the UAS transgene. This methodology was used to manipulate levels of DPP signaling within the clones through the expression of a constitutively active variant of thick veins (the DPP receptor). In imaginal wing discs cellular proliferation was stimulated both within the clones (i.e., autonomous) and in cells adjacent to the clone boundary (i.e., nonautonomous) indicating that cells sense differences in DPP-signaling activity. Therefore, the uniform growth of the entire wing disc in response to DPP signaling, and not just of those cells close to the DPP source, can be explained by the noncell autonomous stimulation of cell proliferation resulting from cells reacting to different levels of DPP signaling in adjacent cells ***(46)***.

3.4. Tetracycline-Transactivator System

Transcriptional activity of the tetracycline-transactivator (tTA), a fusion between the *Escherichia coli* tetracycline repressor (tetR or the reverse tetR for Tet-On) and the strong transcriptional activation domain of the herpes simplex virus VP16, is regulated by tetracycline or its derivatives (e.g., doxycycline). The convenience of inducible expression has led to the widespread use of tTAs in transgenic mouse models and mammalian tissue culture ***(47,48)***. Two versions of the tetracycline systems exist: Tet-On, in which the addition of the drug results in an active reverse-tetR (rtTA) causing transgene activation from the tet operator and Tet-Off, in which addition of the drug inactivates tTA, and in turn, expression from the tet operator is switched off. The introduction of the tetracycline inducible systems, in particular the Tet-On method, to *Drosophila* were initially hindered by a few technical problems, such as the level of transgene induction and adverse effects of high doses of tetracycline ***(49–52)***. A modified version of rtTA, rtTAs-M2-altTA, has largely rectified these problems ***(53,54)***. However, this system is not directly compatible with existing UAS transgenes, such as the existing collection of enhancer promoter (EP) lines (*see* **Subheading 5.**).

To take advantage of the number of established tissue-specific GAL4 lines, both the Tet-On and Tet-Off expression systems have been linked to the GAL4-UAS system (*see* **Fig. 1D**) ***(52,53)***. To do this the tetracycline transactivators were placed under the control of the UAS. Therefore, expression of rTA and rtTAs-M2-altTA can be regulated by crossing the *UAS-rTA* (or *UAS-rtTAs-M2-altTA*) transgenics to a given GAL4 driver ***(52,53)***. This protocol has been used to investigate the role of the calcium/calmodulin protein kinase II (CaMK II) in regulating mating behavior ***(55)***. Expression of a constitutively active CaMKII (T287D CaMKII) in adult neurons was achieved by combining a GAL4 driver, in which GAL4 is expressed in the mushroom body and antennal lobes, regulating *UAS-rTA*, and inhibition of rTA by growth on doxycycline until adulthood. Indeed, this experiment relies on silencing by doxycycline, as expression of T287D CaMKII during development is lethal ***(55)***. Removal of doxycycline permits T287D CaMKII expression. In behavioral assays female flies expressing T287D CaMKII displayed enhanced courtship plasticity during training implying a role for CaMKII in behavioral plasticity.

4. GAL4-Based Methodologies for Improved Spatial Restriction of Gene Expression

4.1. Dual Binary System

A second binary system, which functions in an analogous manner to the GAL4 system, is based on the bacterial lexA protein ***(56,57)***. In a recent report, Lee and Lai have combined the GAL4 and lexA systems to facilitate

more precise cell labeling by MARCM ***(58)***. The lexA DNA-binding domain is fused either to the GAL4 activation domain (lexA::GAD) or the VP16 activation domain (lexA::VP16) resulting in GAL80-sensitive and -insensitive proteins, respectively. All cells can be marked with lexA::VP16 whereas the GAL80 sensitive lexA::GAD can be kept silent until the generation of mutant clones using the MARCM protocol. Using this approach to study the cell lineage in the optic lobe, Lai and Lee ***(58)*** identified a novel neuroglioblast cell type. As use of the lexA system becomes more widespread, modifications of the system are sure to follow. Many of these modifications are likely to be derived from those already in use with the GAL4 system.

4.2. Split GAL4

A new method, "Split GAL4," takes advantage of the modular nature of the GAL4 transcription factor ***(59)***. In this technique the separate DNA binding domain (DBD) and activation domain (AD) of GAL4 are fused to a heterodimerizing leucine zipper motif and each fusion protein is expressed separately. Only when they are present in the same cell can the leucine zippers direct heterodimerization, resulting in the formation of a functional activator (**Fig. 1E**). The fusion proteins, ZIP::GAL4DBD and GAL4AD::ZIP (or a second activation domain VP16AD::ZIP), are transcriptionally inactive when expressed panneuronally using the *elaV* promoter. The inactive constructs are referred to as "hemi-drivers." Transactivation requires the reconstitution of GAL4 by the presence of both ZIP::GAL4DBD and GAL4AD::ZIP, which will only occur in the domains of overlapping expression of the hemi-drivers. Coexpression, through the *elaV* promoter, of the DBD and AD fusion proteins results in nervous system-wide expression of a *UAS-GFP* reporter.

The authors demonstrate that the Split GAL4 system can target gene expression to a restricted cell population at the intersection of the expression patterns of the hemi-drivers ***(59)***. For example, to identify cholinergic neurons within the *Crustacean Cardioactive Peptide* (*CCAP*) neuronal population the GAL4AD::ZIP was expressed in CCAP neurons whereas the *choline acetyltransferase* promoter drove ZIP::GAL4DBD in all cholinergic neurons. The split GAL4 system was able to label a subset of cholinergic neurons within the larger CCAP neuronal population.

5. Genome Wide Screening With GAL4

The GAL4 system has also been used in large-scale gain-of-function genetic screens. A modular misexpression screen has been designed combining P-element mutagenesis and the GAL4 system ***(60)***. The P-elements used contain a GAL4-regulated promoter, consisting of GAL4 DNA-binding sites upstream of a basal promoter, arranged to drive transcription of flanking

genomic sequences after integration ***(60–62)***. Transgenic lines generated by integration of these P-elements are referred to as EP lines and, when GAL4 is present, these P-elements will direct expression of the gene, which lies next to the integration site. Many thousands of EP lines are now available to the research community, from both public and private consortia ***(61,63,64)***. By crossing a driver, which is expressed in the tissue of interest to these libraries one can screen for gain-of-function phenotypes. Such screens have been used to identify genes involved in many processes, including eye morphology ***(65)***, vein formation in the wing ***(66)***, muscle pattern formation ***(64)***, blood cell activation ***(67)***, and axon guidance and synaptogenesis ***(68)***. Furthermore, screens can be performed for modifiers of signaling pathways. For example, Leptin and colleagues screened for modifiers of the fibroblast growth factor (FGF)-signaling cascade by looking for rescue or enhancement of the rough eye phenotype resulting from coexpression of the FGF receptor Breathless and downstream-of-FGF receptor, identifying the small GTPase sar-1 and the cell surface receptor robo-2 as components of the FGF pathway ***(5)***.

Mutations that cause early lethality hinder analysis of the mutated gene's function during later development and adult life. Duffy and colleagues ***(69)*** have developed a GAL4-based technique, termed directed mosaics, which permits the study of recessive lethal mutations by generating mosaic flies in which only select cells or tissues are homozygous for the mutation whereas the rest of the animal is wild-type. This method uses recombination between FRT sites that have been engineering into all five major chromosomal arms in *Drosophila.* FLP is supplied in a tissue-specific manner using characterized GAL4 drivers and *UAS-FLP.* FLP catalyzes mitotic recombination between chromosome arms carrying a particular mutation, resulting in wild-type and homozygous mutant daughter cells. In this manner approx 95% of the genome can be tested in mosaics ***(69)***. With certain modifications, the directed mosaics method can be used to generate an entire tissue made solely of mutant cells ***(70)***. An FRT-marked chromosome is engineered to carry a tissue-specific transgene expressing a dominant cell death gene (e.g., *hid* or *reaper*) ensuring that all daughter cells in the tissue of interest inheriting this chromosome will undergo apoptosis. As a result, the tissue of interest will be entirely made up of cells in which recombination between FRT sites has resulted in homozygous inheritance of the lethal mutation of interest ***(70)*** but not hid or reaper. This has been achieved in the eye using the *ey-GAL4* driver, *UAS-FLP*(EGUF), and a glass multimer reporter (GMR)-hid transgene as a tissue-specific apoptotic factor, and is referred to as the EGUF/hid method ***(70)***. Mutant screens, or examination of existing mutants, can now be performed scoring any aspect of eye biology (e.g., morphology, neurophysiology, and light response) in an otherwise wild-type animal ***(70–72)***.

6. Repressing Genes Using GAL4

Although most studies use GAL4 solely as an activator GAL4 can also be used to switch off a gene of interest. GAL4 can be turned into a repressor by inserting the GAL4-DBD domain into the suppressor of hairy wing isolator domain, which has enhancer-blocking activity. This activity is presumably mediated by, as yet undefined, protein interactions ***(73)***. One can use this chimeric protein in loss-of-function studies in an analogous manner to the gain-of-function studies using the EP lines. In this case the chimera binds to the UAS element that is integrated in the genome, and the suppressor of hairy wing isolator causes a localized inactivation of transcription. The technique offers the promise of performing conditional loss of function screens that switch off gene expression only in the tissue or process of interest.

7. GAL4 Methods are Universally Applicable to Higher Eukaryotes

Early reports demonstrated that GAL4 can activate transcription from the UAS in a range of species including human tissue culture cells and plants ***(18,20)***. The success of the GAL4 system in *Drosophila* has encouraged researchers working on other model organisms, including *Arabidopsis* ***(74,75)***, zebrafish ***(76)***, *Xenopus* ***(77)***, and mouse ***(78,79)***, to adopt the GAL4 system. In mice the GAL4 system was used to analyze both the sonic hedgehog (shh) and BMP-signaling pathways in neural tube development ***(78,79)***. GAL4 was expressed in the roof plate of the neural tube by placing the GAL4 sequence under the control of *Wnt-1* regulatory sequences ***(79)***. Using the Wnt1-GAL4 line to drive expression of *shh* revealed that prolonged exposure to shh results in an increased proliferative rate of neural precursors. Furthermore, at later stages of development differentiation was blocked, demonstrating proliferation and differentiation are separately regulated by shh signaling in the developing spinal cord. Given the success of the GAL4 system, in its various guises in *Drosophila*, it is likely that GAL4-based technologies will continue to be adapted to work in other species.

8. What Does the Future Hold for GAL4?

The GAL4 system is now well-established in the armory of the *Drosophila* geneticist. Modification of the original GAL4 system has led to a more precise level of control of transgene expression in both space and time. To increase the resolution of the GAL4 system it is likely that researchers will combine the GAL4-based reagents listed to manipulate transgene expression in the tissue or cells of interest. For example, the Split GAL4 system can be combined with $GAL80^{ts}$ and MARCM to drive expression in a subset of cells within a given organ. One can ablate given cells within a tissue by driving expression of the proapoptotic protein reaper in clones within an already restricted domain and

examine the biological outcome. In the context of neuronal networks, a similar experiment using tetanus toxin to block activity in selected neurons may reveal precisely which neurons govern a particular behavior.

Other systems of promise are the hormone inducible systems, which use a transactivation domain requiring the presence of a hormone to be active. Obviously, the time delay in switching the transgene off might be a complicating factor for some experiments as a transcriptionally active protein remains after the withdrawal of the drug. This difficulty may be overcome by the introduction of a ligand-sensitive destabilizing domain, which in the absence of ligand results in rapid protein degradation ***(80)***. This approach has been shown to function in a range of mammalian cell lines ***(80)***. However, although such a feature would inactivate GAL4 time would still be required for the degradation of the existing transgenic products. An alternative approach based on fusion to a temperature sensitive intein domain has been demonstrated to function in GAL80 in *Drosophila* and GAL4 in yeast ***(81)***. At permissive temperatures the intein domain is spliced out and the resultant protein is active, whereas at nonpermissive temperatures the intein domain remains resulting in a nonfunctional protein. Incorporation of an intein domain into GAL4 would allow GAL4 activity to be regulated merely by moving the flies to different temperatures, which offers considerable advantages over the presence of a second protein or reliance on the administration of ligands to regulate expression. In particular, the embryonic and pupal life stages are amenable to temperature shift experiments but less easily to drug treatments.

The success of the GAL4 system in addressing basic biological questions has led to the adoption of this technique in more applied situations. In particular, the development of GAL4-based transgenesis in pest insects will help in the identification of new insecticide compounds ***(82)***. More importantly, for diseases where insects are the vectors it is possible that the GAL4 system or one of its variants may provide a mechanism to prevent the transmission of disease or control the population of the insect vector. Finally, *Drosophila* is now used as a screening organism by the pharmaceutical industry ***(83–85)***. Given the flexibility and robust nature of the GAL4 system it is likely that the GAL4 system will be a key methodology for future *Drosophila*-based drug discovery programs.

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References

1. Davis, R. L., Weintraub, H., and Lassar, A. B. (1987) Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell* **51,** 987–1000.
2. Bouwmeester, T., Kim, S., Sasai, Y., Lu, B., and De Robertis, E. M. (1996) Cerberus is a head-inducing secreted factor expressed in the anterior endoderm of Spemann's organizer. *Nature* **382,** 595–601.
3. Southall, T. D., Terhzaz, S., Cabrero, P., et al. (2006) Novel subcellular locations and functions for secretory pathway Ca^{2+}/Mn^{2+}-ATPases. *Physiol. Genomics* **26,** 35–45.
4. Huang, A. M. and Rubin, G. M. (2000) A misexpression screen identifies genes that can modulate RAS1 pathway signaling in *Drosophila melanogaster*. *Genetics* **156,** 1219–1230.
5. Zhu, M. Y., Wilson, R., and Leptin, M. (2005) A screen for genes that influence fibroblast growth factor signal transduction in *Drosophila*. *Genetics* **170,** 767–777.
6. Miklos, G. L. and Rubin, G. M. (1996) The role of the genome project in determining gene function: insights from model organisms. *Cell* **86,** 521–529.
7. Giordano, E., Rendina, R., Peluso, I., and Furia, M. (2002) RNAi triggered by symmetrically transcribed transgenes in *Drosophila melanogaster*. *Genetics* **160,** 637–648.
8. Piccin, A., Salameh, A., Benna, C., et al. (2001) Efficient and heritable functional knock-out of an adult phenotype in *Drosophila* using a GAL4-driven hairpin RNA incorporating a heterologous spacer. *Nucleic Acids Res.* **29,** E55–E55.
9. Van Roessel, P., Hayward, N. M., Barros, C. S., and Brand, A. H. (2002) Two-color GFP imaging demonstrates cell-autonomy of GAL4-driven RNA interference in *Drosophila*. *Genesis* **34,** 170–173.
10. Brand, A. H. and Perrimon, N. (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118,** 401–415.
11. Hashimoto, H., Kikuchi, Y., Nogi, Y., and Fukasawa, T. (1983) Regulation of expression of the galactose gene cluster in *Saccharomyces cerevisiae*. Isolation and characterization of the regulatory gene GAL4. *Mol. Gen. Genet.* **191,** 31–38.
12. Ma, J. and Ptashne, M. (1987) Deletion analysis of GAL4 defines two transcriptional activating segments. *Cell* **48,** 847–853.
13. Ptashne, M. (1988) How eukaryotic transcriptional activators work. *Nature* **335,** 683–689.
14. Lue, N. F., Chasman, D. I., Buchman, A. R., and Kornberg, R. D. (1987) Interaction of GAL4 and GAL80 gene regulatory proteins in vitro. *Mol. Cell Biol.* **7,** 3446–3451.
15. Wu, Y., Reece, R. J., and Ptashne, M. (1996) Quantitation of putative activator-target affinities predicts transcriptional activating potentials. *EMBO J.* **15,** 3951–3963.
16. Bram, R. J., Lue, N. F., and Kornberg, R. D. (1986) A GAL family of upstream activating sequences in yeast: roles in both induction and repression of transcription. *EMBO J.* **5,** 603–608.
17. Giniger, E., Varnum, S. M., and Ptashne, M. (1985) Specific DNA binding of GAL4, a positive regulatory protein of yeast. *Cell* **40,** 767–774.

18. Webster, N., Jin, J. R., Green, S., Hollis, M., and Chambon, P. (1988) The yeast UASG is a transcriptional enhancer in human HeLa cells in the presence of the GAL4 trans-activator. *Cell* **52,** 169–178.
19. Kakidani, H. and Ptashne, M. (1988) GAL4 activates gene expression in mammalian cells. *Cell* **52,** 161–167.
20. Ma, J., Przibilla, E., Hu, J., Bogorad, L., and Ptashne, M. (1988) Yeast activators stimulate plant gene expression. *Nature* **334,** 631–633.
21. Fischer, J. A., Giniger, E., Maniatis, T., and Ptashne, M. (1988) GAL4 activates transcription in *Drosophila*. *Nature* **332,** 853–856.
22. Bellen, H. J., O'Kane, C. J., Wilson, C., Grossniklaus, U., Pearson, R. K., and Gehring, W. J. (1989) P-element-mediated enhancer detection: a versatile method to study development in *Drosophila*. *Genes Dev.* **3,** 1288–1300.
23. O'Kane, C. J. and Gehring, W. J. (1987) Detection in situ of genomic regulatory elements in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **84,** 9123–9127.
24. Hayashi, S., Ito, K., Sado, Y., et al. (2002) GETDB, a database compiling expression patterns and molecular locations of a collection of Gal4 enhancer traps. *Genesis* **34,** 58–61.
25. Hidalgo, A. and Brand, A. H. (1997) Targeted neuronal ablation: the role of pioneer neurons in guidance and fasciculation in the CNS of *Drosophila*. *Development* **124,** 3253–3262.
26. Zhou, L., Schnitzler, A., Agapite, J., Schwartz, L. M., Steller, H., and Nambu, J. R. (1997) Cooperative functions of the reaper and head involution defective genes in the programmed cell death of *Drosophila* central nervous system midline cells. *Proc. Natl. Acad. Sci. USA* **94,** 5131–5136.
27. Martin-Bermudo, M. D., Dunin-Borkowski, O. M., and Brown, N. H. (1997) Specificity of PS integrin function during embryogenesis resides in the alpha subunit extracellular domain. *EMBO J.* **16,** 4184–4193.
28. Brand, A. H., Manoukian, A. S., and Perrimon, N. (1994) Ectopic Expression in *Drosophila*, in Drosophila melanogaster: *Practical Uses in Cell and Molecular Biology,* (Goldstein, L. S. B. and Fyrberg, E. A., eds.), Academic Press, San Diego, pp. 635–654.
29. Lee, T. and Luo, L. (1999) Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. *Neuron* **22,** 451–461.
30. Lee, T. and Luo, L. (2001) Mosaic analysis with a repressible cell marker (MARCM) for *Drosophila* neural development. *Trends Neurosci.* **24,** 251–254.
31. Suster, M. L., Seugnet, L., Bate, M., and Sokolowski, M. B. (2004) Refining GAL4-driven transgene expression in *Drosophila* with a GAL80 enhancer-trap. *Genesis* **39,** 240–245.
32. Vef, O., Cleppien, D., Loffler, T., Altenhein, B., and Technau, G. M. (2006) A new strategy for efficient in vivo screening of mutagenized *Drosophila* embryos. *Dev. Genes Evol.* **216,** 105–108.
33. McGuire, S. E., Le, P. T., Osborn, A. J., Matsumoto, K., and Davis, R. L. (2003) Spatiotemporal rescue of memory dysfunction in *Drosophila*. *Science* **302,** 1765–1768.

34. Matsumoto, K., Toh-e, A., and Oshima, Y. (1978) Genetic control of galactokinase synthesis in *Saccharomyces cerevisiae*: evidence for constitutive expression of the positive regulatory gene gal4. *J. Bacteriol.* **134,** 446–457.
35. Han, D. D., Stein, D., and Stevens, L. M. (2000) Investigating the function of follicular subpopulations during *Drosophila* oogenesis through hormone-dependent enhancer-targeted cell ablation. *Development* **127,** 573–583.
36. Osterwalder, T., Yoon, K. S., White, B. H., and Keshishian, H. (2001) A conditional tissue-specific transgene expression system using inducible GAL4. *Proc. Natl. Acad. Sci. USA* **98,** 12,596–12,601.
37. Roman, G., Endo, K., Zong, L., and Davis, R. L. (2001) P[Switch], a system for spatial and temporal control of gene expression in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **98,** 12,602–12,607.
38. Mao, Z., Roman, G., Zong, L., and Davis, R. L. (2004) Pharmacogenetic rescue in time and space of the rutabaga memory impairment by using Gene-Switch. *Proc. Natl. Acad. Sci. USA* **101,** 198–203.
39. Ferris, J., Ge, H., Liu, L., and Roman, G. (2006) G(o) signaling is required for *Drosophila* associative learning. *Nat. Neurosci.* **9,** 1036–1040.
40. Giannakou, M. E., Goss, M., Junger, M. A., Hafen, E., Leevers, S. J., and Partridge, L. (2004) Long-lived *Drosophila* with overexpressed dFOXO in adult fat body. *Science* **305,** 361.
41. Ito, K., Awano, W., Suzuki, K., Hiromi, Y., and Yamamoto, D. (1997) The *Drosophila* mushroom body is a quadruple structure of clonal units each of which contains a virtually identical set of neurones and glial cells. *Development* **124,** 761–771.
42. Nellen, D., Burke, R., Struhl, G., and Basler, K. (1996) Direct and long-range action of a DPP morphogen gradient. *Cell* **85,** 357–368.
43. Pignoni, F. and Zipursky, S. L. (1997) Induction of *Drosophila* eye development by decapentaplegic. *Development* **124,** 271–278.
44. Struhl, G. and Basler, K. (1993) Organizing activity of wingless protein in *Drosophila*. *Cell* **72,** 527–540.
45. Newsome, T. P., Asling, B., and Dickson, B. J. (2000) Analysis of *Drosophila* photoreceptor axon guidance in eye-specific mosaics. *Development* **127,** 851–860.
46. Rogulja, D. and Irvine, K. D. (2005) Regulation of cell proliferation by a morphogen gradient. *Cell* **123,** 449–461.
47. Gossen, M., Freundlieb, S., Bender, G., Muller, G., Hillen, W., and Bujard, H. (1995) Transcriptional activation by tetracyclines in mammalian cells. *Science* **268,** 1766–1769.
48. Lewandoski, M. (2001) Conditional control of gene expression in the mouse. *Nat. Rev. Genet.* **2,** 743–755.
49. Bello, B., Resendez-Perez, D., and Gehring, W. J. (1998) Spatial and temporal targeting of gene expression in *Drosophila* by means of a tetracycline-dependent transactivator system. *Development* **125,** 2193–2202.
50. Bieschke, E. T., Wheeler, J. C., and Tower, J. (1998) Doxycycline-induced transgene expression during *Drosophila* development and aging. *Mol. Gen. Genet.* **258,** 571–579.

51. McGuire, S. E., Roman, G., and Davis, R. L. (2004) Gene expression systems in *Drosophila*: a synthesis of time and space. *Trends Genet.* **20,** 384–391.
52. Stebbins, M. J. and Yin, J. C. (2001) Adaptable doxycycline-regulated gene expression systems for *Drosophila*. *Gene* **270,** 103–111.
53. Stebbins, M. J., Urlinger, S., Byrne, G., Bello, B., Hillen, W., and Yin, J. C. (2001) Tetracycline-inducible systems for *Drosophila*. *Proc. Natl. Acad. Sci. USA* **98,** 10,775–10,780.
54. Urlinger, S., Baron, U., Thellmann, M., Hasan, M. T., Bujard, H., and Hillen, W. (2000) Exploring the sequence space for tetracycline-dependent transcriptional activators: novel mutations yield expanded range and sensitivity. *Proc. Natl. Acad. Sci. USA* **97,** 7963–7968.
55. Mehren, J. E. and Griffith, L. C. (2004) Calcium-independent calcium/calmodulin-dependent protein kinase II in the adult *Drosophila* CNS enhances the training of pheromonal cues. *J. Neurosci.* **24,** 10,584–10,593.
56. Kidd, S., Lieber, T., and Young, M. W. (1998) Ligand-induced cleavage and regulation of nuclear entry of Notch in *Drosophila melanogaster* embryos. *Genes Dev.* **12,** 3728–3740.
57. Szuts, D. and Bienz, M. (2000) LexA chimeras reveal the function of *Drosophila* Fos as a context-dependent transcriptional activator. *Proc. Natl. Acad. Sci. USA* **97,** 5351–5356.
58. Lai, S. L. and Lee, T. (2006) Genetic mosaic with dual binary transcriptional systems in *Drosophila*. *Nat. Neurosci.* **9,** 703–709.
59. Luan, H., Peabody, N. C., Vinson, C. R., and White, B. H. (2006) Refined spatial manipulation of neuronal function by combinatorial restriction of transgene expression. *Neuron* **52,** 425–436.
60. Rorth, P. (1996) A modular misexpression screen in *Drosophila* detecting tissue-specific phenotypes. *Proc. Natl. Acad. Sci. USA* **93,** 12,418–12,422.
61. Rorth, P., Szabo, K., Bailey, A., et al. (1998) Systematic gain-of-function genetics in *Drosophila*. *Development* **125,** 1049–1057.
62. Toba, G., Ohsako, T., Miyata, N., Ohtsuka, T., Seong, K. H., and Aigaki, T. (1999) The gene search system. A method for efficient detection and rapid molecular identification of genes in *Drosophila melanogaster*. *Genetics* **151,** 725–737.
63. http://genexel.com/eng/index.htm. Accessed January 5, 2007.
64. Staudt, N., Molitor, A., Somogyi, K., et al. (2005) Gain-of-function screen for genes that affect *Drosophila* muscle pattern formation. *PLoS Genet.* **1,** 499–506.
65. Tseng, A. S. and Hariharan, I. K. (2002) An overexpression screen in *Drosophila* for genes that restrict growth or cell-cycle progression in the developing eye. *Genetics* **162,** 229–243.
66. Molnar, C., Lopez-Varea, A., Hernandez, R., and de Celis, J. F. (2006) A gain of function screen identifying genes required for vein formation in the *Drosophila melanogaster* wing. *Genetics* 1635–1659.
67. Zettervall, C. J., Anderl, I., Williams, M. J., et al. (2004) A directed screen for genes involved in *Drosophila* blood cell activation. *Proc. Natl. Acad. Sci. USA* **101,** 14,192–14,197.

68. Kraut, R., Menon, K., and Zinn, K. (2001) A gain-of-function screen for genes controlling motor axon guidance and synaptogenesis in *Drosophila*. *Curr. Biol.* **11,** 417–430.
69. Duffy, J. B., Harrison, D. A., and Perrimon, N. (1998) Identifying loci required for follicular patterning using directed mosaics. *Development* **125,** 2263–2271.
70. Stowers, R. S. and Schwarz, T. L. (1999) A genetic method for generating *Drosophila* eyes composed exclusively of mitotic clones of a single genotype. *Genetics* **152,** 1631–1639.
71. Lee, C. H., Herman, T., Clandinin, T. R., Lee, R., and Zipursky, S. L. (2001) N-cadherin regulates target specificity in the *Drosophila* visual system. *Neuron* **30,** 437–450.
72. Rousset, R., Mack, J. A., Wharton, K. A., Jr., et al. (2001) Naked cuticle targets dishevelled to antagonize Wnt signal transduction. *Genes. Dev.* **15,** 658–671.
73. Pascual, A., Huang, K. L., and Preat, T. (2005) Conditional UAS-targeted repression in *Drosophila*. *Nucleic Acids Res.* **33,** e7.
74. Engineer, C. B., Fitzsimmons, K. C., Schmuke, J. J., Dotson, S. B., and Kranz, R. G. (2005) Development and evaluation of a Gal4-mediated LUC/GFP/GUS enhancer trap system in *Arabidopsis*. *BMC Plant. Biol.* **5,** 9.
75. Laplaze, L., Parizot, B., Baker, A., et al. (2005) GAL4-GFP enhancer trap lines for genetic manipulation of lateral root development in *Arabidopsis thaliana*. *J. Exp. Bot.* **56,** 2433–2442.
76. Scheer, N. and Campos-Ortega, J. A. (1999) Use of the Gal4-UAS technique for targeted gene expression in the zebrafish. *Mech. Dev.* **80,** 153–158.
77. Hartley, K. O., Nutt, S. L., and Amaya, E. (2002) Targeted gene expression in transgenic *Xenopus* using the binary Gal4-UAS system. *Proc. Natl. Acad. Sci. USA* **99,** 1377–1382.
78. Hu, Q., Ueno, N., and Behringer, R. R. (2004) Restriction of BMP4 activity domains in the developing neural tube of the mouse embryo. *EMBO Rep.* **5,** 734–739.
79. Rowitch, D. H., S-Jacques, B., Lee, S. M., Flax, J. D., Snyder, E. Y., and McMahon, A. P. (1999) Sonic hedgehog regulates proliferation and inhibits differentiation of CNS precursor cells. *J. Neurosci.* **19,** 8954–8965.
80. Banaszynski, L. A., Chen, L. C., Maynard-Smith, L. A., Ooi, A. G., and Wandless, T. J. (2006) A rapid, reversible, and tunable method to regulate protein function in living cells using synthetic small molecules. *Cell* **126,** 995–1004.
81. Zeidler, M. P., Tan, C., Bellaiche, Y., et al. (2004) Temperature-sensitive control of protein activity by conditionally splicing inteins. *Nat. Biotechnol.* **22,** 871–876.
82. Handler, A. M. (2002) Prospects for using genetic transformation for improved SIT and new biocontrol methods. *Genetica* **116,** 137–149.
83. Chan, H. Y. (2002) Fly-ing from genes to drugs. *Trends Mol. Med.* **8,** 99–101.
84. Stilwell, G. E., Saraswati, S., Littleton, J. T., and Chouinard, S. W. (2006) Development of a *Drosophila* seizure model for in vivo high-throughput drug screening. *Eur. J. Neurosci.* **24,** 2211–2222.
85. Tickoo, S. and Russell, S. (2002) *Drosophila melanogaster* as a model system for drug discovery and pathway screening. *Curr. Opin. Pharmacol.* **2,** 555–560.