GAL4 System in *Drosophila*: A Fly Geneticist's Swiss Army Knife

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The last decade has seen an enormous expansion in the genetic toolbox of model organisms. This has been particularly apparent in the fruitfly, *Drosophila melanogaster*, in which the development of these tools and their ongoing extension, along with the completion of the genome sequence has allowed for the analysis of most any process. One particularly elegant example of tool development was the creation of the GAL4/UAS system for targeted gene expression in *Drosophila*. In addition to helping make *Drosophila* one of the most genetically tractable metazoans, this system has also helped *Drosophila* attract attention from the biotechnology industry as a viable means to investigate the function of genes implicated in a wide variety of medically and economically important processes. In this article, we review the GAL4/UAS system in *Drosophila* and the numerous extensions that have morphed it into a veritable Swiss army knife for the analysis of gene function.

USER'S MANUAL: GAL4/UAS BASIC TRAINING

GAL4 encodes a protein of 881 amino acids, identified in the yeast *Saccharomyces cerevisiae* as a regulator of genes (e.g., *GAL10* and *GAL1*) induced by galactose (Laughon *et al.*, 1984; Laughon and Gesteland, 1984; Oshima, 1982). In a number of notable studies on transcriptional regulation, the DNA binding and transcriptional activation functions of GAL4 were identified, demonstrated to be separable, and meticulously defined (Ptashne, 1988). GAL4 regulates the transcription of the divergently transcribed *GAL10* and *GAL1* genes by directly binding to four related 17 basepair (bp) sites located between these loci (Giniger *et al.*, 1985). These sites define an Upstream Activating Sequences (UAS) element, analogous to an enhancer element defined in multicellular eukaryotes, which is essential for the transcriptional activation of these GAL4-regulated genes. The DNA binding activity of GAL4 maps to the first 74 residues, while its transcriptional activation function maps to two regions, residues 148–196 and 768–881 (Ma and Ptashne, 1987b). Following upon this work, in 1988 Fischer *et al.* demonstrated that GAL4 expression was capable of stimulating transcription of a reporter gene under UAS control in *Drosophila*. This activity is not limited to *Drosophila*, as GAL4 can function in a wide variety of systems to activate transcription from the UAS element (Kakidani and Ptashne, 1988; Ma *et al.*, 1988; Webster *et al.*, 1988). Importantly, expression of GAL4 in *Drosophila* initially appeared to have no overt deleterious phenotypic effects. These two results helped set the stage for Brand and Perrimon's (1993) landmark article describing the development of the GAL4/UAS system for targeted gene expression in *Drosophila*.

Targeting gene expression in a temporal and spatial fashion has proven to be one of the most powerful techniques for addressing gene function in vivo. In 1993 Brand and Perrimon published a bipartite approach for directing gene expression in vivo (Fig. 1). In this system, expression of the gene of interest, the responder, is controlled by the presence of the UAS element, in this case five tandemly arrayed and optimized GAL4 binding sites (Figs. 1, 2). Because transcription of the responder requires the presence of GAL4, the absence of GAL4 in the responder lines maintains them in a transcriptionally silent state. To activate their transcription, responder lines are mated to flies expressing GAL4 in a particular pattern, termed the driver (Fig. 1). The resulting progeny then express the responder in a transcriptional pattern that reflects the GAL4 pattern of the respective driver (Fig. 1).

This bipartite approach, in which the two components of the system, the responder and the driver, are maintained as separate parental lines, has numerous strengths. First, the transcriptional inactivity of the parental responder line means that transgenic responder lines can be generated for gene products that are toxic, lethal, or have reduced viability when expressed. For example, responder lines currently exist for genes, such as *ricin a*, that encode toxic gene products, for genes, such as *reaper*, that can trigger programmed cell death, and for oncogenes, such as *braf^act*, whose targeted expression often result in reduced viability (Aplin and Kauf-

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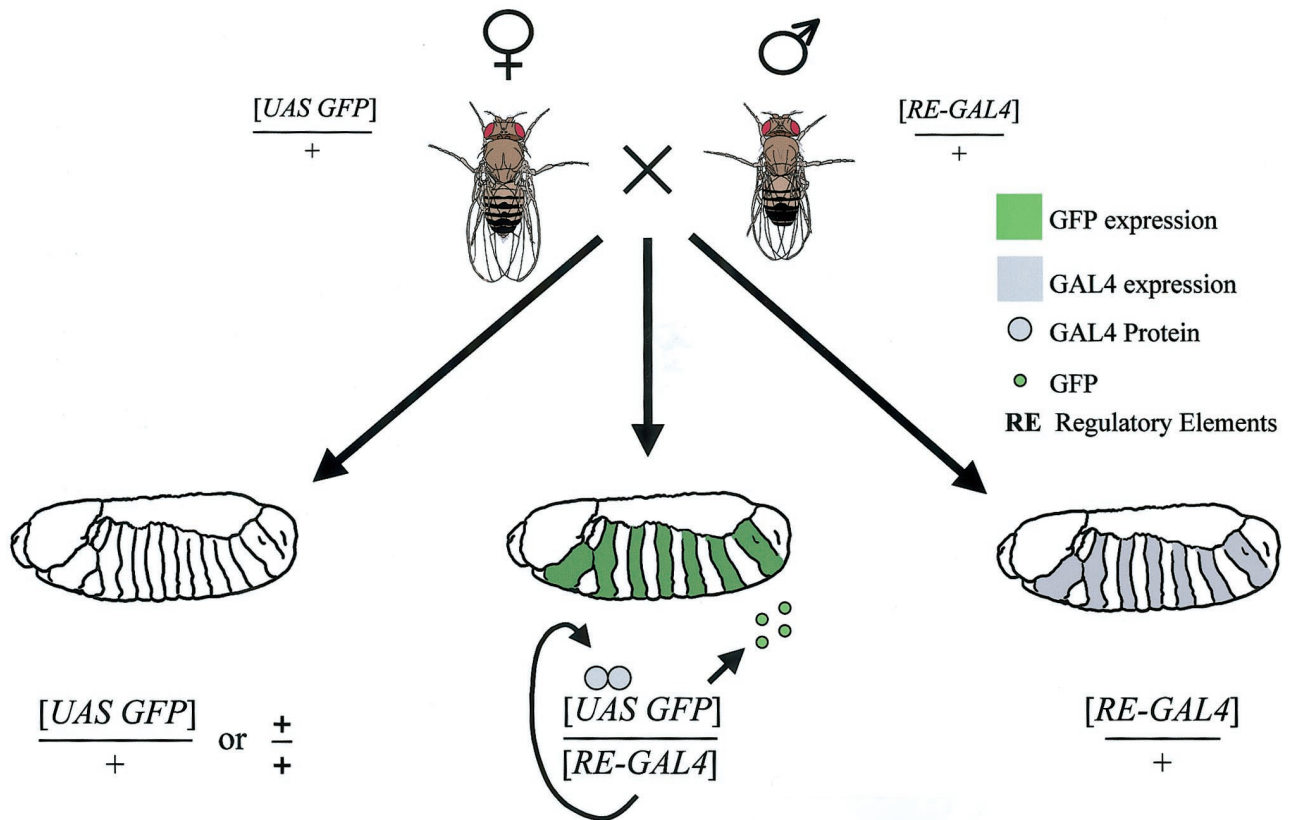
FIG. 1. The bipartite UAS/GAL4 system in *Drosophila*. When females carrying a UAS responder (*UAS-GFP*) are mated to males carrying a GAL4 driver progeny containing both elements of the system are produced. The presence of GAL4 in an alternating segmental pattern in the depicted embryos then drives expression of the UAS responder gene in a corresponding pattern.

man, 1997; Brand and Perrimon, 1994; Zhou *et al.*, 1997). When crossed to a GAL4 driver, induction of such responders results, thereby causing cell death, lethality, or reduced viability. This provides a powerful tool to study the effect of loss of specific cells on the process of interest, as well as the function of these toxic, lethal, or oncogenic genes. An additional strength of the system arises from the ability to target expression of any responder in a variety of spatial and temporal fashions by mating it with distinct GAL4 drivers. Since expression of the responder is dependent on the GAL4 pattern of the driver, Brand and Perrimon cleverly took advantage of the enormous diversity of genomic regulatory sequences and the vigor of the *Drosophila* community by constructing an enhancer-trap GAL4 construct (pGAWB, Fig. 2). This has led to the production of an astounding array of GAL4 drivers for targeting expression to almost every major tissue type.

This has clearly been one of the major strengths of the system, with thousands of drivers having been reported in the literature. However, a highly coordinated effort to report, organize, and deposit such lines at the *Drosophila* stock centers has not been as easily forthcoming. While there has been an effort from a few individual labs

to generate and report driver information, for many GAL4 drivers detailed information on the developmental profile of expression is lacking (Gerlitz *et al.*, 2002; Gustafson and Boulianne, 1996; Manseau *et al.*, 1997; Schupbach and Wieschaus, 1998). Currently, only a limited number of GAL4 drivers are publicly available through the *Drosophila* stock centers. A list of GAL4 lines currently available at the Bloomington *Drosophila* stock center is available online at http://flystocks.bio. indiana.edu/gal4.htm. One goal of this special issue of *genesis* is to provide a service to the community by promoting such an effort for the GAL4/UAS system. In this issue, Hayashi *et al.* report the establishment of a major resource, GETDB, which comprises a database detailing the expression and locations of 4,615 GAL4 enhancer traps.

Although the most common notion of the GAL4/UAS system is as a tool for misexpression studies with a specific gene, this is rapidly changing and the list of alternative uses is diverse and lengthy. Some of the more routine uses of the system include: 1) identification of genes involved in the process of interest via enhancer- or gene-trapping; 2) analysis of cellular autonomy of a gene product through targeted mosaics; 3) cellular marking to

aid in screens for mutations affecting the process of interest; 4) analysis of loss-of-function phenotypes through targeted expression of RNAi and dominant-negative constructs; and 5) genomic approaches to the identification of genes whose misexpression affects the process of interest. Below we cover some of the extensions to the system that have improved its utility in these areas.

USING THE SYSTEM: HANDLING WITH CARE

Temperature Dependence of GAL4

As with any technology, an increased understanding of its nature helps prevent pitfalls in experimental design and often results in the identification of additional inherent and beneficial characteristics. One such discovery was the temperature dependence of GAL4 activity in *Drosophila*. In flies, minimal GAL4 activity is present at 16°C, while 29°C provides a balance between maximal GAL4 activity and minimal effects on fertility and viability due to growth at high temperature. Simply by altering the temperature, a wide range of expression levels of any responder can be achieved, thereby increasing the flexibility of the system. This is particularly advantageous to those studying the postembryonic stages of development. As many drivers express throughout development, undesired lethal effects from targeted expression at early stages may prevent the recovery of the developmental stage of interest. By lowering the temperature, one can reduce activity of the system and bypass such detrimental effects, thereby providing a simple way to obtain the stage of interest for study at higher temperatures.

Defining Targeted Expression of the Responder

Initially, most GAL4 drivers are characterized by crossing to a responder line containing a reporter gene, such as *UAS-β-galactosidase* (*UAS-LacZ*) or *UAS-Green Fluorescent Protein* (*GFP*). However, differences in mRNA and protein stability, cellular localization, and sensitivity and timing of detection between a reporter and the responder gene of interest may lead to differences in the pattern of expression and affect the interpretation of results. Thus, an accurate understanding of the phenotypic consequences of targeting responder expression depends on a concomitant analysis of the expression pattern of the responder when combined with a GAL4 driver. A variety of approaches can be used to determine this for the gene and protein of interest (in situ hybridization or immunohistochemistry with an antibody to the responder protein), but the expanding use of fluorescent protein tagged (FPs) versions of responders promises to provide a versatile future approach.

Determining If a Driver or Reporter Is "Benign"

As mentioned above, a diverse group of responders exists for use as cellular reporters. A powerful use of these "benign" reporters is in the identification and characterization of mutations affecting a specific developmental process. For such uses and by definition, it is essential that the reporter not affect the process under study. While many responders used as reporters do not appear to have deleterious effects, some have been reported to result in phenotypic disruptions. For example, two microtubule-associated reporters, kinesin heavy chain-LacZ fusion protein and tau-green fluorescent protein (GFP), can result in lethality in response to specific drivers (Phelps and Brand, 1998). Likewise, the majority of GAL4 drivers do not appear to have deleterious phenotypic effects on their own. However, instances of a GAL4 driver disrupting aspects of normal development have been reported. For example, effects on ommatidial development have been observed with *P{GAL4-ninaE. GMR}* (Freeman, 1996). For such reasons it is important to ensure that the process of interest is not affected by the presence of either the GAL4 driver or by the presence of a responder being utilized as a cellular reporter.

SYSTEM OPTIONS: WHAT'S AVAILABLE?

From its inception the GAL4/UAS system has undergone numerous refinements and extensions. We first review what the basal elements of the system are and then examine the modifications to the system. To facilitate the generation of transgenic responder and driver lines Brand and Perrimon (1993) constructed a number of vectors (Fig. 2). pUAST is a P-element-based vector that allows one to place the gene of interest under GAL4 control. It contains an upstream UAS element, followed by the *heat shock protein70* (*hsp70*) basal promoter, a multiple cloning site, and the SV40 small t intron and polyadenylation signal. Three separate constructs, pGAWB, pGATB, and pGATN were generated to allow for the expression of GAL4 in distinct patterns. As described above, the pGAWB construct was designed as an enhancer-trap construct. In this construct GAL4 has been inserted downstream of the P transposase promoter and upstream of the *hsp70* terminator. In contrast, pGATB or pGATN provide convenient vectors for placing known regulatory elements upstream of GAL4. Once the regulatory element of interest has been placed upstream of GAL4 in either vector, a fragment containing the regulatory element and GAL4 can be excised and cloned into a P transformation vector. Using this latter approach a number of lines in which specific elements have been placed upstream of GAL4 have been generated and allow for directed expression of target genes in a wide variety of tissues. In this issue, Sharma *et al.* describe a P-element-based transformation vector, pPT-GAL, for placing regulatory sequences directly upstream of GAL4, thereby eliminating the shuttling step necessary with pGATB or pGATN (Fig. 2). Likewise, Roman and Davis (in this issue) describe a similar transformation vector that eliminates a shuttling step by utilizing *lox*-based recombination to insert regulatory sequences directly upstream of GAL4, in this case a hormone-inducible version. With the extensive use of the system,
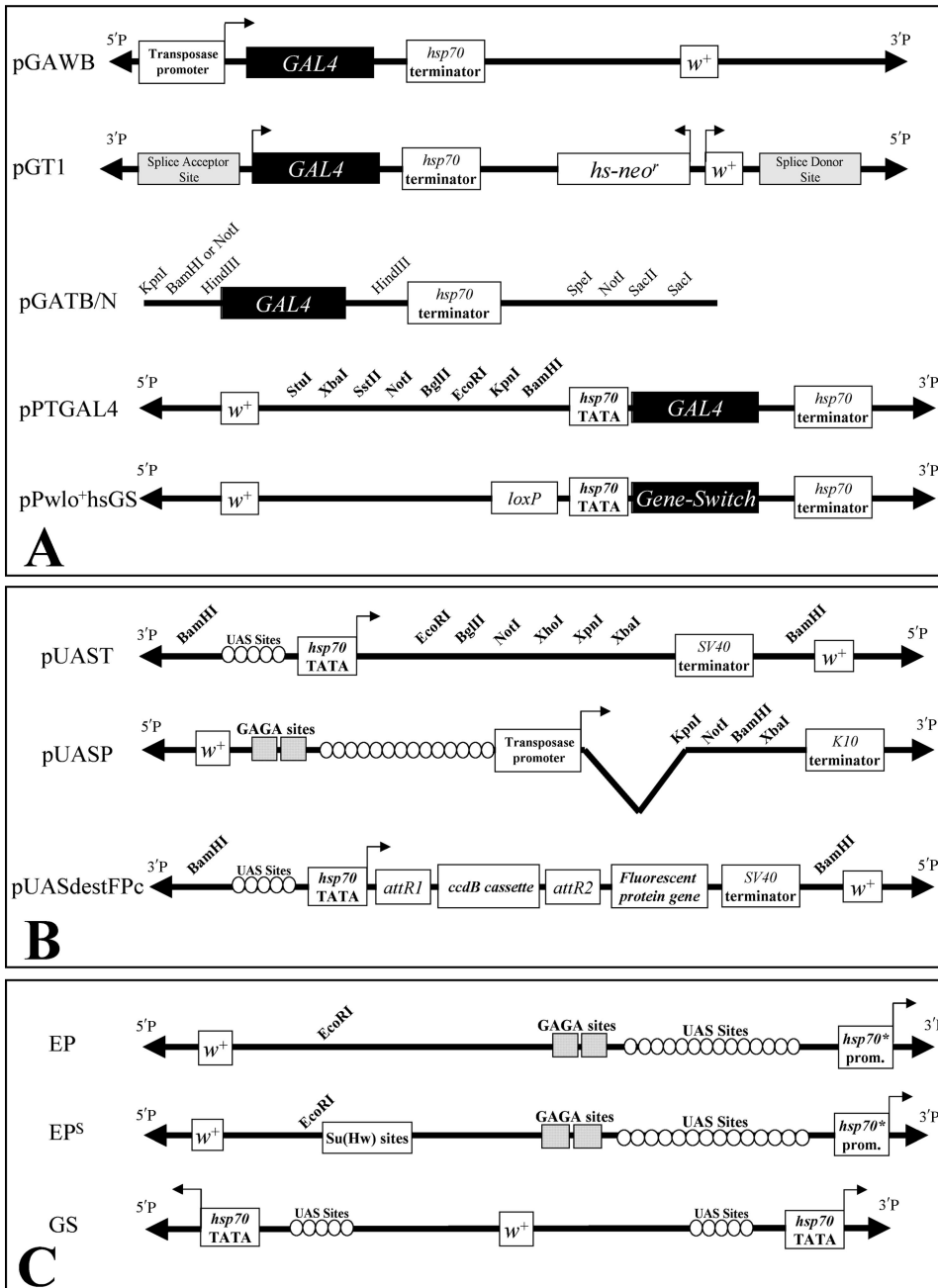
FIG. 2. UAS/GAL4 constructs. Simplified schematics of constructs used in the UAS/GAL4 system are depicted. **A:** pGAWB and pGT1 represent two flavors of enhancer-trap GAL4 P-element transformation vectors used to generate driver lines. pGATB, pGATN, pPTGAL4, and pPwlo+-hsGS are all vectors used to place specific regulatory elements upstream of GAL4. For a more detailed description of pPTGAL4 and pPwlo+hsGS, see the articles by Sharma *et al.* and Roman and Davis, respectively, in this issue. **B:** pUAST, pUASP, and pUASdestFPc are vectors for constructing a UAS responder of the gene of interest. pUASdestFPc represents a set of vectors that allow for the gene of interest to be fused upstream and in-frame to genes encoding a variety of fluorescent protein reporters (Wang, Barkus, and Duffy, unpublished). **C:** EP, EP[S], and GS are vectors that have been used to generate UAS responders of endogenous genes. **D:** Construction of a UAS responder encoding a splice-activated snapback RNA for the gene of interest is depicted using the system described by Nagel *et al.* 2002.

additional modifications have naturally followed. These variations on the GAL4/UAS theme include, among others: vector alterations, germline expression of responders during oogenesis, improved inducibility in flies and cell culture, targeted RNAi, improvements to clonal analysis, and genomic scale generation of responders.

The Screwdriver: Variations on pGAWB

Modifications to GAL4 have also increased the flexibility of the system. An improved pGAWB, called pGALW, has been constructed to increase transposition fre-

quency (Gerlitz *et al.*, 2002). In a laudable effort, Gerlitz *et al.* then used *UAS-GFP* as a reporter and screened approximately 40,000 independent pGALW insertions for wing imaginal disc patterns. From this screen they identified 2,000 insertions that drive expression in the wing imaginal disc. This screen also highlights one of the powerful benefits of the system as an enhancer trap tool. It allows the identification and molecular isolation, via plasmid rescue, of genes expressed in the tissue of interest (e.g., the wing) and, simultaneously, the recovery of a means to drive the expression of responders in said

FIG. 2 (Continued)
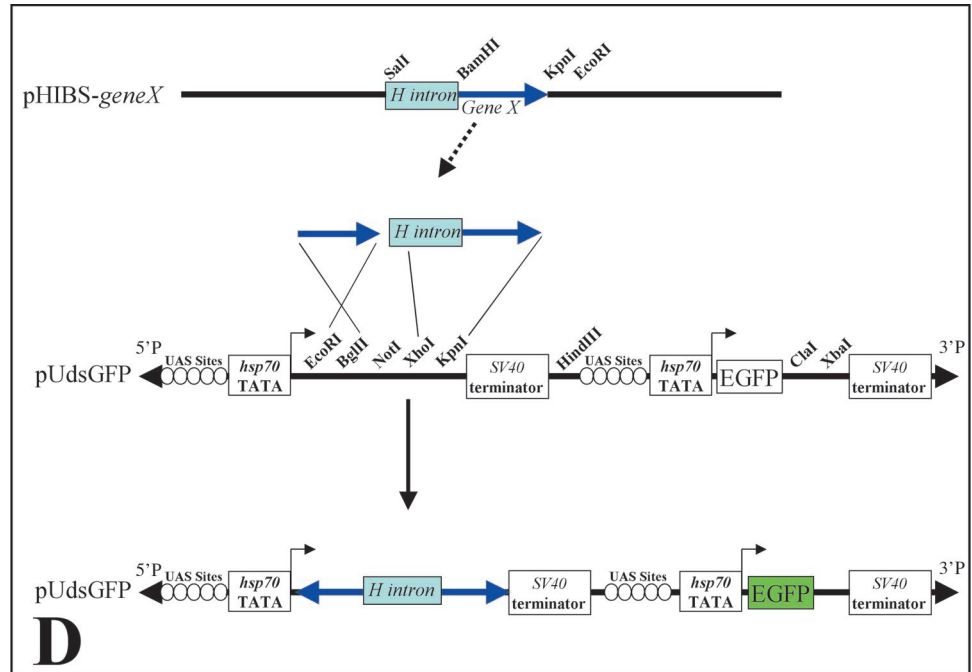
tissue. In this same study, Gerlitz *et al.* noted that both pGAWB and pGALW drivers express GAL4 in the salivary glands, due to the presence of a salivary gland enhancer present in the *hsp70* sequences upstream of the GAL4 coding region. Elimination of these 5′ UTR sequences led to a loss of salivary gland expression, but unfortunately these variants of pGALW had reduced function as enhancer trap vectors limiting their usefulness (Gerlitz *et al.*, 2002). In a related approach, a gene trap vector with GAL4, pGT1, has also been constructed (Fig. 2) (Lukacsovich *et al.*, 2001). A major distinction between the two approaches is that expression of the gene trap vector relies on an endogenous promoter, unlike enhancer traps, which rely upon a promoter present within the enhancer trap vector. A second major distinction is that gene traps utilize a splice acceptor (or donor) site present in the transposon to produce a hybrid mRNA comprised of exon sequences from the gene disrupted and the GAL4 coding sequences.

The Scissors: Expression in the Maternal Germline

The *Drosophila* egg chamber is composed of two main tissue types: the germline derived oocyte and associated nurse cells, and the somatic epithelium. One byproduct of the construction of pUAST was a lack of responder expression in the maternal germline. While a benefit to those wishing to limit responder expression to the somatic epithelium, this was an unanticipated hindrance to those interested in targeting responder expression to the germline during oogenesis. To overcome this, Rørth (1998) developed a modified vector, pUASP, in which the *hsp70* promoter and SV40 terminator of pUAST were replaced with the P transposase promoter and first intron and the *fs(1)K10* 3′ UTR, thereby allowing for efficient responder expression in the maternal germline (Fig. 2). A second benefit, resulting from the development of pUASP, is that early embryonic expression of a responder can be obtained through maternal loading of a responder into the oocyte. It is important to note that pUASP, as with pUAST, efficiently drives expression in other tissues as well. Concomitant with the development of pUASP, a number of GAL4 drivers were developed to efficiently drive responder expression in the maternal germline. A GAL4 driver containing the *nanos* promoter, GAL4 fused to the transcriptional activator VP16 (GAL4-VP16), and the *nanos* 3′ UTR promotes expression of pUASP responders throughout all stages of oogenesis (Rørth, 1998).

The Corkscrew: Increased Inducibility

Although the GAL4/UAS system provided a previously unprecedented degree of temporal and spatial regulation, the lack of absolute temporal and spatial specificity of many drivers can hinder analyses in the tissue of interest. Often this is the result of lethality prior to the developmental stage of interest. As mentioned above, one solution to this takes advantage of the temperature dependence of GAL4 in flies. However, more technically refined improvements to the inducibility of the GAL4/UAS system have also been developed. Currently, at least six different approaches have been developed that provide an additional level of spatial and or temporal control
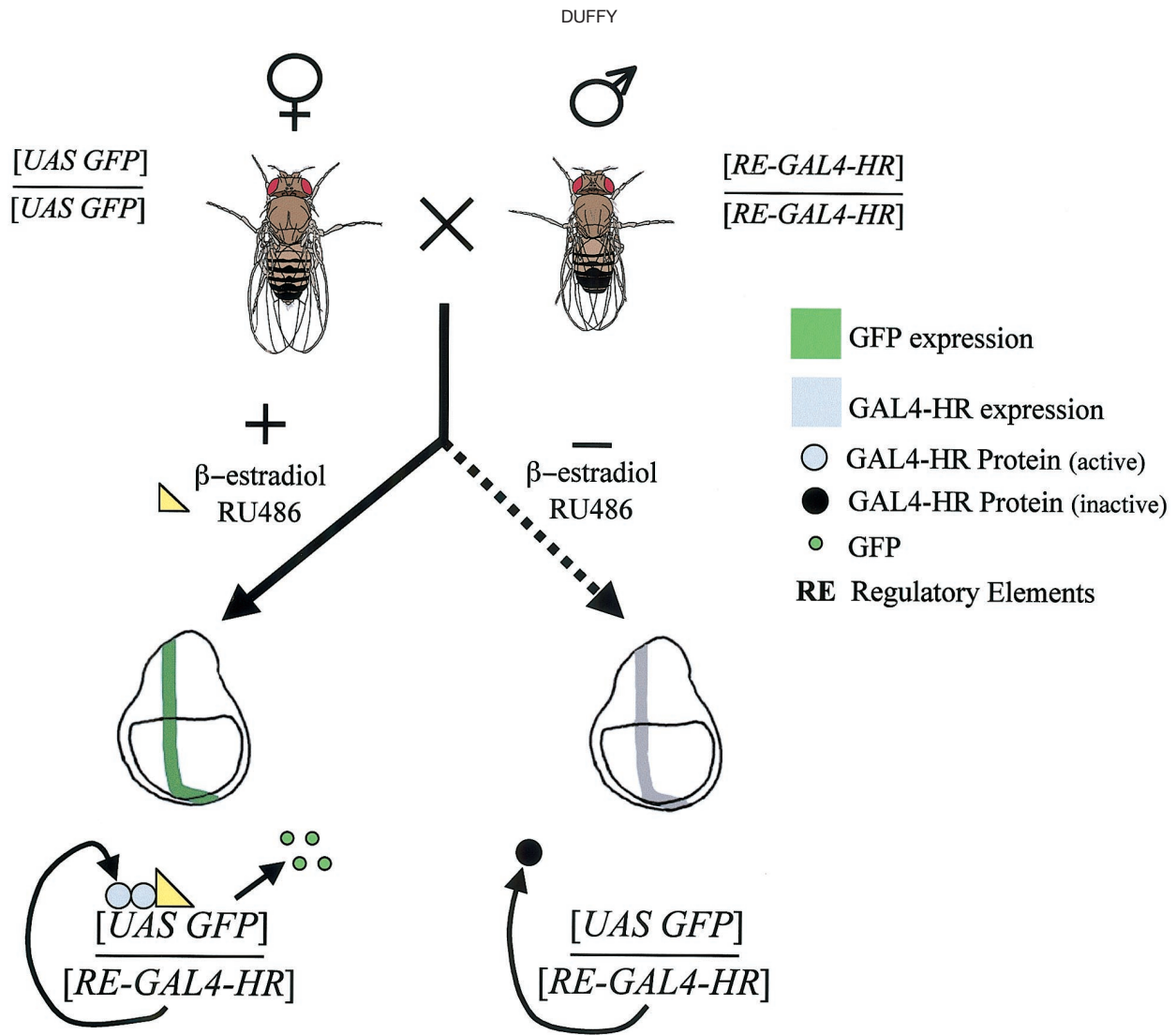
FIG. 3. Hormone-responsive GAL4 induction. Females carrying a UAS responder (*UAS-GFP*) are mated to males carrying a hormone-responsive GAL4 driver (either the GAL4-estrogen receptor chimera or the GAL4-progesterone receptor-human p65 activation domain chimera). In this scheme, the GAL4 driver is expressed in a stripe along the anterior–posterior axis of the wing imaginal disc. In F1 containing both elements of the system, expression of the responder is observed only when these progeny have been exposed to the appropriate hormone.

to GAL4 activation. Below is a brief description of these modifications to the GAL4/UAS system.

Cell-specific induction of a *UAS-LacZ* responder has been achieved with a laser microbeam, which was used to trigger heat shock inducible GAL4 expression (Halfon *et al.*, 1997). Likewise, a caged, inactive version of GAL4-VP16 can be injected into embryos and activated by a beam of UV (365 nm) light, thereby controlling responder expression in a highly specific fashion (Cambridge *et al.*, 1997). Although these latter techniques provide a useful approach to directing desired embryonic GAL4 patterns, their value in addressing later stages of development is limited. The remaining four approaches provide a means of regulating GAL4 activity at most developmental stages.

Not surprisingly, perhaps, because of the well-characterized inducible nature of hormone receptors in regulating gene expression, hormone responsive GAL4 chimeras have been used in *Drosophila* with the GAL4/UAS system. Currently, two such chimeras exist: a GAL4-estrogen receptor chimera and a GAL4-progesterone receptor-human p65 activation domain chimera, termed *GeneSwitch* (Han *et al.*, 2000; Osterwalder *et al.*, 2001; Roman *et al.*, 2001). In both cases, addition of the appropriate ligand results in the induction of GAL4 activity and subsequent expression of the UAS responder (Fig. 3). This provides a straightforward approach to temporally regulate the activity of a GAL4 driver and to circumvent any early expression of a responder that might be detrimental. The limitation of this approach is that it does not take advantage of preexisting
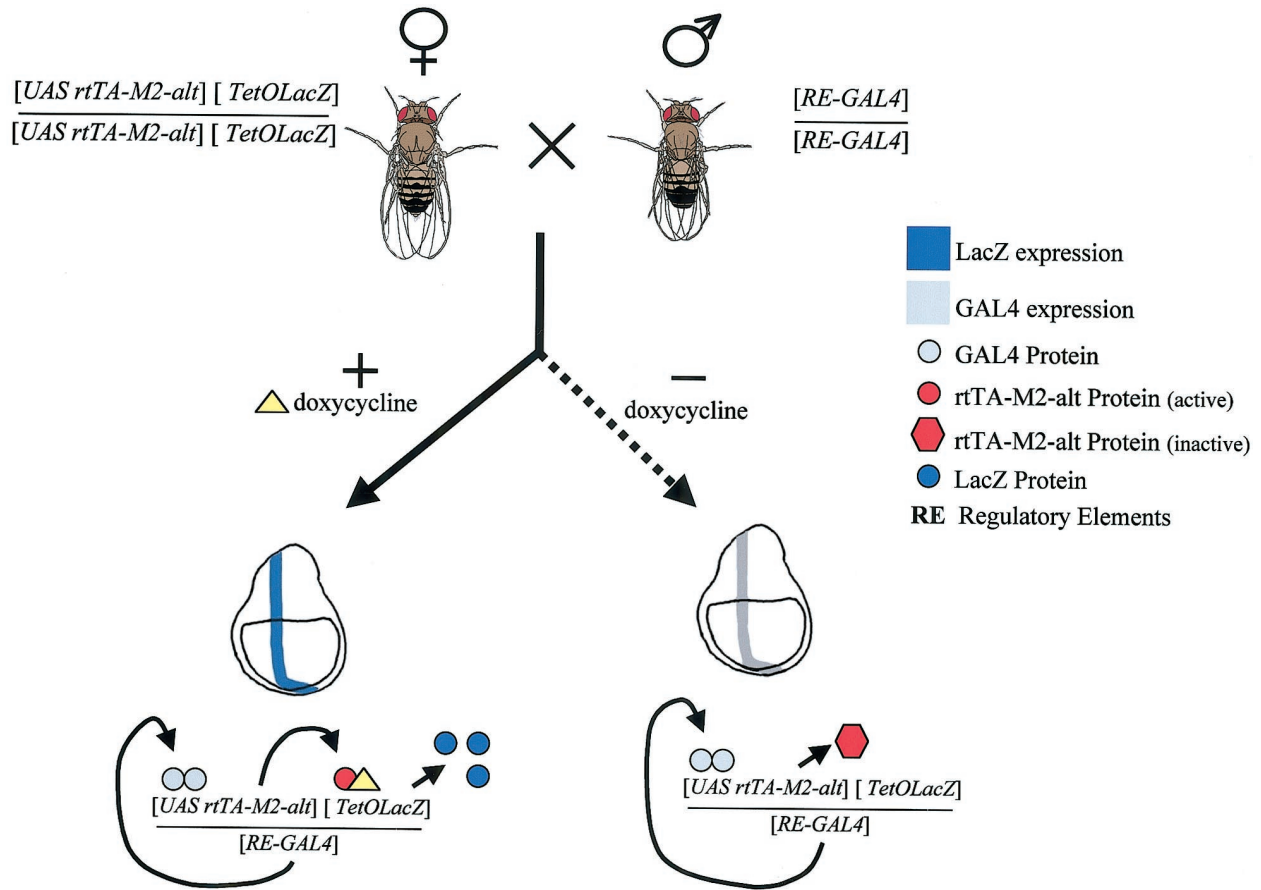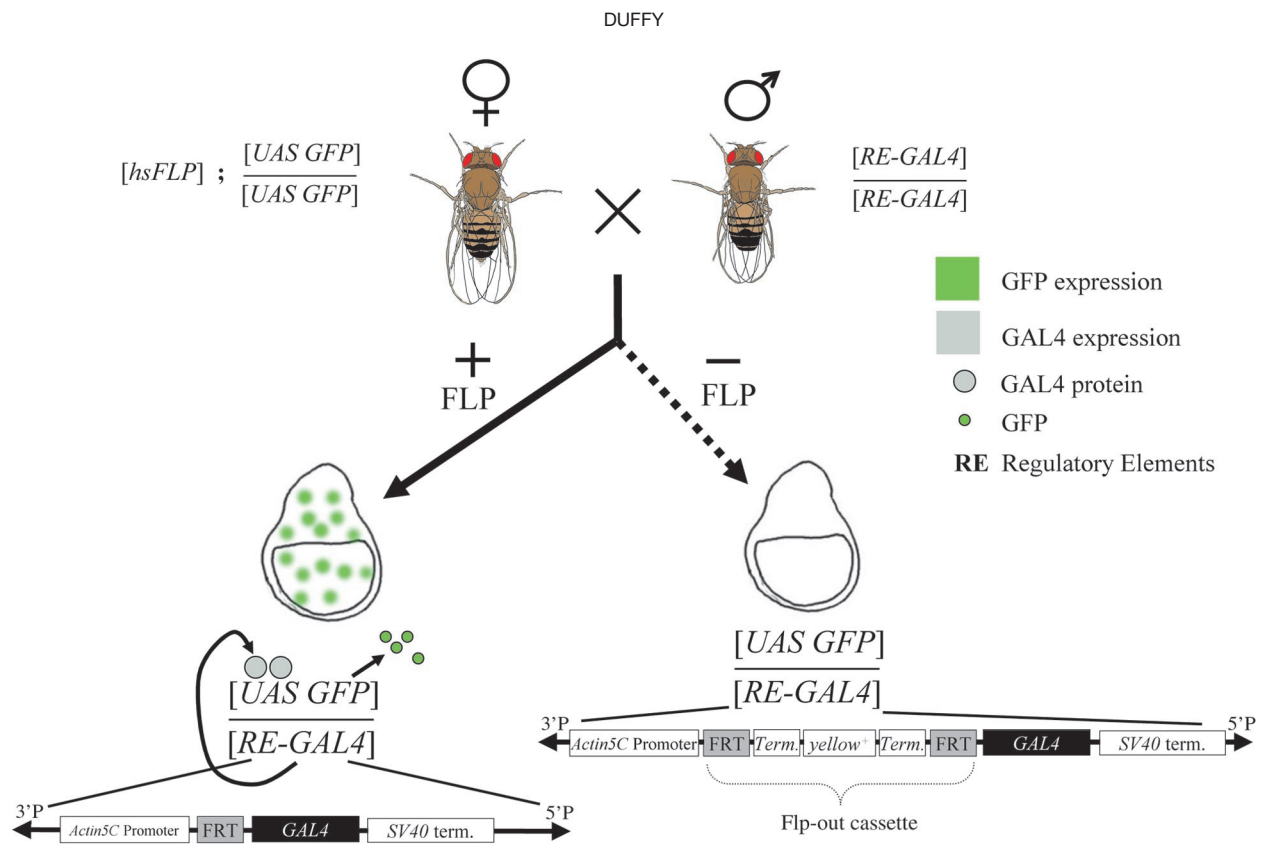
FIG. 4. GAL4 induction with the Tet-On system. In this system, a modified tTA activator, rtTA-M2-alt, is under the control of GAL4, while the *β-galactosidase* (LacZ) gene is under the control of the Tet operator. rtTA-M2-alt activates transcription from the Tet operator only in the presence of tetracycline or its analog doxycycline. As in Figure 3, the GAL4 driver is expressed in a stripe along the anterior–posterior axis of the wing imaginal disc. rtTA-M2-alt is expressed in a corresponding pattern in the wing disc in response to the GAL4 driver, but activates LacZ expression only in the presence of a doxycycline. In this system, the gene of interest is then cloned downstream of the Tet operator to generate an inducible TetO responder.

GAL4 lines, but requires the generation and characterization of additional GAL4-hormone receptor lines.

A related approach combines a modified version of the inducible tetracycline responsive transactivator (rtTA-M2-alt) with the GAL4 system. In the absence of tetracycline (tet) or its analog doxycycline (dox), rtTA-M2-alt is unable to bind to Tet operator sequences (TetO) and activate transcription (Tet-On system). Addition of dox results in binding of rtTA-M2-alt to TetO and transcriptional activation of the target gene. By constructing a *UAS-rtTA-M2-alt* responder, another tier of inducibility to the GAL4 system was added (Stebbins *et al.*, 2001). In this tripartite system, one places the gene of interest under the control of the TetO sequences (TetO responder) and then crosses in both the *UAS-rtTA-M2-alt* responder and the desired GAL4 driver (Fig. 4). GAL4 then drives expression of the *UAS-rtTA-M2-alt* responder, whose activity is then regulated by the presence or absence of the drug. In the absence of tet or dox,

transcription of the TetO responder is off, while addition of tet or dox results in induction of rtTA-M2-alt activity and transcriptional activation of the TetO responder. In contrast to this positive-induction Tet-On system, a Tet-Off system, in which transcription is negatively regulated by the presence of tet or dox, has also been combined with the GAL/UAS system (Stebbins and Yin, 2001).

Use of the yeast FLP recombinase provides another tripartite approach to increasing the resolution of GAL4/ UAS inducibility. Two options, both utilizing the ability of FLP to promote recombination in cis between two FRT sites, have been developed with the GAL4/UAS system (Ito *et al.*, 1997; Nellen *et al.*, 1996; Pignoni and Zipursky, 1997; Zecca *et al.*, 1996). The first option places a similar FRT flanked terminator cassette between the GAL4 gene and its regulatory elements (Fig. 5). The second option places an intervening terminator sequence flanked by FRT sites between the UAS element and the responder gene (Fig. 6). In either case, expres-

FIG. 5. GAL4 induction with the FLP/FRT system. In this modification of the UAS/GAL4 system, the presence of a FLP-out cassette prevents the *Act5C* promoter from triggering GAL4 expression. Upon induction of FLP expression (via a heat-shock FLP recombinase) the intervening FLP-out cassette is excised. This juxtaposes the *Act5C* promoter and the *GAL4* gene leading to *GAL4* transcription and subsequently expression of the *UAS-GFP* responder only in the cells in which the FLP-out cassette has been excised (depicted here as green patches).

sion of the responder in response to the GAL4 driver requires the additional presence of FLP to mediate removal of the intervening terminator cassette (Figs. 5, 6). Thus, an added level of temporal or spatial regulation of the responder can be achieved with a heat shock inducible FLP line or via a limited number of lines in which FLP is expressed under the control of tissue-specific regulatory elements.

An added level of inducibility has also been generated with the GAL4 inhibitor GAL80. Binding of GAL80 to the carboxy-terminal 30 amino acids of GAL4 prevents GAL4-mediated transcriptional activation (Ma and Ptashne, 1987a). Using a ubiquitously expressed GAL80, Lee and Luo (1999) demonstrated that GAL80 is able to inhibit GAL4 activity in *Drosophila* and exhibits no deleterious phenotypic effects on its own. Then, in a clever combination of the GAL80, GAL4/UAS, and FLP/FRT systems, they generated cells lacking GAL80 expression through FLP-mediated mitotic recombination. This resulted in the appearance of GAL4 activity and subsequent expression of UAS responders in these cells (Fig. 7). Thus, a high degree of temporal regulation of UAS responders can be achieved with this system by regulating the loss of GAL80.

The Tweezers: Refined Mosaic Analyses

Historically, two of the major limitations in mosaic analysis have been in producing genotypically distinct cells in the cells or tissue of interest and in distinguishing these distinct cell types. As alluded to above, the work of Lee and Luo (1999) significantly advanced our ability to identify and characterize genotypically distinct cell types. In contrast to systems that mark wild-type cells, their MARCM system (mosaic analysis with a repressible cell marker) combines the GAL4/UAS, GAL80, and FLP/FRT systems to label the mutant cells (Fig. 7). Because these are often the cells of interest for cellular and molecular analysis, this positive marking scheme provides a significant advantage over existing systems that leave mutant cells unlabeled.

In addition to this improved marking system, combination of the GAL4/UAS and FLP/FRT system also enhanced preexisting techniques to produce mutant clones. Using the directed mosaic system, which utilizes a GAL4 responsive FLP, clonal analysis can be restricted to the tissue of interest (Duffy *et al.*, 1998) (Fig. 8). By combining the appropriate GAL4 driver with a *UAS-FLP* responder, FLP expression can be targeted to precursors
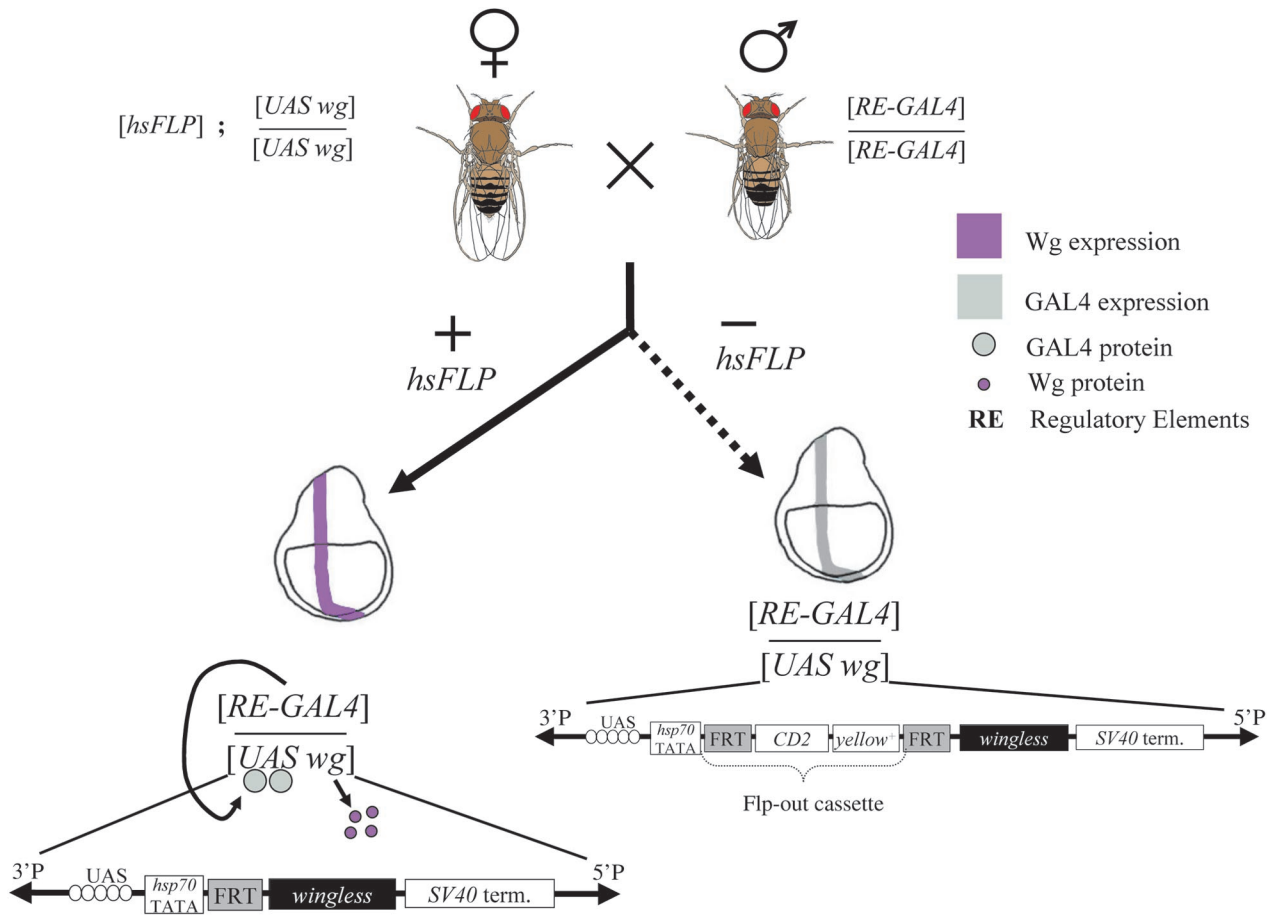
FIG. 6. UAS responder induction with the FLP/FRT system. In a slight variation on the system depicted in Figure 5, the presence of a FLP-out cassette now separates the UAS element from the responder gene (in this case, *wingless (wg)*). This prevents transcription of the responder in response to GAL4 binding to the UAS element. Upon induction of FLP expression (via a heat-shock FLP recombinase) the intervening FLP-out cassette is excised and juxtaposes the UAS element and the responder gene leading to its transcription and subsequent expression. In this figure, the GAL4 driver is expressed in a stripe along the anterior–posterior axis of the wing imaginal disc.

of the tissue of interest. Directed FLP expression then promotes mitotic recombination within this tissue and restricts the production of mutant clones to that tissue. This approach conveniently eliminates the potential confounding effects that the production of clones in many tissues or at earlier stages might cause.

The Saw: Targeted Gene Knockdowns

The GAL4/UAS system is often associated with the analysis of gain-of-function phenotypes. However, its recent combination with RNA-mediated interference (RNAi) technology is emerging as a powerful tool for analysis of loss-of-function phenotypes as well. Currently, a variety of approaches have been adopted and proven successful for the directed expression of constructs that form double-stranded RNA (dsRNA) molecules (Enerly *et al.*, in this issue; Giordano *et al.*, 2002; Kalidas and Smith, 2002; Nagel *et al.*, 2002; Piccin *et al.*, 2001; Reichhart *et al.*, in this issue; Schmid *et al.*, 2002;

van Roessel *et al.*, in this issue). Such dsRNAs are capable of mediating gene-specific RNAi (Enerly *et al.*, in this issue; Giordano *et al.*, 2002; Kalidas and Smith, 2002; Nagel *et al.*, 2002; Piccin *et al.*, 2001; Reichhart *et al.*, in this issue; Schmid *et al.*, 2002; van Roessel *et al.*, in this issue). In one approach, termed splicing-activated RNAi, the formation of the hairpin RNA molecule is controlled through splicing. Inclusion of an intron in the construction of a UAS hairpin responder may aid in formation and transport of the dsRNA molecule (Kalidas and Smith, 2002; Reichhart *et al.*, in this issue) (Fig. 9). A particularly useful vector based on this approach was developed by Nagel *et al.* (2002) and is depicted in Figure 2. In pUdsGFP, a second, UAS-GFP responder was inserted downstream of the position for the UAS-splice-activated hairpin. GFP expression then provides a convenient way to identify cells that are also undergoing RNAi-mediated knockdown of the gene of interest. Alternatively, UAS responder constructs that lack an intron and instead
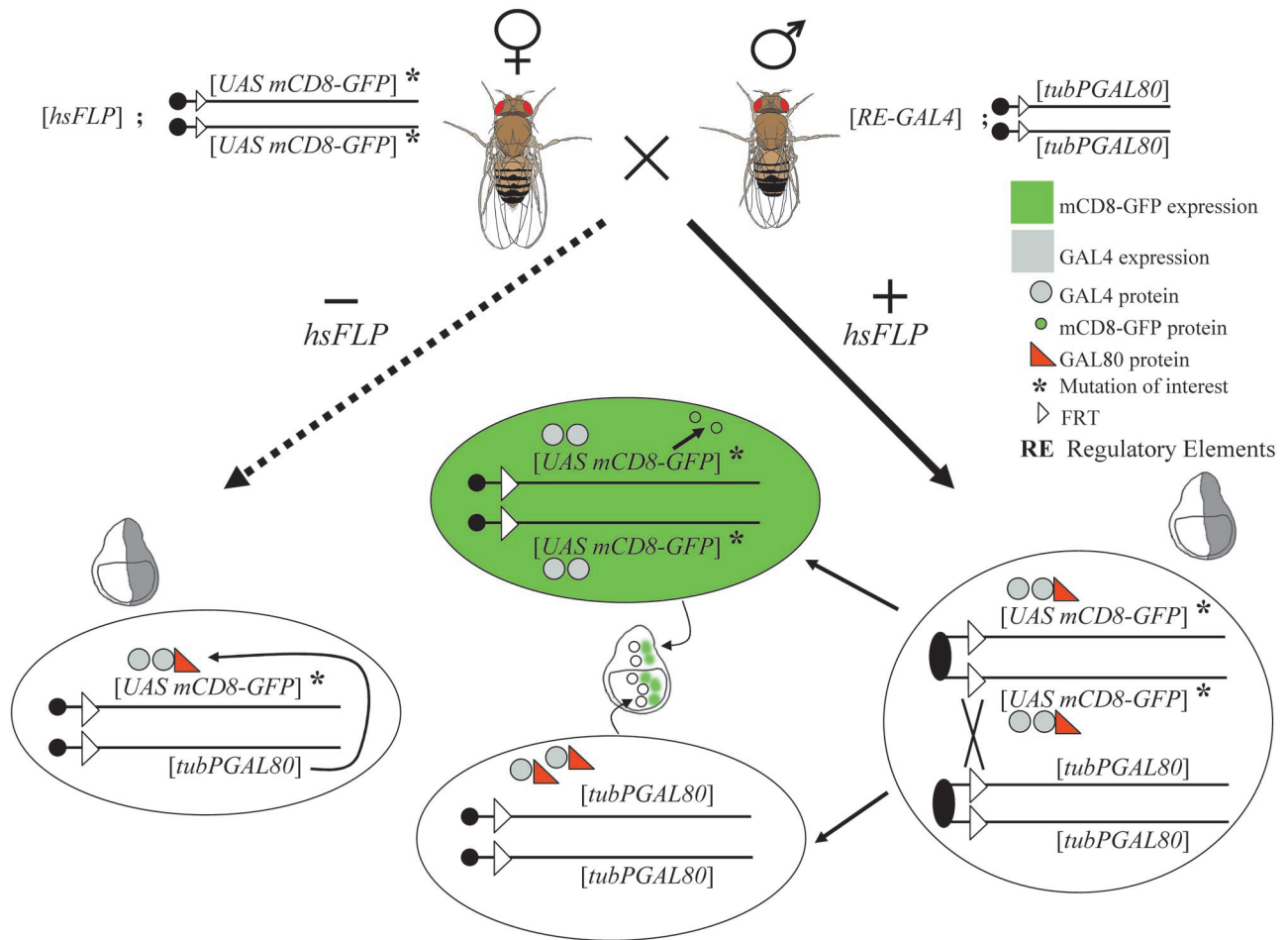
FIG. 7. A positive marking scheme for clonal analysis. In this figure, GAL80 prevents expression of mCD8-GFP in all cells heterozygous for the mutation of interest (*). Mitotic recombination is induced using a heat-shock inducible *FLP* recombinase. During mitosis FLP mediated recombination at the proximally located FRT sequences leads to the production of two daughter cells, each homozygous for the corresponding chromosomes. This results in the production of wing discs that are mosaic for the mutation under study (*). After mitotic recombination, cells that are homozygous for the mutation of interest (*) also now lack GAL80. Consequently, these cells have GAL4 activity and are marked by the presence of the mCD8-GFP, which is under the control of GAL4.

directly form a hairpin have also been utilized to mediate gene-specific RNAi. The absence of direct comparisons between these differing approaches makes it difficult to ascertain their relative efficiencies, but with each method successful gene knockdown effects have been reported.

As an alternative to RNAi-mediated gene knockdowns, dominant-negative or dominant-interfering versions of the protein of interest have also been used extensively in flies with the GAL4/UAS system to disrupt gene activity. In these cases RNAi now provides an independent means to confirm that effects obtained with such molecules accurately represents loss-of-function phenotypes.

The File: Extension to Cell Culture

Although much of the effort on the GAL4/UAS system has been focused on flies, the use of constitutive GAL4 drivers has provided a means to extend the system into *Drosophila* cell culture. Cotransfection of UAS responders with constitutive drivers, such as *Act5C-GAL4* or *arm-GAL4*, efficiently directs responder expression in a variety of *Drosophila* cell lines (Johnson *et al.*, 2000; Klueg *et al.*, in this issue). In addition, inducible responder expression can be achieved with a copper inducible GAL4 under the control of the *metallothionein* promoter (*MtGAL4*) or *hsGAL4* (Klueg *et al.*, in this issue). Extending the GAL4/UAS system to cell culture expands the utility of both systems and provides additional useful attributes. Responders can be rapidly (1–3 days) tested for expression and/or function via transient transfections prior to the more long-term (1–2 months) generation of transgenic flies. It eliminates the need to shuttle the gene of interest into distinct vectors for expression in flies versus cells. Finally, experimenters
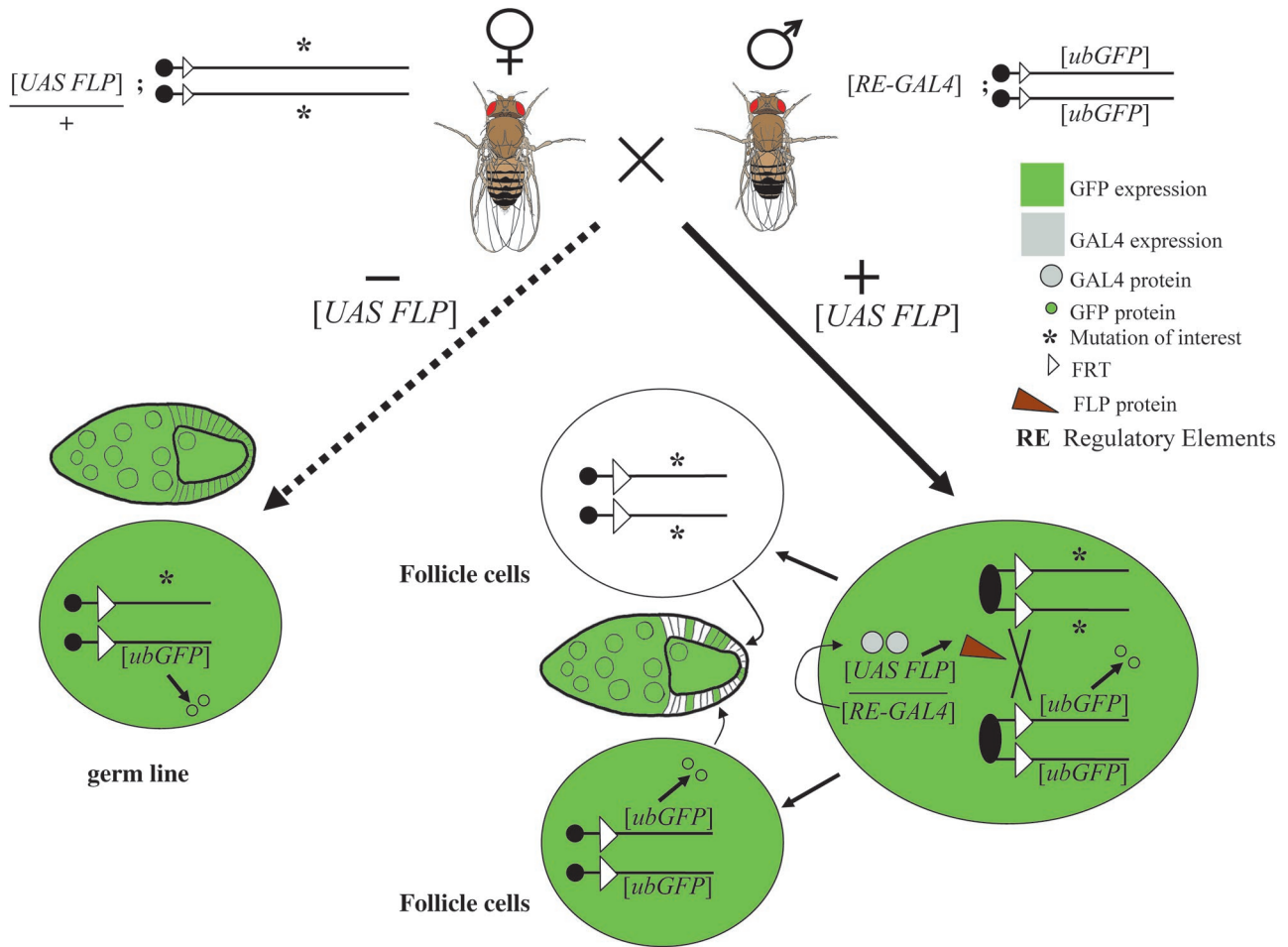
FIG. 8. Generation of directed mosaics. By constructing a *UAS-FLP* responder, mitotic recombination can be driven in a GAL4 dependent fashion. In this figure, GAL4 drives expression of FLP in the stem cells for the somatic follicle cells. Wild-type cells are marked by the presence of GFP, which is expressed under the control of the *ubiquitin* promoter. During mitosis, FLP-mediated recombination in the soma at the proximally located FRT sequences leads to the production of two daughter cells, each homozygous for the corresponding chromosomes. This results in the production of egg chambers that are mosaic within the follicle cell epithelium for the mutation under study (*). These cells, homozygous for this mutation, are then identified by the absence of the GFP marker. In contrast to the soma, FLP activity is absent from the germ line and is therefore composed solely of cells heterozygous for the GFP marker and the mutation under study.

benefit from the vast library of UAS responders already generated by the community.

The Magnifying Glass: Genomic Scale Production of Responders

One of the few limitations in the GAL4/UAS system is the current lack of responder constructs and lines for all genes in the genome. A genetic solution to this problem was initiated by constructing pUAST variants with an altered configuration (Rørth, 1996; Rørth *et al.*, 1998; Toba *et al.*, 1999). One set of these variants (EP and EP^S) contains a UAS element and basal promoter adjacent to one of the P-element inverted repeats. A second variant (GS) has the added benefit of a UAS element and basal promoter adjacent to both of the P-element inverted repeats, allowing for induction of a gene adjacent to either end of the P insertion (Fig. 2). In this configuration, when inserted into the genome these pUAST variants are able to direct the expression of neighboring genes in a GAL4-dependent manner. Currently, a large number of such insertions exist and can be used to assay for misexpression phenotypes with the GAL4 driver of interest. Unfortunately, the insertion specificity of P-elements makes it unlikely that all genes in the genome can be targeted with this approach. However, distinct transposon-based transgenic vectors exist and could be adapted to the GAL4/UAS system to expand the coverage of genes hit by this approach. In addition, the generation of recombination-based cloning technologies and the subsequent adaptation of GAL4/UAS vectors to these
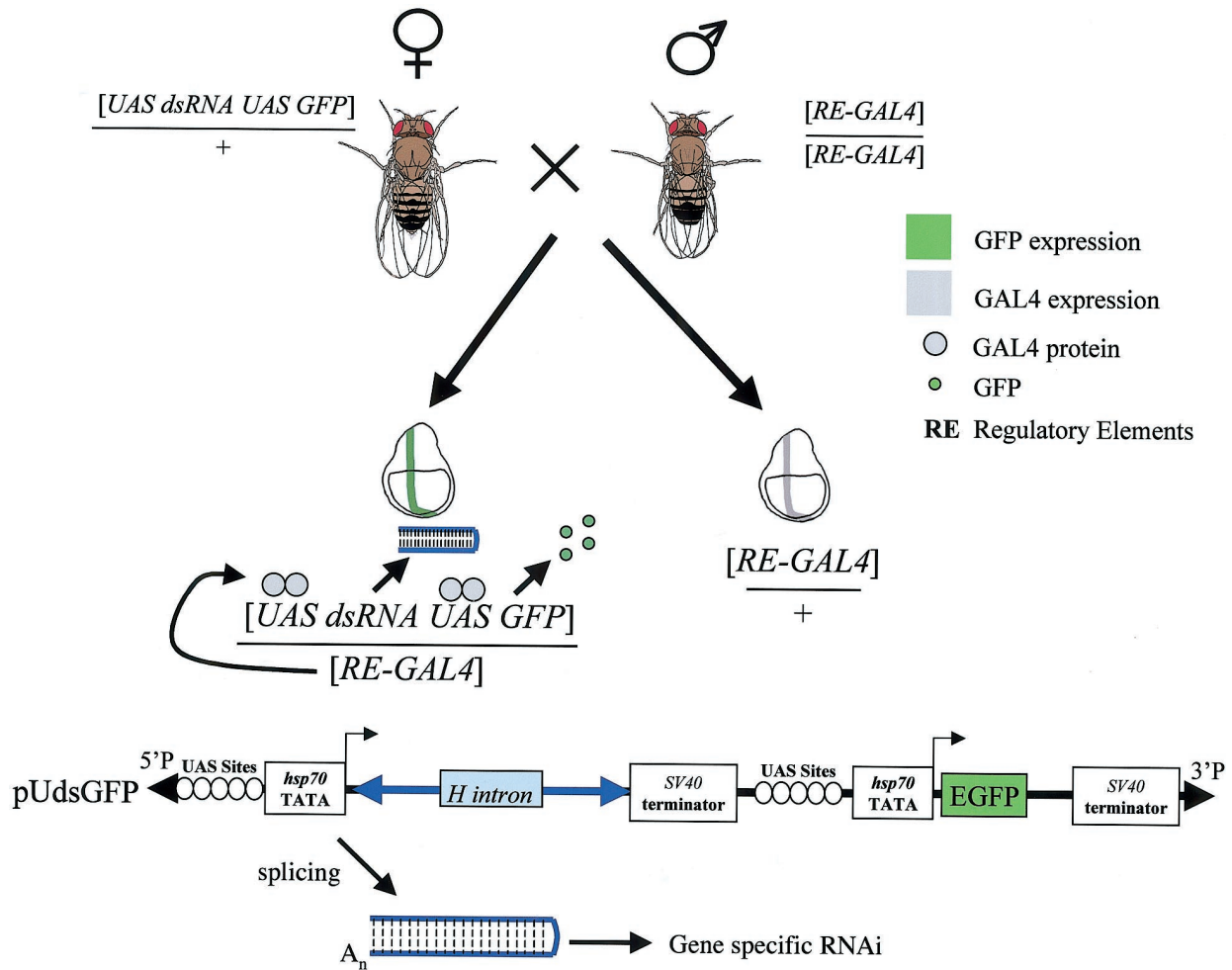
FIG. 9. Splice-activated hairpin formation for directed RNAi. Using the construct pUdsGFP, an intron-separated hairpin is generated for the mRNA to be targeted. When crossed to a GAL4 driver, both the hairpin transcript for the gene of interest and the enhanced GFP marker are expressed in the corresponding GAL4 pattern. Upon removal of the intron (in this case, the 72 bp first intron of the *Hairless* gene) by splicing, hairpin formation occurs and leads to RNAi-mediated knockdown of the gene of interest. Since GFP is under GAL4 control as well, it provides a convenient marker for the cells in which directed RNAi is occurring.

technologies provides an alternative molecular solution to this limitation (see Roman and Davis, this issue, and Fig. 2).

A GENETIC TOOL FOR ALL OCCASIONS

Although the GAL4/UAS system has proven to be a powerful tool for misexpression studies, as described above its utility in other areas has also been extremely beneficial. As a final example, an increasingly powerful use of the system is the identification of loci that interact with the gene of interest through genetic screens for modifiers of a misexpression phenotype. This approach is most commonly based on a "rough eye" phenotype generated by misexpression of a *UAS-gene X* responder using the GAL4 driver *P{GAL4-ninaE.GMR}* (Fig. 10).

This "rough eye" strain is mated to a mutagenized strain and the F1 progeny are screened for dominant mutations that either suppress or enhance the corresponding "rough eye" phenotype. This provides a simple and efficient approach in which large numbers (>100,000) of mutagenized chromosomes can be screened for modifying effects on the phenotype generated by the gene under study. This approach has been used successfully for many *Drosophila* genes and is also being used to address the roles of genes involved in human disease. In a slight twist on this approach, one can also isolate alleles of the gene of interest (*X*) by directly mutagenizing the strain carrying the *UAS-gene X* responder (Guichard *et al.*, 2002; Penton *et al.*, 2002).

Unfortunately, space limitations restrict an adequate coverage of all the uses or adaptations of the system
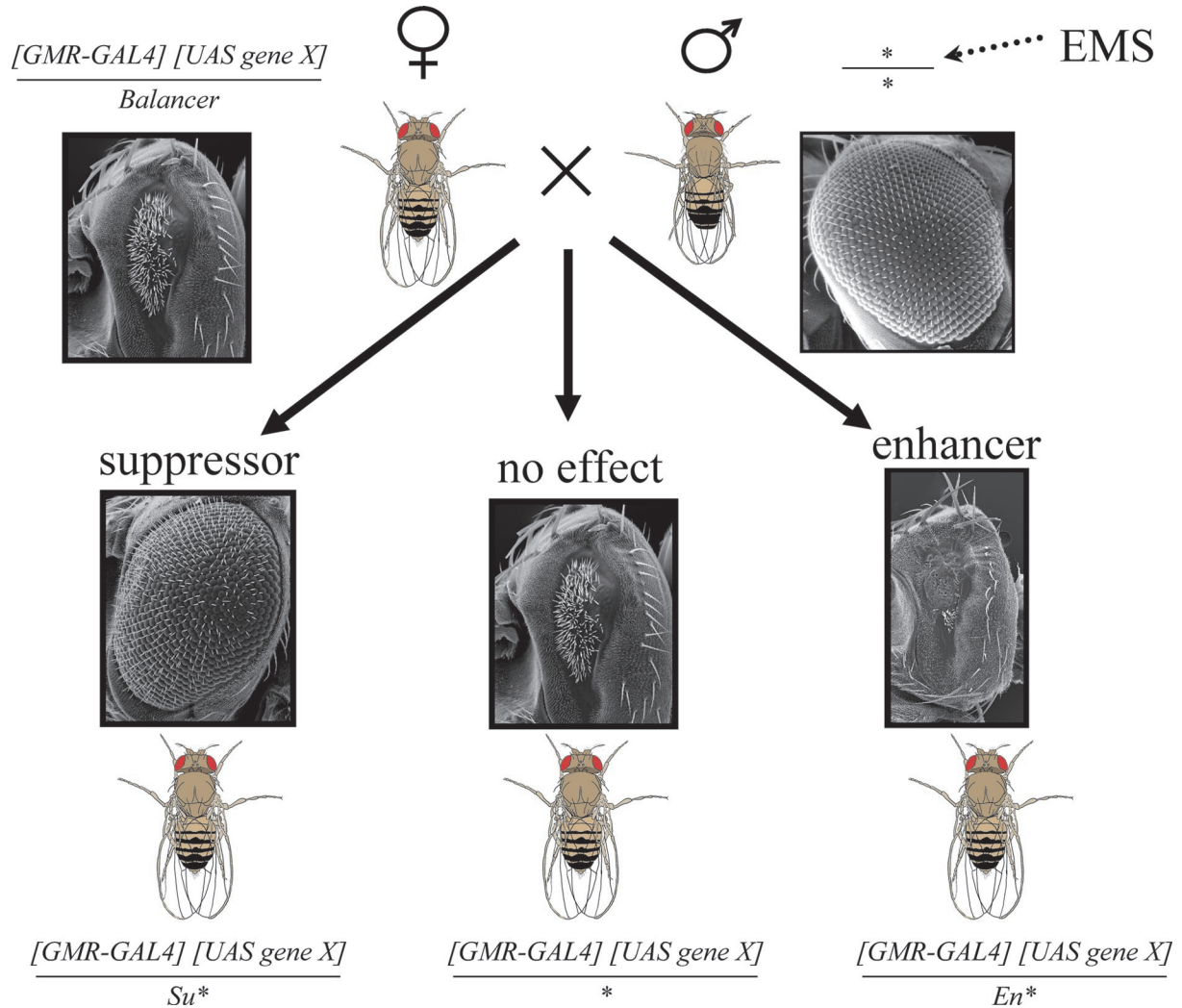
FIG. 10. Screening for modifiers with the GAL4/UAS system. Depicted is an example of screen for modifiers of *gene X* in which GMRGAL4 (*P{GAL4-ninaE.GMR}*) driven expression of *gene X* results in a rough eye phenotype. A recombinant line containing both the GMRGAL4 driver and the UAS *gene X* responder is then crossed to a wild-type strain, which has been mutagenized. The F1 progeny are then screened for modifiers that suppress or enhance the corresponding rough eye phenotype.

here, but the list is extensive and likely to be continually expanded through the creative modifications put in place by the fly community. Briefly, some of these additional uses are: 1) confirmation of gene identity and functional characterization by GAL4/UAS-mediated rescue with wild-type and gene variants as responders; 2) characterization of the physiological or developmental role of specific cell types and populations; 3) cellular marking to aid in screens for mutations affecting the process of interest; 4) production of subcellular markers to investigate cell polarity, vesicular and protein trafficking, chromosomal structure and dynamics, cytoskeletal organization, and membrane structure; and 5) the marking of particular cells and or tissues (using the directed expression of fluorescent reporters) and the creation of opposing phenotypes for use with microarray technologies. This impressive array of uses exemplifies the enormous flexibility of the GAL4/UAS system as an experimental tool and validates its use for the analysis of many biological processes. We can be assured that future additions will continue to make this veritable Swiss army knife an even more essential piece of the fly geneticist's gear.

ACKNOWLEDGMENTS

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