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Targeted gain-of-function screening in *Drosophila* using *GAL4-UAS* and random transposon insertions

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Summary

Alterations in the activity level or temporal expression of key signalling genes elicit profound patterning effects during development. Consequently, gain-of-function genetic schemes that overexpress or misexpress such loci can identify novel candidates for functions essential for a developmental process. *GAL4-Upstream Activating Sequence* (*UAS*)-targeted regulation of gene expression in *Drosophila* has allowed rapid analyses of coding sequences for potential roles in specific tissues at particular developmental stages. *GAL4* has also been combined with randomly mobilized transposons capable of *UAS*-directed misexpression or overexpression of flanking sequences. This combination has produced a genetic screening system that can uncover novel loci refractory to standard loss of function genetic approaches, such as redundant genes. Available libraries of strains with sequenced insertion sites can allow direct correlation of phenotypes to genetic function. These techniques have also been applied to genetic interaction screening, where a *GAL4* driver and *UAS*-regulated insertion collection are combined with an extant mutant genotype. In this article, we summarize studies that have utilized *GAL4-UAS* overexpression or misexpression of random loci to screen for candidates involved in specific developmental processes.

1. Introduction

A major goal of genomic analysis is to identify novel loci and characterize their functions during normal and aberrant cellular processes. However, even for the case of well-established genetic models such as *Drosophila melanogaster* and others, our understanding of the role of most loci is still limited. For this reason, concerted genetic analyses are required to dissect the function and interrelationships of the identified sequences. Conventional genetic studies utilize chemical and radiation mutagenesis to disrupt a process (Greenspan, 1997; Ashburner *et al*., 2005). These mutagens are advantageous as they sample the entire genome and are capable of producing a diverse array of mutations, including both loss-of-function and gain-of-function alleles. Conversely, they are complicated by the cumbersome and time-consuming tasks of mapping and isolating the associated DNA sequences. The advent of *P* transposon-based mutagenesis allowed tagging the mutated sequence that facilitated cloning of the relevant loci (Searles *et al*., 1982; Cooley *et al*., 1988; Yedvobnick *et al*., 1988). However, the original schemes for transposon mutagenesis largely limited the class of mutations to insertional inactivation/loss of function. This limitation excluded production of rare gain-of-function mutations. A transposon-based system that allows both loss-of-function and gain-of-function genetic screening provides significant advantages, since a significant number of loci do not show obvious loss-of-function phenotypes (Miklos & Rubin, 1996). This is possible using specialized transposon vectors, such as those exploiting the *GAL4-Upstream Activating Sequence* (*UAS*) cassette.

The development of the *GAL4-UAS* system in *Drosophila* allowed targeted expression of genes in a wide array of tissues (Brand & Perrimon, 1993).

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The yeast *GAL4* protein is a transcriptional activator that functions in *Drosophila* through interaction with its *UAS* target (Fischer *et al*., 1988). A large number of *Drosophila* strains have been constructed that express *GAL4* in a unique pattern based on the activity of distinct genomic enhancers (Manseau, 1997; Duffy, 2002). When a *GAL4* 'driver' line is crossed to a second transgenic strain containing a coding sequence under *UAS*-regulation, the downstream sequence is expressed in the pattern dictated by *GAL4* expression. This technique has allowed directed expression of mutant versions of proteins in very limited domains, thereby facilitating genetic approaches that would otherwise be precluded by lethality. Brand & Perrimon (1993) validated this idea by expressing an activated form of Dras protein in eye and wing tissues and producing highly penetrant phenotypes that could be subjected to genetic modifier screening.

Rørth (1996) combined *GAL4-UAS* and transposon mobilization to create a misexpression or overexpression genetic screening system. This method required construction of a specialized *P element* transposon named *EP* (Enhancer Promoter) derived from *Casper 4*. *EP* contains multiple copies of the *UAS* target site for *GAL4* upstream of the *hsp70* promoter; it also contains the *mini-white* marker to follow its movements. When *EP* is mobilized to new genomic sites it can position the *GAL4*-regulated *hsp70* promoter upstream of coding sequences. After construction of a library of random *EP* insert lines, each line can be crossed to a specific *GAL4* driver and the F1 progeny scored for phenotypes associated with gene overexpression or misexpression. As changes in the level or temporal expression of key regulatory proteins can elicit profound tissue patterning effects (Fortini *et al*., 1993; Neumann & Cohen, 1996; Nagaraj *et al*., 1999; Hurlbut *et al*., 2007, 2009), this type of screen allows rapid identification of candidate loci necessary for a chosen developmental process. However, as described below, characterization of loss-of-function effects of such candidates is an essential corroboration for overexpression/misexpression analyses. Rørth (1996) first utilized *EP* insertions to study genes involved in eye development through crosses with the *sevenless-GAL4* driver strain that expresses in photoreceptor and cone cells. The *GAL4* line was crossed with a collection of 352 *EP* lines each carrying a single insert. Six sets of progeny exhibited dominant eye phenotypes. Sequencing of the genomic DNA adjacent to the *EP* elements showed that five inserts were close to the transcription start sites of the driven loci. One of these loci was the *Gap1* gene that encodes a Ras GTPase-activating protein, known to function during eye development. This study showed that an overexpression screen is a practical method for finding developmentally relevant genes in a directed, tissue specific manner. After the production of *EP*, additional vectors capable of mediating gene overexpression were designed, including *P*(*Mae-UAS.611*) (Merriam, 1997), *EP yellow* (*EY*) and *EPg* (Bellen *et al*., 2004), *piggyBac WH* (Thibault *et al*., 2004) and the bidirectional *UAS* vectors *Gene Search* (*GS*) (Toba *et al*., 1999) and *XP* (Thibault *et al*., 2004) that are capable of directing *GAL4*-regulated expression from loci on either side of the insertion site (Table 1). The bidirectional vectors offer the obvious advantage of a more rapid scan of loci for potential effects. However, when these insertions land between two loci, more extensive analysis of candidates is required to determine which one is responsible for the effects.

As further demonstrated by Rørth *et al*. (1998) for the case of the *slow border cells* (*slbo*) mutation, overexpression or misexpression screening can be combined with a mutant phenotype to allow searches for genetic modifiers in a mutant/sensitized genetic background. Rørth *et al*. (1998) showed that *slbo* sterility could be suppressed via *EP* directed gene expression mediated by *slbo-GAL4*. Likewise, loss of function phenotypes within the Notch pathway were shown to be enhanced or suppressed by *GAL4*-mediated overexpression of negative or positive regulators respectively, of Notch signalling (Hall *et al*., 2004), shown in Fig. 1. Importantly, overexpression screens can in principle identify both complex pleiotropic loci, and redundant loci that are refractory to most loss of function screening strategies. It has been estimated that two-thirds to three-quarters of *Drosophila* genes are phenotypically silent upon loss-of-function, and some of these effects are due to redundancy (Miklos & Rubin, 1996). Consequently, overexpression screens can complement those based on loss-of-function. These methods now have extraordinary potential for identifying new components of pathways, based on the availability of large collections of transposon inserts at the Bloomington Stock Center, Harvard University and the *Drosophila* Genetic Resource Center at Kyoto. Within these collections insertions in the correct transcriptional orientation and 5′ to the start site are the most likely to produce misexpression/overexpression of a wild-type product. Based on this assumption, it is estimated that from over 29 000 inserts, approximately 5900 protein-coding loci are positioned for *GAL4*-induction within these collections (Table 1). New transposon insertions can also be readily generated using standard mobilization schemes (Alexander *et al*., 2006). However, hotspots for insertion decrease the efficiency of such new screens whereas redundant insertions have been identified in the available collections (Bellen *et al*., 2004; Thibault *et al*., 2004). Here, we review studies utilizing targeted misexpression/overexpression screens in *Drosophila*. The studies are summarized in Table 2.

Table 1. *Summary of potential misexpression/overexpression insertion strains*

Element	Insert no.	Gene no.	Sense orientation	Antisense orientation
XP	8315	2565	1231	1334
GS	6917	2294	1101	1193
WH	7079	3780	617	1279
EY/Epg	3680	3405	2270	568
EP	2437	1645	496	331
pMae-UAS	1206	873	209	226
Total	29 634	–[a]	5924	4931

[a] The overlap of genes between all of the collections has not been determined.
For estimates of insert no. versus gene no. see Bellen *et al*. (2004) and Thibault *et al*. (2004). Insert no. for *XP* and *WH* derives from the original Exelixis collection of Thibault *et al*. (2004). Insert no. for *EY/EPg* represents a selected subset within the Gene Disruption Project strains at Bloomington. Sense orientation was estimated from fraction of inserts 5′ to start site of loci (Bellen *et al*., 2004; Thibault *et al*., 2004), and the fraction of those inserts in the correct orientation to express the locus. This is a conservative estimate since insertions within certain exons and introns of loci can also drive a wild-type gene product. Antisense orientation was estimated from the fraction of inserts 3′ to start site of loci, and the fraction of those inserts in the orientation predicted to produce antisense RNA.
Locations of collections: Bloomington: Gene Disruption Project strains and Exelixis strains (*XP*, *WH*, *EY/EPg*, *EP and pMae*); Harvard: Exelixis strains (*XP*, *WH*); Kyoto Drosophila Genetic Resource Center strains (*GS*, *pMae*); Szeged strains (*EP*). Note: the Szeged Stock Center is closing and the remaining *EP* strains will be re-located.
Updated information on the availability and number of the above strains can be found on the Bloomington Stock Center webpage, the Harvard Medical School Exelixis Collection webpage and the Kyoto Drosophila Genetic Resource Center webpage.

2. General considerations for screens

There are several issues to consider when designing and interpreting a misexpression/overexpression screen. Most importantly, identification of a *GAL4*- induced locus that affects development of a particular tissue does not establish a role for the locus within that tissue. Misexpression or overexpression can produce phenotypes irrelevant to wild-type function (Tseng & Hariharan, 2002; Molnar *et al*., 2006; Mindorff *et al*., 2007; Gregory *et al*., 2007; Stofanko *et al*., 2008), and it is likely that any large-scale screen will produce examples of this effect. Consequently, a normal function for the gene in the identified tissue needs to be corroborated through loss-of-function analysis. Genetic tests for loss-of-function can utilize canonical alleles, imprecise excision-induced deletions of the insertion, or strains derived from several other recent methods (see section 5). Additionally, based on the phenotypes associated with gene induction, a locus may appear to impact a well-described process or developmental pathway. In those cases, effects of expression on mutant phenotypes associated with known pathway components can be used to integrate the locus within the pathway (Abdelilah-Seyfried *et al*., 2000; Kankel *et al*., 2007; Franciscovich *et al*., 2008). If normal expression of the tagged locus has been described in the tissue under study that can provide additional support for a role (Maybeck & Roper, 2009). Further, it is possible that background mutations in an insertion strain contribute to the phenotype. Evidence that induced expression of the tagged locus is solely responsible for the phenotype can be obtained by demonstrating the same phenotype after expression of a defined *UAS*-cDNA or gene construct (Stofanko *et al*., 2008).

Insertion elements can also induce loss-of-function mutations via the insertion process. Such insertions should produce knockout effects independent of *GAL4*, and will be enhanced by other alleles or deletions for the locus. However, another critical consideration in these screens is the potential effects of RNA interference elicited by *GAL4*-induced antisense transcripts. A *GAL4*-regulated transposon that inserts within a transcription unit in the antisense orientation may be capable of producing such an effect, and examples have been discussed (Gregory *et al*., 2007; Maybeck & Roper, 2009). We surveyed the major collections for inserts with such position and orientation, including *EP* (Rørth, 1996), *WH* and *XP*

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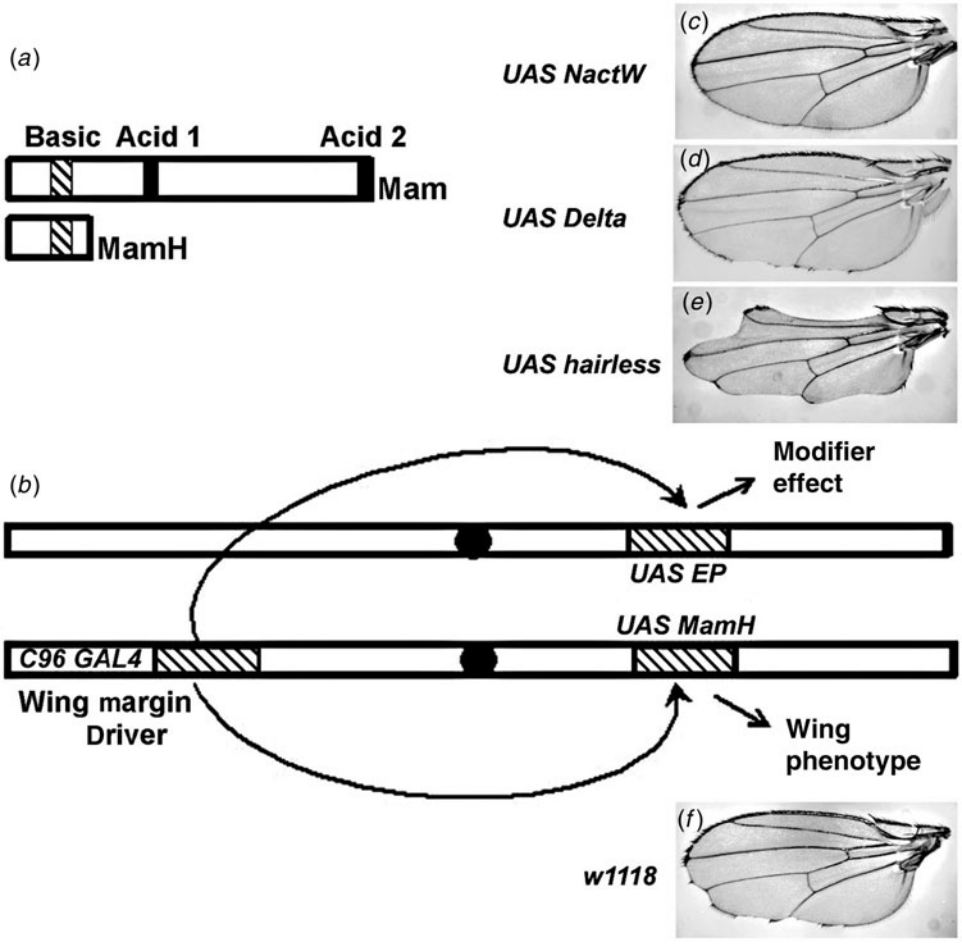

Fig. 1. Combined overexpression-genetic modifier screen using *GAL4-UAS* and *EP*. (*a*) Diagram of full length and truncated forms of Mastermind (Mam) protein. The truncated MamH polypeptide is a potent inhibitor of Notch pathway function when expressed under *GAL4-UAS* regulation in various tissues (Helms *et al*., 1999). (*b*) A genotype constitutively expressing *UAS-MamH* across the dorsoventral margin of the wing imaginal disc is created by recombination with the *C96-GAL4* driver transgene. This recombinant chromosome produces a 100% penetrant, dominant wing nicking phenotype, shown in panel (*f*). In a typical screen individual *EP* strains are crossed to the *C96-GAL4* + *UAS-MamH* recombinant strain and the F1 progeny are scored for changes in the wing phenotype (Hall *et al*., 2004; Alexander *et al*., 2006; Kankel *et al*., 2007). The sensitized screening system can be pre-tested with known pathway components to determine if wing modifications follow predictions. For example, the loss of function *MamH* phenotype is suppressed by coincident expression of activated Notch (*c*) or wild-type Delta proteins (*d*), both of which elevate Notch signalling. Conversely, co-expression of the negative Notch pathway regulator hairless enhances the wing phenotype (*e*).

(Thibault *et al*., 2004), *EY* and *EYg* (Bellen *et al*., 2004) and *GS* and *P*(*Mae-UAS.611*) (Kyoto collection) and estimate that a significant fraction may elicit an RNAi effect (Table 1). Up to one-third of the *GAL4*-targeted loci in these collections may have the potential for RNA-mediated loss-of-function effects rather than misexpression or overexpression. Consequently, it is essential to perform genetic analyses to determine the basis for the *GAL4*-induced phenotypes. An RNA interference phenotype should be *GAL4*-dependent, in contrast to an insertion that produces a phenotype by damaging the locus.

For screens in a sensitized genetic background there are additional considerations. Most importantly, what appears to be a novel genetic interaction needs to be distinguished from a simple misexpression/overexpression effect. This can be checked by crossing the *GAL4* driver strain with the candidate insertion modifier in an otherwise wild-type genetic background. Secondly, effects of insertions on the function of *GAL4* activity itself need to be examined. For example, an insertion that generally decreases or increases transcription can impact the screen's baseline phenotype if that phenotype is produced via *GAL4-UAS*, which is a transcription-based system. Consequently, a suppression or enhancement effect may be elicited through changes in the ability of *GAL4* to activate, rather than by an effect on a gene relevant to

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Table 2. *Summary of screens*

Tissue/process	Element	No. of lines	No. of modifier lines	Reference
Wild-type background screens				
CNS: axon guidance	*GS*	1127	56	Mindorff *et al.* (2007)
CNS/PNS dendrite formation	*GS*	1127	60/43	Ou *et al.* (2008)
Larval motor axon guidance and synaptogenesis	*EP*	2293	114	Kraut *et al.* (2001)
Neuroendocrine remodelling	*EP*, *EY*, *WH*, *XP*	6097	58	Zhao *et al.* (2008)
Adult PNS: external sensory organ development	*EP*	2293	105	Abdelilah-Seyfried *et al.* (2000)
Myogenic development	*EP*	2293	84	Bidet *et al.* (2003)
Muscle patterning	*EP*	4500	78	Staudt *et al.* (2005)
Wing vein	*GS*	12 853	500	Molnar *et al.* (2006)
Eyes: cell cycle regulation	*EP*	2296	46	Tseng & Hariharan (2002)
Cytoskeleton	*EP*	1001	51	Maybeck & Roper (2009)
Haemocyte development	*EP and EY*	3412	108	Stofanko *et al.* (2008)
Anoxia sensitivity	*EP*	1600	4	Huang & Haddad (2007)
Sensitized background screens				
Notch wing margin	*EP*	2300	8	Hall *et al.* (2004)
Notch wing margin	*EP*	2000	3	Alexander *et al.* (2006)
Notch wing margin	*EP*, *WH*	15 500	610	Kankel *et al.* (2007)
Notch eye	*EP*, *WH*	10 447	170	Shalaby *et al.* (2009)
Suppressors of polyQ toxicity	*EP*	7000	59	Kazemi-Esfarjani & Benzer (2000)
Eye cell growth and tumorigenesis	*GS*	Unreported	1	Ferres-Marco *et al.* (2006)
Neuronal plasticity	*EP*	4300	303	Franciscovich *et al.* (2008)
FGF signalling	*EP*	2100	50	Zhu *et al.* (2005)
Chk2 signalling	*EP*	2240	25	Park & Song (2008)
Neuromuscular junction	*GS*	3000	99	Laviolette *et al.* (2005)
Oxidative stress resistance	*P{Mae UAS.611}*	2500	9	Monnier *et al.* (2002)
Rho signalling	*GS*	2190	112	Gregory *et al.* (2007)

the phenotype. Modifiers of *GAL4* can be identified by crossing candidate strains to a *GAL4* driver strain that shows a phenotype by itself within an otherwise wild-type background, such as *GMR-GAL4* (Kankel *et al.*, 2007). Alternatively, the candidate modifier can be tested against an independent *GAL4-UAS* phenotype that is unrelated to the screen phenotype. These are important controls since effects on *GAL4* can be prevalent in screens. For example, in the large-scale screen of the Exelixis library of 15 500 insertions, 294/610 tested insertions (48 %) altered the *GMR-GAL4* eye phenotype (Kankel *et al.*, 2007). In contrast, a screen of a *GS* collection only found 7 % of modifiers acting through a *GAL4* effect (Gregory *et al.*, 2007). Modifiers of *GAL4* phenotypes can include loci encoding global transcription or translation factors, among other examples.

3. Misexpression/overexpression screening in wild-type genetic backgrounds

Transposon-based misexpression/overexpression screening has been used to identify candidates for loci involved in a wide variety of processes such as nervous system and muscle patterning, wing and eye development and immune system development among others. These studies assay the phenotypic effects of random gene overexpression or misexpression in an otherwise wild-type genetic background. Such screens are also referred to as gain-of-function, reflecting the fact that gene expression can be driven at anomalously high levels in a normal site of expression, or alternatively, the gene may be driven at a time or in a tissue that it is normally not expressed.

(i) *Nervous system*

The development of the central nervous system (CNS) requires an intricate and complicated system of regulation, specifying cell fate and creating proper axon guidance. In particular, axons are guided to their correct targets by many environmental and signalling cues. Since the nervous system depends on specific patterning of synaptic connections, appropriate cues for axon development and synaptic partner formation are crucial.

Mindorff *et al.* (2007) used the *scrt*$^{11\text{-}6}$*-GAL4* driver, a *PGawB* insertion in *scratch*, which expresses *GAL4* throughout the CNS and peripheral nervous system (PNS) of the developing embryo. Over 1000 *GS* lines were tested and 142 insertions caused lethality. Lethal embryos were analysed by staining for Fasciclin 2,

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which is expressed strongly on all motorneurons and on a set of interneurons. Immunostaining of the nervous system showed that 56 (39%) of the lethal lines produced axonal defects, representing 51 unique genes. Thirty-eight of the genes had been previously studied, and 17 were implicated in the development of the nervous system. This set includes loci essential for neurogenesis, such as *mam* and *H*, and neural differentiation, including *mirr* and *pnt*. The identification of *leak* (*roundabout 2*), encoding the Slit receptor validated the scheme, which also found 13 novel genes, predicted to encode secreted and transmembrane proteins. The screen identified a large number of loci encoding transcription factors, including the NFκB product of *dorsal*. Additional analyses of *dorsal* misexpression in the visual system using *GMR-GAL4* revealed mistargeting of photoreceptors cells R2–R5 to the medulla, rather than the lamina plexus layer of the brain optic lobe. However, a role for *dorsal* in axon targeting was not corroborated with loss-of-function mutations, indicating that high levels of *dorsal* expression may perturb the normal function of other essential loci.

Ou *et al*. (2008) used an overexpression screen to find candidate genes for dendrite morphogenesis in the CNS and PNS. Although many factors have been implicated in dendrite development, including transcription factors, GTPases, and cytoskeleton-associated proteins, current knowledge of their morphogenesis is incomplete (Grueber & Jan, 2004; Williams & Truman, 2004; Parrish *et al*., 2007). Additionally, dendrites of the CNS differ from those of the PNS, as the former are not specialized for receiving external stimuli (Hughes & Thomas, 2007). Thus, it is important to ascertain if the development of these distinct dendrite types involve the same mechanisms. Two overexpression screens were conducted using 141 *GS* lines, one in the da neurons of the PNS and the other in RP2 motor neurons of the CNS. These 141 derived from a prescreen of 1127 lines for those producing lethality when expressed in the CNS (Mindorff *et al*., 2007). Multiple *GAL4* drivers were used, including *ppk1.9-GAL4*, which expresses in dendritic sensory neurons tiling the larval body wall. The gain-of-function screen in da neuron dendrites found 35 unique loci causing mutant phenotypes of five classes. These five classes resemble mutant phenotypes of known genes involved in dendrite growth. Inserts at *tramtrack*, encoding a zinc finger protein, resembled *shrub* mutants, which have reduced embryonic dendrite growth (Brenman *et al*., 2001; Sweeney *et al*., 2006). Inserts at six loci, including *cbt*, encoding a zinc finger transcription factor, are similar to mutations of Polycomb Group genes (Parrish *et al*., 2007). Six additional targeted loci including *abrupt* and *Hr38* elicited phenotypes related to *cut* mutants, and one *GS* line induced phenotypes similar to mutations of *spineless* with both increases and reductions in dendrite growth (Kim *et al*., 2006*a*). A last class included one line that resembled constitutive activation of the GTPase Cdc42, leading to reduced embryonic dendritic branching (Gao *et al*., 1999). In the RP2 CNS neuron screen, 51 genes elicited phenotypes, including a large fraction of loci encoding transcription factors such as *mastermind* and *pointed* (Bilder *et al*., 1998; Helms *et al*., 1999). None of these genes have been previously shown to affect dendrite growth, but further testing indicated that at least 43 of the 51 candidates are normally expressed in the CNS during periods of dendritic development. Overall, 39% of the genes identified were the same in the two screens, although the varying phenotypes suggest that mechanisms of development for dendrites of the CNS and PNS only partially overlap.

Kraut *et al*. (2001) designed a gain-of-function screen to alter synaptic connections between motor neurons and muscles. Two thousand two hundred and ninety-three *EP* insertions were driven in motor neurons via *elav GAL4*, which expresses in all post-mitotic neurons. To observe the neuromuscular junction (NMJ), the *elav-GAL4* driver strain was combined with *UAS-Green Fluorescent Protein* (*GFP*). This allowed the NMJ to be visualized in live third instar larvae or after dissection and staining with anti-*GFP*. One hundred and fourteen *EPs* produced strong mutant phenotypes. Three classes were noted: errors in pathfinding, morphologically altered, reduced or missing synapses, and excess or ectopic synapses. Most insertions produced phenotypes in more than one class. The *EPs* landed adjacent to 41 known genes, of which more than three quarters are required for nervous system development based on loss-of-function analyses. Sixteen of these loci affect axon or synapse formation, including *Fasciclin 2*, *neurexin*, *Laminin A* and also *roundabout 2*. Thirty-five new genes, without a characterized loss of function phenotype were also identified, including sequences encoding kinases, phosphatases, ATPases, Rho family GTPases and others involved in protein modification or degradation.

Zhao *et al*. (2008) used misexpression in a screen for candidate genes involved in metamorphic remodelling of the neuroendocrine system. The processes of molting and metamorphosis require the action and precise timing of the juvenile hormones and ecdysteroids family of hormones (Nijhout, 1994). Proper levels of ecdysis-triggering hormone (ETH) and crustacean cardioactive peptide (CCAP) are required for normal ecdysis (Kim *et al*., 2006*b*). Following ecdysis, neurons secreting CCAP and other neuropeptides such as bursicon, which functions during wing expansion, undergo major changes during the pupal stage (Luan *et al*., 2006). Though it is understood that a myriad of extensive hormone and neuropeptide changes occur throughout metamorphic remodelling,

the mechanisms are not fully described. The four *GAL4* drivers: *EH-GAL4*, *CCAP-GAL4*, *c929-GAL4* and *386Y-GAL4* directed transposon expression to various neuronal and peptidergic cells. This includes neurons in the brain and ventral nerve cord, endocrine cells in the corpora cardiaca, endocrine Inka cells, midgut cells and PNS neurons. These were tested with 6000 insertion lines, including *EP*, *EY*, *WH* and *XP* to identify potential regulators of ecdysteroid-dependent metamorphosis of neuropeptidergic cells. The screen revealed over 50 loci whose misexpression caused defects either in ecdysis or wing expansion. Additionally, 14 loci were found to disrupt the CCAP/bursicon neuron cell projections during metamorphosis. Of these loci, genes known to be involved in neuronal development were found, including *cabut*, an ecdysteroid-response gene, and *fat facets*, encoding an ubiquitin-specific protease (Hegde, 2004; Munoz-Descalzo *et al*., 2005). Several of the loci had not been previously implicated in neuronal remodelling. This includes three novel inserts driving expression of micro-RNAs, small non-coding RNAs that play diverse regulatory roles (Kloosterman & Plasterk, 2006). Additionally, two loci *mip120* and *stonewall* code for proto-oncoprotein-like transcription factors responsible for signalling by Myb-like protein complexes.

A PNS screen was conducted to find genes involved in adult mechanosensory bristle development (Abdelilah-Seyfried *et al*., 2000). External sensory (es) organs are comprised of a neuron and support cells. The es organ develops from a single sensory organ precursor (SOP) cell that is chosen from an equipotent proneural cluster during the process of lateral inhibition (Ghysen & Dambly-Chaudiere, 1989). The SOP cell divides and asymmetrically differentiates into distinct daughter cells, with the help of Notch signalling (Hartenstein & Posakony, 1989) and other genetic regulators. Over 2000 *EP* insertions were misexpressed in the SOP and surrounding cells using *scabrous-GAL4* (*sca-GAL4*). Abdelilah-Seyfried *et al*. (2000) observed that 105 (4·6%) of the *EPs* produced mutant phenotypes, exhibiting either a loss of external support cells, increase in es organs or support cells, or increase/decrease in a cell type after cell transformation. The 105 *EPs* tagged 78 unique loci, associated with lateral inhibition, cell differentiation, cell cycle regulation and es organ formation. Thirty-seven loci were previously characterized, including those implicated in es organ development. These include *emc* and *big brain*, known to have roles in lateral inhibition (Skeath & Carroll, 1991; Rao *et al*., 1992). *Big brain*, a member of the aquaporin family, influences endosome maturation and Notch activity (Kanwar & Fortini, 2008). Other candidates not previously associated with es organ development were also found, such as *hedgehog*, originally defined for its roles in segment polarity and eye morphogenesis (Mohler & Vani, 1992; Heberlein *et al*., 1993) and *yan*, which encodes a transcription factor identified for its role in photoreceptor cell development (Lai & Rubin, 1992).

(ii) *Muscle*

EPs have been used to drive loci in mesodermal cells with the goal of finding regulators of muscle and heart cell fate specification (Bidet *et al*., 2003). Normal myogenic development involves an array of cell–cell signalling and mesoderm-specific transcription factors, which create distinct competence domains. These domains eventually transform into clusters of equipotent cells via receptor tyrosine kinases (RTKs), epidermal growth factor receptor (EGFR) and fibroblast growth factor receptor (FGFR), which impinge on the mitogen-activated protein kinases (MAPKs) pathway (Carmena *et al*., 1998). In these domains, one cluster will express the homeodomain transcription factor Even-skipped (Eve). In the same competence domain are cells that express the homeobox gene *ladybird* (*lb*). In normal development, *lb* and *eve* are never co-expressed, as they specify cell lineages. Bidet *et al*. (2003) compared the expression pattern of these homeobox genes in *EP* overexpression genotypes relative to wild-type. *24B-GAL4* was used to drive expression of 2293 *EP* insertions ubiquitously in mesodermal cells, including myogenic cell precursors. After scoring protein accumulation, 84 (3·7%) of the *EPs* were found to alter expression profiles in *lb* and/or *eve*. The insertions were proximal to 31 known genes and 18 predicted genes, and 80% of the identified candidates have vertebrate orthologues, suggesting essential functions. Among them is *rhomboid*, which is required for maturation and release of EGF signals, *yan*, which encodes an ETS transcription factor/negative RTK effector and *rac2*. Rhomboid and Yan are hypothesized to interact with Rac2, a Rho GTPase in the RTK signalling pathway during diversification of cardiac and muscle cell lineages. The majority of the other candidate genes encode DNA/RNA binding factors or proteins involved in signalling. For example, the *extra macrochaetae* (*emc*) gene, encodes a helix-loop-helix (HLH) transcription factor that does not contain a basic DNA-binding domain (Garrell & Modolell, 1990; Cubas *et al*., 1994). The absence of the DNA binding domain allows Emc to dimerize with other bHLH proteins and down-regulate their activity. This is consistent with an Emc role in binding and sequestering general myogenic bHLH factors in the mesoderm.

Staudt *et al*. (2005) utilized *EP*-elements driven in the epidermis to find genes implicated in muscle patterning. Muscle cell development involves processes where founder cells fuse with undetermined muscle cells to form myotubes; these myotubes migrate

towards and attach with a set of epidermal border cells termed apodemes (Schnorrer & Dickson, 2004). These processes are guided by a variety of genes, including those involved in transcription such as *stripe*, and cell communication, including *slit*/*robo* and *fibroblast growth factor* (FGF). To find additional loci involved in this process, Staudt *et al*. constructed *stripe-GAL4* drivers to express in apodeme precursors and a group of cells that serve as substrate for muscle cell migration. Combining the drivers with 4500 *EPs* (3700 unique *EP* insertions), they looked for inserts that caused lethality, as disruption of muscle patterning prevents hatching. Antibody (anti-Myosin) staining of the differentiated but unhatched embryos was used to analyse for muscle pattern defects. Sixty-six (1·5%) *EPs* caused unambiguous gain-of-function phenotypes. This included 13 genes that code for membrane associated and secreted factors, eight coding for factors involved in protein modification, six coding for transcription factors, three coding for cytoskeleton binding proteins, and seven coding for functions involved with cell cycle control and biosynthesis. The screen also identified known muscle pattern formation genes such as *Toll*, *gut feeling*, and *sulphateless* and genes involved in cell migration and/or embryonic cell targeting including *esg* and *sdc*.

(iii) *Wing and eye*

Wing and eye development are popular choices for genetic screens since alterations in both structures are readily scored in adults, and formation of the wing and eye requires numerous signalling pathways that are used reiteratively during development (Neumann & Cohen, 1996; Nagaraj *et al*., 1999; Freeman, 2005; de Iongh *et al*., 2006; Jemc & Rebay, 2006). Thus, gain-of-function screens in these tissues can allow detection of adult phenotypes associated with key loci that are normally early lethals when mutated. Molnar *et al*. (2006) screened for genes involved in wing vein development. They expressed 13 000 *GS* insertions using *GAL4-shv*$^{3\ kpm}$, a *GAL4* driver expressing in the developing pupal veins. This screen uncovered 493 (4·2%) insertions that elicited modified wing vein phenotypes. The elements mapped to 254 insertion sites with 149 being single hits. Remarkably, of the identified genes, ~60% belong to the Notch, EGFR and decapentaplegic (Dpp) signalling pathways, previously known to affect vein formation. The remaining classes of loci, including numerous uncharacterized sequences, were enriched for putative functions in transcription and cell signalling.

Tseng & Hariharan (2002) screened for loci that restrict cell growth or cell-cycle progression in the eye by looking for a small eye phenotype in adults. They employed *EP* lines driven throughout eye development using *eyeless-GAL4*. Of the 2296 *EP* lines tested, 46 (2·0%) displayed reduced eye size of varying severity. The 46 *EP* lines identified 32 different loci and 13 were in previously characterized genes. Some of these loci, such as *hedgehog, dpp* and *fringe* were known to be involved in eye development (Cho & Choi, 1998). Two putative transcriptional regulators, *Kruppel-homolog 1* and *elbowB*, and two regulators of the cytoskeleton, *Rac2* and *pebble*, (Lehner, 1992; Harden *et al*., 1995) were also identified. Nineteen of the loci were only identified via sequence as novel open reading frames.

(iv) *Other tissues*

A study of cytoskeleton formation employed 1001 *EP* insertions to identify genes required for salivary gland tubulogenesis (Maybeck & Röper, 2009). The *EP* strains were crossed to *forkhead-GAL4,* which targets expression in the embryonic salivary glands, and also combined with either a *GFP* marker of the microtubule cytoskeleton (*GFP-EFGas2*) or a cell shape marker (*SrcGFP*). Fifty-one (5%) of the *EPs* produced significant mutant phenotypes when misexpressed, classified as either invagination defects, gland shape and lumen defects, position defects, or gland sub-fate defects. Of the 51 lines, seven corresponded to genes previously implicated in salivary gland morphogenesis: *chickadee*, *tec29*, *doughnut on 2*, *rhomnoid1 and spitz*, *tap* and *slit*. Forty-four *EP* lines corresponded to genes that encode proteins not previously associated with tubulogenesis, such as Egalitarian, Traf-4 and RanGAP, having functions in microtubule-based transport, Toll-like receptor signalling and nuclear transport, respectively (Minakhina *et al*., 2005; Takeshita *et al*., 2005). In addition, 14 uncharacterized sequences were found with orthologues in other species. The range of potential functions identified in the screen was broad, including cytoskeletal and transcription factors, proteins involved in signalling, protein synthesis and degradation, membrane proteins, trafficking proteins and micro RNAs of the *mir-310* cluster, previously implicated in epithelial morphogenesis.

Stofanko *et al*. (2008) analysed the immune system by screening for genes regulating larval haemocyte migration and differentiation. Haemocytes mediate defence against foreign pathogens. There are three types: plasmatocytes, crystal cells and lamellocytes and their differentiation requires numerous transcription factors such as Lozenge, Collier and the GATA factor Serpent. In the open circulatory system of *Drosophila* larvae, the majority of haemocytes freely circulate within the haemocoel; the rest remain sessile in patches or 'islets.' Haemocyte migration is regulated by either a response to signals from growth factors or a response to chemicals released due to wounding. However, the regulators that control

haemocyte differentiation and attachment to the sessile islets are not well described. Using a *Peroxidasin-GAL4* driver combined with *UAS GFP*, over 3400 *EP* and *EY* lines were expressed in plasmatocytes and crystal cells. One hundred and eight insertions, representing 101 loci were found to affect haemocyte development. The insertions caused disruption of sessile haemocyte compartments, changes in haemocyte number, improper positioning of haemocytes and changes in lymph gland size. Fifty-five *EPs* perturbed the formation of dorsal sessile haemocyte compartments; affected loci included *RhoGeF2*, involved in cell shape and mesodermal invagination during gastrulation (Barrett *et al*., 1997; Hacker & Perrimon, 1998) and *C3G*, a Ras family nucleotide exchange factor (Ishimaru *et al*., 1999). Overexpression of 36 loci increased haemocyte numbers, whereas 53 loci increased lymph gland size. Classification of the 101 candidate genes by gene ontology (GO) showed that more than half of the genes had no previously described function. Of the genes that did have described functions, the most abundant encoded transcription factors/nucleic acid binding proteins, such as *Kr*, *esg*, *chn* and *broad*.

Huang & Haddad (2007) used overexpression to screen for genes involved in anoxia sensitivity. *Drosophila* embryos can survive in severe hypoxic conditions for days and adult flies can survive without oxygen for hours without apparent injury (Haddad *et al*., 1997; Krishnan *et al*., 1997). Though *Drosophila* is known to be tolerant to hypoxia, the mechanisms to sense and respond to low O_2 are relatively unknown. Using *daughterless-GAL4* (*da-GAL4*), which expresses ubiquitously, 1600 *EPs* were overexpressed throughout development. The screen measured changes in the time required for flies to recover after oxygen deprivation. Four *EP* lines showed significantly longer recovery times. Remarkably, each of these *EPs* drove the same downstream transcript, CG14709, encoding a protein in the multidrug resistance protein (MRP) subfamily, a member of the ATP-binding cassette (ABC) transporter superfamily (Dean *et al*., 2001; Dean & Annilo, 2005). Other studies have suggested that CG14079 is an orthologue of MRP4/ABCC4, responsible for nucleotide transport (Dean *et al*., 2001). However, a role for CG14079 (renamed *Drosophila anoxia-sensitive dMRP4*) in response to hypoxia had not been characterized. CG14079 was overexpressed only in neurons using the *elav-GAL4* driver and a similar delayed recovery to oxygen deprivation was observed, demonstrating that overexpression in neurons alone causes the anoxia-sensitive phenotype. Interestingly, CG14079 had been identified in an earlier gene overexpression screen (Monnier *et al*., 2002). This screen of 2500 *P*(*Mae-UAS-6.11*) elements driven ubiquitously by *da-GAL4* was designed to find loci that affected lifespan in the presence of excess reactive oxygen species via exposure to H_2O_2. Five lines were characterized, with overexpression of CG14079 leading to increased sensitivity to oxidative stress. These studies suggest a potential link between responses to both anoxia and oxidative stress.

4. Misexpression/overexpression screening in sensitized genetic backgrounds

GAL4-regulated overexpression/misexpression is also applicable to genetic interaction screening. To implement a sensitized screen, a *GAL4* driver and *UAS*-regulated insertion collection are combined with a canonical mutant genotype, or a *UAS*-regulated construct that produces a phenotype readily scoreable for changes, such as a dominant-negative eye or wing phenotype. In designing a sensitized screen, it is crucial to select a phenotype that can be pretested for predicted responses to gain-of-function and loss-of-function modifiers. For example, expression of a dominant-negative construct should down regulate a pathway and produce a loss-of-function phenotype. This phenotype is predicted to be suppressed by overexpression of positive effectors of that pathway, or enhanced by overexpression of negative effectors (Fig. 1). Likewise the phenotype associated with the construct should respond appropriately to loss-of-function mutations in pathway components. Thus, sensitized screens that investigate processes that are genetically well described are more likely to succeed.

(i) *Wing*

Hall *et al*. (2004) and Alexander *et al*. (2006) screened for Notch pathway components by each testing approximately 2000 *EP* insertions for their ability to modify a wing nicking phenotype (Fig. 1). The wing phenotype derived from dorsal–ventral wing margin expression of a truncated version of the Mastermind (Mam) protein. Mam contains an amino terminal charge cluster of basic amino acids that mediates a physical association with the intracellular domain of Notch (Kitagawa *et al*., 2001). A shortened version of Mam (*UAS-MamH*) that terminates directly after the basic domain acts as a dominant negative for Notch pathway function when expressed under *GAL4* regulation (Helms *et al*., 1999). The *C96-GAL4* driver was used to express *UAS-MamH* across the developing wing margin and to simultaneously drive the library of single *EP* inserts. These screens identified canonical Notch pathway components such as Delta and Mam, as well as other functions known to impact Notch signalling, including the glycosyltransferase Fringe (Moloney *et al*., 2000) and the negative regulator of EGF receptor function, Kek1 (Ghiglione *et al*., 1999). More novel loci that influence Notch signalling were also identified, such as *domino*, which encodes

products of the SW12/SNF2 class of DNA-dependent ATPases (Ruhf *et al*., 2001), *poils aux pattes* (*pap*) which encodes a protein related to the TRAP 240 component of the mediator transcription complex (Boube *et al*., 2000) and a novel *Minute* locus encoding ribosomal protein L13a.

The *GAL4-UAS*-Mam truncation-based screen for Notch pathway modifiers was greatly expanded by Kankel *et al*. (2007), who utilized the Exelixis collection of transposon insertions as the source of modifiers (Thibault *et al*., 2004). This collection derives from several transposable elements with different insertion sequence site preferences. It contains 15 500 unique insertions, in or near 53% of *Drosophila* coding regions. As above, dominant negative Mam was driven via *C96-GAL4* across the wing margin and tested against the insert library. Insertions targeting 408 specific loci identified 31 previously characterized Notch interactors. Additionally, this screen identified 160 novel candidates for loci that affect Notch signalling, including those with RNA recognition motifs, linking Notch to RNA processing. Associations between Notch, cell proliferation and other pathways such as EGF receptor were also uncovered. Significantly, a novel class of interactors was found that appeared specific to loss of *mam* function, rather than Notch signalling. One example of this involved the *armadillo* (*arm*) locus, encoding the *Drosophila* β-catenin homolog. A *mam*-specific interaction with *arm* was also seen cross-species using a cell culture assay.

(ii) *Eye*

Kazemi-Esfarjani & Benzer (2000) screened for suppressors of polyglutamine (polyQ) toxicity mediated by a *UAS*-driven run of 127 CAG codon repeats. The transgene encoding a polyQ product was driven in all cells of the retina by *GMR-GAL4*, which contains the *rhodopsin1* gene enhancer. Flies co-expressing these constructs exhibit a severely disrupted eye morphology that is associated with aggregates of polyQ. To look for modifiers of this rough eye phenotype, 7000 *EP* transpositions were generated and each was tested as a transheterozygote for modification of the *GMR-GAL4 + UAS-polyQ* phenotype. Fifty-nine modifiers were identified and two suppressor loci were characterized further: *dHDJ1* that encodes a protein homologous to the human HSP40/HDJ1 and *dTPR2* that encodes a product related to human Tetratricopeptide 2. These results were of interest since both proteins contain a J domain, found in chaperone proteins, and their activity could influence polyQ aggregate formation.

Ferres-Marco *et al*. (2006) employed Delta overexpression in the eye to screen for factors that contribute to cell growth and tumorigenesis. *GAL4* under *eyeless* (*ey*) regulation was used to drive Delta anterior to the morphogenetic furrow and create a large eye phenotype. The *ey-GAL4* construct simultaneously drove random inserts of the bidirectional *GS* vector. The screen led to identification of an insert that overexpressed both the *longitudinals lacking* (*lola*) and *pipsqueak* (*psq*) loci, which produced invasive tumours derived from eye tissue when co-expressed with *Delta*. The *lola* and *psq* loci each encode multiple forms of transcription factors that contain a BTB domain. The BTB domain has been previously associated with epigenetic silencing of loci through recruitment of Polycomb Group and HDAC complexes (Melnick *et al*., 2002). Ferres-Marco *et al*. (2006) identified the *Retinobastoma family protein* (*Rbf*) as one key target for gene silencing in these eye tumours. They also found that Delta-Notch signalling contributes to *Rbf* silencing through hypermethylation of DNA around the promoter, and suggested that both gene-silencing events contribute to malignancy.

A sensitized misexpression screen was done to study genes involved in neuronal plasticity, the process in which neural circuits change in response to environmental alterations (Franciscovich *et al*., 2008). Stable changes in neurons involve transcription or translation of specific products, and a crucial transcription factor AP-1, a heterodimer of Fos and Jun, plays diverse roles in their plasticity (Sanyal *et al*., 2002; Etter *et al*., 2005). However, despite knowledge of the various roles of AP-1, little is known about the upstream or downstream factors that mediate its effects in neurons. This study used a *UAS*-activated dominant negative truncation of the Fos gene, which inhibits AP-1, to generate a small adult eye phenotype when combined *eyeless-GAL4* (Eresh *et al*., 1997; Sanyal *et al*., 2002). Four thousand three hundred *EPs* were tested for rescue or enhancement of the eye phenotype. Genes known to function solely in eye development were not further investigated. Candidates from this screen were also driven with $elav^{C155}$*-GAL4* in post-mitotic neurons to examine effects in neural development. The screen found over 300 candidates, with 249 verified and 54 predicted genes. Several products previously implicated with AP-1 function were found, such as Ras85D, Bsk (dJNK), CycB and Men, confirming the validity of this screen. Ten suppressors and 15 enhancers were further tested in the NMJ. Thirteen of these resulted in mutant synaptic phenotypes. Two genes producing the strongest phenotypes, *sprouty* and *shaggy*, are inhibitors of signalling cascades. *Shaggy*, a GSK3-β kinase, was shown to inhibit AP-1-dependent synaptic growth via interactions with the Jun-N-terminal kinase pathway.

Zhu *et al*. (2005) screened for genes involved in the FGF signalling pathway. The two FGF transmembrane receptors, Breathless (Btl) and Heartless (Htl),

phosphorylate target kinases upon ligand binding. Signalling involves the cytoplasmic molecule downstream-of-FGF-receptor (Dof) and others such as Corkscrew (Battersby *et al*., 2003; Petit *et al*., 2004; Wilson *et al*., 2004). The signalling mechanism from the activation of the FGF receptor to its intracellular targets was investigated. Although FGF signalling is not required for eye development, Zhu *et al*. genetically targeted this tissue for their modifier screen. *GMR-GAL4* (Freeman, 1996) was used to drive constitutively activated Btl plus Dof in the eye, eliciting a rough eye phenotype (Casci *et al*., 1999). This genotype was then combined with 2100 *EPs*, and 26 enhancers' plus 24 suppressors of the rough eye phenotype were identified. The candidate genes encode proteins of diverse functions, including kinases, transcription factors, membrane proteins and mitochondrial proteins. Only a minority of these modifiers interacted with an eye phenotype produced by ectopic EGFR expression, suggesting that the loci scored did not overlap in function with this pathway. In contrast, more significant interactions were observed with an eye phenotype produced from an activated form of PVR, the receptor for both platelet-derived and vascular endothelial growth factors. In follow up analyses, two of the modifier genes, *sar1* and *robo2*, encoding a GTPase and a protein involved in axon guidance respectively were further examined. Loss of function for these loci produced genetic interactions with a *dof* hypomorphic allele, affecting endogenous FGF signalling within the trachea of embryos, validating the genetic scheme.

Park & Song (2008) screened for genes involved in the *checkpoint* (*Chk2*) signalling pathway, which plays key roles in DNA damage response. Upon phosphorylation following DNA damage, the Chk2 receptor protein kinase relays signals to a variety of downstream proteins to bring about DNA repair, cell cycle arrest and apoptosis (Sancar *et al*., 2004). Due to the critical functions of Chk2 signalling, pathway genes are involved in tumorigenesis or have oncogenic properties. To search for undocumented members of the Chk2 pathway, Park & Song (2008) used the *GMR-GAL4* driver to express *UAS-Chk2*, producing a rough eye phenotype, and to simultaneously drive *EPs*. The screen was validated using a mutation in the ATM-kinase, which is required for activation of Chk2. The mutation suppressed the rough eye phenotype. A counterscreen of *EPs* against a rough eye phenotype derived from *UAS-Notch* expression was also employed to eliminate modifiers common to Notch signalling. Using 2240 *EPs*, they identified 25 candidate genes not previously associated with Chk2 signalling; 22 *EPs* suppressed and three enhanced the original phenotype. Several of the inserts led to defects in G2 arrest after irradiation, implicating the loci in the response to DNA damage. One of the enhancers, *ballchen* (Aihara *et al*., 2004), encodes a protein kinase that phosphorylates histone H2A, implicated in the DNA repair response (Tanaka *et al*., 2007). The suppressors included *calmodulin*, which may modulate cell cycle progression via spindle effects (Goshima *et al*., 2007), *kayak* (*Drosophila* Fos), which functions at the G2-M transition (Hyun *et al*., 2006) and *melted*, which regulates cell growth via effects on the insulin-signalling pathway (Teleman *et al*., 2005).

Shalaby *et al*. (2009) used the Exelixis collection in a screen for new Notch pathway modifiers. Their study utilized a Delta gain-of-function phenotype mediated via *GMR-GAL4* driven Delta overexpression posterior to the morphogenetic furrow. This expression results in a dominant eye phenotype that derives from cell fate alterations associated with aberrant Notch signalling. After outcrossing to the insertion library they obtained 170 candidates, including loci known to function in Notch signalling, such as *numb* and *kuzbanian* as well as a number of novel candidates. Two hormone receptor loci, *Hr38* and *Hr39*, and others involved in intracellular trafficking, including *Vha68-2*, which encodes a component the v-ATPase proton pump complex were found. A large number of eye modifiers were not identified in the Kankel *et al*. (2007) screen, possibly reflecting the different tissue contexts (eye versus wing). One modifier was studied in some detail: CG2446 (*Amun*). *Amun* is predicted to encode a DNA glycosylase, an activity associated with base excision repair processes, and also transcriptional regulation. Evidence was presented linking *Amun* to the regulation of Achaete, a transcription factor necessary for specification of sensory cells within equivalence groups associated with Notch activity.

(iii) *Other tissues*

Laviolette *et al*. (2005) performed a gain-of-function-modifier screen for loci involved in the formation of the larval NMJ. N-ethylmaleimide sensitive factor (NSF) has roles in vesicular trafficking and can influence neurotransmitter receptors levels and synaptic strength. The starting phenotype for this study was overgrowth of the neuromuscular synapse, elicited by expression of a dominant negative form of NSF2. The construct was driven in neurons by *elav-GAL4* and the NMJ visualized with a GFP fusion to the CD8 protein transmembrane domain and the C-terminus of the Shaker channel. Three thousand *GS* insertion lines were analysed and 99 suppressors were obtained, with 89 being near identifiable gene sequences. The 89 loci specify numerous products previously associated with nervous system function, for example, the *lola*, *nejire* and *E2F* loci encode transcription factors. Loci encoding cytoskeletal components, including *actin5C*, *myosin binding protein* and signalling proteins, such as

methuseleh, which functions in G protein receptor coupled signalling, were also uncovered. The signalling class of modifier loci included *frizzled 4*, encoding a Wnt receptor, implicating Wg signalling in NMJ formation.

Gregory *et al.* (2007) used a sensitized overexpression screen to find genes that modulate Rho signalling during cytokinesis. Rho is a GTPase with crucial roles during cytokinesis in animal cells (Lu *et al.*, 2009). In *Drosophila*, Rho is activated at the cell equator by Pebble (Pbl), a Rho GTP exchange factor, initiating cytokinesis (Hime & Saint, 1992; Lehner, 1992). Other proteins involved include RacGAP50c (Jantsch-Plunger *et al.*, 2000) and Pavarotti (Pav-KLP) (Somers & Saint, 2003). Expression of a *UAS*-driven dominant negative version of Pbl via *GMR-GAL4* produces a rough eye phenotype through failure of Rho activation. Two thousand one hundred and ninety *GS* lines were tested for effects in this hypomorphic Rho signalling background and 112 modifiers were analysed. More than half of the loci encode cell cycle or signalling proteins, and others encode enzymes involved in metabolism, particularly regulators of phospholipids. The screen found known genes encoding proteins involved in cytokinesis, such as Diaphanous, a Rho effector involved in actin organization (Afshar *et al.*, 2000) and Four wheel drive, a phosphoinositide-4 kinase involved in cytokinesis during spermatogenesis (Brill *et al.*, 2000). Four genes not previously implicated in cytokinesis were scored: *thread/diap1*, *cdc 14*, *Pitslre* and *phosphoinositide-dependent kinase 1* (*PDK1*). Thread/Diap1 is an inhibitor of apoptosis (Lisi *et al.*, 2000) and Cdc 14 is a protein phosphatase regulator of late mitotic events in *Caenorhabditis elegans* (Stegmeier & Amon, 2004). Pitslre is a homolog of cyclin-dependent kinase 11 (CDK11) known to promote centrosome maturation and spindle formation during mitosis (Petretti *et al.*, 2006), whereas PDK1 is involved in cell growth regulation (Bimbo *et al.*, 2005).

5. Conclusions

GAL4-UAS-mediated overexpression/misexpression allows rapid screening of coding sequences in *Drosophila* for potential roles in specific tissues. This method has the capacity to uncover novel loci refractory to standard loss-of-function genetic approaches, such as redundant or pleiotropic genes. Additionally, the large extant libraries of strains harbouring sequenced transposon insertion sites often allow direct correlation of phenotypes to gene functions. Studies outlined in this review have validated this type of genetic screen by uncovering loci previously associated with the processes targeted. Further, in each case novel loci were also identified. It is important to stress that overexpression or misexpression alone cannot be used to implicate a gene in a developmental process. However, the large and growing list of genetic resources in *Drosophila*, including mutations in many loci, allow further tests for loss-of-function phenotypes associated with the candidate loci. For example, nested sets of chromosomal deficiencies are available that cover the majority of the *Drosophila* genome (Parks *et al.*, 2004; Tweedie *et al.*, 2009), allowing rapid dosage effect assays for loci with no mutant alleles. Additionally, the generation of imprecise excisions (Xu *et al.*, 2006), targeted gene knockouts (Gong & Golic, 2004), libraries of RNAi strains (Dietzl *et al.*, 2007; N. Perrimon, personal communication [http://www.flyrnai.org/TRiP-HOME.html]) and zinc finger nucleases (Beumer *et al.*, 2008) provide methods to inactivate specific loci if canonical alleles are not available. The possibility of rapid candidate gene identification through overexpression or misexpression analysis, in combination with these techniques for gene inactivation, provides a powerful tool for analysis of gene function.

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