



Genes with ectopic expression phenotypes are common, not rare.

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Traditionally, gene function in model organisms has been studied through analysis of loss-of-function mutant phenotypes. Gene redundancy, however, poses the limitation that not all genes have a loss-of-function phenotype. To the extent that overexpression of genes causes detrimental phenotypes, ectopic or mis-expression is a viable method of identifying genes and the initial determination of the gene function. The GAL4-UAS system developed for *Drosophila* by Brand and Perrimon (1993) can be used to overexpress genes in an ectopic, or cell-and-tissue-specific manner. Since the target gene and its activator are initially separated in two distinct lines, this allows study even of ectopic expression that causes lethality (Phelps and Brand, 1998).

Numerous ectopic expression studies have been carried out with the GAL4-UAS system in *Drosophila*. One of the most impressive experiments was the induction of ectopic eyes in *Drosophila* by targeted gene expression of the eyeless gene (Halder *et al.*, 1995). When the GAL4 driver line E132 (which expresses GAL4 in wing, haltere, leg, and antennal imaginal discs) was crossed to the responder line UAS eyeless, ectopic eyes were observed in the wings, legs, antennae, and halteres (Halder *et al.*, 1995). These results could be observed in the few animals that lived to the pharate adult stage when E132 expressed only in small patches. Any greater expression resulted in early death.

While misexpression of transcription factors, such as eyeless, usually have detrimental effects, many proteins do not disturb the normal development and function of the cells when they are produced in excess, and thus show no ectopic expression phenotype (*e.g.*, lacZ-coded beta-galactosidase). Indeed, the cell biology of systems usually does not suggest models in which overexpression of normally occurring proteins disturbs cell function. Perhaps as a result, the frequency of gene products that will have a harmful effect when misexpressed is largely unknown.

The purpose of this study is to estimate the percentage of the genes in *Drosophila* that lead to a detectable phenotype when overexpressed. To get an estimate, the GAL4-UAS 2 element driver and responder system was used to cause tissue-specific overexpression. Genes were sampled for an overexpression phenotype through adjacency to P element inserts designed to be responders for an EP screen as described by Rorth (1996). We assume as a first approximation that P elements tend to insert preferentially near the 5' ends of genes at roughly equal rates for all genes. The development of a y+ marked EP line (P{6.11, y+}#4, Merriam and Poole, unpublished) makes possible the rapid generation and screening of a large number of lines since neither balancing nor homozygosis of the UAS lines is necessary to score the results of crosses to w+ marked drivers in a y w background. We observed that almost 30% of the lines generated have some Gal4-conditional overexpression phenotype.

A total of 628 y+UAS exceptional males of independent origin were obtained from the screen described by Lengyel and Merriam (2001). An initial cross of each male to y w; P{w+ byn-Gal4} females identified 52 that are either lethal (*i.e.*, no y+ w+ progeny) or semi-lethal (<1/2 expected y+ w+ progeny). The byn-Gal4 driver is expressed in the embryonic hind gut and some later stages (Iwaki and Lengyel, unpublished). The interpretation of these 52 lines is that they are inserted in correct orientation

Table 1. Descriptions of ectopic expression phenotypes with tissue specific Gal4 drivers.

UAS line #	Embryonic hind gut adult viability scored	Post mitotic adult bristles	Developing eyes scored in adult
A 23	normal	normal	partial pigment loss
A 43	normal	normal	small, pigment loss
B 71*	normal	thin, short	normal
C 45	normal	thin, short, few	normal
C 75	normal	thin, short, few	normal
D10*	normal	normal	rough
D 11	normal	normal	small, rough?
D 12	normal	normal	partial pigment loss
G 5	lethal	very short, thin	lethal
G 6	lethal	short, pointy	semi-lethal
H 47	lethal	very short	rough, pigment loss
H 66	lethal	two shafts	lethal
H 70	semi-lethal	short	pigment loss cell death
H 71	normal	normal	smaller?
H 84	normal	normal	darker?
I 10	normal	normal	larger, darker
I 16	lethal	normal	lethal
J 18*	normal	normal	small
K 34	normal	normal	lethal
M 8	normal	normal	small, pigment loss, cell death
N 9	semi-lethal	very short, thin	lost pigment
N 12	normal	normal	larger, darker
O 4	normal	normal	small, rough?
O 18	semi-lethal	normal	pigment loss
Q 7	normal	normal	small
Q 9	normal	very short	normal
R 13	normal	normal	semi-lethal pigment loss cell death
R 19	semi-lethal	lethal	lethal
U 4	semi-lethal	normal, few	small eye, pigment
U 12	lethal	very short, pointy bristles	normal, few
U 14	lethal	very short, few	lighter
V 8	normal	normal	pigment loss
W 10	normal	normal	lethal

to genes that cause problems when overexpressed in the hind gut (and other tissues), causing death in all or most of the  $y^+ w^+$  animals receiving both driver and responder.

The lines with normal  $y^+ w^+$  progeny were rescreened by crosses to a driver expressing Gal4 under the Actin5C promoter. Under these conditions of widespread constitutive Gal4 expression, 133 of the  $y^+UAS$  lines had a lethal or semi-lethal ectopic expression phenotype. The remainder were indistinguishable from wild type (*i.e.*, normal). The larger number showing a phenotype with the Actin promoter Gal4, that previously were normal with the *byn*-Gal4 driver, is thought to reflect the cumulative effects of ectopic expression of their weaker phenotypes summed over many more tissues. In agreement with this, a set of 13 lines characterized as lethal or semi-lethal with *byn*-Gal4 were also crossed to the Actin-Gal4 driver; all were lethal. Thus, 186, or almost 30%, of the 628 lines are interpreted as being adjacent to genes whose products are capable of harming the cell or tissue when overexpressed. This figure is in line with other estimates, either from smaller samples (Minye and Merriam, 2001) or with different EP constructs (Toba *et al.*, 1999). Since the P element orientation is expected to be appropriate for overexpression in only half the cases, this suggests that a large fraction, almost 60% perhaps, of genes code for products that cause problems when mis or overexpressed.

That the 186 lines described above can be separated into different classes by their different responses to the 2 drivers likely means that genes differ in the kind and severity of their ectopic expression phenotypes. An alternative explanation is that gene responses are more uniform but that details of where the P elements are inserted contribute to variation in responses to these drivers. In order to obtain more qualitative descriptions of the ectopic expression effects, 158 of these lines were further crossed to 2 additional drivers with largely tissue specific expression patterns. These crosses used the B11 driver that predominantly expresses Gal4 in the adult trichogen and tormogen (Merriam and Guild, unpublished), for assays in post-mitotic cells, and the GMR driver that expresses Gal4 in waves in the developing eye tissue. Some 33 of the lines showed abnormal bristles and/or abnormal eyes. This amounts to roughly 1/5th of lines suggested to be inserts near genes with overexpression phenotypes, or approximately 1/20th of all lines.

These 33 lines and their ectopic expression phenotypes are listed in Table 1. 30 of the lines were characterized as lethal with the Actin-Gal4 driver; the other 3 that are indicated with asterisks were characterized as semi-lethal. As a conclusion, stronger ectopic expression responses were more likely to also show tissue specific effects. 7 of 10 tested byn lethal lines are listed in Table 1, as are 5 or 16 byn semi-lethal lines. 18 of 109 tested byn normal-actin-Gal4 lethal lines made it to Table 1, but only 3 of 24 tested actin-Gal4 semi-lethal lines. The stronger byn lethal lines were also more likely to affect both bristles and eyes, although exceptions were noted (*e.g.*, I16 and R19).

Ectopic expression effects that are not constant between the different tissue specific drivers might in some cases suggest the kinds of functions characteristic of those genes. The most common bristle phenotype was shorter, thinner or more pointed shafts, although one case of doubled shafts suggests a differentiation change. The eye phenotypes mostly showed degrees of roughness, from a classic rough-like appearance through more extreme stages with smaller, lozenge-like features to completely eyeless. Among the most interesting eye phenotypes are 3 with larger ommatidia and bulging eyes. Several of these suggest growth control changes. Thus, these lines are a valuable resource to screen for specific phenotypes as a next step to identify new genes affecting a process, such as growth control. Sequencing the genome adjacent to the P element should inform which gene, or putative open-reading-frame, is capable of causing the ectopic expression phenotype.

References: Brand, A.H., and N. Perrimon 1993, *Development* 118: 401-415; Halder, G., P. Callaerts, and W.J. Gehring 1995, *Science* 267: 1788-1792; Lengyel, J., and Merriam, J., 2001, *Dros. Inf. Serv.* (this issue); Minye, H., and Merriam, J., 2001, *Dros. Inf. Serv.* (this issue); Phelps, C. B., and A.H. Brand 1998, *Ectopic Gene Expression in Drosophila Using GAL4 System. A Companion to Methods in Enzymology* 14: 367-379; Rorth, P., 1996, *Proc. Natl. Acad. Sci. USA* 93: 12418-12422; Toba, G., T. Ohsako, N. Miyata, T. Ohtsuka, K.-H. Seong, and T. Aigaki 1999, *Genetics* 151: 725-737.