High-Frequency Generation of Conditional Mutations Affecting Drosophila melanogaster Development and Life Span

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ABSTRACT

Genome sequencing reveals that a large percentage of Drosophila genes have homologs in humans, including many human disease genes. The goal of this research was to develop methods to efficiently test Drosophila genes for functions in vivo. An important challenge is the fact that many genes function at more than one point during development and during the life cycle. Conditional expression systems such as promoters regulated by tetracycline (or its derivative doxycycline) are often ideal for testing gene functions. However, generation of transgenic animals for each gene of interest is impractical. Placing the doxycycline-inducible (“tet-on”) promoter directed out of the end of the Ptransposable element produced a mobile, doxycycline-inducible promoter element, named Pdl. Pdl was mobilized to 228 locations in the genome and was found to generate conditional (doxycycline-dependent), dominant mutations at high frequency. The temporal control of gene overexpression allowed generation of mutant phenotypes specific to different stages of the life cycle, including metamorphosis and aging. Mutations characterized included inserts in the α-mannosidase II (dGMII), ash1, and pumilio genes. Novel phenotypes were identified for each gene, including specific developmental defects and increased or decreased life span. The Pdl system should facilitate testing of a large fraction of Drosophila genes for overexpression and misexpression phenotypes at specific developmental and life cycle stages.

SEQUENCING of the Drosophila genome reveals ~13,600 genes (Adams et al. 2000). At least half of these are homologous to human genes, including more than half of known human disease genes (Rubin et al. 2000). The powerful molecular and genetic tools available for Drosophila have made this organism a highly successful model for determining the functions of conserved genes. However, such research is often labor intensive and new functional genomic research methods are required to study the large number of interesting genes identified by the genome sequence. A critical obstacle to this research is the fact that many genes function in multiple processes, at multiple stages of the life cycle, and may be required for viability. Simple loss-of-function mutations or gene “knockouts” often do not allow the organism or cells to proceed to the stage of interest. Conditional mutations, such as ones expressed only at permissive temperatures, overcome this obstacle by providing temporal control of gene function. Temperature-sensitive mutations have been essential for the analysis of diverse biological processes including yeast and mammalian cell cycle and Drosophila and Caenorhabditis elegans development. However, temperature-sensitive mutations are not ideal because they are rare and the temperature shift often has confounding effects on other genes and processes. For example, temperature-sensitive mutations are particularly problematic for study of Drosophila aging, as life span is profoundly affected by temperature (Baker et al. 1989).

Conditional gene expression systems provide a type of conditional, dominant misexpression “mutation.” Gene expression can be activated with temporal control in transgenic animals using systems based on recombination and systems triggered by hormones or other chemicals. For example, tetracycline [or doxycycline (DOX)]-regulated promoters have been used to facilitate numerous studies of gene function in mammals and, more recently, in Drosophila (Gossen and Bujard 1992; Gossen et al. 1995; Bello et al. 1998; Bieschke et al. 1998). Conditional systems are particularly well suited to the study of aging (Bieschke et al. 1998; Sun and Tower 1999; Tower 2000; J. Sun, D. Folk, T. J. Bradley and J. Tower, unpublished data). They allow the investigator to avoid toxic or other effects of gene expression during development and determine gene effects in the aging adult, such as increased or decreased life span. In addition, like other quantitative traits, life span is sensitive to genetic background. Conditional systems provide powerful controls for genetic background, as control and overexpressing animals are genetically identical. A drawback of this approach is that generation of transgenic animals for each gene of interest is labor intensive and is not practical for large scale functional genomics.

The Drosophila P transposable element can be readily
mobilized to generate hundreds or thousands of Drosophila lines with unique insertions (COOLEY et al. 1988). The P element has been engineered to facilitate many types of genetic manipulation, including insertional mutagenesis, enhancer-trapping, and generation of gene misexpression mutations (O’KANE and GEHRING 1987; BELLEN 1989; SPRADLING et al. 1995; RORTH 1996; HAY et al. 1997; RORTH et al. 1998; TOBA et al. 1999; LUCASOVICH et al. 2001). In the experiments presented here the usefulness of P-element mutagenesis has been extended by engineering a P element (PdL) that creates conditional (DOX-dependent) mutations at high frequency. The system combines the benefits of the tet-on conditional gene expression system with the utility of P-element mutagenesis.

MATERIALS AND METHODS

Drosophila strains: All D. melanogaster strains are as described (LINDSLEY and ZIMM 1992; http://flybase.bio.indiana.edu/).

Drosophila culture and life-span assays: Drosophila were cultured on standard agar/molasses/cornmeal/yeast media (ASHBURNER 1989). To obtain adult flies of defined age, the indicated PdL lines and Oregon-R control strain were crossed to rta lines and stocked at 25°C in urine specimen bottles. Prior to eclosion of the majority of pupae, bottles were cleared of adults and newly eclosed flies were allowed to emerge over the next 48 hr. The majority of the males will have mated during this time. The males only were then removed and were designated 1 day old and were maintained at 25°C at 40 per vial in culture vials with food. At 4 days of age the males were split into control and experimental groups of 200 males each, with experiments (+DOX) supplementing 250 μg/ml DOX. Dead flies were counted at each passage, and the number of vials was progressively reduced to maintain ~40 flies per vial. To calculate mean life spans for the experimental (+DOX) and control (−DOX) cohorts, each fly’s life span was tabulated, their life spans were averaged, and the SEM was calculated. Statistical significance of differences in mean life span was calculated for each experiment using unpaired two-sided t-tests.

Construction and transformation of PdL: A 560-bp EcoRI fragment containing seven tetO repeats and the hsp70 core promoter from −40 to +86, was excised from plasmid pTT40 (BIESCHKE et al. 1998) and cloned into the polylinker of PcASper-4 transformation vector (THUMMEL and PIROTTA 1992). Multiple independent germ-line transformants of the PdL construct were generated using standard methods (RUBIN and SPRADLING 1982), using the γ-actin/HIN recipient strain (PATTON et al. 1992).

Southern analysis of PdL copy number: DNA was isolated from PdL lines and restriction digested with XbaI, HinIII, and PstI. DNA was transferred to Southern blot and hybridized with a radiolabeled 172-bp fragment from the 3′ end of PdL. This probe fragment was generated by PCR amplification with primers located within the 3′ end IR (atgtagataataacaaaggtcctg) and P3MSCREV (atgtagatattcaacaggcactgttggggtttgaat) and cloned into the pCR2.1-TOPO cloning vector (Invitrogen, San Diego). Dideoxy sequencing was carried out using the Sequenase version 2.0 DNA sequencing kit (United States Biochemical, Cleveland) and the T7 and M13 reverse sequencing primers.

DNA sequence analyses: PdL flanking DNA sequences were used to query GenBank databases using BLASTN program with default settings as provided at the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/).

Northern analyses: Messenger RNA was isolated from adult Drosophila using the RNAsqueous kit (Ambion, Austin, TX), fractionated on 10% agarose gels and transferred to Gene Screen membranes (DuPont/NEC, Boston, MA). The DNA probe for exon 4 of the dGMII gene was generated by PCR amplification from Drosophila genomic DNA using primers GL178478-5 (ggataaagcagaaactgaagccaag) and GL178890-3 (tggtatcgactcttgcagctggcat). The dGMII intergenic region probe was generated using primers GL178748-5 (gtgaaaatgagaatgcttgaagc) and GL1789890-3 (gctgactgctgctagttctgac). The dGMII intragenic region probe was generated using primers GL179311-5 (ctgataagcgagcaactaatcctg) and GL179719-3 (tgggacagggcttgtttttgttggcag). The probe for exon 1 of the ash-f gene was generated using primers ASH1FWD (tgctgtagtcttttaggtc) and ASH1REV (tgatgctgcttggtttgtttttttttt). The probe for pum exon 9 +10 was a 700-bp EcoRI to SacI fragment of Drosophila cDNA clone SD30602 (Genome Systems). The probe for pum intron 8 was generated using line 3B2 genomic DNA template and primers IR (cgggacactctgttattgcaatg) and pum120121 (gtgaaacatttagctttcgacggatgt). The loading control was ribosomal protein gene Rp49 (O’CONNELL and ROSBACH 1984). DNA probes were 32P-labeled using the Prime-it II DNA labeling kit (Stratagene, La Jolla, CA). Hybridization signals were visualized by autoradiography. Transcript size was determined by comparison with 1-kb RNA ladder (GIBCO-BRL, Gaithersburg, MD) according to the manufacturer’s instructions.

Electron microscopy: Scanning electron microscopy was carried out at the University of Southern California Center for Electron Microscopy and Microanalysis using a Cambridge 360 SEM. Samples were prepared using standard methods, except that critical point drying was replaced by a 15-min treatment with hexamethyldisilazane (ADAMS et al. 1987).

RESULTS

Construction, transformation, and transposition of the novel P-element mutagen PdL: The tet-on tetracycline inducible system (GOSSEN et al. 1995) was recently adapted to transgenic Drosophila (BIESCHKE et al. 1998). The cytoplasmic actin (actin5C) promoter was used to drive constitutive, tissue-general expression of the reverse tetracycline trans activator (rtTA) in a transgenic construct called rTA. A tetracycline (DOX)-inducible promoter was constructed by placing seven repeats of the rtTA binding site (“tetO”) upstream of a core promoter. The core promoter consisted of hsp70 gene sequences from −40 to +86, including the TATAA box, the transcriptional initiation site, and 86 bp of 5′-untranslated region (UTR) sequences. The hsp70 core

at 16°C. PCR amplification was performed using primers Pyr1 (ctttatcgacgtctgatggttgataa) and IR (ccgcgacctatgttatttcactcatt). PCR protocol was performed as follows: step 1, 95°C for 5 min; step 2, 95°C for 30 sec; step 3, 51°C for 1 min; step 4, 72°C for 1 min; step 5, repeat steps 2–4, 40 times; step 6, 72°C for 10 min. The PCR product was subcloned into the pCR2.1-TOPO cloning vector (Invitrogen, San Diego). Dideoxy sequencing was carried out using the Sequenase version 2.0 DNA sequencing kit (United States Biochemical, Cleveland) and the T7 and M13 reverse sequencing primers.

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The 215 new insertions of PdL into Drosophila were transformed using standard methods to create 13 independent transgenic lines. Three of the PdL transformant lines were tested for frequency of transposition by appropriate crosses to Δ2-3 transposase source (ROBERTSON et al. 1988). Line PdL(X)A yielded the highest frequency (27%) and was used to generate 215 new insertions on the second and third chromosomes, using standard methods (COOLEY et al. 1988; TOWER and KURAPATTI 1994).

**Identification of conditional, dominant mutations affecting metamorphosis:** The 215 new insertions of PdL were analyzed along with the 13 original transformant lines. Each of the total 228 PdL insert lines were crossed to flies containing the rtTA transactivator construct, using culture media ±DOX. DOX was not present inside the eggshell because the mothers were not prefed DOX. In this way gene misexpression will be specific to the larval and pupal stages. Progeny containing both PdL and rtTA were scored for viability and visible phenotypes. Out of 228 PdL lines, nine lethal and five visible mutations were identified (Table 1). As expected, the mutations were both conditional (DOX dependent) and dominant. Visible phenotypes included curled wings, blistered wings, and rough eyes (Figure 2). For each of the five visible mutations, zero flies exhibited the mutant phenotype in the absence of DOX. Therefore there is no detectable leakiness of this system with regard to mutant phenotypes. For both the visible and lethal mutations in the presence of DOX, penetrance of the phenotype was high, and varied from 67 to 100%, with most at 100% (Table 1).

Each of the PdL insertions was made homozygous in the absence of rtTA to assay for recessive phenotypes. Both visible (curled wing) and lethal phenotypes were observed (Table 1).

**Identification of conditional, dominant mutations affecting life span:** Thirteen PdL lines were tested for conditional, dominant effects specific to the aging period of the life cycle. These were the 13 lines where DOX feeding and gene overexpression during development was found to cause lethal or visible phenotypes. Each of the 13 lines (as well as Oregon-R wild-type controls) was crossed to rtTA in the absence of DOX, to generate 400 age-synchronized male flies containing both constructs. At 4 days of age the males were split into control and experimental groups, with experimentals placed on culture media supplemented with 250 μg/ml DOX. The flies were transferred to fresh vials every 2 days and the number dead was recorded. Mean life span was calculated, and the percentage difference between control and experimental groups is presented (Table 1). For several lines exhibiting a change in life span with DOX, the experiment was repeated and the results of both experiments are presented. Half of the lines exhibited a conditional, dominant phenotype of reduced life span, with decreases ranging from ~4.7 to ~32%. The high frequency of negative effects on life span observed is likely due to the fact that this set of lines was not random, but rather one where expression during development had been found to be disruptive or lethal. In contrast, line PdL(3)19B3 exhibited a reproducible increase in life span of ~10%, while the control of Oregon-R wild type crossed to rtTA gave no significant change in life span.

**Molecular characterization of PdL mutations:** Southern analysis was used to determine the copy number of new PdL inserts in each line (data not shown; summarized in Table 1). Three lines containing single inserts were chosen for molecular analysis. For each of these lines excision of the PdL insert by crossing to Δ2-3 transposase source reverted the mutation. Chromosomal regions flanking the 5′ end of the insert were amplified by inverse PCR and sequenced. Comparison of the flanking sequences with the Drosophila genome database allowed mapping of the site of PdL insertion and the identification of the mutated gene. Line PdL(3)19B3 (rough eye/increased life span) contained an insert in the 5′-UTR of the α-mannosidase II (dGMII) gene, 67 bp 5′ of the ATG translation start codon (Figure 3A). α-Mannosidase II is a Golgi apparatus enzyme involved in protein glycosylation (RABOUILLE et al. 1999). RNA was isolated from flies containing the PdL(3)19B3 insert and rtTA, cultured ±DOX. Northern analysis revealed a 4-kb transcript that hybridized to a probe derived from the dGMII exon 4 and that was induced 15-fold by DOX (Figure 3D). As expected, the DOX-induced transcript is slightly larger than the endogenous dGMII transcript. This is because PdL is inserted near +1 of dGMII, and PdL contributes an extra 86 bp of 5′-UTR sequences.

It was of interest to determine if overexpression was limited to the dGMII gene or whether some DOX-induced transcription might read through to adjacent downstream gene(s). The Northern blot was hybridized.
<table>
<thead>
<tr>
<th>Line name</th>
<th>No. of insertions (gene mutated)</th>
<th>Developmental phenotype</th>
<th>Penetrance(^b) (%)</th>
<th>Life-span phenotype(^a)</th>
<th>Recessive phenotype</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PdL(3)3G</td>
<td>2 (pumilio)</td>
<td>Curled wing</td>
<td>91</td>
<td>-DOX 44.30 ± 0.515</td>
<td>Curled wing</td>
<td>Allelic to PdL(3)3B2</td>
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<tr>
<td>PdL(3)3B2</td>
<td>1 (pumilio)</td>
<td>Curled wing</td>
<td>67</td>
<td>+DOX 45.72 ± 0.490</td>
<td>Curled wing</td>
<td>Allelic to PdL(3)3G, reverted</td>
</tr>
<tr>
<td>PdL(3)58A2/TM3</td>
<td>3 (pumilio)</td>
<td>Curled wing</td>
<td>95</td>
<td>% change ((P)) -1.3 (0.416)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PdL(3)19B3/TM3</td>
<td>1 (dGMI)</td>
<td>Rough eye</td>
<td>100</td>
<td>WT Complements PdL(3)3G</td>
<td></td>
<td></td>
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<tr>
<td>PdL(3)35A2/TM3</td>
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<tr>
<td>PdL(3)53A/TM3</td>
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<td>Lethal</td>
<td>100</td>
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<tr>
<td>PdL(3)2B1</td>
<td>3</td>
<td>Lethal</td>
<td>100</td>
<td></td>
<td></td>
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<tr>
<td>PdL(3)43C1/TM3</td>
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<td>Lethal</td>
<td>100</td>
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<tr>
<td>PdL(3)72A3/TM3</td>
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<td>Lethal</td>
<td>100</td>
<td></td>
<td></td>
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<tr>
<td>PdL(3)73A1/TM3</td>
<td>3</td>
<td>Lethal</td>
<td>100</td>
<td></td>
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<tr>
<td>PdL(3)75A3/TM3</td>
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<td>Lethal</td>
<td>100</td>
<td></td>
<td></td>
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<tr>
<td>PdL(2)14B1 Sp/SM5</td>
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<td>Lethal</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PdL(2)45C1 Sp/SM5</td>
<td>3</td>
<td>Lethal</td>
<td>92</td>
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<td></td>
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<tr>
<td>Oregon-R</td>
<td></td>
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</table>

WT, wild type; ND, not done.

\(^b\) Penetrance calculations: For visible mutations penetrance = 100\(^a\) [mutant/(WT + mutant)]; for lethal mutations penetrance = 100\(^a\) [1 - (no. of escapers/no. of expected)].

\(^a\) Mean life span in days at 25\(^\circ\), ±SEM, \(P\) value for unpaired, two-sided \(t\)-test.
Drosophila Conditional Mutants

and the rtTA transactivator, genotype \( w;PdL(3)3B2/\text{rtTA}(3)E2 \), cultured throughout larval and pupal development in the presence of \( 250 \mu g/ml \) DOX. Control flies cultured without DOX were wild type and were identical to those in A (data not shown). (D) Compound eye of control flies containing PdL insert 19B3 in the dGMII gene and the rtTA transactivator, genotype \( w;PdL(3)19B3/\text{rtTA}(3)E2 \). The flies were cultured throughout larval and pupal development in the presence of \( 250 \mu g/ml \) DOX, in parallel with the control flies in A. (E) Compound eye of experimental flies containing PdL insert 19B3 in the dGMII gene and the rtTA transactivator, genotype \( w;PdL(3)19B3/\text{rtTA}(3)E2 \). The flies were cultured throughout larval and pupal development in the presence of \( 250 \mu g/ml \) DOX, in parallel with the control flies in D.

to a probe specific for \( CG16765 \), which is the next downstream ORF predicted by the Drosophila genome sequence (Figure 3A). The probe hybridized only to a very faint band of approximately the size predicted for the \( CG16765 \) transcript (2.2 kb) that was not detectably altered by DOX (Figure 3D and additional data not shown). A probe specific for the intergenic region gave no detectable hybridization (data not shown). While this does not rule out the possibility that a small amount of transcription might read through the dGMII gene, any such transcription that might be occurring does not appear to result in detectable stable RNA.

Line \( PdL(3)11A3 \) (lethal/decreased life span) contained an insert in the \( \text{absent, small or homeotic discs 1} \) (ash1) gene, 206 bp 5’ of the normal ash1 transcription initiation site (Figure 3B). ash1 is a member of the trithorax group of genes and encodes an RNA polymerase II transcription factor that positively regulates expression of homeotic genes during development (LaJeunesse and Shearn 1995; Tripoulas et al. 1996). RNA was isolated from flies containing the \( PdL(3)11A3 \) insert and rtTA, cultured \( \pm \) DOX. Northern analysis revealed an 8.1-kb transcript that hybridized to a probe derived from the ash1 exon 1 and that was induced fourfold by DOX (Figure 3E). For both the dGMII and ash1 mutations, the size of the DOX-induced transcripts suggests that they are correctly terminated and processed.

Line \( PdL(3)2B2 \) (curled wing/unchanged life span) contained an insert near the middle of the 120-kb intron 8 of the pumilio gene (Figure 3C). This intron is one of the largest known in Drosophila and contains an \( \sim 500 \) bp “hot spot” for Pelement insertion (Paris and Lin 1999). Pumilio is an RNA binding protein involved in regulation of translation and is required both maternally and zygotically for multiple stages of development (Forbes and Lehmann 1998; Wharton et al. 1998). Two other \( PdL \) mutant lines exhibited a similar curled wing phenotype and were found to have insertions at the same location within pumilio (Figure 3C). Northern analysis of these lines revealed a complex pattern of endogenous and DOX-induced pumilio transcripts common to each of the three lines (Figure 3F). A probe derived from pumilio exons 9 + 10 hybridized to the native 6.9-kb pumilio RNA and a 0.4-kb RNA. A probe derived from pumilio intron 8 revealed that part of the signal at 6.9 kb results from a second RNA originating just downstream of the Pelement hotspot. This transcript begins in the intron, was rare or absent in adult males, and was induced 3- to 10-fold by DOX. Two smaller RNAs were also strongly induced by DOX. Searches of the Drosophila cDNA database with sequences from this region of pumilio intron 8 identified several cDNAs derived from wild-type Drosophila tissue culture cells (diagrammed in Figure 3C). These cDNAs also initiate just downstream of the hot spot, contain an ATG start codon in an alternative exon spliced in frame to pumilio exon 9, and encode a potential alternative pumilio protein. Taken together, the data suggest that in the three mutant lines examined, \( PdL \) has inserted at the 5’ end of a previously unidentified pumilio internal promoter and activated expression of alternative pumilio gene product(s).

**DISCUSSION**

\( PdL \) was found to generate conditional, dominant mutations at high frequency. Approximately 7% of mu-
Figure 3.—Molecular characterization of selected mutations. (A–C) Intron and exon boundaries of the mutated genes are indicated, with numbering according to DNA sequences obtained from NCBI web page (http://www.ncbi.nlm.nih.gov/). Locations for transcriptional initiation and ATG translation start codons are indicated by arrows. PdL inserts are indicated by triangles and each is oriented 5' to 3', as indicated by internal arrows. DNA fragments used as probes in Northern analyses are indicated above each diagram. (A) dGMII (sequence accession no. AJ132715; genomic scaffold sequence AE003682 (Adams et al. 2000)). (B) ash1 (sequence accession no. U49439). (C) pumilio (sequence accession no. L07943; intron sequence and numbering are from Drosophila genomic scaffold sequence AC no. AE003681; SD10525 cDNA sequence is from AC no. AI549655). (D–F) Northern analysis of selected lines. Oregon-R wild-type strain and the indicated PdL mutant strains were crossed to rTA. Total RNA was isolated from progeny cultured DOX, transferred to Northern blots, and hybridized with the gene-specific probes indicated (A–C). Ribosomal protein 49 gene Rp49 was used as control for loading. Two amounts of RNA were loaded for each sample (1X and 2X, as indicated), and signals were quantitated by phosphoimager. (D) dGMII mutant strains and controls. (E) ash1 mutant strain and controls. (F) pumilio mutant strains and controls.
tated chromosomes yielded visible or lethal phenotypes when *PdL* was activated by DOX feeding during larval and pupal development. It is likely that the frequency at which *PdL* inserts cause gene overexpression is significantly higher than the frequency at which mutations were identified in these experiments. Genes affecting
embryogenesis would not have been detected because DOX is not present inside the eggshell. If desired, such genes could potentially be detected by prefeeding the mothers DOX. In addition, not all overexpressed genes are expected to produce lethal or obvious visible phenotypes, and therefore overexpression of such genes would not have been detected.

In most of the mutant lines, the mutated chromosome had multiple PdL insertions, and there are likely two reasons for this result. First, the starting insert line selected for mobilization had a relatively high transposition frequency, which reduces the effort required to identify new insertions but favors multiple inserts. Second, chromosomes with multiple inserts will be more likely to have a new mutation. The chromosomes examined were ones selected for mutant phenotype, and this selection may have enriched for multiple insert chromosomes.

The temporal control of gene overexpression and mutant phenotype provided by PdL extends the usefulness of P-element mutagenesis. The conditional nature of the PdL mutations allowed identification of stage-specific gene misexpression phenotypes that it had not previously been possible to study. For example, the PdL insertions in pumilio produced a novel curled wing phenotype and suggested the existence of a novel pumilio internal promoter. Misexpression of ashl specifically in the adult revealed a previously unknown negative effect on life span. Finally, the PdL insertion in dGMII yielded some of the first mutant phenotypes described for this gene. Feeding of DOX during larval and pupal development disrupted eye development, while feeding of DOX only during adulthood resulted in an ~10% increase in life span. α-Mannosidase II is involved in protein glycosylation and the likely mechanism for either phenotype in Drosophila is currently unclear. In mouse, mutation of the homologous gene encoding α-mannosidase II causes a systemic autoimmune disease that becomes more severe with increasing age, and that resembles human lupus erythematosus (Chui et al. 2001). Very recently another group has used a P-element “gene-trap” method to generate a lethal insertion in the Drosophila dGMII gene (Lucasovich et al. 2001).

In previous studies, P elements with outwardly directed promoters generated (nonconditional) mutations at frequencies ranging from 2 to 64%, depending on the particular promoters used to drive expression (Rorth 1996; Rorth et al. 1998; Toba et al. 1999). In those studies the elegant GALA/UAS system (Brand and Perrimon 1993) was used to provide tissue-specific gene misexpression and tissue-specific phenotypes resulting from a promoter directed out of the end of an engineered P element, called “EP.” Activation of EP line gene misexpression in larval imaginal tissues was often associated with wing and eye phenotypes similar to those observed here for PdL. The large pumilio intron 8 is a hot spot for P-element insertion (Parisi and Lin 1999); however, no mutant phenotypes were observed for multiple EP insertions at this site (Rorth et al. 1998; P. Rorth, personal communication). Because of its unique temporal regulation, PdL mutagenesis is expected to identify gene functions not detected with other methods.

The conditional mutations caused by PdL are dominant gain-of-function mutations, as opposed to the more common loss-of-function mutations resulting from gene disruptions. The gain-of-function phenotypes could result from expression of the gene in the wrong place or in the wrong amount, and both situations have been observed with EP mutagenesis (Rorth et al. 1998). An important consideration is that inappropriate expression of a protein by PdL might disrupt development or life span due to a nonspecific effect, such as by preventing cell differentiation or causing a novel, nonspecific toxicity. One way to confirm that the gene is involved in a particular process or pathway of interest is to also examine the loss-of-function phenotype for the gene. This is facilitated by the fact that the PdL insertion may be located such that it disrupts the gene and thereby generates a loss-of-function phenotype when made homozygous. This appears to be the situation with lines PdL(3)3G and PdL(3)3B2 where both the overexpression and recessive phenotypes were a curled wing, and in nine PdL lines where the recessive phenotype was lethality. If a PdL insertion is located such that it does not disrupt the gene, such disruptions can often be generated by imprecise excision or local transposition of the element (Salz et al. 1987; Tower et al. 1993; Spradling et al. 1995). The dominant and conditional nature of PdL mutations should therefore allow for the relatively rapid and efficient identification of genes of potential interest, which can then be confirmed to be involved in the relevant process or pathway by more traditional analyses. This general strategy has been shown to be highly productive in studies of Drosophila development using EP misexpression mutagenesis (Rorth et al. 1998, 2000; Huang and Rubin 2000; Mata et al. 2000).

The conditional nature of the mutations generated by PdL makes them particularly well suited to studies of aging and life span. The temporal control of gene overexpression allows the investigator to avoid any toxic or confounding effects during development and study gene functions specifically in the adult. In addition, like other quantitative traits, life span is greatly affected by genetic background, and PdL provides powerful controls for this variable. Control and mutant (overexpressing) flies have identical genetic backgrounds, and therefore any differences observed must be due to DOX and the subsequent gene overexpression or misexpression. Several lines were identified where activation of PdL in the adult caused reductions in life span, with decreases up to ~32%. This high frequency of negative effects was not surprising, given that these were lines where activation of PdL during development was disruptive or lethal.
Increased life span is expected to be a more rare phenotype of gene misexpression. A small but reproducible increase in life span of ~10% was associated with the PdL insertion in the dGMII gene; however, it is not possible to predict how common this phenotype is likely to be based on this one example. It is known that induced overexpression of at least two additional genes, Cu/ZnSOD and MnSOD, can extend adult Drosophila life span based on experiments using different gene expression systems (Parkes et al. 1998; Sun and Tower 1999; J. Sun, D. Folk, T. J. Bradley and J. Tower, unpublished data). It seems likely that additional genes will exist that can extend life span when overexpressed in the adult. The potential for PdL mutagenesis to test large numbers of genes should provide an efficient way to identify additional genes that regulate aging and life span.

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