An EP overexpression screen for genetic modifiers of Notch pathway function in Drosophila melanogaster

LAUREN E. HALL, SHAUNA J. ALEXANDER, MICHAEL CHANG, NATHANIEL S. WOODLING AND BARRY YEDVOBNICK*
Department of Biology, Emory University, Atlanta, GA, 30322, USA
(Received 11 November 2003 and in revised form 16 December 2003)

Summary

The Notch pathway comprises a signal transduction cascade required for the proper formation of multiple tissues during metazoan development. Originally described in Drosophila for its role in nervous system formation, the pathway has attracted much wider interest owing to its fundamental roles in a range of developmental and disease-related processes. Despite extensive analysis, Notch signaling is not completely understood and it appears that additional components of the pathway remain to be identified and characterized. Here, we describe a novel genetic strategy to screen for additional Notch pathway genes. The strategy combines partial loss of function for pathway activity with Enhancer-promoter (EP)-induced overexpression of random loci across the dorsoventral wing margin. Mastermind (Mam) is a nuclear component of the Notch signaling cascade. Using a GAL4-UAS-driven dominant-negative form of Mam, we created a genotype that exhibits a completely penetrant dominant wing-nicking phenotype. This phenotype was assayed for enhancement or suppression after outcrossing to several thousand EP lines. The screen identified known components or modifiers of Notch pathway function, as well as several potential new components. Our results suggest that a genetic screen that combines partial loss of function with random gene overexpression might be a useful strategy in the analysis of developmental pathways.

1. Introduction

The Notch pathway is one of a small group of signaling systems that regulate metazoan development (Gerhart, 1999; Barolo & Posakony, 2002). These systems share the general feature of receiving an extrinsic signal and transmitting an intracellular signal involving multiple components, and they also exhibit common principles involving specific activation and repression (Barolo & Posakony, 2002). Notch functions during numerous cellular interactions within vertebrates and invertebrates. The pathway is composed of membrane receptors (Drosophila Notch, Caenorhabditis elegans Glp-1 and Lin-12, and mammalian Notch1–Notch4), ligands of the Delta/Serrate class and multiple cytoplasmic and nuclear proteins that are involved in signal transmission and the regulation of target gene expression. Notch pathway genes, originally designated as neurogenic loci, were first associated with lateral inhibition of neuroblast formation in the Drosophila central nervous system (CNS) (Lehman et al., 1983). Subsequently the pathway was shown to act at other stages of CNS formation (Skeath & Doe, 1998; Buescher et al., 1998; Schuld & Brand, 1999; Van Der Bor & Giangrande, 2001), as well as within a wide spectrum of tissues throughout Drosophila/invertebrate development (Hartenstein et al., 1992; Artavanis-Tsakonas et al., 1999; Weinmaster, 1997) and vertebrate/mammalian development (Gridley, 1997; Mumm & Kopan, 2000). Moreover, as additional activities of the Notch pathway were characterized, it became apparent that a much wider array of gene products was involved in its function (Baron et al., 2002). In various contexts, Notch mediates inductive cell interactions, cell proliferation and apoptosis (Artavanis-Tsakonas et al., 1999). Evolutionarily conserved components of the Notch pathway have been implicated in human diseases and dysmorphic syndromes (Gridley, 1997, 2003), including Alzheimer’s disease (Haas, 1997; Ye et al., 1999),

* Corresponding author. Tel: + 1 (404) 727, 4203. Fax: + 1 (404) 727, 2880. e-mail: Biolby@biology.emory.edu

DOI: 10.1017/S0016672304006731 Printed in the United Kingdom
Alagille syndrome (McCright et al., 2002), spondylo-costal dysostosis (Gridley, 2003) and CADASIL (Villa et al., 2001), and two mammalian Notch genes were first characterized as oncogenes. The human Notch1 locus was associated with a translocation present in T lymphoblastic leukemia (Ellisen et al., 1991); excessive activity of Notch1, Notch2 or Notch3 predisposes to T-cell leukemia (Aster & Pear, 2001). Moreover, the mouse Notch4/int-3 locus is a Mouse Mammary Tumor Virus (MMTV) integration site, and thus associated with mammary tumors (Van Leeuwen & Nusse, 1995). It is now hypothesized that dysregulation of Notch signaling is associated with additional human neoplasms, including those of the cervix, lung, skin and salivary gland (Maillard & Pear, 2003; Tonon et al., 2003).

Despite intensive study, the genetic and biochemical details of Notch signaling are only partially understood and it appears that a more complete description will require the identification of additional pathway components. For example, genetic screens (Levitan & Greenwald, 1995; Verheyen et al., 1996; Yedvobnick et al., 2001; Jarriault & Greenwald, 2002) and biochemical strategies (Royet et al., 1998; Hubbard et al., 1996; Lamar et al., 2001; Negerie et al., 2002) continue to uncover loci involved in Notch function, strongly suggesting that others remain undiscovered. We described previously a Drosophila GAL4 upstream activation sequence (GALA-UAS) (Brand & Perrimon, 1993) genetic system that has been used to depress the Notch pathway in a tissue-specific manner, leading to typical loss of function phenotypes in imaginal tissues (Helms et al., 1999; Yedvobnick et al., 2001) or embryos (Yedvobnick et al., 2004). This was accomplished through overexpression of truncated forms of Mastermind (Mam), an essential nuclear component (Smoller et al., 1990; Bettler et al., 1996) of the Notch activation complex (Petcherski & Kimble, 2000; Wu et al., 2000; Kitagawa et al., 2001; Fryer et al., 2002).

In this study, we genetically combined a GALA-UAS-driven dominant-negative Mam truncation with a collection of Enhancer-promoter (EP) overexpression lines (Rorth, 1996). The truncation we used, named MamH, terminates shortly after the basic Mam domain that mediates a physical association with nuclear Notch. MamH protein presumably competes with Notch. MamH protein presumably competes with Notch. MamH, terminates shortly after the basic Mam domain. All crosses were performed at 25 °C. The C96-GAL4 driver line expresses across the dorsoventral wing margin. This transgene was recombined onto a chromosome containing the UAS-MamH transgene and the recombinant chromosome balanced over TM3 Sb strain was obtained from the Artavanis lab (Massachusetts General Hospital Cancer Centre, Charleston, MA) and DpBx/TM6c was obtained from the Muskaevitch lab (Boston College, Chestnut Hill, MA). The fringe1×/TM3 Sb strain was obtained from K. Irvine (Rutgers University, Waksman Institute, Piscataway, NJ). The remaining strains were obtained from the Bloomington Stock Center.

### 2. Methods

(i) Mam truncation constructs

Construction of Mam truncations in pUAST is described in Helms et al. (1999). UAS-MamH terminates at nucleotide 1489 of cDNA B4 (Smoller et al., 1990). Mam residue 245, which is 55 residues C-terminal of the basic charge cluster.

(ii) Drosophila strains

Wing margin (C96-GAL4) and proneural (309-GAL4) driver lines, and UAS-NactW, UAS-Heartless and UAS-Delta have been described previously (Helms et al., 1999). The collection of approximately 2300 EP lines (Rorth et al., 1996) was obtained from Exelixis (http://flystation.exelixis.com/). The nd1 line was obtained from the Artavanis lab (Massachusetts General Hospital Cancer Centre, Charleston, MA) and DlBX9/TM3 Sb from the Artavanis lab (Massachusetts General Hospital Cancer Centre, Charleston, MA) and DlBX9/TM3 Sb. The fringe1×/TM3 Sb strain was obtained from K. Irvine (Rutgers University, Waksman Institute, Piscataway, NJ). The remaining strains were obtained from the Bloomington Stock Center.

(iii) Genetic screen

All crosses were performed at 25 °C. The C96-GAL4 driver line expresses across the dorsoventral wing margin. This transgene was recombined onto a chromosome containing the UAS-MamH transgene and the recombinant chromosome balanced over TM3 Sb. The sequence of EP insertion sites and their distance and orientation relative to transcription units were obtained from FlyBase. EP lines producing wing modification of C96-MamH were subsequently mated to a C96-GAL4 strain to examine overexpression effects in the absence of the Mam truncation. Phenotypic interactions resulting from background modifiers in the EP strains were investigated by crossing the EP lines to other Notch pathway mutations exhibiting wing phenotypes. Potential loss-of-function effects...
at the sites of EP insertion were tested by use of chromosomal deficiencies that spanned the insertion site or a loss-of-function allele of the relevant gene.

(iv) Mounting of wings and notums

Wings were dehydrated in isopropanol and mounted in Euparol. Notums were positioned on slides and photographed directly under a dissecting microscope.

3. Results

(i) EP modifier screen

The $w^+\ EP$ element (Rorth, 1996) contains $UAS(E)$ sequences immediately 5’ to the $hsp70$ promoter ($P$). When the $EP$ element is mobilized, in addition to insertional inactivation of loci, it can activate downstream genes when GAL4 is present. In screens, the $w^+\ EP$ element is transposed to random genomic
sites and the new site of residence of the element is tested for phenotypic effects when driven by an Enhancer-GAL4 construct. Therefore, using a tissue-specific GAL4 driver, the genome can be screened for loci that produce phenotypes when overexpressed (Abdelilah-Seyfried et al., 2000). Alternatively, when combined with a pre-existing mutant phenotype, the screen can be used to identify loci that bypass, suppress or enhance the mutant phenotype (Rorth et al., 1998; Kazemi-Esfarjani & Benzer, 2000). In the present screen, the C96-GAL4 element present on the same chromosome as UAS-MamH (C96-MamH) can simultaneously create the mutant phenotype and drive the random EP insertion and its downstream gene (Fig. 1D). The C96-MamH chromosome elicits a 100% penetrant dominant wing-nicking phenotype when outcrossed to w^{118}, as shown in Fig. 1E. Genotypes that also contain an EP P element hop can be assayed for changes in wing phenotype. This approach was validated in genotypes containing the C96-MamH truncation chromosome and UAS-regulated Notch pathway loci that function as positive or negative effectors. For example, overexpression of the Delta ligand or an activated form of Notch is expected to elevate pathway signaling, and we observed suppression of the C96-MamH wing phenotype in these genotypes (Fig. 1A, B). By contrast, overexpression of Hairless, which tethers the Su(H) repression complex to promoters and negatively regulates the pathway (Barolo & Posakony, 2002) enhances C96-MamH (Fig. 1C). Therefore the nicked-wing phenotype produced in the C96-MamH genotype is sensitive to alterations in Notch pathway signaling level, and should be capable of detecting additional pathway components.

(ii) Screening the Rorth EP collection

We tested the EP strategy by screening the Rorth (1996) collection. This collection contains approximately 2300 EP inserts. Each of the lines was mated with the C96-MamH/TM3 Sb strain and the non-Sb progeny were scored for alteration of the C96-MamH/+ wing phenotype. Several modifier lines were obtained and the data is summarized in Figs 2, 3 and Table 1. Unless stated otherwise, the EP inserts are oriented to overexpress the most proximal locus. Three enhancers are inserted in or upstream of known loci associated with the Notch pathway or a target locus of Notch activation. One of these, EP 3082, inserted upstream of the fringe gene. Fringe is a glycosyltransferase that modifies the Notch protein and alters its responsiveness to Delta and Serrate (Moloney et al., 2000). EP 3082 is associated with a minor distal wing nick when combined with C96-GAL4 (Fig. 2B2), but it produces a severe synergistic phenotype in combination with the C96-MamH truncation (Fig. 2B1). EP 0509 is inserted upstream of the kekkon-1 (kek1) locus, which encodes a component of epidermal growth factor (EGF) signaling. Kek1 is a transmembrane protein that negatively regulates the EGF receptor (Ghiglione et al., 1999). The EGF pathway functions at the dorsoventral wing margin to activate Vestigial expression, and reduced EGF receptor activity leads to nicked wings (Nagaraj et al., 1999). Therefore, overexpression of Kek1 is expected to synergize with depressions in Notch signaling at the margin. Although we observe a minor wing-nick effect with the EP 0509 and C96-GAL4 combination (Fig. 2C2), an enhanced and highly penetrant phenotype derives from the combination with the Mam truncation (Fig. 2C1). EP 2127 inserts into the first intron of the Rpl19 gene, which encodes ribosomal protein L19. EP 2127 enhances the C96-MamH phenotype, but no phenotype is observed in combination with C96-GAL4 (Fig. 2D1, D2). EP 2127 is oriented opposite to the normal transcription of Rpl19 and is probably associated with loss of function. We corroborated this by testing a deficiency for the Rpl19 region for interaction with C96-MamH and observed enhancement (Fig. 2D5). Mutation of this locus was previously isolated in a screen for Delta modifiers (Klein and Campos-Ortega, 1992). Given the structural role of Rpl19 in the ribosome, EP 2127 is probably a nonspecific modifier. However, the targeting of fringe and kek1 provide validation that the EP screen is capable of uncovering more relevant loci.

Several other strong and highly penetrant interactions were identified in the collection (Fig. 3). Two of these, EP 2371 and EP 0684, targeted genes that encode the Domino and Escargot transcription factors, respectively. Mutations of domino were isolated in a screen for disorders in hematopoiesis, a process that also requires the Notch pathway (Duvic et al., 2002). Domino encodes two proteins of the SW12/SNF2 class of DNA-dependent ATPases that provide a repressed structure to chromatin (Ruhf et al., 2001). The proteins are widely expressed during development, including all imaginal discs and the CNS. Genotypes containing EP 2371 (Domino) and C96-MamH exhibit a strong wing enhancement (Fig. 3B1), whereas no phenotype is observed in the control C96-GAL4 plus EP 2371 class (Fig. 3B2). Escargot is a zinc-finger transcription factor that functions redundantly with Snail protein during embryonic wing disc formation and neuroblast asymmetric division (Fuse et al., 1996; Cai et al., 2001). Overexpression of Escargot alone produces a strong phenotype; genotypes carrying EP 0684 and C96-GAL4 show a striking distal wing curvature phenotype (Fig. 3C2). The wing is also wider across its anterior-posterior dimension. C96-MamH combined with EP 0684 suppresses the distal curvature phenotype and enhances loss of wing blade material from distal regions (Fig. 3C1).
Fig. 2. EP line modifiers include previously identified loci that interact with the Notch pathway or a pathway target. Panels show wings prepared from genotypes containing C96-MamH (row 1), C96-GAL4 (row 2), nd1 (row 3), DfBX9 (row 4) or chromosomal deficiencies spanning the insertion site of EP elements, or a loss of function mutation for the insertion locus (row 5). Strains in rows 1–4 were mated with w1118 (column A) or EP lines EP3082, fringe (column B), EP0509, kekkon 1 (column C) and EP2127 RpL19 (column D). In the case of deficiency/loss-of-function (lof) mutations, row 5 shows transheterozygotes with the C96-MamH chromosome: B5, fng13; C5, Df(2L)prd1.7; D5, Df(2R)M60E. All genotypes are transheterozygous except for the hemizygous nd1/Y males.
Fig. 3. Additional EP line modifiers include novel loci. Wings are prepared from genotypes containing C96-MamH (row 1), C96-GAL4 (row 2), nd¹ (row 3), Df/BX9 (row 4) or chromosomal deficiencies spanning the insertion site of EP elements, or a loss of function mutation for the insertion locus (row 5). Strains in rows 1–4 were mated with w¹¹¹8 (column A) or EP lines EP 2371, domino (column B), EP 0684, escargot (column C), EP 3375, pap (column D), EP 2630 (column E) and EP 3084 (column F). In the case of deficiency/loss-of-function (lof) mutations, row 5 shows transheterozygotes with the C96-MamH chromosome: B5, Df/(2R)Pu-D17; C5, Df/(2L)TE35BC-8; D5, pap<sup>k760</sup>; E5, Df/(2L)/N22-5. All genotypes are transheterozygous except for the hemizygous nd¹/Y males.
is a strong suppressor of C96-MamH that is not associated with any phenotype in combination with C96-GAL4 (Fig. 3D1, D2). EP 3375 inserts within the second exon of the poils aux pattes (pap) locus, oriented opposite to its transcription, and is probably associated with a loss of function. Consistent with this idea, the papK760 mutation also suppresses the C96-MamH phenotype (Fig. 3D5). The pap locus encodes a protein related to human TRAP 240, a component of the Mediator transcription complex (Boube et al., 2000).

Two other sites of EP insertion might affect uncharacterized genes. EP 2630 inserts within the first intron of the taiman locus but is oriented to drive expression opposite to transcription of taiman. EP 2630 shows a strong and completely penetrant enhanced phenotype in combination with C96-MamH, producing extensive loss of wing blade material (Fig. 3E1). To determine whether EP 2630 produces the wing enhancement through inactivation of taiman, we combined the C96-Mam truncation with several mutations of taiman (data not shown), as well as a deficiency for the taiman region, Df(2L)N22-14. No enhancement was observed in these genotypes, indicating that loss of function for taiman is not responsible for the phenotypic interaction (Fig. 3E5). Additionally, the combination of EP 2630 with C96-GAL4 does not elicit a detectable phenotype (Fig. 3E2). EP 2630 could be driving an uncharacterized sequence within the first intron or upstream of taiman that is responsible for the interaction with C96-MamH. EP 3084 is a strong suppressor of the C96-MamH phenotype that is not associated with a phenotype in combination with C96-GAL4 (Fig. 3F1, F2). The insertion site of EP 3084 is within repeated sequence of chromosome 3 heterochromatin and we have not been able to identify the sequences responsible for the interaction.

### (iii) Secondary characterization of EP lines

EP lines can modify the C96-MamH wing phenotype for reasons unrelated to overexpression. As discussed above, an EP insert may inactivate a locus. EP inactivation of a locus should elicit a modifier phenotype that resembles a deficiency or loss-of-function mutation interaction for that region (Yedvobnick et al., 2001), as we observe for EP 2127 and EP 3375. Our analysis suggests that modifier EP lines oriented to drive a downstream locus do not act through insertional inactivation. As shown in Figs 2 and 3 (row 5), mutations or deficiencies that span the site of EP insertion for lines 0509, 2271, 0684 and 2630 do not enhance the C96-MamH phenotype. Although we do observe some enhancement with loss of fringe function (Fig. 2B5), the effect is very different from the overexpression effect of EP 3082 (Fig. 2B1).

An interaction apparently caused by an EP might actually derive from a background mutation induced elsewhere in the genome. Background mutations can be expected to modify other Notch pathway wing mutations and would not act through the GAL4 system. Therefore, we tested EP lines for genetic interactions in males hemizygous for the ndI allele of Notch, and also in flies heterozygous for the dominant DeltaBX9 allele. The ndI wings exhibit minor nicks along the margin and wing vein thickening. Phenotypes were compared with those observed after outcrosses of ndI and DeltaBX9 to the control w1118 strain. EP lines oriented to overexpress genes did not exhibit interactions (Figs 2 and 3, rows 3 and 4), but those associated with insertional inactivation showed ndI enhancement (EP 2127, Fig. 2D3) or ndI suppression (EP 3375, Fig. 3D3). An enhancement of the DeltaBX9 phenotype was detected for EP 2127 (Fig. 2D4). However, the suppressor EP 3375 also exhibited enhancement of DeltaBX9 (Fig. 3D4).

Finally, EP wing modifiers might elicit similar effects within other contexts of Notch function. Therefore, EP lines were mated to the 309-MamH/Cy strain to investigate interaction in a different tissue (Fig. 4, Table 2). This strain contains a recombinant chromosome that drives the UAS-MamH truncation within proneural clusters on the notum. Notums from 309-MamH flies contain additional macrochaetes.

### Table 1. Summary of EP modifiers

<table>
<thead>
<tr>
<th>EP line</th>
<th>Cyto site</th>
<th>Gene</th>
<th>Function</th>
<th>C96-GAL4</th>
<th>ndI</th>
<th>DI</th>
<th>Df/lof</th>
<th>309-MamH</th>
</tr>
</thead>
<tbody>
<tr>
<td>3082 (E)</td>
<td>78A1</td>
<td>fringe</td>
<td>Glycosyltransferase</td>
<td>WE</td>
<td>NE</td>
<td>NE</td>
<td>E</td>
<td>S</td>
</tr>
<tr>
<td>0509 (E)</td>
<td>33F4</td>
<td>kekkon-1</td>
<td>Tyrosine phosphatase</td>
<td>WE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>E</td>
</tr>
<tr>
<td>2127 (E)</td>
<td>60E10</td>
<td>RpL19</td>
<td>Ribosomal protein</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>E</td>
</tr>
<tr>
<td>2371 (E)</td>
<td>57D11</td>
<td>domino</td>
<td>Transcription</td>
<td>SE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>E</td>
</tr>
<tr>
<td>0684 (E)</td>
<td>35C4</td>
<td>escargot</td>
<td>Transcription</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>E</td>
</tr>
<tr>
<td>3375 (S)</td>
<td>78A5</td>
<td>poils aux pattes</td>
<td>Transcription</td>
<td>SE</td>
<td>S</td>
<td>E</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2630 (E)</td>
<td>30A2</td>
<td>Unknown</td>
<td>Unknown</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>E</td>
<td>E</td>
</tr>
<tr>
<td>3084 (S)</td>
<td>Ch3 hetero</td>
<td>Unknown</td>
<td>Unknown</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>?</td>
<td>S</td>
</tr>
</tbody>
</table>

* The interaction of each EP line with the C96-MamH phenotype is designated as enhancer (E) or suppressor (S). Abreviations: NE, no effect; SE, strong effect; WE, weak effect. These designations also apply to ndI, DI, Df/lof and 309-MamH columns.
Several sites (Helms et al., 1999); the posterior notopleural site is particularly sensitive. For example, compared with control w^{1118} notums, there is a single pn bristle at 100% of the sites. (B) In 309-MamH/w^{1118} or 309-MamH/Cy notums, a duplication of the pn bristle is observed 75–80% of the time. (C) EP 3082 suppresses the 309-MamH duplication; a single np is observed in 89% of sites. (D) EP 3082 driven by 309-GAL4 is associated with loss of the pn bristle 24% of the time and a single bristle is observed in the remaining sites. (E) EP 2127 enhances the 309-MamH phenotype, with 97% of sites showing multiple bristles and 39% show three or four bristles. (F) EP 0509 enhances the 309-MamH phenotype because multiple np bristles are observed at every site. Clusters of three or more bristles are observed at 86% of the sites. (G) EP 2371 enhances the 309-MamH phenotypes; multiple np bristles are observed at 99% of the sites. (H) EP 3375 (pop allele) suppresses the 309-MamH phenotype; single np bristles are observed at 49% of the sites. (I) P[PZ]paprK760 strongly suppresses the 309-MamH phenotype; single np bristles are observed at 95% of sites. (J) EP 0684 eliminates bristle formation in combination with 309-MamH. Identical effects are observed when EP 0684 is driven by 309-GAL4 (data not shown). (K) EP 2630 enhances the 309-MamH phenotype because multiple np bristles are observed at 97% of the sites. Clusters of three or more bristles are observed at 43% of the sites. (L) EP 3084 suppresses the 309-MamH phenotype; single np bristles are observed at 83% of the sites.

at several sites (Helms et al., 1999); the posterior notopleural site is particularly sensitive. For example, compared with control w^{1118} notums, in which we never observe a duplication of the posterior notopleural bristle, the 309-MamH strain exhibits duplication at 79% of the sites (Fig. 4A, B). EP line, 3082 (fringe) suppresses the 309-MamH-associated bristle duplication (89% single bristle, Fig. 4C). Furthermore, genotypes in which EP 3082 is driven by 309-GAL4 alone exhibit loss of the posterior notopleural bristle 24% of the time (Fig. 4D). Similar bristle effects have been reported previously after Fgron overexpression (Klein & Martinez-Arias, 1998). The EP 2127 line (Rpl19) showed an enhancement of the bristle duplication phenotype, with 97% of the sites showing multiple bristles (Fig. 4E). EP 0509 (kek1) enhanced the 309-MamH phenotype, because 100% of the sites exhibited additional posterior notopleural bristles and, in many cases, clusters of bristles were evident (Fig. 4F). A similar but somewhat weaker enhancement was observed with EP 2371 (domino) (Fig. 4G). The EP 3375 (pop) wing suppressor also suppressed the bristle duplication phenotypes to 51% of the sites (Fig. 4H). However, a nearly complete suppression
Table 2. EP modifier interaction with 309-MamH, showing the percentages of posterior notopleural bristle sites with 0, 1, 2 or more bristles

<table>
<thead>
<tr>
<th>Strain</th>
<th>Posterior notopleural bristle number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>w1118</td>
<td>100</td>
</tr>
<tr>
<td>309-MamH</td>
<td>21</td>
</tr>
<tr>
<td>EP 3082</td>
<td>89</td>
</tr>
<tr>
<td>EP 0509</td>
<td>0</td>
</tr>
<tr>
<td>EP 2127</td>
<td>3</td>
</tr>
<tr>
<td>EP 2371</td>
<td>1.5</td>
</tr>
<tr>
<td>EP 0684&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–</td>
</tr>
<tr>
<td>EP 3375</td>
<td>49</td>
</tr>
<tr>
<td>pap&lt;sup&gt;K760&lt;/sup&gt;</td>
<td>95</td>
</tr>
<tr>
<td>EP 2630</td>
<td>3</td>
</tr>
<tr>
<td>EP 3084</td>
<td>83</td>
</tr>
</tbody>
</table>

The 309-MamH value is in transheterozygotes with Cy Balancer chromosome. The values for EP lines and the pap<sup>K760</sup> allele represent genotypic combination with 309-MamH chromosome.

<sup>a</sup> N is the number of posterior notopleural sites scored.

<sup>b</sup> Transheterozygote for EP 0684 and 309-MamH or 309-GAL4 eliminated nearly 100% of all notum bristles, so the posterior notopleural site could not be scored in these genotypes.

Mam truncactions in imaginal tissues (Helms et al., 1999; Yedvobnick et al., 2001) or during embryogenesis (Yedvobnick et al., 2004) produces dominant negative effects on Notch signaling. Dominant Mam truncactions retain the polypeptide’s basic charge cluster (Smoller et al., 1990), which is required for physical association with the intracellular domain of Notch and complex formation with Su(H) protein. However, they lack more C-terminal regions of the polypeptide that function in gene activation (Helms et al., 1999; Petcherski & Kimble, 2000; Wu et al., 2000; Kitagawa et al., 2001; Fryer et al., 2002). Using a recombinant C96-GAL4 + UAS-MamH (C96-MamH) chromosome, we tested single EP insertion transheterozygotes for modification of truncation-induced wing phenotypes. In these genotypes, the C96-GAL4 transgene drives both the Mam truncation and sequences downstream of the EP element (Fig. 1). This strategy can detect either suppressed or enhanced wing phenotypes, as shown with UAS strains expressing activated Notch, Delta or Hairless transgenes (Fig. 1). Furthermore, EP candidates are readily tested for overexpression phenotypes in the absence of the Mam truncation by outcrossing the EP line with the appropriate GAL4 driver strain. Potential loss-of-function effects associated with EP insertion and background mutations within EP lines that can modify Notch pathway function can also be distinguished through test crosses to relevant mutants (Figs 2–4, Table 1).

We identified EP inserts in or near loci previously associated with the Notch pathway or a target of the pathway (Fig. 2), and other inserts that might represent new pathway components (Fig. 3). The EP 3082 and EP 0509 enhancers insert upstream of the fringe and kekkon-1 genes, respectively. Fringe modifies Notch receptor activity at the wing margin through its glycosyltransferase activity (Montgomery et al., 2000), whereas Kek1 is involved in negative regulation of the EGF receptor. EGF signaling is involved in Vestigial expression in the wing (Nagaraj et al., 1999). Thus, in neither case is it surprising that overexpression impacts Notch pathway processes. However, it is noteworthy that, in each case, simple overexpression produces a very minor phenotype that synergizes with the C96-MamH-associated phenotype (Fig. 2). This highlights the likelihood that a combined screen that uses overexpression in conjunction with loss-of-function will be useful in uncovering new loci. This conclusion is further supported by other EP strains that exhibit no phenotype upon outcrossing to GAL4 drivers, yet strongly enhance the C96-MamH phenotype (see below). In the case of EP 2127, the insert appears to disrupt function of the RpL19 locus, previously identified as a Notch pathway modifier (Klein & Campos-Ortega, 1992). Thus, the EP screen can also detect modifiers through insertional inactivation of dosage-sensitive loci.
The EP 2371 enhancer is inserted upstream of the domino locus, which encodes SW12/SNF2-related proteins that are involved in gene repression (Ruhf et al., 2001). Because Mam truncation expression reduces Notch target expression (Helms et al., 1999; Yedvobnick et al., 2001), one hypothesis to explain the EP 2371 genetic enhancement effect is that overexpression of Domino further downregulates the pathway or its targets. However, it is presently unknown whether Domino normally functions to repress the Notch pathway or its targets at the wing margin. EP 0684 inserts upstream of escargot, and we observed a strong wing phenotype when this line was crossed to the control C96-GAL4 strain. The phenotype appears to derive, in part, from additional growth in the distal wing blade, and this effect is blocked by coexpression of the MamH truncation. Strong overexpression effects with other GAL4 constructs driving Escargot have been observed (Rorth et al., 1998), suggesting that multiple tissues are sensitive to elevated levels of this protein. It is unclear why the MamH truncation diminishes the overexpression effect of Escargot, but the Escargot phenotype might require normal levels of Mam function. In this regard, it is interesting that Mam and Escargot are involved in both wing development and asymmetric cell division in the CNS (Fuse et al., 1996; Cai et al., 2001). EP 3375 inserts within the second exon of the pap locus, which encodes a Drosophila homolog of the TRAP240 protein, and this results in a suppressor phenotype. TRAP240 forms part of a large transcription complex linking specific factors to the RNA polymerase II core complex (Boube et al., 2000). The EP 3375 insert appears to produce a loss-of-function mutation in pap, because it behaves similarly to other pap mutations. The dosage-sensitive interaction observed between pap mutations and the MamH truncation effect in wing and notum could reflect a negative regulatory role for the Pap protein in the nuclear arm of the Notch pathway.

The modifier effects for two of the identified EP inserts could not be associated with a gene. EP 2630 is the strongest enhancer we found and it is inserted within the first intron of the taiman locus, which encodes a protein related to AIB1, a steroid receptor coactivator (Bai et al., 2000). EP 2630 is orientated to drive expression opposite to transcription of taiman, suggesting that the enhancement could be due to loss of function for taiman. However, deficiencies for the taiman locus do not enhance the C96-MamH wing phenotype (Fig. 3E5), arguing that loss of taiman function does not underlie the effect. Additionally, the EP 2630 strain does not enhance nd¹ or DIB⁴⁹ mutations, indicating that the effect is not due to a background mutation in the EP 2630 strain. Finally, w⁻ revertants of the EP 2630 element no longer enhance C96-MamH wings (data not shown). The basis for the strong enhancement of EP 2630 could derive from overexpression of a locus within the first intron of taiman, or possibly upstream of taiman. Interestingly, control genotypes containing C96-GAL4 and EP 2630 do not exhibit any wing phenotypes. Therefore, if EP 2630 enhancement requires overexpression of a locus, its effect is only apparent in the sensitized genotype produced through Mam truncation coexpression.

Finally, our assay for the effects of EP inserts in a different context of Notch function, proneural clusters on the notum, was very consistent with the wing margin data. In a genotype that produces additional bristles (309-MamH), we observed enhancement or suppression of the phenotype in transheterozygotes with EPs (Fig. 4, Table 2). The notable exception was EP 3082, which drives the Fringe protein, in which we observed enhancement in the wing assay (Fig. 2) but suppression in the bristle assay (Fig. 4). An explanation for this derives from the effect of driving EP 3082 in the notum with 309-GAL4 alone, where loss of bristles is observed (Fig. 4D). In an earlier report, Klein and Martinez-Arias (1998) showed that overexpression of Fringe could lead to loss of bristles through lack of sensory organ precursor formation. Thus, it appears that the effect of Fringe overexpression is epistatic to that of MamH truncation expression during the formation of sensory organ precursors.

In conclusion, we have used a genetic system in which GAL4-UAS drives the expression of a Mam truncation in combination with random EP inserts. The system was used to screen for loci involved with Notch pathway function, and several candidates were obtained. Because of hot spots for insertion, screening with a P-element-based strategy requires many transpositions (Spradling et al., 1999). Consequently, we are presently generating a collection of new EP insertions to screen for additional pathway components.

Supported by NSF (IBN, 9904411), an Emory URC Award and a gift from the Ammerman Foundation. We thank Exelixis for providing the EP collection.

References


Van Der Bor, V. & Giangrande, A. (2001). Notch signaling represses the glial cell fate in fly PNS. *Development* 128, 1381–1390.


