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Discovery of genes with highly restricted expression patterns in the *Drosophila* wing disc using DNA oligonucleotide microarrays

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SUMMARY

The *Drosophila* wing disc is divided along the proximal-distal axis into regions giving rise to the body wall (proximal), wing hinge (central) and wing blade (distal). We applied DNA microarray analysis to discover genes with potential roles in the development of these regions. We identified a set of 94 transcripts enriched (two fold or greater) in the body wall and 56 transcripts enriched in the wing/hinge **region. Transcripts that are known to have highly restricted expression patterns, such as *pannier, twist* and *Bar-H1* (body wall) and *knot, nubbin* and *Distal-less* (wing/hinge), showed strong differential expression on the arrays. In situ hybridization for 50 previously uncharacterized genes similarly revealed that transcript enrichment identified by the array analysis was consistent with the observed spatial expression. There was a broad spectrum of patterns, in some cases suggesting that the genes could be targets of known signaling pathways. We show that three of these genes respond to *wingless* signaling. We also discovered genes likely to play specific roles in tracheal and myoblast cell types, as these cells are part of the body wall fragment. In summary, the identification of genes with restricted expression patterns using whole genome profiling suggests that many genes with potential roles in wing disc development remain to be characterized.**

Supplementary data available online

Key words: *Drosophila*, Imaginal wing disc, Microarray, *wingless*

INTRODUCTION

The imaginal discs of *Drosophila melanogaster*, which form the adult exoskeleton, offer an accessible model system for examining epithelial spatial patterning. In the larva, the discs show a prepattern of gene expression presaging the development of the body region and its specific structures. Knowing the number and identity of all genes involved is critical for a coherent understanding of genetic mechanisms operating to pattern a given structure.

Identifying genes has largely relied on the isolation of mutants with pattern defects, but strategies that detect genes by their expression patterns, enhancer and protein trapping using P elements with reporter genes (Bellen et al., 1989; Bier et al., 1989), or a GFP tag (Morin et al., 2001), have also identified genes involved in tissue-specific differentiation. However, P-elements show specificity in their insertion sites (Liao et al., 2000), so that screening the entire genome in this way may remain incomplete.

The annotated genome sequence (Adams et al., 2000) offers a systematic way to investigate spatial patterns of expression of all genes in the imaginal discs. Because the predicted gene number is approximately 14,000, this seems a large undertaking, but is being done for embryos that lend themselves more readily to high throughput in situ hybridization (http://www.fruitfly.org/cgi-bin/ex/insitu.pl). To identify a subset of genes that may have important roles in wing disc development, we used hybridization to high-density DNA-oligonucleotide arrays to define genes that show enriched spatial expression patterns. Cluster analysis of gene expression throughout the *Drosophila* life cycle has led to the identification of muscle-specific transcripts (Arbeitman et al., 2002) and genes expressed in specific imaginal discs, including the wing disc, have been discovered by profiling individual discs (Klebes et al., 2002). Here we compared RNA profiles from two complementary wing disc fragments, the presumptive wing/hinge and presumptive body wall regions (Fig. 1). These regions are separated by a lineage restriction that occurs in the first larval instar and genes preferentially expressed in one region may be important for that fate.

Our analysis identified many genes with uncharacterized roles in development that have striking spatial expression domains. We discuss the results in the light of the sequence information available for these genes and the role of known genes expressed in similar patterns. Some expression patterns suggest regulation by key morphogens. We have shown that

three genes, with robust expression in the wing pouch, are sensitive to *wingless* (*wg*) signaling. This work makes a significant contribution to the goal of finding all the genes involved in wing disc development, by identifying a collection of genes that have not been implicated previously but which have expression patterns suggestive of potential region-specific roles.

MATERIALS AND METHODS

Target preparation and array hybridization

Approximately 200 wild-type (Berlin strain) wandering third-instar larvae were dissected and the wing discs were collected in a drop of PBS on Sylgard (Dow Corning). Discs were cut between the presumptive hinge and the body wall regions using a 30-gauge syringe needle, and fragments were lysed in separate groups in RLT buffer (Qiagen). Total RNA was extracted from the tissue lysate using an RNeasy kit (Qiagen). Approximately 8 μg of total RNA from each of six independent samples (three wing/hinge and three body wall) was processed to produce biotinylated cRNA targets, which were hybridized to *Drosophila* Genechip 1 arrays following standard Affymetrix procedures (http://www.affymetrix.com).

Data analysis

Six arrays were used and each demonstrated control parameters within recommended limits (Raw Q<=30, background <=100, GAPDH 3′/5′ ratios below four). To allow comparison between chips, each was analyzed using global scaling with a target intensity of 300. The scaling factors used to normalize to the target value were within four-fold of each other in all comparisons (range 0.36 to 1.34). Affymetrix Microarray Suite version 5.0 software was used to make each pair-wise comparison between the three wing/hinge and the three body wall arrays. The data were then exported to Excel, in which the 'Signal Log Ratios' were converted to fold changes. Only transcripts called as present in at least two samples, and showing a two-fold or greater difference ($P \geq 0.95$) between the wing/hinge and body wall samples in at least six out of the nine comparisons are included. The data were sorted by average fold-change to produce the lists of genes shown in Tables 1 and 2. This filtering of the data means that some genes with spatially restricted patterns may be excluded; this is especially likely to be the case for genes with low expression levels. Therefore, the Excel spreadsheet with the full comparison set is given at http://dev.biologists.org/supplemental/, and the array data have been deposited into the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) (series accession number GSE93 and sample accession numbers GSM2583, GSM2584, GSM2585, GSM2586, GSM2587 and GSM2588).

In situ hybridization

In situ hybridization of DIG-labeled antisense RNA probes to dissected wandering third instar larvae followed a standard protocol (Sturtevant et al., 1993). Probes were generated using the appropriate RNA polymerase (SP6, T7 or T3) following the manufacturer's protocol (Roche). Synthesis was assessed by gel electrophoresis and the precipitated probe was dissolved in 50% formamide in TE. The DIG-labeled probes were diluted and tested over a broad range, as there was a great deal of variation in the effective probe concentration. Discs were mounted in Aquapolymount (Poly Sciences) and photographed using bright field or Nomarski optics.

cDNA clones and genomic exon fragments used to generate probes for in situ hybridization

When possible, DIG-labeled RNA probes were generated from cDNA clones obtained from the Drosophila Gene Collection (DGC, http://www.fruitfly.org/DGC/index.html) or from published sources. Most clones used were from the DGC1 release. DGC clones belonging to the unreleased DGC2 set were ordered from the *Drosophila* EST collections maintained by ResGen (Invitrogen). Clones used are listed in descending order according to the ranked lists (Tables 1 and 2), with the DGC clone identification number or literature citation as appropriate. Clones were linearized in the 5′ multiple cloning site (except where noted) and transcribed with the appropriate RNA polymerase.

Notum-enriched list

pOT2a-tailup (GH12431), pOT2a-CG11100 (SD10763), pFlc-CG15064 (RE70039), pFlc-CG15353 (RH63135), pBS-CG6921 (LD14839), pFlc-BM40/SPARC (RH45818), pFlc-Obp56a/CG11797 (RE46170), pOT2a-zfh1 (SD06902), pFlc-viking (RE68619), pBS-Ef1alpha100E (RE68984), pFlc-Obp99a/CG18111 (RH70762), pOT2a-CG10126 (GH22994), pOT2a-CG9338 (GH07967), pBS-Cg25C (GM04010) (this clone failed to grow and a PCR product was generated, see below), pBS-Act57B (LD04994) (linearized with AflIII to generate a 3′UTR probe specific to Actin57B that does not cross-hybridize with other Actins), pOT2a-CG5397 (GH04232), pFlc-Idgf4 (RE30918), pOT2a-CG4386 (LD47230), pFlc-CG3244 (RH18728), pFlc-CG2663 (RE73641), pOT2a-CG10275 (LD31354), pOT2a-CG8689/CG30359 (GH18222), pOT2a-tsp/CG11326 (GH27479), pOT2a-Gs2 (GH14412), pFlc-Gld (RE20037).

Wing/hinge-enriched list

pOT2a-CG17278 (SD04019), pBS-pdm2 (Poole, 1995), pFlc-CG8780 (RE33994), pOT2a-Nep1/5894 (GH03315), pBS-opa (Benedyk et al., 1994), pOT2a-Cyp310a1 (LD44491), pFlc-CG14534 (RE71854), pOT2a-CG9008/BG:DS00797.2 (GH14910), pOT2a-zfh2 (GH11902,), pFlc-Doc2/CG5187 (RE40937) (linearized with AvaII to produce a specific 3′-end probe that does not cross-hybridize with Doc1), pOT2a-ana (GH07389), pOT2a-CG8381/CG30069 (LP06813), pOT2a-CG8483 (LD39025), pCR-TOPOII-dorsocross (Lo and Frasch, 2001) (linearized with AflII to produce a specific 3′-end probe that does not cross-hybridize with Doc2/CG5187), pFlc-wengen/CG6531(RE29502).

For genes without cDNA clones available, gene-specific fragments were generated by PCR from genomic DNA using either Taq polymerase (Invitrogen) or Pfu Turbo polymerase (Stratagene). Genomic DNA was generated from adult Canton-S flies using the Qiagen DNeasy Kit. Primers were chosen that would specifically amplify exonic sequence from the gene. The primers were designed using the annotated gene information from GadFly, FlyBase and NCBI/GenBank. Exon numbers mentioned below refer to exon predictions or experimental information from the above sources. Exon fragments were cloned into pBS-KS and verified by sequencing. All clones have a 3′ T7 promoter. The fragments generated, the corresponding gene region and the primers used are listed below (written 5′ to 3′ with any 5′/3′ restriction sites introduced underlined) in the order that they appear on the wing/hinge enriched list (Table 2):

dve exon 5, bp 520-769; primers 5Pdve CATGCACC-ACGATCACCATCATGG and Rdve CGTGCTGACTCAGATAGCC-GTTCATGG

CG15001 single exon, bp 1-321 (+5bp 5′UTR); primers 5P15001 GCGAATTCGCAATGGAGGCGAGCTCGAATCC; and 3P15001 GCTCTAGACTTGTTCCATTCGCATTCCTTCC

CG15489 single exon, bp 38-727; primers 5PN15489 GCGAATTCAGCAACGGCAGCAGCGTGTTGC and 3PN15489 GCTCTAGACTGTTGGCGCCTTCAATGTTGTCG

CG15488 single exon, bp 8-404; primers 5PN15488 GCGAATTCATGTCAGCGCAAATCACAGCAGCTCC and 3PN15488 GCTCTAGACACTGCTCTAAATCTCGCACATCC

CG15000 exon 3, bp 22-840; primers 5PN15000 GCGAATTCAACCGGCATTGATCTTCAATCCGG and 3PN15000 GCTCTAGAGGAGAGTCGCTGTCGCTTGGAGG

CG4861/CG31094 exon 3, bp 288-1374; primers 5P4861

CAACCAAGGAGGATGCAACGCAACC and 3P4861 GCTCTAGAACTTGTTCGCCTTGAAGACGG

CG6469 exon 2, bp 24-346; primers 5P6469 GCGAATTCGATGTGAAGGACGACG and 3P6469 GCTCTAGACTGGTGGTACTGTGGCTGCTGC

CG14301 exon 4, bp 2-227; primers 5P14301 GCGAATTCCTGGCATTACTGCGACATCGATGG and 3P14301 GCTCTAGATCAGGTGAAGATGTCCTCGACC

Ugt86Di/CG6658 exon 8, bp 23-417; primers 5PUgt GCACTCACCCAACATGATTGAAGTGG and RUgt CCAGTCGGAAATGTAGACATTCTCCG

CG5758 exon 8, bp 77-471; primers 5P5758N GCGAATTCTGCTGCTGAACCACTTCGTTAAGG and 3P5758N GCTCTAGATCCTCGAAGTCCTCGTCATCACCG

Cg25C exon 4, bp 90-900; primers 5P25C GCGAATTCAGTTACGACATTGTAGACTCTGC and 3P25C GCTCTAGAGTCCAGTGTCGCCTTCAGGTCC

Fly stocks

The following transgenic lines were used: *71B-GAL4* (Brand and Perrimon, 1993), *C96-GAL4* (Gustafson and Boulianne, 1996), *UAS-wg* and *UAS-DNdTCF* (van de Wetering et al., 1997). Larvae from crosses were raised at 29°C to enhance GAL4 activity and produce more extreme phenotypes.

RESULTS AND DISCUSSION

Microarrays were used to identify differentially expressed transcripts

To identify genes with expression patterns enriched in the presumptive wing/hinge or body wall regions, wing imaginal discs were cut into two fragments at the boundary between the body wall and the wing hinge (Fig. 1). Folds associated with the hinge provide morphological features to allow precise cutting (Fig. 1). RNA expression profiles of these samples were determined using oligonucleotide microarrays representing approximately 13,500 known and predicted genes in the *Drosophila* genome (Genechip *Drosophila* Genome Array 1, Affymetrix). The transcripts were ranked by average fold-change and those showing a two-fold or greater enrichment are shown in Tables 1 and 2. Information for all genes is available at http://dev.biologists.org/supplemental/. Ninety-four transcripts show two-fold or greater enrichment in the body wall (Table 1) and 56 transcripts show two-fold or greater enrichment in the wing/hinge (Table 2). Several of these genes were also discovered by Klebes et al. as being more highly expressed in wing discs than leg discs or eye-antennal discs, suggesting they may also have appendage-specific roles (Klebes et al., 2002).

Genes with known restricted expression patterns show enrichment on the arrays

The rank order of transcripts correlates well with the spatial expression patterns of characterized genes. In the body wall, *pannier* (*pan*), *twist* (*twi*) and *BarH1*, which are enriched in the body-wall sample (Table 1), are known to be highly expressed in the presumptive body wall (Bate et al., 1991; Ramain et al., 1993; Sato et al., 1999). In the wing, *knot* (*kn*), *nubbin* (*nub*) and *Distal-less* (*Dll*) are expressed at levels greater than 10-fold above those in the body wall (Table 2). *kn* is expressed in the wing 3/4 intervein and hinge regions (Mohler et al., 2000; Vervoort et al., 1999), *nub* is strongly expressed in the entire wing pouch (Ng et al., 1995) and *Dll* is expressed along the dorsal-ventral (DV) margin exclusively in the wing pouch (Campbell et al., 1993; Gorfinkiel et al., 1997).

Other genes, known to have important roles in disc development, appear lower down the rank order (Table 2). *vestigial* (*vg*), a key gene for development of the wing and hinge regions (Williams et al., 1991), shows only two-fold enrichment but this is consistent with the expression pattern of *vg* in the wing disc that extends into the body wall region (Williams et al., 1991). Transcripts with expression patterns restricted to the posterior compartment, *engrailed* (*en*), *invected* (*inv*) and *hedgehog* (*hh*) (Coleman et al., 1987; Kornberg et al., 1985; Tabata et al., 1992), showed approximately two-fold enrichment in the wing/hinge sample (Table 2). The anterior-posterior compartment boundary splits the wing/hinge region into two equally sized compartments, but the position of the boundary in the body wall region produces a small posterior compartment representing approximately one-quarter of the total tissue (Fig. 1B). This is consistent with the approximately two-fold enrichment of posterior-specific transcripts found in the wing/hinge tissue sample. The *E(spl)-Complex* genes are expressed in developing sensory organs found in both the body wall and wing margin regions. Hence, these genes are not enriched in any one sample (see http://dev.biologists.org/supplemental/). The *m6* gene is an exception (enriched in the body wall sample) (Table 1) and is known to be expressed only in the body wall region

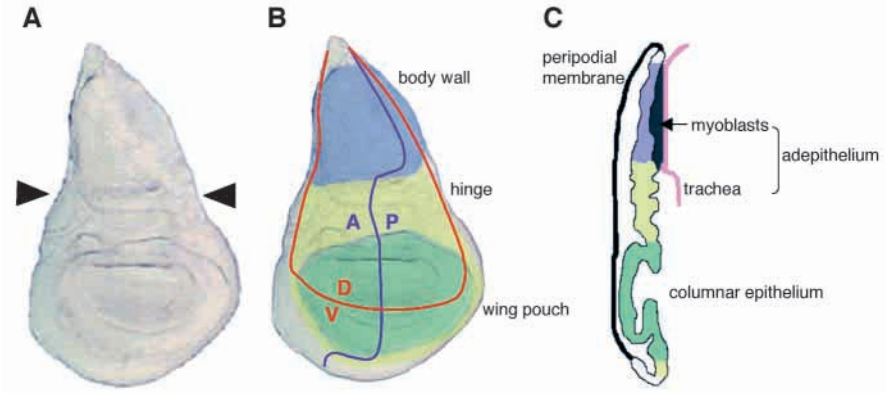

Fig. 1. *Drosophila* wing disc and fate maps. (A) Third instar wing disc. To generate the wing/hinge and body wall fragments, the discs were cut across above the folds corresponding to the hinge region (arrowheads). (B) Fate map of the wing disc showing the anterior-posterior (AP) and dorsal-ventral (DV) compartment boundaries and major regions in the disc. In the adult, the wing pouch (green) gives rise to the wing blade, the hinge (yellow) constricts to form a mobile link to the body wall (blue) or mesonotum of the fly. (C) Cell layers of the wing disc. There are three cell layers: the squamous epithelium or peripodial membrane, the columnar epithelium that gives rise to the adult epidermis, and the adepithial layer comprised of myoblasts, which develop into the flight muscles of the thorax, and tracheal cells of the larval and future adult airways.

Table 1. Body wall enriched transcripts

Gene/synonym	FC	Signal	Function/homology	Expression pattern/Ref	Rank
Transcription factors (17)					
pannier	29.4	497	Zinc-finger (GATA-type)	Ramain et al., 1993	1
tailup	23.9	237	LIM domain/homeobox	Fig. 2A	3
twist	22.5	763	bHLH	Bate et al., 1991	4
CG11835	12.8	976	Similar to bicaudal	n.d.	7
BarH1	9.7	401	Homeobox	Sato et al., 1999	11
stripe	8.7	645	Zinc-finger (C2H2)	Fernandes et al., 1996	14
nervy	7.5	185	TAF domain	n.d.	18
zfh1	7	411	Zinc-finger (C2H2); homeobox	Fig. 2H	20
eyegone	6.3	475	Paired box	n.d.	22
C15	4.1	488	Homeobox	n.d.	41
toe/CG10704	3.7	946	Pax domain/homeobox	n.d.	44
drumstick/CG10016	3.7	508	Zinc-finger (C2H2)	n.d.	45
spineless	3.1	336	bHLH-PAS	Duncan et al., 1998	54
Sox102F/CG11153	2.7	125	HMG-box	n.d..	60
Mef2	2.6	305	MADS box	Ranganayakulu et al., 1995	65
odd skipped	2.4	194	Zinc-finger (C2H2)	n.d	72
sob	2.1	1100	Zinc-finger (C2H2)	n.d.	87
Enzymes (15)					
Ance	25.5	7184	Metallopeptidase	n.d.	2
wunen-2/CG8805	6.8	446	Phosphatidate phosphatase	n.d.	21
CG5397	5	406	O-acyltransferase	Fig. 2P	33
Idgf4	4.6	4778	Glycoside hydrolase	Fig. 2Q	37
CG4386	4.5	190	Trypsin family serine protease	Fig. 2R	39
dHS6ST/CG17188	3.3	176	Heparan sulfate 6-O-sulfotransferase	Kamimura et al., 2001	50
CG30360/CG8689/CG30359	3.2	179	α-amylase	n.d.	51
Gs2	3	279	Cytoplasmic glutamine synthetase	n.d.	55
Gld	2.6	1056	Glucose dehydrogenase	Cox-Foster et al., 1990; Fig. 2Y	62
CG6199	2.4	841	Lysyl hydroxylase	n.d.	73
Ugt58Fa/CG4414	2.4	702	UDP-glucuronosyltransferase	n.d.	74
SP1029/CG11956	2.3	1514	Aminopeptidase	n.d.	76
CG9747	2.2	451	Fatty acid desaturase	n.d.	85
CG7860	2.1	1301	Asparaginase	n.d.	86
Traf1	2.1	168	TNF receptor-associated factor 1	Preiss et al., 2001	91
Cell adhesion proteins (12)					
BM-40/SPARC	7.6	2295	Ca^{2+}-binding glycoprotein	Fig. 2F	17
viking	6.3	390	Collagen IV alpha2 chain	Fig. 2I	23
Cg25C	5.4	867	Collagen IV alpha-chain	Fig. 2N	31
trol/CG7981/perlecan	4.4	340	Basement membrane proteoglycan	n.d.	40
CG3244	3.4	278	C-type lectin domain	Fig. 2S	47
CG10275	3.4	543	Laminin G domain,	n.d.	49
Tsp/CG11326	3.1	537	Thrombospondin-3 like	Fig. 2W	53
CG3624	2.4	3410	Ig domain	n.d.	70
Fas2	2.3	335	NCAM homolog	n.d.	80
ImpL2	2.1	1254	Secreted Ig-family	Klebes et al., 2002; Osterbur et al., 1988	90
scab/alphaPS3	2.1	159	PS-integrin, alpha-subunit	Brower et al., 1984	92
CG10323	2	158	Fibulin/fibrillin-like	n.d.	94
Ligand binding/carrier proteins (12)					
heartless	9.1	194	Fibroblast growth factor receptor	Emori and Saigo, 1993	13
Obp56a/CG11797	7.2	650	Odorant binding	Fig. 2G	19
Obp99a/CG18111	5.6	1338	Odorant binding	Fig. 2K	28
CG10126	5.6	435	EF-hand family Ca-binding	Fig. 2L	29
CG2663	3.4	658	α-tocopherol transfer protein-like	Fig. 2T	48
CG3896	2.8	434	Ferric reductase-like	n.d.	59
CG16820	2.7	176	Odorant binding	n.d.	61
Fbp1	2.6	421	Protein transporter	n.d.	63
regucalcin/CG1803	2.6	1347	SMP30 Ca^{2+}-binding domain	n.d.	64
CG9358	2.5	6517	Odorant binding	n.d.	68
Obp56d/CG11218	2.1	152	Odorant binding	n.d.	88
Tre1/CG3171	2	430	G-protein-coupled taste receptor	n.d.	93
Structural proteins (4)					
CG9593	6.1	286	Fibrinogen C-terminal domain-like	n.d.	24
Act57B	5.2	1255	Actin filament component	Fig. 2O	32
Lcp1	2.2	637	Larval cuticle protein	n.d.	81
CG12505	2.2	280	Zn-finger (CCHC)	n.d.	82
Drip	3.9	582	Aquaporin-like, water transporter	n.d.	42

Table 1. Continued

Gene/synonym	FC	Signal	Function/homology	Expression pattern/Ref	Rank
Signal transduction proteins (5)					
stumps	10.5	380	Pioneer, FGFR pathway	n.d.	9
E(spl)m6	9.3	151	Brd gene family	Lai et al., 2000; Wurmbach et al., 1999	12
eiger/CG12919	4.7	124	TNF-family ligand	n.d.	36
RhoGAP18B/CG7481	2.4	750	GTPase activation domain	n.d.	71
Pvf2/CG13780	2.2	275	Ligand for Pvr RTK	n.d.	84
Miscellaneous proteins (5)					
Ef1alpha100E	5.8	769	Translation elongation factor	Fig. 2J	27
CG5980	3.7	628	PDZ domain	n.d.	46
apontic	2.8	420	RNA binding	n.d.	58
BcDNA:LD28120/CG8062	2.5	183	Monocarboxylate transporter	n.d.	67
Unknown (24)					
CG11100	15.7	140		Fig. 2B	5
CG15064	15.5	471		Fig. 2C	6
CG15353	12.3	11612		Fig. 2D	8
CG6921	9.7	145		Fig. 2E	10
CG13023	8.5	453		n.d.	15
CG9192	7.7	178		n.d.	16
CG13044	6.1	1100		n.d.	25
CG12443	6.1	458		n.d.	26
CG9338	5.5	367		n.d.	30
CG15785	5	464		n.d.	34
CG15786	4.8	219		n.d.	35
CG12481	4.6	328		n.d	38
CG13194	3.9	490		n.d	43
CG9812	3.2	595		n.d	52
CG1572	3	768		n.d	56
CG11370	2.9	638		n.d	57
BcDNA:GH07269/CG6528	2.6	380		n.d	66
CG10200	2.4	2351		n.d	69
CG9312	2.3	490		n.d	75
CG5391	2.3	349		n.d	77
CG17549	2.3	829		n.d	78
CG3770	2.3	542		n.d	79
CG13986	2.2	976		n.d	83
CG13053	2.1	1076		n.d	89

The transcripts are listed in rank order of fold change grouped by the predicted function of the encoded protein. The average signal of the transcript on the three arrays, and its overall rank by fold change are shown. Only transcripts showing a twofold or greater difference ($P \geq 0.95$) between the wing and body wall samples in at least six out of the nine comparisons are included.

(Wurmbach et al., 1999). In contrast, genes that show ubiquitous expression such as Ras or tubulin show no enrichment on the arrays (see http://dev.biologists.org/supplemental/).

Microarray analysis can therefore identify transcripts known to be differentially expressed in the wing/hinge and body wall regions of the disc. Further, the rank order of these by fold change reflects the level of enrichment so that transcripts with more restricted domains appear higher on the list. Few expression patterns of the genes on our list have been described, so to verify the validity of the approach, and to discover more genes with potential roles in the development of these specific regions, we made in situ hybridizations for some of these uncharacterized genes.

In situ hybridization confirms the restricted expression of previously uncharacterized genes

We analyzed 50 transcripts, shown in Tables 1 and 2, that had strong enrichment (mostly three-fold or greater). For the body wall-enriched transcripts, the larger set, we analyzed only transcripts for which clones are available in the *Drosophila* gene collections (DGC1 and DGC2, Berkeley Drosophila Genome Project). For the wing/hinge region, we examined transcripts with three-fold or greater enrichment, systematically in rank order from the top, and generated PCR probes when clones were not available. We found that all transcripts tested showed expression patterns that were consistent with the microarray data providing confirmation that the microarray analysis mirrors the spatial distribution of transcripts in vivo. Body wall-specific expression patterns are shown in Fig. 2 and wing/hinge-specific patterns are shown in Fig. 3.

Genes with elevated expression in the body wall

The wing disc comprises three cell layers; the squamous epithelium of the peripodial membrane, the columnar epithelium that becomes the adult epidermis, and the adepithelial layer that includes myoblast cells that give rise to adult thoracic muscles and tracheal cells that form air passages (Fig. 1C). The adepithelial layer extends from the proximal disc dorsally into the hinge region (Fig. 1C). The body wall fragment includes cells of all three layers, so the arrays also identified transcripts specific to muscle and tracheal cells.

pan and *BarH1*, which encode transcription factors, are expressed in the body wall epidermis and are involved in bristle patterning (Ramain et al., 1993; Sato et al., 1999). Both transcripts were highly enriched on the arrays (Table 1). Also highly enriched was *tailup* (*tup*) (Thor and Thomas,

Table 2. Wing/hinge enriched transcripts

Gene/synonym	FC	Signal	Function/homology	Expression pattern/Ref.	Rank
Transcription factors (18)					
dve	60.3	809	Homeobox	Fig. 3B	2
pdm2	58.7	1329	POU/homeobox	Fig. 3C	3
kn	42.8	353	HLH	Mohler et al., 2000; Vervoort et al., 1999	4
Pox-n	30.4	191	Paired box	Dambly-Chaudiere et al., 1992	6
opa	16.8	161	Zinc-finger (C2H2)	Fig. 3G	9
nubbin	16.4	2039	POU/homeobox	Ng et al., 1995	10
Dll	12.4	780	Homeobox	Campbell et al., 1993; Gorfinkiel et al., 1997	13
CG15000	8.4	686	NABP homolog	Fig. 3L	16
zfh2	6.5	798	Zinc-finger (C2H2); homeobox	Klebes et al., 2002	19
Sox15	4.7	670	HMG-box	Cremazy et al., 2001	22
bifid/omb	4.3	321	T-box	Grimm and Pflugfelder, 1996	24
Doc2/CG5187	4.2	312	T-box	Fig. 3R	25
rotund/roe/CG10040	4.2	687	Zinc-finger (C2H2)	St Pierre et al., 2002	26
vvl	3.3	651	POU/homeobox	de Celis et al., 1995	31
engrailed	2.4	910	Homeobox	Kornberg et al., 1985	41
dorsocross	2.3	1307	T-box	Fig. 3X	44
vg	2.0	1597	Novel	Williams et al., 1991	55
inv	2.0	591	Homeobox	Coleman et al., 1987	56
Enzymes (8)					
CG17278	68.2	9821	Serpin	Fig. 3A	1
CG3132	28.7	302	β-galactosidase	Fig. 3E	7
Nep1/CG5894	19.3	315	Neprilysin metalloprotease	Fig. 3F	8
Cyp310a1	8.9	571	Cytochrome P450	Fig. 3J	14
Ugt86Di/CG6658	3.8	797	UDP-glucosyl transferase	Fig. 3S	27
pipe	2.4	110	Heparin sulfotransferase	n.d.	40
mas	2.2	1521	Serine-type endopeptidase	n.d.	51
Inos	2.1	3695	Myo-inositol-1-phosphate synthase	n.d.	53
Cell adhesion proteins (5)					
CG5758	3.6	686	β-Ig-H3/Fasciclin	Fig. 3U	29
CG8381/CG30069	3.5	2294	Proline-rich/PEVK motif	Fig. 3V	30
inflated	2.9	284	α-PS2 integrin	Brower et al., 1984	33
Netrin-A	2.4	107	Laminin EGF	n.d.	42
echinoid/CG12676	2.6	393	Tmb-protein/Egfr antagonist	Bai et al., 2001	37
Ligand binding/carrier proteins(3)					
CG4861/CG31094	6.2	200	ldl-receptor	Fig. 3O	20
CG9057	2.5	2854	Lipid associated protein	n.d.	38
wengen/CG6531	2.3	277	TNFR-family receptor	Fig. 3Y	47
Structural proteins (4)					
CG6469	4.8	341	Larval cuticle	Fig. 3P	21
CG14301	4.5	459	Chitin binding	Fig. 3Q	23
CG7160	2.5	640	Larval cuticle	n.d.	39
Gasp	2.1	272	Chitin-binding protein	n.d.	54
Signal transduction proteins (2)					
cv-2/CG15761	2.3	260	Secreted or transmembrane	Conley et al., 2000	43
hh	2.2	399	Secreted ligand	Tabata et al., 1992	49
Miscellaneous proteins (8)					
CG14534	8.2	206	DUF243 domain	Fig. 3M	17
ana	3.6	1081	Novel secreted glycoprotein	Fig. 3T	28
CG8483	2.9	804	Venom allergen	Fig. 3W	33
geko/CG13696	2.6	388	Olfaction	n.d.	36
CG17357/CG32048/CG3179	2.3	570	Phosphotyrosine interaction domain	n.d.	45
CG12063	2.3	446	PAN domain	n.d.	46
CG3307	2.2	185	SET domain	n.d.	48
CG6166	2.1	261	Bacterial ABC transporter	n.d.	52
Unknown proteins (8)					
CG8780	31.0	596		Fig. 3D	5
CG15001	13.0	156		Fig. 3H	11
CG15489	12.8	341		Fig. 3I	12
CG15488	8.7	503		Fig. 3K	15
BG:DS00797.2/CG9008	6.8	1124		Fig. 3N	18
CG16868	2.9	623		n.d.	34
CG6234	2.8	2079		n.d.	35
EG0002.3/CG2904	2.2	1277		n.d.	50

The transcripts are listed in rank order of fold change (FC) grouped by the predicted function of the encoded protein. The average signal of the transcript on the three arrays, and its overall rank by fold change are shown. Only transcripts showing a twofold or greater difference ($P \geq 0.95$) between the wing and body wall samples in at least six out of the nine comparisons are included.

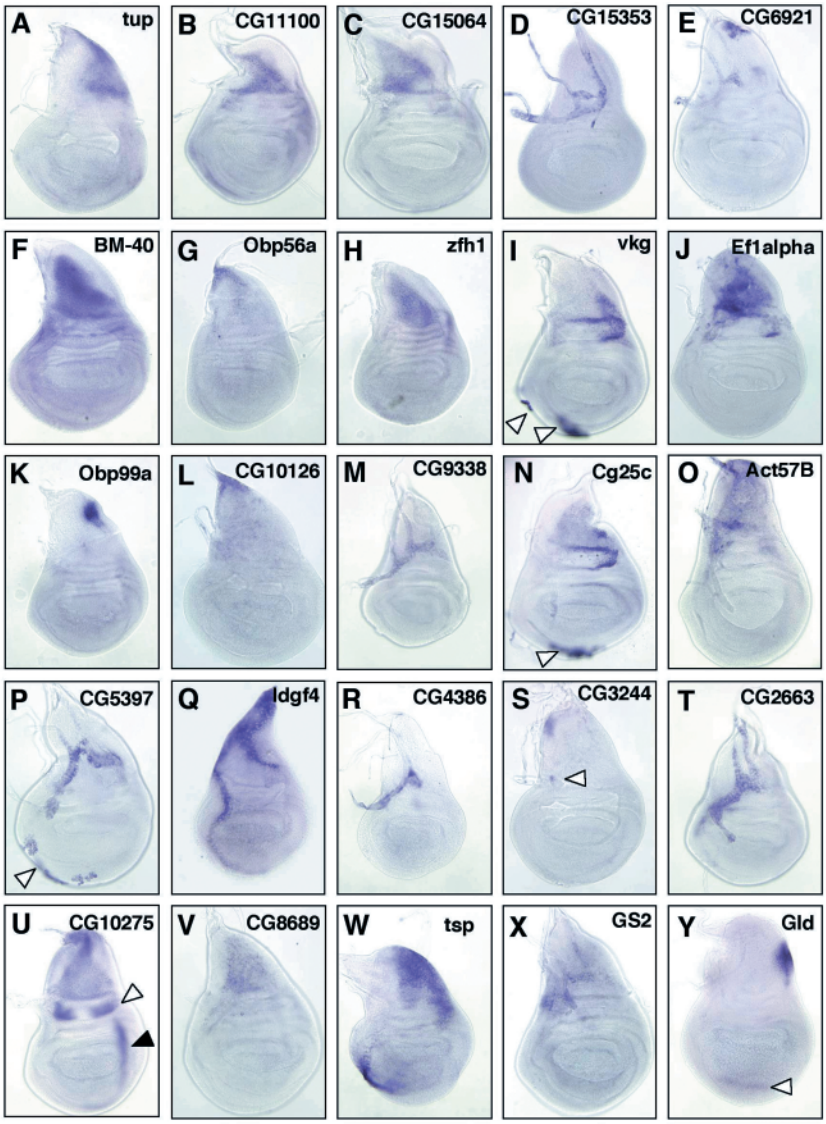

Fig. 2. Expression patterns of transcripts enriched in the body wall sample. The expression patterns of the transcripts are shown in the overall rank order according to fold change with the most highly enriched shown first (Table 1). (A) *tailup* (*tup*) is expressed in cells of the dorsal posterior epithelium. (B) *CG11100* is expressed in the myoblasts. (C) *CG15064* is expressed in the myoblasts. (D) *CG15353* is expressed in tracheal cells. (E) *CG6921* is highly expressed at the proximal tip of the disc and weakly in tracheal cells. (F) *BM-40/SPARC* is expressed in the myoblasts. (G) *Obp56a/CG11797* is expressed in stalk cells at the proximal tip of the disc. (H) *zinc finger homology 1* (*zfh1*) is expressed in the myoblasts. (I) *viking* (*vkg*) is expressed in myoblasts and blood cells (arrowheads, one group of cells is out of the plane of focus). (J) *Elongation factor 1 alpha100E* (*Ef1 alpha*) is expressed in the myoblasts. (K) *Obp99a/CG18111* is expressed in the epithelium in cells of the presumptive scutellum. (L) *CG10126* is expressed in stalk cells at the proximal tip of the disc. (M) *CG9338* is expressed in tracheal cells. (N) *Cg25C* is expressed in myoblasts and blood cells (arrowhead). (O) *Actin 57B* (*Act57B*) is expressed in myoblasts. (P) *CG5397* is expressed in tracheal cells and blood cells (arrowhead). (Q) *Imaginal disc growth factor 4* (*Idgf4*) is expressed in the dorsal peripodial membrane. (R) *CG4386* is expressed in the dorsal branch of the tracheal system. (S) *CG3244* is expressed in two patches of cells in the prescutum (the smaller cluster is indicated with an arrowhead). (T) *CG2663* is expressed in the trachea. (U) *CG10275* is expressed in the myoblasts, hinge columnar epithelium (white arrowhead), and peripodial membrane (black arrowhead). (V) *CG8689* is expressed in the myoblasts. (W) *thrombospondin/CG11326* (*tsp*) is expressed in the dorsal posterior body-wall region and the anterior ventral wing hinge. (X) *Glutamine synthetase* (*GS2*) is expressed in tracheal cells. (Y) *Glucose dehydrogenase* (*Gld*) is expressed in the posterior dorsal epithelium in cells of the presumptive postnotum and a line of cells in the hinge region (arrowhead).

1997), which encodes a LIM domain homeobox protein, and is expressed in the epithelium in a large region of the posterior body wall encompassing the presumptive postnotum, scutellum and scutum (Fig. 2A). No role for *tup* in patterning the mesothorax has been described. Another transcript with broad expression was *thrombospondin/CG11326 (tsp)*, which is expressed in a similar region of the body wall to *tup* (Fig. 2W). *tsp* is also expressed in the ventral hinge and hence shows lower enrichment on the arrays. The other genes found to be specific to the epithelium showed highly localized expression: *Obp56a*/*CG11797* (Fig. 2G), *CG10126* (Fig. 2L), *CG3244* (Fig. 2S) and *Glucose dehydrogenase* (Fig. 2Y). *Obp56a*/*CG11797* encodes an odorant-binding protein and interestingly three other odorant-binding proteins showed enrichment on the arrays (Table 1): *Obp99a*/*CG18111* (Fig. 2K), *CG9358* and *Obp56d/CG1128*. We found *Idgf4*, encoding an imaginal disc growth factor (Kawamura et al., 1999), is expressed in the peripodial membrane, primarily in dorsal cells (Fig. 2Q). Presumably secretion of Idgf4 could influence development of the columnar epithelium.

Myoblast cells of the adepithelial layer develop into the direct and indirect flight muscles of the thorax, and genes involved in the development of these muscles have been shown to be expressed in the myoblasts during wing disc development. Several of these transcripts are enriched on the arrays (Table 1): *Mef2* (Cripps et al., 1998), *twist* (*twi*) (Bate et al., 1991) and *heartless* (*htl*) (Cripps et al., 1998). *Act57B* is known to be regulated by *Mef2* in the embryo (Kelly et al., 2002), and we show *Act57B* is expressed in the myoblasts (Fig. 2O), suggesting this relationship also exists in these adult muscle precursors. *Mef2* expression is activated by *twi* (Cripps et al., 1998) and may be inhibited by the transcriptional repressor, *zinc finger homology 1* (*zfh1*) (Postigo et al., 1999). *zfh1* is expressed in the myoblasts (Fig. 2H). *stumps* is also enriched on the arrays (Table 1) and expressed in the myoblasts (Sato and Kornberg, 2002). Together with *htl*, *stumps* has a role in the development of the tracheal cells (Imam et al., 1999; Sato and Kornberg, 2002) (see also below). *Viking* (*Vkg*) encodes a component of collagen type IV and is known to be coexpressed with *Cg25C*, another collagen IV subunit in the embryo and in blood cells (Yasothornsrikul et

al., 1997). Both transcripts are enriched on the arrays (Table 1) and show similar expression patterns in the adepithelial myoblasts and blood cells (Fig. 2I,N). Other genes showing specific expression in the myoblasts are *BM-40/SPARC*, a calcium-binding glycoprotein, which is expressed in the embryonic mesoderm (Furlong et al., 2001; Martinek et al., 2002) (Fig. 2F), *Elongation factor 1 alpha 100E* (*Ef1 alpha*) (Hovemann et al., 1988) (Fig. 2J), *CG8689*, an alpha-amylase (Fig. 2V), and two transcripts encoding predicted proteins with unknown function *CG11100* (Fig. 2B) and *CG15064* (Fig. 2C).

In the wing disc, cells of the larval and developing adult tracheal systems require activity of genes in the FGF pathway (Sato and Kornberg, 2002). Some of the key genes are expressed in the myoblasts (for example, *htl* and *stumps*), others in the epithelium (for example, *branchless*, *bnl*), and others in the tracheal cells themselves (for example, *breathless*, *btl*) (Sato and Kornberg, 2002). *htl* and *stumps* showed enrichment on the arrays but *bnl* and *btl* were not detectable. For *bnl* this may be because expression is highly localized and apparently at very low levels (Sato and Kornberg, 2002). However, it is not clear why the arrays failed to detect *btl* expression because we did identify six genes that are also expressed specifically in tracheal cells. These are *CG5397*, an O-acyltransferase (Fig. 2P), *CG4386*, a serine-type endopeptidase (Fig. 2R), *CG2663*, an alpha-tocopherol transfer-like protein (Fig. 2T), and *CG15353* (Fig. 2D), *CG6921* (Fig. 2E) and *CG9338* (Fig. 2M) that have no known homologies. In particular, *CG4386* is interesting as it is only expressed in the dorsal branch (Fig. 2R), and *CG6921* is distinguished because it is very strongly expressed in the most proximal cells (Fig. 2E).

Genes with elevated expression in the wing pouch and hinge regions

The wing/hinge fragment of the wing disc primarily contains cells of the peripodial membrane and the columnar epithelium (Fig. 1C), with only a few myoblasts that extend into the hinge region. Thus the genes detected by the arrays as enriched in this disc fragment were expressed in cells of one of the two epithelial layers.

Transcription factors comprise the largest category of genes (18/56) with elevated expression in the wing/hinge region. These are expected to have regulatory roles in patterning the region. Transcription factors with known expression domains and roles in wing development are present: *kn*, *pox-n*, *nub*, *Dll*, *bifid/optomotor blind*, *rotund*, *ventral veins lacking*, *en*, *vg* and *in* (Awasaki and Kimura, 2001; Cohen et al., 1989; Coleman et al., 1987; de Celis et al., 1995; Grimm and Pflugfelder, 1996; Kornberg et al., 1985; Mohler et al., 2000; Ng et al., 1995; St Pierre et al., 2002; Tabata et al., 1992; Vervoort et al., 1999). *pdm2*, which is highly related to *nub*, also shows wing-enriched expression on the arrays and is expressed in a similar domain to *nub* (Fig. 3C) (Ng et al., 1995). *pdm2* apparently has no significant function in the wing (Yeo et al., 1995). The roles of the remaining seven predicted transcription factors is unknown, although the expression pattern of *zinc finger homology 2 (zfh2)* and *Sox 15* have been described and both are expressed specifically in the hinge region (Cremazy et al., 2001; Klebes et al., 2002). *defective proventriculous* (*dve*), which encodes a homeodomain protein (Nakagoshi et al., 1998), and *CG15000*, which is similar to NGFI-A-binding protein 2, are broadly expressed in the wing pouch, although *dve* is downregulated at the DV compartment boundary (Fig. 3B,L). *odd paired* (*opa*), known for a role in embryonic segmentation (Benedyk et al., 1994), is discretely expressed in cells of the presumptive mesopleura and dorsal hinge (Fig. 3G). No role for *opa* in wing disc development has been reported. *Dorsocross1* (*Doc1*) (Lo and Frasch, 2001) and *Doc2*/*CG5187* are T-Box related factors that are expressed in what appears to be an identical domain in the wing disc (Fig. 3R,X). Both transcripts also accumulate in body wall cells and this probably lowers their position in the overall ranked list (Table 2).

Eight transcripts encoding enzymes are enriched two-fold or greater in the wing/hinge region (Table 2). This group includes the most highly enriched transcript detected in the analysis, a kazal-type serpin gene *CG17278* (68-fold, Table 2). *CG17278* shows a strong and specific expression pattern in the wing encompassing most of the wing pouch. One of the potentially most interesting wing-enriched enzymes is a cytochrome P450 gene, *Cyp310a1*. This gene is strongly expressed in the dorsal and ventral parts of the wing pouch but excluded from the DV and AP boundaries (Fig. 3J). Variable expression in anterior body wall cells is also observed which is consistent with the array data that indicate *Cyp310a1* transcripts are also present in body wall RNA. The role of cytochrome P450 genes in development has recently been reviewed (Stoilov, 2001), and the list of these genes with roles in development is growing. Surprisingly, the β-galactosidase gene (*CG3132*) was found to be enriched in the wing/hinge region (Table 2). β-gal expression in *Drosophila* has been analyzed and although it is expressed in some discs, wing expression was not reported (Schnetzer and Tyler, 1996). We did find weak expression in a cluster of cells in the hinge but the majority of expression is in blood cells, which adhere preferentially to the distal disc margin (Fig. 3E). Thus the β-gal transcript probably appears as wing/hinge enriched primarily because it is expressed in blood cells. We also determined the expression pattern of two other enzymes; the metalloendopeptidase *Nep1*/*CG5894* (Fig. 3F) and *UDP-glucosyl transferase* (*Ugt86Di*) (Fig. 3S).

The α-integrin, *inflated*, which has a role in cell adhesion, is expressed in the ventral compartment (Brower et al., 1984) and is thus enriched on the wing/hinge arrays (Fig. 1, Table 2). A novel gene, *CG5758*, is potentially involved in cell adhesion as it encodes a predicted protein with β-Ig-H3/Fas domains and its expression is restricted to the dorsal hinge (Fig. 3U). *CG8381* encodes a proline-rich protein with repeated 'PEVK' motifs also found in titin. This gene is strongly expressed in the wing pouch but repressed in cells of the future veins and cells at the DV margin (Fig. 3V). Despite intense expression in the wing pouch, *CG8381* shows only modest enrichment on the arrays (Table 2), probably reflecting the fact that the gene is also expressed in several groups of cells in the body wall region (Fig. 3V).

The expression of two receptors was determined. *CG4861* encodes an ldl-receptor-like protein and is expressed at very low levels throughout the wing pouch (Fig. 3O). *wengen/CG6531*, which is a receptor of the TNFR family (Kanda et al., 2002), is expressed strongly in the wing pouch and weakly in the body wall (Fig. 3Y). On the arrays, its ligand, *eiger* (Kanda et al., 2002), was undetectable in the wing/hinge region sample but enriched in the body wall sample (Table 1).

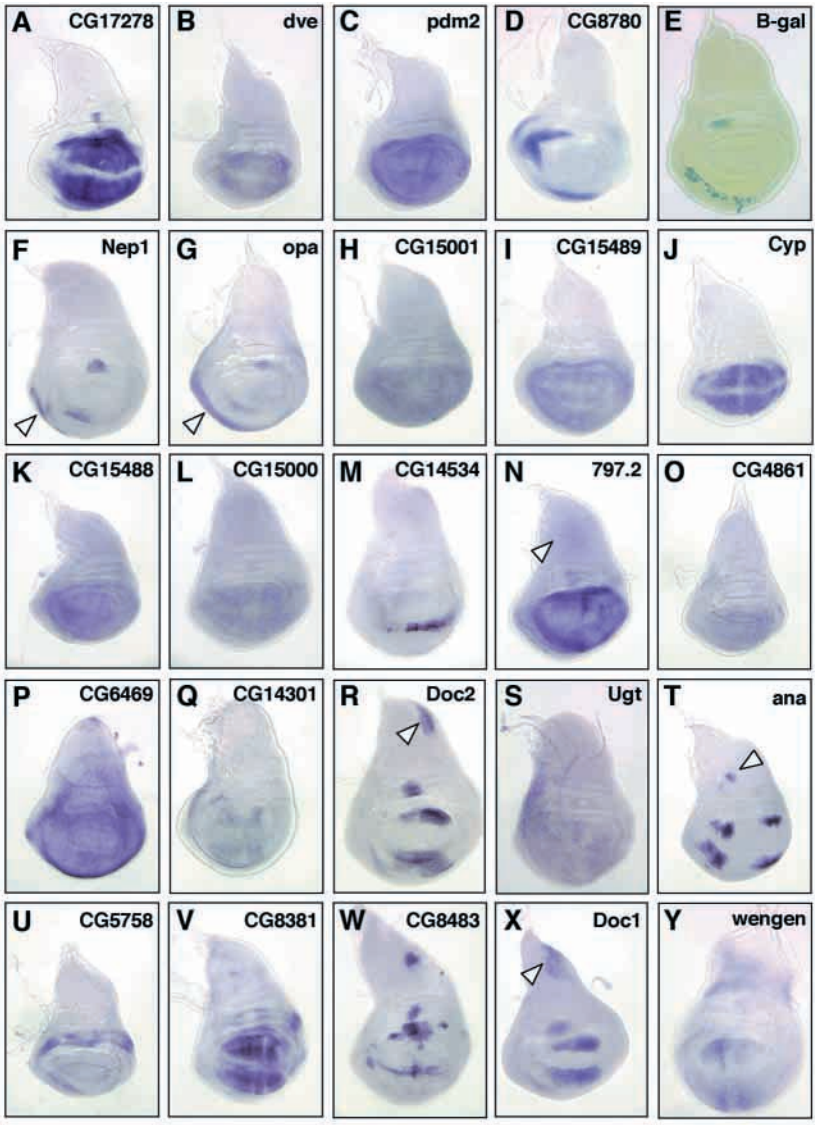

Fig. 3. Expression patterns of transcripts enriched in the wing/hinge sample. The expression patterns of the transcripts are shown in the overall rank order according to fold change with the most highly enriched shown first (Table 2). All genes are expressed in the columnar epithelium except where noted. (A) *CG17278* is strongly expressed throughout the wing pouch, but not in cells at the DV margin, and expressed in a small cluster of cells in the dorsal hinge. (B) *defective proventriculous (dve)* is weakly expressed throughout the wing pouch, but not in cells at the DV margin. (C) *POU domain protein 2 (pdm2)* is expressed throughout the wing pouch. Expression levels vary with higher expression in cells probably corresponding to vein precursors. (D) *CG8780* is expressed in cells of the mesopleura and ventral hinge. (E) *β-galactosidase/CG3132* (*β-gal*) is expressed in a cluster of cells in the dorsal hinge and in blood cells that adhere to the anterior ventral margin of the disc. (F) *Nep1/CG5894* is expressed in the mesopleura (arrowhead) and dorsal and ventral hinge. (G) *odd paired* (*opa*) is expressed in the mesopleura (arrowhead) and dorsal and ventral hinge. (H) *CG15001* is weakly expressed throughout the wing pouch. (I) *CG15489* is expressed weakly throughout the wing pouch. Expression levels vary with higher expression in cells probably corresponding to vein precursors. (J) *Cyp310a1* (*Cyp*) is expressed throughout the wing pouch except in cells at the DV and AP margins. (K) *CG15488* is expressed throughout the wing pouch. Expression levels vary with higher expression in cells probably corresponding to vein precursors. (L) *CG15000* is weakly expressed throughout the wing pouch. (M) *CG14534* is specifically expressed in cells that give rise to the posterior margin of the wing. (N) *BG:DS00797.2/CG9008* (*797.2*) is strongly expressed throughout the wing pouch with some modulation in levels that does not appear to correlate with known features such as veins. Transcripts are also present at low levels in adepithial cells (arrowhead, out of the plane of focus). (O) *CG4861* is expressed very weakly throughout the wing pouch. (P) *CG6469* is expressed in peripodial cells with higher levels of expression in ventral cells. (Q) *CG14301* expression is weak but elevated expression was seen in cells at the anterior disc margin and in four clusters of cells in the wing pouch and overlying peripodial membrane. (R) *Doc2*/*CG5187* is expressed in four patches of cells including a group of cells in the body wall (arrowhead). (S) *UDP-glucosyl transferase 86Di/CG6658* (*Ugt*) is expressed in a diffuse pattern with elevated expression along the anterior disc margin, extending into the body wall region, and in intervein regions. (T) *anachronism* (*ana*) is expressed in clusters of cells and individual cells, probably neuroblasts. A cluster of cells in the body wall region express *ana* (arrowhead). (U) *CG5758* is expressed in the hinge region. Expression is modulated so that the pattern has a patch-like appearance. (V) *CG8381* is expressed strongly in the wing pouch with modulation at the DV margin and the presumptive veins. The gene is also expressed at low levels in several clusters of cells in the body wall region. (W) *CG8483* is expressed along the DV margin and in several clusters of cells in the hinge and body wall regions. (X) *Dorsocross 1* (*Doc1*) is expressed in four patches of cells including a group of cells in the body wall (arrowhead). (Y) *wengen/CG6531* is expressed in the wing pouch with stronger expression in future veins and weakly in the body wall region (cells out of plane of focus).

Two structural proteins, *CG6469*, a larval cuticle protein, and *CG14301*, a chitin-binding protein, are the only genes we identified as being expressed in the ventral peripodial membrane. *CG6469* is expressed broadly in the peripodial membrane but at a higher level in the ventral region (Fig. 3P). *CG14301* is expressed in cells of both epithelial layers, in the columnar epithelium at the anterior disc margin and in four patches of cells in the wing pouch and the overlying peripodial membrane (Fig. 3Q).

In a group of genes with miscellaneous functions (Table 2) we determined the expression of three genes. *anachronism* (*ana*), a secreted glycoprotein (Ebens et al., 1993), is expressed in five clusters of cells including one in the body wall region and in some individual neuroblasts (Fig. 3T). *ana* null mutants are viable and have no observable defects suggesting it is not required, or functions redundantly, in the wing (Park et al., 1997). *CG14534*, which has a domain that has been recognized in several proteins but has an unknown function (DUF243), is expressed only in cells that will give rise to the posterior wing margin (Fig. 3M). *CG8483*, which

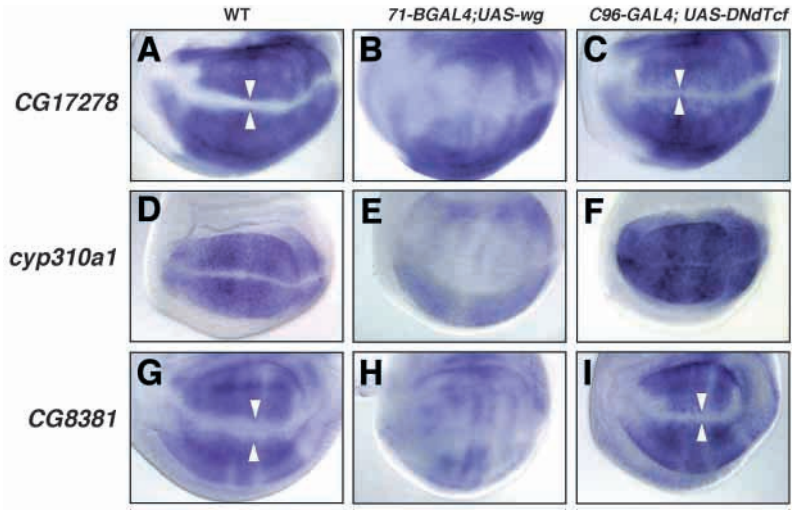

Fig. 4. Regulation by *wg*. In wild-type discs, *CG17278* (A), *Cyp310a1* (D) and *CG8381* (G) are expressed broadly in the wing pouch but excluded from cells at the DV boundary. Misexpression of *wg* in the dorsal and ventral wing pouch (*71B-GAL4; UAS-wg*) inhibits expression of the genes in a broad band of cells in the wing pouch (B,E,H). Inhibition of Wg signaling at the DV margin (*C96-GAL4; UAS-DNdTcf*) induces more cells at the margin to express the genes (C,F,I). At the center of a wild-type disc approximately four rows of cells do not express *CG17278* (A, arrowheads), whereas expressing cells are adjacent at this point when Wg is inhibited (C, arrowheads). When Wg is inhibited, *Cyp310a1*, expression spans the DV boundary for its entire length (F). Expression of *CG8381* in the wild-type wing pouch is excluded from a band of six to seven cells at the DV boundary (G, arrowheads) and this is reduced to approximately two cells at the equivalent position when Wg is inhibited (I, arrowheads).

has homology to a venom allergen, is expressed in a complex pattern suggestive of expression in peripheral sense organ precursors (Fig. 3W).

We determined the expression pattern for five of eight genes for which the sequence reveals no homology to known protein domains. *CG15489* and *CG15488* (Fig. 2I,K) are in a cluster of genes also including *nub* and *pdm-2* that are expressed in similar domains and are adjacent in the genome. *CG15001*, consisting of only a single exon, is adjacent to another gene (*CG15000*), also discovered on the arrays, with a similar expression domain (Fig. 3H,L). *BG:DS00797.2/CG9008* is expressed strongly in the wing pouch and also in the adepithelial cell layer (Fig. 3N). *CG8780* is highly enriched on the arrays (31-fold, Table 2) and expressed specifically in the hinge and ventral pleura (Fig. 3D).

Regulation by *wg* signaling

The genes, *CG17278*, *Cyp310a1* and *CG8381* all show very intense expression in the wing pouch but reduced expression at the DV margin (Fig. 4A,D,G). Wg is expressed at the DV margin forming a gradient that regulates the expression of target genes in a concentration-dependent manner (Strigini and Cohen, 2000). To determine whether Wg signaling represses the expression of *CG17278*, *Cyp310a1* and *CG8381*, we ectopically expressed *wg* in the dorsal and ventral wing-pouch regions (*71B-gal4*; *UAS-wg*), or inhibited Wg function at the DV margin by expressing a dominant-negative form of TCF (van de Wetering et al., 1997), a transcription factor required for Wg-signal transduction (*C96-GAL4*; *UAS-DN-dTCF*). With higher levels of Wg activity in the wing pouch, expression of all three genes was inhibited (Fig. 4B,E,H). In contrast, inhibition of Wg signaling at the DV margin allowed ectopic expression of *Cyp310a1* in all margin cells and increased the number of cells expressing *CG17278* and *CG8381* (Fig. 4F,C,I). In the presumptive margin, cells continue to express *wg* in the absence of Wg activity, cell replication increases (Phillips and Whittle, 1993), and ectopic expression of *dmyc* appears in margin cells (Johnston et al., 1999). Therefore, ectopic expression of the genes studied here is caused by loss of Wg-dependent repression rather than loss of the non-expressing cells from the presumptive margin. This does not imply that Wg-dependent repression must be direct. Without functional data on these potential target genes, their relationship to *wg* and their role in wing patterning remain unknown.

Concluding remarks

We have shown that microarray analysis of RNA profiles, followed by in situ hybridization, can rapidly identify candidate genes that warrant investigation for their role in wing disc development. Most genes identified here are not represented by mutant alleles. This may reflect any of several situations. (1) Some genes may be refractive to mutagenesis. This is unlikely to be the case for chemical mutagens or ionizing radiation, but P elements show specificity in insertion site (Liao et al., 2000). As P elements are the mutagen of choice in most current screens, for example, the Berkeley Drosophila Genome Project (BDGP) screen (Spradling et al., 1999), some genes may not be susceptible. (2) Redundancy masks gross phenotypes. This may be a factor for highly related genes such as *Doc1* and *Doc2/CG5187*, which are also expressed in very similar patterns (Fig. 3R,X), and for members of multi-gene families such as the cytochrome, *Cyp310a1* and the serpin, *CG17278*. (3) The genes have no crucial function. Despite having a localized expression pattern, some genes may play a minor role or no role in the cells in which they are expressed.

The challenge will be to decide among these possibilities in a new round of genetic analysis that uses techniques such as RNA silencing or homologous recombination to reduce function of specific genes (Fortier and Belote, 2000; Kennerdell and Carthew, 1998; Kennerdell and Carthew, 2000; Martinek and Young, 2000; Piccin et al., 2001; Rong and Golic, 2000; Rong et al., 2002), and if necessary simultaneously in several genes in a family, to determine if phenotypic change occurs. Whole genome profiling is a powerful method to identify genes that then become a high priority for such analysis.

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REFERENCES

Adams, M. D., Celniker, S. E., Holt, R. A., Evans, C. A., Gocayne, J. D., Amanatides, P. G., Scherer, S. E., Li, P. W., Hoskins, R. A., Galle, R. F. et al. (2000). The genome sequence of *Drosophila melanogaster*. *Science* **287,** 2185-2195.

Arbeitman, M. N., Furlong, E. E., Imam, F., Johnson, E., Null, B. H., Baker, B. S., Krasnow, M. A., Scott, M. P., Davis, R. W. and White, K. P. (2002). Gene expression during the life cycle of *Drosophila melanogaster*. *Science* **297,** 2270-2275.

Awasaki, T. and Kimura, K. (2001). Multiple function of *poxn* gene in larval PNS development and in adult appendage formation of *Drosophila*. *Dev. Genes Evol.* **211,** 20-29.

Bai, J., Chiu, W., Wang, J., Tzeng, T., Perrimon, N. and Hsu, J. (2001). The cell adhesion molecule Echinoid defines a new pathway that antagonizes the *Drosophila* EGF receptor signaling pathway. *Development* **128,** 591-601.

Bate, M., Rushton, E. and Currie, D. A. (1991). Cells with persistent twist expression are the embryonic precursors of adult muscles in *Drosophila*. *Development* **113,** 79-89.

Bellen, H. J., O'Kane, C. J., Wilson, C., Grossniklaus, U., Kurth Pearson, R. and Gehring, W. J. (1989). P-element-mediated enhancer detection: a versatile method to study development in *Drosophila*. *Genes Dev.* **3,** 1288-1300.

Benedyk, M. J., Mullen, J. R. and DiNardo, S. (1994). odd-paired: a zinc finger pair-rule protein required for the timely activation of engrailed and wingless in *Drosophila* embryos. *Genes Dev.* **8,** 105-117.

Bier, E., Vaessin, H., Shepherd, S., Lee, K., McCall, K., Barbel, S., Ackerman, L., Carretto, R., Uemura, T., Grell, E. et al. (1989). Searching for pattern and mutation in the *Drosophila* genome with a P-lacZ vector. *Genes Dev.* **3,** 1273-1287.

Brand, A. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118,** 401-415.

Brower, D. L., Wilcox, M., Piovant, M., Smith, R. J. and Reger, L. A. (1984). Related cell-surface antigens expressed with positional specificity in *Drosophila* imaginal discs. *Proc. Natl. Acad. Sci. USA* **81,** 7485-7489.

Campbell, G., Weaver, T. and Tomlinson, A. (1993). Axis specification in the developing *Drosophila* appendage: the role of *wingless*, *decapentaplegic*, and the homeobox gene *aristaless*. *Cell* **74,** 1113-1123.

Cohen, S. M., Bronner, G., Kuttner, F., Jurgens, G. and Jackle, H. (1989). Distal-less encodes a homoeodomain protein required for limb development in *Drosophila*. *Nature* **338,** 432-434.

Coleman, K. G., Poole, S. J., Weir, M. P., Soeller, W. C. and Kornberg, T. (1987). The invected gene of *Drosophila*: sequence analysis and expression studies reveal a close kinship to the engrailed gene. *Genes Dev.* **1,** 19-28.

Conley, C. A., Silburn, R., Singer, M. A., Ralston, A., Rohwer-Nutter, D., Olson, D. J., Gelbart, W. and Blair, S. S. (2000). Crossveinless 2 contains cysteine-rich domains and is required for high levels of BMP-like activity during the formation of the cross veins in *Drosophila*. *Development* **127,** 3947-3959.

Cox-Foster, D. L., Schonbaum, C. P., Murtha, M. T. and Cavener, D. R. (1990). Developmental expression of the glucose dehydrogenase gene in *Drosophila melanogaster*. *Genetics* **124,** 873-880.

Cremazy, F., Berta, P. and Girard, F. (2001). Genome-wide analysis of Sox genes in *Drosophila melanogaster*. *Mech. Dev.* **109,** 371-375.

Cripps, R. M., Black, B. L., Zhao, B., Lien, C. L., Schulz, R. A. and Olson, E. N. (1998). The myogenic regulatory gene *Mef2* is a direct target for transcriptional activation by Twist during *Drosophila* myogenesis. *Genes Dev.* **12,** 422-434.

Dambly-Chaudiere, C., Jamet, E., Burri, M., Bopp, D., Basler, K., Hafen, E., Dumont, N., Spielmann, P., Ghysen, A. and Noll, M. (1992). The paired box gene *pox neuro*: a determinant of poly-innervated sense organs in *Drosophila*. *Cell* **69,** 159-172.

de Celis, J. F., Llimargas, M. and Casanova, J. (1995). *Ventral veinless*, the gene encoding the Cf1a transcription factor, links positional information and cell differentiation during embryonic and imaginal development in *Drosophila melanogaster*. *Development* **121,** 3405-3416.

Duncan, D. M., Burgess, E. A. and Duncan, I. (1998). Control of distal antennal identity and tarsal development in *Drosophila* by spineless-aristapedia, a homolog of the mammalian dioxin receptor. *Genes Dev.* **12,** 1290-1303.

Ebens, A. J., Garren, H., Cheyette, B. N. and Zipursky, S. L. (1993). The *Drosophila* anachronism locus: a glycoprotein secreted by glia inhibits neuroblast proliferation. *Cell* **74,** 15-27.

Emori, Y. and Saigo, K. (1993). Distinct expression of two *Drosophila* homologs of fibroblast growth factor receptors in imaginal discs. *FEBS Lett.* **332,** 111-114.

Fernandes, J. J., Celniker, S. E. and Vijay Raghavan, K. (1996). Development of the indirect flight muscle attachment sites in *Drosophila*: role of the PS integrins and the stripe gene. *Dev. Biol.* **176,** 166–184.

Fortier, E. and Belote, J. M. (2000). Temperature-dependent gene silencing by an expressed inverted repeat in *Drosophila*. *Genesis* **26,** 240-244.

Furlong, E. E., Andersen, E. C., Null, B., White, K. P. and Scott, M. P. (2001). Patterns of gene expression during *Drosophila* mesoderm development. *Science* **293,** 1629-1633.

Gorfinkiel, N., Morata, G. and Guerrero, I. (1997). The homeobox gene *Distal-less* induces ventral appendage development in *Drosophila*. *Genes Dev.* **11,** 2259-2271.

Grimm, S. and Pflugfelder, G. O. (1996). Control of the gene *optomotor-blind* in *Drosophila* wing development by decapentaplegic and wingless. *Science* **271,** 1601-1604.

Gustafson, K. and Boulianne, G. L. (1996). Distinct expression patterns detected within individual tissues by the GAL4 enhancer trap technique. *Genome* **39,** 174-182.

Hovemann, B., Richter, S., Walldorf, U. and Cziepluch, C. (1988). Two genes encode related cytoplasmic elongation factors 1 alpha (EF-1 alpha) in *Drosophila melanogaster* with continuous and stage specific expression. *Nucleic Acids Res.* **16,** 3175-3194.

Imam, F., Sutherland, D., Huang, W. and Krasnow, M. A. (1999). *stumps*, a *Drosophila* gene required for fibroblast growth factor (FGF)- directed migrations of tracheal and mesodermal cells. *Genetics* **152,** 307-318.

Johnston, L. A., Prober, D. A., Edgar, B. A., Eisenman, R. N. and Gallant, P. (1999). *Drosophila myc* regulates cellular growth during development. *Cell* **98,** 779-790.

Kamimura, K., Fujise, M., Villa, F., Izumi, S., Habuchi, H., Kimata, K. and Nakato, H. (2001). *Drosophila heparan sulfate 6-O-sulfotransferase (dHS6ST)* gene. Structure, expression, and function in the formation of the tracheal system. *J. Biol. Chem.* **276,** 17014-17021.

Kanda, H., Igaki, T., Kanuka, H., Yagi, T. and Miura, M. (2002). Wengen, a member of the *Drosophila* tumor necrosis factor receptor superfamily, is required for Eiger signaling. *J. Biol. Chem.* **277,** 28372-28375.

Kawamura, K., Shibata, T., Saget, O., Peel, D. and Bryant, P. J. (1999). A new family of growth factors produced by the fat body and active on *Drosophila* imaginal disc cells. *Development* **126,** 211-219.

Kelly, K. K., Meadows, S. M. and Cripps, R. M. (2002). *Drosophila* MEF2 is a direct regulator of Actin57B transcription in cardiac, skeletal, and visceral muscle lineages. *Mech. Dev.* **110,** 39-50.

Kennerdell, J. R. and Carthew, R. W. (1998). Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway. *Cell* **95,** 1017-1026.

Kennerdell, J. R. and Carthew, R. W. (2000). Heritable gene silencing in *Drosophila* using double-stranded RNA. *Nat. Biotechnol.* **18,** 896-898.

Klebes, A., Biehs, B., Cifuentes, F. and Kornberg, T. B. (2002). Expression profiling of *Drosophila* imaginal discs. *Genome Biol.* **3**, 0038.

Kornberg, T., Siden, I., O'Farrell, P. and Simon, M. (1985). The *engrailed* locus of *Drosophila: in situ* localization of transcripts reveals compartment-specific expression. *Cell* **40,** 45-53.

Lai, E. C., Bodner, R. and Posakony, J. W. (2000). The enhancer of split complex of *Drosophila* includes four notch-regulated members of the Bearded gene family. *Development* **127,** 3441-3455.

Liao, G. C., Rehm, E. J. and Rubin, G. M. (2000). Insertion site preferences of the P transposable element in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **97,** 3347-3351.

Lo, P. C. and Frasch, M. (2001). A role for the COUP-TF-related gene *seven-up* in the diversification of cardioblast identities in the dorsal vessel of *Drosophila*. *Mech. Dev.* **104,** 49-60.

Martinek, N., Zou, R., Berg, M., Sodek, J. and Ringuette, M. (2002). Evolutionary conservation and association of SPARC with the basal lamina in *Drosophila*. *Dev. Genes Evol.* **212,** 124-133.

Martinek, S. and Young, M. W. (2000). Specific genetic interference with behavioral rhythms in *Drosophila* by expression of inverted repeats. *Genetics* **156,** 1717-1725.

Mohler, J., Seecoomar, M., Agarwal, S., Bier, E. and Hsai, J. (2000). Activation of knot (kn) specifies the 3-4 intervein region in the *Drosophila* wing. *Development* **127,** 55-63.

Morin, X., Daneman, R., Zavortink, M. and Chia, W. (2001). A protein trap strategy to detect GFP-tagged proteins expressed from their endogenous loci in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **98,** 15050-15055.

Nakagoshi, H., Hoshi, M., Nabeshima, Y. and Matsuzaki, F. (1998). A novel homeobox gene mediates the Dpp signal to establish functional specificity within target cells. *Genes Dev.* **12,** 2724-2734.

Ng, M., Diaz-Benjumea, F. J. and Cohen, S. M. (1995). Nubbin encodes a POU-domain protein required for proximal-distal patterning in the *Drosophila* wing. *Development* **121,** 589-599.

Osterbur, D. L., Fristrom, D. K., Natzle, J. E., Tojo, S. J. and Fristrom, J. W. (1988). Genes expressed during imaginal discs morphogenesis: *IMP-L2*, a gene expressed during imaginal disc and imaginal histoblast morphogenesis. *Dev. Biol.* **129,** 439-448.

Park, Y., Caldwell, M. C. and Datta, S. (1997). Mutation of the central nervous system neuroblast proliferation repressor ana leads to defects in larval olfactory behavior. *J. Neurobiol.* **33,** 199-211.

Phillips, R. G. and Whittle, J. R. (1993). *wingless* expression mediates determination of peripheral nervous system elements in late stages of *Drosophila* wing disc development. *Development* **118,** 427-438.

Piccin, A., Salameh, A., Benna, C., Sandrelli, F., Mazzotta, G., Zordan, M., Rosato, E., Kyriacou, C. P. and Costa, R. (2001). Efficient and heritable functional knock-out of an adult phenotype in *Drosophila* using a GAL4-driven hairpin RNA incorporating a heterologous spacer. *Nucleic Acids Res.* **29,** E55-55.

Poole, S. J. (1995). Conservation of complex expression domains of the pdm-2 POU domain gene between *Drosophila virilis* and *Drosophila melanogaster*. *Mech. Dev.* **49,** 107-116.

Postigo, A. A., Ward, E., Skeath, J. B. and Dean, D. C. (1999). zfh-1, the *Drosophila* homologue of ZEB, is a transcriptional repressor that regulates somatic myogenesis. *Mol. Cell. Biol.* **19,** 7255-7263.

Preiss, A., Johannes, B., Nagel, A. C., Maier, D., Peters, N. and Wajant, H. (2001). Dynamic expression of *Drosophila* TRAF1 during embryogenesis and larval development. *Mech. Dev.* **100,** 109-113.

Ramain, P., Heitzler, P., Haenlin, M. and Simpson, P. (1993). *pannier*, a negative regulator of achaete and scute in *Drosophila*, encodes a zinc finger protein with homology to the vertebrate transcription factor GATA-1. *Development* **119,** 1277-1291.

Ranganayakulu, G., Zhao, B., Dokidis, A., Molkentin, J. D., Olson, E. N. and Schulz, R. A. (1995). A series of mutations in the D-MEF2 transcription factor reveal multiple functions in larval and adult myogenesis in *Drosophila*. *Dev. Biol.* **171,** 169-181.

Rong, Y. S. and Golic, K. G. (2000). Gene targeting by homologous recombination in *Drosophila*. *Science* **288**, 2013-2018.

Rong, Y. S., Titen, S. W., Xie, H. B., Golic, M. M., Bastiani, M., Bandyopadhyay, P., Olivera, B. M., Brodsky, M., Rubin, G. M. and Golic, K. G. (2002). Targeted mutagenesis by homologous recombination in *D. melanogaster*. *Genes Dev.* **16,** 1568-1581.

Sato, M., Kojima, T., Michiue, T. and Saigo, K. (1999). Bar homeobox genes are latitudinal prepattern genes in the developing *Drosophila* notum whose expression is regulated by the concerted functions of *decapentaplegic* and *wingless*. *Development* **126,** 1457-1466.

Sato, M. and Kornberg, T. (2002). FGF is an essential mitogen and chemoattractant for the air sacs of the *Drosophila* tracheal system. *Dev. Cell* **3**, 195.

Schnetzer, J. W. and Tyler, M. S. (1996). Endogenous beta-galactosidase activity in the larval, pupal, and adult stages of the fruit fly, *Drosophila melanogaster*, indicates need for caution in lacZ fusion-gene studies. *Biol. Bull.* **190,** 173-187.

Spradling, A. C., Stern, D., Beaton, A., Rhem, E. J., Laverty, T., Mozden, N., Misra, S. and Rubin, G. M. (1999). The Berkeley Drosophila Genome Project gene disruption project: single P-element insertions mutating 25% of vital *Drosophila* genes. *Genetics* **153,** 135-177.

St Pierre, S. E., Galindo, M. I., Couso, J. P. and Thor, S. (2002). Control of *Drosophila* imaginal disc development by rotund and roughened eye: differentially expressed transcripts of the same gene encoding functionally distinct zinc finger proteins. *Development* **129,** 1273-1281.

Stoilov, I. (2001). Cytochrome P450s: coupling development and environment. *Trends Genet.* **17,** 629-632.

Strigini, M. and Cohen, S. M. (2000). Wingless gradient formation in the *Drosophila* wing. *Curr. Biol.* **10,** 293-300.

Sturtevant, M. A., Roark, M. and Bier, E. (1993). The *Drosophila rhomboid* gene mediates the localized formation of wing veins and interacts genetically with components of the EGF-R signaling pathway. *Genes Dev.* **7,** 961-973.

Tabata, T., Eaton, S. and Kornberg, T. B. (1992). The *Drosophila hedgehog* gene is expressed specifically in posterior compartment cells and is a target of engrailed regulation. *Genes Dev.* **6,** 2635-2645.

Thor, S. and Thomas, J. B. (1997). The *Drosophila islet* gene governs axon pathfinding and neurotransmitter identity. *Neuron* **18,** 397-409.

van de Wetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Loureiro, J., Ypma, A., Hursh, D., Jones, T., Bejsovec, A. et al. (1997). Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene *dTCF*. *Cell* **88,** 789-799.

Vervoort, M., Crozatier, M., Valle, D. and Vincent, A. (1999). The COE transcription factor Collier is a mediator of short-range *Hedgehog*-induced patterning of the *Drosophila* wing. *Curr. Biol.* **9**, 632-639.

Williams, J. A., Bell, J. B. and Carroll, S. B. (1991). Control of *Drosophila* wing and haltere development by the nuclear vestigial gene product. *Genes Dev.* **5,** 2481-2495.

Wurmbach, E., Wech, I. and Preiss, A. (1999). The enhancer of split complex of *Drosophila melanogaster* harbors three classes of notch responsive genes. *Mech. Dev.* **80,** 171-180.

Yasothornsrikul, S., Davis, W. J., Cramer, G., Kimbrell, D. A. and Dearolf, C. R. (1997). *viking*: identification and characterization of a second type IV collagen in *Drosophila*. *Gene* **198,** 17-25.

Yeo, S. L., Lloyd, A., Kozak, K., Dinh, A., Dick, T., Yang, X., Sakonju, S. and Chia, W. (1995). On the functional overlap between two *Drosophila* POU homeo domain genes and the cell fate specification of a CNS neural precursor. *Genes Dev.* **9**, 1223-1236.