Autosomal Mutations Affecting Adhesion Between Wing Surfaces in Drosophila melanogaster

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ABSTRACT

Integrins are evolutionarily conserved transmembrane αβ heterodimeric receptors involved in cell-to-matrix and cell-to-cell adhesions. In Drosophila the position-specific (PS) integrins mediate the formation and maintenance of junctions between muscle and epidermis and between the two epidermal wing surfaces. Besides integrins, other proteins are implicated in integrin-dependent adhesion. In Drosophila, somatic clones of mutations in PS integrin genes disrupt adhesion between wing surfaces to produce wing blisters. To identify other genes whose products function in adhesion between wing surfaces, we conducted a screen for autosomal mutations that produce blisters in somatic wing clones. We isolated 76 independent mutations in 25 complementation groups, 15 of which contain more than one allele. Chromosomal sites were determined by deficiency mapping, and genetic interactions with mutations in the βPS integrin gene myospheroid were investigated. Mutations in four known genes (blistered, Delta, dumpy and mastermind) were isolated. Mutations were isolated in three new genes (pioppio, thea and steamer duck) that affect myo-epidermal junctions or muscle function in embryos. Mutations in three other genes (kakapo, kiwi and moa) may also affect cell adhesion or muscle function at hatching. These new mutants provide valuable material for the study of integrin-dependent cell-to-cell adhesion.

INTEGRINS are a versatile family of evolutionarily conserved transmembrane α, β heterodimeric receptors involved in cell-to-matrix and cell-to-cell adhesions (BURRIDGE 1986; HYNES 1987). Sites of integrin-mediated adhesion to the extracellular matrix serve as nucleation foci for cytoskeletal organization and assembly. The study in mammalian cells of focal adhesions, sites of integrin aggregation at cell-substrate attachments, has demonstrated that the cytoplasmic domains of locally aggregated integrin molecules interact with a constellation of proteins associated with the actin cytoskeleton (α-actinin, vinculin, talin, paxillin, tensin, zyxin) (SCHWARZ 1992; MIMAMOTO et al. 1995). The mechanical connection of integrins to the actin cytoskeleton can explain adhesion-modulated changes in cell migration and morphogenesis. A burgeoning body of evidence also implicates integrins in conventional signal transduction pathways, exemplified by receptor tyrosine kinases that initiate a cascade of events ultimately leading to the activation of transcription factors regulating the functions of specific genes (reviewed in DAMSKY and WEBB 1992; HYNES 1992; SCHwarz 1992; JULIANO and HASKILL 1993; CLARK and BRUGGE 1995). Integrins lack intrinsic kinase activity but can activate protein tyrosine kinases within adhesion complexes. In particular, the focal adhesion kinase (FAK), a novel kinase, localizes to focal adhesions and is activated when integrin becomes bound to ligand (BURRELL et al. 1992; BAKER et al. 1994; CLARK and BRUGGE 1995). Recent genetic evidence demonstrates the essential nature of integrin function during mammalian development. Loss of β1 integrins in knock-out mutants in mice results in lethality shortly after implantation (FASSELLER and MEYER 1995; STEPHENS et al. 1995). Loss of FAK in mice leads to a general failure in mesoderm development (ILIC et al. 1995, 1996).

Integrins are also essential for development in Drosophila (LEPTIN 1989; VOLK et al. 1990; ZUSMAN et al. 1990, 1993; BROWER et al. 1995; MARTIN-BERMUDO and BROWN 1996). In contrast to mammals, where 16 α and eight β chains have been identified (HYNES 1992), only three α- and two β-integrins are known in Drosophila (GOTWALS et al. 1994b). Three of these integrin chains were originally identified as position specific (PS) antigens in wings (WILCOX et al. 1981; BROWER et al. 1984) and are referred to as αPS1, αPS2, and βPS. αPS1 is encoded by multiple edematous wings (mew) (BROWER et al. 1995), αPS2 by inflated (if) (WILCOX et al. 1989), and βPS by lethalt(1)myospheroid (mys) (MACKRELL et al. 1988). Recently described β1 integrin is exclusively expressed in endodermal cells (YEE and HYNES 1993). Mutants of the α3 gene (volado) may affect the nervous system (described in GOTWALS et al. 1994b). Further variation in integrin structure is provided by alternate splicing of the αPS2 and βPS genes (BROWN et al. 1989; HYNES 1992). βPS, the most widely expressed Drosophila β integrin, is present in epidermal and mesodermal cells...
and forms heterodimers with all three α chains. Complementary distributions of αPS1βPS and αPS2βPS occur at the junctional attachments between muscle and epidermis and between the dorsal and ventral wing surfaces. αPS1βPS is expressed in embryonic epidermal cells and in the dorsal wing epithelium; αPS2βPS is expressed in embryonic mesodermal cells and in the ventral wing epithelium (Brower et al. 1984). Two extracellular ligands have been identified for the Drosophila integrins. Drosophila laminin is a ligand for αPS1 (Gotwals et al. 1994a) and tiggrin, a novel Drosophila extracellular matrix protein, is a ligand for αPS2 (Fogerty et al. 1994). Tiggrin is present in both myo-epidermal and wing junctions (Fogerty et al. 1994; Fessler and D. Frisch, unpublished results).

Null mutations in PS integrin genes cause several distinct phenotypes (Brower et al. 1995; Roote and Zusman 1995). Two of these phenotypes are of particular relevance here: (1) the detachment of the muscles from the epidermis that results from a failure in the myo-epidermal junction when embryonic muscle contraction begins (Wright 1966; Newman and Wright 1981; Martin-Bermudo and Brown 1996) and (2) the formation of wing blisters in homozygous somatic clones resulting from the disruption of the basal junctions that hold the two wing surfaces together (Brower and Jaffe 1989; Brabant et al. 1996). Both of these phenotypes are produced by mys (βPS) and if (αPS2) mutations. Null neuv (αPS1) mutations, surprisingly, fail to cause embryonic muscle detachment but cause post-hatching larval lethality. Wing blistering is the only phenotype common to mutations in the three PS integrin genes.

Normal developmental and adhesive functions of integrins depend on numerous other proteins including extracellular ligands, cytoskeletal proteins, and proteins that function in integrin-dependent signaling pathways. In mammals, effective mutational dissection of integrin-dependent processes depends on disruption of known genes via gene “knockouts,” and consequently on prior knowledge of roles of particular gene products in integrin-dependent processes. In Drosophila, however, mutational dissections can be effectively pursued without prior knowledge of encoded gene products by recovering mutations that disrupt integrin-dependent developmental processes. In particular, because wings are not essential for viability, recovering mutations that produce separation of the wing surfaces, putatively by disrupting integrin-dependent formation or maintenance of basal junctional complexes, is possible. Furthermore, because mutations in both αPS1 and αPS2 genes (if and neuv) produce wing blisters, a mutational screen based on identifying mutations through the wing blister phenotype could identify genes whose products function with both αPS1βPS and αPS2βPS. Thus, mutations affecting a wide spectrum of integrin-related functions could be recovered using this approach (see DISCUSSION).

We have conducted a screen on the second and third chromosomes designed to identify genes essential for maintenance of the epithelial bilayer in wings. The X chromosome was not screened because the genes encoding αPS1, αPS2 and βPS are all located on the X, and we did not wish to recover additional alleles of integrin genes. To make the screen highly efficient, we identified mutations that cause blisters in somatic wing clones of F1 offspring from X-rayed adult males, obviating the need to do a traditional three generation screen. The somatic clones arose from stage-specific crossing over at specific centromere proximal chromosomal sites (FRT sites) on each autosomal arm in response to expression of the yeast site-specific FLP recombinase, driven by the hsp70 promoter (Golic and Lindquist 1989; Golic 1991; Xu and Rubin 1993). Heat-shock dependent crossing over at each FRT site produces wing clones at frequencies that approach 90%. The easy detection of wing blisters, the nonessential nature of wings, and the high frequency at which somatic clones are produced using the FLP recombinase system provided an exceptional opportunity to recover mutations in genes whose function is required to form or maintain the epithelial bilayer of the wing. We have taken advantage of this opportunity to isolate 76 independent mutations in 25 complementation groups that cause wing blisters in somatic clones or cause dominant wing blisters. We report here the isolation and the initial phenotypic properties of the mutants.

MATERIALS AND METHODS

Strains and media: All stocks were maintained on standard corn meal, molasses, agar medium at 18 and 25 °C. The FRT/FLP stocks used for mutagenesis (40-w, 40-y F, 42-w, 42-y F, 80-w, 80-y F, 82-w, 82-y F, X) were generously provided by G. M. Rubin and are described in Xu and Rubin (1993). These stocks all contain FRT sites near to the centromere on each major autosomal arm; the F stocks contain hsFLP. The multiple wing hair (mash; cell marker was used to make an isogenic mash P(white-us1)70C P(neoFRT)/80B stock. Delta (D) and mash stocks were obtained from the Bloomington stock center. A dumpy" (dp") mutation was present on zipper chromosomes obtained from D. L. Kiehart. Several blistered (hs) and the mys" mutations, already present in our laboratory, have been previously described (Gotwals and Frisch 1991; Frisch et al. 1994). The mys" stock was obtained from D. Brower. Stocks carrying deficiencies for various regions of the second and third chromosomes and a mastermind (mam) stock were obtained from G. M. Rubin.

Mutant screen: The key step in the screen is the identification in F1 progeny of new mutations that cause wing blisters in somatic clones. In general, 10 groups of 10–15 3–5-day-old y w males homozygous for a P(white-us1)1 [=P{Fpy}; w+] in Xu and Rubin (1993)] insertion and a basal P(neoFRT) [=P{Fpy}; hs-neo; FRT] in Xu and Rubin (1993)] insertion near to the centromere on the same chromosome arm received ~4000 r of X-rays over a period of 1000 sec. These mutagenized males were immediately mated at 25 °C to ~20 5–10-day-old y w P(hsFLP)/1 y w P(hsFLP)/1 females that were also homozygous for P(Car20y1) [=P{Fpy}; y+] in Xu and Rubin (1993) P(neoFRT) insertions on the same chromosomal arm as those
in the males. Five or six cohorts of progeny were recovered by transferring the parents every 24 hr to fresh vials. The parents were then discarded. To induce the FLP recombinase, the w P{hsFLP}1/Y (or w P{hsFLP}1/yw); P{white-urn} P{nedFRT} / P{Par20y} P{nedFRT} progeny were heat shocked for 1 hr in a 37°C water bath 48 and 72 hr after mating began. Hence, most animals received at least one heat shock before or near the start of the second instar when the wing imaginal discs each contain ~100 cells or less. Because the mature wing disc contains ~50,000 cells, somatic recombinants induced at this time are expected to produce clones that contain 50 or more cells. Adults in each vial were scored daily or every second day for the presence of wing blisters until no further flies emerged. Emerging adults were occasionally also scored for white (w/w) clonal sectors to confirm that somatic recombination had occurred. Initially, animals with any wing blister-like aberration were recovered and retested. With experience, we limited recovery to adults with wing blisters like those shown in Figure 1 (see RESULTS) because only these proved to be heritable. Both single males or single females carrying putative mutations were retested by mating to y w P{hsFLP}1/ y w P{hsFLP}1; P{Car20y} P{nedFRT} animals and the progeny were recovered and exposed to the same heat shock protocol. Because flies with wing blisters sometimes got stuck in the food, these initial matings were often done in vials to which no dried yeast was added and that were laid on their sides until eggs were seen on the surface of the food. If the putative mutation retested by producing numerous offspring with wing blisters, w+ males with wing blisters were mated to a yw stock carrying an appropriate balancer chromosome (CyO, P{act-lacZ} or TM6B, Tb y w P{mini-w" Abd-A+gal}) either typically to balance the lethal mutation or, occasionally, to establish a homozygous stock. Once balanced, each stock was retested to confirm that the balanced lethal mutant chromosome still produced clonal wing blisters.

There were two exceptions to this protocol. The nonmutagenized XR chromosome did not carry a P{Car20y} insertion, but instead carried a P{P[im] / P{mini-w"} hs-M] in Xu and Rubin (1993) insertion. Also, the mutagenized Y chromosome in some cases carried w+. This allowed us to identify homozygous clonal patches independent of the blister phenotype for one mutant isolated on this chromosome (rhea). Only the y marker was easily available for clonal analysis with the rest of the mutations, y proved to be difficult to distinguish in clonal patches in the wings, so for most of the mutations, we were unable to determine dorsal or ventral wing surface specificity, or whether the blisters were confined within clonal boundaries. Although w- chromosomes were always mutagenized, in some instances (15 out of 77) the mutation was recovered upon retesting in G2 progeny on a w y chromosome. The vast majority of these (14 out of 15; one was on 2L) are mutations on chromosome 2R that are distal to the 47A w+ and the 44B y+ insertions on the mutagenized and nonmutagenized chromosomes, respectively. Many of the mutations on the y+ chromosome were initially recovered in G1 males, suggesting that these exceptions did not arise because of normal meiotic recombination. We do not understand the origin of these mutant chromosomes. However, because all of these mutations are distal to the w- insertion, we believe these exceptions arose because of recombination due in part to the presence of extra, perhaps cryptic, FRT sites on both the y+ and w- chromosomes just distal to the site of the w- insertion. 

Deficiency mapping: Complementation tests were performed at 25°C between mutant chromosomes and chromosomes deficient in various segments of the second and third chromosome. When possible, in instances where failure of complementation between a mutant and deficiency chromosome were found, additional alleles of the same complementation group were tested against the same deficiency to eliminate the possibility that the failure in complementation was due to the unfortunate presence of an additional lethal on the mutant chromosome. This does not eliminate the possibility that failure in complementation might be due to a secondarily mutations on the deficiency chromosome and not to the deficiency itself. We assume, however, that failure of complementation is a result of the deficiency, per se. When available, additional deficiencies from the same chromosomal region were tested to confirm the initial result and/or to determine more precisely the chromosomal location of the gene.

Genetic interactions: Possible genetic interactions with hypomorphic mys mutations were tested by crossing mys+/+ mys- females with males heterozygous for a new mutation and a balancer chromosome (e.g., CyO/Gyo; or rhea/ TM6B) or with males homozygous for the progenitor chromosomes. The frequency of wing blisters and held-out wings typical of some mys alleles (De La Pompa et al. 1988; Wilcock 1990) was then determined in male progeny heterozygous for a new mutation and compared to the frequencies in mys males heterozygous for a progenitor chromosome or for a balancer chromosome. All crosses were done at 25°C.

Phenotypic characterization: For recessive lethals, we determined if the mutant is an embryonic, larval, or pupal lethal. Animals heterozygous for a mutant chromosome and a Canton-S chromosome were mated to each other and allowed to lay eggs for ~4-6 hr. One hundred embryos were then transferred and aligned on a collection plate. Embryos were aged for 24 hr at 25°C, and the number of unhatched eggs counted. When most eggs (>90%) hatched, the larvae were fed and observed daily through pupation and eclosion to establish the approximate stage of lethality. When possible, two different alleles were crossed to each other to avoid affects from second site lethals. For embryonic lethals we collected eggs for 2-hr periods at 25°C. These were aged for 20-22 hr and then dechorionated in bleach, fixed in a 50:50 mixture of 4% formaldehyde and heptane, devitellinized in a 50:50 mixture of methanol and heptane, and transferred gradually to 100% PBS. Embryos were mounted in 70% glycerol in PBS and viewed under phase and polarized light optics.

RESULTS

Recovery of wing blister mutants: We conducted a one-generation mutational screen for X-ray-induced mutations that cause wing blisters in somatic clones of wing tissue, using the yeast FLP recombinase system (Golic and Lindquist 1989; Golic 1991; Xu and Rubin 1993). The results of the mutational screens, which were limited to the second and third chromosomes to avoid isolation of new mutations in the X-linked PS integrin genes, are summarized in Table 1. In total, 92,784 chromosomes were screened. Seventy-six independent mutations in 25 complementation groups distributed on all four arms were established. Among the 25 complementation groups, 15 are represented by two or more alleles. Multiple new mutations were recovered in four known genes (bs, Dl, dp and man), all of which were initially identified because of their clonal and nonclonal phenotypes and subsequently confirmed by crosses to known alleles of each gene. Eleven newly identified genes defined by more than one allele have been named after flightless birds, as were three other genes, each defined by one new mutation and a single
deficiency. As described below, the efficiency of recovery of mutations and the number of mutations recovered varied greatly among the four chromosome arms.

2L: Twenty-four independent mutations in six complementation groups were established from screens of 40,888 chromosomes. The efficiency of recovery was one established mutation per 1704 screened chromosomes. Four of the six complementation groups are composed of two or more alleles [cassowary (cas), dp, Ostrich (Ost), penguin (pen)]. All the new dumpy alleles recovered are lethal and include examples of dp\(^d\), dp\(^b\) and dp\(^{ab}\) mutations (Carlson 1959). Ost alleles cause dominant blisters but are lethal in homozygous and heteroallelic combination. We have been unable to identify a deficiency on 2L or 2R that does not complement Ost, and so have not excluded the possibility that Ost is located on 2R. A single mutant chromosome defining one complementation group (2L-A) is not complemented by a chromosome deficient for 21A1-21B6,7 (Df(2L)net-PM47C) and two chromosomes deficient for 28B2-28D1 (Df(2L)XE2750; Df(2L)XE3801). Whether the 2L-A chromosome carries an inversion with breakpoints in the 21A6,7 and 28B2-D1 regions, or two independent lethals has not been established. The 2L-F mutation complements for viability a chromosome deficient for 38A6,B1-40A4,Bl (Df(2L)lw161). The heterozygous adults, however, exhibit the held-out wing phenotype.

2R: Forty-one independent mutations in 12 complementation groups were established from screens of 16,969 chromosomes. Another mutation defining another complementation group was initially established but subsequently lost. The efficiency of recovery was one established mutation per 414 screened chromosomes. Eight of the 12 complementation groups are composed of two or more alleles [auk, bs, kakapo (kak), kitikete (kit), kiwi, mam, moa and piopio (pio)]. Two complementation groups, xenicid (xen) and takehe (tak), are each identified, respectively, by a single mutation that fails to be complemented by a single deficiency (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Arm</th>
<th>Name</th>
<th>Alleles</th>
<th>Description</th>
<th>Blister(^a)</th>
<th>Lethal phase</th>
<th>Non-comp. deficiency</th>
<th>Interactions</th>
<th>mys(^92)</th>
<th>mys(^99)</th>
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<tr>
<td>2L</td>
<td>2L-A</td>
<td>1</td>
<td>r, hl</td>
<td>A</td>
<td>Larva</td>
<td>See text</td>
<td>Moderate</td>
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<td>None</td>
</tr>
<tr>
<td></td>
<td>2L-F</td>
<td>1</td>
<td>r, hl</td>
<td>B</td>
<td>Pupa</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>cassowary</td>
<td>5</td>
<td>r, hl</td>
<td>A</td>
<td>Pupa</td>
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<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>dumpy</td>
<td>9</td>
<td>r, hl</td>
<td>A</td>
<td>Embryo/larva</td>
<td>25A1, 4</td>
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</tr>
<tr>
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<td>Ostrich</td>
<td>2</td>
<td>D, hl</td>
<td>A'</td>
<td>Pupa</td>
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<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>penguin</td>
<td>6</td>
<td>r, hl</td>
<td>A</td>
<td>Larva</td>
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<td>None</td>
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<td>None</td>
</tr>
<tr>
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<td>r, hv</td>
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<td>Adult</td>
<td>None</td>
<td>Weak</td>
<td>None</td>
<td>None</td>
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<td></td>
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<td>Larva</td>
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<td>Weak</td>
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</tr>
<tr>
<td></td>
<td>auk</td>
<td>3</td>
<td>r, hl</td>
<td>A</td>
<td>Pupa</td>
<td>Df(2R)txix, 51A1, 2-51B6</td>
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<td>None</td>
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<tr>
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<td>2</td>
<td>D, hl</td>
<td>C</td>
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<td>None</td>
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<tr>
<td></td>
<td>kakapo</td>
<td>5</td>
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<td>A</td>
<td>Early larva</td>
<td>Df(2R)C1,1,4-50C23,2D</td>
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<td>None</td>
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<td>kitikete</td>
<td>3</td>
<td>r, hl</td>
<td>A</td>
<td>Larva</td>
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<tr>
<td></td>
<td>mam</td>
<td>2</td>
<td>r, hl</td>
<td>D</td>
<td>Embryo</td>
<td>Df(2R)C1,1,4-50C23, 2D</td>
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<td>None</td>
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<tr>
<td></td>
<td>moa</td>
<td>4</td>
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<td>A</td>
<td>Early larva</td>
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<td>None</td>
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<td></td>
<td>piopio</td>
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<td>r, hl</td>
<td>C</td>
<td>Embryo</td>
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<tr>
<td></td>
<td>takehe</td>
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<td>r, hl</td>
<td>A</td>
<td>Pupa</td>
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<td>A</td>
<td>Early larva</td>
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<tr>
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<td>D, hv</td>
<td>d</td>
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</tr>
<tr>
<td></td>
<td>rhea</td>
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<td>A</td>
<td>Embryo</td>
<td>Df(3L)Wr10, 75A6,7-75C1,2</td>
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<td>r, hv</td>
<td>B'</td>
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</tr>
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<td></td>
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<td></td>
<td>stk</td>
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<td>A</td>
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<tr>
<td></td>
<td>struthio</td>
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<td>Weak</td>
<td>Strong</td>
<td>None</td>
</tr>
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</table>

ND, not determined.

\(^a\) r, recessive; D, dominant; hl, homozygous lethal; hv, homozygous viable.
\(^b\) Wing blister phenotypic classes are described in the text.
\(^c\) Heterozygotes and homozygotes have fluid filled or collapsed wings.

Blister phenotype determined in homozygotes (3L-B) or heterozygotes (Ost).
**3L**: Three independent mutations in two complementation groups were established from screens of 9593 chromosomes. The efficiency of recovery was one established mutation per 3198 screened chromosomes. 3L-B has a dominant phenotype of short wings with blisters. As with *Ost*, we have not identified a deficiency that fails to complement 3L-B, so it could be located on 3L or *rhea* is defined by two X-ray-induced alleles obtained in this screen and a third EMS-induced allele (provided by M. Brabant from a screen conducted by M. Diamond).

**3R**: Eight independent mutations in five complementation groups were established from screens of 25,334 chromosomes. The efficiency of recovery was one established mutation per 3167 screened chromosomes. Two of the complementation groups are composed of two or more alleles [Dl and *steamer duck* (*sik*)]. One complementation group, *struthio* (*str*), contains a single mutation that fails to be complemented by a single deficiency (Table 1).

**Phenotypic characterizations of mutants**: The phenotypes of the mutants were characterized in terms of the properties of the blisters, lethality, and when lethal, the stage(s) of lethality. Embryonic lethals (except *Dl, dp* and *mam*) or mutants that died at the embryonic/larval interface were examined to determine if any obvious anatomical aberration is caused by a mutation. When possible, heteroallelic combinations were used (see Materials and Methods).

**Properties of blisters**: The blisters that arose, when a heat-shock was applied around the end of the first instar, were classified in terms of location, shape, and vein abnormalities (Table 1) as follows:

Type A blisters (Figure 1, a and b) are discrete, more or less round blisters of variable size located anywhere on the wing. Venation is normal. Blisters of this type are like those associated with clones of *mys* lethal mutations. Sixteen mutants had type A blisters.

Type B blisters (not shown) are like type A blisters but are limited to the distal half of the wing. Venation is normal. Only two mutants, *2L-F* and *3R-B*, had type B blisters. Some alleles of an X-linked gene, *vesiculated*, also have blisters limited to the distal third of the wing (Wilcox 1990).

Type C blisters (Figure 1c) tend to be large and lack sharp boundaries. Large blisters occur even when clones are induced late in development (92–118 hr after egg laying). Wings are sometimes rumpled. Venation is normal. Two mutants, *auk*, and *pio* exhibit type C blisters.

Type D blisters (Figure 1d) are like type A blisters but are also associated with venation abnormalities. Mutants with this phenotype are *3R-C, bs, Dl*, and *mam*. Like *Ost, bs* can cause dominant wing blisters. The *bs* gene has been implicated in the specification of intervein regions (Frister et al. 1994). *bs*, which is allelic to *pruned*, encodes the Drosophila serum response factor (Guillemin et al. 1996; Montagne et al. 1996). One of the two new *bs* alleles described here (2R-19) is a chain-terminating base-substitution (Montagne et al. 1996). New mutations in the neurogenic genes, *Dl* and *mam* were recovered. Dominant blisters are present with high penetrance in some *Dl* stocks and not all of the blisters seen here occur in homozygous *Dl* clones (see Discussion). *Dl* and *mam* clones that include the wing margin cause scalloping, because of cell death. Thoracic bristle clusters arise in some *Dl* clones. Thoracic clones of *mam* lack bristles (Dietrich and Campos-Ortega 1984).

**Lethality and lethal stage**: Among the 25 mutant genes identified, only three are viable as homozygotes. These three (2R-F, *3L-B* and *3R-B*) are each defined by a single mutant chromosome and produce homozygous adults with wing blisters. Blisters are seen in *3L-B* heterozygotes. Among the lethals, six are embryonic lethals (including *Dl, mam*, and most of the *dp* mutations), five are early larval (posthatching) lethals, five are larval lethals (including *bs*), and six are pupal lethals.

**Morphology of embryonic and early larval lethals**

*rhea*: *rhea* is a late embryonic lethal in which the somatic musculature completely detaches from the epidermis (Figure 2, d–f). This phenotype is not evident until very late in embryogenesis suggesting that muscles
detach from the epidermis when muscle contractions begin. However, in contrast to mys mutants where the muscles separate from each other as well as from the epidermis, attachments between the muscles in rhea mutants are maintained so that the somatic musculature forms a continuous birefringent tube surrounding the internal organs.

piopio (pio) and steamer duck (stk): pio is a late embryonic lethal. stk primarily causes early larval lethality. Both mutants are characterized by apparent excessive contraction of the segmental muscles just before hatching. The onset of this phenotype is presumably delayed or less severe in stk than in pio, so that most stk animals hatch. However, stk animals that hatch fail to grow. The phenotype is characterized by very short thick, highly birefringent longitudinal and oblique muscles in the posterior of the embryo (Figure 2, h and i). Three observations suggest that the phenotype is due to hypercontraction of the musculature rather than a defect in muscle development per se. (1) At stage 17 (immediately before hatching but not earlier) entire segments shorten as the muscles shorten and thicken and outward bulges in the integument occur (Figure 2). (2) pio embryos stained with anti-myosin antibody (courtesy of D. Kiehart) appear normal through stage 15. (3) Newly hatched stk embryos elongate to wild-type length when compressed under a coverslip. This phenotype may arise because of a failure in communication between intersegmental muscles (see Discussion).

dumpy: The various allele-dependent lethal stages of dp have been described (Metcalf 1971). Homozygous dpO animals are embryonic lethals, dpO and dpH animals die at the embryonic/larval interface and dpH animals die during the first larval ecdysis. No gross anatomical abnormalities are associated with embryonic lethality of dp animals (Metcalf 1971).

kakapo, kiwi, moa, and xenicid: Mutations in kak, kiwi, moa, and xea primarily cause early larval lethality. The homozygous larvae of each mutant exhibit no gross morphological abnormalities at hatching. All hatched larvae fail to grow. Although most kiwi alleles act as early larval lethals, some heteroallelic combinations cause more embryonic than larval lethality.

Delta and mastermind: The early embryonic neurogenic phenotypes of Dl and mnn have previously been described (for review, see Campos-Ortix 1993).

struthio (stru): stru was evaluated as a homozygote and over Df(3R)N19. stru is a late embryonic lethal, which lacks any gross abnormalities.

Deficiency mapping of mutations: Because of the nature of genetic screens using the yeast FLP-recombinase system, new recessive mutations are isolated on one autosomal arm at a time. Thus, the chromosomal arm on which each mutation resides was usually known at the time of recovery (see above). To place the mutations at more precise locations on each autosomal arm, alleles representative of each complementation group were tested for complementation with chromosomes deficient for various segments of each chromosomal arm (see Materials and Methods). Chromosomal locations of the four previously identified genes (bs, Dl, dp, and mnn) were already known. In addition to these, the approximate chromosomal locations were determined for 10 of the newly identified genes (auk, kak, kit, moa, pio, rhea, stk,
stru, tak, and xen). We report in Table 1 the smallest deficiency that fails to complement a particular complementation group. Knowing the approximate locations of particular genes allowed us to consider whether any of the new mutations might affect previously described genes in that region and, of course, to eliminate other genes. Of particular note, kak resides in a region that contains groovin, a gene whose product, along with the extracellular αPS ligand, tiggerin, has been implicated in the structure of the embryonic myoepidermal junction and in adhesion between wing surfaces (FOGERTY et al. 1994; VULK and VIJAYRAGHAVAN 1994). However, alleles of kak do not cause detachment of the embryonic musculature from tendon cells in homozygous embryos. kak animals, however, fail to grow after hatching.

**Genetic interactions:** Interactions, suppression or enhancement, between nonallelic mutations have often served to implicate independent genes in common processes. It has previously been reported that some bs alleles can act as dominant enhancers of mys by causing in mys hemizygous males an increased frequency of wing blisters (WILCOX 1990; FRISTROM et al. 1994). An interaction with mys is also signaled by an increase in the penetrance of the "held out" wing phenotype (WILCOX 1990). The held out phenotype results from aberrations in the indirect flight muscles (DE LA POMPA et al. 1989). Consequently, we investigated whether any of our newly isolated autosomal mutants are able to dominantly enhance the penetrance or expressivity of wing blisters and of the held out phenotype of two viable X-linked β-integrin mutations, mys or mys. This was accomplished by determining the frequency of wing blisters and held out wings in males hemizygous for a mys allele and heterozygous for the mutant chromosome (see MATERIALS AND METHODS). These results were compared with the frequency of wing blisters and held out wings in sibling males heterozygous for the balancer chromosome and with results obtained simultaneously in separate vials using the progenitor chromosomes. In general, the mys control males had a frequency of wing blisters in a range of 5-10%, and of held out wings in a range of 30-50%. For mys control males the frequency of wing blisters was 1-2%, and the frequency of held out wings was in a range of 60-80%. An interaction was considered weak when the frequency of wing blisters in mys was increased from ~10 to ~20% (in auh, kak, kit, kiwi, mam, tak, xen and 2RL) or when the frequency of held-out wings was moderately increased (e.g., from ~50% in sibs and progenitors to ~70% in mys; stk/+ animals). The held-out phenotype was enhanced by stk in both mys and mys males but pio only enhanced the held-out phenotype in mys. Neither stk nor pio enhanced the penetrance of blisters in crosses with either mys allele (see DISCUSSION). The interaction was considered moderate for mys and 2LA (the frequency of males with blisters increased from ~10 to ~40%). Both bs and rhea mutations interacted strongly with both mys alleles. For example, both new bs alleles increased the frequency of blistered mys males from 1-2 to ~30% (but there was no enhancement of the held-out phenotype). Both rhea alleles strongly enhanced the penetrance of both the blistered and held-out phenotypes in mys and mys males. For example, rhea increases the frequency of blisters in mys males from 1-2 to 50-60%. The held-out phenotype increases from 60-80 to 90%. Male viability is also reduced.

**DISCUSSION**

Stable junctions between the embryonic segmental muscles and tendon cells and between the epidermal cells of the two surfaces of the wing require integrins. The structural similarities between myo-epidermal junctions and wing junctions led us to expect that in addition to integrins, many other junction-associated components will also be shared by tendon cells and wing cells. In mammals, the cytoplasmic domains of locally aggregated integrin molecules interact with a constellation of proteins associated with the actin cytoskeleton (α-actinin, vinculin, talin, paxillin, tensin, zyxin) (SCHWARZ 1992; MIYAMOTO et al. 1995). None of these components have been identified in epidermal cells in Drosophila. However, the transmembrane protein, Groovin, and the extracellular αPS ligand, Tiggerin, have been implicated in the structure of the embryonic myoepidermal junction and in adhesion between wing surfaces (FOGERTY et al. 1994; VULK and VIJAYRAGHAVAN 1994). Furthermore, in both epidermal tendon cells (LAIFOOK 1967; FYRBERG et al. 1990) and intervein cells of wings (TUCKER et al. 1986; MOGENSEN and TUCKER 1987, 1988) arrays of microtubules and microfilaments stretch from apical hemidesmosomes attached to the overlying cuticle to the basal junctions. The similarity in these structures suggest that some similar cytoskeletal proteins will be shared between myo-epidermal and basal wing junctions.

In the wing, these cytoskeletal (transalar) arrays arise some 20 hr after the basal junctions are first evident (FRISTROM et al. 1993) and assemble from apex to base (MOGENSEN and TUCKER 1987, 1988). The differentiation of the tendon cell cytoskeleton in embryos also occurs after the myo-epidermal junction has formed (NEWMAN and WRIGHT 1981). In mys/mys embryos, neither junctional undercoats nor ordered cytoskeletal arrays differentiate in the putative tendon cells (S. M. NEWMAN, personal communication). Similarly, in severe blistered mutants, transalar arrays fail to differentiate in areas where the wing surfaces have failed to form basal junctions (FRISTROM et al. 1994). Thus, it is not clear whether the failure in mys mutants of the attachment between the muscle and tendon cell, and of the attachment between wing surfaces is due to the absence of the junction per se, to the absence of the associated
cytoskeletal array, or to both. In any case, it is likely that a failure in the structure of the actin cytoskeleton could also result in loss of adhesion. Such a condition has precedence in mice where loss of BPAG1, a protein that connects integrin-containing hemidesmosomes to intermediate filaments, can cause blisters because of ruptures between the filament network and the hemidesmosomes (Guo et al. 1995). In conclusion, there is good reason to expect that mutations in genes other than those in the PS integrin genes will affect both myoepidermal junctions and basal wing junctions.

Wing mys clones deficient in βPS integrin, mew clones deficient for αPSI integrin, and if clones deficient for αPS2 integrin can all cause wing blisters. Embryos deficient for αPS2 and βPS die without hatching primarily because of the failure of myoepidermal junctions (Newman and Wright 1981; Bramant and Brower 1993). Animals (mew/mew) deficient for αPSI die after hatching without exhibiting any gross muscle abnormalities (Brower et al. 1995). Wing epithelial clones deficient in other gene products essential for the formation or maintenance of junctions between wings could also result in wing blisters. Consequently, using the FLP recombinase system (Golic and Lindquist 1989; Xu and Rubin 1993) to produce somatic crossing over, we have recovered mutations on the four major autosomal arms that cause clonal wing blisters. As discussed below, at least four of these genes are also implicated in normal muscular function during development. In addition to these, mutations in other genes may identify components common to both embryonic epidermal and wing junctions. Thus, this genetical approach has yielded valuable tools that can be used for the study of muscle and junction function during development.

Mutant recovery: We have recovered and established mutations in 25 different genes whose products are essential for the maintenance of adhesion between wing surfaces. Over 70% of the genes identified in this screen are located on chromosome 2. At least 12 of these, almost half of the total, are located on the right arm of chromosome 2. The low level of recovery of mutations on chromosome 3 is not caused by a failure in the test system, particularly on 3L, because homozygous clonal patches of the cell-marker mabh on 3L were readily found in ~60% of the progeny from irradiated flies (data not shown) and because clones were readily induced in flies carrying new mutations on both arms of chromosome 3. Multiple alleles were recovered for 15 of the genes. Three additional genes, stru, xen, and tak, are each defined by a single mutation and a single deficiency. Additional genes with products necessary for the formation or maintenance of wing junctions exist. For example, we would not have recovered null mutations in genes whose products are essential for cytokinesis or cell viability and for maintenance of wing junctions (e.g., in a gene that encodes a component of the actin cytoskeleton). In such genes, we might have recovered rare hypomorphic mutations that can still cause wing blisters in homozygous clonal patches. As a group, genes represented by single mutations are often viable or have late lethal phases (of a total of nine in which the lethal phase has been determined, two are viable; three are pupal lethals). These mutations may be hypomorphic alleles of genes essential for cell viability or cell division. Likewise, we could not have recovered mutations in some genes that encode components of the extracellular matrix. Some extracellular matrix molecules (collagen, laminin, tiggrin) that are demonstrated or putative ligands for integrins (Gotwals et al. 1994a) are probably synthesized by hemocytes and not by epidermal cells (L. Fessler, personal communication).

Mutations in known genes: At least four of these genes have been previously identified. As expected, we recovered new mutations in bs, a gene implicated in the specification of intervein cells (Fristrom et al. 1994). We also recovered new mutations affecting the neurogenic genes Dl and mam, genes implicated in the specification and maintenance of epidermal cells (for review, Campos-Ortega 1993). Although dominant blisters are penetrant in some balanced Dl/+ strains, these are preferentially localized in the distal third of the wing (data not shown). Following heat shock of Dl frt/+ frt larvae, we find proximal wing blisters as well as distal ones. We infer that the proximal blisters arise in Dl/Dl clones. Neurogenic genes are essential for the specification of the embryonic ectoderm. The neurogenic phenotype arises from mutations in these genes because all cells in the neuroectoderm take on a neural fate by default (Campos-Ortega 1993). We believe that blisters arise in Dl and mam clones because of a failure to maintain the normal properties of ectodermal cells within the clonal boundaries. We do not know the specific defects that are responsible for blistering, but it is unlikely that either the Dl (an apical protein) or Mam (a nuclear protein) products function directly in the formation or maintenance of basal junctions. Finally, we recovered new mutations in dp, a gene known to affect the maintenance of myoepidermal junctions in the indirect flight muscles (Metcalfe 1970). We will comment further on dp below.

Mutations in genes affecting muscle function or attachment: The larval body wall musculature in embryos attaches to tendon cells in the ectoderm at segment boundaries. The attachment is tripartite (Figure 3). A muscle from each adjacent segment is attached to a common tendon cell. In addition, the adjacent muscles attach to each other. Both of these attachments depend on αPS2βPS integrins. As a consequence, mutations in either the mys or if genes produce the myospheroid phenotype because upon contraction at hatching the muscles detach both from the tendon cell and from each other to produce contracted balls (Newman and Wright 1981). Two other possible attachment defects
could arise at embryonic myoepidermal junctions. (1) Muscles could detach from the tendon cell but remain intersegmentally attached to each other. Upon contraction, this should create a tube of muscle separate from the body wall. An example of this phenotype is seen in embryos mutant for both invected and engrailed where tendon cells are not specified (Martin-Bermudo and Brown 1996). (2) Muscles could detach from each other at the segmental boundary, but remain attached to the tendon cells. This should not result in a gross muscle detachment phenotype, but might lead to aberrant function of the larval musculature.

**rhea:** We conclude that mutations in *rhea* exemplify the first phenotype described above. *rhea* is a late embryonic lethal in which at the time of hatching the larval musculature is seen as a birefringent tube well separated from the larval integument (Figures 2, d–f and 3). We also infer that *rhea* is directly involved in integrin function because among all of the new mutations isolated, only the two *rhea* alleles strongly enhance both hypomorphic *mys* alleles (Table 1). This suggests that myo-epidermal junctions *per se* fail in *rhea* animals. Because the musculature contracts away from the epidermis as a continuous birefringent tube, we infer that the intersegmental connections between muscles remain intact. The *rhea* chromosome was marked with *mwh* allowing us to determine that clones on either the dorsal or ventral wing surface produce blisters. Blisters in the adult wing are always slightly larger than the clones, similar to blisters observed in α or β integrin mutant clones (Brabant et al. 1996). We have not examined pupal wings, where clonal and blister boundaries tend to correlate closely. We are currently cloning and characterizing this gene.

**pio-pio and steamer duck:** Although *pio* is a late embryonic lethal and *shh* is a late embryonic/early larval lethal, the phenotypes of homozygotes of each mutant are very similar (Figures 2, g–i, and 3). In both, the posterior body wall musculature appears contracted, resulting in short, fat animals with bulging posterior segments. During muscle contractions involved in hatching and crawling, contraction of the longitudinal muscles occurs in an anterior/posterior wave; as one segment contracts, the adjacent one relaxes. This presumably requires communication between the muscles of one segment and the next via the junctions between muscles (Figure 3). Thus, a mechanical or physiological disruption of those junctions might result in the observed phenotype. Although mutations in both genes cause wing blisters in homozygous somatic clones, the phenotypes differ. *pio* produces type C blisters. *shh* produces type A blisters similar to those caused by *myospheroid*. Both mutations also interact genetically with *mys* mutations but only to enhance the held-out phenotype, not the wing blister phenotype. The held-out phenotype results from defects in the function or attachment of the indirect flight muscles (De La Pompa et al. 1989). Because both the embryonic phenotype and the held-out phenotype suggest a muscle defect rather than an epidermal defect, it is possible that *pio* and *shh* gene products interact with αEC βPC integrin. If so, only ventral clones may result in wing blisters.

**dumpy (dp):** *dumpy* is a complex genetic locus, with both viable and lethal alleles. Among the viable *dp* alleles are oblique (σ) alleles that produce truncated, oblique wings, and vortex (υ) alleles that produce vortices on the thorax at positions of muscle insertions. There are also two viable alleles that produce both wing and thoracic phenotypes. Early reports (Waddington 1941; King 1964) indicated an association between the thoracic abnormalities and defects in the indirect flight muscles in *σ*/*σ* homozygotes. Mosaic analyses (King 1964) and developmental analyses (Metcalfe 1970) suggest, but do not prove, that the site of action of the
defect is in the thoracic epidermis and that the defects in the indirect flight muscles arise secondarily.

In addition to the viable alleles, there are also lethal alleles at the locus. These alleles can lack $\alpha'$ function (olv alleles), $\nu'$ function (lu alleles) or both functions (olvimento alleles). Lethal dp alleles also exist that provide $\alpha'$ and $\nu'$ functions (lalleles). The lethal alleles are viable in heteroallelic combination with the viable alleles, producing the expected adult phenotypes, e.g., ov/olv flies exhibit both wing and thoracic mutant phenotypes, while ov/l flies are wild type in appearance. In our current somatic genetic screen we only recovered lethal alleles (five olv alleles, three lv alleles, and one l allele).

We infer from our own data and previously published data that only lethal alleles cause failure of adhesion between wing surfaces in homozygous somatic clones, i.e., that the $\Gamma$ function is essential for the formation or maintenance of wing junctions. The stage of lethality is allele specific (METCALF 1971). olv homozygotes are embryonic lethals, ol and lv homozygotes die at the egg-larval interface (muscular contraction occurs but batching usually fails), and l/l animals die at the first molt. According to METCALF (1971), no gross abnormalities were seen in olv/olv embryos, but muscular contraction was absent. Muscular contraction was reported in ol/ol and lv/lv embryos, but most animals failed to hatch from the egg. We found no obvious genetic interaction between either of the two mys alleles and a newly isolated dp$^{x+}$ allele (Table 1).

**Mutations that allow hatching but prevent further development:** Mutations in four genes, kiwi, moa, kak, and xen, cause no gross anatomical abnormalities and allow hatching. The mutant animals, however, fail to grow and soon are distinct from their wild-type sibs due to their small size. Homozygous meow/meow animals, with defects in the $\alpha_{psi}$ gene, also lack gross anatomical abnormalities, are able to hatch, but then fail to grow as first instar larvae (BROWER et al. 1995). Also some lethal dp/dp animals that hatch fail to grow (METCALF 1971). The bases of the common phenotype, the failure to grow after hatching, among these mutants may be quite disparate. Nevertheless, mutations in three of these genes (kiwi, moa, and kak) interact genetically with mys mutations (Table 1), suggesting a role in the formation or maintenance of basal junctions in wings.

To summarize, we have identified 15 genes with two or more alleles, whose normal functions are autonomously required for the in vivo maintenance of adhesion between epidermal wing surfaces, as revealed by the effects of mutations in somatic wing clones. Mutations in four of these genes ($\alpha_{psi}$, pio, rhoa, and stk) cause additional demonstrated defects in the structure of myo-epidermal junctions or in muscular function. Mutations in three others (kiwi, moa, kak, and some dp mutations) exhibit a phenotype (hatching followed by failure to grow) similar to that of meow/meow animals. We believe mutations in these genes also affect proteins necessary for the normal function of myo-epidermal or other junctions. So, at least four, and perhaps several more of the 25 genes identified in the current screen may function directly in the formation or maintenance of integrin-dependent junctions between cells or may function in other mechanisms of cell to cell adhesion. The nature of the gene products is, of course, unknown, but may include products such as FAK, talin, paxillin, and others that have not previously been identified in Drosophila. We hope other workers will take advantage of these mutations to study junction formation and function.

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