Development 108, 391–402 (1990)
Printed in Great Britain © The Company of Biologists Limited 1990

Requirements for integrins during *Drosophila* development

SUSAN ZUSMAN, RAMILA S. PATEL-KING, CHARLES FFRENCH-CONSTANT and RICHARD O. HYNES

Howard Hughes Medical Institute and Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Summary

The common β subunit of the PS antigens of *Drosophila* is homologous with vertebrate integrins and is encoded by the *lethal(1)myospheroid* gene. We have generated flies mosaic for wild-type and mutant alleles of *lethal-(1)myospheroid* using adult gynandromorphs and radiation-induced somatic crossing over. The defects observed in the gynandromorphs demonstrate widespread requirements for PS integrins during development especially in ventrally derived structures, which also show strong expression of PSβ integrin. Smaller *lethal(1)myospheroid* clones induced during larval development result in blister and vein defects in the wings and aberrant development of photoreceptor cells, demonstrating roles for PS integrins during development of both wings and eyes. PS integrins are required for the close apposition of the dorsal and ventral wing epithelia and for the proper arrangement of photoreceptor cells. However, many other adhesive and morphogenetic processes proceed normally in the absence of integrins containing the β subunit encoded by *lethal(1)myospheroid*.

Key words: integrin, *Drosophila*, *lethal(1)myospheroid* gene, mosaics, eye–antennae disc, photoreceptor.

Introduction

The sequence of morphogenetic events observed during the formation of a multicellular organism often requires changes in cell adhesion, shape, migration and proliferation. In order to understand such basic developmental mechanisms, it is important to identify the molecules that mediate them. The integrins, a family of transmembrane glycoprotein receptors, have been associated with these functions. Integrins are heterodimers consisting of noncovalently associated α and β subunits. Their extracellular domains bind to adhesive molecules such as fibronectin, laminin and collagen, and their intracellular domains interact with the cytoskeleton (see Hynes, 1987; Buck and Horwitz, 1987; Ruoslahti and Pierschbacher, 1987; and Ruoslahti, 1988 for reviews). The combination of a specific α subunit with a specific β subunit has been shown to be important in determining the affinity for specific ligands. As many as 11 distinct α subunits and 5 distinct β subunits have been identified in vertebrate cells (Hynes, 1987; Ruoslahti and Pierschbacher, 1987; Sonnenberg *et al.* 1988; Cheresh *et al.* 1989; Hemler *et al.* 1989; Kajiji *et al.* 1989).

The *Drosophila* position-specific (PS) antigens are integrin subunits. PS1 and PS2 are α subunits (Leptin *et al.* 1987; Bogaert *et al.* 1987) each of which binds to a common β subunit known as PS3 or PSβ (Wilcox *et al.* 1984; Leptin *et al.* 1987, 1989). PS integrins are present during most of embryonic development, but are concentrated in specific embryonic tissues. PS2α is mainly found in mesodermal derivatives, while PS1α is in ectodermal and endodermal derivatives and PSβ is found in all three germ layers (Bogaert *et al.* 1987; Leptin *et al.* 1989).

During later stages, the PS integrins localize to specific regions of the eye–antennae, wing and leg disk epithelia, as well as to other larval and adult tissues (Wilcox *et al.* 1981; Brower *et al.* 1984, 1985; and Wilcox and Leptin, 1985). In the wing and eye–antennae imaginal discs, PS1α and PS2α are found in complementary patterns, which suggest they may cooperate in their functions. In the wing disc, PS1α and PS2α are expressed on the dorsal and ventral surfaces respectively, while PSβ is found throughout the disc (Brower *et al.* 1985). This distribution suggests that integrins may be involved in bringing together or maintaining the apposition of the dorsal and ventral surfaces of the wing at metamorphosis. This event is thought to be necessary for the proper shaping and organization of the wing as well as the normal patterning of wing crossveins (Waddington, 1940; Garcia-Bellido, 1977). Development of the eye is accompanied by an intriguing shift in PS integrin expression. During the third larval instar, a morphogenetic furrow travels across the eye disc; undifferentiated epithelium ahead of this furrow develops after passage of the furrow into the organized pattern of ommatidia characteristic of the adult eye

(Ready *et al.* 1976). PS1α and PS2α are expressed on opposite sides of this morphogenetic furrow (Brower *et al.* 1985) suggesting roles for integrins in the normal development of the eye disc.

The advantages of genetic analysis in *Drosophila* make it possible to study the functions of integrins *in vivo*. Genomic DNA clones of the *lethal(1)myospheroid* locus have been isolated (Digan *et al.* 1986). Sequence analysis of cDNA clones corresponding with this gene has shown that it encodes a protein homologous to vertebrate β integrins (MacKrell *et al.* 1988) and studies of PS antigen expression in *l(1)mys* embryos show that this gene encodes the PSβ subunit (Leptin *et al.* 1989). The *l(1)mys* mutation has allowed an initial examination of the role of β integrins in *Drosophila* development. Embryos hemizygous or homozygous for loss of function *l(1)mys* mutations can develop approximately normally through a large portion of embryonic development. The first defects in mutant embryos are observed when the first muscle contractions normally occur. During this time the somatic muscles pull away from their attachment sites. Also, distortion of visceral mesoderm and gut epithelia, herniation of brain tissue and abnormal dorsal closure are observed in these embryos. These abnormalities result in embryonic death (Wright, 1960; Newman and Wright, 1981). In addition to the previously mentioned defects, removal of both maternal and zygotic *l(1)mys* expression, resulting in a complete absence of β integrin, causes abnormal germband retraction (Wieschaus and Noell, 1986; Leptin *et al.* 1989).

Because of the embryonic lethal phenotype of *l(1)mys*, it has not been possible to study the functions of integrins in later stages of *Drosophila* development. Cell adhesion undoubtedly plays important roles in later morphogenesis and the presence of PS integrins in imaginal discs (Brower *et al.* 1985) suggests that they function there. In order to address questions of possible functions of integrins in later stages of development we have used *Drosophila* genetic methods to generate mosaic animals that contain patches of wild-type and *l(1)mys* tissue. In this way, the functions of integrins in particular regions can be examined in the context of an organism with normal integrin expression elsewhere.

In the experiments described below, we produced adult *l(1)mys* gynandromorphs and *l(1)mys* somatic clones and have examined the requirements for integrins during development. The location of these requirements in the developing embryo is compared with *l(1)mys* transcription patterns and with the distribution of β integrin in the developing embryo. We show widespread requirements for *l(1)mys* expression in the developing embryo and demonstrate roles for integrins in the development of both wings and eyes.

Materials and methods

Mutant strains and definition of PSβ integrin

The *lethal(1)myospheroid* alleles used in these experiments, $l(1)mys^{XG43}$ and $l(1)mys^{XB87}$, were induced by EMS (Wieschaus *et al.* 1984). Late embryos homozygous for $l(1)mys^{XG43}$ or $l(1)mys^{XB87}$ do not produce immunologically detectable PS3 β subunit (Leptin *et al.* 1989) and behave as loss-of-function alleles in various complementation tests (Zusman, unpublished results). Mutant chromosomes carrying $l(1)mys^{XG43}$, $l(1)mys^{XB87}$ or the marker mutations, *yellow*, *white* or *forked*, alone or in various combinations, were kept over the balancer *Fm7a* (Merriam and Duffy, 1972). For a detailed description of the marker mutations, see Lindsley and Grell (1968). PSβ integrin will refer to the β subunit which is encoded by the *l(1)mys* gene.

Clones for lethal(1)myospheroid

A genomic phage encompassing the *lethal(1)myospheroid* gene was generously provided by Dr S. Haynes (Digan *et al.* 1986) and used to isolate cDNA clones from a λgt11 library prepared from 0–16 h embryos (a generous gift of Drs K. Zinn and C. Goodman). Genomic and cDNA clones were analyzed by restriction mapping, subcloning and sequencing (Patel-King and Hynes, unpublished data). Sequences match those previously described by MacKrell *et al.* (1988).

RNA isolation, Northern blot analysis

RNA was isolated from staged wild-type embryos using a phenol/chloroform extraction method (Ayme and Tissieres, 1985). Total RNA from each of the indicated stages was electrophoresed in 1.0% agarose/formaldehyde gels containing 50 mM Hepes pH 7.8 and 1 mM EDTA, and RNA sizes were determined by comparison with an RNA ladder (Bethesda Research Laboratories). Separated RNAs were transferred to nylon membranes (Zetaprobe, Biorad.) and hybridized as described in DeSimone and Hynes (1988) with a [^{32}P]-labeled 3.3 kb *l(1)mys* cDNA probe covering the entire coding region. Blots were washed in 2×SSC/0.1% SDS at 65°C for 1 h prior to autoradiography. Equal loading and transfer of the RNA samples was checked by ethidium bromide staining of the gel and the blotted nylon filter.

In situ *hybridization to sectioned embryos*

Embryos were collected from females on agar plates coated with yeast paste and were dechorionated, fixed, dehydrated and embedded in paraffin as described in Ingham *et al.* (1985). 7 micron sections were prepared and processed as described in ffrench-Constant and Hynes (1988) using either ^{35}S-labeled antisense or sense RNA transcripts of a 1.1 kb. *l(1)mys* cDNA probe from the coding region as hybridization probes.

Preparation of embryos for whole mounts and antibody staining

0–16 h embryos were collected from Oregon R females at 25°C and were dechorionated, fixed and prepared for antibody reaction as described by Zusman and Wieschaus (1987), except that fresh paraformaldehyde was used for fixation. Embryos were mounted in a 3:1 solution of methyl salicylate and Canada Balsam, and were viewed under bright-field optics on a Zeiss Axiophot microscope.

The β-specific antiserum reported by Marcantonio and Hynes (1988) was used in this study to examine the distribution of β integrin on *Drosophila* embryos. This antiserum was raised against a synthetic peptide corresponding to the COOH-terminal end of chicken β_1 integrin. Previous studies have shown that this antiserum recognizes $\alpha\beta$ integrin complexes in *Drosophila* cells (Marcantonio and Hynes, 1988). Staining performed in the absence of primary serum or with immune serum in the presence of competitor peptide gave very little signal.

Production of adult gynandromorphs

l(1)mys gynandromorphs were collected from crosses in which males carrying the unstable ring X chromosome $In(1)w^{vc}$ (Hinton, 1955; Hall *et al.* 1976) were mated to *y l(1)mys/Fm7a* females. The ring X males also had a Y-linked duplication of the *Notch* region to increase survival ($y^+w^+N^+Y$), although this is not essential to the experiment. Random loss of the ring X chromosome during early development produces patches of cells, which are *y l(1)mys/−*, that is, are male cells with the yellow cuticle marker and are lacking a wild-type *l(1)mys* gene. *l(1)mys* gynandromorphs in which both maternal and zygotic $l(1)mys^+$ expression were removed from mutant tissue were produced by crossing *y l(1)mys/*Ovo^D females containing homozygous *y* $l(1)mys^{XG43}$ *f* germ cells to ring X males. Ovo^D is a dominant female sterile that prevents egg formation (Jimenez and Campos-Ortega, 1982; Garcia-Bellido and Robbins, 1983; Perrimon *et al.* 1984). When *l(1)mys/*Ovo^D female larvae are irradiated to induce mitotic recombination, some germ cells become homozygous for *l(1)mys* and lose the Ovo^D gene. Germline clones were identified using the procedures described by Zusman and Wieschaus (1985). A *y w f/Fm7a* or *y w f/*Ovo^D control cross was run simultaneously with each experiment using ring X individuals from the same bottles used in the experimental crosses.

All adult gynandromorphs were examined under a dissecting microscope or prepared for microscopic examination using the methods described by Szabad *et al.* (1979). The genotype of 20 landmark structures was recorded and the left and right sides of each individual were scored independently. A 'maleness average' score was determined for each landmark structure as the number of times a structure was mutant divided by the number of times that structure was scored. Significant differences between values were detected using χ^2 contingency tables ($P<0.05$).

Mitotic clones

Mitotic recombination was induced by gamma irradiation (1500 rads) of *y* $l(1)mys^{XG43}$ *f/*Ovo^D, *y* $l(1)mys^{xB87}$*/*Ovo^D, *w* $l(1)mys^{XG43}$*/*Ovo^D or *y w f/*Ovo^D larvae, between 48 and 66 h old. The Ovo^D chromosome was used in these experiments, since flies containing germline clones could also be used to generate gynandromorphs (see above). Wings from flies containing chromosomes marked with *yellow* and *forked* were embedded in Faure's mountant and examined under bright-field optics on a Zeiss Axiophot microscope. Heads containing *w l(1)mys* clones were removed, submerged in immersion oil and examined under antidromic illumination (Francescini, 1975) or were fixed and embedded in JB4 (Polysciences Inc,) as described in Mahowald *et al.* (1979). 4 micron sections were cut using a Leitz 1516 microtome, stained with methylene blue, and viewed under bright-field or Nomarski optics.

Results

Expression of the lethal(1)myospheroid *gene*

The *lethal(1)myospheroid* gene is expressed both maternally and zygotically (Digan *et al.* 1986; Wieschaus and Noell, 1986). To investigate further the pattern of *l(1)mys* expression during embryonic development, Northern blots of RNA from various embryonic stages were probed with a 3.3 kb *l(1)mys* cDNA. These blots reveal two major mRNAs of sizes 3.8 and 4.4 kb in 0–4 h embryos. These two mRNA species could represent the existence of multiple transcripts or alternative splicing. During subsequent stages of development, we observed only the 4.4 kb mRNA. The level of *l(1)mys* RNA drops significantly in 4–8 h embryos (extended germbands) and again becomes abundant in 8–14 h embryos (shortened germbands, dorsal closure; Fig. 1). *l(1)mys* RNA continues to be observed throughout embryonic development.

To examine the spatial distribution of *l(1)mys* mRNA during embryonic development, we hybridized ^{35}S-labeled *l(1)mys* antisense and sense RNAs to paraffin sections of 0–24 h embryos. Hybridization was seen with the antisense probe at all stages examined, while none was seen in control experiments using the sense probe. Fig. 2 shows that, during the first 9 nuclear divisions of development (see Foe and Alberts, 1983 for review of nuclear divisions), *l(1)mys* RNA is uniformly distributed throughout the embryo (Fig. 2A, B). As the

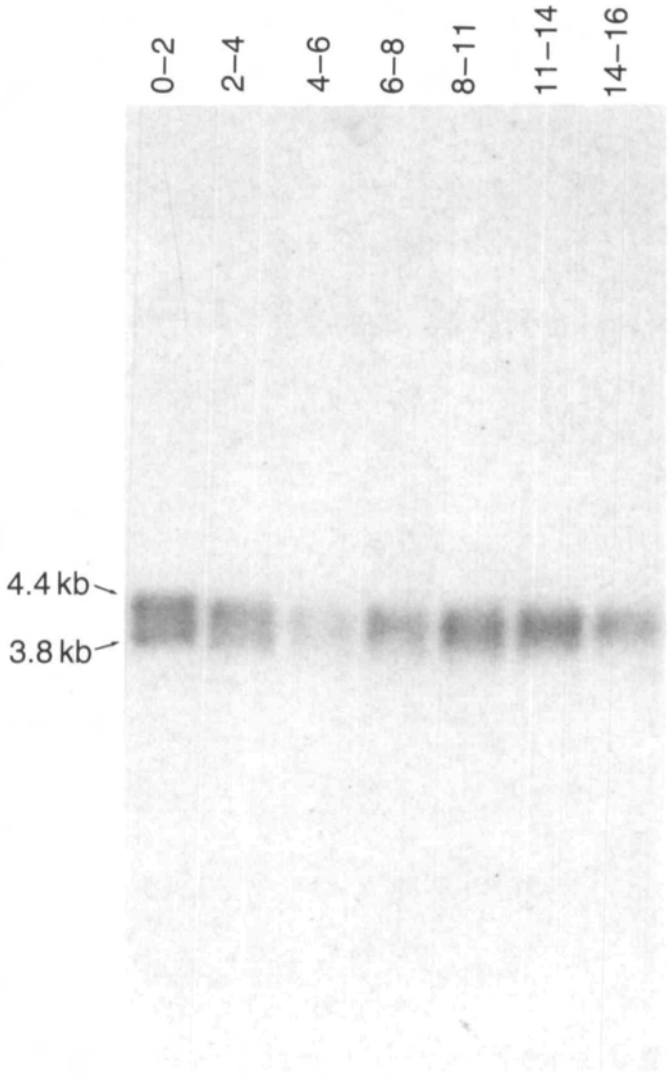


Fig. 1. Northern blot of embryonic RNAs probed with a 3.3 kb ^{32}P-labeled *l(1)mys* cDNA. Each lane contains 10 μg total RNA. The number at the top of each lane represents the age of embryos (hours after fertilization) from which RNA was extracted. RNA sizes are given in kb and were determined by comparison with the migration of marker RNAs.

nuclei migrate to the periphery of the embryo (nuclear division 12–13), the transcripts also move to the periphery of the embryo (Fig. 2C, D). By the cellular blastoderm stage, *l(1)mys* transcripts are localized to all the newly formed cells. As in the Northern blot results, the *in situ* hybridization experiments also demonstrate a temporary decrease in RNA levels when the germband is fully extended (Fig. 2E, F). Abundant RNA levels are again observed during germband retraction (Fig. 2G, H) and also during later stages (data not shown). Thus *l(1)mys* transcripts were found in most, if not all, embryonic tissues.

The distribution of β integrin in the developing embryo

Antiserum against a synthetic vertebrate β integrin peptide (Marcantonio and Hynes, 1988) was used to examine the location of β integrin protein in developing embryos. 0–16 h embryos were fixed, incubated with the antibodies, followed by anti-rabbit antibody complexed to HRP and examined in whole-mount preparations. The staining patterns described below were found only when embryos were incubated with immune serum and were not observed when embryos were incubated with preimmune serum or with immune serum plus the peptide used in the production of antisera (data not shown).

The first observable β integrin appears during the early stages of gastrulation. In wild-type embryos, gastrulation begins with the formation of the ventral furrow, which brings the future mesoderm into the interior of the embryo. The resulting ventral tube of presumptive mesoderm and the immediately surrounding ectoderm comprise the germband. Also, during this stage of development the posterior and anterior midgut primordia become apparent. The earliest definitive staining for β integrin is seen about 20 min after the

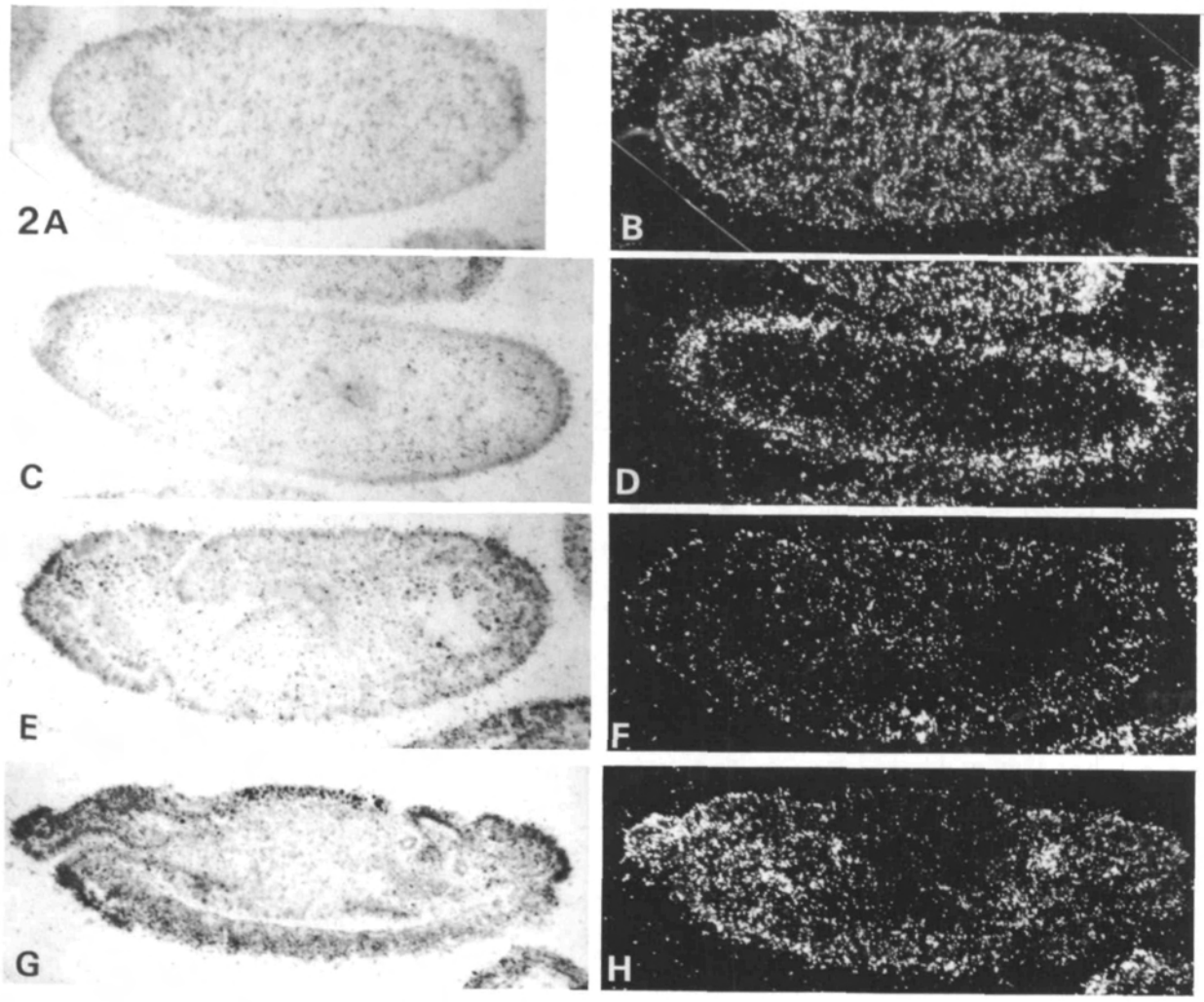


Fig. 2. Localization of *l(1)mys* transcripts in embryos. Bright-field (left) and corresponding dark-field (right) photographs were taken after autoradiography. Autoradiography and exposure for all embryos was 10 days. The montage shows appropriately staged embryos from a single experiment photographed and enlarged at the same magnification so that RNA levels at particular stages can be compared. Orientation of embryos is anterior left and dorsal up. (A,B) syncytial blastoderm (stage 2); (C,D) cellular blastoderm (stage 5); (E,F) gastrulation with a fully extended germband (stage 10); (G,H) embryo with shortened germband (stage 13). See Campos-Ortega and Hartenstein (1985) for a description of the stages.

onset of gastrulation (Fig. 3A). A dispersed staining pattern is especially obvious in the germband and posterior and anterior midgut primordia. As the germband extends, β integrin appears to become concentrated in the area of the germband between mesoderm and ectoderm (Fig. 3B). Previous studies of sections of embryos stained with PSβ antibodies suggest that this staining pattern reflects a large concentration of β integrin on opposing basal surfaces of these two epithelial sheets (Leptin *et al.* 1989). β integrin is also observed during this stage around the stomodeum and proctodeum, the primordia of the larval hindgut and foregut, respectively (Fig. 3C). As the germband shortens, β integrin becomes concentrated in the round balls of somatic mesoderm (Fig. 3D). During this and subsequent stages, it is also concentrated around the foregut, hindgut and midgut (Fig. 3E, 3F). Little or no staining, however, was observed in the lateral hypoderm. As previously described by Leptin *et al.* (1989), we observed β integrin in approximately 14 h old embryos localizing to the attachment sites of the body wall muscles (not shown). Little staining was observed in the central nervous system. These data indicate widespread expression of β integrin in the embryo.

Localized requirements for zygotic and maternal gene activity

The mRNA detected at the early stages is presumably largely maternal, whereas later stages also contain embryonic transcripts. Both maternal and embryonic expression can contribute to development. The maternal mRNA is not absolutely required since *lethal(1)myospheroid*$^{+}$ embryos derived from homozygous *l(1)mys* oocytes are normal (Leptin *et al.* 1989). Nevertheless, maternal *l(1)mys* expression has been shown to produce functional β integrin that can contribute to the developing embryo. This is demonstrated by the observations that: (1) extra maternal copies of *l(1)mys*$^{+}$ can partially rescue the phenotype resulting from loss of zygotic expression (Wieschaus and Noell, 1986); and (2) removal of the maternal contribution produces a more severe defect in embryos that are *l(1)mys* homozygotes (Wieschaus and Noell, 1986; Leptin *et al.* 1989).

We conducted two series of experiments to address the role of zygotic and maternal PS integrin expression. To determine whether the zygotic expression of *l(1)mys* is required in all regions of the embryo for normal viability, gynandromorphs with *l(1)mys*$^{+}$ and *l(1)mys*^{XG43} or *l(1)mys*^{XB87} patches of tissue were produced from ring X/ *y l(1)mys* flies by random loss of the ring X chromosome containing the wild-type *l(1)mys* gene (Hall *et al.* 1976, see Materials and methods). Only 7% of the *l(1)mys* gynandromorphs survived to the adult stage, whereas control *l(1)mys*$^{+}$ gynandromorphs showed normal viability. In the surviving *l(1)mys* mosaic animals, mutant patches were small and covered no more than 10% of the fly, while control patches constituted an average of 50% of the fly. Mutant tissue observed in the head, thorax and abdomen was often normal in appearance. However, wing blisters, missing legs or leg parts, tergite defects and missing pieces of cuticle were frequently observed in *l(1)mys* gynandromorphs, whereas such defects were rare in *l(1)mys*$^{+}$ gynandromorphs (Table 1). The largest mutant patches constituting approximately 10% of the fly were in the dorsally located tergites and could be normal or defective in appearance. These observations suggest that, while PSβ integrin is not required in every cell of the developing fly for viability, it appears to influence or be necessary for the development of many adult structures including the wings, legs and tergites.

Table 1. *Abnormalities in gynandromorphs*

Abnormality	*In(1)w*^{vc} *y w f* gynandromorphs*	*In(1)w*^{vc} *y l(1)mys*^{XG43} gynandromorphs*
Total no. scored	100	100
Wing blisters	0	19
Legs or leg parts missing	0	7
Tergite defects or missing cuticle	1	30

* *In(1)w*^{vc}=Ring X chromosome, which can be randomly lost during early development.

Gynandromorphs were scored by the presence of patches of *yellow* cuticle. These patches are male (X0) and hemizygous for *l(1)mys*.

To determine localized requirements for PSβ integrin in particular regions of the embryo, we measured the frequency with which 20 adult structures in *l(1)mys*^{XG43} and control mosaics were mutant. Since such mutant patches are made up of male (XO) cells, this is called the maleness average score. The maleness average scores calculated for structures in control mosaics give the probability that a particular structure will be mutant, if there is no lethality associated with loss of the wild-type gene; reduced scores in the *l(1)mys* gynandromorphs indicate lethality. Table 2 shows significant lethality associated with all scored structures in *l(1)mys* mosaics suggesting that most large patches of mutant tissue result in lethality. There is a particularly strong bias for lethality in those mosaics that have mutant cells in ventrally derived structures. For example, in the abdominal segments of surviving mosaics, the ventrally located sternites were found to be mutant at a significantly lower frequency than the more dorsally located tergites. In addition, marked clones lacking *l(1)mys* expression were not observed in the legs, the most ventrally located structures scored. Finally, no *l(1)mys* mosaic borders were observed to cross the ventral midline; however, they crossed the dorsal midline in 10% of the surviving mosaics.

The conclusion that not every cell in the developing embryo requires the normal level of *l(1)mys* expression for viability is complicated by the fact that maternally derived PSβ integrin may still be present in mutant cells. To determine whether these cells can survive throughout development without either maternally or zygotically derived PSβ integrin, we performed an additional series of experiments in which both the maternal and zygotic components were eliminated. Gynandromorphs were produced by crossing ring-X

Table 2. *Calculated maleness average scores for landmarks in control and* l(1)mys^{XG43} *adult gynandromorphs*

	Control cross[a]	l(1)mys crosses[a]
Nonmosaic[b]	32	438
Mosaics	100	100
Sides scored[c]	200	200
Mosaic survival[d]	1.00	0.07
Lethality[e]	0.0	0.93
Mean maleness average	0.41	0.10

Embryonic location	Landmarks	Maleness average score	Maleness average score*
Anterior	Ocellus	0.33	0.09
Dorsal	Proboscis	0.42	0.10
Dorsal	Humerus	0.35	0.10
Lateral	Scutellum	0.42	0.09
Ventral	Leg-1	0.43	0.00
Ventral	Leg-2	0.42	0.00
Ventral	Leg-3	0.42	0.00
Dorsal	Tergite-1	0.39	0.15
Dorsal	Tergite-2	0.40	0.16
Dorsal	Tergite-3	0.39	0.15
Dorsal	Tergite-4	0.41	0.15
Dorsal	Tergite-5	0.43	0.24
Dorsal	Tergite-6	0.43	0.18
Dorsal	Tergite-7	0.43	0.18
Ventral	Sternite-2	0.42	0.05
Ventral	Sternite-3	0.41	0.05
Ventral	Sternite-4	0.43	0.04
Ventral	Sternite-5	0.43	0.04
Ventral	Sternite-6	0.41	0.04
Posterior	Genitalia	0.44	0.08

[a] Combined data from two sets of experiments; one (70 flies) in which only zygotic *l(1)mys* expression was lost and one (30 flies) in which both zygotic and maternal *l(1)mys* expression were lost from mutant cells in the gynandromorphs (See Methods). Since the mean maleness average score and mosaic survival were the same in both experiments, the data were combined.

[b] Nonmosaics include *In(1)w^{vc}/y l(1)mys^{XG43}*, *In(1)w^{vc}/y l(1)mys^{XG43} f* and *In(1)w^{vc}/y w f* flies.

[c] Since the right and left halves of each mosaic animal were scored independently, the sample size for each landmark is double the number of mosaic animals obtained.

[d] Survival=(number of *mys* mosaics)/(number of *mys* nonmosaics)×(number of control nonmosaics)/(number of control mosaics).

[e] Lethality associated with *l(1)mys* in mosaics; 1-survival rate of the mutant.

* Maleness average=

$$\frac{\text{the number of times a structure is scored as mutant}}{\text{the number of times the structure was scored}}$$

All *l(1)mys* maleness average scores were significantly smaller than the corresponding control scores ($P<0.05$).

males to *y l(1)mys^{XG43}f/Ovo^{D}* females containing homozygous *y l(1)mys^{XG43} f* germline clones (See Materials and methods). 30 adult gynandromorphs were observed with *l(1)mys^{-}* patches. As in the previous experiment, there was a 95% lethality associated with these mosaics and the same bias against mutant patches in the ventral tissue of surviving gynandromorphs was observed. Also, wing blisters, missing leg parts, missing cuticle and tergite defects were found only in *l(1)mys* mosaic flies. Because the level of mosaic survival and the mean maleness average score for this second series of experiments were the same as for the first series, both sets of data are combined in Table 2. The fact that the data from the two series are the same indicates that the maternal expression of *l(1)mys* does not appear to play a role different from that of zygotic expression.

Collectively, the combined data suggest that, although certain localized regions of the embryo can develop in the complete absence of PSβ integrin (maternal or zygotic), expression of PSβ integrin is required in many parts of the developing fly. There is an especially large requirement for *l(1)mys^{+}* expression (PSβ integrin) in ventrally derived tissues.

Comparison of the data on gynandromorphs with the immunolocalization data suggests a correlation between regions of the embryo that have a large concentration of β integrin and regions that show the greatest requirement for normal levels of β integrin for viability. Such regions include the ventrally derived mesoderm and surrounding ectoderm and perhaps part of the gut. In contrast, we detected much less β integrin staining in most dorsally and laterally derived regions of the embryo, which appear to have a smaller requirement for local expression of PSβ integrin as indicated by the gynandromorph studies.

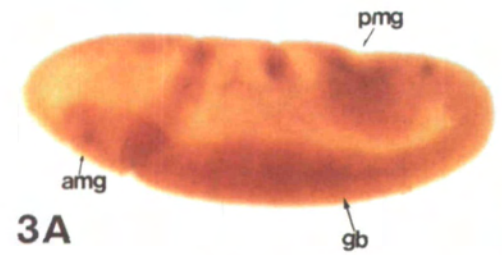
Requirements during wing development

Earlier studies have demonstrated the presence of PSβ

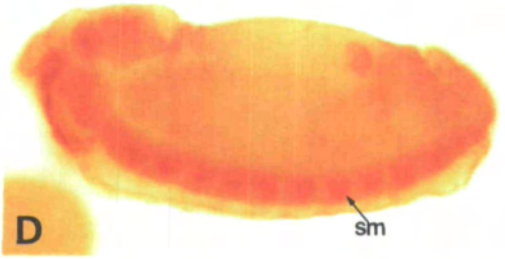
Fig. 3. Whole mounts of wild-type embryos stained with an antiserum produced against a β integrin peptide. Antibody localization was visualized using biotinylated goat anti-rabbit antibodies bound to an HRP complex. Note the large concentration of β integrin in the germband (gb) and its derivatives (i.e. sm; arrows) and in the gut primordia (i.e. pr, st, amg, and pmg; arrows). Orientation of embryos is anterior left and dorsal up. (A) gastrula approximately 20 min after the onset of gastrulation; (B) gastrula with a fully extended germband; (C) embryo shortly after the onset of segmentation; (D and E) embryos with shortened germbands; (F) embryo shortly after dorsal closure. gb, germband; amg, anterior midgut; pmg, posterior midgut; st, stomodeum; pr, proctodeum; sm, somatic mesoderm; mg, midgut; hg, hindgut.

Fig. 4. Requirements for β integrin in wing disc development. (A) Wild-type wing; (B–D) wings containing *l(1)mys* clones and increasingly severe wing blisters. Note brown cuticle around blister in B, abnormal venation in C, and folds around the wing margin in D (arrows).

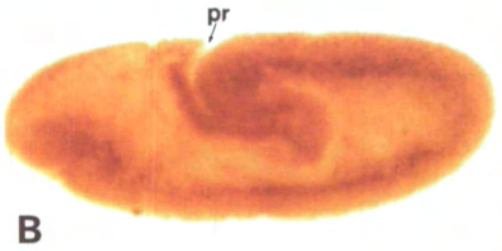
Fig. 7. Parasagittal sections of eyes containing *w l(1)mys* clones. (A) Parasagittal section of an eye showing the borders of a *w l(1)mys* clone (large thin arrows). Note the normal lamina of the optic ganglion in the area of the clone (small thin arrow). (B and C) Higher magnification of sections containing *w l(1)mys* clones. Note the disoriented and fragmented rhabdomere bundles (small thin arrows) in the *w l(1)mys* ommatidia. Also note the holes on the basal side of mutant ommatidia (large thin arrows). *w l(1)mys* lens cells usually form and appear generally normal (small thick arrows). Orientation of the sections is apical down and basal up.



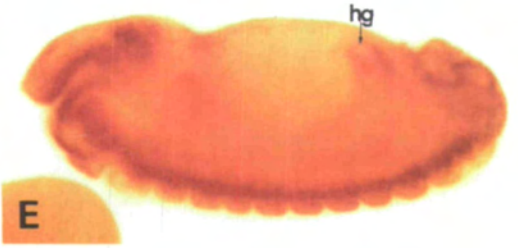
3A



D

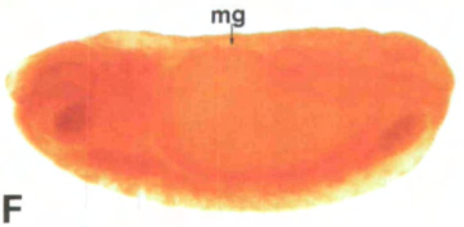


B

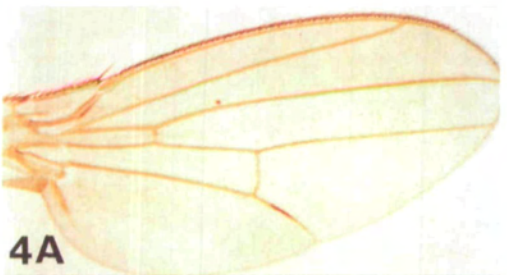


E

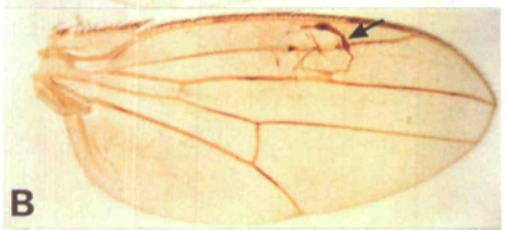
C



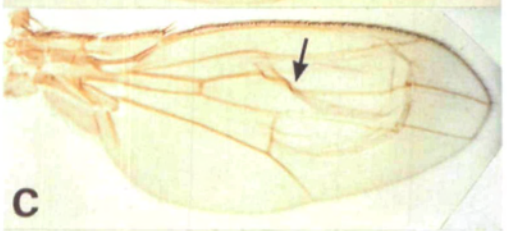
F



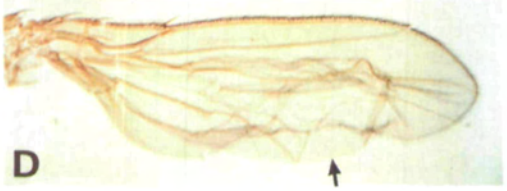
4A



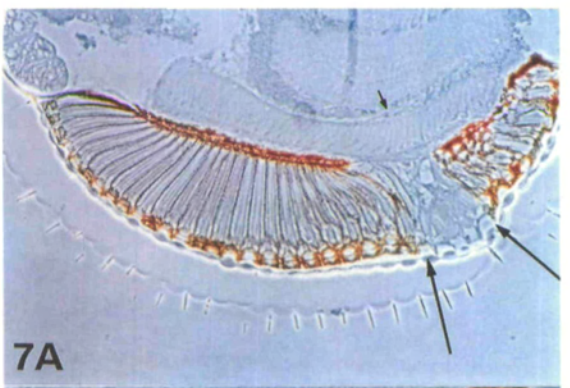
B



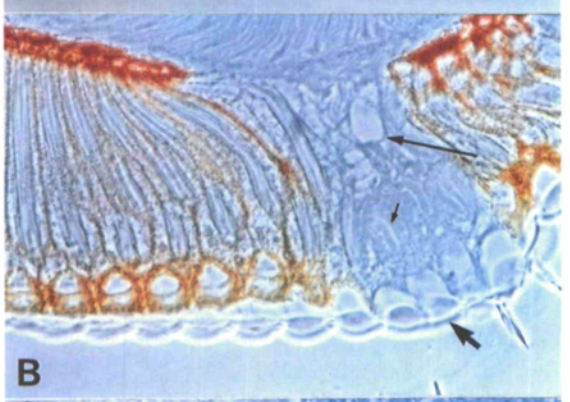
C



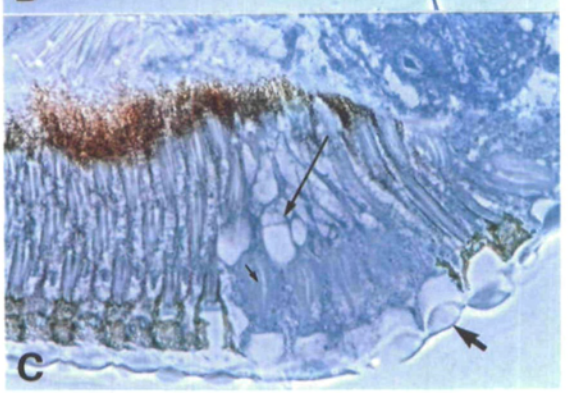
D



7A



B



C

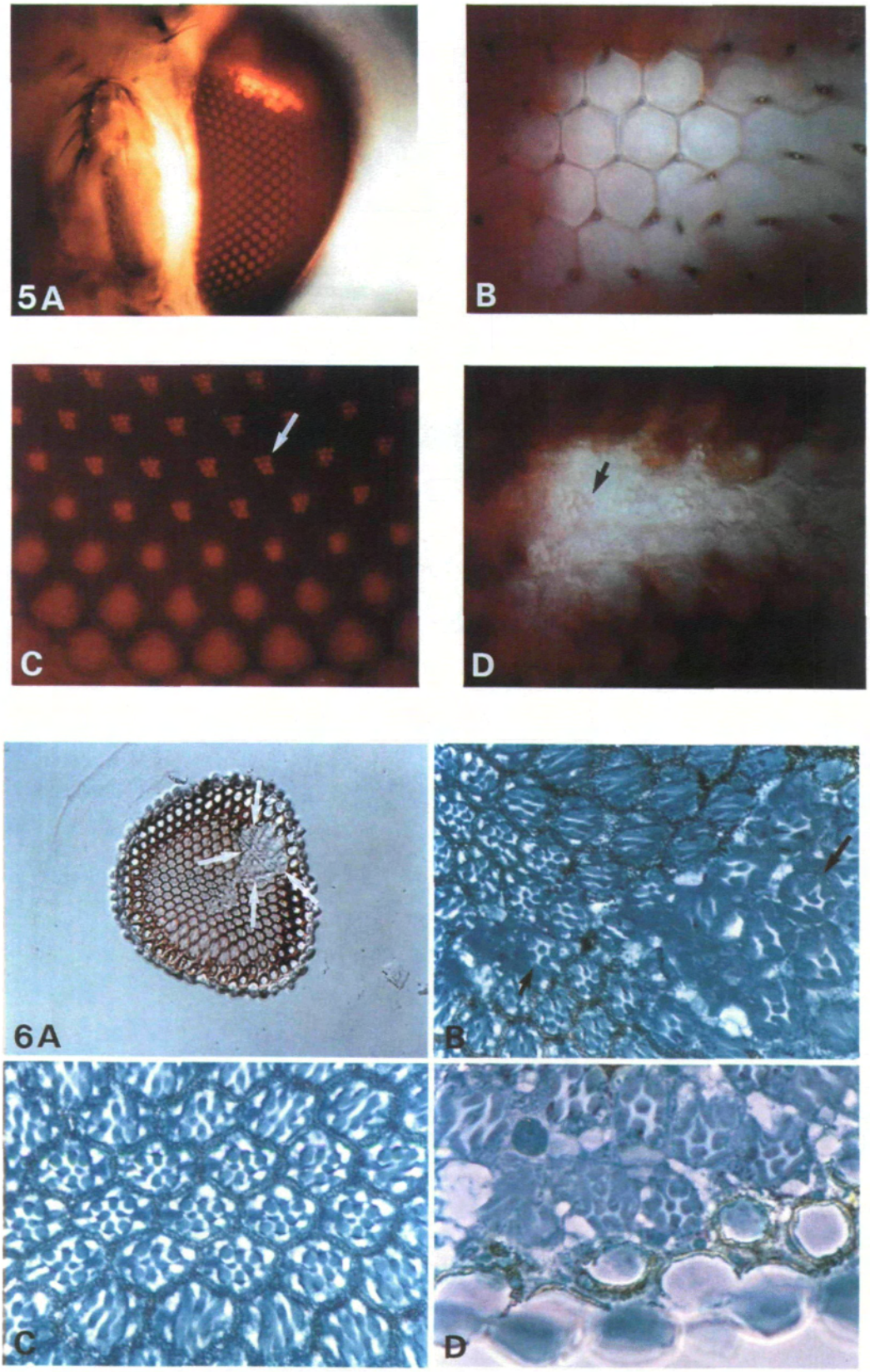


Fig. 5. Requirements for β integrin during eye disc development. Eyes were submerged in oil and viewed under antidromic illumination. (A) Eye containing a *w l(1)mys* clone. (B) Ommatidia from the clone shown in A). Plane of focus at the level of the lens. Note the normal shape and arrangement of ommatidia and bristles. (C) Enlargement of wild-type ommatidia in A. Plane of focus at the level of the rhabdomeres. (D) Enlargement of *l(1)mys* ommatidia in A. Plane of focus at the level of the rhabdomeres. Note the abnormal shape and size of the rhabdomeres (arrow). C and D were photographed at the same magnification and were enlarged to the same size.

Fig. 6. Cross sections of eyes containing *w l(1)mys* clones. (A) Cross section of an eye showing the borders of a *w l(1)mys* clone (arrows). (B) Higher magnification of the boundary between wild-type (left) and mutant (right) tissue. Compare the regular size and arrangement of the wild-type rhabdomeres with the abnormally large and irregular shape of the *l(1)mys* rhabdomeres and their irregular arrangement (arrows). (C) Enlargement of *l(1)mys*$^+$ ommatidia illustrating the regular pattern of rhabdomeres. (D) Enlargement of *w l(1)mys* ommatidia showing derangement, large size and occasional duplication or folding of mutant rhabdomeres. C and D were photographed at the same magnification and were enlarged to the same size.

integrin in the developing eye–antennae, leg and wing discs (Brower *et al.* 1985). To examine the effect of PSβ integrin loss on the development of these and possibly other imaginal discs, larvae heterozygous for a *l(1)mys*^{XG43} chromosome marked with *yellow (y)* and *forked (f)* or *white (w)* were irradiated with gamma rays during larval stages to generate clones of cells homozygous for *y l(1)mys f* or *w l(1)mys*.

20 % of the 597 irradiated *y l(1)mys*^{XG43} *f* heterozygous flies examined contained wing defects, including blisters (the most common defect), folds in one or both surfaces of the wing, vein abnormalities and missing or enlarged halteres. In contrast, these defects were not observed in nonirradiated *y l(1)mys f* heterozygotes, or in irradiated *y w f* heterozygotes, but were observed when flies containing another *l(1)mys* loss of function allele, *l(1)mys*^{XB87}, were irradiated. These data suggest that the observed wing defects were not due to dominant affects of *l(1)mys*, other mutations, or irradiation damage, but rather were due specifically to the loss of PSβ integrin in a group of wing disc cells.

This hypothesis was confirmed by mounting 85 wings with detectable abnormal morphology and examining them for clones. Virtually all of these wings contained detectable mutant *l(1)mys* cells. However, most blisters or defects encompassed a significantly larger area of the wing than that of the associated clones. In addition, *l(1)mys* wing clones often extended in the proximal–distal direction and were confined to either the posterior or anterior compartment (Garcia-Bellido *et al.* 1973, 1976), while blisters were typically oval or circular in shape and often crossed the compartment boundary.

Fig. 4 shows that the severity of the wing blisters can range from small separations of the dorsal and ventral epithelia (Fig. 4B) to large blisters (Fig. 4C) and folds causing larger, more general disruption of dorsal and ventral epithelia (Fig. 4D). The trichomes (hairs, derived from individual cells in the wing epithelium) develop normally and in apparently normal patterns in the blister, but are often more sparsely distributed than in the surrounding regions of the wing, as if the epithelium constituting the blister is stretched. Cell death and/or cell proliferation do not appear to play a major role in the blistering effect since *y l(1)mys f* clones were as large (average of 80 cells) and as abundant (20 % of irradiated flies) as in the control *y w f* clones. Wing veins included in the blisters were often abnormal, and associated with brown cuticle, while the overall vein pattern in the wing was usually maintained (Fig. 4). The differentiation of bristles within blisters remained normal. No apparent relationship was observed between the morphology of the wing and whether the clone was dorsally or ventrally located.

Since PS1 and PS2 integrin α subunits are expressed in the mature wing imaginal disc on dorsal and ventral epithelium, respectively, we examined clones crossing the wing margin, which is derived from the boundary of PS1 and PS2 expression. Most of these clones, however, leave the pattern of marginal bristles looking normal and do not produce blisters.

These data indicate that PSβ integrin is required for the normal development of the wing and suggest that it is necessary for keeping the dorsal and ventral wing epithelia apposed and for normal wing vein development.

We did not observe any abnormalities in the legs of irrradiated *y l(1)mys f* heterozygotes. However, since the expected leg clones are small we would not necessarily have expected any gross defects and more detailed analysis would be required to identify subtle defects.

Requirements during eye development

The differentiation of the *Drosophila* eye disc into the adult compound eye occurs during the late larval and early pupal stages of development. The adult eye consists of several hundred ommatidia, each containing 8 photoreceptor cells (R1–R8) at its core and twelve surrounding accessory cells. Cross sections show a reiterated asymmetrical arrangement of 6 peripheral rhabdomeres (R1–R6) surrounding 2 central ones (R7, R8). R1–R6 span the entire thickness of the retina, while R7 and R8 are set one above the other, with R7 more apically located and R8 more basally located. Above the photoreceptor unit lies the lens system, consisting of the corneal lens, the pseudocone, 2 primary pigment cells and 4 cone cells. Around the photoreceptor cells and lens system is a ring of secondary and tertiary pigment cells, surrounding each ommatidial unit. These pigment cells are shared by neighboring units and therefore sections of the adult compound eye show a honeycomb-like array of hexagonal ommatidia (see Tomlinson, 1988 for a recent review of adult eye structure).

15 % of the 384 heterozygous *w l(1)mys*^{XG43} irradiated flies and 14 % of the 454 heterozygous *y w f* irradiated flies were observed to have *white* eye clones constituting 5–30 % of the eye (Fig. 5A). In order to examine the effect of the absence of PSβ integrin on ommatidial shape and rhabdomere organization, eyes of 28 *w l(1)mys* flies and 21 *y w f* flies that contained clones were immersed in oil and examined under antidromic illumination (Francescini, 1975). In all eye clones examined, the hexagonal array of ommatidia appeared normal and continuous with that of neighboring wild-type ommatidia (Fig. 5B). However, more detailed examination showed that the organization and structure of the rhabdomeres in *l(1)mys* ommatidia was abnormal. Unlike the regular organization of wild-type or *y w f* rhabdomeres (Fig. 5C), *l(1)mys* rhabdomeres showed a more random arrangement and irregular structure (Fig. 5D). Under antidromic illumination, *l(1)mys* photoreceptor cells often appeared to be larger than wild-type cells, and at times, showed fewer than 7 rhabdomeres in a particular optical plane. In addition, in 16 out of the 28 *l(1)mys* clones examined, no rhabdomeres were detectable under antidromic illumination. These data suggest that PSβ integrin is not required for establishment of the hexagonal pattern of ommatidia, but is required for the normal arrangement of rhabdomeres within each ommatidium. The fact that we were unable to observe rhabdomeres in certain *l(1)mys* clones further suggests that loss of PSβ integrin

might result in areas without rhabdomeres or cause rhabdomeres to be oriented in such a way that they are no longer visible under antidromic illumination.

To investigate further the nature of the defects in the *l(1)mys* eye clones, 15 additional eyes containing one or more *l(1)mys* clones were embedded in plastic and sectioned. In sections transverse relative to the ommatidia (Fig. 6), the wild-type ommatidia showed the typical trapezoidal array of rhabdomeres, whereas the *l(1)mys* clones showed abnormally large and disorganized rhabdomeres. Although the number of rhabdomeres per ommatidial unit appeared to be approximately normal, the deformed shapes frequently made accurate counts difficult. In some cases, rhabdomeres appeared to be duplicated, at least in cross section (Fig. 6D). In sections perpendicular to the plane of the retina (Fig. 7), the rhabdomeres remained together as units, but they failed to span the thickness of the retina and were disorganized. This disorganization was more severe at basal positions where holes were frequently observed. However, the lamina, the first optic ganglion, was intact and appeared to have a normal morphology. The apical segments of the rhabdomeres appeared more wild-type in organization than the basal segments and the arrangement of the corneal lens cells appeared relatively normal. In both transverse and longitudinal sections, the ommatidial units closest to the boundaries of the clones often looked the most normal (Figs 6, 7), which might be expected since ommatidia are not clonal units.

In summary, the overall arrangement of ommatidia and the lamina is normal in *l(1)mys* clones, rhabdomeres are induced in normal or near normal numbers, but fail to develop their normal shape and organization within each ommatidial unit. These results indicate that PS integrins are necessary for some, but not all aspects of development of ommatidial arrays (See Discussion).

Discussion

Earlier studies have described the defects in embryogenesis of *l(1)mys* embryos (Wright, 1960; Newman and Wright, 1981; Wieschaus and Noell, 1986; Leptin *et al.* 1989). These defects include incomplete germband extension and contraction, aberrant attachment of visceral muscles, detachment of somatic muscles from their attachment sites and failure of dorsal closure leading to herniation. Many of these defects are more severe when the maternal component of *l(1)mys* is also deleted; additionally, elevation of the maternal contribution can partially restore the deficit in *l(1)mys* zygotes (Wieschaus and Noell, 1986). These results demonstrate that both maternal and zygotic expression of *l(1)mys* can participate in normal development, although the maternal component is dispensable. It is tempting to speculate that the two *l(1)mys* mRNA species detected in 0–4 h embryos (Fig. 1, see also Digan *et al.* 1986) are maternally derived and that they decay over the first 6–8 h of development and are replaced by a zygotic transcript at later times (Fig. 1). However, we cannot at present distinguish maternal from zygotic transcripts. The *l(1)mys* mRNAs become segregated to all the cells of the blastoderm (Fig. 2 C,D) and mRNA is detectable in all or most tissues throughout development (Fig. 2). The PSβ integrin encoded by the mRNA is first readily detectable at the time of gastrulation (Fig. 3) and is detectable in many tissues, including the germband and the gut throughout development. Integrins are concentrated in muscle attachment sites (Bogaert *et al.* 1987; Leptin *et al.* 1989; our unpublished results) and their absence in *l(1)mys* mutants is likely the cause of much of the mutant phenotype. Given the known functions of integrins in vertebrates, namely cell–matrix and cell–cell adhesion (Hynes, 1987; Ruoslahti and Pierschbacher, 1987; Buck and Horowitz, 1987), it is likely that they play a similar role in *Drosophila* and that, in their absence, cell attachments are defective.

The mutations we have used in the generation of mosaics appear to be null alleles of *l(1)mys*, which produce no PSβ integrin subunit. The two associated PSα subunits (PS1α and PS2α) fail to be processed to the cell surface in the absence of PSβ (Leptin *et al.* 1989). Thus, cells lacking *l(1)mys*$^+$ lack both of these PS integrins as well as any others that share the same β subunit. If other subfamilies of integrins exist in *Drosophila*, which is not yet known, they presumably persist.

The gynandromorphs (Tables 1 and 2) contain large clones derived from cells that lose *l(1)mys*$^+$ and PSβ integrin relatively early in development. As would be expected, many (93%) of these gynandromorphs die before the adult stage. Although our gynandromorph data do not provide any information on the age or phenotype of dead mosaics, the surviving mosaics provide indirect information on the requirements for PSβ integrin in different regions of the organism. The data (Table 2) indicate that the greatest requirement is in ventral structures. For instance, we never observed mutant patches on the legs and legs were frequently missing, suggesting either that development of legs has a strong requirement for PS integrins, or that adjacent ventral structures have such a requirement, or both. The selection against survival of gynandromorphs lacking PS integrins in ventral structures (Table 2) is concordant with the high level of expression of PSβ integrin in the germband and its derivatives (Fig. 3) and suggests a primary role for PS integrins in the development or maintenance of one or more of these structures. In addition, our results suggest that the abnormalities (i.e. dorsal rupture) observed along the dorsal midline of *l(1)mys* embryos (Wright, 1960; Newman and Wright, 1981; Wieschaus and Noell, 1986) might be secondary and result from the lack of integrins in ventrally derived cells. While some mutant patches in the *l(1)mys* gynandromorphs appeared to develop normally, many others gave rise to defects of varying severity, such as wing blisters and tergite defects. These results also indicate a requirement for PS integrins in the development of tergites and wings.

Somatic clones generated by radiation-induced mi-

totic crossing-over produce smaller mutant patches and lead to defects in the development of wings (Fig. 4) and eyes (Figs 5–7). The nature of the defects in the wings and eyes provides information as to which aspects of development require the presence of PS integrins and which do not. The very existence of wing and eye clones at the same frequency and size in both *l(1)mys* and wild-type crosses indicates that PS integrins are not required for cell viability or proliferation or for maintenance of cell sheets. The defects produced by loss of PS integrins appear, rather, to arise from defects in interactions between sheets or groups of cells.

The blisters that form in the wings appear to result from a failure of apposition of the two surfaces of the wing, each of which is derived from an epithelial sheet. While these sheets appear relatively normal in the blisters, they are not properly attached to each other and the areas of detachment are larger than the areas of the clones. This suggests that a defect in attachment in the area of the clone may generate a point of weakness allowing separation of the two epithelial sheets over a large area. During development of the wing disc, the dorsal and ventral epithelial layers separate and rejoin several times (Waddington, 1940; Milner and Muir, 1987). During the separations, the two epithelial layers remain attached via long cellular processes, which contain microfilaments and microtubules (Mogensen and Tucker, 1988) and are connected at their ends by cell–cell attachments. Since PS1α is expressed in the dorsal epithelium and PS2α in the ventral epithelium (Brower *et al.* 1985), it seems quite likely that these two integrins participate in the attachment of the two epithelial layers either directly or via intervening extracellular matrix. Similar wing blisters have been reported by Wilcox *et al.* (1989) in flies heterozygous for a hypomorphic allele ($l(1)mys^{nj42}$) of the *l(1)mys* locus and for an amorphic allele ($l(1)mys^{XG43}$) and in flies homozygous for *inflated*, the gene for PS2α. Flies doubly homozygous or hemizygous for mutations in *l(1)mys* and *inflated* show even more extreme wing defects. These observations are in good agreement with our results. Absence of $l(1)mys^{+}$, and the PSβ integrin that it encodes, would lead to defects in interepithelial attachment, the failure to maintain the correct apposition of dorsal and ventral wing surfaces and the development of the blisters and folds (Fig. 4).

Interpretation of the eye defects is a little more complex, but previous descriptions of pattern formation during eye development provide a good basis for analyzing potential roles for PS integrins. The process of ommatidial development involves cell–cell adhesion, cell movements and cell-matrix interactions, exactly those processes in which integrins have been implicated in vertebrates (see Introduction). The defects seen in *l(1)mys* somatic eye clones indicate that PS integrins are necessary for the proper organization of ommatidial units, although many aspects of eye development proceed in the absence of PS integrins.

Examination of *l(1)mys* somatic eye clones first allows identification of the processes for which PS integrins are *not* necessary. For example, the fact that lenses, and the hexagonal array of ommatidia, develop essentially normally in clones (Figs 5–7) indicates that the cone cells, and the secondary and tertiary pigment cells, develop in the normal positions. Also an apparently normal lamina, the first optic ganglion, forms in mutant *l(1)mys* areas. Together with the appearence of photoreceptor cells with recognizable rhabdomeres, albeit significantly distorted (Figs 5–7), these results suggest that the development of the patterned array of ommatidial units, initial differentiation of photoreceptors and the projection of their axons into the lamina all proceed along approximately normal paths without PS integrins. Thus, the 'preclusters' of photoreceptor precursors that appear just behind the morphogenetic furrow and form the core of the developing ommatidial units (Ready *et al.* 1976, 1986; Tomlinson, 1988) must appear in their normal array. That is, the clustering of these cells from the previously undifferentiated epithelial layer happens in the absence of PS integrins despite the switch of PS1α integrins in front of the furrow to PS2α integrins behind it (Brower *et al.* 1985). These integrins also appear to be unnecessary for the cell movements that position subsequent members of the ommatidial unit in relation to the core of precluster-derived cells. The cells that move apically along the sides of the precluster include the precursors of the cone cells (Ready *et al.* 1986; Tomlinson, 1988) and, as noted above, cone cells develop normally in the correct apical location. Since an apparently normal lamina forms in mutant *l(1)mys* areas, integrins also appear not to be absolutely required for projection of photoreceptor axons, as previous studies (Meinertzhagen, 1973; Meyerowitz and Kankel, 1978; Fischbach and Technau, 1984) have shown that proper retinal innervation is required during late larval stages for the normal development of the optic ganglia. Thus, one can conclude that PS integrins are not necessary for segregation of preclusters, for many of the apicobasal movements of the cells of the ommatidial units, for the projection of photoreceptor axons or for determination or differentiation of cone cells, photoreceptor cells, secondary and tertiary pigment cells or lamina.

The fact that the photoreceptors are disorganized within the mutant ommatidia indicates that integrins *are* needed for the normal patterning of these cells. The disorganization precludes the identification of individual photoreceptors by their positions within the ommatidia. Thus, while it appears that each of the mutant ommatidia contains an approximately normal number of photoreceptors (Fig. 6), our current level of analysis does not rule out deficiencies or excesses in photoreceptor number and does not allow one to define exactly which of the usual eight receptors in each unit is correctly formed, let alone correctly positioned. Since a 'default' pathway for R7 is to develop into cone cells (Tomlinson and Ready, 1986, 1987), we also cannot rule out aberrant determination of certain photoreceptor cells.

The simplest interpretation of the current data is that precluster formation, recruitment, induction and differentiation of photoreceptors, cone cells and pigment

cells all proceed relatively normally without PS integrins. Therefore, it appears that photoreceptor cells and rhabdomeres depend on the expression of PS integrins predominantly during later stages of development, perhaps as late as the pupal stages when the ommatidial units elongate. The enlarged and distorted profiles of rhabdomeres in transverse section (Figs 5 and 6) and their apparent basal retraction in the parasagittal sections (Fig. 7) suggest that the attachments of these cells at the basal surface of the retina depend on integrins. The relatively normal array at the apical end of each ommatidial unit could rely on apical cell–cell interactions among the photoreceptor cells. A more detailed analysis will be necessary to define more precisely the nature of the defects caused by the absence of specific PS integrin subunits.

The lethality and defective development of both embryonic and adult structures, consequent upon loss of PS integrins, is not unexpected given their presumed involvement in multiple cell adhesion phenomena. It is striking, however, how much development can occur in the absence of these proteins. This point is particularly clear from the analysis of eye development. The extensive development of ommatidial units and their normal overall patterning in *l(1)mys* eye clones must involve cell adhesion events not dependent on PS integrins. Other molecules, such as cadherins (Takeichi, 1988), immunoglobulin-related adhesion molecules such as N-CAM (Cunningham *et al.* 1987) or fasciclins (Patel *et al.* 1987; Zinn *et al.* 1988), and possibly other integrins with β subunits distinct from that encoded by *l(1)mys* may be involved. The same could be true for early developmental processes, such as germband extension. Dual involvement of two subfamilies of integrins in a given biological process has precedent in the case of fibroblast adhesion (Singer *et al.* 1988; Dejana *et al.* 1988), as does the dual involvement of integrins and cell–cell adhesion molecules in the case of neurite outgrowth (Tomaselli *et al.* 1988). In both these instances, ablation of both classes of cooperating adhesive receptors is necessary to block the biological process. Further work will be necessary to investigate potential cooperation between PS integrins and other adhesive receptor systems, perhaps including other integrins.

In summary, the data discussed above demonstrate requirements for PS integrins in several parts of the early embryo and especially in ventral structures. They are also required for the normal development of the wing and the eye. The defects occurring in the absence of PS integrins can all be interpreted in terms of loss of adhesion of a cell layer and of individual cells, consistent with the suspected functions of integrins. However, other adhesions are completely normal in the absence of PS integrins and must rely on other molecules.

We thank Eric Wieschaus, Ted Wright and the stock center at Bowling Green for *Drosophila* stocks, Susan Haynes and Igor Dawid for the *l(1)mys* genomic clone, Kai Zinn and Corey Goodman for the cDNA library and Mary Lou Pardue and Jim Garbe for providing embryos and advice on RNA preparation. We also wish to thank Norbert Perrimon for his useful comments and for the use of his sectioning equipment and Linda Ambrosio for all her technical help, information and encouragement. We are grateful to Ruth Lehmann, Terry Orr-Weaver, Hermann Steller, Elizabeth George, Gene Huh, Pamela Norton and Larry Zusman for their helpful comments on this manuscript and Sue Bergin, John Cohan, Spenser Ladd and David Smouse for their photographic assistance and general support. This work was supported by the Howard Hughes Medical Institute.

References

AYME, A. AND TISSIERES, A. (1985). Locus 67B of *Drosophila melanogaster* contains seven, not four closely related heat shock genes. *EMBO J.* **4**, 2949–2954.

BOGAERT, T., BROWN, N. AND WILCOX, M. (1987). The *Drosophila* PS2 antigen is an invertebrate integrin that, like the fibronectin receptor, becomes localized to muscle attachments. *Cell* **51**, 929–940.

BROWER, D. L. (1985). The sequential compartmentalization of *Drosophila* segments revisited. *Cell* **41**, 361–364.

BROWER, D. L., PIOVANT, M. AND REGER, L. A. (1985). Developmental analysis of *Drosophila* position-specific antigens. *Devl Biol.* **108**, 120–130.

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 disks. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7485–7489.

BUCK, C. A. AND HORWITZ, A. F. (1987). Cell surface receptors for extracellular matrix molecules. *Ann. Rev. Cell Biol.* **3**, 179–205.

CAMPOS-ORTEGA, J. A. AND HARTENSTEIN, V. (1985). *The Embryonic Development of* Drosophila melanogaster. Springer Verlag.

CHERESH, D. A., SMITH, J. W., COOPER, H. M. AND QUARANTA, V. (1989). A novel vitronectin receptor integrin ($\alpha_v\beta_x$) is responsible for distinct adhesive properties of carcinoma cells. *Cell* **57**, 59–69.

CUNNINGHAM, B. A., HEMPERLY, J. J., MURRAY, B. A., PREDIGER, E. A., BRACKENBURY, R. AND EDELMAN, G. M. (1987). Neural cell adhesion molecule: structure, immunoglobin-like domains, cell surface modulation and alternative RNA splicing. *Science* **236**, 799–806.

DEJANA, E., COLELLA, S., CONFORTI, G., ABBADINI, M., GABOLI, M. AND MARCHISO, P. C. (1988). Fibronectin and vitronectin regulate the organization of their respective Arg-Gly-Asp adhesion receptors in cultured human endothelial cells. *J. Cell Biol.* **107**, 1215–1223.

DESIMONE, D. W. AND HYNES, R. O. (1988). *Xenopus laevis* integrins. *J. biol. Chem.* **263**, 5333–5340.

DIGAN, M. E., HAYNES, S. R., MOZER, B. A., DAVID, I. B., FORQUIGNON, F. AND GANS, M. (1986). Genetic and molecular analysis of *fs(1)h*, a maternal effect homeotic gene in *Drosophila*. *Devl Biol.* **114**, 161–169.

FFRENCH-CONSTANT, C. AND HYNES, R. O. (1988). Patterns of fibronectin gene expression and splicing during cell migration in chicken embryos. *Development* **104**, 368–382.

FISCHBACH, K. F. AND TECHNAU, G. (1984). Cell degeneration in the developing optic lobes of the *sine oculis* and *small optic-lobes* mutants of *Drosophila melanogaster*. *Devl Biol.* **104**, 219–239.

FOE, V. E. AND ALBERTS, B. M. (1983). Studies of the nuclear and cytoplasmic behaviour during the five mitotic cycles that precede gastrulation in *Drosophila* embryogenesis. *J. Cell Sci.* **61**, 31–70.

FRANCESCINI, N. (1975). Sampling of the visual environment by the compound eye of the fly: Fundamentals and applications. In *Photoreceptor Optics* (ed. Synder, A. W. and Menzel, R.). pp. 98–125.

GARCIA-BELLIDO, A. (1977). Inductive mechanisms in the process of vein formation in *Drosophila*. *Wilhelm Roux's Arch. devl Biol.* **182**, 93–106.

GARCIA-BELLIDO, A., RIPOLL, P. AND MORATA, G. (1973).

Developmental compartmentalization of the wing disc of *Drosophila*. *Nature New Biol.* **245**, 251–253.

GARCIA-BELLIDO, A., RIPOLL, P. AND MORATA, G. (1976). Developmental compartmentalization in the dorsal mesothoracic disc of *Drosophila*. *Devl Biol.* **98**, 132–147.

GARCIA-BELLIDO, A. AND ROBBINS, L. G. (1983). The viability of female germline cells homozygous for zygotic lethals in *Drosophila melanogaster*. *Mol. gen. Genet.* **192**, 253–263.

HALL, J., GELBART, W. AND KANKEL, D. (1976). Mosaic systems. In *The Genetics and Biology of Drosophila*, vol. 1, (eds. M. Ashburner and E. Novitsky), pp. 265–314.

HEMLER, M. F., CAROUSE, C. AND SONNENBERG, A. (1989). Association of the VLA α^6 subunit with a novel protein. *J. biol. Chem.* **264**, 6529–6535.

HINTON, C. W. (1955). The behaviour of an unstable ring-chromosome of *Drosophila melanogaster*. *Genetics* **40**, 951–961.

HYNES, R. O. (1987). Integrins: a family of cell surface receptors. *Cell* **48**, 549–554.

INGHAM, P. W., HOWARD, K. K. AND ISH-HOROWICZ, D. (1985). Transcription pattern of the *Drosophila* segmentation gene *hairy*. *Nature* **318**, 439–445.

JIMENEZ, F. AND CAMPOS-ORTEGA, J. A. (1982). Maternal effects of zygotic mutants affecting early neurogenesis in *Drosophila*. *Wilhelm Roux's Arch. devl. Biol.* **191**, 191–201.

KAJIJI, S., TAMURA, R. N. AND QUARANTA, V. (1989). A novel integrin ($\alpha_E\beta_4$) from human epithelial cells suggests a fourth family of integrin adhesion receptors. *EMBO J.* **8**, 673–680.

LEPTIN, M., AEBERSOLD, R. AND WILCOX, M. (1987). *Drosophila* position-specific antigens resemble the vertebrate fibronectin-receptor family. *EMBO J.* **6**, 1037–1043.

LEPTIN, M., BOGAERT, T., LEHMANN, R. AND WILCOX, M. (1989). The function of PS integrins during *Drosophila* embryogenesis. *Cell* **56**, 401–408.

LINDSLEY, D. L. AND GRELL, E. H. (1968). *Genetic Variations of* Drosophila melanogaster. Carnegie Inst. Washington Publ. 627.

MACKRELL, A. J., BLUMBERG, B., HAYNES, S. R. AND FESSLER, J. H. (1988). The *lethal myospheroid* gene of *Drosophila* encodes a membrane protein homologous to vertebrate integrin β subunits. *Proc. natn. Acad. Sci. U.S.A.* **85**, 2633–2637.

MAHOWALD, A. P., CAULTON, J. H. AND GEHRING, W. J. (1979). Ultrastructural studies of oocytes and embryos derived from female flies carrying the *grandchildless* mutation in *Drosophila subobscura*. *Devl Biol.* **69**, 118–132.

MARCANTONIO, E. E. AND HYNES, R. O. (1988). Antibodies to the conserved cytoplasmic domain of the integrin β1 subunit react with proteins in vertebrates, invertebrates and fungi. *J. Cell Biol.* **106**, 1765–1772.

MEINERTZHAGEN, I. A. (1973). Development of the compound eye and optic lobe of insects. In *Developmental Neurobiology of Arthropods*, (ed. D. Young) pp. 51–104.

MERRIAM, J. R. AND DUFFY, C. (1972). First multiple seven now contains sn^{x2} better balancing. *Drosophila Inform. Serv.* **48**, 43.

MEYEROWITZ, E. M. AND KANKEL, D. R. (1978). A genetic analysis of visual system development in *Drosophila melanogaster*. *Devl Biol.* **62**, 112–142.

MILNER, M. J. AND MUIR, J. (1987). The cell biology of *Drosophila* wing metamorphosis in vitro. *Roux's Arch. devl Biol.* **195**, 63–73.

MOGENSEN, M. M. AND TUCKER, J. B. (1988). Intermicrotubular actin filaments in the transalar cytoskeletal arrays of *Drosophila*. *J. Cell Sci.* **91**, 431–438.

NEWMAN, S. M. AND WRIGHT, T. R. (1981). Histological and ultrastructural analysis of developmental defects produced by the mutation *lethal (1) myospheroid* in *Drosophila melanogaster*. *Devl Biol.* **86**, 393–402.

PATEL, N. H., SNOW, P. M. AND GOODMAN, C. S. (1987). Characterization and cloning of Fasciclin III: a glycoprotein expressed on a subset of neurons and axon pathways in *Drosophila*. *Cell* **48**, 975–988.

PERRIMON, N., ENGSTROM, L. AND MAHOWALD, A. P. (1984). The effects of zygotic lethal mutations on female germline functions in *Drosophila*. *Devl Biol.* **105**, 404–414.

READY, D. F., HANSON, T. E. AND BENZER, S. (1976). Development of *Drosophila* retina, a neurocrystalline lattice. *Devl Biol.* **53**, 217–240.

READY, D. F., TOMLINSON, A. AND LEBOVITZ, R. M. (1986). Building an ommatidium: geometry and genes. In *Development Of Order In The Visual System*, (eds. Hilfer, S. R. & Sheffield, J. B.). pp. 97–125.

RUOSLAHTI, E. (1988). Fibronectin and its receptors. *Ann. Rev Biochem.* **57**, 375–413.

RUOSLAHTI, E. AND PIERSCHBACHER, M. D. (1987). New perspectives in cell adhesion: RGD and integrins. *Science* **238**, 491–497.

SINGER, I. I., SCOTT, S., KAWKA, D. W., KAZAZIS, D. M., GAILIT, J. AND RUOSLAHTI, E. (1988). Cell surface distribution of fibronectin and vitronectin receptors depends on substrate composition and extracellular matrix accumulation. *J. Cell Biol.* **106**, 2171–2182.

SONNENBERG, A., HOGERVORST, F., OSTEROP, A. AND VELTMAN, F. E. M. (1988). Laminin receptor on platelets is the integrin VLA-6. *Nature, Lond.* **336**, 487–489.

SZABAD, J., SCHUBACH, T. AND WIESCHAUS, E. (1979). Cell lineage and development in the larval epidermis of *Drosophila melanogaster*. *Devl Biol.* **73**, 256–271.

TAKEICHI, M. (1988). The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. *Development* **102**, 639–655.

TOMASELLI, K. J., NEUGEBAUER, K. M., BIXBY, J. L., LILIEN, J. AND REICHARDT, L. F. (1988). N-cadherin and integrins: two receptor systems that mediate neuronal process outgrowth on astrocyte surfaces. *Neuron* **1**, 33–43.

TOMLINSON, A. (1988). Cellular interactions in the developing *Drosophila* eye. *Development* **104**, 183–193.

TOMLINSON, A. AND READY, D. F. (1986). *Sevenless*: A cell specific homeotic mutation of the *Drosophila* eye. *Science* **231**, 400–402.

TOMLINSON, A. AND READY, D. F. (1987). Cell fate in the *Drosophila* ommatidium. *Devl Biol.* **123**, 264–275.

WADDINGTON, C. H. (1940). The genetic control of wing development in *Drosophila*. *J. Genet.* **41**, 75–139.

WIESCHAUS, E. AND NOELL, E. (1986). Specificity of embryonic lethal mutations in *Drosophila* analyzed in germline clones. *Roux's Arch. devl Biol.* **195**, 63–73.

WIESCHAUS, E., NUSSLEIN-VOLHARD, C. AND JURGENS, G. (1984). Mutations affecting the pattern of the larval cuticle in *D. melanogaster*. III. Zygotic loci on the X-chromosome. *Wilhelm Roux's Arch. devl Biol.* **193**, 296–307.

WILCOX, M., BROWER, D. L. AND SMITH, R. J. (1981). A position-specific cell-surface antigen in the *Drosophila* wing imaginal disc. *Cell* **25**, 159–164.

WILCOX, M., BROWN, N., PIOVANT, M., SMITH, R. J. AND WHITE, R. A. H. (1984). The *Drosophila* position-specific antigens are a family of cell surface glycoprotein complexes. *EMBO J.* **3**, 2307–2313.

WILCOX, M., DIANTONIO, A. AND LEPTIN, M. (1989). The function of PS Integrins in *Drosophila* wing morphogenesis. *Development* **107**, 891–898.

WILCOX, M. AND LEPTIN, M. (1985). Tissue-specific modulation of a set of related cell surface antigens in *Drosophila*. *Nature* **316**, 351–354.

WRIGHT, T. R. F. (1960). The phenogenetics of the embryonic mutant, *lethal (1) myospheroid*, in *Drosophila melanogaster*. *J. exp. Zool.* **143**, 77–79.

ZINN, K., MCALLISTER, L. AND GOODMAN, C. S. (1988). Sequence analysis and neuronal expression of Fasciclin I in grasshopper and *Drosophila*. *Cell* **53**, 577–587.

ZUSMAN, S. B. AND WIESCHAUS, E. F. (1985). Requirements for zygotic gene activity during gastrulation in *Drosophila melanogaster*. *Devl Biol.* **111**, 359–371.

ZUSMAN, S. B. AND WIESCHAUS, E. F. (1987). A cellular marker system and mosaic patterns during early embryonic development in *Drosophila melanogaster*. *Genetics* **115**, 725–736.

(*Accepted 6 December 1989*)

Note added in proof

While this paper was under review, Brower, D. L. and Jaffe, S. M. reported wing blisters caused by *l(1)mys* somatic clones and *inflated* mutations. *Nature* **342**, 285–287 (1989).