Genetic Analysis of the Drosophila α_{PS2} Integrin Subunit Reveals Discrete Adhesive, Morphogenetic and Sarcomeric Functions

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ABSTRACT

The integrin family of cell surface receptors mediates cell-substrate and cell-to-cell adhesion and transmits intracellular signals. In Drosophila there is good evidence for an adhesive role of integrins, but evidence for integrin signalling has remained elusive. Each integrin is an αβ heterodimer, and the Drosophila β_{PS} subunit forms at least two integrins by association with different α subunits: $\alpha_{PS1}\beta_{PS}$ (PS1) and $\alpha_{PS2}\beta_{PS}$ (PS2). The complex pattern of PS2 integrin expression includes, but is more extensive than, the sites where PS2 has a known requirement. In order to investigate whether PS2 integrin is required at these additional sites and/or has functions besides mediating adhesion, a comprehensive genetic analysis of *inflated*, the gene that encodes α_{PS2}, was performed. We isolated 35 new *inflated* alleles, and obtained 10 alleles from our colleagues. The majority of alleles are amorphs (36/45) or hypomorphs (4/45), but five alleles that affect specific developmental processes were identified. Interallelic complementation between these alleles suggests that some may affect distinct functional domains of the α_{PS2} protein, which specify particular interactions that promote adhesion or signalling. One new allele reveals that the PS2 integrin is required for the development of the adult halteres and legs as well as the wing.

A feature of many cell surface receptors is the ability to elicit multiple responses at different times or places within the organism. This diversity of function is reflected by the genetic complexity of the loci that encode these receptors. In Drosophila this is particularly well documented for the loci encoding Notch and the epidermal growth factor (EGF) receptor, which are both complex genes displaying interallelic complementation (*e.g.*, Foster 1975; Portin 1975; Clifford and Schüpbach 1989). Such complementation can occur when the gene product has multiple functions and particular alleles eliminate or alter single functions; an individual transheterozygous for alleles that mutate different functions will still retain wild-type function for both activities and therefore appear to be wild type. The characterization of the molecular lesions associated with particular classes of Notch and EGF receptor alleles has greatly contributed to our understanding of how the different segments of these proteins contribute to the function of these receptors (Hartley *et al.* 1987; Kelley *et al.* 1987; Xu *et al.* 1990; Clifford and Schüpbach 1994). In this work we have examined whether a similar genetic complexity is found for the PS2 integrin cell surface receptor.

Integrins are a family of cell surface adhesion molecules found in both vertebrates and invertebrates (reviewed in Hynes 1992). Integrins were first identified by their ability to promote cell adhesion to the extracellular matrix and to other cells. Each integrin is a heterodimer composed of a single α subunit noncovalently linked to a single β subunit. The heterodimers form during synthesis, and the subunits must form a heterodimer to become transported from the endoplasmic reticulum to the plasma membrane, suggesting that single subunits have no activity (Cheresh and Spiro 1987; Kishimoto *et al.* 1987; Leptin *et al.* 1989). Both subunits contribute to extracellular ligand binding and, therefore, the specific αβ combination defines the ligand(s) bound by a particular integrin, which include both secreted extracellular matrix proteins and other types of cell surface protein (reviewed in Hynes 1992). The ability of integrins to bind their ligands can be modulated by the cell through an increase in the affinity of integrins for their ligands, which appears to occur by a combination of integrin aggregation and conformational changes. In addition, there is increasing evidence that integrin binding to extracellular ligands results in the transduction of signals within the cells, such as changes in tyrosine phosphorylation (Clark and Brugge 1995). These signals may not only play a role in the reorganization of the cytoskeleton, but may also lead to changes in gene expression. As a link between the extracellular environment and the inside of the cell, integrins also interact with cytoplasmic proteins via their cytoplasmic tails, which are unusually short (15–45 amino acids *vs.* 600–1200 amino acids in the extracellular domains). Current data suggests that the α cytoplasmic tail regu-

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lates the accessibility of the β cytoplasmic tail for interaction with a variety of proteins, both cytoskeletal proteins such as talin and α-actinin, and signaling molecules such as focal-adhesion kinase and integrin-linked kinase (Craig and Johnson 1996). While a large number of molecules colocalize with integrins at sites of function, such as focal adhesions where the cell attaches to an extracellular substrate, it is not yet clear which molecules are normally linked directly to the integrins.

In Drosophila five integrin subunits have been identified to date: the β_{PS} subunit, which forms heterodimers with three α subunits, α_{PS1}, α_{PS2} and α_{PS3}; and a novel β subunit, β_v, whose α subunit partner has yet to be characterized (Brown 1993; Gotwals *et al.* 1994). Genetic loci have been identified for three of the integrin subunits: the β_{PS} subunit is encoded by the *myospheroid* (*mys*) locus (MacKrell *et al.* 1988; Leptin *et al.* 1989), α_{PS1} by the *multiple edematous wings* (*mew*) locus (Brower *et al.* 1995), and α_{PS2} by the *inflated (if)* locus (Brower and Jaffe 1989; Wilcox *et al.* 1989; Brabant and Brower 1993; Brown 1994). Amorphic mutations at each of these loci cause lethality when hemizygous or homozygous, and to date all mutations at these loci are recessive. As expected, the phenotype of an amorphic *mys* allele, which lacks the function of all three integrins, is more severe than the phenotypes of mutations in single α subunits (Wright 1960; Newman and Wright 1981; Brabant and Brower 1993; Brown 1994; Brower *et al.* 1995; Roote and Zusman 1995). The phenotype of embryos mutant for *mys* has several features: the somatic muscles detach and round up; the midgut fails to elongate, and the gastric caecae and proventriculus are defective; the nerve cord fails to condense; and dorsal closure is defective, resulting in a dorsal hole in the cuticle. Embryos mutant for the α_{PS2} subunit (*if*) have a phenotype that is similar to *mys*, except that the onset of muscle detachment is later, the midgut phenotype is milder, and dorsal closure occurs normally. In contrast, amorphic mutations in the α_{PS1} subunit (*mew*) do not cause complete embryonic lethality, and most of the mutant embryos hatch with a defective gut. In the embryo, the two α subunits have complementary patterns of expression, with α_{PS2} restricted to the mesoderm and α_{PS1} expressed in the epidermis and endoderm (Bogaert *et al.* 1987; Leptin *et al.* 1989; Wehrli *et al.* 1993).

Our current interpretation of the phenotypes described above is that the integrins are required for adhesion between the different embryonic cell layers. For example, the $\alpha_{PS2}\beta_{PS}$ integrin is required in the somatic muscles to maintain their attachment to the body-wall epidermis and in the visceral mesoderm to mediate interactions with the midgut endoderm. However, we do not know to what degree the integrins directly mediate adhesion—by adhesion to extracellular matrix or cell surface proteins, or by the more indirect role of signaling to other adhesion molecules. The two integrins $\alpha_{PS1}\beta_{PS}$ (PS1) and $\alpha_{PS2}\beta_{PS}$ (PS2) also show complementary expression in the developing wing (Wilcox *et al.* 1981; Brower *et al.* 1984), with PS1 expressed in the presumptive dorsal surface and PS2 expressed in the ventral surface. Disruption of integrin function in the developing wing, either in animals homozygous for viable integrin mutations or those containing clones of cells in the wing homozygous for lethal amorphic alleles, results in a failure of adhesion between the two layers of the wing blade and formation of a wing blister (Brower and Jaffe 1989; Wilcox *et al.* 1989; Zusman *et al.* 1990; Brabant and Brower 1993; Brower *et al.* 1995). The PS2 integrin is not only expressed in the wing imaginal disc; it is also expressed in specific regions of the haltere, eye-antennal, and leg imaginal discs (Brower *et al.* 1985). However, defects in these tissues have not yet been characterized.

Because the β_{PS} subunit associates with different α subunits, it seems likely that one could generate alleles of this locus that specifically disrupt a subset of its functions; however, it is not clear that this would be true for a single α subunit. Like Notch and the EGF receptor the α_{PS2} subunit is also a large protein (1263 amino acids) that is modular in structure; in common with other α subunits, it contains seven repeated domains that have recently been proposed to form a β-propeller structure (Springer 1997). Prior to this work only two types of allele of *inflated*, which encodes the α_{PS2} subunit, had been recovered: spontaneous adult viable alleles and embryonic lethal amorphic alleles (Weinstein 1918; Curry 1939; Wilcox *et al.* 1989; Brabant and Brower 1993; Brown 1994). We have therefore generated new mutations in the *inflated* locus with two aims in mind. First, can we find evidence for more than one type of function for this cell surface receptor, which would be consistent with the modular structure of the α subunit and the proposed functions of integrins in both adhesion and signaling. Such multiple functions would be revealed by the isolation of alleles that show different subsets of the null phenotype and interallelic complementation; this would also reveal that *inflated* is a complex gene. Second, can we find mutants that reveal functions of the PS2 integrin in other adult structures, which might be predicted from the specific patterns of expression of this integrin in the other imaginal discs. Through the isolation of 35 *inflated* alleles we show that the PS2 integrin does have additional functions during development and that *inflated* is a complex locus.

MATERIALS AND METHODS

The Tubingen fly food recipe (Steward and Nüsslein-Volhard 1986) was used in all experiments. New *inflated* alleles are shown in Table 1; other mutations are described in Lindsley and Zimm (1992), with the exception of the newly characterized *enhancer of inflated, e(if)1*, which we found in our $cn^1; ry^{506}$ stock, and has been mapped to 2L. This mutation enhances the penetrance of the if^3 phenotype, rather than its

severity. The various screens we performed for new *inflated* alleles are described in results, and mutagenesis with 25–50 mm EMS in 1% sucrose was as described in Grigliatti (1986). The F1 progeny were either directly screened or individually crossed to three virgin attached *X* females that were obtained from the stock $shi^{ts}/C(1)DX$, *y w f*/*Y*, following a shift to 28° to kill the males and ease the collection of virgin females. In one F1 screen we mutagenized with 3.3 mm ENU in 10% acetic acid. In the F1 screen using *e(if)1*, we used 25 mm EMS in 1% sucrose and 0.002% acetic acid to provide a more reproducible pH in the EMS solution. We have previously described an F1 screen using γ-ray mutagenesis at a dose of 4000 r (Brown 1994). For the FLP-FRT screen, the flies were mutagenized with X rays at a dose of 4500 r. This screen will be described in detail elsewhere (E. P. Walsh and N. H. Brown, unpublished results).

Phenotypic analysis: In order to be able to unambiguously determine the genotype of the mutant *inflated* male embryos, *if*/*Y*, we crossed y^1 onto those alleles that did not have it, and balanced the *if* alleles with an *FM6* balancer chromosome containing an insertion of a y^+ *P*-element construct, $FM6y^+$ (Martín-Bermudo *et al.* 1997). When females that are $y\ if^{B4}/FM6y^+$ are crossed to y^+ males, only the *inflated* mutant males will be *yellow*, which can be distinguished at the end of embryogenesis by the pale mouth hooks and denticle belts. We characterized the *inflated* muscle phenotype in two ways: by examination of embryos under polarized light as described in Drysdale *et al.* (1993), and by staining with embryos with

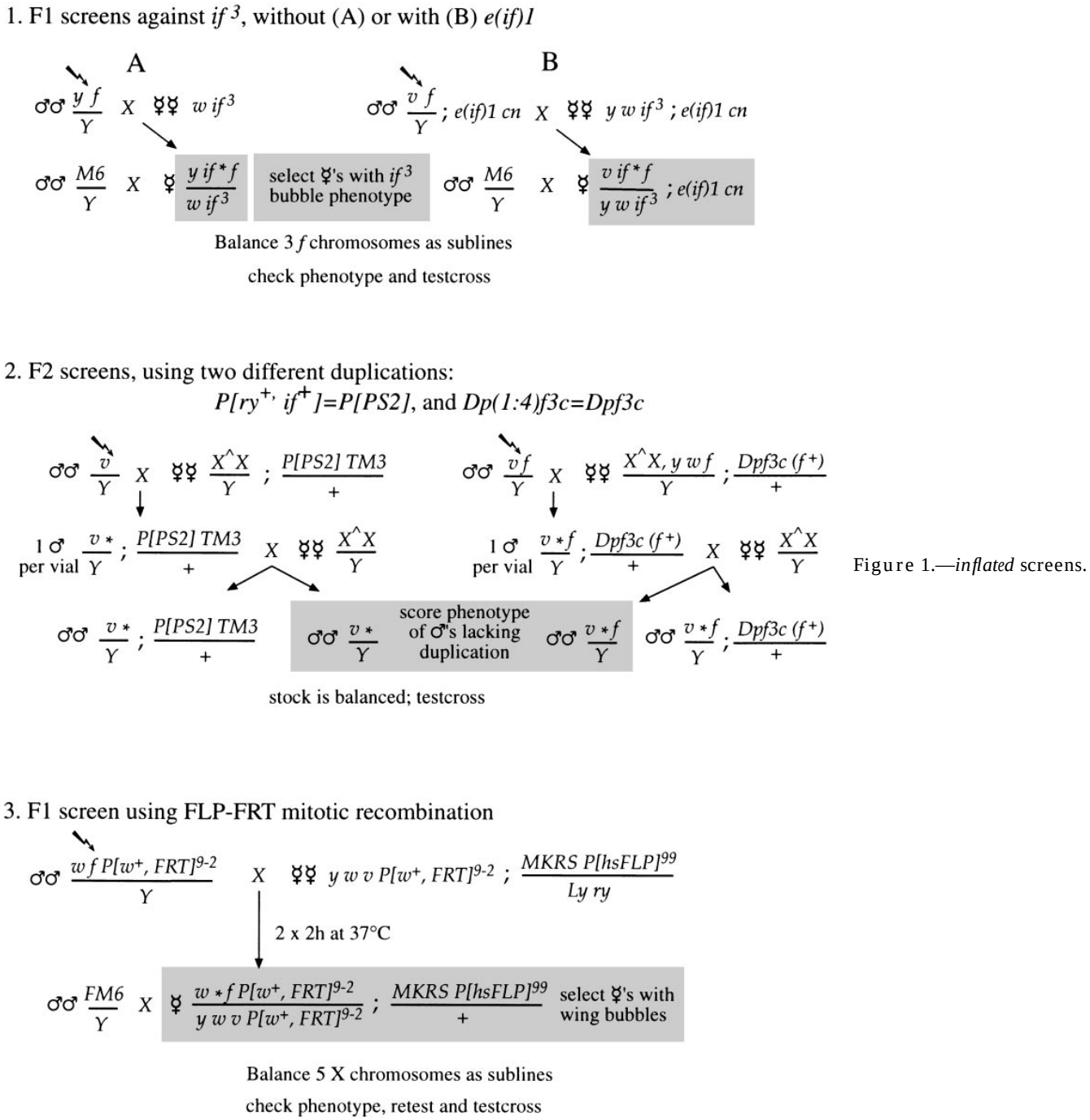

Figure 1.—*inflated* screens.

phalloidin conjugated to rhodamine. Staining of embryos and imaginal discs with anti-PS2 antibodies was performed using standard conditions (*e.g.*, Bogaert *et al.* 1987; Martín-Bermudo *et al.* 1997). A polyclonal antisera was prepared commercially against a peptide of the COOH terminal 15 amino acids of α_{PS2}. To examine the gut phenotype and the extent of nerve-cord condensation, embryos were dissected with tungsten needles, the midguts and nerve cords mounted in phosphate-buffered saline, and examined with Nomarski optics (Carl Zeiss, Thornwood, NY). To examine the sarcomeric phenotype of the muscles at the end of stage 17, embryos were dissected, fixed with 4% formaldehyde, and stained with phalloidin conjugated to rhodamine. These embryos were then examined by confocal microscopy (MRC 1000; Bio-Rad, Richmond, CA). Micrographs were scanned using a Nikon (Garden City, NY) Coolscan, and the scanned or confocal images were assembled in Photoshop 3.0 (Adobe Systems, Mountain View, CA) and labeled with FreeHand 5.5 (Macromedia, San Francisco).

RESULTS

Isolation of new *inflated* alleles: To determine whether *inflated* is a complex gene, it was initially essential to isolate additional mutant alleles. We chose to use a variety of different screening approaches in case a single style of screen would miss certain types of mutations, which did turn out to be the case. The first approach used was an F1 screen for mutations that fail to complement the viable if^3 allele, an example of which is shown in Figure 1. Flies heterozygous for if^3/Df are fully viable and fertile, and contain a large centrally positioned bubble in one or both wings. Two EMS alleles isolated by this method have already been described (Brabant and Brower 1993), as have three γ-ray alleles (Brown 1994). We isolated 3 additional alleles from a screen of 30,000 F1 females that were the progeny of ENU mutagenized males, and 2 from 30,000 F1 females arising from EMS mutagenized males (see Table 1). This frequency of new mutations was low, and lower than the rate of mutation of the *white* or *yellow* locus, which we also scored during the F1 screen (data not shown). As confirmed by subsequent screens, the low frequency of point mutations recovered by this screen is because of a combination of two factors: the incomplete penetrance of the if^3 phenotype and the fact that some *inflated* alleles complement the if^3 allele. We tried to circumvent these problems in four ways: by screening over the stronger semi-lethal allele if^{V2}, by performing F2 screens; by utilising a fortuitously identified enhancer of *inflated*, which increases the penetrance of if^3; and by performing FLP-FRT-induced mosaic screens.

In our previous γ-ray screen for *inflated* alleles we recovered a semiviable allele, if^{V2}, with a very strong adult phenotype described in more detail below. Cytological analysis showed that this allele is a translocation between the *X* and the fourth chromosome (15A;101F, data not shown; *inflated* maps at 15A4). The break in the fourth chromosome does not appear to have generated a mutation with a visible phenotype, as we see no difference in the phenotype of $T(1:4)if^{V2}/Y$; + males compared with $T(1:4)if^{V2}/T(1:4)if^{V2}$ females. This stronger allele was used in an F1 screen similar to the one described above (M. D. Martín-Bermudo and N. H. Brown, unpublished results) and one new allele was recovered.

We performed F2 lethal screens for new *inflated* alleles by screening for mutations in the intervals covered by one or other of two different duplications of the *inflated* locus. One duplication is a *P*-element construct, P[PS2, ry+], containing 39 kb of genomic DNA encompassing the *inflated* gene, inserted on a *TM3*, *Sb* balancer chromosome (Brown 1994). This construct rescues null *inflated* alleles (Brown 1994); however, because it was possible that it would not cover every *inflated* allele, we used a larger duplication for the bulk of the F2 screen. We used *Dp(1:4)f3c* [also known as *Dp(1)80f3c* and *Df(1)80f3c*], which extends 110 kb downstream of the *inflated* gene and extends a division upstream (14E; 16A1–2; Falk *et al.* 1984). This duplication fully complements a deletion of the entire *inflated* transcription unit, *Df(1)rif* (Brown 1994), and therefore must complement all loss-of-function *inflated* alleles. The two screens are shown in Figure 1; five *inflated* alleles were recovered from 2529 *X* chromosomes screened against P[PS2, ry+] and seven from 11,945 *X* chromosomes screened against *Dp(1;4)f3c*.

Another approach made use of a fortuitously identified enhancer of *inflated*, *e(if)1*, which increases the penetrance of the if^3 phenotype. Stocks containing this mutation were used in an F1 screen (Figure 1) and 10 new *inflated* alleles were isolated from a screen of less than 15,000 F1 females. The effectiveness of this enhancer is highlighted by the fact that one of the alleles we isolated in this screen almost fully complements if^3 in the absence of *e(if)1* (Table 2).

The final approach was to use the FLP-FRT system for mitotic recombination (Golic 1991; Xu and Rubin 1993) to generate mutant clones in the F1 generation. Clones homozygous for an embryonic lethal *inflated* allele in the wing cause bubbles if they are on the ventral surface (Brabant and Brower 1993). Therefore, one can use the FLP-FRT system to isolate new alleles of *inflated* and other genes involved in the adhesion of the two surfaces of the wing by screening for mutations that give bubbles in clones. A screen of this type on the *X* chromosome was performed (E. P. Walsh and N. H. Brown, unpublished results; see Figure 1), which produced four *inflated* alleles. Similar screens were performed by Brower *et al.* (1995) who have sent us the six *inflated* alleles that they recovered.

With these different screens we have isolated 35 *inflated* alleles; combining these with the 4 preexisting alleles and 6 alleles donated by our colleagues provides a bank of 45 alleles (Table 1) for our genetic analysis of the *inflated* locus.

***inflated* is a complex locus:** Through phenotypic analysis of single alleles and by complementation-testing of

TABLE 1

***inflated* alleles**

Allele	Class	Screen	Mutagen	Discoverer	Reference
if^{3}	IV		spontaneous	1	a
if^{k27e}	0			2	b
if^{A7}	0	F1 over *if*^{3}	EMS	3	c
if^{B2}	0	F1 over *if*^{3}	EMS	3	c
if^{B4}	0	F1 over *if*^{3}	γ rays	4	d
if^{B6}	0	F1 over *if*^{3}	γ rays	4	d
if^{V2}	IV	F1 over *if*^{3}	γ rays	4	e
if^{C1A}	0	F1 over *if*^{3}	ENU	5	e
if^{C2B}	III	F1 over *if*^{3}	ENU	5	e
if^{C3C}	0	F1 over *if*^{3}	ENU	5	e
if^{C4D}	0	F1 over *if*^{3}	EMS	5	e
if^{C5E}	0	F1 over *if*^{3}	EMS	5	e
if^{L13}	0	F1 over *if*^{V2}	MES	6	e
if^{2B1}	III	F1 over *if*^{3}; *e(if)1*	EMS	4	e
if^{2B2}	0	F1 over *if*^{3}; *e(if)1*	EMS	4	e
if^{2B3}	0	F1 over *if*^{3}; *e(if)1*	EMS	4	e
if^{N18R}	0	F1 over *if*^{3}; *e(if)1*	EMS	4	e
if^{N19S}	0	F1 over *if*^{3}; *e(if)1*	EMS	4	e
if^{02B}	0	F1 over *if*^{3}; *e(if)1*	EMS	4	e
if^{09I}	0	F1 over *if*^{3}; *e(if)1*	EMS	4	e
if^{011K}	0	F1 over *if*^{3}; *e(if)1*	EMS	4	e
if^{017Q}	0	F1 over *if*^{3}; *e(if)1*	EMS	4	e
if^{Q2B}	0	F1 over *if*^{3}; *e(if)1*	EMS	4	e
if^{SEF}	II	F2 against *P[PS2]*	EMS	7	e
if$^{4.5}$	0	F2 against *P[PS2]*	EMS	7	e
if^{A2}	0	F2 against *P[PS2]*	EMS	7	e
if^{M2}	0	F2 against *P[PS2]*	EMS	7	e
if^{O2}	0	F2 against *P[PS2]*	EMS	7	e
if^{9}	0	F2 against *Dpf3c*	EMS	7	e
if^{13ts}	I	F2 against *Dpf3c*	EMS	7	e
if^{16}	0	F2 against *Dpf3c*	EMS	7	e
if^{17}	I	F2 against *Dpf3c*	EMS	7	e
if^{21}	I	F2 against *Dpf3c*	EMS	7	e
if^{35}	I	F2 against *Dpf3c*	EMS	7	e
if^{38}	0	F2 against *Dpf3c*	EMS	7	e
if^{K13}	0	FLP-FRT	EMS	8	e
if^{K23}	0	FLP-FRT	EMS	8	e
if^{L6}	0	FLP-FRT	EMS	8	e
if^{L10}	0	FLP-FRT	EMS	8	e
if^{L326}	0	FLP-FRT	EMS	9	e
if^{L648}	0	FLP-FRT	EMS	9	f
if^{6}	0	FLP-FRT	X rays	10	e
if^{8}	0	FLP-FRT	X rays	10	e
if^{26}	0	FLP-FRT	X rays	10	e
if^{64}	0	FLP-FRT	X rays	10	e

Discoverers: 1) Curry (1939), 2) Falk *et al.* (19840, 3) Brabant and Brower (1993), 4) Brown (1994), 5) Brown, Bloor and Duncan (1992), 6) Martín-Bermudo (1993), 7) Bloor (1996), 8) Brower (1995), 9) Zusman (1995), 10) Walsh (1994).

References: a) Curry (1939), b) Falk *et al.* (1984), c) Brabant and Brower (1993), d) Brown (1994), e) the present article, f) Brower *et al.* (1995).

the alleles against each other we have divided the 45 *inflated* alleles into five classes (see Tables 1 and 2). We cannot fit all the alleles into a simple hypomorphic series: there is a "branch" caused by some alleles showing complementary subsets of the null phenotype, and this is confirmed by interallelic complementation between alleles on the two branches. Previous work has shown that amorphic *inflated* alleles are embryonic-lethal when hemi- or homozygous (Brabant and Brower 1993; Brown 1994). The epidermis and the resultant secreted cuticle appear normal in these mutant embryos, but defects in three internal embryonic tissues are observed:

TABLE 2

Interallelic complementation

Class		if^{B4}	if^{C4D}	if^{13ts}	if^{17}	if^{21}	if^{35}	if^{SEF}	if^{C2B}	if^{2B1}	if^{V2}	if^{3}
0	if^{B4}		no	no	no	no	no	no	no	no	no	no
0	if^{C4D}	no		no	no	no	no	no	no	no	no	no
I	if^{13ts}	no	no		no	no	no	no	yes	yes	yes	yes
I	if^{17}	no	no	no		no	no	no	esc	esc	yes	yes
I	if^{21}	no	no	no	no		no	no	esc	esc	yes	yes
I	if^{35}	no	no	no	no	no		no	no	esc	yes	yes
II	if^{SEF}	no	no	no	no	no	no		yes	yes	yes	yes
III	if^{C2B}	no	no	yes	esc	esc	no	yes		no	no	no
III	if^{2B1}	no	no	yes	esc	esc	esc	yes	no		part	yes
IV	if^{V2}	no	no	yes	yes	yes	yes	yes	no	part		no
IV	if^{3}	no	no	yes	yes	yes	yes	yes	no	yes	no	

The number of *if* transheterozygotes that eclose and look wild type was scored relative to the *if*/*FM6* siblings. Alleles were scored as complementing (yes) when the normal-looking transheterozygotes were greater than 50% of the siblings; partially complementing (part) when the transheterozygotes were between 20–50%; escapers (esc) when the transheterozygotes were between 0–20%; noncomplementing (no) when no normal transheterozygotes eclosed (compared to at least 100 siblings).

(1) The somatic muscles detach and round up; (2) gut morphogenesis is defective in that the anterior midgut does not become a slender tube and only two fat gastric caeca are formed rather than the normal four slender caeca; and (3) the ventral nerve cord does not fully condense. The first two defects can be attributed to the loss of *inflated* function in the somatic and visceral muscles, respectively. The third defect may be a result of loss of *inflated* function in the mesodermal neurons, and seems unlikely to be a secondary effect of the muscle detachment because embryos mutant for other loci that also cause severe muscle abnormalities can undergo nerve cord condensation normally (unpublished observations). The only adult phenotype that has been described to date is the separation of the two surfaces of the wing to produce a bubble. This is observed in the viable alleles if^{1} and if^{3}, and when clones homozygous for amorphic *inflated* alleles are produced on the ventral wing surface (Weinstein 1918; Curry 1939; Brower and Jaffe 1989; Wilcox *et al.* 1989; Brabant and Brower 1993).

The majority (36) of the *inflated* alleles isolated are amorphs or strong hypomorphs (class 0). This group of alleles includes the null allele if^{B4}, which is a small deficiency within the gene (Brown 1994). When hemizygous, the other 35 alleles in this group exhibit the embryonic *inflated* amorphic phenotype and are phenotypically indistinguishable from the if^{B4} mutation (as determined by observation of the somatic muscles under polarized light and dissection of the midgut and nerve cord). The ten *inflated* alleles isolated from the clonal screens of the wing all fall into this amorphic class. The remaining amorphic alleles have not been systematically tested for their ability to produce wing bubbles when homozygous mutant clones are generated on the ventral wing surface, but those that have been tested do so (Brabant and Brower 1993; Brower *et al.* 1995; our unpublished observations), and they all fail to complement the wing bubble phenotype of if^{3}.

Four of the new alleles (class I) show weaker phenotypes in all three tissues when compared to the amorphs, although they remain embryonic lethal. All the phenotypes are enhanced over *Df(1)rif* and so we define this class as hypomorphs. The weakest class I allele, if^{13ts}, is temperature sensitive. Three of the new alleles (class II and class III) are phenotypically unusual; we have separated these into two classes that complement each other and, thus, define the "branches" in the allelic series. The single class II allele (if^{SEF}) particularly affects the structure of the sarcomeres within the striated somatic muscles, whereas the class III alleles (if^{C2B}, if^{2B1}) particularly affect the gut. Finally, for simplicity we have put the adult viable alleles into a single group, class IV, even though the new allele if^{V2} is semilethal and gives a much stronger phenotype than if^{3}. Two other published viable alleles would also be members of this group, if^{1} and if^{N} (Weinstein 1918; Lindsley and Zimm 1992), but they appear to be lost.

The different classes of *inflated* alleles show interallelic complementation (Table 2). Both class I and class II alleles fully complement class IV alleles, and the class II allele fully complements the class III alleles. The if^{13ts} class I allele also fully complements class III alleles, but the transheterozygotes between the other class I alleles and the class III alleles generally die, although a few adult escapers are observed. Our working model for these results (taking into account results discussed below) is that the complementation between class II and III alleles arises because these alleles are mutant in separate *inflated* functions. The class II function is not required in the development of the adult epidermis, and therefore this allele also complements the visible pheno-

TABLE 3

Wing phenotypes and genetic interactions of *inflated* alleles

Class	Allele	% of expected *mys*^{XR04}/*if* heterozygotes	Wing phenotype over *if*^{V2}	% of wings with *y if* clone and bubble	Minigene rescue wing phenotype
0	*if*^{B4}	—	bubble	72	bubble
0	*if*^{C1A}	3	bubble		
I	*if*^{17}	23	wt	0	wt
I	*if*^{21}	—	wt	0	wt
I	*if*^{35}	39	wt	—	wt
II	*if*^{SEF}	80	wt	0	wt
III	*if*^{C2B}	89	bubble	53	bubble
III	*if*^{2B1}	100	bubble/wt	4	wt/bubble

types of the class IV alleles. The ability of the class I hypomorphic alleles to complement viable class IV alleles suggests that the level of *inflated* activity in these mutations is sufficient to mediate the adult functions of *inflated*. Our results emphasize that the class of *inflated* mutant one can recover is dependent on the type of screen used to isolate the mutations, because screening for new mutations in the wing will fail to isolate class I and class II alleles as these alleles are able to support normal wing development.

We also examined the extent of the dominant genetic interaction of the different classes of *inflated* alleles with the antimorphic *myospheroid* allele, *mys*^{XR04}. With amorphic *inflated* alleles, 90–100% of *mys*^{XR04} +/+ *if* flies die (Wilcox 1990; Brabant and Brower 1993). One rationale for this is that the α_{PS2} subunit expression is limiting and PS2 integrin heterodimers containing the mutant β_{PS} subunit encoded by the *mys*^{XR04} allele are stable but impaired in function, and, therefore, in the double heterozygote the level of the active PS2 heterodimer will be reduced to one-fourth (Bunch *et al.* 1992). We tested the different classes to find out if they were altered in their interaction compared with amorphic alleles (Table 3). The amorphic allele *if*^{C1A} behaves as expected, with only 3% of the double heterozygotes surviving, and the class I hypomorphic alleles *if*^{17} and *if*^{35} show a partial genetic interaction, with 23% and 33% survival, respectively, suggesting that these alleles produce some active protein and supporting the identification of this class as hypomorphs. The class II and III alleles show almost no genetic interaction with *mys*^{XR04} suggesting that the level of the α_{PS2} subunit is not reduced in these mutations, just particular functions.

Embryonic functions of *inflated*: An examination of the phenotypes of the new classes of *inflated* alleles demonstrates that the *inflated* gene has separate functions in the somatic musculature *vs.* the gut and nerve cord. The class II allele, *if*^{SEF}, is embryonic lethal yet the phenotype is surprisingly mild: the nerve cord is fully condensed (not shown), midgut morphogenesis occurs normally, and the vast majority of muscles remain attached to the epidermis (Figure 2). The somatic muscles in *if*^{SEF} mutant embryos, however, do exhibit a defect in the contractile ultrastructure. Examination by polarized light shows little evidence of sarcomeric structure in these muscles, and staining for filamentous actin with rhodamine-phalloidin reveals that the f-actin fails to become properly organized (Figure 2; see also below). We examined embryos at earlier times during stage 17 with polarized light and did not observe the appearance of striations in *if*^{SEF} mutant embryos, suggesting that the PS2 integrin is required for the formation of muscle sarcomeric structure rather than for its maintainance. The strong waves of muscle contraction that normally accompany hatching from the vitelline membrane and chorion are not observed in these mutants, although some residual muscle function is present, as weak muscle contractions occur if the mutant animal is poked with a needle (not shown). Therefore, the *if*^{SEF} mutant appears to be unable to form normal contractile somatic muscles. When we stained *if*^{SEF} mutant embryos with the PS2hc/2 monoclonal antibody (Bogaert *et al.* 1987), we did not detect any staining; however, we could detect wild-type staining with a polyclonal antisera directed against the C-terminal 15 amino acids of the α_{PS2} subunit (data not shown). This suggests that this mutant alters the conformation of the PS2 integrin (and the PS2hc/2 epitope) rather than its expression.

The muscle phenotype of the *if*^{SEF} allele is not enhanced when placed over a deficiency and the midgut and nerve cord remain wild type in appearance (results not shown), demonstrating that this allele is amorphic for a subset of *inflated* function. Additionally, the muscle phenotype is partially ameliorated when *if*^{SEF} is placed in *trans* with the hypomorphic alleles, demonstrating that the hypomorphs supply some of the function missing in the *if*^{SEF} mutant.

Examination of the phenotype of the class I hypomorphic alleles shows that all embryonic *inflated* activities, including the sarcomeric function of *inflated*, are perturbed in these mutants. Detachment of somatic muscles from the epidermis occurs in embryos mutant for

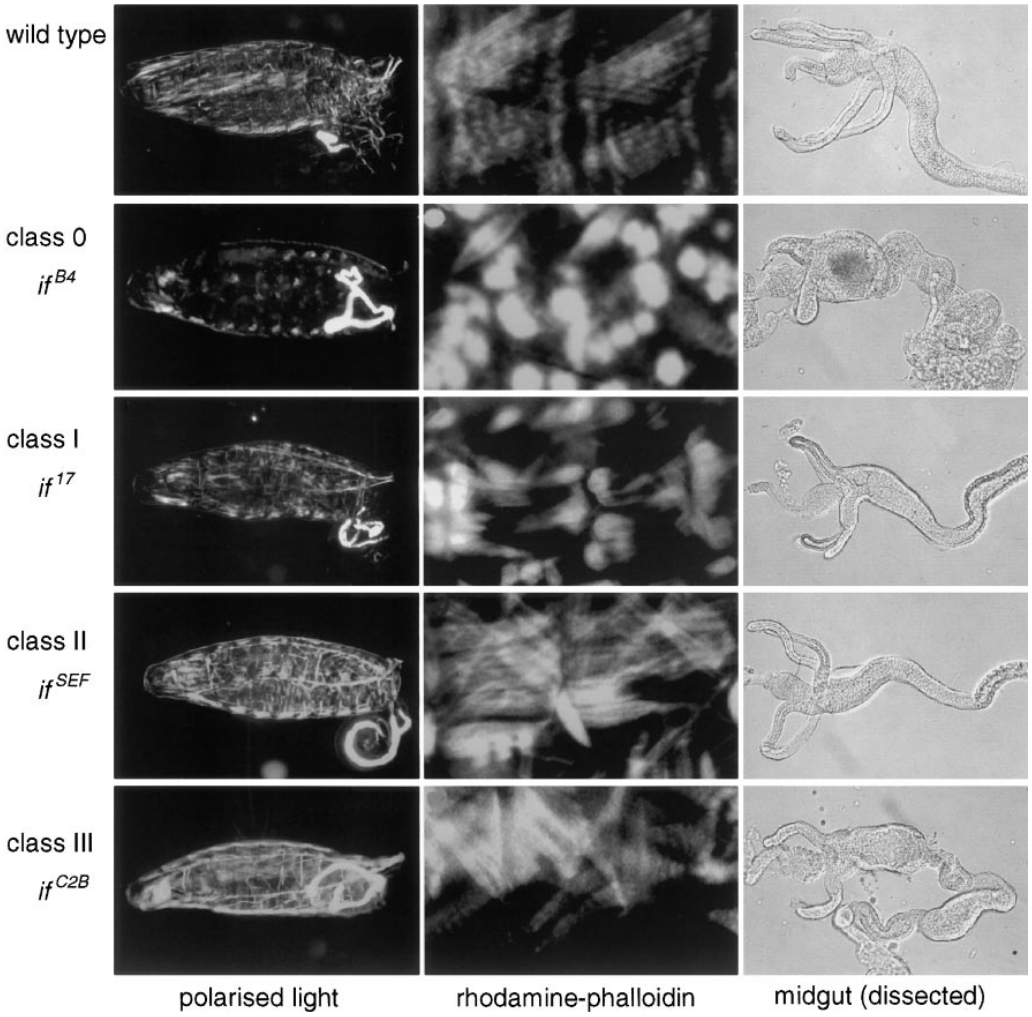

Figure 2.—*inflated* mutant phenotypes. Embryos at the end of embryogenesis (stage 17, 20–24 hr at 25°) that are mutant for the different classes of inflated allele were examined by polarized light, staining for filamentous actin with rhodamine phalloidin, or the guts dissected and examined with Nomarski optics. In each case anterior is to the left.

class I alleles, but in addition many of the muscles that remain attached lack or show disturbed sarcomeric structure (Figure 2). These mutants also display a mild disruption of midgut morphogenesis (Figure 2) and incomplete nerve cord condensation (not shown). The muscle detachment and midgut and nerve cord phenotypes are all increased in severity when class I alleles are placed over the deficiency *Df(1)if* (not shown). Within this class an allelic series ($if^{13ts} < if^{21} < if^{17} < if^{35}$) can be identified based upon observations of mutant stage 17 embryos (not shown).

A significant fraction of embryos mutant for the class III *inflated* alleles if^{C2B} and if^{2B1}, hatch to first instar larvae. Approximately one-fifth of the mutant individuals carrying the if^{C2B} allele hatch, and these larvae slowly become less motile and die over the next 48 hr. if^{C2B} mutant embryos that failed to hatch were examined by polarized light and rhodamine-phalloidin staining (Figure 2 and see below). We observed that the muscles remained attached and had normal sarcomeric structure. In contrast, the midgut fails to elongate and only two fat gastric caecae are formed (Figure 2). Staining of the visceral muscles in the mutant midguts shows that there is some detachment of the visceral muscle layer, but that the sarcomeric structure is not perturbed. Hatched if^{C2B} larvae possess the same phenotypic characteristics as their unhatched counterparts, that is, wild-type muscles and abnormal midguts, and it seems likely that the larval lethality is a result of their inability to feed, but we do not know why some of the mutant embryos fail to hatch. The if^{2B1} allele appears to be a weaker class III allele because a greater proportion of mutant embryos hatch (80%); however, no mutant third instar larvae have been observed (the mutant larvae are marked with *y*; see materials and methods). As with if^{C2B} the lethal if^{2B1} embryos and a proportion of hatched larvae have a defective gut, but normal muscles. However, some if^{2B1} larvae appear to have normal guts and survive longer. These individuals survive until the second instar stage when they start to show some muscle detachments (not shown). The lethal class III embryos also have defective nerve cord condensation (not shown). When these alleles are placed over *Df(1)rif*, the gut and nerve cord phenotypes are enhanced, and rare muscle detachments are observed (not shown). Unfortunately, our attempts to examine the expression of the

PS2 integrin in the midguts of these mutants proved inconclusive, due to the difficulty in getting reproducible staining of the visceral muscles in stage 16 embryos with the PS2 antibodies.

To get a better view of the effect of the different classes of inflated mutant on sarcomeric structure, we dissected class I, II, and III *if* mutant embryos at the end of embryogenesis, stained the muscles and midguts with phalloidin conjugated to rhodamine, and examined them by confocal microscopy. In the somatic muscles of wild-type (not shown) and if^{C2B} embryos (Figure 3C) the actin filaments are aligned and visible as bright stripes and the H bands, containing just myosin filaments, appear as the dark stripes (see Figure 3G). In the if^{SEF} embryos the actin filaments are seen to be continuous strands, with no intervening H bands (Figure 3B). Somatic muscles from the hypomorphic mutation if^{17} show a mixture of striated and nonstriated muscles (Figure 3A), and the defects in the sarcomeric structure can occur in muscles that remain attached. None of the mutants appears to disrupt the sarcomeric structure of the visceral muscles (Figure 3, D–F), although both if^{17} and if^{C2B} affect the integrity of the visceral muscle layer. However, it is clear from the phalloidin staining and previous ultrastructural examinations (Sandborn *et al.* 1967; Goldstein and Burdette 1971) that the sarcomeric structure of visceral muscles is different from the somatic muscles (Figure 3G), and therefore it is not so suprising that the PS2 integrin is required for one but not the other. The circumference of the midgut is covered by hemi-circular sets of mononucleate visceral muscles, which attach end to end, and an outer layer of longitudinal muscles. One of the two "seams" where the circular muscles attach end to end can be seen in the if^{SEF} gut (Figure 3E, white arrow), which is indistinguishable from a wild-type gut (not shown). Within each visceral muscle the phalloidin stains rectangles of actin, which are interspersed by a dark region containing a bright dot of phalloidine staining (Figure 3, D–G). Ultrastructural analysis has shown that there are no H bands in these muscles and unusual filaments link the punctate Z bands to the thin filaments that overlap the myosin filaments (Sandborn *et al.* 1967; Goldstein and Burdette 1971). Therefore, our interpretation of the phalloidin staining is that the Z bands stain as the bright dot, the gap represents the unusual filaments (which could be actin that does not bind phalloidin or some other protein), and the rectangles represent actin filaments (see Figure 3G). The length of the rectangles varies depending on whether the muscle is relaxed or contracted (*e.g.*, Figure 3E *vs.* F; both are seen in wild-type guts). The number of thin filaments surrounding each thick filament in cross section is larger than in the somatic muscles (Sandborn *et al.* 1967; Goldstein and Burdette 1971), supporting the idea that the actin filaments overlap. Consistent with the morphogenetic defects observed in the midgut in class I and III alleles (Figure 2), we see that the circumferential visceral muscles fail to surround the gut in these mutants (Figure 3, D and F). The if^{17} midgut has a small gap at the seam, whereas in if^{C2B} midguts the circular visceral muscles are found as two plates of muscles that are almost completely separated, and the longitudinal muscles are also disordered. This phenotype could arise if the layers of the mesoderm have failed to migrate across the midgut and thus never meet, or by failure of the muscle-muscle attachment, so that they detach along the seams following contraction.

The analysis of these new classes of *inflated* allele has shown that in addition to the known function of the PS2 integrin in mediating the attachment of the muscles, it also has a role in the formation or maintainence of the muscle contractile ultrastructure. We have also found that the function of the PS2 integrin in the morphogenesis of the midgut and the nerve cord is distinct from its function in muscle attachment and sarcomeric structure. From our existing data it seems likely that the if^{SEF} mutant affects the structure of the protein, but we have not been able to determine whether the class III mutations disrupt *cis*-regulatory regions or protein domains.

Functions of *inflated* in the adult: Our current data suggest that viable *inflated* alleles arise only when mutations specifically alter expression in the imaginal tissues. We have been unable to generate viable wing bubble alleles of *inflated* with EMS. The one viable allele we recovered is the γ-ray allele if^{V2}, which is a translocation between the *X* and the fourth chromosome (15A;101F; data not shown). This allele causes a substantial reduction of the expression of the α_{PS2} subunit in the third instar wing imaginal disc (Figure 4) and is semilethal with a very strong adult phenotype. There is some larval lethality (but no embryonic lethality; not shown), judging by the reduced numbers of if^{V2} pupae and adults relative to their siblings, and the majority of the mutant individuals die while eclosing: they get their head and legs out but then become stuck. We think that this is due to the *inflated* wings sticking to the pupal case. A few mutant individuals do successfully eclose and have severe adult abnormalities (Figure 4), although they are viable and fertile. The two layers of the wing blade are completely separated and the wings appear as hemolymph filled balloons. The hemolymph often becomes dried and blackened within the wing. In addition to this extreme version of the wing blister phenotype previously observed for *inflated*, two novel phenotypes are observed in this mutant. The halteres are distorted, appearing longer and less rounded than in wild type and having a rougher surface (Figure 4). The legs are also misshapen, with a kink in the femur of particularly the second and third legs (Figure 4). The latter phenotype is not due to detachment of leg muscles as no detached muscles are observed in the mutant legs (not shown), nor is it an injury that arises during eclosion as the leg kinks can be seen in the pupa prior to eclosion (not shown).

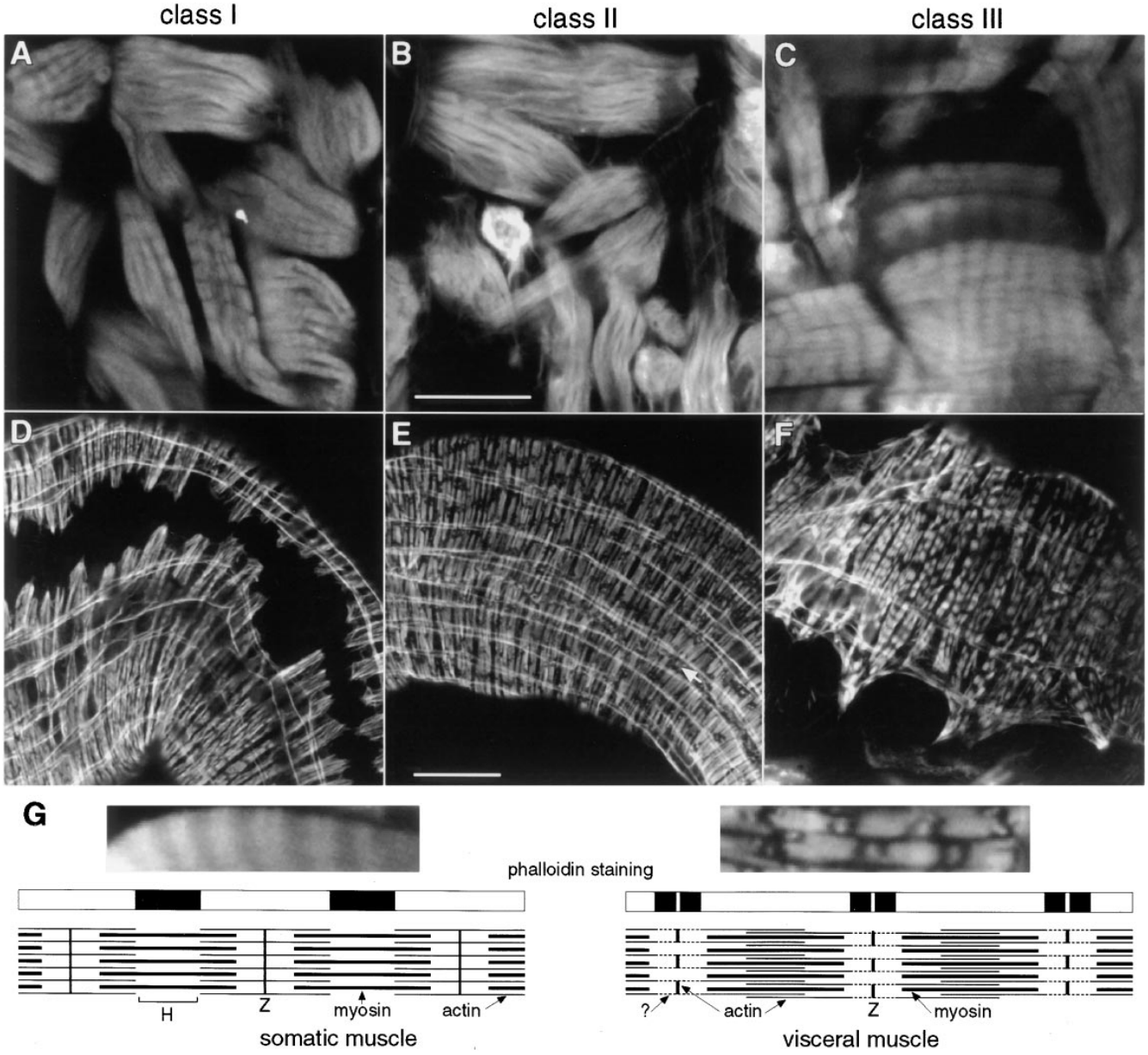

Figure 3.—Sarcomeric phenotypes of *inflated* mutants. Mutant late stage 17 embryos/first instar larvae were dissected open, fixed, and stained with rhodamine-phalloidin. Some disruption of the somatic muscles occurred during the dissection. The comatic muscles are shown in A–C, with partial disruption of the sarcomeres seen in the class I mutant if^{17}, complete disruption in the class II mutant if^{SEF}, and wild-type appearance in the class III mutant if^{C2B}. The region of the midgut just posterior to the gastric caecae is shown in D–F. None of the mutants affects the sarcomeric structure of the circular visceral muscles, but in the class I and class III mutants the visceral muscles do not completely surround the gut, with a gap observed at the position where the two layers of muscles normally attach end-to-end at a "seam," indicated by a white arrow in the class II gut in E (which is indistinguishable from wild type). Bar, 20 μm. The proposed relationship between the phalloidin staining and the underlying sarcomeric structure is shown in G, with enlargements corresponding to a 20-μm long segment of the muscles, a schematic of the phalloidin staining below and models of muscle structure underneath. The staining of the somatic muscles is consistent with standard models of sarcomeric structure; however, the visceral muscle pattern is puzzling. The lack of a dark H band region, containing just myosin, is consistent with the complete overlap of the myosin filaments by actin filaments (see text for details), but the regions that do not stain with phalloidin suggest an unusual filament between the punctate Z disc and the bulk of the actin filaments. The bright thin band within the dark region suggests that the Z discs contain actin filaments.

The if^{V2} allele has no phenotype in the eye, as expected from the lack of any phenotype in the eye of clones of cells homozygous for *inflated* amorphic alleles (Brower *et al.* 1995). The antennae and mouth parts also appear normal.

The breakpoint of the if^{V2} allele is approximately 5 kb downstream of the *inflated* transcription unit (data not shown). This mutation does not remove essential regulatory elements since a *P*-element construct, which contains 36 kb of genomic DNA but extends only 2 kb

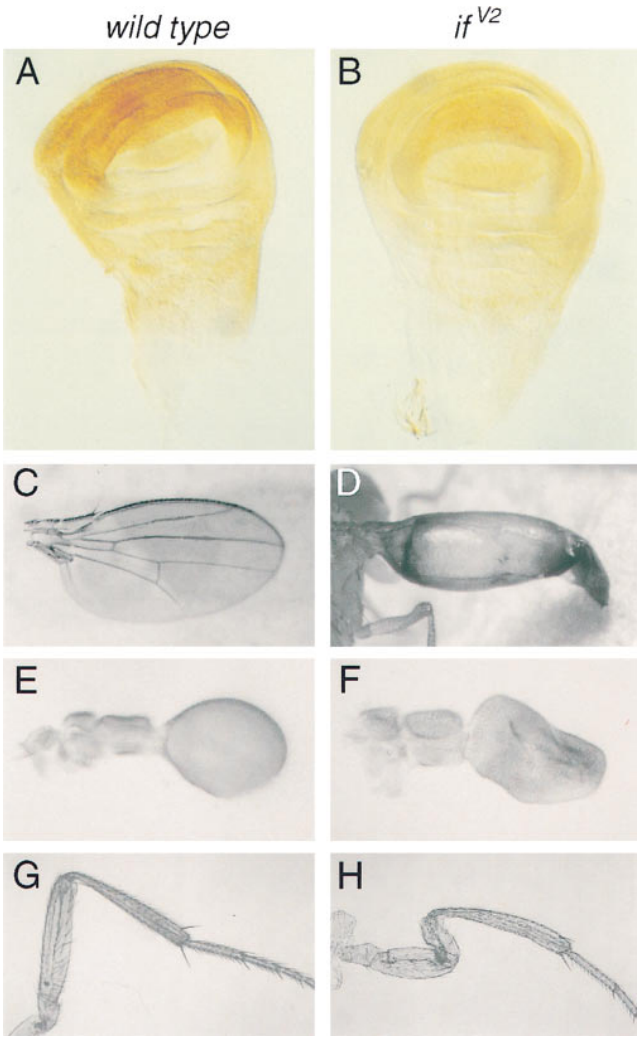

Figure 4.—The class IV if^{V2} allele has reduced expression of the α_{PS2} subunit and defects in wing, haltere, and legs. Late third instar imaginal discs from wild-type (A) and if^{V2} (B) larvae were dissected and stained together with an antibody against the α_{PS2} subunit. Anterior is to the left and ventral is at the top. Wings (C, D), halteres (E, F), and third leg (G, H) are shown from wild-type (C, E, G) and if^{V2} mutant (D, F, H) adults.

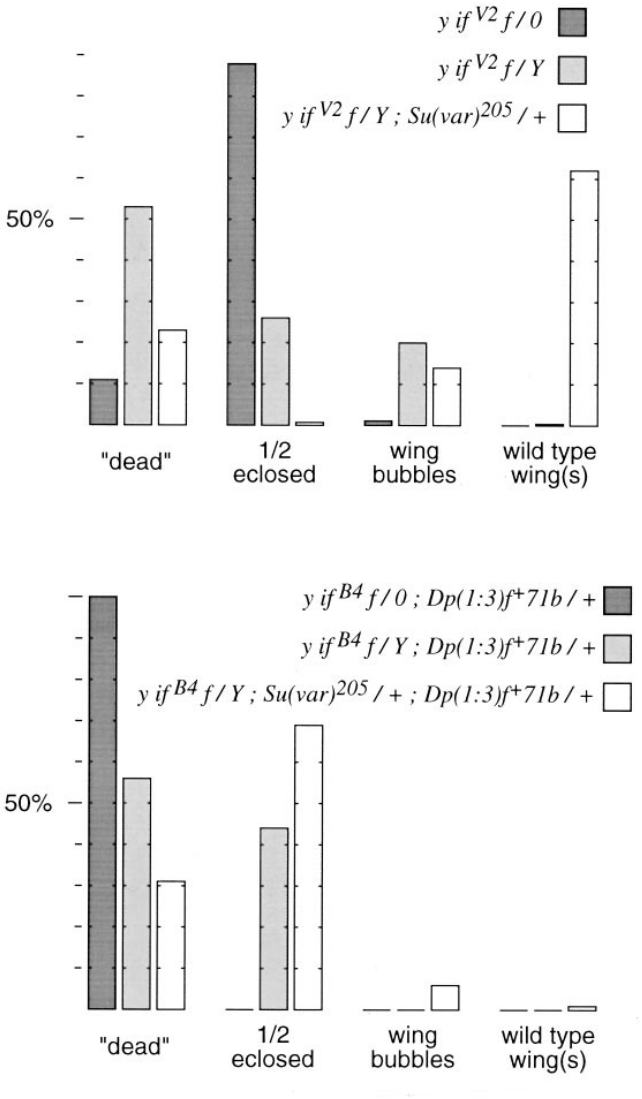

Figure 5.—The strong adult phenotypes of if^{V2} and if^{B4}; $Dp(1:3)f^{+}71b$ are due to position effect variagation. The effect of the loss of the Y, which enhances PEV, and the dominant suppressor of PEV, *Su(var)205*, were examined. Adults were compared to their siblings to determine those that died prior to puparation (the missing adults = "dead"), those that partially eclosed (one-half eclosed), and those that eclosed and had wing bubbles of wild-type wings. We do not understand the suppression of lethality of if^{V2} by the loss of the Y, but it may be strain variation.

3′ to the poly A site, fully rescues *inflated* mutations (Brown 1994). Instead the defect in *inflated* function in the if^{V2} allele arises from position effect variegation that suppresses the enhancers within the transcription unit. Thus, the adult phenotypes are enhanced by lower temperature (not shown) and the removal of the Y (Figure 5; in this experiment the removal of the Y improved the survival of mutant larvae), and it is suppressed by the suppressor of position effect variagation *Su(var)205* (Sinclair *et al.* 1983; Figure 5). We can also generate a very similar phenotype from another genetic combination using the duplication part of $Tp(1:3)f^{+}71b$ (Craymer and Roy 1980): if^{B4}/Y; $Dp(1:3)f^{+}71b/+$. $Dp(1:3)f^{+}71b$ extends from far upstream of the *inflated* transcription unit (16C2) to just 3′ of it (15A4) and is inserted in the centric heterochromatin of the third chromosome (80-81). The if^{B4}/Y; $Dp(1:3)f^{+}71b/+$ phenotype is also suppressed by *Su(var)205* and enhanced by the loss of the Y (Figure 5).

Class I alleles and the class II allele if^{SEF} fully complement the if^{V2} allele (Tables 2 and 3). In order to determine whether this is because these alleles have wild-type function in the wing or whether it represents some more complicated genetic interactions, we made clones of some of these alleles by FLP-FRT-induced recombination (Table 3). While clones of the null allele if^{B4} and the class III alleles if^{C2B} and if^{2B1} give bubbles in the wing, no such bubbles were observed with the if^{SEF} allele

or two of the class I alleles. The difference in wing function between the allelic classes was confirmed when we attempted to rescue *inflated* alleles with a shortened *P*-element if^+ construct that lacks *cis*-elements required for adult function. In an effort to shorten the *P*-element rescue construct, we initially removed sequences from both 5′ and 3′ to the gene (Figure 6), and found that this shorter gene still fully rescues the if^{B4} null allele (data not shown). We then deleted three of the introns about 15 kb 3′ to the start of transcription to generate an if^+ "minigene" (Figure 6). When we attempted to rescue the null if^{B4} allele with this minigene, we only obtained a small number of rescued adults, which had eclosed with severe defects in the wings, halteres, and legs, similar to if^{V2} (not shown). In contrast, the minigene fully rescues if^{SEF} and the hypomorphs (Table 3). The minigene rescues the lethality of if^{C2B}, but the adults have bubbles in the wing of variable severity, depending on the minigene insertion site. The minigene almost fully rescues if^{2B1} but a few adults are observed with bubbles in the wing. Finally, the minigene does not rescue the if^{V2} phenotype at all (not shown). Thus, these results confirm that if^{V2}, if^{C2B}, and if^{2B1} (weakly) are mutant in *inflated* function in the adult, while if^{SEF} and the hypomorphs have wild-type function in the adult.

DISCUSSION

In this study of the *inflated* gene we have analyzed the phenotypes and allelic interactions of 45 *inflated* alleles (summarized in Table 4). We have found that *inflated* is a complex locus and contains at least two separably mutable activities. These results suggest that the integrin α_{PS2} subunit, encoded by *inflated*, has different kinds of activities in the different tissues of the developing animal, as indicated by the five classes of alleles we have recovered. We have examined the role of the PS2 integrin in the attachment of the somatic muscles, the formation of muscle sarcomeres, the morphogenesis of the midgut, gastric caecae and the contraction of the ventral nerve cord, and the morphogenesis of the adult, especially the adhesion between the two surfaces of the wing. Amorphic *inflated* alleles affect all these processes, while the other four classes of allele only affect a subset of them. The class I hypomorphs are complementary to the class IV viable alleles: the partial activity of the class I alleles is sufficient for function in the adult but not for any of the other functions. Therefore, it appears that the only way to generate adult viable class IV alleles is by making mutations that specifically perturb expression of the PS2 integrin in the imaginal discs. This contrasts to many other loci where weak alleles give a phenotype in the adult but not in the embryo, or strong alleles give a dominant adult phenotype (see Lindsley and Zimm 1992). The class II allele is complementary to the class III alleles: it produces a PS2 integrin that is largely wild-type except that it is unable to aid in the formation of the somatic muscle sarcomeres (sarcomeric function), whereas class III alleles eliminate a PS2 function that is required in midgut and gastric caecae morphogenesis, as well as the contraction of the nerve cord and morphogenesis of the adult structures (morphogenetic function). Both class II and class III alleles still retain the ability to mediate muscle attachment (adhesive function).

The single class II allele, if^{SEF}, is one of the 12 alleles recovered in the F2 screens, and it has a unique phenotype. It is embryonic lethal, but the only defect we have observed is a failure in the formation of the normal somatic muscle sarcomeric structure, thus confirming the existence of a sarcomeric function of the PS2 integrin. The existence of this defect would have been predicted from the study of Volk *et al.* (1990) who noted that the PS2 integrin is localized at the Z-discs of somatic muscles cultured *in vitro*, and who observed that muscles from embryos mutant for the β_{PS} subunit did not form a normal sarcomeric structure. However, this sarcomeric defect could have been a secondary effect of the rounding up of the muscles due to their detachment. This has been resolved by the if^{SEF} mutant where the sarcomeric structure is disrupted in somatic muscles that remain attached, clearly demonstrating that the

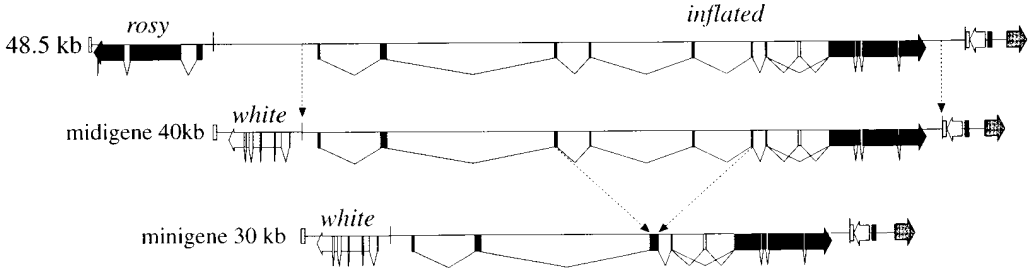

Figure 6.—Construction of shorter *inflated* rescue constructs. At the top is the initial rescue construct containing 36 kb of genomic DNA that fully rescues *inflated* mutants (Brown 1994) and was used as the duplication for some of our F2 lethal screens (see Figure 1). This was shortened by removing sequences 5′ and 3′, as shown by the dotted lines, and transferring to a *white*$^+$ marked vector; this midi-gene also fully rescues. The introns 3, 4, and 5 were removed by replacing a segment of genomic DNA with a fragment from a cDNA clone, shown by the dotted lines; this only partially rescues (see text and Table 3).

TABLE 4

Summary of different classes of *inflated* alleles

	Complementation					Functions				
Class	0	I	II	III	IV	No. of alleles	Muscle attachment	Sarcomeric structure	Midgut morphogenesis[a]	Adult morphogenesis
0	−	−	−	−	−	36	−	−	−	−
I		−	−	±	+	4	−	−	−	+
II			−	+	+	1	+	−	+	+
III				−	−	2	+	+	−	−
IV					−	2	+	+	+	−

[a] This category includes elongation of the midgut, formation of gastric caecae, and condensation of the ventral nerve cord.

PS2 integrin itself is required for the formation of the normal sarcomeric structure in the somatic muscles. In contrast, PS2 does not appear to be required for the formation of the visceral muscle sarcomeres, which differ in structure. This difference may aid in the elucidation of the role of the PS2 integrin in organizing the sarcomeres of the somatic muscles.

The muscle sarcomeric defect is also observed in some of the muscles in embryos mutant for hypomorphic alleles, raising the possibility that if^{SEF} might be simply an even weaker hypomorph. However, there are several arguments against this. The if^{SEF} allele does not behave like a hypomorph because its phenotype is unaltered when *in trans* to a deficiency. In addition, the phenotype of if^{SEF} gets weaker *in trans* to the hypomorphs, instead of stronger as it would if it were a weak hypomorph, showing that in fact the hypomorphic alleles are weaker for the PS2 sarcomeric function than if^{SEF}. Finally, the hypomorphic alleles have a fully penetrant gastric caecae phenotype and an incompletely penetrant sarcomeric phenotype, suggesting that the development of the gastric caecae is a developmental event that is very sensitive to a reduction in PS2 integrin activity, and the if^{SEF} allele has wild-type development of these structures. Thus it seems that the if^{SEF} allele specifically disrupts the sarcomeric function of the PS2 integrin.

The sarcomeric function of PS2 could work by playing a role in the organization of each unit of the contractile cytoskeleton, consistent with the localization of PS2 to the Z discs of muscle cultured *in vitro* (Volk *et al.* 1990). This would be similar to the role of integrins in the formation of muscle sarcomeric structure in *C. elegans*, even though the muscles form in a different way. In the body wall muscles of this organism, multiple structures per muscle, called dense bodies, anchor the contractile ultrastructure through the membrane to the hypoderm (Hresko *et al.* 1994). Dense bodies appear to be equivalent to the Z discs of vertebrate and Drosophila striated muscle, and like Z discs, they are rich in actin and α-actinin. The *pat-3* gene encodes a β integrin subunit, which is localized to the muscle membrane underlying the dense bodies and is required for the subsequent localization of talin and vinculin to them (Williams and Waterston 1994; Gettner *et al.* 1995). Not only do mutants that lack this integrin subunit fail to localize either talin or vinculin, they also do not form distinct dense bodies and fail to organize their contractile cytoskeleton. If the PS2 integrin works in a similar way, then at each Z disc it would link the sarcomeres to the membrane, to help organize the sarcomeric structures.

The class III alleles are rare alleles recovered from F1 screens for wing bubble phenotypes; only 2 were recovered from a total of 24 EMS- or ENU-induced mutants. Their phenotype is distinct from the amorphic and hypomorphic alleles and complementary to the if^{SEF} phenotype, as indicated by their ability to fully complement if^{SEF} genetically. Thus, the class III alleles specifically disrupt the morphogenetic PS2 function, at the same time retaining the adhesive and sarcomeric functions. An alternative interpretation of the class III alleles is that they are regulatory mutations in an enhancer that drives *inflated* expression in the visceral mesoderm, imaginal discs, and whatever cells require *inflated* to mediate nerve cord condensation. However, this is not consistent with the little we know about *inflated cis*-regulatory elements. The second intron contains an enhancer for expression in the embryonic somatic and visceral mesoderm, which contains sites for the transcription factors Twist (A. Dokidis, N. H. Brown and F. C. Kafatos, unpublished observations) and D-MEF2 (Ranganayakulu *et al.* 1995). When we constructed the minigene we retained this intron, but in deleting introns 3–5 we appear to have deleted *cis*-regulatory elements that are important for *inflated* expression in the imaginal tissues because the minigene does not rescue the adult phenotypes. Thus, unless there are additional essential enhancers it would be difficult to generate a mutation that removed both mesodermal and imaginal disc enhancers while retaining all *inflated* coding exons. Therefore, we favor the view that the class III mutations disrupt a specific morphogenetic function of the PS2 integrin protein.

The PS2 morphogenetic functions are required for midgut morphogenesis, nerve cord condensation, and the adult phenotypes, but not for muscle attachment or the formation of muscle sarcomeric structure. The wing and the gut share the feature that the interaction between the two layers of cells occurs over a large surface, in contrast to the specific points of muscle attachment. We do not have a clear picture of how the loss of the PS2 morphogenetic function in the visceral mesoderm leads to the defects in the morphogenesis of the midgut and gastric caecae. We have found that PS2 function is required to maintain the integrity of the visceral muscle layer, but do not know whether it is required when the visceral muscles initially surround the endoderm or later to mediate the end-to-end attachment of the visceral muscles or their lateral adhesion to the endoderm. The defects in the shape of the gut epithelia that occur in the absence of PS2 function could be a consequence of the loss of the continuous contractile layer of muscles, which is required mechanically to change the shape of the gut, or they could be due to a loss of signal transduction between the mesoderm and gut. A failure in the close apposition of the visceral mesoderm and midgut could indirectly cause defects in signal transmission between these cell layers. It is also possible that the PS2 integrin morphogenetic role is itself part of the signaling machinery necessary for midgut morphogenesis.

Underlying the adult phenotypes observed in mutations in members of the PS integrin family is their expression in the imaginal discs. This is particularly so in the wing imaginal disc where they are expressed in a striking reciprocal pattern; PS2 is expressed in the cells destined to become the ventral layer of the wing blade and PS1 is expressed in the cells destined to become the dorsal layer (Wilcox *et al.* 1981; Brower *et al.* 1984). In large clones, loss of α_{PS2} from the ventral cells, α_{PS1} from the dorsal cells, or β_{PS} from either set of cells leads to a failure in attachment between the two cell layers and the formation of a wing bubble in the adult (Brower and Jaffe 1989; Zusman *et al.* 1990; Brabant and Brower 1993; Brower *et al.* 1995). Surviving adults mutant for the class IV *inflated* allele if^{V2} presumably have no PS2 integrin-mediated adhesion function in the cells of the ventral wing blade, resulting in the formation of balloon-like wings. These survivors also display defects in the morphogenesis of the adult legs and halteres. In the haltere the PS2 integrin is expressed in a small patch similar in position to the presumptive ventral wing blade in the wing disc (Brower *et al.* 1985), but how this mediates the formation of a round haltere is unclear. In the third instar larval leg discs, PS2 is only weakly expressed (Brower *et al.* 1985); however, it may be more strongly expressed during pupal development and therefore account for the phenotype we have observed. Distinct patterns of PS2 expression are observed in the eye-antennal imaginal disc (Brower *et al.* 1985); however, the mature structures are unaffected in if^{V2} mutants (not shown). This confirms the previous reports that clones of null *inflated* alleles in the eye disc have no phenotypic consequence (Brower *et al.* 1995).

In the absence of data on the sequence changes of the mutations we can only speculate as to the nature of these classes of allele. Because integrins do not have any inherent enzymatic activity they are thought to function through their binding to other proteins inside and outside the cell. Therefore, it is likely that the Class II and Class III mutations alter the ability of PS2 integrin to bind to specific proteins. All *inflated* mutations have the highest probability of being in the extracellular domain of the α_{PS2} subunit since 96% of the amino acids (1307/1363) are extracellular. However, mutations in the extracellular domain could alter the binding of integrins to either extracellular ligands or intracellular proteins. By altering the ligand-binding pocket, the mutations could destroy the ability of the integrin to bind to specific extracellular ligands. The α_{PS2} integrin is alternatively spliced; exon 8 encodes a 25 amino acid exon that is either omitted or inserted into the extracellular part of α_{PS2}, near to the region where the ligand-binding domain resides (Brown *et al.* 1989). The suggestion that this alternative splicing alters the ligand specificity and/or affinity has been demonstrated by the different ability of these two forms of the $\alpha_{PS2}\beta_{PS}$ integrin to bind to vertebrate ligands (Zavortink *et al.* 1993) and Tiggrin (Fogerty *et al.* 1994). Furthermore, recent site-directed mutagenesis of the vertebrate α4 subunit has identified three residues specifically required for ligand binding (Irie *et al.* 1995), and the homologous residues in α_{PS2} are the last three residues in exon 7, adjacent to the alternatively spliced region. Thus, one of the new mutations could alter this region of α_{PS2} and specifically block binding to some but not all ligands. Expression of the single isoforms of the α_{PS2} subunit using the GAL4 system allows rescue of *inflated* phenotypes (Roote and Zusman 1996; Martín-Bermudo *et al.* 1997), but perhaps defects would be observed if wild-type levels of the single isoforms were expressed. Mutations in the extracellular domain of the α_{PS2} subunit could alter the interaction of the PS2 integrin with cytoplasmic proteins by altering the ability of extracellular ligand-binding-induced conformational changes to be transmitted to cytoplasmic tails. This could alter the ability of the integrins to bind to specific cytoskeletal proteins involved in cell shape changes or linkage of the contractile apparatus to the membrane. It could also alter the interaction of the integrin with intracellular signaling molecules. Clearly, a rare mutation in the cytoplasmic tail could also cause defects in the intracellular interactions.

This genetic analysis of *inflated* alleles suggests that PS2 functions can be categorized as adhesive, sarcomeric, or morphogenic. The different functions could all involve adhesion to extracellular ligands to promote selective cell attachment. For example, a model in which

PS2 integrin occurs in two interchangable activation states, such as low and high affinity forms, can account for these multiple functions. In this model both activation states are able to mediate general adhesion, but a PS2 integrin locked into the high affinity state (class III) is unable to mediate the morphogenetic, functions, and a PS2 integrin locked into a low affinity state (class II) is unable to mediate the sarcomeric function. Alternatively, the class I allele could be defective in PS2 integrin binding to a ligand specifically involved in constructing the muscle sarcomeres, and the class III alleles could specifically block integrin signaling. Although still requiring extracellular ligand binding, the morphogenetic function could be achieved by the initiation of an intracellular signaling pathway. We are currently sequencing the class I, II, and III mutants to see if the different classes map to discrete areas of the α_{PS2} subunit. We anticipate that this will help reveal the mechanistic basis of the different integrin activities.

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