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The *Serrate* locus of *Drosophila* and its role in morphogenesis of the wing imaginal discs: control of cell proliferation

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SUMMARY

The *Drosophila* gene *Serrate* encodes a transmembrane protein with 14 EGF-like repeats in its extracellular domain. Here we show that loss-of-function mutations in this gene lead to larval lethality. Homozygous mutant larvae fail to differentiate the anterior spiracles, exhibit poorly developed mouth-hooks and show a severe reduction in the size of the wing and haltere primordia, which is not due to cell death. The few homozygous mutant escapers that pupariate develop into pharate adults that almost completely lack wings and halteres. Clonal analysis in the adult epidermis demonstrates a requirement for *Serrate* during wing and haltere development. Targeted ectopic expression of *Serrate* in the imaginal discs using the yeast transcriptional activator Gal4 results in regionally restricted induction of cell proliferation, e. g. the ventral tissues in the case of the wings and halteres. The results suggest that the wild-type function of *Serrate* is required for the control of position-specific cell proliferation during development of meso- and metathoracic dorsal discs, which in turn exerts a direct effect on morphogenesis.

Key words: *Serrate*, EGF-like protein, imaginal discs, cell proliferation, *Drosophila*

INTRODUCTION

The imaginal discs of *Drosophila melanogaster* are an ideal system in which to study mechanisms of pattern formation and morphogenesis. The discs arise as small invaginations of the epidermis during embryogenesis (Auerbach, 1936; Nöthiger, 1972; Bate and Martinez Arias, 1991). During larval stages, the discs grow and each adopts a characteristic morphology; finally, during metamorphosis, they evaginate and give rise to invariant parts of the adult epidermis. Many genes have been identified that are involved in the control of cell proliferation, cell fate determination or pattern formation (Shearn et al., 1971; Garcia-Bellido, 1975; see also Shearn, 1978, Wilkins and Gubb, 1991, Whittle, 1990, Garcia-Bellido and de Celis, 1992; Williams and Carroll, 1993, and Cohen, 1993 for reviews). Several of these, including *decapentaplegic* (*dpp*) (Posakony et al., 1991), *wingless* (*wg*) (Couso et al., 1993) and *patched* (*ptc*) (Phillips et al., 1990), exhibit spatially restricted expression patterns in the imaginal discs, induce patterning defects upon mutation and encode signalling or receptor molecules. These are thought to provide landmarks for the establishment and/or maintenance of positional information, guiding position-specific proliferation and differentiation of cells. Many of the genes required for pattern formation in the imaginal discs are also required for embryonic development, implying that similar mechanisms are required for pattern formation both during larval and imaginal development. (For further discussions of the roles of segment polarity genes during pattern formation in the embryo and the imaginal discs the reader is referred to Martinez Arias, 1989; Wilkins and Gubb, 1991; Bryant, 1993).

The molecular analysis of proteins involved in aspects of cell communication during development has revealed the occurrence of similar protein motifs in molecules regulating quite different processes. One such motif is similar to the epidermal growth factor, EGF. Several genes encoding proteins with EGF-like repeats have been shown to control cell fate decisions during development, such as *Notch* and *Delta* of *Drosophila* (Wharton et al., 1985; Kidd et al., 1986; Vässin et. al., 1987; Kopczynski et al., 1988) or *lin-12* and *glp-1* of *Caenorhabditis elegans* (Yochem and Greenwald, 1989), while others are assumed to act on various aspects of pattern formation in *Drosophila*, e. g. the gene *slit* encodes a secreted protein necessary for CNS development (Rothberg et al., 1988, 1990), *crumbs* is required for epithelial development (Tepaß et al., 1990; Tepaß and Knust, 1990) and *fat* is necessary for imaginal disc morphogenesis (Bryant et al., 1988; Mahoney et al., 1991).

The *Drosophila* gene *Serrate* (*Ser*) is a member of this family and encodes a transmembrane protein with 14 EGF-like repeats in the extracellular domain (Fleming et al., 1990; Thomas et al., 1991) that shows a complex expression pattern

during embryogenesis. However, *Ser*$^{-}$ embryos appear phenotypically wild type; indeed such embryos hatch (Thomas et al., 1991). The observation that two existing alleles, *Ser*^{D} and *Ser*^{Bd}, cause abnormal phenotypes in the wing margin and the finding that the Serrate protein is expressed in the prospective wing margin (Thomas et al., 1991), imply a function for this gene the wing imaginal disc. Here we provide evidence that the gene *Ser* is required to control position-specific cell proliferation in the anlage of the wing blade and the halteres, and may thus contribute to the control of patterning during imaginal disc development.

MATERIALS AND METHODS

Drosophila stocks

Flies were grown on standard medium and crosses were performed at room temperature or at 25°C. Descriptions of balancer chromosomes and markers can be found in Lindsley and Zimm (1992). Oregon R was used as wild-type stock. *Ser* alleles used in this work are listed in Table 1. If not specified, *Ser*$^{-}$ means *Ser*^{RX82} or *Ser*^{RX106} in *trans* over a deficiency.

Somatic clones

Somatic clones were produced by X-ray-induced mitotic recombination (Becker, 1976), using the genotypes *mwh red e Ser*^{Rx106} and *mwh red e Ser*^{Rx82}. Eggs from the cross *Ser*^{Rx}*/TM6* × *Ki Sb M(3)w/TM2* were irradiated at 36±12 hours (first instar larvae), 60±12 hours (second instar larvae) and 132±12 hours (late third instar) (25°C) with 1000 rad (0.3 mm Al filter). As a control, *mwh red e/Ki Sb M(3)w* larvae were irradiated using the same conditions. The size, distribution and phenotype of clones on head, thorax, legs, wings and abdomen of adults were analyzed.

Staining of imaginal discs and immunohistochemistry

Imaginal discs were prepared from mutant and wild-type larvae and stained with toluidine blue solution (1% toluidine blue, 0.3 M $Na_2B_4O_7$, 0.16 M boric acid, pH 8.0), fixed in Bodian´s fixative (4% formaldehyde, 5% acetic acid in 72% ethanol), dehydrated and mounted in GMM (Lawrence et al., 1986). Pictures were taken with a Zeiss microscope equipped with Nomarski optics.

Antibody staining was done as described by Tepaß et al. (1990), using the polyclonal α-Ser serum directed against part of the extracellular domain (s4566; Thomas et al., 1991).

For histological sections, late second or early third instar larva were cut open and fixed in 4% glutaraldehyde/2% OsO_4 in 0.1 M phosphate buffer. After dehydration in ethanol and acetone, the imaginal discs were embedded in Araldite. Semi-thin sections (2 μm) were cut on a Reichert Mikrotom and stained with 1% toluidine blue in 1% borate.

Scanning electron micrographs

Flies were dehydrated in an ethanol series, critical point dried in a Beckman CP drying device, mounted for scanning electron microscopy and coated with a 20 nm layer of gold. Pictures were taken on a Hitachi S-520 scanning electron microscope at 10 kV.

Germ-line transformation and ectopic expression

The UAS-*Ser* mini-gene was constructed as follows. The *Ser* cDNA N10-29 (Thomas et al., 1991), cloned as an *Eco*RI fragment in the Bluescript vector, was partially digested with *Nru*I and then with *Sal*I, using a site in the polylinker. The resulting fragment of 5 kb, which comprised 4073 bp of the ORF and the complete 3′-trailer, was joined to a 0.65 kb genomic *Pst*I-*Nru*I fragment, containing the remaining 151 bp of the ORF and extending 499 bp upstream of the putative translational start site. This construct was cloned as a *Sma*I-*Sal*I fragment into the S2 cell expression vector pRmHa3 (Bunch et al., 1988), thus providing an *Eco*RI site within the vector immediately ahead of the mini-gene, which was used for cloning into the *Eco*RI site of the pUAST vector (Brand and Perrimon, 1993). This *Ser* mini-gene was named mg5603. Germ-line transformation experiments were carried out essentially as described in Spradling (1986). Transposase was supplied by co-injection of the Δ2-3 helper plasmid (Laski et al., 1986). Transgenic stocks were established over the appropriate balancers or kept as homozygotes.

The Gal4 activator lines used in this study for induction of ectopic expression originate from an enhancer trap screen (Hinz et al., 1994). Here, two activator lines were used, Gal4$^{540.3}$, which bears an insertion on the third chromosome and drives Gal4 expression under the control of the *hairy* promoter, and Gal4$^{559.1}$, which carries the insert on the second chromosome and mimics the expression pattern of the gene *patched*.

Gal4$^{540.3}$/UAS-Ser^{mg5603} transheterozygotes were subjected to temperature shifts from 18 to 25°C and vice versa. To determine the onset of the phenocritical period, 12 hour egg collections were allowed to develop at 25°C for various periods and were then shifted to 18°C. To determine the end of the phenocritical period, a population of transheterozygous flies comprising all developmental stages was raised at 18°C and then placed at 25°C. Staging was according to Ashburner (1989).

RESULTS

Phenotypic characteristics of *Ser* mutants

In a mutagenesis screen for null alleles of the *Ser* locus, we recovered ten X-ray-induced revertants of the dominant allele *Ser*^{D} (Thomas et al., 1991). Some characteristics of these alleles and two previously known dominant alleles, *Ser*^{D} and *Ser*^{Bd}, are summarized in Table 1. Whereas the gain-of-function mutation *Ser*^{D} is homozygous viable (Lindsley and Zimm, 1992; Fleming et al., 1990; Thomas et al., 1991), the remaining alleles are homozygous lethal. The embryonic lethality associated with *Ser*^{Rx3} and *Ser*^{Rx120} is not related to the loss of *Ser*, since only larval lethality is seen in *trans* with other *Ser*$^{-}$ alleles. Animals homozygous for most of the other *Ser* alleles die as larvae; only *Ser*^{Rx119} homozygotes die at

Table 1. *Ser* alleles used in this study

Allele	Source	Cytology	Lethality[1]	Reference
Ser^{D}	spontaneous	normal[3]	v	a
Ser^{Bd}	spontaneous	normal[3]	l[2]	a
Ser^{Rx3}	X-ray	Df(3R)97E7-11; 97F3-11	e[4]	b
Ser^{Rx50}	X-ray	normal	l	b
Ser^{Rx82}	X-ray	normal[3]	l	b
Ser^{Rx103}	X-ray	normal	l	b
Ser^{Rx106}	X-ray	normal[3]	l	b
Ser^{Rx107}	X-ray	normal[3]	l	b
Ser^{Rx111}	X-ray	nd	l	b
Ser^{Rx112}	X-ray	nd	l	b
Ser^{Rx119}	X-ray	nd	p	this work
Ser^{Rx120}	X-ray	nd	e[4]	this work

[1]Stage at which homozygous animals die. e, embryonic; l, larval; p, pupal; v, viable.

[2]A few percent of homozygous lethal embryos can be observed; see ref. b

[3]Defect mapped at the molecular level; see ref. b.

[4]Embryonic lethality not associated with a mutation at *Ser*, since this allele is larval lethal with other *Ser*.

References: [a]Lindsley and Zimm, 1992; [b]Thomas et al., 1991.

pupal stages, and pharate Ser^{Rx119} flies look morphologically normal. Ser^{Rx119} can be regarded as hypomorphic, because most animals hemizygous for this allele are larval lethal; only a few pupae develop, which exhibit the phenotype characteristic of amorphic escapers (see below). Only Ser^{D} is viable in *trans* to all the other *Ser* alleles, while the remaining alleles listed in the Table are lethal in inter se combinations, with one exception: Ser^{Rx119} is viable over Ser^{Bd}.

Besides Ser^{Bd} and Ser^{D}, three of the revertants (Ser^{Rx82}, Ser^{Rx106} and Ser^{Rx107}) were characterized at the molecular level (Thomas et al., 1991). The polymorphisms mapped in Ser^{Rx82} and Ser^{Rx106} are consistent with the presence of deletions of 0.5 kb and about 9 kb, respectively, whereas mapping data do not unambiguously allow us to determine the aberration in Ser^{Rx107}. All three alleles express no protein detectable with an antibody directed against part of the extracellular domain (s4566; Thomas et al., 1991; data not shown) as expected for amorphic alleles.

As previously described (Thomas et al., 1991), and in contrast to data published by Fleming et al. (1990) embryos homozygous for a deletion of the 97F region (e. g. Ser^{Rx3}) look perfectly wild type in cuticle preparations and when stained with various organ-specific antibodies, directed against antigens in the epidermis or the nervous system. The stage at which other Ser^{-} revertants die could not be determined unambiguously, because larvae could not be accurately staged for two reasons: (1) a few homozygous mutant animals remain in the larval stage for up to 18 days after egg laying; (2) although some of the mutant larvae reach the size of wild-type third instar larvae, the anterior spiracles and mouth hooks, prime indicators of larval age (Bodenstein, 1950), were abnormal. Anterior spiracles neither developed the knob-like structure typical for first and second instar stages, nor the retractable, finger-like structures characteristic of the third instar wild-type larva. The tracheal trunk terminated without opening to the exterior anteriorly (Fig. 1A,B) but the posterior spiracles always looked normal. The defect in the anterior spiracle may explain the reduced viability of most of the larvae, since second and third instar larvae use both the anterior and the posterior spiracles for respiration (Whitten, 1980). The mouth hooks always exhibited features characteristic of first instar larvae, at least in mutants homozy-

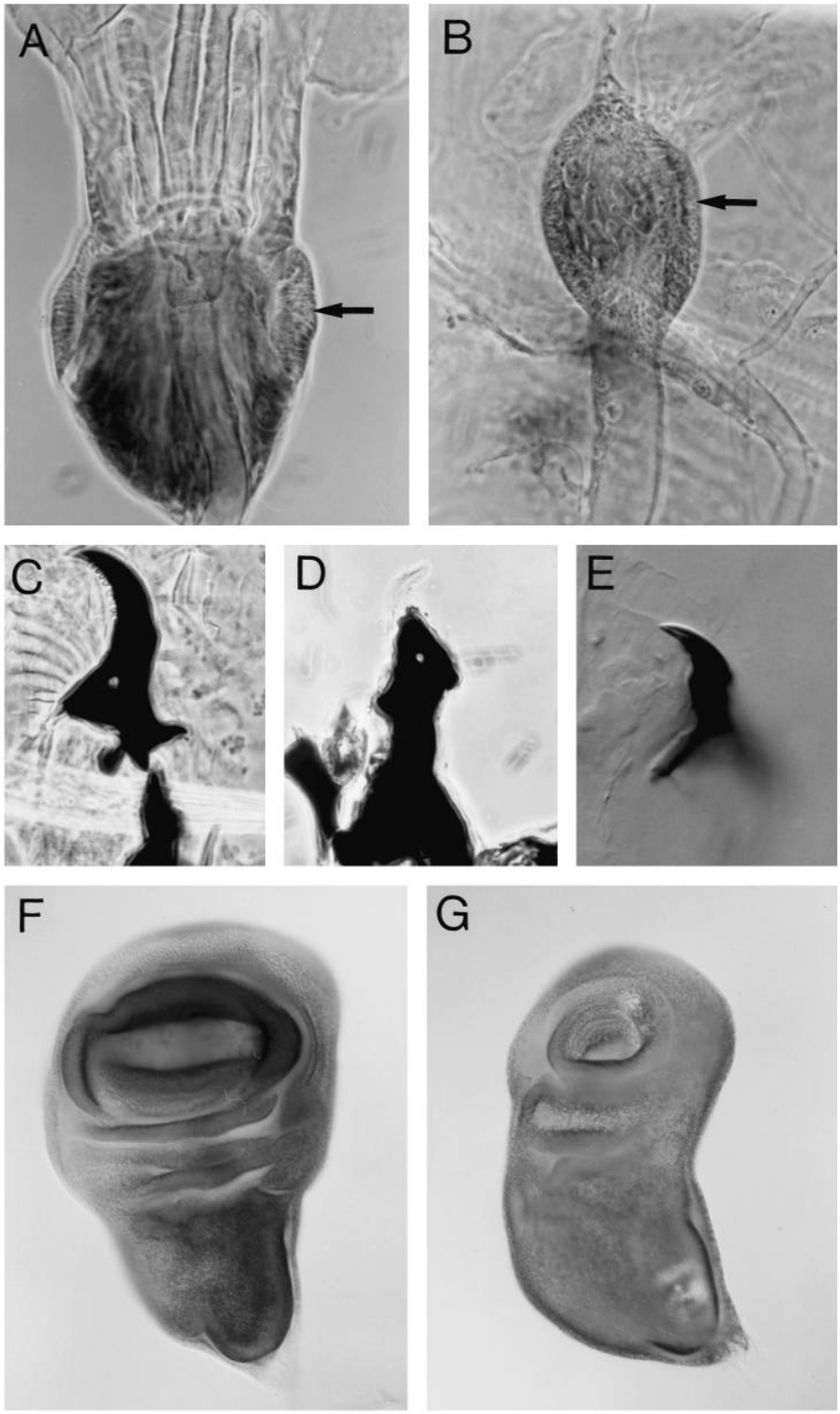

Fig. 1. Phenotypic characteristics of homozygous Ser^{-} larvae. (A,B) Anterior spiracles of wild-type (A) and *Ser* (B) third instar larvae. The wild-type anterior spiracles are characterized by eight retractable, finger-like structures, which are completely missing in the mutant, in which the trachea terminates without any differentiated structure. The anterior spiracle is embedded in the humeral disc (layer of small cells at the periphery; arrows), the size of which appears normal in the mutant. (C-E) Mouth hooks of wild-type third instar larvae (C), Ser^{-} third instar larvae (D) and, for comparison, wild-type first instar larvae (E). In the mutant, the hooks are smaller, poorly differentiated (note the lack of teeth) and fused to the head skeleton. (F,G) Wing imaginal discs of third instar wild-type (F) and Ser^{-} (G) larvae. While the parts of the disc that give rise to dorsal and ventral structures of the notum are comparable in size in both discs, the primordium of the wing is much smaller in the mutant disc.

gous for amorphic alleles (Fig. 1C-E). This fact may explain why homozygous *Ser* larvae feed less than their wild-type siblings. Furthermore, some mutant imaginal discs were smaller than those of the wild type. Particularly in the dorsal mesothoracic discs, the relative size of the wing primordium was severely reduced in comparison to the notal portion of the disc (Fig. 1F,G). In contrast to third instar discs from larvae bearing other mutations, in which reduction of wing size is caused by more or less extensive cell death, e. g. *vestigial* or *apterous* (Fristrom, 1969; Williams et al., 1993), no sign of degeneration could be observed in Ser^{-} discs of late second or early third instar larvae (Fig. 2), suggesting that lack of proliferation is the reason for the rudimentary wing anlage. The size of the humeral disc appeared normal (Fig. 1A,B). These observations suggest that at least some of the mutant larvae reach the third instar stage, although their development is greatly impaired, such that in most cases pupariation does not take place.

A few escapers homozygous for amorphic alleles occasionally developed into pharate adults, and inspection revealed that their wings and halteres were more or less completely absent (Fig. 3A,B). Most of the mesothoracic dorsal appendage rudiments represent parts of the hinge region, which connects the wing to the notum and looked fairly normal; the genital apparatus and the anal plates of the flies were small or absent, some of the tarsal segments were fused (not shown) and the compound eyes were rough and reduced in size (Fig. 3C,D).

Clonal analysis of *Ser* mutations in adults

To analyze *Ser* function in imaginal discs and to test for cell autonomy, clonal analysis was performed. Clones homozygous for two amorphic alleles, Ser^{Rx106} or Ser^{Rx82}, were found in adult flies on head, thorax, legs and abdomen; phenotype, frequency and size of mutant clones showed no significant difference from the controls (*mwh red e*).

In addition to clones at various locations, which developed perfectly normally, we observed in many of the irradiated animals scalloping of the anterior or posterior wing margin and of the halteres. Depending on the time of irradiation, the extents of deletions varied considerably, ranging from small notches to areas covering approximately a third of the wing blade (Fig. 4). We suppose that the notches reflect the positions of homozygous *Ser* clones, the cells of which failed to develop. As no appropriate markers are available on the third chromosome to analyze the corresponding twin spots, the presence of *Ser* clones could only be assessed indirectly. However, the occurrence of this phenotype exclusively in the mutant and the correlation between the stage irradiated and the size and frequency of the notches strongly indicate that these represent mutant clones. Furthermore, the failure of these clones to develop is compatible with the almost complete lack of wings and halteres in Ser^{-} adult escapers (see above). At the borders of the notches on the first vein we never found wild-type bristles, which would indicate rescue of genotypically Ser^{-} cells by neighboring heterozygous cells. This suggests that *Ser* is required autonomously in the cells of the developing wing.

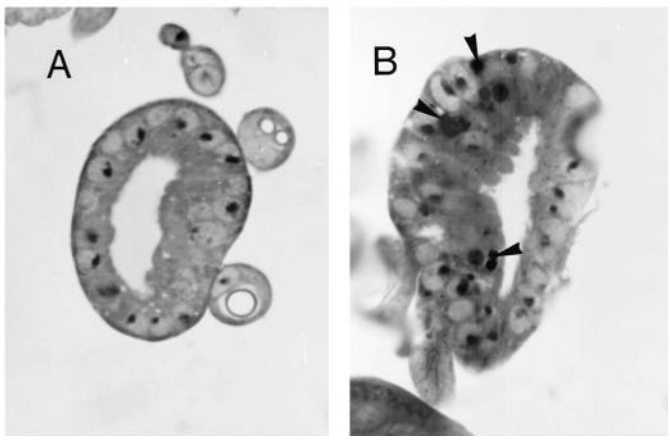

Fig. 2 Histological sections of wing discs of late second instar larvae. Section through a Ser^{-} (A) and ap^{56f} disc (B). While dead cells are detectable in the *ap* disc (arrowheads), no cell death is visible in the Ser^{-} disc.

Serrate is expressed in the embryonic dorsal thoracic discs

A requirement for *Ser* function for the development of the wing and haltere discs is further supported by the observation that the Serrate protein is expressed in the dorsal thoracic discs, i. e. the humeral, wing and haltere discs from embryo stage 13 onward (Fig. 5). The invagination in T1, which is associated with the anterior spiracle and lies slightly more dorsally than the other two thoracic discs, probably represents the humeral disc. Wing and haltere discs, located in the posterior halves of T2 and T3, respectively, consist of small groups of cells with elongated extensions reaching into the posteriorly located segment (Bate and Martinez Arias, 1991).

Ectopic expression of *Ser*

We used the two component system to drive ectopic expression of Serrate (Brand and Perrimont, 1993). Five transgenic effector lines were obtained, which carried the *Ser* minigene downstream of Gal4-responsive UAS_G elements. All behaved similarly in the experiments described below. To induce ectopic expression of the *Ser* mini-gene, we used activator lines $Gal4^{540.3}$ and $Gal4^{559.1}$, which express the Gal4 protein under the control of the *hairy* and *patched* promoters, respectively. These lines were chosen because the striped expression patterns in the embryonic ectoderm of *hairy* (Carroll et al., 1988; Hooper et al., 1989) and *patched* (Hooper and Scott, 1989; Nakano et al., 1989) are at least partially complementary to the stripes in which *Ser* is expressed (Thomas et al., 1991). As compared with the amount of endogenous Serrate protein, both activator lines induce strong overexpression of Serrate in the embryo (data not shown) and the imaginal discs (Fig. 6C,G). Although the embryonic expression patterns of both lines clearly differ from the endogenous *Ser* expression pattern, ectopic expression of *Ser* did not result in any abnormal embryonic phenotype or in embryonic lethality. Rather, with both activator lines, animals developed to late pupal stages and some even hatched.

The most obvious phenotype exhibited by transheterozygous flies (activator/effector) that developed at 25°C comprised a locally restricted, extensive overgrowth of wing (Fig. 6D,H), haltere and leg tissues (data not shown). In the following, we focus on the wing. Outgrowth in the wing is restricted to parts of the ventral surface. While the bulk of additional tissue induced by the $Gal4^{540.3}$ activator line protrudes on the ventral

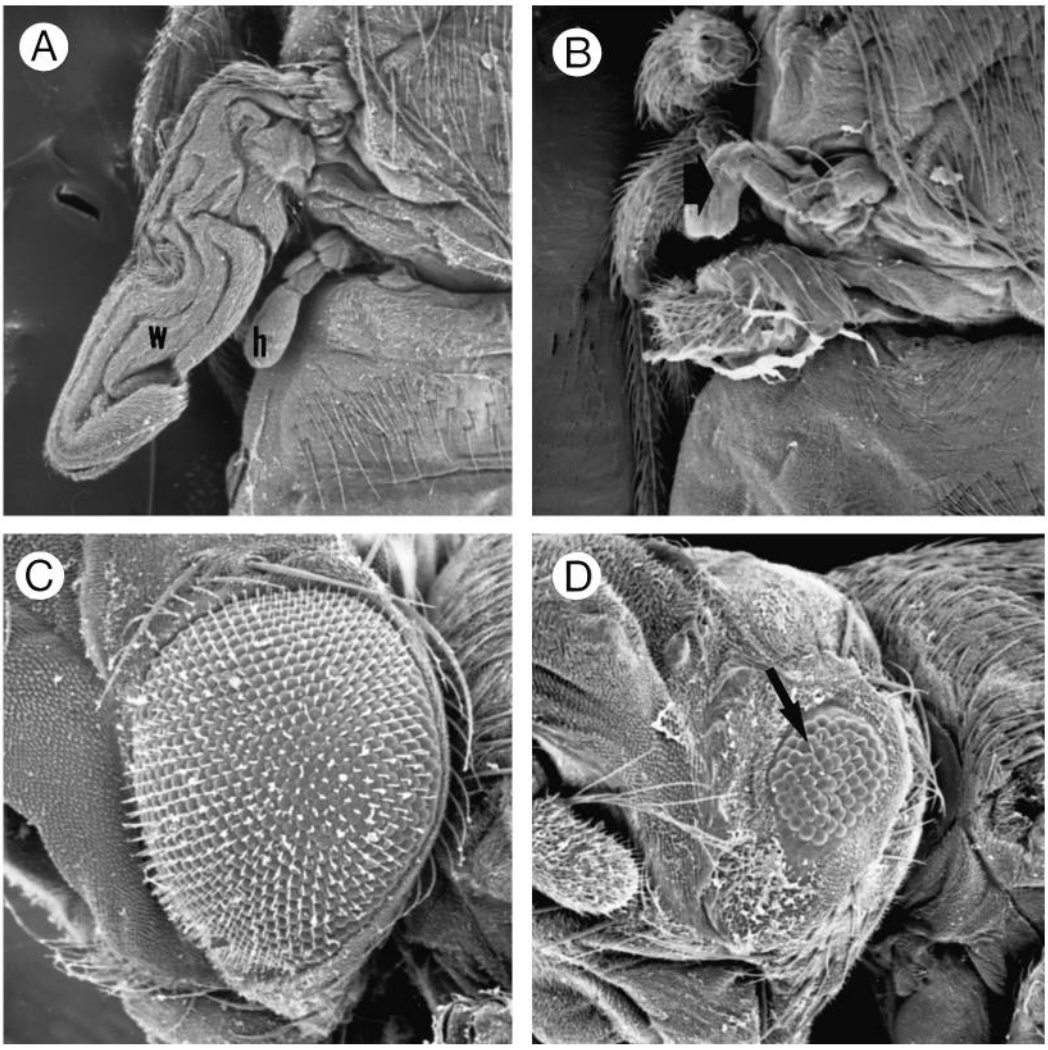

Fig. 3. Scanning electron micrographs of wild-type and homozygous *Ser*$^{-}$ pharate adults. Wild-type (A,C) and mutant (B,D) pharate adults were dissected from their pupal cases at 4 days after puparium formation. A and B show dorsal views of the left side of the thorax and part of the abdomen. The wing (w) in the wild-type is still folded and the haltere (h) is clearly visible. In the mutant, only a rudimentary wing (arrow) can be detected, while the haltere is absent. C and D show the compound eyes of a wild-type and a mutant fly, respectively. The eye phenotype of the mutant is variable; this represents the most extreme case, in which the eye consists only of a few ommatidia, which are irregularly arranged and occasionally fused (arrow), resulting in a rough appearance. The bristles in the eye are reduced in number.

surface close to the anterior hinge region (Fig. 6D), the additional wing tissue obtained with the Gal4$^{559.1}$ activator line is found between the third and the fourth wing vein, usually forming a large blister on the ventral wing blade (Fig. 6H). In general, the additional tissue is accompanied by rows or patches of bristles.

The phenotypes observed in the wings can be unambiguously related to the expression patterns and morphological defects in imaginal discs of mutant third instar larvae. Ectopic expression of Serrate in a stripe along the anterior-posterior compartment boundary of the wing-thoracic disc (Gal4$^{559.1}$ line; Fig. 6F) caused drastic extension of the wing disc along the proximodistal axis (Fig. 6G). The endogenous Serrate expression, which is still detectable in the anlage of the wing margin (compare Fig. 6A with Fig. 6G) allowed us to conclude that the primordia of the notum and the dorsal wing blade are of more or less normal proportions, while the primordium of the ventral wing surface is enlarged relative to the other two. This is in good agreement with the final wing phenotype, characterized by an outgrowth of mainly the ventral surface. In case of the Gal4$^{540.3}$ line, abnormal outgrowth of the disc can be correlated with a patch of strong expression that is consistently detected in the anterior part of the ventral wing pouch, explaining the wing phenotype described above (Fig. 6B,C).

The additional tissue obtained in the imaginal wing disc after ectopic expression of Serrate is composed of cells of normal size and regular epithelial morphology and shows no sign of the neoplasic overgrowth described for mutations in some of the tumor suppressor genes, e. g. *l(1)discs large* (Woods and Bryant, 1991). Furthermore, as judged by the density of the hairs (trichomes) on the wing blades, each of which corresponds to an individual cell (Dobzhansky, 1929), no difference in cell size was observed in the ectopic tissue versus the wild-type wing tissue. This leads us to conclude that overgrowth is mainly the result of an increased rate of cell proliferation rather than of cell growth.

Pupal lethality and the severity of overgrowth after ectopic expression of Serrate was strictly temperature-dependent and was greatly reduced at 18°C in comparison to 25°C. With the Gal$^{540.3}$ activator line in particular, the overgrowth phenotype was almost entirely absent and only consistently spread wings (held at an angle of up to 90°) still point to slight defects in the hinge region. We took advantage of this temperature sensitivity to determine the phenocritical period for these effects. Animals raised at 18°C and shifted to the restrictive temperature (25°C) 6 days or less before eclosion did not show an overgrowth phenotype. However, shifting one day earlier resulted in severe hyperplasia in many cases. The reverse shift experi-

ment produced no mutant phenotype when animals were raised for up to 3 days after egg laying at 25°C, whereas one more day at the restrictive temperature usually resulted in a strong phenotype. From these data, we conclude that the period sensitive for the induction of hyperplasic growth of wings by ectopic expression of Serrate lies between mid-second and mid-third larval instar.

DISCUSSION

Results presented in this paper lead to the conclusion that *Ser* has pleiotropic functions during larval stages. The reduced

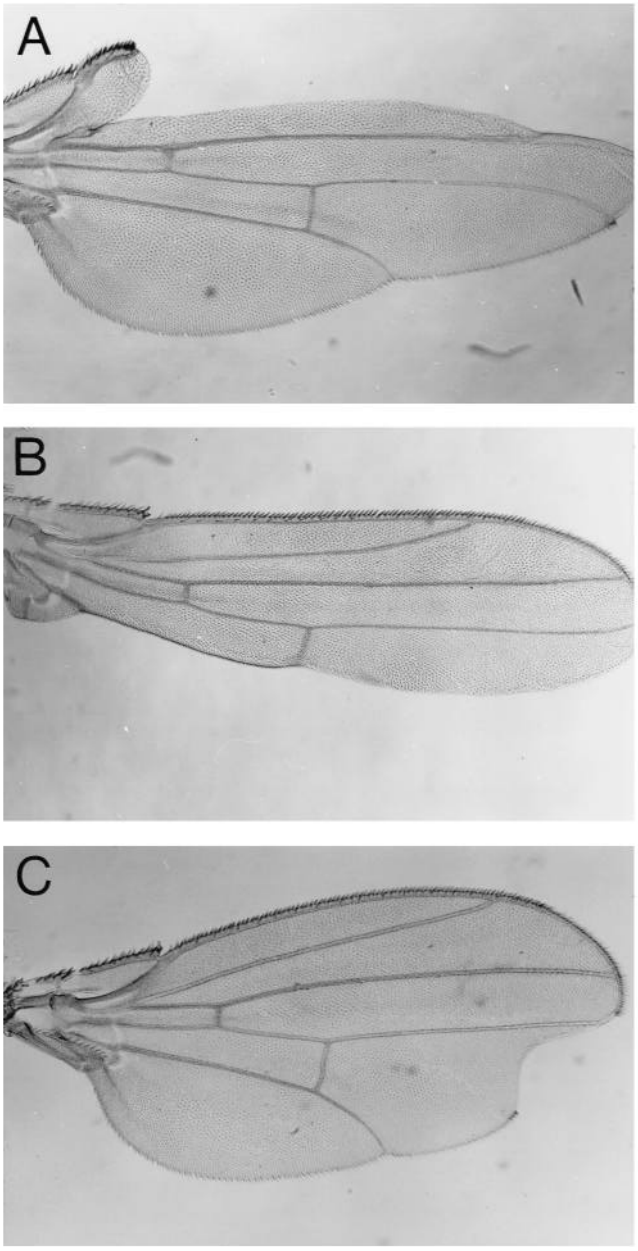

Fig. 4. *Ser*$^{-}$ clones in the wing produced by mitotic recombination. *Ser*^{Rx82} clones were induced in the first (A,B) or second (C) larval instar. They appear at various sites of the wing and the extent of scalloping depends on the time of irradiation.

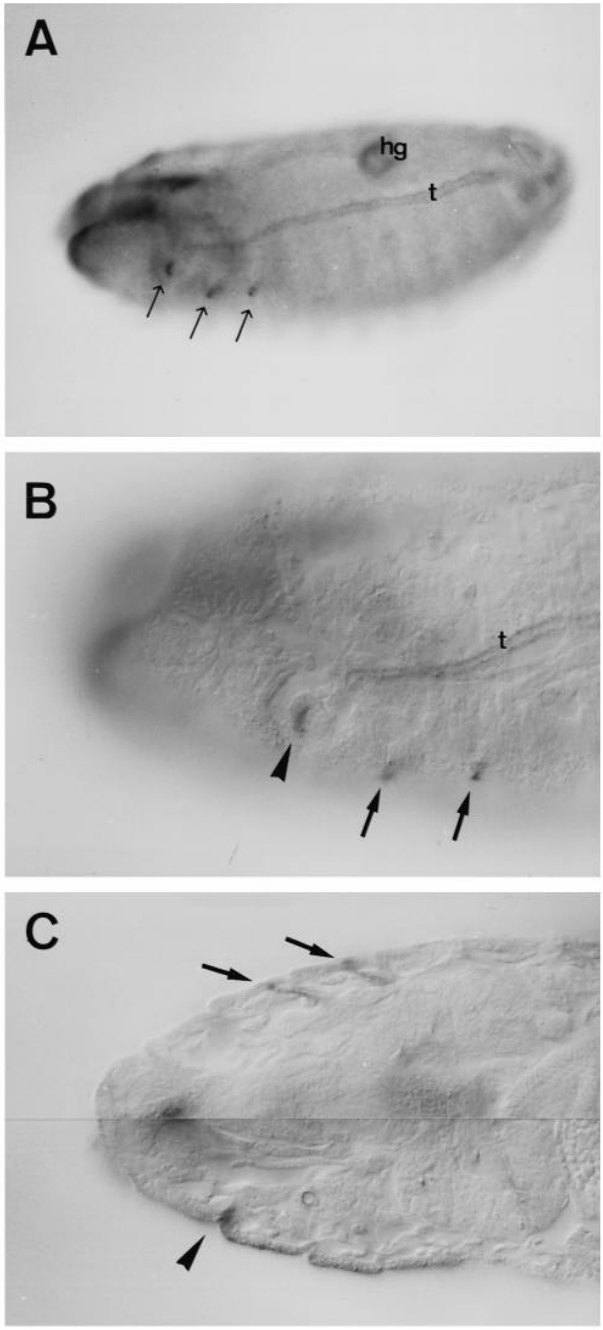

Fig. 5. Whole-mount staining of embryos with α-Serrate antibody. (A) Dorsolateral view of a stage 15 embryo. Strong Serrate expression can be detected in the tracheal trunk (t), in part of the hindgut (hg; out of focus) and in three clusters of cells in each of the thoracic segments (arrows), which, based on their position, represent the humeral, wing and haltere discs, respectively. (B) Higher magnification of the anterior region of an embryo of the same stage as in A. In addition to the wing and haltere discs (arrows), the antibody also stains the humeral disc (arrowhead), which lies anteriorly and slightly dorsally to the other two and is localized in T1, immediately below the end of the tracheal trunk, where the anterior spiracle will develop. (C) Combined horizontal optical sections of a stage 17 embryo. This photograph depicts the extension of the wing and haltere disc into the next posterior segment (arrows; described by Bate and Martinez Arias, 1991) and the association of the humeral disc with the anlage of the anterior spiracle (arrowhead). Stages according to Campos-Ortega and Hartenstein (1985). In A-C, anterior is to the left.

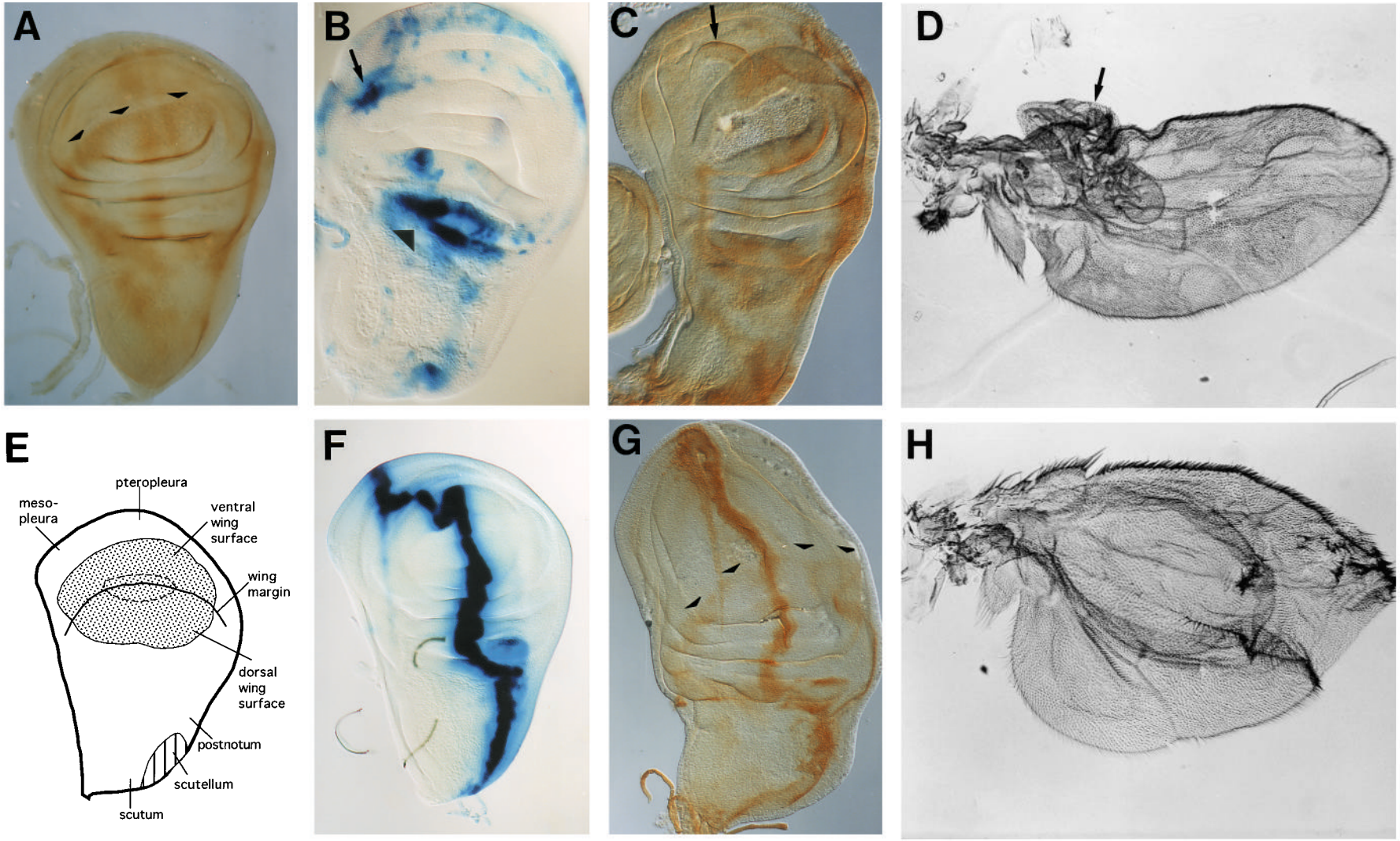

Fig. 6. Consequences of ectopic Serrate expression in the dorsal mesothoracic imaginal discs. The figure shows third instar wing discs stained for Serrate expression (A,C,G) or *lacZ* activity (B,F) and the corresponding wing phenotypes (D,H) in the activator lines Gal4$^{540.3}$ (B-D) and Gal4$^{559.1}$ (F-H). (**A**) A wild-type disc of a third instar larvae to document the endogenous Serrate expression. The wing margin is marked by arrowheads. (**B**) Within a dynamic pattern, consistently strong expression of the reporter gene can be observed in a patch of the prospective ventral wing blade (arrow) and the hinge region (arrowhead). (**C**) The phenotype of the disc resulting from ectopic Serrate expression. A protrusion of the anterior part of the ventral wing blade is clearly visible (arrow). (**D**) The ventral side of such a wing. In accordance with the disc phenotype, a mass of additional tissue appears in an anterior-proximal position (arrow). (**E**) Fate map of the wing imaginal disc according to Bryant (1975). (**F**) The Gal4$^{559.1}$ activator line drives *lacZ* expression in a narrow stripe along the anterior-posterior compartment boundary in the entire disc. (**G**) A disc expressing Serrate in a corresponding pattern is clearly enlarged. Using the endogenous expression of Serrate in the wing margin as marker (arrowheads), it is obvious that this extension is confined to the future ventral wing pouch. (**H**) As a consequence, the resulting wing exhibits a large blister due to enlargement of the ventral surface. Patches or rows of bristles can be observed.

viability of homozygous mutant larvae may be due to the fact that the anterior spiracles are absent or non-functional. Larval lethality might also be caused by subtle defects in the central nervous system, since the Serrate protein can be observed on a subset of axons in the embryo (Thomas et al., 1991). Aspects of the defects observed in larval stages can be correlated with the embryonic expression pattern, since expression of Serrate is observed in the anlagen of the anterior spiracles and the dorsal thoracic imaginal discs in the wild-type (Thomas et al., 1991; this work). The complex expression pattern in the embryonic epidermis, however, has no phenotypic counterpart in the mutant larvae.

The most obvious function of the wild-type *Ser* gene is to ensure correct morphogenesis of wing and haltere discs. However, lack of wings and halteres cannot account for pupal lethality, since other mutations causing similar defects are perfectly compatible with normal viability, e. g. some alleles of *apterous* (Wilson, 1981), *vestigial* (Williams et al., 1991) or *wingless* (Sharma and Chopra, 1976). Deletion of wings and halteres in *Ser* mutants never results in duplications of thoracic structures, indicating that the gene is not involved in the specification of wing or halteres versus thorax, or in the specification of the disc anlage per se, but is required for growth and/or differentiation of these structures. Thus the size of the mutant wing primordium is reduced with respect the entire disc, as is also observed in larvae homozygous for Ser^{Bd} (data not shown). The relative absence of cell death suggests that this is the result of reduced cell proliferation; this primordium later differentiates to a certain extent, giving rise to the rudimentary wing in Ser^{-} animals.

Results obtained from ectopic expression of *Ser* are in full agreement with the hypothesis that *Ser* is involved in the control of position-specific cell proliferation, since overgrowth is not induced in all sites with high levels of ectopic expression of Serrate. Thus, Serrate expression along the anterior-posterior compartment boundary of the entire dorsal thoracic disc or even ubiquitously (data not shown) induces overgrowth only in the ventral wing blade. The restricted responsiveness of tissues to ectopic Serrate further suggests that additional factors are required for proper function of *Ser*, which are expected to exhibit spatially restricted expression patterns, such as *wg* or *ap*, expression of which is restricted to the ventral compartment in the second larval instar or the dorsal compartment in the second and third larval instar, respectively (Couso et al., 1993; Williams et al., 1993; Cohen et al., 1992). The temperature-shift results further imply that the capacity to induce additional cell proliferation is temporally restricted to stages between mid-second and mid-third instar. The failure of ectopic expression in the embryo to provoke any defect can similarly be explained as being due to the absence of a suitable partner or of non-responsiveness of most of the cells.

Partners of Serrate may include the proteins encoded by *N* or *Dl*, two other members of the EGF-like protein family. Results of studies of genetic interactions between *N*, *Dl* and *Ser* point to close interactions between these genes (Thomas et al., 1991; de Celis et al., 1993). Indeed, Rebay et al. (1991) have shown that Serrate-expressing S2 cells are capable of forming aggregates with Notch-expressing cells via heterophilic adhesion. Participation of *N* in the control of wing growth was demonstrated by using a temperature-sensitive allele of *N*, N^{ts1} (Shellenbarger and Mohler, 1978). These authors showed that N^{ts1} flies develop small wings, partial wing margin deletions or notches, depending on the stage at which the larvae were subjected to the restrictive temperature. An active role in cell proliferation processes has also been discussed for the human (*TAN1*) and mouse *N* homologs in the context of the development of distinct forms of T-cell lymphoma and mammary tumours, respectively (Ellisen et al., 1991; Jhappan et al., 1992).

The importance of regional differences in mitotic activities for the control of pattern formation in the imaginal discs has already been suggested by the analysis of the clone sizes obtained by mitotic recombination in various parts of the adult epidermis (Garcia-Bellido and Merriam, 1971; Ripoll, 1972). Furthermore, analysis of regenerating tissues strongly supports a close correlation between cell proliferation and pattern formation in the imaginal discs. Several genes have been described, whose wild-type function is complementary to that of *Ser*: they restrict cell growth of imaginal discs and other imaginal tissues. Loss-of-function mutations in *fat* (Bryant et al., 1988), *lethal (2) giant discs* [*l(2)gd*; Bryant and Levinson, 1985] or $l(3)c43^{hs}$ (Martin et al., 1977), for example, result in overgrowth of imaginal discs. In contrast to the hyperplasia induced by ectopic expression of Serrate, however, overgrowth in these mutants is neither temporally nor spatially restricted and seems to reflect a general inability of the tissue to respond to signals controlling cell proliferation.

Proliferation in imaginal discs is thought to be stimulated when cells are confronted with neighbors carrying different positional values. Thus one has to postulate localized factors that are able to mediate interactions between the cells of a tissue in order to provide spatially restricted clues for growth induction or termination. During regeneration, for example, limited growth is induced in order to compensate for the discontinuity of positional values, and cell proliferation terminates when the missing values are intercalated (French et al., 1976). Whether ectopic Serrate expression gives rise to a discontinuity in positional values, which then induces local cell proliferation, is still unknown. The structural features of Serrate and its restricted function in a subset of imaginal discs makes it an attractive and amenable system for dissecting the various steps in this pattern formation process.

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