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 halters. 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 halters. 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 evaporinate 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 (Tepaš et al., 1990; Tepaš 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, SerD and SerBl, 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 SerR382 or SerR106 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 SerR106 and mwh red e SerR382. Eggs from the cross SerR382TM6 × Ki Sb M(3)wmTM2 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 Na2B4O7, 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 extra-membrane domain (s4566; Thomas et al., 1991).

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 SerD (Thomas et al., 1991). Some characteristics of these alleles and two previously known dominant alleles, SerD and SerBl, are summarized in Table 1. Whereas the gain-of-function mutation SerD 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 SerR382 and SerR120 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 SerR119 homozygotes die at

Table 1. Ser alleles used in this study

<table>
<thead>
<tr>
<th>Allele</th>
<th>Source</th>
<th>Cytology</th>
<th>Lethality</th>
<th>Reference</th>
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<tr>
<td>SerD</td>
<td>spontaneous</td>
<td>normal3</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>SerBd</td>
<td>spontaneous</td>
<td>normal3</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>SerR3</td>
<td>X-ray</td>
<td>DH(3R)97E7-11; 97F3-11</td>
<td>e4</td>
<td>b</td>
</tr>
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<td>X-ray</td>
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</tr>
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<td>b</td>
</tr>
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<td>X-ray</td>
<td>nd</td>
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<tr>
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<td>this work</td>
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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.

pupal stages, and pharate SerRx119 flies look morphologically normal. SerRx119 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 SerP 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: SerRx119 is viable over SerRd.

Besides SerRd and SerP, three of the revertants (SerRx82, SerRx106 and SerRx107) were characterized at the molecular level (Thomas et al., 1991). The polymorphisms mapped in SerRx82 and SerRx106 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 SerRx107. 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. SerRx3) 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.](image-url)
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<sup>Rx106</sup> or Ser<sup>Rx82</sup>, 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<sup>−</sup> 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<sup>−</sup> cells by neighboring heterozygous cells. This suggests that Ser is required autonomously in the cells of the developing wing.

**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 mini-gene downstream of Gal4-responsive UAS<sub>G</sub> elements. All behaved similarly in the experiments described below. To induce ectopic expression of the Ser mini-gene, we used activator lines Gal4<sup>540.3</sup> and Gal4<sup>559.1</sup>, 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<sup>540.3</sup> activator line protrudes on the ventral

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**Fig. 2** Histological sections of wing discs of late second instar larvae. Section through a Ser<sup>−</sup> (A) and ap<sup>56</sup>/disc (B). While dead cells are detectable in the ap disc (arrowheads), no cell death is visible in the Ser<sup>−</sup> disc.
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 neoplastic 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 Gal4^{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 hyperplastic 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. SerR82 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 Gal4540.3 (B-D) and Gal4559.1 (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 Gal4559.1 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 spec-
ification of wing or halteres versus thorax, or in the specifica-
tion 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 SerBd (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 compart-
ment 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 DI, two other members of the EGF-like protein family..
Results of studies of genetic interactions between N, DI and
Ser point to close interactions between these genes (Thomas et
have shown that Serrate-expressing S2 cells are capable of
forming aggregates with Notch-expressing cells via het-
rophic adhesion. Participation of N in the control of wing
growth was demonstrated by using a temperature-sensitive
allele of N, Nn1 (Shellenberger and Mohler, 1978). These
authors showed that Nn1 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 (TANI) and mouse N homologs in the
context of the development of distinct forms of T-cell
lymphoma and mammary tumours, respectively (Ellisen et al.,

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 (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 dis-
continuity of positional values, and cell proliferation termi-
nates when the missing values are intercalated (French et al.,
1976). Whether ectopic Serrate expression gives rise to a dis-
continuity 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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