Phenotypic and Molecular Characterization of Ser^D, a Dominant Allele of the Drosophila Gene *Serrate*

Ulrich Thomas,[1] Franziska Jönsson, Stephan A. Speicher and Elisabeth Knust

Institut für Entwicklungsbiologie, Universität zu Köln, 50923 Köln, Germany

Manuscript received April 1, 1994
Accepted for publication September 10, 1994

ABSTRACT

The Drosophila gene *Serrate* (*Ser*) encodes a transmembrane protein with 14 epidermal growth factor–like repeats in its extracellular domain, which is required for the control of cell proliferation and pattern formation during wing development. Flies hetero- or homozygous for the dominant mutation Ser^D exhibit scalloping of the wing margin due to cell death during pupal stages. Ser^D is associated with an insertion of the transposable element *Tirant* in the 3′ untranslated region of the gene, resulting in the truncation of the *Ser* RNA, thereby eliminating putative RNA degradation signals located further downstream. This leads to increased stability of *Ser* RNA and higher levels of Serrate protein. In wing discs of wild-type third instar larvae, the Serrate protein exhibits a complex expression pattern, including a strong stripe dorsal and a weaker stripe ventral to the prospective wing margin. Wing discs of Ser^D third instar larvae exhibit additional Serrate protein expression in the edge zone of the future wing margin, where it is normally not detectable. In these cells expression of wing margin specific genes, such as *cut* and *wingless*, is repressed. By using the yeast Gal4 system to induce locally restricted ectopic expression of Serrate in the edge zone of the prospective wing margin, we can reproduce all aspects of the Ser^D wing phenotype, that is, repression of wing margin–specific genes, scalloping of the wing margin and enhancement of the *Notch* haplo-insufficiency wing phenotype. This suggests that expression of the Serrate protein in the cells of the edge zone of the wing margin, where it is normally absent, interferes with the proper development of the margin.

PATTERN formation in the wing imaginal discs requires the regulated activities of many genes, which control proliferation, cell fate determination and morphogenesis (for reviews see SHEARN 1978; WHITTLE 1990; WILKINS and GUBB 1991; GARCIA-BELLIDO and DE CELIS 1992; COHEN 1993; WILLIAMS and CARROLL 1993; *Vis. cit* WILLIAMS *et al.* 1993). One of the genes involved is *Serrate* (*Ser*), which encodes a transmembrane protein with 14 epidermal growth factor (EGF)-like repeats in its extracellular domain (FLEMING *et al.* 1990; THOMAS *et al.* 1991). Besides having other functions in the larvae, *Ser* is required for the control of position-specific cell proliferation in the meso- and metathoracic dorsal appendages of the adult: loss-of-function mutations result in the nearly complete lack of the wing and halteres. This is due to lack of cell proliferation in the imaginal discs rather than to cell death and leaves the notal portions of the discs unaffected. Conversely, ectopic expression of *Ser* leads to spatially and temporally restricted induction of additional cell proliferation, which suggests that *Ser* may provide important clues for patterning the imaginal discs (SPEICHER *et al.* 1994). In fact, *Ser* is expressed on the dorsal side of the wing disc of second instar larvae and is, in conjunction with *wingless* (*wg*), required to induce the expression of *vestigial* (*vg*) at the dorsal/ventral compartment boundary (J. P. COUSO, S. B. CARROLL, E. KNUST and A. MARTINEZ-ARIAS, unpublished results). The dominant allele Ser^D (used as marker on several third chromosomal balancers) is homozygous viable and produces scalloping of the wing margin, which is more severe in homozygous than in heterozygous condition (BELT 1971; FLEMING *et al.* 1990; THOMAS *et al.* 1991) (Figure 1). This dominant phenotype is not a result of haplo-insufficiency, because a deficiency in *trans* with the wild-type gene does not induce this phenotype (THOMAS *et al.* 1991).

Scalloping of the wing margin is produced by mutations in many genes, *e.g.*, *cut* (*ct*) (JOHNSON and JUDD 1979), *Beadex* (*Bx*) (FRISTROM 1969; SANTAMARIA and GARCIA-BELLIDO 1975), *Notch* (*N*) (SHELLENBARGER and MOHLER 1978), *Lyra* (*Ly*) (ABBOT 1986) and *scalloped* (*sd*) (JAMES and BRYANT 1981; SIMPSON *et al.* 1981) and is generally due to local cell death. The wing margin includes the dorsal/ventral compartment boundary, which is established by the end of the first instar as revealed by clonal analysis (GARCIA-BELLIDO *et al.* 1976) and is reflected by the expression of the gene *apterous* (*ap*) (BLAIR 1993; DIAZ-BENJUMEA and COHEN 1993). *ap* expression is restricted to the dorsal compartment from early second instar on (COHEN *et al.* 1992;

Corresponding author: Elisabeth Knust, Institut für Entwicklungsbiologie, Universität zu Köln, 50923 Köln, Germany.

[1] *Present address:* Institut für Neurobiologie, Abt. Neurochemie und Molekularbiologie, Brennecke Str. 6, 39118 Magdeburg, Germany.

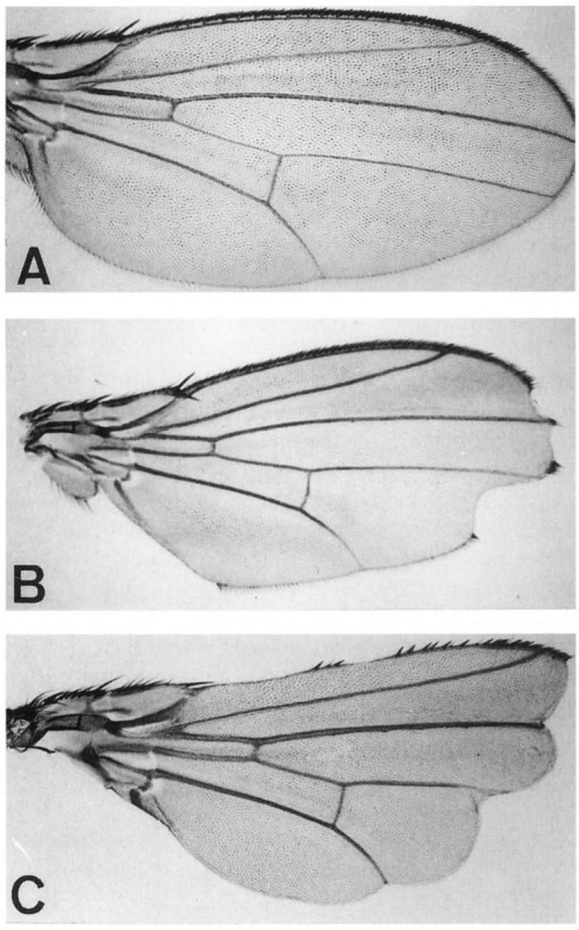


FIGURE 1.—Wing phenotypes of wild-type (A), $Ser^D/+$ (B) and Ser^D/Ser^D (C) flies. Note that there is thickening of the third and, less pronounced, the fifth wing vein visible in C, a phenotypic trait also characteristic for *N* alleles. In the homozygous condition, nearly all hairs on the posterior wing margin and most of the bristles on the anterior margin are missing.

WILLIAMS *et al.* 1993), and the border between *ap*-expressing and *ap* nonexpressing cells acts as an organizing center for the whole disc (BLAIR 1993; DIAZ-BENJUMEA and COHEN 1993) by inducing the expression of *vestigial* (WILLIAMS *et al.* 1994).

Differentiation of the wing margin itself starts in the middle of the third instar; at this time, cells of the margin are morphologically distinct from the surrounding tissue (BROWER *et al.* 1982) and exhibit a distinct proliferation behavior ("zone of nonproliferating cells") (O'BROCHTA and BRYANT 1985; SCHUBIGER and PALKA 1987). The differentiation of the marginal structures depends on the development of a stripe of a few cells, which express *wingless* and *cut* (BLAIR *et al.* 1993; BLOCHLINGER *et al.* 1993; called the "edge" zone by COUSO *et al.* 1994). During third instar and early pupal stages, structures characteristic for the wing margin develop, *i.e.*, a triple row of sensory organs anteriorly, a double row of sensory organs at the tip and noninnervated bristles on the posterior wing margin (HARTENSTEIN and POSAKONY 1989; CUBAS *et al.* 1991). The loss of *wg* function during third larval instar leads to the failure to establish the edge properly and results in the complete lack of sensory organs (PHILIPPS and WHITTLE 1993; COUSO *et al.* 1994).

To understand the wing phenotype of Ser^D flies, we studied this allele both phenotypically and at the molecular level. We show that the mutation is caused by the insertion of a retrotransposon in the 3′ untranslated region of the gene, which increases the stability of *Ser* RNA and, consequently, allows synthesis of more protein. We also present evidence to show that, probably due to the longer half-life of the RNA, Serrate protein can be detected in the edge zone of the wing margin in third instar larvae, at a time when it is normally not detectable in these cells. We discuss this result with respect to the development of wing notches and the function of *Ser* for the differentiation of the margin.

MATERIALS AND METHODS

Drosophila stocks: Flies were grown on standard medium and crosses were performed at room temperature or at 25°. Descriptions of balancer chromosomes and markers can be found in LINDSLEY and ZIMM (1992). Oregon R was used as wild-type stock. The following *Ser* and *N* alleles were used: Ser^D (THOMAS *et al.* 1991; LINDSLEY and ZIMM 1992) and N^{55e11} (LINDSLEY and ZIMM 1992). Also used were $Gal4^{459.2}$, a Gal4-expressing line characterized in an enhancer trap screen (kindly provided by U. HINZ); UAS-*Ser*, a transgenic line carrying a *Ser* minigene (mg5603) under the control of five UAS-elements (UAS_G), the binding sites for Gal4 (SPEICHER *et al.* 1994); *ctwHZ-2*, a transgenic line carrying a reporter *lacZ* gene, which expresses β-galactosidase under the control of the wing margin-specific enhancer of *cut* (*ct*) (JACK and DELOTTO 1992; kindly provided by J. JACK); *CyO*, wg^{en11} is a transgenic line carrying a *lacZ* reporter gene on the balancer, which expresses β-galactosidase under the control of the *wingless* promoter (SIEGFRIED *et al.* 1992; kindly provided by A. MARTINEZ-ARIAS) and *vg-lacZ*, a transgenic line carrying the *lacZ* reporter gene expressed under the control of the wing margin-specific enhancer of *vestigial* (*vg*) (WILLIAMS *et al.* 1994; kindly provided by S. B. CARROLL).

Standard DNA techniques: Restriction site mapping, Southern blotting, subcloning, library screening by colony hybridization with ^{32}P-labeled probes and isolation of genomic DNA were carried out essentially as described by SAMBROOK *et al.* (1989). Gravity flow columns from QIAGEN were used for plasmid DNA preparation according to the instructions of the manufacturers.

Construction of a partial genomic library in a plasmid vector: About 200 μg of genomic DNA from homozygous Ser^D flies was digested with *Hinc*II and subsequently size fractionated by centrifugation in a sucrose gradient (SW 41 rotor, 12 hr, 30,000 rpm, 20°). The gradient was prepared as follows: SW 41 tubes were filled with 10 ml 12.5% sucrose in 0.2 M NaCl, 2 mM EDTA and 20 mM Tris-HCl (pH 7.5), frozen overnight at −70° and thawed at room temperature shortly before centrifugation. After centrifugation, fractions of 400 μl each were collected from the bottom of the tube by means of a syringe. Fractions enriched for DNA fragments of between 1.4 and 2.0 kb were pooled, diluted and the DNA was

precipitated. After resuspension, the DNA was digested with *Bam*HI, ligated into Bluescript KS^{M13+} vector (linearized with *Sma*I and *Bam*HI) and transformed into *Escherichia coli* DH5α. Among more than 6000 colonies, 1 (g9922) was found to hybridize with a 1.8-kb *Hinc*II-*Eco*RI cDNA fragment containing the last exon of *Ser*.

Sequencing and sequence analysis: A set of overlapping subclones of the clone g9922 containing genomic DNA of Ser^D was generated in the Bluescript vector using various restriction enzymes. The complete sequence was determined by dideoxy chain-termination reactions (SANGER *et al.* 1977), using the T7 polymerase (Pharmacia) and following the Pharmacia protocol. Besides the T3, T7, SK and KS primers specific for Bluescript sequences, we also used two *Ser*-specific oligonucleotides for priming. The *Fast*-A program (LIPMAN and PEARSON 1985) was used to search the EMBL databank for homologous sequences.

***In situ* hybridization and immunohistochemistry:** *In situ* hybridizations to wholemount embryos were performed with digoxygenin-labeled probes essentially as described by TAUTZ and PFEIFLE (1989). For antibody staining, imaginal discs were fixed for 20 min in 4% formaldehyde/phosphate-buffered saline (PBS), washed three times in PBS/1% Triton and once in PBS/1% Triton/2% goat serum and incubated overnight at 4° with the primary antibody [mouse monoclonal anti-β-galactosidase antibody (Boehringer) at dilutions of 1:500 to 1:1000 or affinity-purified rabbit anti-Serrate serum directed against the extracellular domain (R4566; 1:10)]. After three washes and blocking with PBS/1% Triton/2% goat serum, discs were incubated for 2 hr at room temperature with rhodamine-coupled goat anti-rabbit or fluorescein-coupled anti-mouse secondary antibodies (Boehringer). In some cases, double stainings with both the Serrate specific antibody and the antibody against β-galactosidase were performed. The primary antibodies were distinguished by using fluorescein- or rhodamine-coupled secondary antibodies.

In addition to the polyclonal affinity-purified α-SER serum directed against part of the extracellular domain (s4566, from rabbit) (THOMAS *et al.* 1991), a polyclonal mouse serum was raised against part of the cytoplasmic domain (s3865). For this purpose, a TrpE fusion protein containing amino acids 1232 to 1408 of Serrate was expressed in *E. coli* (SPINDLER *et al.* 1984). After partial purification (RIO *et al.* 1986), ~5 μg of the fusion protein was emulsified with Freund's complete adjuvant for the initial immunization of Balb/c mice and with Freund's incomplete adjuvant for all further boosts. The presence of *Ser*-specific antibodies was revealed by the typical *Ser* expression pattern after immunostaining of wholemount embryos.

We used the *ctwHZ-2* line (JACK and DELOTTO 1992), the *CyO, wg*$^{en11\text{-}lacZ}$ line (SIEGFRIED *et al.* 1992) and the *vg-lacZ* line, which express β-galactosidase under the control of wing margin-specific regulatory elements of the *cut, wingless* and *vestigial* promoter, respectively. Staining for *lacZ* activity was performed essentially as described by WAGNER-BERNHOLZ *et al.* (1991). Discs were dissected in PBS and fixed in 1% glutaraldehyde and washed three times for 20 min in phosphate-buffered Tween (PBT). Staining buffer (10 mM phosphate buffer, pH 7.2, 150 mM NaCl, 1 mM $MgCl_2$, 3 mM K_4[FeII(CN)$_6$] × 3 H_2O, 3 mM K_3[FeIII(CN)$_6$], 0.3% Triton X-100) was prewarmed to 37°, 1:40 vol 10% X-Gal stock solution (in DMSO) was added and the discs were incubated. After washing in PBT and PBT/50% glycerol, discs were mounted in 100% glycerol.

Induction of ectopic Serrate expression: We used an *Ser* minigene (mg5603), which was cloned downstream of five UAS_G sequences (upstream activating sequences; binding sites for the yeast transcription factor *Gal4*), a hsp70 TATA box and a transcriptional start site (for detailed description see SPEICHER *et al.* 1994). For the induction of ectopic expression of the *Ser* minigene, flies bearing the UAS_G-*Ser* construct ("effector" strain) were crossed to flies that carried a transgene for the yeast transcription factor Gal4 ("activator" stock). Ectopic expression is only obtained in progeny carrying both the gene encoding the yeast transcription factor Gal4 and the gene of interest (in our case *Ser*) (BRAND and PERRIMON 1993).

Acridine orange staining: We used essentially the protocol described by MASUCCI *et al.* (1990). Imaginal discs were dissected from wandering third instar larvae or prepupae in Drosophila Ringer solution (128 mM NaCl, 2 mM KCl, 35.5 mM sucrose, 5 mM HEPES, 1.8 mM $CaCl_2$, 4 mM $MgCl_2$, pH 7.1). They were stained for 5 min in acridine orange (1×10^{-6} M) in Ringer solution. After briefly washing three times in Ringer solution, the discs were mounted in Ringer and viewed immediately by fluorescence microscopy.

RESULTS

Molecular characterization of Ser^D: *Partial cloning of the mutant gene:* The only aberration within the genomic region of Ser^D detected by Southern blot analysis consists in a 5.5-kb insertion within the last exon (FLEMING *et al.* 1990; THOMAS *et al.* 1991). This exon encodes amino acids 1158–1408 of the Serrate protein and includes 901 bp of 3′ untranslated sequences (THOMAS *et al.* 1991). For a more detailed analysis of this region, we cloned a 1.5-kb *Bam*HI-*Hinc*II fragment from a partial plasmid library constructed from DNA of homozygous Ser^D flies. Sequencing of this fragment, which contained part of the insertion together with preceding *Ser* sequences, allowed us precisely to map the insertion site 81 bp downstream of the translational stop codon (Figure 2A). The preceding 1024 bp are identical to the corresponding wild-type sequence, except from a single base pair exchange within the last 117 bp intron. In addition, the cloned fragment carries 505 bp of insertion sequence (Figure 2B). According to results obtained from a homology search in the EMBL Data Bank, the insertion is a representative of the so-called "Tirant" element, a middle repetitive element first described in the context of the dominant mutation *Achaetous* of the gene *extramacrochaetae* (*emc*) (GARRELL and MODOLELL 1990). Short sequences of 152 and 122 bp from the two termini of this element were previously published, and both sequences appear within the 505-bp sequence presented here. As depicted in Figure 2A, the finding is compatible with the assumption that *Tirant* contains long terminal repeats (LTRs), which themselves terminate in inverted repeats of 5 bp. Thus, *Tirant* can be regarded as a typical retrotransposon (SMITH and CORCES 1991).

Expression of Ser RNA in the mutant: Based on these data, a transcript with an altered 3′ untranslated region would be expected in the case of Ser^D. As shown by *in situ* hybridizations carried out with different *Tirant*-

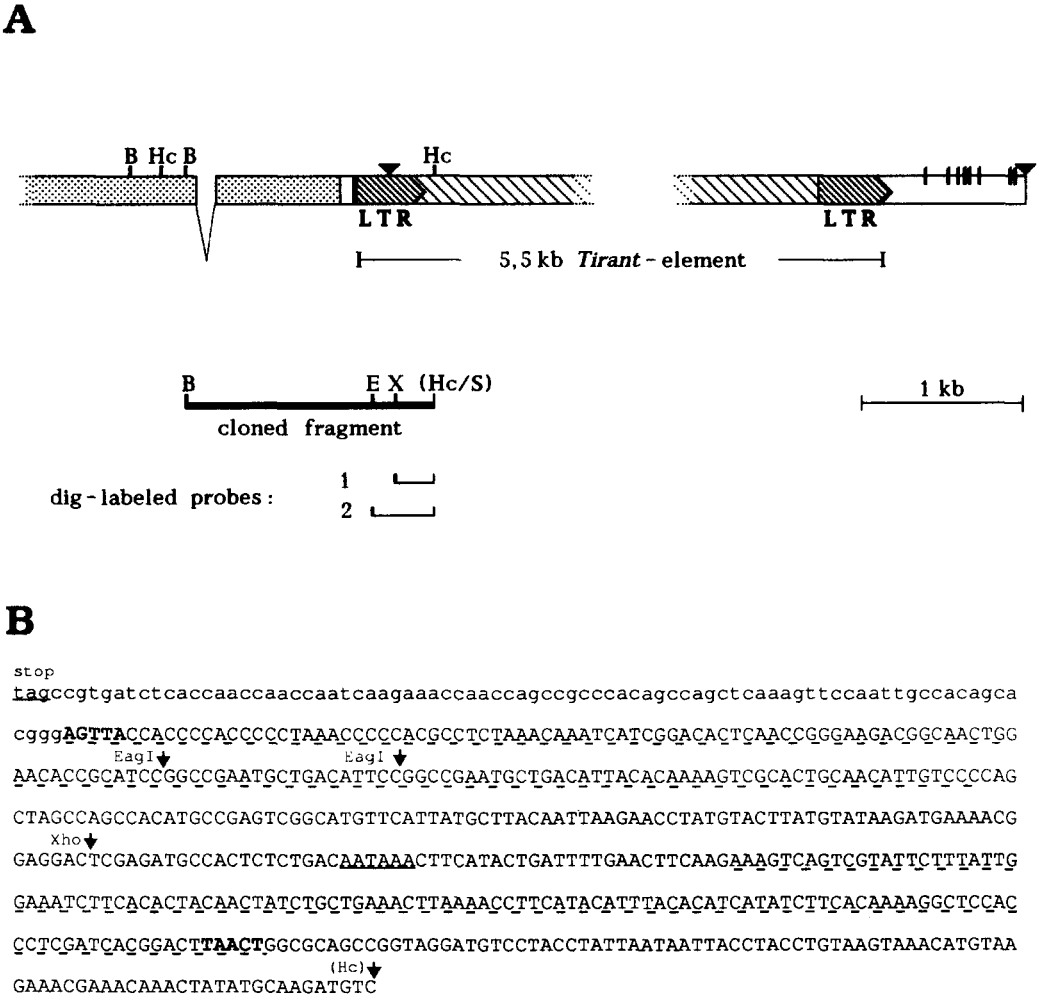


FIGURE 2.—Molecular characteristics of Ser^D. (A) Schematic representation of the genomic region affected in Ser^D. As deduced from sequence analysis of the cloned fragment, the *Tirant* element is inserted 81 bp downstream of the translation stop codon. (▨) Coding sequence within the last two exons of *Ser*; (□) (on both sides of the insertion) the 3′-UTR of the *Ser* gene, interrupted by the insertion. Potential polyadenylation signals in the 5′-LTR and at the end of the wild-type trailer are marked by triangles. Bold vertical dashes indicate the clustered RNA degradation motifs $ATTT_nA$, which are eliminated from the Ser^D transcript. Subfragments nos. 1 and 2 were used for *in situ* hybridizations described in Figure 3. Restriction sites are abbreviated as follows: B, *Bam*HI; E, *Eag*I; Hc, *Hinc*II; S, *Sma*I; X, *Xho*I. (B) Partial sequence of Ser^D. The inserted *Tirant* element of 505 bp is shown in capital letters, preceded by 84 bp of *Ser* 3′-UTR (starting with the translation stop codon; small letters). Within the first 416 bp of the insertion, two stretches of DNA are indicated by dashed lines, each of which is identical to one of the terminal sequences of *Tirant* published previously (GARRELL and MODOLELL 1990). This leads us to conclude that *Tirant* carries 416-bp LTRs terminating with 5 bp inverted repeats (bold letters). The identification of the insert as *Tirant* was further confirmed by mapping *Sal*I and *Eco*RI sites at the appropriate locations.

specific probes, termination of transcription within the LTR could indeed be demonstrated in Ser^D embryos, whereby the hexanucleotide AAUAAA within the LTR probably serves as polyadenylation signal (Figure 2B). Using a probe that contained only sequences of the *Tirant* element flanking both sides of the polyadenylation signal (*Eag*I-*Hinc*II fragment; probe no. 2 in Figure 2A), a *Ser*-specific expression pattern was observed in Ser^D but not in wild-type embryos (Figure 3, A and B). Moreover, this pattern was hardly detectable with a subfragment of this probe (*Xho*I-*Hinc*II fragment; probe no. 1 in Figure 2A), which mostly contains LTR sequences 3′ of the putative polyadenylation signal (data not shown), confirming that termination of transcription occurs within the LTR sequence. Using either of these probes, strong background hybridization was consistently detected in embryos of both genotypes, which probably reflects the sum of transcription from several *Tirant* elements scattered in the genome. A particularly strong expression was detected in the anlagen of the gonads (Figure 3B), apparently restricted to one sex, because it occurred in only ~50% of the embryos. The high expression level of this transposon in the anlage of the gonads is reminiscent of the expression of the

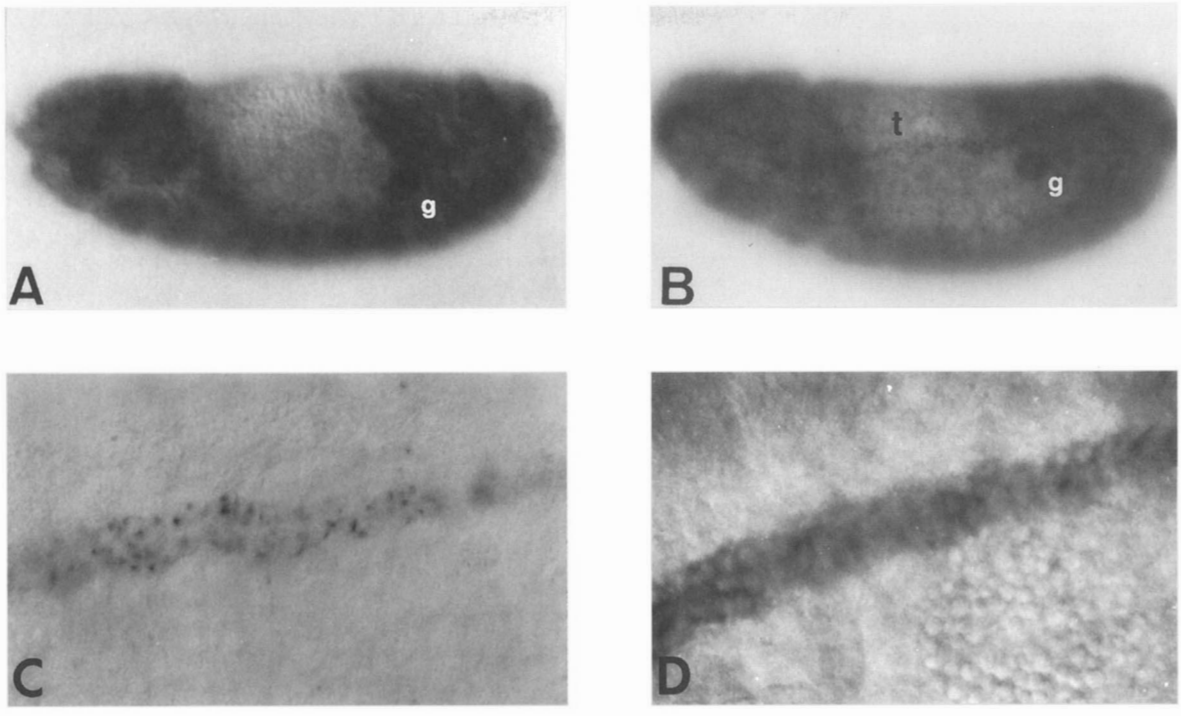


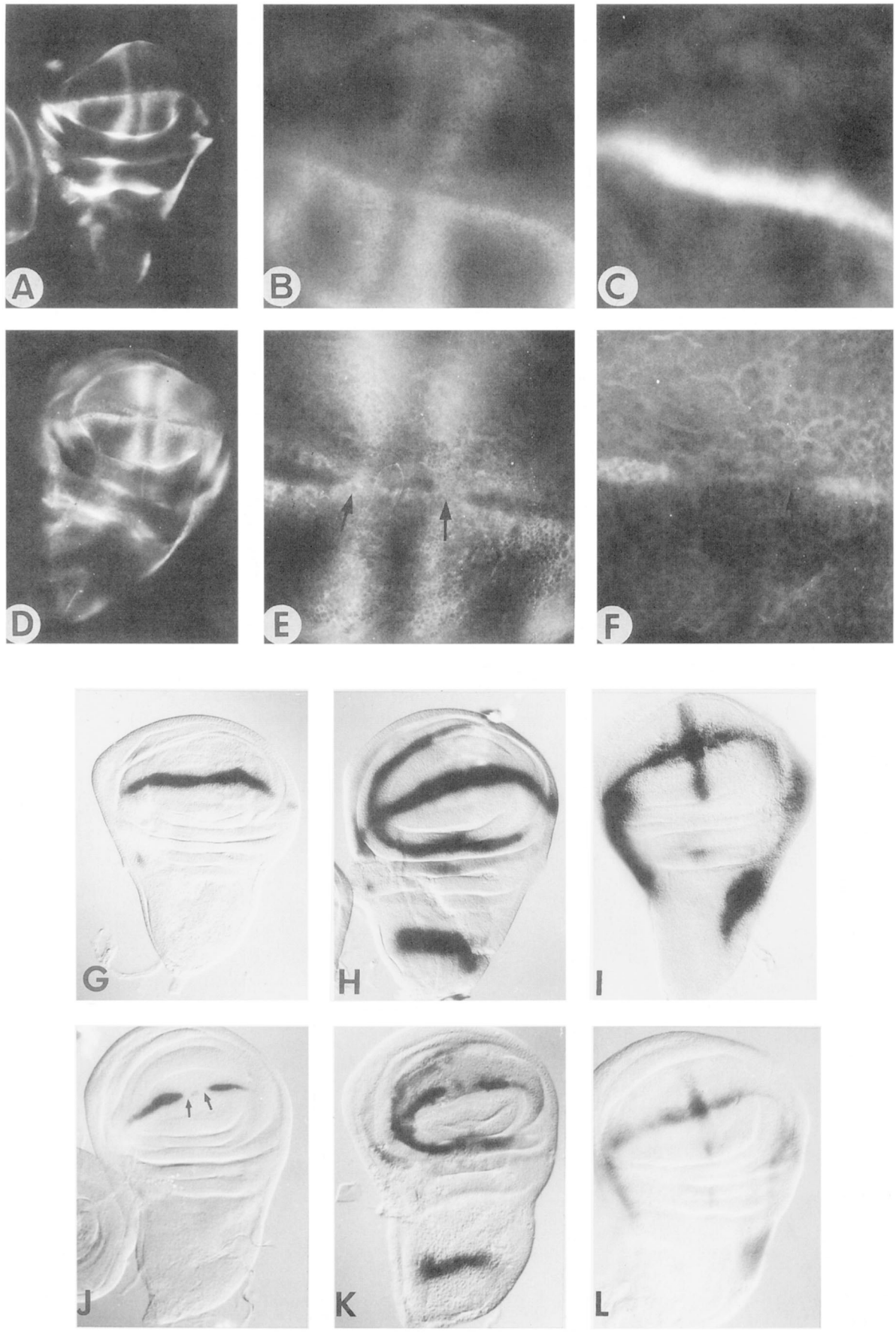
FIGURE 3.—*In situ* hybridizations with *Tirant*- and *Ser*-specific probes. Using probe no. 2 (see Figure 2A), which contains sequences of the *Tirant* element located 5′ and 3′ of the poly(A) site of the LTR, a *Ser*-specific expression pattern was revealed in Ser^D (B) but not in wild-type embryos (A). The difference is best (though not exclusively) detectable in the main tracheal stems (t). Both in wild-type and Ser^D embryos, the reproducible background staining probably reflects the overlapping transcription domains of various *Tirant* elements present in the genome. The prominent expression in the anlagen of the gonads (g) seems to be sex-specific, because only ~50% of the embryos exhibited this staining. (C and D) *In situ* hybridizations with a *Ser*-specific probe to wild-type (C) and Ser^D (D) embryos to document the higher stability of the *Ser* RNA in the mutant. Shown are parts of the main tracheal stems. Although the nuclear staining in both embryos is comparable, the intensity of cytoplasmic staining is significantly higher in the mutant (D) than in wild-type (C).

412 retrotransposon, which is expressed in the gonadal mesoderm (BROOKMAN *et al.* 1992).

As a consequence of the premature termination of the *Ser* transcript, the AU-rich trailer sequence of the *Ser* message, which contains a 600-bp region with eight potential RNA degradation signals ($AU(U)_nA$) (SHAW and KAMEN 1986; JONES and COLE 1987; SHYU *et al.* 1989), is missing in the mutant version of the transcript; hence, the truncated transcript should have a longer half-life than the wild-type RNA. This assumption was confirmed by a series of *in situ* hybridizations and immunohistological stainings performed in parallel on wild-type and mutant tissues. The levels of both *Ser* RNA and protein are considerably increased in homozygous Ser^D embryos and imaginal discs (see below). As judged from the staining intensity, expression of the mutant gene results in much more than twice the amount of protein in comparison with the wild type. The spatial patterns of expression were essentially the same in wild type and mutant tissues at any given stage of development (see below). As in the wild type, the protein is associated with the membrane but can also be detected in intracellular vesicles, which, however, often appear larger in the mutant than in the wild type (THOMAS *et al.* 1991).

To test whether the observed overexpression is in fact caused by increased mRNA stability rather than by an increased transcription rate, a genomic fragment including exon as well as intron sequences was used for *in situ* hybridizations (map units 0–3.5 according to THOMAS *et al.* 1991). This allowed us to discriminate between nascent transcripts (reflected by nuclear staining) and mRNA in the cytoplasm (revealed by staining at the cell borders). Whenever wild-type and Ser^D tissues showed comparable nuclear staining, the intensity of cytoplasmic staining was always higher in the mutant (Figure 3, C and D). On the other hand, tissues with comparable staining at the membrane always gave more intense staining in the nuclei of wild-type than of Ser^D tissues. This led us to conclude that the increased abundance of *Ser* RNA in Ser^D cells is the result of greater stability of the transcript, and not due to a higher transcription rate.

Developmental analysis of Ser^D: Serrate protein in wing imaginal discs of early and mid-third instar wild-type larvae can be detected in two stripes running parallel to the anterior-posterior compartment boundary, which, based on the examination of older discs, correlate with the future third and fourth wing veins, respectively, whereas staining in the prospective fifth vein is


A
B
C
D
E
F
G
H
I
J
K
L

of lesser intensity (Figure 4A). These stripes become more prominent in the evaginating disc of early prepupae (Figure 5A). In addition, Serrate-positive cells in the wild type can be detected in two stripes four to five cells wide, situated dorsal and ventral to the prospective wing margin. Staining of the dorsal stripes is much more intense than in the ventral. The location of these stripes with respect to the wing margin was determined by simultaneously analyzing the expression of *cut* and *Ser* in the same disc. Expression of *cut* was monitored by means of a *lacZ* line (*ctwHZ-2*), in which β-galactosidase expression is under the control of the wing margin–specific enhancer of *cut* (*ct*) (JACK and DELOTTO 1992), *Ser* expression by a *Ser*-specific antibody. In the wing discs of transgenic *ctwHZ-2* third instar larvae, a band, four to five cells wide, in the prospective margin expresses β-galactosidase from the third instar stage until 24 hr after puparium formation (Figure 4, B and C), which correlates well with the expression pattern of the cut protein (JACK *et al.* 1991; BLOCHLINGER *et al.* 1993; our own observations); this region defines the so-called "edge" zone, the proper development of which is crucial for patterning of the wing margin (BLAIR 1993; BLOCHLINGER *et al.* 1993; COUSO *et al.* 1994). The stripe of *cut*-expressing cells is flanked on the dorsal and ventral sides by Serrate expressing cells, whereas the cells in the edge itself seem to express very low amounts of Serrate, if at all (Figure 4, B and C).

Wing imaginal discs prepared from early and mid-third instar homozygous Ser^D larvae exhibit normal morphology. Two differences with respect to the spatial expression pattern of Serrate are particularly striking. First, the staining is much more intense in the mutant than in the wild type, which is in agreement with the conclusion drawn from the transcriptional analysis of *Ser* in the mutant (see above; *cf.* Figure 4, A and B with D and E). The second, and probably more important, difference between wild type and Ser^D is that in Ser^D discs, Serrate protein is found in cells of the distal portion of the edge zone; we never detected any staining at this location in the wild type (Figure 4, D and E, arrows). However, the distal-most cells of the edge in Ser^D are, as in wild type, devoid of Serrate protein. In the wing margin of evaginating discs of prepupae, indentations are occasionally visible between the stripes of Serrate expression on the wing blade, which correspond to the notches seen in the adult wing (Figure 5B). Staining mutant discs of similar age with acridine orange confirmed that cells in these regions undergo cell death (Figure 5C). We did never observe cell death to this extent in wild-type discs of comparable age nor in third instar discs of Ser^D (data not shown), suggesting that the notches seen in Ser^D wings are the result of cell death occurring during pupariation.

The analysis of the expression of *cut-lacZ* in discs of Ser^D third instar larvae revealed that the stripe of *lacZ* expressing cells in the edge zone is interrupted in the distal region of the wing margin (Figure 4, F and J). As revealed by double stainings, cells in which *cut*-expression is abolished correspond to those cells that ectopically express the Serrate protein. This becomes particularly obvious in a small group of cells in the distal-most edge that still show *lacZ* expression: here no Serrate protein is visible in the mutant disc (Figure 4, E and F). This pattern can be observed both in heterozygotes and homozygotes. The repression of a wing margin-specific gene by ectopic Serrate expression is not restricted to *cut* but also affects the expression of other genes, *e.g.*, *wingless*. Using a *wg-lacZ* line, we found lack of expression in the same regions that fail to express *ct-lacZ* expression (*cf.* Figure 4, H and K). In the pupa, the disruption of *cut-lacZ* expression is more pronounced (not shown), as has already been described by JACK and DELOTTO (1992).

To exclude the possibility that the lack of *ct* and *wg* expression is merely due to the fact that, though not detectable by acridine orange staining, a small portion of cells in the edge zone have died, we used another marker, which is, unlike *ct* and *wg*, turned on in the future margin already in the second instar larvae, namely a *lacZ* reporter gene driven by the wing margin–specific enhancer of *vg* (WILLIAMS *et al.* 1994). In Ser^D, this marker is expressed without any interruption throughout the wing margin as in wild type, demonstrating that the cells not expressing *ct* and *wg* are present (Figure 4, I and L). Taken together, these data demonstrate that in Ser^D the Serrate protein is ectopically expressed in the distal portion of the edge zone of the margin, in cells in which it is not detectable in wild-type discs of this stage, and suggest that this in turn abolishes *wg* and *cut* expression, resulting in cell death and finally scalloping of the wing margin.

FIGURE 4.—Expression patterns of *Ser*, *ct* and *wg* in wild-type and Ser^D wing discs. Wing discs of wild-type (A–C and G–I) and homozygous Ser^D (D–F and J–L) larvae, carrying the *ct-lacZ* (A–F, G and J), the *wg-lacZ* (H and K) or the *vg-lacZ* (I and L) reporter genes. (A–C) Same disc (Ser^+); (D–F) Same disc (Ser^D) stained simultaneously with both α-serrate and the α-β-galactosidase antibodies. Rhodamine fluorescence reveals the Serrate expression (A, B and D, E), fluorescein fluorescence the *lacZ* expression (C and F). Discs in G–L were stained for β-galactosidase activity. Note that *ct* is expressed in a stripe of cells (C, F, G and J), corresponding to the edge of the wing margin (BLAIR 1993; BLOCHLINGER 1993; COUSO *et al.* 1994), whereas the Serrate protein is localized in adjacent cells dorsal and ventral to this stripe (B and E). Ectopic expression of Serrate in Ser^D is visible in a small group of cells in the edge zone of the wing margin of homozygous Ser^D larvae (E; arrows), leading to repression of *cut* and *wingless* expression in these cells (F, J and K). However, the expression of *vg-lacZ* is not abolished in these cells (*cf.* I, wild-type, with L, Ser^D), excluding the possibility that these cells have undergone cell death.

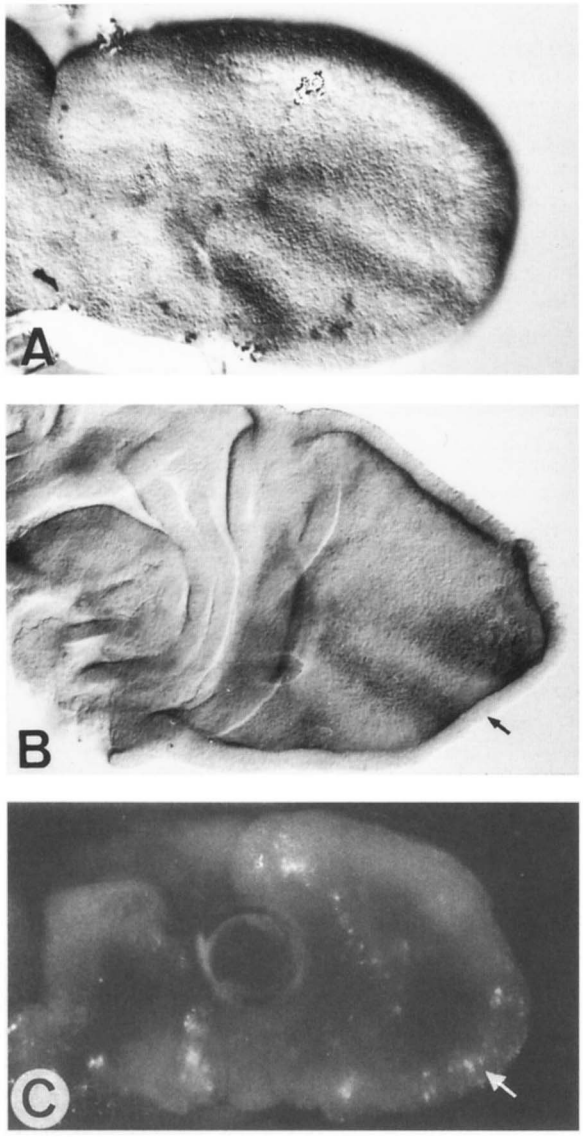


FIGURE 5.—Phenotypic traits of wings from wild-type and Ser^D early pupae. Wild-type (A) and homozygous Ser^D (B and C) wing discs of early pupae stained with the α-Serrate antibody (A and B) or acridine orange (C). Note the strong staining of the prospective veins in both discs (A and B). The future notches are clearly visible in the mutant disc (B) and can be correlated with regions of extensive cell death (C; arrow).

Overexpression of Serrate in the edge zone of the wing margin leads to scalloping of the wing: To analyze whether the notches in the wing margin of Ser^D flies are causally related to overexpression of a wild-type Serrate protein in the edge zone of the margin, we used the Gal4 system (BRAND and PERRIMON 1993) to overexpress the wild-type Serrate protein (from an UAS_G-*Ser* construct) (SPEICHER *et al.* 1994). Besides a strong expression in major parts of the future dorsal notum, the activator line $Gal4^{459.2}$ drives UAS_G-*Ser* (or UAS_G-*lacZ*) expression in a broad stripe across the disc perpendicular to the prospective wing margin, roughly between the second and fifth future wing veins (Figure 6A). While staining of the dorsal part of the disc is already visible in discs of early second instar larvae, the stripe only appears in discs of early third instar larvae (not shown). Driving Serrate expression by means of the $Gal4^{459.2}$ activator results in scalloping of the wing margin (Figure 6C). Analysis of *ct* expression in this genetic background ($Gal4^{459.2}$/+; UAS_G-*Ser*/+) revealed inhibition of *ct* expression in a region within the distal edge zone, which covers the central two thirds of the stripe of ectopic serrate expression that crosses the edge (Figure 6B). In contrast, the *vg-lacZ* line expresses the reporter gene in this genetic background as in wild-type, that is, without any interruption in the margin (data not shown).

The results described above demonstrate that the phenotypic consequences of the Ser^D mutation (scalloping of the wing, repression of margin-specific genes) can be mimicked by expression of the wild-type Serrate protein in the edge of the wing margin of third instar larvae, where it is normally not detectable at this stage. To extend this similarity further and to include other phenotypic traits, we analyzed wings obtained after driving Serrate expression with the $Gal4^{459.2}$ activator in an *N* heterozygous background. This enhances the mutant phenotype (Figure 6D), just as the Ser^D phenotype is enhanced in an *N* heterozygous background (FLEMING *et al.* 1990; THOMAS *et al.* 1991). This supports our previous argument (THOMAS *et al.* 1991) that the scalloping phenotype in Ser^D is the result of titration of active *N* gene product by excess Serrate and thus reflects haploinsufficiency of *N*. The fact that the Ser^D phenotype is attenuated by an additional wild-type copy of *N* is compatible with this view (FLEMING *et al.* 1990; THOMAS *et al.* 1991).

DISCUSSION

The dominant allele Ser^D is associated with an insertion of the middle repetitive element *Tirant*. The insertion of a mobile element into a transcription unit can affect its regulation (SMITH and CORCES 1991) or the nature of its gene product. In the case of the *emc* allele *Achaetous*, the *Tirant* element has integrated into the coding region, leading to expression of a shortened protein (GARRELL and MODOLELL 1990). In contrast, in the Ser^D allele the *Tirant* insertion maps within the 3′ untranslated region and leads to synthesis of a truncated but stable transcript, lacking putative RNA degradation motifs. Disruption of such AUUUA motifs in the 3′ UTR of granulocyte monocyte colony stimulating factor (GM-CSF) mRNA has been shown to result in a 30- to 40-fold increase in RNA levels (SAVANT-BHONSALE and CLEVELAND 1992). As a consequence, the Serrate protein accumulates to much higher concentrations in the mutant than in the wild type. As revealed by se-

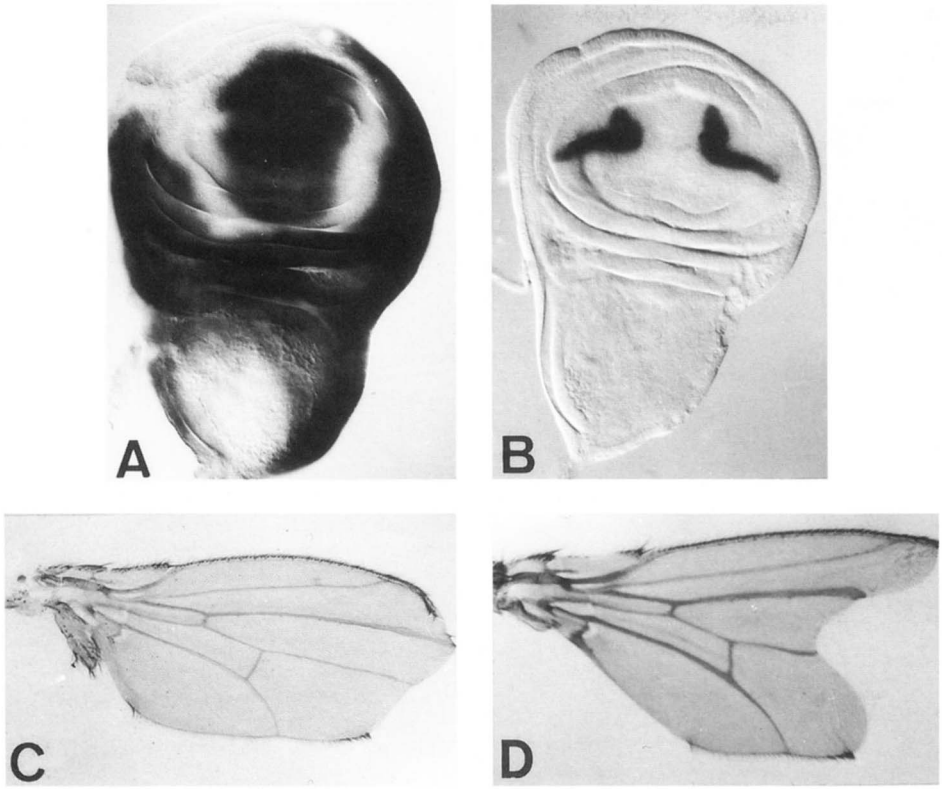


FIGURE 6.—Ectopic expression of *Serrate*. (A) Wing disc of third instar larvae of the genotype $Gal4^{459.2}$; UAS_G-*lacZ* stained for β-galactosidase activity to demonstrate the pattern of expression. There is a predominant stripe of expression crossing the prospective wing margin. (B) Wing disc of third instar larvae of the genotype $Gal4^{459.2}$; UAS-*Ser*, which also contain the *ct-lacZ* reporter gene, stained for β-galactosidase activity. Note that the gap in the *ct* expression domain in the prospective wing margin corresponds to the site at which the stripe of ectopic Serrate expression crosses the prospective wing margin (*cf.* A) (*cf.* Figure 4G for *ct* expression in wild type). (C) Wing from a fly of the genotype $Gal4^{459.2}$; UAS_G-*Ser*. Note the occurrence of notches mainly between the third and fourth and the fourth and fifth wing veins. (D) Wing from a fly of the genotype $N^{55e11}/+$; $Gal4^{435}$; UAS_G-*Ser*. The notches are clearly enhanced compared with those in C.

quence analysis of part of the mutant gene, the Ser^D gene product does not exhibit alterations within the carboxyterminal fifth of the protein. Furthermore, the overall integrity of the protein was confirmed by its normal immunoreactivity with antibodies directed against its extracellular and cytoplasmic portions. The results of ectopic expression of the wild-type protein are consistent with the assumption that overexpression of Serrate in the edge zone of the margin, where it is normally absent at this stage of development, rather than the synthesis of a modified protein, leads to the scalloping of the wing seen in Ser^D flies.

The presence of RNA degradation motifs in the 3′ end of the *Ser* mRNA as well as the fairly high rate of protein internalization suggested by its presence in large intracellular vesicles (THOMAS *et al.* 1991) point to a rapid downregulation of *Ser* expression at certain stages of development. Comparable observations have been made in the context of the dynamic regulation of *Dl* expression (VÄSSIN *et al.* 1985; KOPCZYNSKY and MUSKAVITCH 1989; KOOH *et al.* 1993). We therefore propose that, in the case of Ser^D, expression of the Serrate protein in the edge zone of the prospective wing margin is due to abnormal persistence of RNA, and hence protein, in cells of the edge. Alternatively, albeit less likely, it is possible that the 5.5-kb *Tirant* insertion may release *Ser* from control by inhibitory elements located in the 3′ part of the gene. It has recently been shown that the Serrate protein is expressed in the dorsal side of the wing disc of second instar larvae, including the prospective wing margin, and resolves into the dorsal and ventral stripes (Figure 4, A and B) during third larval instar (J. P. COUSO, S. B. CARROLL, E. KNUST and A. MARTINEZ-ARIAS, unpublished data). However, it remains to be analyzed why only the distal-most region of the edge exhibits increased levels of Serrate protein in Ser^D.

Results obtained from studies of genetic interactions and gene dosage studies between *N*, *Dl* and Ser^D led us to speculate that the Ser^D phenotype could be the consequence of a reduction in functional Notch protein, due to titration by the Serrate protein (THOMAS *et al.* 1991). This, in turn, would affect the balanced interaction of *N* and *Dl* (VÄSSIN *et al.* 1985; XU *et al.* 1990). In fact, REBAY *et al.* (1991) have shown that Serrate-expressing S2 cells are capable of forming aggregates with Notch-expressing cells via heterophilic adhesion, which is mediated by the same two EGF-like repeats of the Notch protein that also interact with Delta.

We have recently shown that ectopic expression of Serrate can provoke additional growth of part of the wing disc and that the lack of Serrate protein results in the development of no wings at all (SPEICHER *et al.* 1994). At first sight, these results seem to contradict the observations presented in this paper, namely that ectopic overexpression of Serrate (in the case of Ser^D or by means of the Gal4-activated expression of Serrate)

results in loss of tissue, that is, scalloping of the wing. We cannot explain this apparent contradiction at present, but we propose that the different effects obtained by ectopic expression reflect differential requirements for *Ser* at different stages in wing development. In fact, both notching and overgrowth are observed in the same wing when a Gal4 activator controlled by the *patched* promoter (PHILIPPS *et al.* 1990) is used to drive Serrate expression (U. THOMAS and E. KNUST, unpublished observation). Generally, ectopic expression of Serrate early in development results in the induction of ectopic proliferation at the ventral side of the wing (SPEICHER *et al.* 1994), which is mediated by induction of *vg* expression at a newly formed boundary between Serrate-expressing and Serrate-nonexpressing cells (J. P. COUSO, S. B. CARROLL, E. KNUST and A. MARTINEZ-ARIAS, unpublished data). In contrast, effects produced by later overexpression of Serrate in cells of the edge zone of the prospective margin can be explained as being the result of sequestration of functional Notch^{+} product, preventing interactions with its appropriate ligand(s). In fact, although the *ptc*-Gal4 activator used previously is expressed in the wing disc already in the second instar, the Gal4$^{459.2}$ activator used here turns on ectopic expression of Serrate in the prospective ventral wing only from the early third instar (data not shown). This suggests that cells are only competent to the signal mediated by Serrate for a restricted period of time. With respect to the early function, *Ser* and *N* probably operate in the same direction (and perhaps synergistically), because *N*, too, is believed to promote cell proliferation during disc development (SHELLENBARGER and MOHLER 1978). This assumption is further supported by results from genetic interaction studies, involving an antimorphic *N* allele and *Ser*^{RX106}, a loss-of-function allele (DE CELIS *et al.* 1993). At later stages, the binding of Notch by excess Serrate interferes with patterning of the wing margin, which is reflected by the repression of *wg* and *ct*. Expression of *ct* itself in the edge zone is dependent on *wg* activity (COUSO *et al.* 1994), and the repression of *ct* by ectopic Serrate is likely to be a consequence of *wg* repression in these cells. It remains to be elucidated, however, whether the influence of Serrate on *wg* expression is a consequence of reduced levels of functional *N* product or is the result of more direct interactions.

We thank BIRGIT BAYER for technical assistance and J. A. CAMPOS-ORTEGA and P. HARDY for critical comments on the manuscript. This work was supported by a graduate studentship of the Graduiertenförderung of Nordrhein-Westfalen to U.T. and a grant from the Deutsche Forschungsgemeinschaft to E.K.

LITERATURE CITED

ABBOT, L. A., 1986 Restoration of wing margins in *Lyra* mutants of *Drosophila melanogaster*. Dev. Biol. **115:** 233–248.

BELT, A. L., 1971 A non-lethal allele of *Serrate*? Dros. Inf. Serv. **46:** 116.

BLAIR, S., 1993 Mechanisms of compartment formation: evidence that nonproliferating cells do not play a critical role in defining the D/V lineage restriction in the developing wing of Drosophila. Development **119:** 339–351.

BLOCHLINGER, K., L. Y. JAN and Y. N. JAN, 1993 Postembryonic patterns of expression of *cut*, a locus regulating sensory organ identity in Drosophila. Development **117:** 441–450.

BRAND, A., and N. PERRIMON, 1993 Targeted expression as a means of altering cell fates and generating dominant phenotypes. Development **118:** 401–415.

BROOKMAN, J. J., A. T. TOOSY, L. S. SHASHIDHARA and R. A. H. WHITE, 1992 The 412 retrotransposon and the development of gonadal mesoderm in Drosophila. Development **116:** 1185–1192.

BROWER, D. L., R. J. SMITH and M. WILCOX, 1982 Cell shapes on the surface of the *Drosophila* wing imaginal disc. J. Embryol. Exp. Morphol. **67:** 137–151.

COHEN, B., M. E. MCGUFFIN, C. PFEIFLE, D. SEGAL, and S. M. COHEN, 1992 *apterous*, a gene required for imaginal disc development in Drosophila encodes a member of the LIM family of developmental regulatory proteins. Genes Dev. **6:** 715–729.

COHEN, S. M., 1993 Imaginal disc development, pp. 747–841 in *Development of Drosophila*, edited by A. MARTINEZ-ARIAS and and M. Bate. Cold Spring Harbor Press, Cold Spring Harbor, NY.

COUSO, J. P., S. A. BISHOP and A. MARTINEZ-ARIAS, 1994 The wingless signalling pathway and the patterning of the wing margin in Drosophila. Development **120:** 621–636.

CUBAS, P., J. F. DE CELIS, S. CAMPUZANO and J. MODOLELL, 1991 Proneural clusters of *achaete-scute* expression and the generation of sensory organs in the Drosophila imaginal wing disc. Genes & Dev. **5:** 996–1008.

DE CELIS, J. F., R. BARRIO, A. DEL ARCO and A. GARCIA-BELLIDO, 1993 Genetic and molecular characterization of a Notch mutation in its Delta- and Serrate-binding site. Proc. Natl. Acad. Sci. USA **90:** 4037–4041.

DIAZ-BENJUMEA, F. J., and S. M. COHEN, 1993 Interaction between dorsal and ventral cells in the imaginal disc directs wing development in Drosophila. Cell **75:** 741–752.

FLEMING, R. J., T. N. SCOTTGALE, R. J. DIEDERICH and S. ARTAVANIS-TSAKONAS, 1990 The gene *Serrate* encodes a putative EGF-like transmembrane protein essential for proper ectodermal development in *Drosophila melanogaster*. Genes & Dev. 4: 2188–2201.

FRISTROM, D., 1969 Cellular degeneration in the production of some mutant phenotypes in *Drosophila melanogaster*. Mol. Gen. Genet. **103:** 363–379.

GARCIA-BELLIDO, A., and J. F. DE CELIS, 1992 Developmental genetics of the venation pattern of Drosophila. Annu. Rev. Genet. **26:** 277–304.

GARCIA-BELLIDO, A., P. RIPOLL and G. MORATA, 1976 Developmental compartmentalization in the dorsal mesothoracic disc of Drosophila. Dev. Biol. **48:** 132–147.

GARRELL, J., and J. MODOLELL, 1990 The *Drosophila extramacrochaetae* locus, an antagonist of proneural genes that, like these genes, encodes a helix-loop-helix protein. Cell **61:** 39–48.

HARTENSTEIN, V., and J. W. POSAKONY, 1989 Development of adult sensilla on the wing and notum of *Drosophila melaogaster*. Development **107:** 389–405.

JACK, J., and Y. DE LOTTO, 1992 Effect of wing scalloping mutations on *cut* expression and sense organ differentiation in the Drosophila wing margin. Genetics **131:** 353–363.

JACK, J., D. DORSETT, Y. DE LOTTO and S. LIU, 1991 Expression of the *cut* locus in the Drosophila wing margin is required for cell type specification and is regulated by a distant enhancer. Development **113:** 735–747.

JAMES, A. A., and P. J. BRYANT, 1981 Mutations causing pattern deficiencies and duplications in the imaginal wing disk of *Drosophila melanogaster*. Dev. Biol. **85:** 39–54.

JOHNSON, T. K., and B. H. JUDD, 1979 Analysis of the *cut* locus of *Drosophila melanogaster*. Genetics **92:** 485–502.

JONES, T. R., and M. D. COLE, 1987 Rapid cytoplasmic turnover of c-myc mRNA: requirement of the 3′untranlated sequence. Mol. Cell. Biol. **7:** 4513–4521.

KOOH, P. J., R. G. FEHON and M. A. T. MUSKAVITCH, 1993 Implications of dynamic patterns of Delta and Notch expression for

cellular interactions during Drosophila development. Development **117:** 493–507.

KOPCZYNSKI, C. C., and M. A. T. MUSKAVITCH, 1989 Complex spatiotemporal accumulation of alternative transcripts from the neurogenic gene *Delta* during Drosophila embryogenesis. Development **107:** 623–636.

LINDSLEY, D. L., and G. G. ZIMM, 1992 *The Genome of Drosophila.* Academic Press, Inc., San Diego, CA.

LIPMAN, D. J., and W. R. PEARSON, 1985 Rapid and sensitive protein similarity searches. Science **227:** 1435–1441.

MASUCCI, J. D., R. J. MILTENBERGER and F. M. HOFFMANN, 1990 Pattern-specific expression of the *Drosophila decapentaplegic* gene in imaginal discs is regulated by 3′-cis regulatory elements. Genes Dev. **4:** 2011–2023.

O'BROCHTA, D. A., and P. J. BRYANT, 1985 A zone of non-proliferating cells at a lineage restriction boundary in Drosophila. Nature **313:** 138–141.

PHILLIPS, R. G., and J. R. S. WHITTLE, 1993 *wingless* expression mediates determination of peripheral nervous system elements in late stages of Drosophila wing disc development. Development **118:** 427–438.

PHILLIPS, R. G., I. J. H. ROBERTS, P. W. INGHAM and J. R. S. WHITTLE, 1990 The Drosophila segment polarity gene *patched* is involved in a position-signalling mechanism in imaginal discs. Development **110:** 105–114.

REBAY, I., R. J. FLEMING, R. G. FEHON, L. CHERBAS, P. CHERBAS *et al.*, 1991 Specific EGF repeats of Notch mediate interactions with Delta and Serrate: implications for Notch as a multifunctional receptor. Cell **67:** 687–699.

RIO, D. C., F. A. LASKI and G. RUBIN, 1986 Identification and immunochemical analysis of biologically active Drosophila P element transposase. Cell **44:** 21–32.

SAMBROOK, J. M., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual,* Ed. 2, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

SANGER, F., S. NICKLEN and A. R. COULSON, 1977 DNA sequencing with chain termination inhibitors. Proc. Natl. Acad. Sci. USA **74:** 5463–5467.

SANTAMARIA, P., and A. GARCIA-BELLIDO, 1975 Developmental analysis of two wing scalloping mutants ct^6 and Bx^J of *Drosophila melanogaster.* Roux's Arch. Dev. Biol. **178:** 233–245.

SAVANT-BHONSALE, S., and D. W. CLEVELAND, 1992 Evidence for instability of mRNA containing AUUUA motifs mediated through translation-dependent assembly of a 20S degradation complex. Genes Dev. **6:** 1927–1939.

SCHUBIGER, M., and J. PALKA, 1987 Changing spatial patterns of DNA replication in the developing wing of Drosophila. Dev. Biol. **123:** 145–153.

SHAW, G., and R. KAMEN, 1986 A conserved A_n sequence from the 3′untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell **46:** 659–667.

SHEARN, A., 1978 Mutational dissection of imaginal disc development, pp. 443–510 in *The Genetics and Biology of Drosophila,* Vol. 2c, edited by M. ASHBURNER, and T. R. F. WRIGHT. Academic Press, New York.

SHELLENBARGER, D. L. and J. D. MOHLER, 1978 Temperature-sensitive periods and autonomy of pleiotropic effects of $l(1)N^{ts1}$, a conditional Notch lethal of Drosophila. Dev. Biol. **62:** 432–446.

SHYU, A.-B., M. E. GREENBERG and J. G. BELASCO, 1989 The c-fos transcript is targeted for rapid decay by two distinct mRNA degradation pathways. Genes Dev. **3:** 60–72.

SIEGFRIED, E., T.-B. CHOU and N. PERRIMON, 1992 *wingless* signalling acts through *zeste-white 3,* the *Drosophila* homolog of glycogen synthase kinase-3, to regulate *engrailed* and establish cell fate. Cell **71:** 1167–1179.

SIMPSON, P., P. A. LAWRENCE and F. MASCHAT, 1981 Clonal analysis of two wing-scalloping mutants of Drosophila. Dev. Biol. **84:** 206–211.

SMITH, P. A., and V. G. CORCES, 1991 Drosophila transposable elements: mechanisms of mutagenesis and interactions with the host genome. Adv. Genet. **29:** 229–300.

SPEICHER, S. A., U. THOMAS, U. HINZ and E. KNUST, 1994 The *Serrate* locus of Drosophila and its role in morphogenesis of wing imaginal discs: control of cell proliferation. Development **120:** 535–544.

SPINDLER, K. R., D. S. E. ROSSER and A. J. BERK, 1984 Analysis of adenovirus transforming proteins from early regions 1A and 1B with antisera to inducible fusion antigens produced in *Escherichia coli.* J. Virol. **49:** 132–141.

TAUTZ, D., and C. PFEIFLE, 1989 A non-radioactive in situ hybridization method for the localization of specific RNAs in Drosophila embryos reveals a translational control of the segmentation gene *hunchback.* Chromosoma **98:** 81–85

THOMAS, U., S. A. SPEICHER and E. KNUST, 1991 The Drosophila gene *Serrate* encodes an EGF-like transmembrane protein with a complex expression pattern in embryos and wing discs. Development **111:** 749–761.

VÄSSIN, H., J. VIELMETTER and J. A. CAMPOS-ORTEGA, 1985 Genetic interactions in early neurogenesis of *Drosophila melanogaster.* J. Neurogenet. **2:** 291–308.

WAGNER-BERNHOLZ, J. T., C. WILSEN, G. GIBSEN, R. SCHUH and W. J. GEHRING, 1991 Identification of target genes of the homeotic gene Antennapedia by enhancer detection. Genes & Dev. **5:** 2467–2480.

WHITTLE, J. R. S., 1990 Pattern formation in imaginal discs. Semin. Cell Biol. **1:** 241–252.

WILKINS, A. S., and D. GUBB, 1991 Pattern formation in the embryo and imaginal discs of Drosophila. Dev. Biol. **145:** 1–12.

WILLIAMS, J. A., and S. B. CARROLL, 1993 The origin, patterning and evolution of insect appendages. BioEssays **15:** 567–577.

WILLIAMS, J. A., S. W. PADDOCK and S. B. CARROLL, 1993 Pattern formation in a secondary field: a hierarchy of regulatory genes subdivides the developing Drosophila wing disc into discrete subregions. Development **117:** 571–584.

WILLIAMS, J. A., S. W. PADDOCK, K. VORWERK and S. B. CARROLL, 1994 Organization of wing formation and induction of a wing-patterning gene at the dorsal/ventral compartment boundary. Nature **368:** 299–305.

XU, T., I. REBAY, R. J. FLEMING, T. N. SCOTTGALE and S. ARTAVANIS-TSAKONAS, 1990 The *Notch* locus and the genetic circuitry involved in early Drosophila neurogenesis. Genes Dev. **4:** 464–475.

Communicating editor: T. SCHÜPBACH