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The Phenotype of *engrailed* Mutations in the Antenna of *Drosophila*

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The eye-antenna imaginal disc of *Drosophila* is subdivided into anterior and posterior compartments and it is expected therefore that the *engrailed* gene would be locally required in the posterior compartment. Here we describe the phenotype of *engrailed* mutations in the antenna. Clones of cells which were mutant for en^1/en^{C2} were produced in wild-type antennae by mitotic recombination. The clones showed the typical syndrome of cells mutant for *engrailed*, being normal in the anterior compartment and showing partial posterior-to-anterior transformation in the posterior compartment.

INTRODUCTION

Most, perhaps all, of the body segments of *Drosophila* are subdivided into anterior and posterior compartments (Garcia-Bellido *et al.*, 1973, 1976; Steiner, 1976; Morata and Lawrence, 1978, 1979; Struhl, 1977, 1981; Kornberg, 1981a). The *engrailed* gene product appears to be specifically required in the cells of posterior compartments of the thorax and abdomen (Morata and Lawrence, 1975; Lawrence and Morata, 1976; Kornberg, 1981a; Lawrence and Struhl, 1982). Mutant anterior cells are completely unaffected, while mutant posterior cells form abnormal patterns which consist of anterior elements arranged in partial mirror symmetry. The eye-antennal part of the head is also subdivided into two compartments which were deemed to be anterior and posterior by homology with compartments in the thorax (Morata and Lawrence, 1978, 1979). If this allocation is correct the posterior compartment should show some abnormal phenotype in flies mutant for *engrailed*. While no clear phenotype was detected in en^1 antennae, the antennae of $engrailed^1/engrailed^{C2}$ (en^1/en^{C2}) flies were abnormal, with a partial transformation (to give anterior structures) of the posterior region. This observation was interpreted as supporting the designation of the posterior compartment (Morata and Lawrence, 1979). However, the lethal en^{C2} chromosome used in those experiments is known to carry other mutations and hence the antennal phenotype might be due to them, and not to *engrailed* (Kornberg, 1981b). Here we test this possibility using a small duplication for the *engrailed* region which allows us to make cellular clones of the en^1/en^{C2} genotype in a background of wild-type cells. We show that the en^1/en^{C2} phenotype in the antenna and wing is due to alteration in function of only the *engrailed* gene. We also reexamine $engrailed^1$ and find it shows a weaker but similar phenotype.

MATERIALS AND METHODS

To measure the expression of en^1/en^1 and en^1/en^{C2} in the antenna and other parts, different stocks carrying en^1 (*lt stw* $en^1/SM5$, *pk cn* $en^1/SM5$, *cn* $en^1/SM5$; $M(3)i^{55}/TM1$) were crossed *inter se* and also to the same en^{C2} chromosome. Each combination was reared at 17, 25, and 29°C; the en^1/en^{C2} flies were mounted for examination in the light microscope and the phenotype in antennae, wings, and legs was recorded.

For the production of en^1/en^{C2} clones in wild-type antennae, we chose the second chromosome and the temperature (29°C) that gave the strongest phenotype. The clones were made by crossing $en^{C2}/SM5$; *mwh* females to *lt stw* $en^1/SM5$; *Su(en)28* $M(3)i^{55}/TM2$ males (*Su(en)28* was generously provided by Dr. M. Russell; he has informed us that it is located at 62C and is a small duplication of about two bands probably translocated from the *engrailed* region) and irradiating their offspring during the larval period with a dose of 1000 rad (Phillips Be 151, 300 rad/min).

The descendants were separated into two groups: (1) the genotype *lt stw* en^1/en^{C2}; *Su(en)28* $M(3)i^{55}/mwh$ where *mwh* clones would be mutant for *engrailed* and (2) the control genotypes *lt stw* $en^1/SM5$; *Su(en)28* $M(3)i^{55}/mwh$ and $en^{C2}/SM5$; *Su(en)28* $M(3)i^{55}/mwh$ where *mwh* clones remain wild type for *engrailed* (see Lindsley and Grell (1968) for the rest of the nomenclature). Two other *en* alleles used in this study, en^{I0} and en^{IK}, were kindly provided by Drs. C. Nüsslein-Volhard

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and E. Wieschaus. The allele en^{C2} (Kornberg, 1981b) was referred to earlier as en^2 (Morata and Lawrence, 1978, 1979).

RESULTS

The Phenotype of en^1/en^1 in the Antenna

We reared en^1/en^1 flies at 17, 25, and 29°C. At 17 and 25°C no phenotype was observed but at 29°C, although the phenotype in the wing was much suppressed, there was a clear phenotype in the antenna. The third segment was bigger and more rounded in shape and the "bristle of doubt," which is close to the border and can have anterior or posterior provenance (Morata and Lawrence, 1979), was often considerably enlarged—in some cases it became the largest bristle on the antenna (Fig. 1). This phenotype was more extreme when the flies carried a *Minute* mutation, $M(3)i^{55}$. Then the third segment was sometimes very large and the arista was grossly thickened. Thus *engrailed*^{1} itself affects the antenna.

The Phenotype of en^1/en^{C2}

In en^1/en^{C2} antenna, although there is a considerable variation between left and right sides of one individual (Fig. 2) there is a very clear mutant phenotype which is affected by temperature. Whereas in flies cultured at 17°C most of the antennae were normal or showed only a slight effect, those that were reared at 25°C and particularly at 29°C presented a clear, but variable phenotype (Table 1). At 29°C the second antennal segment was almost always (90%) abnormal in the region of the posterior compartment. Here there were extra anterior-like bristles which were often patterned in mirror-image symmetry with respect to those in the anterior compartment (Fig. 3). In a sample of 20 control antennae we counted 5 ± 1 bristles in this posterior area while in a similar number of en^1/en^{C2} antennae we found an average of 16 ± 3 bristles. By contrast the number of bristles in the anterior region was unaffected (controls 15 ± 1, en^1/en^{C2} 17 ± 2; n = 20). Most third antennal segments were clearly bigger than normal and often bilobed. About half of those that carried a bilobed third antennal segment also had an extra arista (Table 1). In some cases at 29°C, the posterior cuticle of the second and third segment was segregated into separate vesicles (Figs. 4, 5). All these effects are consistent and support the previous interpretation (Morata and Lawrence, 1979) that en^1/en^{C2} produces a partial posterior-to-anterior transformation. However, in some cases (Fig. 6) the number of extra bristles in the posterior region was greater than that usually found in the anterior compartment. This suggests some extra growth (see Discussion).

The wings of en^1/en^{C2} also showed a mutant phenotype affected by temperature. At 17°C they were grossly enlarged, there was scalloping of the posterior margins, and the triple rows of anterior-like bristles were poorly formed. The phenotype was very similar to that produced by the interaction between *engrailed* and *Minutes* (Lawrence and Morata, 1976). At 29°C the wings were not

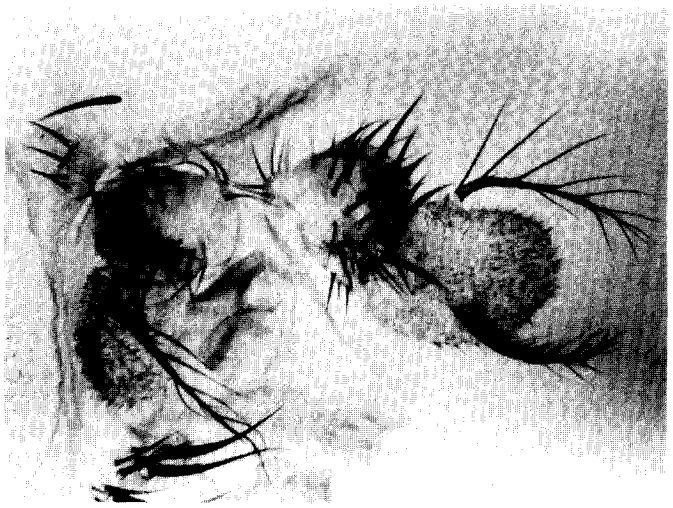

FIG. 2. Antennae of a en^1/en^{C2} fly grown at 29°C. The right antenna is abnormal, with enlarged second and third segments and duplicated aristae. The left antenna is little affected by the *engrailed* mutation. ×120.

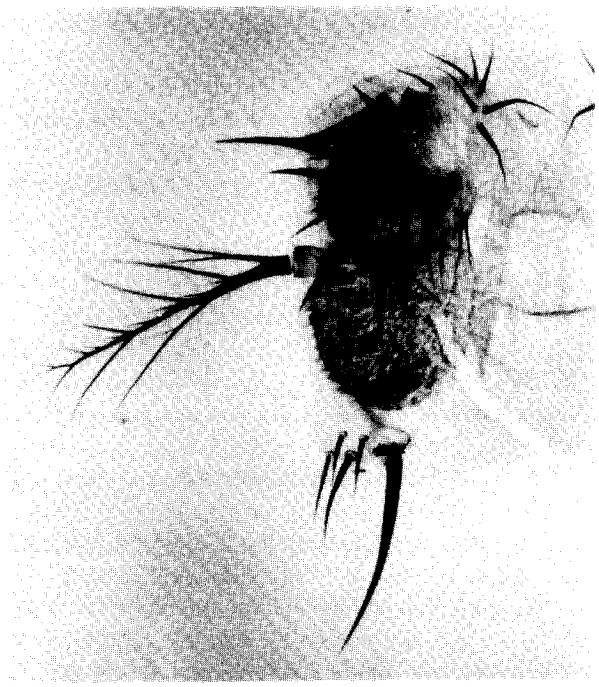

FIG. 1. Antenna of a en^1/en^1 fly reared at 29°C. Note that the antenna is somewhat different from wild type (compare Fig. 7, right); the "bristle of doubt" (arrow) is considerably enlarged and there are extra bristles on segments I and II. ×145.

TABLE 1
EFFECT OF TEMPERATURE ON THE *engrailed* PHENOTYPE IN THE ANTENNAE

Genotype	Temperature (°C)	*n*	Adventitious bristles of anterior type in segment II	Enlargement of segment III	Bilobed segment III	Extra arista
lt stw en^1/en^{C2}	17	100	31	0	0	0
lt stw en^1/en^{C2}	25	100	55	7	0	0
lt stw en^1/en^{C2}	29	100	94	59	32	14
lt stw en^1/en^{C2}; *Su(en)28/+* (Control)	29	90	1	0	0	0

so enlarged and developed a well-formed triple row of bristles on the posterior margin. This is very similar to the phenotype of en^1/en^1 wings at 25°C (Lawrence and Morata, 1976). The legs of en^1/en^{C2} were also abnormal (Kornberg, 1981b) having fused tarsi and a variable number of extra bristles. In some cases it was possible to identify anterior elements such as sexcomb teeth in the posterior region.

All different aspects of en^1/en^{C2} phenotype in the antennae, wings, and legs are completely rescued by the small en^+ duplication which also covers the phenotype of en^1/en^1 and rescues the lethal phenotype of two other *engrailed* alleles, en^{IO} and en^{IK}. This shows that the translocated element includes the *engrailed*$^+$ gene and demonstrates that the defects observed in en^1/en^{C2} cannot be due to any mutations outside these two chromosomal bands.

The engrailed Mutant Phentoype Is Restricted to the Posterior Compartment

The phenotype of en^1/en^{C2} antennae suggests a local effect of the *engrailed* mutation on only the posterior antennal compartment, but is not conclusive as other factors can cause an "engrailed" phenotype in the wing (*apterous-blot* (Whittle, 1979), and cell death and regeneration (Szabad *et al.*, 1979)). A better test is to eliminate the *engrailed*$^+$ gene from clones of cells in either the anterior or the posterior compartment and then to compare the effect of the mutation on the different compartments. The clones were generated at 48–72 and 72–96 hr (*Minute* time at 29°C) after egg laying which cor-

FIG. 3. Antenna of a en^1/en^{C2} fly grown at 29°C. The boundary between anterior and posterior cells should be near the position marked by the dotted line. Note the presence of large bristles (arrows) characteristic of the anterior compartment but in the posterior region and arranged in mirror-image symmetry with respect to the anterior ones. ×300.

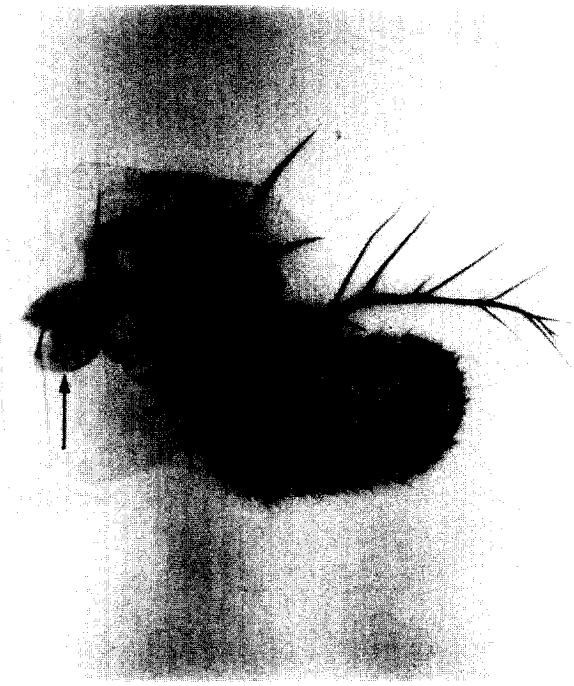

FIG. 4. Antenna of a en^1/en^{C2} fly grown at 29°C. Note that the posterior cuticle of the second segment (arrow) containing tooth bristles has sorted out into a vesicle that is barely attached to the rest of the antenna. ×145.

FIG. 5. Antenna of a en^1/en^{C2} fly grown at 29°C. Note that the posterior cuticle of the third segment has formed separate vesicles (arrow). ×145.

responds to about the middle of the larval period. The results are presented in Table 2. Those clones restricted to the anterior compartment were usually large, gave the normal bristle pattern, and often defined the anteroposterior boundary. By contrast posterior clones (23 in total, including those which also extended to the anterior compartment) were very small; in the second antennal segment they never included more than three bristles. However, these clones reproduced in patches the phenotype seen in entirely en^1/en^{C2} antennae. Examples were additional large bristles in the posterior side of the second segment (Figs. 7, 8) and enlargement of the third segment (Fig. 9). In general they were located near the anteroposterior boundary. In control flies both anterior and posterior clones differentiated and developed normally, posterior clones were usually large and often filled the entire compartment.

TABLE 2
NUMBER AND LOCATION OF *mwh* CLONES FOUND IN ANTENNAE

Genotype	Age at irradiation	*n*	*A*	*P*	*A* + *P*
en^1/en^{C2}; *Su(en)28* $M(3)i^{55}/mwh$	48-72	620	45	3	9
en^1/en^{C2}; *Su(en)28* $M(3)i^{55}/mwh$	72-96	450	37	6	5
$en^{C2}/+$; *Su(en)28* $M(3)i^{55}/mwh$ (Control)	48-72	470	23	15	7

We observed a significant reduction in the number of en^1/en^{C2} clones recovered in the posterior compartment when compared to the number of anterior clones. In control flies we found (Table 2) 30:22 *mwh* (*en*/+) clones extending to the anterior and posterior compartments, respectively. In experimental flies the comparable figures were 54:12 ($P = <0.01$) considering only the clones generated at the same time as in controls.

Clones in the Wing

For comparison with the antenna we studied the behavior of en^1/en^{C2} cells in the wing at 29°C (irradiated at 84 ± 12 hr after egg laying). Anterior clones were normal, could almost fill the anterior compartment, and often defined the anteroposterior boundary (16/69). Posterior clones were usually abnormal, showed a typical *engrailed* phenotype (abnormal veins, ectopic campaniform sensilla, triple row bristles on the posterior margin) and those located in the middle of the wing crossed into anterior territory (17/70 clones). We have also observed several cases of en^1/en^{C2} clones in the posterior compartment that were sorting out from surrounding en^+ cells. Our best example is shown in Fig. 10 where the *mwh* clone formed a vesicle that had almost completely separated from surrounding posterior cells.

FIG. 6. Multiplication of bristles in the posterior regions of en^1/en^{C2} antennae. The number of extra bristles exceeds the usual number of anterior bristles. ×120.

DISCUSSION

The justification for studying *engrailed* in detail is that it is one of the few genes yet identified that seems to have a role which is geographically confined to cells

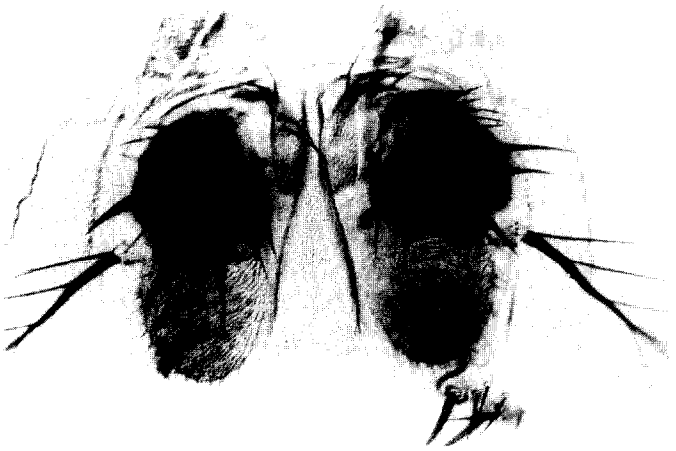

FIG. 7, 8. Antennae of genotype 1 (see Materials and Methods), the left bears a clone of cells, marked with *mwh*, that is mutant for en^1/en^{C2}. ×145. Figure 8 shows in detail the *mwh* trichomes (*m*) and the extra bristles (e.g., arrow) that are associated with the clone. ×490.

occupying precisely defined regions or compartments (Morata and Lawrence, 1975). These compartments can be independently defined as units of cell lineage (Garcia-Bellido *et al.*, 1973). The main part of the head of *Drosophila* is made by one disc and is divided into two compartments, designated as anterior and posterior by homology with the leg compartments (Morata and Lawrence, 1979). The *engrailed* syndrome (a partial transformation of posterior-to-anterior pattern, loss of the anteroposterior compartment boundary due to transgression by posterior cells) has been found in the wing (Morata and Lawrence, 1975; Lawrence and Morata, 1976; Kornberg, 1981b), the legs and proboscis (Tokunaga, 1961; Lawrence and Struhl, 1982); the abdomen (Kornberg, 1981a), and the eye-antenna (Morata and Lawrence, 1979). The evidence for the antenna, however, was based only on the phenotype of en^1/en^{C2} antennae which might be produced by other factors present in en^{C2} chromosome (Kornberg, 1981b) or perhaps be the indirect result of a disruption of the growth of mutant appendages (Whittle, 1979; Szabad *et al.*, 1979).

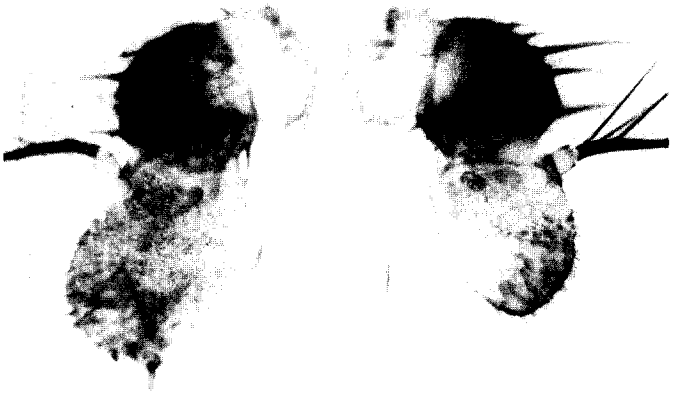

FIG. 9. Antennae of genotype 1 (see Materials and Methods); the left one bears a en^1/en^{C2}; *mwh* clone in the third segment which causes hypertrophy of that segment (compare with the right antenna). ×145.

Here we present evidence that the *engrailed* gene in the antenna performs a role homologous to that in the wing, leg, proboscis, and abdomen. In the first place, we

FIG. 10. A wing of genotype 1 (see Materials and Methods) bearing a en^1/en^{C2}; *mwh* clone (arrow) in the posterior compartment. The clone is separating into a vesicle. ×90.

have reexamined the phenotype of en^1/en^{C2} at different temperatures and genetic backgrounds and confirmed and extended the previous description (Morata and Lawrence, 1979). The different aspects of en^1/en^{C2} phenotype can be adequately interpreted as a posterior-to-anterior transformation; the appearance of extra bristles in the posterior region of the second antennal segment is expected because the anterior compartment bears many more bristles than the posterior. Further the extra bristles are of anterior type, which are larger and arranged in a specific pattern that is partially reproduced in the posterior compartment in mirror-image fashion (Fig. 3). Similarly the increase in size of third antennal segment is expected if the posterior compartment is transformed towards the anterior. Also, as the anterior compartment extends dorsoventrally while the posterior is exclusively ventral (Morata and Lawrence, 1979), the transformation of posterior-to-anterior can produce two surfaces in the ventral side of the segment which consequently becomes bilobed. In the cases of extreme expression, the arista, which is entirely anterior (Morata and Lawrence, 1979), is duplicated in mirror-image symmetry (Fig. 2). There is one aspect of the phenotype which cannot be so explained: In some cases the number of extra bristles that appear in the posterior region is actually greater than the number of bristles present in the normal anterior compartment (Fig. 6). A similar phenomenon has been observed in posterior en^1/en^1 wings which are enlarged and contain duplications of dorsal veins; a phenotype that is enhanced by *Minutes* (Lawrence and Morata, 1976). This is not simple homeosis; however, it is worth emphasizing that all aspects of en^1/en^{C2} syndrome in wings, legs and antennae are completely suppressed by the two-band duplication called *Su(en)28* which indicates very strongly that homeosis and extra growth both result from the same genetic defect in en^+ function.

The behavior of en^1/en^{C2} clones strengthens the evidence that the role of *engrailed* is limited to all posterior compartments, including that of the eye-antenna. The clones found in the anterior compartment are normal and often define the anteroposterior boundary while the posterior clones are associated with the mutant phenotype. The observation that the number of *engrailed* mutant clones found in the posterior compartment is unexpectedly small is the same result found in the proboscis (Lawrence and Struhl, 1982) and indicates that posterior clones are lost during development. The reason for the loss of these clones is not clear. We favor the hypothesis that they are lost during development through sorting out. This idea is supported by antennae that are entirely en^1/en^{C2} where the posterior structures sometimes become isolated into separate vesicles (Figs. 4, 5). Further support comes from the clones in the posterior wing which are apparently segregating from nearby territory (Fig. 10). A similar observation has been made for *bithorax* clones which are transformed into wing and segregate from haltere territory (Morata and Garcia-Bellido, 1976). Sorting out is the result of cells acquiring different affinities (Nöthiger, 1964) and our interpretation is in line with the published evidence that mutant cells for *engrailed* acquire anterior affinities (Garcia-Bellido and Santamaria, 1972; Morata and Lawrence, 1975; Lawrence and Morata, 1976; Kornberg, 1981a,b; Lawrence and Struhl, 1982).

However, the loss of posterior en^1/en^{C2} clones together with the extra growth of en^1/en^{C2} antennae could be interpreted differently as due to local cell death and subsequently disruption of growth in the posterior compartment. In this view en^1/en^{C2} phenotype in antennae results from duplication of structures due to abnormal growth, not genuine homeosis. We believe this hypothesis is wrong for three reasons: (1) In en^1/en^{C2} antennae there are adventitious structures that are undoubtedly of anterior type such as the big second antennal bristles (Fig. 3) or the arista. We have shown here and previously (Morata and Lawrence, 1979) that these structures can be produced by cells of the posterior compartment. Typical posterior structures like tooth bristles never appear to be duplicated. (2) From the results shown in Table 2, it is clear that many posterior en^1/en^{C2} clones generated by mitotic recombination are missing in the adult antennae. At least a fraction of the missing clones should have induced the duplicative process and hence we should find duplicated structures not associated with marked *mwh* clones. We found none after carefully examining hundreds of irradiated antennae. (3) The homeotic transformation produced by en^1/en^1 or en^1/en^{C2} clones in the wing is not mediated by abnormal growth or cell death: Even clones of one or two cells can autonomously show the anterior transformation (Garcia-Bellido and Santamaria, 1972; Lawrence and Morata, 1976). In short the abnormal growth hypothesis makes the implausible demand that homologous homoeotic transformations in the head and in the wing are produced in two unrelated ways by the same genetic defect.

One point which requires further discussion is the difference in expression of en^1/en^{C2} and the *engrailed*-lethals recently studied in mosaics by Lawrence and Struhl (1982). In this study no effect was found in the antenna although the mutant clones showed other aspects of *engrailed* phenotype. We believe that the reason for this apparent discrepancy is that neither the *engrailed*-lethals nor the viable mutations are complete null alleles and that they are partial mutations expressed differently in the various regions of the body, depending on the allele. Kornberg (1981b) observed that the majority of *engrailed*-lethals showed in the wing a very

weak expression over en^1, certainly weaker than en^1 homozygotes or en^1/en^{C2}. He also pointed out that lethality in *engrailed* does not imply strong mutant syndrome; different lethal combinations of en^{LA9} die during embryogenesis and yet show very slight expression on the cuticle. It seems that certain alleles affect preferentially certain regions while having little effect on others. For example, in the report of Lawrence and Struhl (1982), the *engrailed*-lethal clones have no effect in the antenna, but affect the male genitalia and the humerus, structures that appear normal in en^1/en^{C2} flies. Thus, both phenotypes are incomplete. The function of *engrailed* is probably complex and only a mosaic analysis of the entire deletion of the gene would reveal the total extent of the phenotype. That analysis, however, is yet to be done.

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