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The function of *engrailed* and the specification of *Drosophila* wing pattern

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SUMMARY

The adult *Drosophila* wing (as the other appendages) is subdivided into anterior and posterior compartments that exhibit characteristic patterns. The *engrailed (en)* gene has been proposed to be paramount in the specification of the posterior compartment identity. Here, we explore the adult *en* function by targeting its expression in different regions of the wing disc. In the anterior compartment, ectopic *en* expression gives rise to the substitution of anterior structures by posterior ones, thus demonstrating its role in specification of posterior patterns. The *en*-expressing cells in the anterior compartment also induce high levels of the *hedgehog* (*hh*) and *decapentaplegic (dpp)* gene products, which results in local duplications of anterior patterns. Besides, *hh* is able to activate *en* and the *engrailed*-related gene *invected* (*inv*) in this compartment. In the posterior compartment we find that elevated levels of en product result in partial inactivation of the endogenous *en* and *inv* genes, indicating the existence of a negative autoregulatory mechanism. We propose that *en* has a dual role: a general one for patterning of the appendage, achieved through the activation of secreted proteins like *hh* and *dpp*, and a more specific one, determining posterior identity, in which the *inv* gene may be implicated.

Key words: *Drosophila*, wing disc, compartment, *engrailed*, *hedgehog*, *invected*

INTRODUCTION

One basic feature of the organization of *Drosophila* is the subdivision of the body into metameres called parasegments, which appear to define the limits of patterning processes in the embryo (see Lawrence, 1992, for a general overview). The *engrailed (en)* gene plays an essential role both in the establishment of the parasegmental subdivision of the embryo (Kornberg, 1981a; Ingham et al., 1985) and in the maintenance of parasegmental borders in adult appendages (Morata and Lawrence, 1975; Lawrence and Morata, 1976).

The function of *en* was originally studied in the adult structures (Garcia-Bellido and Santamaria, 1972; Morata and Lawrence, 1975; Lawrence and Morata, 1976), especially the wing, where anterior and posterior compartments were first found (Garcia-Bellido et al., 1973). Subsequent work indicated that it performs a similar role in other structures such as the antenna and the abdominal histoblasts (Morata and Lawrence, 1979; Kornberg, 1981b; Morata et al. 1983). It was found that the maintenance of the anteroposterior compartment border in wing cells requires normal *en* function; wings of en^1 mutants failed to fix the lineage boundary that normally separates the anterior and posterior compartments (Morata and Lawrence, 1975). Furthermore, mosaic analysis demonstrated that this requirement is specific to posterior compartment cells. In addition to the lack of a proper anteroposterior border, the posterior wing compartment of the en^1 (or en^1/en^2) mutants shows a vein and bristle pattern resembling that of the anterior compartment (Garcia-Bellido and Santamaria, 1972; Lawrence and Morata, 1976). These observations led to the proposal (Lawrence and Morata, 1976) that *en* is active in the posterior compartments where it specifies "posterior" identity, be this in the wing, leg or antenna. The implication of this idea is that the critical difference between an anterior and a posterior compartment is provided by the en product; its presence would determine posterior patterns while its absence would specify anterior ones. It is implicit in this hypothesis that, for example in the wing, the complete loss of *en* function will produce a complete transformation of the posterior compartment into the anterior one, while ectopic expression in the anterior compartment would transform it into a posterior compartment. Studies on the expression of the en product (DiNardo et al., 1985; Fjose et al., 1985; Brower, 1986; Kornberg et al., 1985) supported some features of the model; it was found that *en* encodes a DNA binding protein restricted to the posterior compartments of embryonic and adult segments, although there is a late *en* expression in some cells of the anterior compartment close to the border (Blair, 1992).

However, other results indicated a genetic and functional complexity not foreseen by the model. One complication is that lethal (presumably null) *en* mutations do not show a complete transformation of posterior into anterior patterns (Lawrence and Struhl, 1982; Gubb, 1985). Besides, mutant *en* clones sometimes produce extra growth in the wing blade (Lawrence

and Morata, 1976; Lawrence and Struhl, 1982). The situation is further complicated by the finding (Coleman et al., 1987) that there are two similar genes arranged in tandem, *en* and *invected (inv)*, which encode products of similar sequence. While the existence of *inv* may help to explain the incomplete transformation of mutant cells for lethal *en* alleles (Lawrence and Struhl, 1982), assuming *en* and *inv* may encode partially redundant functions, the fact is that no individual *inv* mutation has so far been isolated and so its role, if any, remains unknown. Only very recently a chromosome deficient for both *en* and *inv* has been made and mosaic analysis using this chromosome has shown that in the posterior wing margin, doubly mutant cells show a complete transformation of posterior into anterior pattern (Hidalgo, 1994).

Although its expression is restricted to posterior compartments, an indirect participation of *en* in anterior adult patterns has been suggested. Recent experiments have shown that *hh*, a posterior specific gene controlled by *en* (Tabata et al., 1992; Lee et al., 1992), activates *decapentaplegic (dpp)*, an anterior gene involved in the patterning of anterior and posterior compartments (Tabata and Kornberg, 1994; Basler and Struhl, 1994; Capdevila and Guerrero, 1994). These studies have led to a better understanding of the role of *en* in the patterning of anterior compartments, but it is unknown if *en* is sufficient to provide posterior identity in imaginal discs.

Some of the problems concerning the function of *en* can be approached by studying the morphogenetic consequences of its ectopic expression in anterior compartments. We have made use of the GAL4 method (Brand and Perrimon, 1993) to express the en product ectopically in the anterior wing disc compartment. We find that it causes a transformation toward posterior patterns in the region containing the product, but also a duplication of anterior patterns in the adjacent region. The anterior to posterior transformation is accompanied by activation of the *inv* gene, while the anterior duplications correlate with high ectopic levels of *dpp*. The hh product appears to be associated with these two roles, inducing expression of *en, inv* and *dpp*. We propose that *en* has a dual function: (1) a non-cell autonomous function necessary for the proliferation and patterning of the entire appendage, achieved through the control of *dpp* activity and (2) a cell autonomous function responsible for the determination of posterior compartment identity, to which both *en* and *inv* may contribute. Our results also indicate that, in the wing imaginal disc, *en* negatively regulates its own expression.

MATERIALS AND METHODS

Fly stocks

Wild-type flies were from the Oregon R strain (Lindsley and Zimm, 1992). GAL4 lines were as follows: MS 1096 from F. Jimenez (Capdevila and Guerrero, 1994), 71B, 30A (Brand and Perrimon, 1993), C-765,C-734, C-743, C-580 from A. Brand. The UAS-*hh* line was described by Capdevila and Guerrero, (1994). The mutant stocks were as follows: en^1, en^2 are described by Lindsley and Zimm (1992); Lawrence and Morata (1976) and Morata et al. (1983). The *dpp-lacZ* (BS3.0, Blackman et al., 1991), *en-lacZ* (ry xho 25/CyO, Hama et al., 1990) and *hh-lacZ* (J413, Ma et al., 1993) stocks were kindly provided by W. Gelbart, T. Kornberg and K. Moses respectively.

Generation of UAS-*en* transgenic fly lines

For the production of the UAS-*en* transgenic fly lines, a *Eco*RI DNA fragment of 2 kb containing the entire *en* ORF was isolated from a plasmid (D2B) (Poole et al., 1985) and cloned into the *Eco*RI site of the pUAST plasmid. The recombinant plasmid containing the *en* cDNA in the correct orientation relative to the UAS sequences was used to transform *Drosophila* embryos from the stock w^{118} by standard procedures of microinjection (Roberts, 1986). Several independent lines were obtained, all of them showing a similar level of gene expression as judged by immunostaining of imaginal discs using anti-en/inv monoclonal antibody that recognizes both en and inv antigens (Patel et al., 1989).

Whole-mount immunostaining of imaginal discs

Immunostainings using anti-ptc (Capdevila et al., 1994b), anti-en (Patel et al., 1989), anti-hh (Capdevila and Guerrero, 1994), and anti-ci (Slusarski et al., 1995) antisera were performed essentially as described by Capdevila et al. (1994a). The imaginal discs from wandering third instar larvae were fixed in 4% paraformaldehyde in PBS for 20 minutes at room temperature and washed in PBS. For diaminobenzidine (DAB) staining, PBS containing 0.2% BSA, 0.1% saponin and 5% goat serum was used for blocking and for antibody incubation. Tissue was blocked for 1 hour and incubated overnight at 4°C in a dilution of the primary antibodie, washed and incubated in a 1/300 dilution of biotinylated anti-mouse (for en and ptc antibodies) or anti-rat (in the case of anti-hh and anti-ci antibodies) for 1 hour at room temperature. Discs were then washed in PBT (PBS containing 0.1% Tween 20) and incubated for 30 minutes in Vector AB elite solution in PBT. After several washes in PBT, the reaction was developed in 0.5 mg/ml DAB (Sigma) in PBS containing 0.06% H_2O_2. Discs were mounted under coverslips in eponaraldite (Fluka) after dehydration.

Imaginal discs were observed and photographed under a Zeiss Axiophot microscope.

X-Gal staining

Imaginal discs were first fixed in 4% paraformaldehyde in PBS for 20 minutes at room temperature, fixed again in 0.5% glutaraldehyde (Fluka) in PBS on ice for 2 minutes and washed in PBS. The reaction was developed in 5 mM $K_4[Fe^{II}(CN)_6]$, 5 mM $K_3[Fe^{III}(CN)_6]$, 1 mM $MgCl_2$ and 0.2% X-Gal in PBS containing 0.3% Triton X-100. Discs were mounted and observed as described for diaminobenzidine staining.

Whole-mount RNA in situ hybridization

An *inv* DNA fragment of 684bp (7-690 bp) from the first exon of the gene was obtained by PCR from genomic DNA using two synthetic oligonucleotides as primers (5′GGAAGCTTACCTTGGCCAGCACTCG 3′, and 5′ CCGGATCCCAGCTGAGCAAGTGCATC 3′) and subcloned in the Bluescript vector. This DNA fragment does not hybridize to the *en* DNA sequence. Synthesis of both *inv*- and *en*-specific probes was achieved by unidirectional PCR. The following synthetic oligonucleotides were used as primers: 5′ AGCAGTGCTGCTGAATGC 3′ for the *inv* probe and 5′ GCTCTAGAGCGTGGAAACTCATGTCAC 3′ for the *en* probe. Standard PCR conditions and DIG-labeled nucleotides (Boehringer Mannheim) were used. In situ hybridization (Tautz and Pfeiffle, 1989) was done as previously described (Capdevila et al., 1994a).

RESULTS

Ectopic *engrailed* expression leads to a transformation of anterior into posterior patterns in the wing

We have obtained transformed flies carrying a construct in which an *en* cDNA is under UAS control (see Materials and Methods) in order to express the en product ectopically using the GAL4 system (Brand and Perrimon, 1993). As ectopic expression of *en* during embryogenesis is bound to

be lethal, we used some GAL4 lines driving expression of the GAL4 protein restricted to portions of the imaginal discs, which allow survival to adulthood. Some of them have already been described (Brand and Perrimon, 1993; Capdevila and Guerrero, 1994). As they act in different regions of the wing disc and with different levels, positional and quantitative differences in *en* expression can be tested. We have crossed the UAS-*en* flies to the following GAL4 lines: MS 1096, (gift from F. Jimenez, see also Capdevila and Guerrero, 1994), 30A, 71B (Brand and Perrimon, 1993), C-765, C-580, C-734, C-743 (gifts from Andrea Brand) to direct the en product in the anterior and the posterior wing compartments.

In the MS 1096/UAS-*en* line there is en product all over the dorsal wing pouch (Fig. 1D), although there is also some expansion into the ventral part of the disc at the end of the third instar. As shown in Fig. 1E,F, the anterior dorsal wing develops a posterior pattern. Characteristic posterior pattern elements such as the double row hairs and the axillary cord appear in the anterior margin. We find that the transformed dorsal compartment is substantially smaller than the untransformed ventral one (Fig. 1E), suggesting that the control of size and proliferation is, at least to a certain degree, independent in dorsal and ventral wing compartments.

In the 71B line the GAL4 expression is localized in dorsal and ventral regions of the wing pouch but it does not extend to the dorsoventral border (Fig. 1G). The 71B/UAS-*en* flies present the vein pattern of part of the anterior compartment transformed into the corresponding posterior pattern (Fig. 1H,I), indicating that not only the margin, but also other regions of the compartment can be transformed by *en*. The C-734 line produces dorsal and ventral expression away from the dorsoventral border and also results in a similar transformation in the vein pattern. These results clearly argue for the instructive property of the en product being in the specification of posterior patterns.

The transformation induced by *engrailed* is associated with a change in the expression of specific anterior and posterior patterning genes

The homeotic transformations described above are accompanied by changes in the expression of genes that are normally transcribed specifically in the anterior or posterior compartments. The anteriorly expressed genes *cubitus interruptus (ci)*, *patched (ptc)* and *dpp*, (whose expression patterns are shown in Fig. 2A,C,E) are repressed in the area of *en* expression, (Fig. 2B,D,F), although they become active in the surrounding region (see below). The posterior gene *hh* (Fig. 2J) becomes activated in *en*-expressing cells. Moreover, using a specific probe for the posterior gene *inv* (see Material and Methods), we have been able to study *inv* expression in lines with ectopic *en* expression. In normal development inv protein has the same distribution as en in both embryos and imaginal discs (Coleman et al., 1987; Fig. 2K). We find that the presence of the en product in the anterior compartment of UAS-*en*/MS 1096 flies produces an ectopic expression of *inv* (Fig. 2L) in the same *en*-expressing cells. This indicates a regulatory interaction between *en* and *inv*.

invected and *engrailed* are activated by *hedgehog*

When ectopically expressing *hh* in MS1096/UAS-*hh* flies (Fig. 3B), we have observed posterior transformation of the anterior margin (Fig. 3A) similar to that found in MS1096/UAS-*en*, suggesting derepression of *en* and/or *inv*. This is visualized by the staining with the monoclonal antibody mAb4D9 (Patel et al., 1989), which recognizes both products. The staining extends to the anterior compartment of MS1096/UAS-*hh* wing discs (Fig. 3D). The possibility of a specific effect on either *en* or *inv* was checked by in situ hybridization using specific probes for each gene. The result is that both *en* (Fig. 3G) and *inv* (Fig. 3F) are derepressed. Surprisingly, we found no alteration of *lacZ* expression when using the *en-lacZ* line Xho25 (Hama et al., 1990) as a marker of *en* activity, indicating that this line does not respond to *hh* as the endogenous gene. Additionally, we have also found (using the *hh-lacZ* line J413; Ma et al., 1993) that, as a consequence of *en* activation, the *hh* endogenous gene is in turn activated.

The ectopic expression of *en* and *inv* as a response to *hh* has also been found in other GAL4 lines (30A and C-765) which also exhibit posterior transformation in the anterior compartments. These transformations are sometimes difficult to detect, perhaps because *dpp* and *ptc*, both induced by *hh* (Fig. 3C), would counteract the posterior transformations induced by *en*, or because the levels of *en* induced by UAS-*hh* are in some cases insufficient to produce a posterior transformation.

Ectopic activation of *engrailed* also produces duplications of anterior wing patterns

In addition to the transformations of anterior into posterior wing described above, in some lines we also find duplications of anterior wing patterns associated with these transformations. This is especially clear in those lines that drive ectopic expression in restricted regions of the dorsal and ventral compartments along the dorsoventral border. The best example is the 30A line where the en product is induced in the proximal regions of the posterior and anterior compartments, both in the dorsal and ventral sides (Fig. 4A). In 30A/UAS-*en* flies, the costa, located in the proximal anterior wing, is transformed into axillary cord, a region located in an homologous position along the proximal-distal axis of the posterior compartment, but this is often accompanied by a duplication of adjacent anterior patterns (Fig. 4D). Since wing duplications are often associated with ectopic expression of *dpp* (Capdevila and Guerrero, 1994), we have checked for a possible alteration of *dpp* in these wings. We find an area of high levels of dpp product just adjacent to the region of ectopic *en* expression (Fig. 4B). This activation of *dpp* is associated with high levels of *ptc* and *ci* in the same region, as is shown in Fig. 2D,F, for the MS 1096 line. This recreates the situation along the anteroposterior border, and as in this position, the *dpp* gene initiates the signaling mechanism which eventually leads to a duplication of part of the anterior pattern (Capdevila et al., 1994; Tabata and Kornberg, 1994; Basler and Struhl, 1994; Capdevila and Guerrero, 1994). An induction of *dpp* is also found by GAL4-driven expression of *hh* (Capdevila and Guerrero, 1994), but in this case the number of *dpp*-expressing cells is greater, since *hh* and *dpp* can be coexpressed in the same cells (Fig. 3B,C). This results in bigger duplications and outgrowths of the wing.

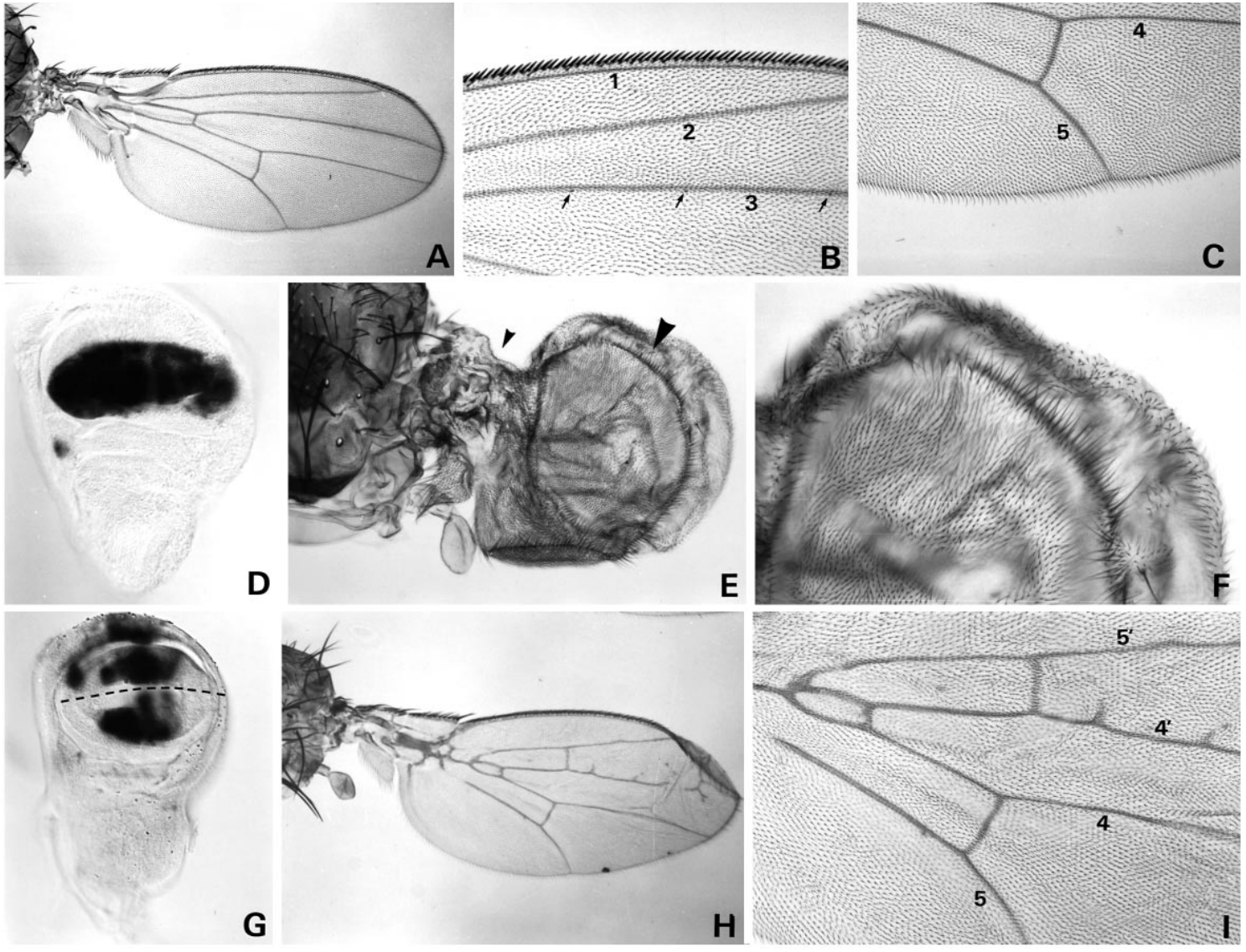

Fig. 1. Posterior transformation of the anterior compartment induced by targeted expression of *en* in specific regions of the anterior wing disc. (A) Wild-type wing. (B) Detail of part of a wild-type anterior margin, with the characteristic stout bristles, veins 1, 2 and 3, and specific sensillae in vein 3 (arrows). (C) Posterior compartment margin, containing characteristic thin hairs, very different from the socketed bristles of the anterior margin. Veins 4 and 5 are indicated. (D) Distribution of the GAL4 protein in the MS 1096 line, as shown by X-gal staining in the wing disc. It is restricted mainly to the dorsal wing pouch. (E) An MS 1096/UAS-*en* fly wing, showing the anterior compartment transformed into posterior; the costa region is replaced by axillary cord (small arrowhead) and the anterior wing margin by posterior wing margin (large arrowhead). Notice that the transformed (dorsal) wing region is reduced in size. (F) Detail of the transformed anterior margin showing the characteristic long hairs typical of the posterior margin. (G) Distribution of the GAL4 protein in the 71B line. It appears in the dorsal and ventral regions but not in the dorsoventral border (dotted line). (H) Transformed wing of a 71B/UAS-*en* fly showing a transformation of veins 2 and 3 into 4′ and 5′ and giving rise to a mirror image arrangement. (I) Higher magnification of the same wing to show the mirror image transformation. Notice the lack of sensillae in the transformed vein 3 (4′).

Excess of *engrailed* function, but not of *hedgehog*, leads to a transformation of posterior into anterior patterns

One surprising result found in most of the lines examined is that the excess of en product in the posterior compartment gives rise to a transformation towards anterior pattern. This leads to some paradoxical phenotypes like the one shown in Fig. 5D,E corresponding to an MS 1096/UAS-*en* wing, in

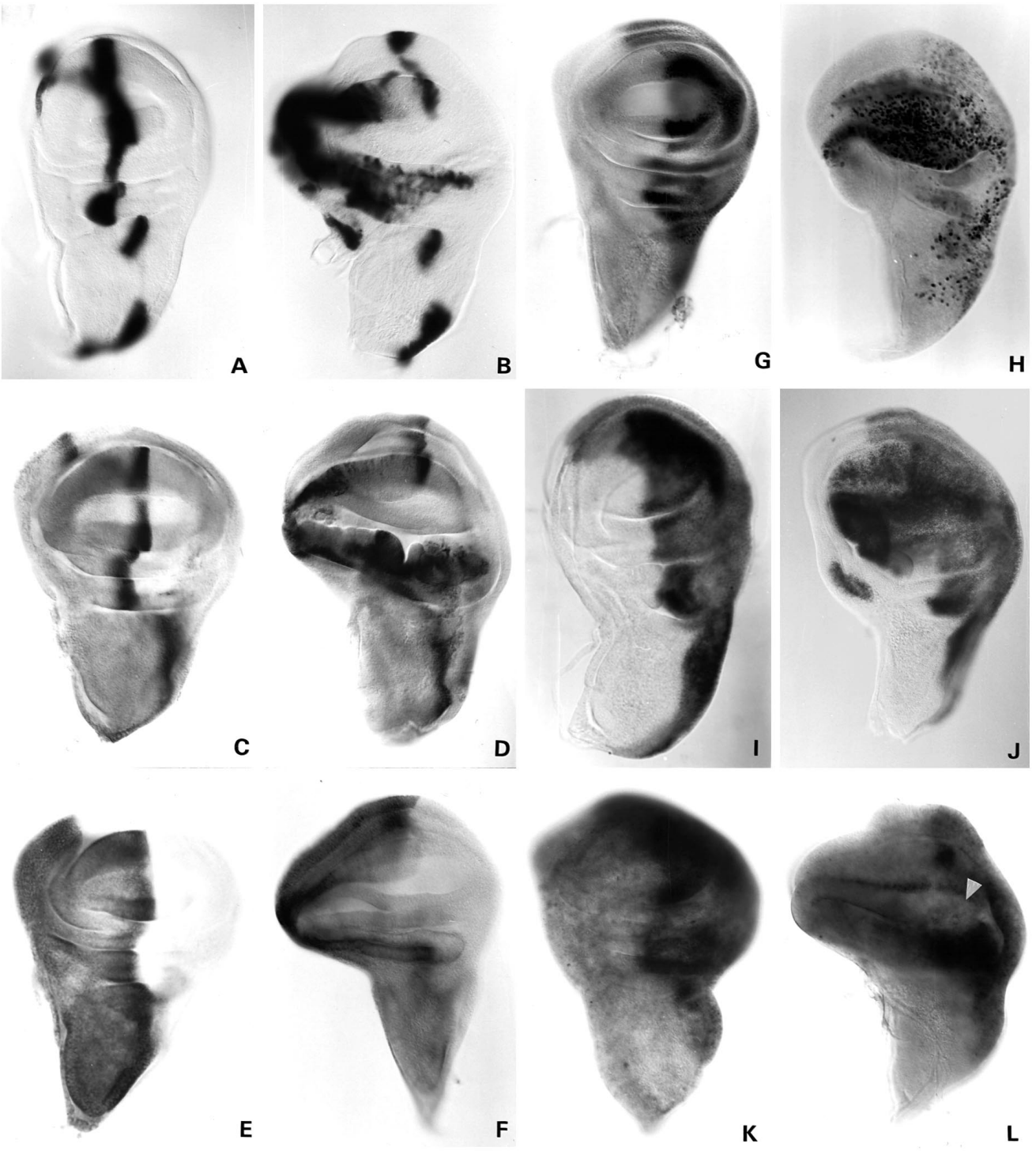

Fig. 2. Ectopic en activity modifies the expression pattern of anterior and posterior specific genes in the wing disc. (A,C,E) Wild-type expression of *dpp* (X-gal staining), *ptc* (anti-ptc antibody staining) and *ci* (anti-ci antibody staining) respectively. (B,D,F) Expression of the same genes in MS 1096/UAS-*en* flies. Notice the repression of the three genes in the area of *en* ectopic expression together with gain of function in the surrounding region. (G,I,K) Wild-type expression of *en/inv* (anti-en/inv antibody staining), *hh* (X-gal staining) and *inv* (in situ hybridization) compared with (H,J,L) expression of the same genes in MS 1096/UAS-*en* flies. Note that *hh* and *inv* expressions are induced in the same area as *en*. The arrowhead indicates a region in the posterior compartment showing loss of *inv* expression.

which the anterior compartment is transformed into posterior, but the posterior one is transformed into anterior. This anterior transformation of the posterior compartment can occur anywhere along the proximodistal margin of the adult wings (Fig. 5B-E compare with Fig. 1C) and also in internal regions of the wing blade. In several instances the posterior compartments closely resemble those of en^1/en^1 or en^1/en^2 mutants (Fig. 5A).

Since the only difference between the anterior and the posterior compartments is the amount of en product, due to the summation in the posterior compartment of the endogenous plus the exogenous proteins, the result suggests that the excess of product is impairing the function of the endogenous *en*, and perhaps also *inv*, function. We have checked the expression of *en* and *inv* in MS 1096/UAS-*en* (Fig. 5G for *en* and Fig. 2L for *inv*) and in en^1/en^1 discs, and found that the levels of expression of the two genes are variably reduced in the posterior compartment. In addition, we have observed derepression of endogenous *en* gene in some cells of the anterior compartment (Fig. 5G). Occasionally (Fig. 4B), we also observe derepression of *dpp* in the posterior compartment, probably due to the local loss of *en* (Raftery et al., 1991; Sanicola et al., 1995). However, we fail to see a reduction in *hh* expression, which remains apparently normal. We do not know if the activation of the endogenous *en* gene is mediated by *hh* or if it can occur by direct activation by the exogenous en product. The anterior transformation of the posterior compartment is not found in the MS 1096/UAS-*hh* flies (compare Fig. 5D,E with Fig. 3A). This suggests that the high levels of en product are directly responsible for the reduction in *inv* expression in the posterior compartments of MS 1096/UAS-*en* flies.

The anterior transformation of the posterior compartment by excess of en product is hard to explain because the GAL4 driven en product should be sufficient to rescue the loss of the endogenous gene. In principle, one explanation could be that *inv*, which is also down regulated by the excess of en product,

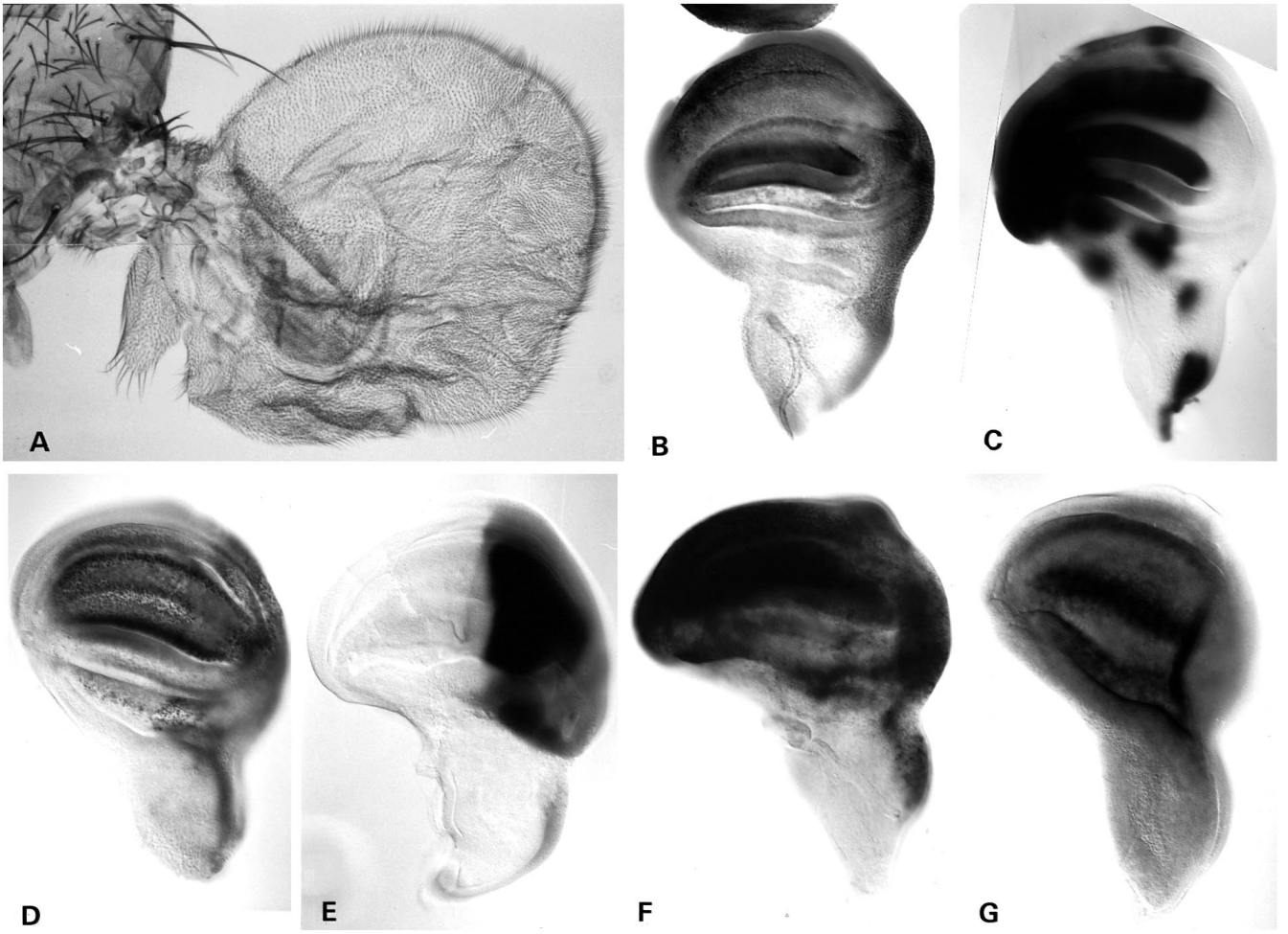

Fig. 3. Transformation of anterior to posterior wing by ectopic expression of *hh* in the MS 1096/UAS-*hh* genotype. (A) A wing exhibiting the posterior transformation clearly visible along the wing margin, now of posterior identity. (B) Distribution of the hh protein in the wing disc, where it extends to the anterior compartment. (C) *dpp* expression as reported by *dpp-lacZ* (BS-3.0) line; *dpp* is expressed in most of the cells in the anterior compartment that contain hh product. (D) Distribution of the en-inv antigen in a wing disc. The staining extends to the anterior compartment whereas normally it is restricted to the posterior compartment. (E) *en* expression as visualized by the *en-lacZ* line. β-gal distribution is confined to the posterior compartment. (F) *inv* expression as shown by the specific *inv* probe. (G) *en* expression as indicated by a specific en probe. Both *en* and *inv* are ectopically expressed in the anterior compartment and their expression patterns mimic that of *hh* in the same line.

has a major role in the posterior wing pattern which cannot be covered by the GAL4 driven en product.

DISCUSSION

The function of *engrailed* in the wing

In the experiments reported here we have used the GAL4 method to express ectopically the en product in anterior wing compartments. This produces a homeotic transformation of the region in the anterior wing where the en product is expressed in a posterior wing region located in a similar proximodistal position (see below). This clearly demonstrates the ability of the en product to specify adult posterior patterns and indicates that *en* is responsible for posterior adult identity in normal development (Morata and Lawrence, 1975).

The change in identity of the anterior cells is associated with changes in the expression patterns of genes specific for the anterior and posterior compartments. Anteriorly expressed genes, like *ci*, *ptc* and *dpp*, are repressed in the area where *en* is ectopically expressed, while posterior genes such as *hh* and *inv* are activated. Thus, one conclusion from our results is that *inv* is regulated by its closely linked gene *en*, as recently suggested by Goldsborough and Kornberg (1994). It is, however, unclear whether *inv* contributes to the posterior transformation. A possible role of *inv* is suggested by the results of Hidalgo (1994), who showed that posterior cell clones lacking both *en* and *inv* produce a stronger transformation than those lacking only *en*. The isolation of specific mutations in the *inv* locus may settle the issue about the specific role of *inv*.

In addition to the posterior transformations of anterior patterns discussed above, we often observe duplications of anterior wing patterns just adjacent to the regions exhibiting posterior transformations. Analysis of gene expression in the vicinity of the *en*-expressing region shows that *dpp* is ectopically activated just outside this region. This is also accompanied by high local levels of ptc and ci proteins, very much resembling the situation in the anteroposterior boundary. The ectopic activation of *hh* in the area of *en* ectopic expression generates an interface of *hh*- and non-*hh*-expressing cells which results in local activation of *dpp* as a consequence of blocking the repressive effect of *ptc* upon *dpp* expression. This produces a transformation similar to that achieved by direct ectopic expression of *dpp* (Capdevila and Guerrero, 1994). Pattern duplications are only found in those GAL4 lines inducing local areas of *dpp* transcription within the disc. It is possible that a graded distribution of dpp product is a requisite for the generation of wing patterns.

Altogether, our experiments suggest that the overall function of *en* in wing disc development can be separated into two distinct roles.

The first one is the primordial function in the cells of the posterior compartment to specify the characteristic posterior pathway (Morata and Lawrence, 1975; Lawrence and Morata, 1976). This is presumably achieved through the activation of posterior genes (and/or the repression of the anterior ones), although the mechanism is not known. The second role is indirect and connected with the overall specification of pattern in the appendage. It is achieved by the interface between cells of the posterior compartment expressing hh product (as activated by *en*) with anterior cells not transcribing it. The hh product is passed to the adjacent anterior cells (Tabata and Kornberg, 1994; Basler and Struhl, 1994; Capdevila and Guerrero, 1994), which respond by activating *dpp* (Capdevila and Guerrero, 1994). The

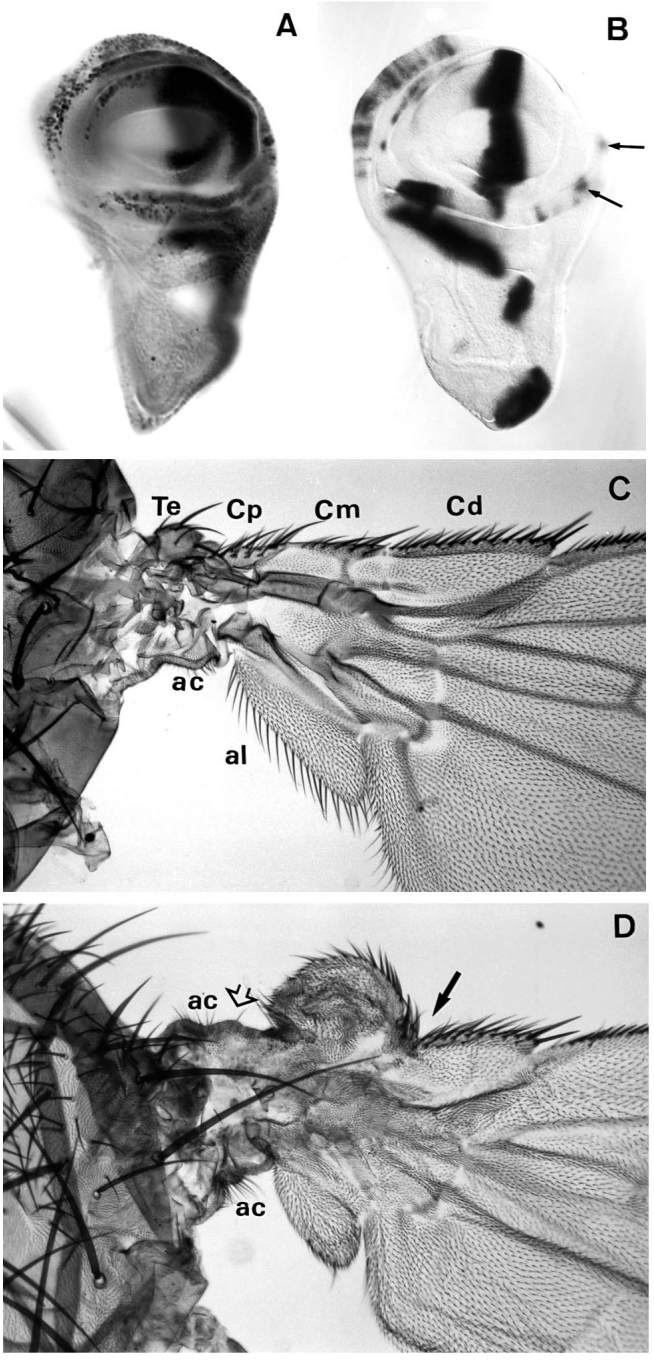

Fig. 4. Effect of ectopic *en* expression in 30A/UAS-*en* flies. (A) Distribution of en and inv proteins in a 30A/UAS-*en* wing imaginal disc. (B) Ectopic *dpp* expression in a disc of the same genotype. The arrows indicates *dpp* derepression in the posterior compartment. (C) Proximal part of a wild-type wing. Note the tegula (Te), and proximal (Cp), medial (Cm) and distal (Cd) costa, in the anterior compartment, and axillary cord (ac) and alula (al) in the posterior compartment. (D) Mirror image duplication in the anterior compartment of a 30A/UAS-*en* wing, showing the replacement of Te, Cp and Cm by ac (open arrow) and the duplication axis of the Cd distal pattern (arrow).

expression of *dpp* restricted to the anteroposterior boundary has to be precisely controlled and is achieved through the function of *ptc*, *ci* and other genes (Capdevila and Guerrero, 1994). From this region the morphogenetic influence of *dpp* spreads to anterior and posterior compartment cells. In the absence of any other modifying factor, the two wing compartments would develop anterior patterns in mirror image symmetry (e.g. as in an en^1 homo-zygous wing). These two distinct roles of *en* can be clearly visualized in the 30A/UAS-*en* flies in which the en product is planted in the proximal anterior wing region: the proximal costa is transformed into an axillary cord, a characteristic posterior structure, and in addition, there is a duplication of anterior wing patterns (Fig. 4). Changes in both patterns are caused by the presence of the en product.

The activation of *en* and *inv* by *hh*

The activation of *en* and *inv* by *hh* observed in several GAL4 lines (1096, 30A, C-765) is an unexpected result, the functional significance of which is unclear, but that could be responsible for the late activation of *en* observed in some cells of the anterior compartment in third instar wing imaginal discs (Blair, 1992). This late anterior *en/inv* expression may have a patterning function in the region between vein 3 and the compartment border (Hidalgo, 1994). The activation of *en/inv* by *hh* could be achieved by an unknown mechanism or perhaps a consequence of blocking the repressive effect of *ptc*, as occurs for *dpp* and *ptc* itself (Capdevila et al., 1994a; Tabata and Kornberg, 1994; Basler and Struhl, 1994; Capdevila and Guerrero, 1994).

Although the activation of *en* and *inv* by *hh* has only been

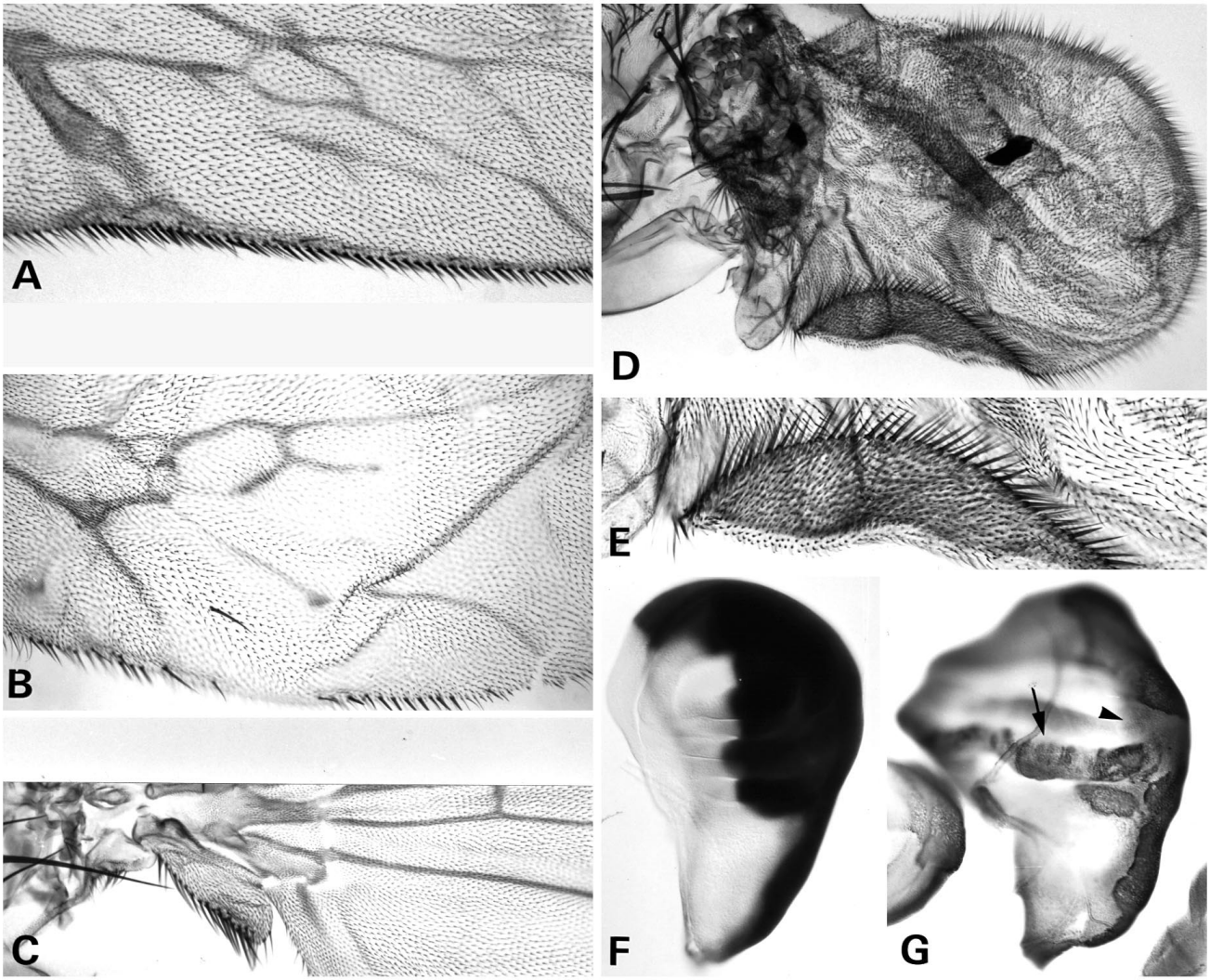

Fig. 5. Anterior transformation of the posterior compartment induced by high levels of en product. (A) Part of the posterior compartment of an en^1/en^2 wing showing anterior transformation, especially visible by the appearance of the stout bristles characteristic of the anterior margin (see Fig. 1B). (B) Posterior margin of a C-743/UAS-*en* wing displaying a similar transformation. (C) Proximal part of a 30A/UAS-*en* wing showing socketed costa-like bristles in the alula region, also indicating an anterior transformation. (D) Transformed wing of an MS 1096/UAS-*en* fly. (E) Detail of the transformed posterior margin showing the appearance of stout bristles all over the edge. (F) Normal *en* expression as monitored by X-gal staining in the *en-lacZ* line in an untransformed disc. (G) *en* expression in an MS 1096/UAS-*en* wing disc, also carrying the *en-lacZ* gene and developed using anti-β-gal antibody staining. It shows a reduction in the amount of β-gal in the dorsal posterior region of the wing pouch (arrowhead), precisely the region where GAL4-induced en product adds to the endogenous product. A derepression of *en* endogenous gene is also found in some cells of the anterior compartment (arrow).

observed in the anterior compartment, it is possible that it also occurs in the posterior compartment, where it might have a role, connected, for example, with the maintenance of *en* activity. However, all identified elements of the *hh* signal involved in *dpp* activation in the wing disc, like *ptc* or protein *kinase A* (Capdevila et al., 1994; Jiang and Struhl, 1995; Li et al., 1995; Pan and Rubin, 1995; Lepage et al., 1995) have anterior compartment activity. Therefore, the possible activation of *en/inv* by *hh* in the posterior compartment may suggest the existence of an unidentified receptor and/or a different transduction pathway specific to the posterior compartment.

Excess engrailed product causes loss of function *engrailed* phenotypes

The excess of en product in the posterior compartment of some GAL4/UAS-*en* discs, results in phenotypes similar to those of mutations like en^1 or en^2 (Fig. 5A), known to cause partial loss of *en* function (Brower, 1986; Raftery et al., 1991; our own observations). As in the case of en^1 mutants, we also observe a loss of the endogenous *en* and *inv* gene products in GAL4/UAS-*en* discs. Here, there is a situation in which normal levels of *en* activate *inv*, but inappropriate high levels produce the opposite effect, perhaps by direct repression on *en* and *inv* transcription. It has recently been suggested (Goldsborough and Kornberg, 1994) that *en* and *inv* share regulatory elements, which may explain why the two genes are repressed by the excess of *en* function. Apparently, this repression does not involve *hh*, for we do not see a reduction in *hh* expression in the posterior compartments of MS 1096/UAS-*en* flies. Moreover, the excess of hh product in the posterior compartments of MS 1096/UAS-*hh* flies does not affect the posterior pattern, also arguing against an involvement of *hh*.

The functional significance of this phenomenon is uncertain at present; it obviously indicates that the amount of en product has to be precisely regulated and also provides an explanation for the observation that heat-shock-induced high levels of en product in embryogenesis produce a phenotype resembling that of en^- embryos (Poole and Kornberg, 1988). There are some cases reported in which different levels of the regulatory product may also have opposite effects; the Antp product up-regulates *Scr* at moderate levels but suppresses its expression at high levels (Pelaz et al., 1993).

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