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Evidence for *engrailed*-Independent *wingless* Autoregulation in *Drosophila*

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Proper spatial expression of the *wingless* (*wg*) gene in the *Drosophila* embryonic epidermis is crucial to intrasegmental patterning. Single cell wide *wg* expression is initiated at the blastoderm stage in response to combinatorial regulation by the pair rule genes. Later, during gastrulation, when the epidermal expression of the pair rule genes has disappeared, *wg* becomes regulated by the activity of the segment polarity genes. The segment polarity gene *engrailed* (*en*) is expressed in cells adjacent to the *wg*-expressing cells and is required to maintain *wg* transcription. Since *wg* is in turn required to maintain *en* expression, *wg* appears to autoregulate its own expression through an *en*-dependent paracrine feedback loop. In this paper, we demonstrate that wild-type *wg* expression requires *wg* activity during stage 9, prior to its requirement for *en* maintenance, indicating that *wg* has an autoregulatory role that is distinct from its paracrine feedback loop through *en*. In addition, by misexpressing Wg and En in distinct spatial patterns in the epidermis, we find that En is capable of inducing expression from the endogenous *wg* gene only in immediate adjacent cells which have been exposed to Wg. Furthermore, exogenous Wg expression enables maintenance of endogenous *wg* transcription in both *wg* and *en* mutant embryos. Our results support the model that in the wild-type embryo, *wg* has an autoregulatory function which is distinct and separable from paracrine regulation via *en*. We also provide evidence that late, localized Wg expression is crucial for the asymmetric patterning of epidermal cell types as reflected in the larval cuticle.

INTRODUCTION

The establishment of asymmetry during embryonic development is an important aspect of the generation and patterning of diverse cell types in a mature organism (Ingham and Martinez-Arias, 1992; Gurdon, 1992). In *Drosophila melanogaster*, anteroposterior asymmetry is initiated by the differential distribution of maternal products and the responding zygotic factors in the syncytial blastoderm (Akam, 1987; Ingham, 1988; St. Johnston and Nusslein-Volhard, 1992). The precise transcription domains of downstream genes, in particular the segment polarity genes *wingless* (*wg*) and *engrailed* (*en*), are established by the pair rule class of zygotic genes. *wg* and *en* are transcribed in adjacent stripes of cells that, respectively, mark the most posterior and anterior regions of each of the 14 parasegments in the epidermis, thereby displaying the first sign of intrasegmental polarity (Baker, 1987; DiNardo *et al.*, 1988).

The initiation and early maintenance of *wg* and *en* expression appears to be a cell autonomous process, controlled at different times by putative transcription factors. Initiation of *wg* transcription at embryonic stage 5 requires activity of the *paired* (*prd*) and *odd-paired* (*opa*) genes (Ingham and Hidalgo, 1993; Fregorio *et al.*, 1986; Benedyk *et al.*, 1994). Later the proteins encoded by the *sloppy-paired* (*slp*) locus are responsible for maintaining *wg* expression during stages 7-9 (Grossniklaus *et al.*, 1992; Cadigan *et al.*, 1994). By embryonic stage 8, the early pair rule activators have disappeared and regulation of *wg* and *en* becomes dependent on segment polarity genes (Martinez-Arias *et al.*, 1988; Peifer and Bejsovec, 1992; Perrimon, 1994). Some of the segment polarity genes act nonautonomously, indicating that cell-cell communication is involved in maintenance of *wg* and *en* expression.

wg is the *Drosophila* homologue of *Wnt-1*, a vertebrate proto-oncogene and a member of a large family of secreted glycoproteins (Baker, 1987; Rijsewijk *et al.*, 1987; for reviews see McMahon, 1992; Nusse and Varmus, 1992). Wg protein is produced in the epidermis in single cell wide stripes and appears to be secreted (van den Heuvel *et al.*, 1989; Gonzalez *et al.*, 1991). Null *wg* mutations result in embryos that fail to secrete the smooth, or "naked," cuticle normally found ventrally at the pos-

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terior of each larval segment. In this *wg* mutant "lawn" phenotype, the entire ventral surface of the cuticle is covered with denticles (Nusslein-Volhard and Wieschaus, 1980). Thus even though its expression is restricted to a narrow stripe of cells in each metamere of the epidermis, *wg* activity is required for the proper generation of diverse cell types in the entire segment (Bejsovec and Wieschaus, 1993).

A number of results indicate that *wg* has a role in maintaining its own transcription through cell-cell signaling. Even though *en* is expressed in adjacent cells, *wg* is required for the maintenance of *en* expression. In mutants lacking *wg* activity, *en* striped expression initiates normally but then fades during stage 9 (DiNardo *et al.*, 1988; Martinez-Arias *et al.*, 1988, Bejsovec and Martinez-Arias, 1991, Heemskerk *et al.*, 1991). In turn, the activity of the homeodomain containing protein En is required for *wg* maintenance in adjacent cells (Fjose *et al.*, 1985; Poole *et al.*, 1985; Martinez-Arias *et al.*, 1988; Bejsovec and Martinez-Arias, 1991). As a transcription factor, En presumably regulates a pathway that signals the neighboring *wg* cells. This pathway has been proposed to include the secreted factor Hedgehog (Ingham *et al.*, 1991; Mohler and Vani, 1992; Lee *et al.*, 1992; Tabata *et al.*, 1992). Wg therefore can be regarded as autoregulatory since it maintains *en* expression, which is in turn required for *wg* maintenance. However, the *en*-dependent paracrine signal serves to maintain *wg* expression in the cells adjacent and anterior to the *en* cells (where *wg* transcription was initiated) but does not induce *de novo wg* expression in cells posterior to the *en* stripes. This is not simply because these posterior cells do not respond to *en* activity: the *patched* (*ptc*) gene is expressed in the cells just posterior to *en* stripes and expression of *ptc* responds positively to paracrine *en*-dependent signaling (Hidalgo and Ingham, 1990).

In order to explain how the *en*-dependent signal can maintain but not activate *wg* transcription in neighboring cells, two models have been put forward (Fig. 1). First, it has been postulated that the epidermis is divided into domains that are predisposed to express either *wg* or *en* (Ingham *et al.*, 1991; Fig. 1A). According to this model, only "*wg*-competent" cells that receive the *en*-dependent signal can transcribe *wg*. The products of the *slp* locus, which encodes two putative transcription factors, appear to define these *wg* competence domains (Grossniklaus *et al.*, 1992; Cadigan *et al.*, 1994). Cells located posteriorly to each *en* stripe may thus be incompetent to express *wg*, due to the absence of *slp* activity. The second model states that, in order to express *wg*, epidermal cells require exposure to Wg protein itself in addition to the signal from the *en*-expressing cells (Hooper and Scott, 1992; Fig. 1B). According to this model, only the cells in which *wg* expression was initiated by the pair

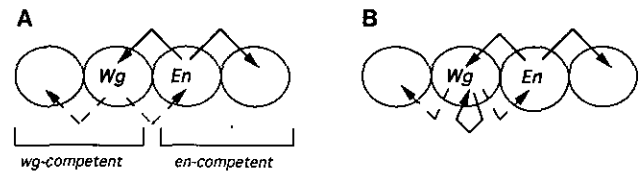


FIG. 1. Two models for the maintenance of asymmetric gene expression (adapted from Hooper and Scott, 1992). Depicted are a row of four ventral epidermal cells, one representing a *wg* stripe and another representing an adjacent *en* stripe (anterior is left). (A) In one model (Ingham *et al.*, 1991), distinct domains of cells in each metamere are competent to express either *wg* or *en*, but not both. These domains appear to be designated by activity of the *slp* genes (Cadigan *et al.*, 1994). (B) In the second model (Hooper and Scott, 1992), *wg* expression is limited by a requirement for both Wg protein (looped solid arrow) and a paracrine signal dependent on en (solid arrows). The latter is regarded as autoregulation since *en* expression requires paracrine *wg* activity for its maintenance (dashed arrows). In the second model, although the *en*-dependent signal is received by cells posterior to the *en*-expressing cells (Hidalgo and Ingham, 1990), these posterior cells will not express *wg* due to lack of exposure to Wg.

rule genes can transcribe *wg*. In other words, *wg* might autoregulate by two distinct mechanisms, an "*en*-dependent autoregulatory" pathway that operates through paracrine signaling and an "*en*-independent autoregulatory" pathway that may reflect an autocrine activity of Wg (Hooper, 1994).

Since both *wg* and *en* expression cease in mutants that make nonfunctional Wg protein (Bejsovec and Martinez-Arias, 1991; Ingham and Hidalgo, 1993; van den Heuvel *et al.*, 1993; this work), it has been difficult to clearly assess the *en*-independent contributions of Wg to *wg* transcription. Evidence for distinct *wg* autoregulatory pathways has been reported (Ingham and Hidalgo, 1993; Bejsovec and Wieschaus, 1993; Hooper, 1994). These analyses involved double mutant embryos and demonstrated an absolute requirement for Wg only in cells away from the parasegment borders (and the normal *wg* expression domains). Therefore an autoregulatory requirement for *wg* in the wild-type *wg*-expression domain at the parasegment borders—juxtaposed with the *en*-expressing cells—has not yet been demonstrated.

To examine the role of *wg* in its own regulation, we have compared the timing of disappearance of *wg* RNA and En protein in double-labeled *wg* mutant embryos. In addition, in order to avoid the potential complications of double mutant analysis, we have used the *GAL4* system (Brand and Perrimon, 1993) to directly misexpress Wg in distinct patterns in the embryonic epidermis. These experiments have allowed us to analyze in detail the effects of exogenous Wg on *en* and *wg* expression. Our data support the model that the maintenance of restricted *wg* transcription during late gastrulation requires *en*-independent *wg* autoregulatory activity. We

also demonstrate the importance of spatially localized Wg in the asymmetric patterning of epidermal cell fates.

MATERIALS AND METHODS

Fly Strains

Flies were raised on standard *Drosophila* media at 25°C. Descriptions of balancers and mutations that are not described below can be found in Lindsley and Zimm (1992). Description of wg^{IG22}, a protein null allele of *wg*, can be found in van den Heuvel *et al.* (1993). *Cyo;*wg^{en11}, a *CyO* balancer chromosome with a *lacZ* enhancer trap inserted in *wg*, is described in Kassis *et al.* (1992). en^{CX1}, a phenotypically null allele of *engrailed*, is described in Heemskerk *et al.* (1991). The third chromosome *hs-wg* line, where the *wg* gene is under the control of the heat shock promoter, is described in Noordermeer *et al.* (1992).

UAS lines. The *UASwg* (M7-2.1) line is described in Wilder and Perrimon (1995). The line is homozygous for an insertion on the third chromosome of the P-element pUAST (Brand and Perrimon, 1993), into which the *wg* cDNA has been cloned. The *wg* cDNA encodes a temperature-sensitive product identical to that made by wg^{IL114}, which is active at or below 17°C (Nusslein-Volhard *et al.*, 1984; van den Heuvel *et al.*, 1993). The *UASlacZ* line used in this study is a homozygous viable insertion on the second chromosome (Brand and Perrimon, 1993). To construct *UASen* (plasmid F135), pUAST was digested with *Eco*RI and ligated to the *en* cDNA, isolated as a 2-kb *Eco*RI fragment from D2Ben. D2Ben was kindly provided by Steve DiNardo. Transgenic lines were generated by injection into embryos of genotype *yw;* Δ2-3, Sb/TM6 using standard procedures (Spradling, 1986; Robertson *et al.*, 1988). Two *UASen* transformant lines were used in these experiments: $UASen^{4-3}$, an insertion on the second chromosome which is homozygous viable, and $UASen^{4-1}$, an insertion on the *TM6* chromosome. $UASen^{4-1}$ is kept as *TM6,* $UASen^{4-1}$*/h-lacZ. h-lacZ*, also known as *l(3)6531*, was obtained from Allan Spradling and is a lethal insertion of an enhancer trap *lacZ* element at the *hairy* locus.

GAL4 lines. The *hairyGAL4* (*hGAL4*) line is a homozygous third chromosome line (described in Brand and Perrimon, 1993, as *1J3*). The *pairedGAL4/TM3* (*prdGAL4*) line was kindly provided by Laurent Fasano and Claude Desplan. Expression patterns for these *GAL4* lines are described in the Results section.

Experimental Crosses and Embryo Staging

prdGAL4/UASwg embryos were generated by crossing *prdGAL4/TM3* flies with *UASwg* flies; 50% of the progeny embryos are of the experimental genotype. *hGAL4/UASwg* embryos were generated by crossing *hGAL4* flies with *UASwg* flies; all the progeny embryos are of the desired genotype. *prdGAL4/UASen* embryos were generated by crossing *prdGAL4/TM3* with $UASen^{4-1}$, *TM6/h-lacZ* flies; 25% of the progeny embryos are of the experimental genotype. Embryos bearing the *UASen* transgene were identified by the presence of alternating broad *en* stripes.

In all experiments involving *UASwg*, the embryos were raised at 16–17°C, a permissive temperature for the *UASwg* product (Wilder and Perrimon, 1995). Control crosses were performed at 25°C. Since development is greatly retarded at 16°C (compared to 25°C), in the text the ages of embryos are described by stages (according to Campos-Ortega and Hartenstein, 1985) rather than by hours following egg deposition.

For the *UASen;prdGAL4/hswg* experiment, $UASen^{4-3}$/+;*prdGAL4*/+ and $UASen^{4-3}$/+;*hswg*/+ flies were crossed together. Repeated heat shock treatment of *hs-wg* embryos results in ectopic *wg* expression following a broadening of every *en* stripe (Noordermeer *et al.*, 1992). To avoid this, *UASen;prdGAL4/hs-wg* embryos were heat-shocked under a protocol in which *hs-wg* alone does not induce ectopic *wg*. Three- to five-hr-old embryos were incubated at 37°C in a water bath for 10 min, aged an additional 2 hr and 30 min at 25°C, and then fixed. *UASen;prdGAL4* embryos, which represent $\frac{3}{8}$ of the total progeny, could be identified by the presence of the broad odd *en* stripes. *UASen;prdGAL4/hswg* embryos ($\frac{3}{16}$ of progeny) were identified by alternating broad *en* stripes and the novel *wg* expression anterior and/or posterior to these *en* stripes (see Results), which is never observed in *prdGAL4/UASen* alone. Under our heat shock regimen very few embryos (<5%; n = 198) contained ectopic *wg* attributable to *hs-wg* alone (i.e., ectopic *wg* and *en* in even segments).

Antibody Staining and in Situ Hybridization

Fixation and preparation of embryos for *in situ* hybridization to transcripts and/or antibody detection of protein(s) were performed as described in Manoukian and Krause (1992). Detection of endogenous *wg* transcripts was accomplished using a PCR-generated digoxigenin-labeled 5′ *wg* untranslated sequence-specific probe, which is unique to only endogenous *wg* transcripts. The PCR primer sequence is 5′-CTGTTCGACGGCACACACAC-3′. This allowed the detection of the endogenous *wg* transcripts without detection of *UASwg* transcripts, since this untranslated sequence was deleted from *UASwg* during cloning. In experiments involving the *Cyo,*wg^{en11} chromosome (Kassis *et al.*, 1992), endogenous *wg* transcription was monitored by probing

for *lacZ* transcripts, since this line expresses *lacZ* in the *wg* pattern (Siegfried *et al.*, 1992). Anti-Wingless antiserum (van den Heuvel *et al.*, 1989) was used at 1:100 dilution, and anti-Engrailed antiserum (Patel *et al.*, 1989) was used at 1:2 dilution.

Cuticle Preparations

Cuticles were cleared in Hoyers-Lactic acid solution and mounted as described by Struhl (1989) and were then photographed under dark-field optics.

RESULTS

Wg Activity is Required Prior to En Activity for wg Transcription

It has been previously shown that *wg* expression ceases in the absence of *wg* activity (Bejsovec and Martinez-Arias, 1991; Ingham and Hidalgo, 1993; van den Heuvel *et al.*, 1993). This could be solely a secondary consequence of the decay of *en* expression that occurs in *wg* mutant embryos during stage 9 (Martinez-Arias *et al.*, 1988; DiNardo *et al.*, 1988; Bejsovec and Martinez-Arias, 1991; Heemskerk *et al.*, 1991). If this were the case, then the loss of En protein should precede the loss of *wg* transcription in *wg* mutant embryos. However, in wg^{IG22} embryos (which produce *wg* transcript but lack functional protein; van den Heuvel *et al.*, 1993) that have been double-labeled for *wg* transcripts and En protein, nearly all epidermal *wg* expression disappears in the epidermis at stage 9, prior to the decay of epidermal En (Fig. 2B). This result, together with the observation that *wg* expression does not cease in *en* mutants until stage 10 (Bejsovec and Martinez-Arias, 1991; Fig. 6I), suggests that the loss of *wg* expression in *wg* mutants is not a secondary consequence of the loss of En and is consistent with a more direct, *en*-independent autoregulatory function of *wg*.

Induction of Ectopic wg Depends on the Localization of Exogenous Wg

It has been demonstrated that ubiquitous Wg misexpression from a heat shock promoter (*hs-wg*) can result in ectopic stripes of endogenous *wg* expression in every segment (Noordermeer *et al.*, 1992) (In the text, *ectopic* will refer to misexpression of the endogenous *wg* gene and *exogenous* will refer to misexpression from a transgene). In these *hs-wg* experiments, the width of each *en* stripe broadens significantly. Because the ectopic *wg* stripes are induced adjacent and posterior to the broadened *en* stripes, it is not possible to determine whether exogenous Wg has *en*-independent autoregulatory activity (Fig. 1B) or whether the *de novo* induction of the ectopic *wg* is strictly a secondary consequence of ectopic *en*. In the latter case, some other factor besides Wg (e.g., *slp*) must determine the cells that express ectopic *wg* (Fig. 1A). To attempt to study the effects of exogenous Wg on endogenous *wg* expression more specifically, we have used the *GAL4* system of targeted misexpression (Brand and Perrimon, 1993). This has allowed us to uncouple the effects of exogenous Wg on endogenous *wg* and *en* expression.

If the direct exposure of cells to Wg is required for maintenance of *wg* transcription, then endogenous *wg* transcription should be activated ectopically in cells adjacent and posterior to *en* stripes only if exogenous Wg were directed specifically to these cells. To test this, flies carrying the GAL4 responsive transgene *UASwg* (Wilder and Perrimon, 1995) were crossed to two different lines of flies expressing GAL4 in distinct patterns during embryogenesis. The results are shown in Fig. 4 and schematized in Fig. 7. The *hGAL4* line drives GAL4 expression from stage 8 to late stage 10 of embryogenesis in the odd-numbered parasegments (Figs. 3A, 3C, and 3E). In *hGAL4/UASwg* embryos, exogenous Wg is expressed in seven broad stripes of cells, overlapping the even numbered, or even, endogenous *wg* stripes and the adjacent odd numbered, or odd, *en* stripes, but mainly lying posterior to the odd *en* stripes (see Fig. 7B). In *hGAL4/UASwg* embryos, endogenous *wg* transcription is activated ectopically during stage 10 (Fig. 4A), as revealed with a probe that detects endogenous versus exogenous *wg* RNA (see Materials and Methods). This effect is not fully penetrant, as ectopic *wg* is not induced posterior to all of the odd *en* stripes. By late stage 11, ectopic *wg* expression is present in stripes that are nearly as well defined as their wild-type counterparts (Fig. 4B). Although by this time exogenous Wg expression has faded (Figs. 3C and 3E), the ectopic *wg* stripes persist throughout the remainder of embryogenesis.

The ectopic *wg* stripes found in *hGAL4/UASwg* embryos are invariably located immediately adjacent and posterior to the odd *en* stripes (Figs. 4A and 4B). These *en* stripes are broadened from their normal width of two to three cells, due to the positive effect of exogenous Wg on *en* expression (Noordermeer *et al.*, 1992). The size of the *en* stripes in *hGAL4/UASwg* embryos is variable, ranging from three to six cells wide at late stage 10. It is possible then that the ectopic *wg* stripes in these embryos are strictly a secondary consequence of ectopic *en* and are not dependent on the presence of Wg in these cells. The misexpression of Wg by *prdGAL4*, described below, suggests, however, that this is not the case.

prdGAL4 drives *UASwg* expression from stages 8 to relatively late in embryogenesis, past stage 13 (Figs. 3B, 3D and 3F). In *prdGAL4/UASwg* embryos, Wg is targeted to the odd *en* stripes and also to cells that are

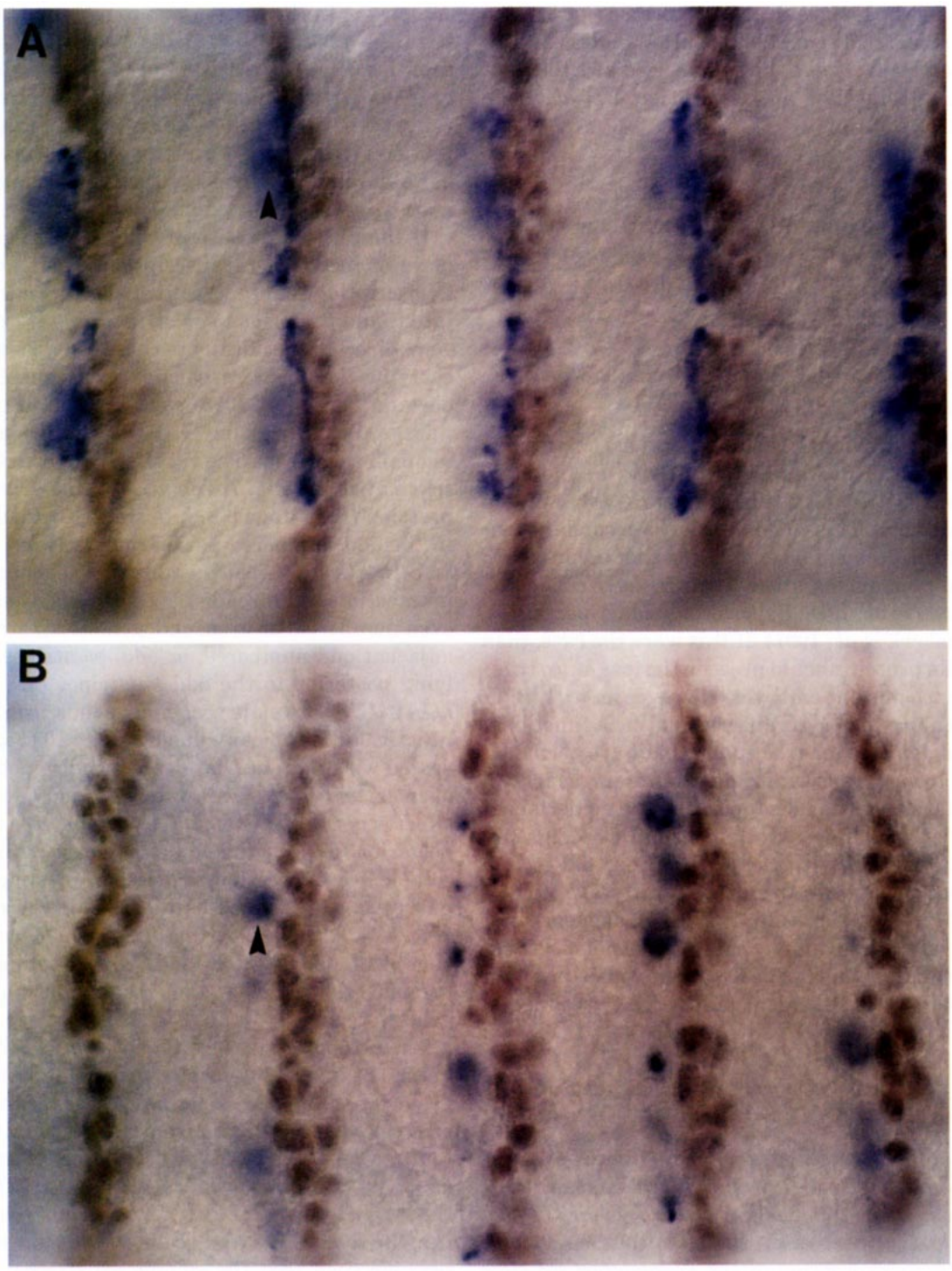
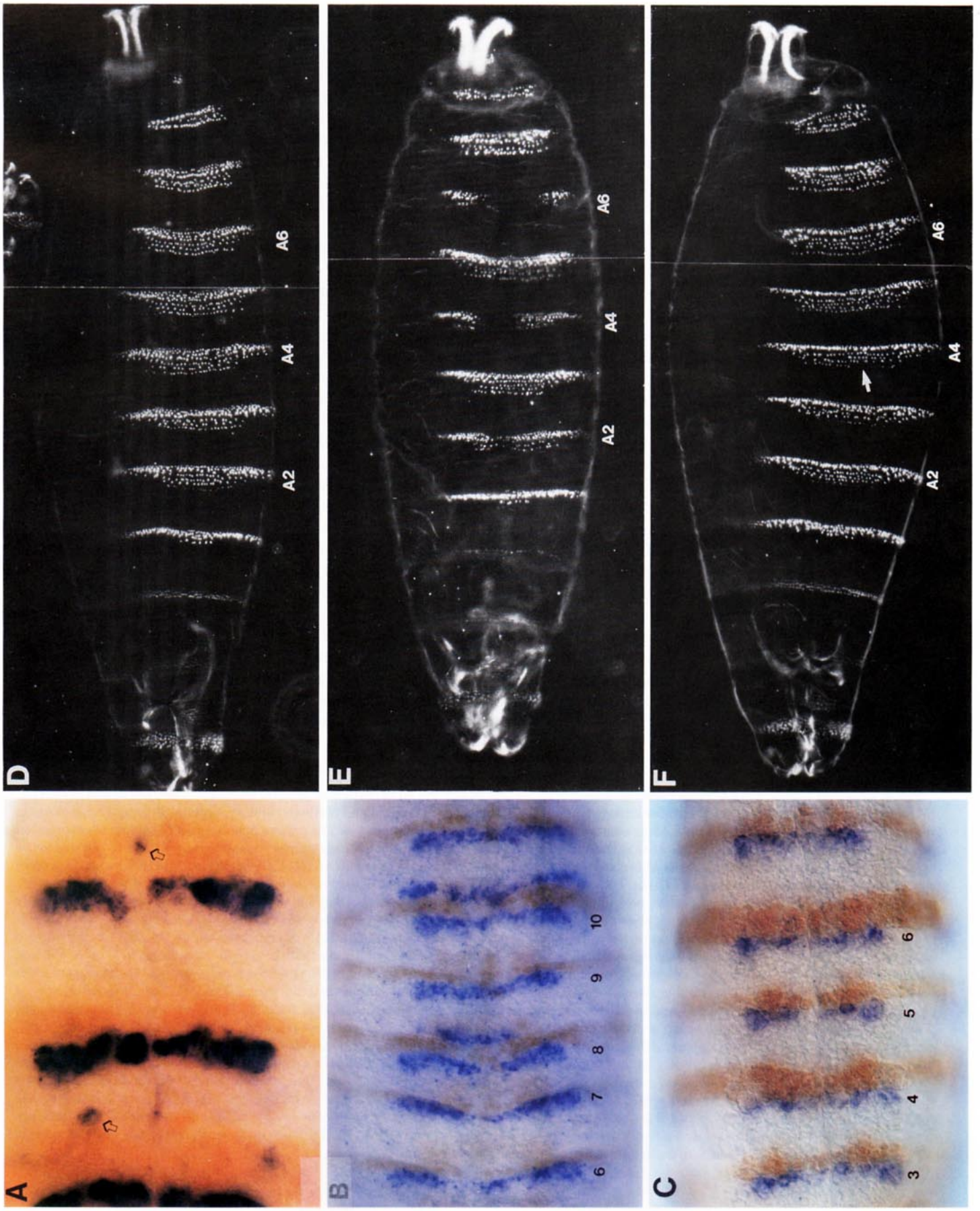


FIG. 2. Expression of *wg* and En in the ventral epidermis of wild-type and *wg* mutant embryos. Wild-type (A) and wg^{IG22} mutant (B) embryos, which produce transcript but no detectable Wg protein (van den Heuvel *et al.*, 1993), were double labeled for *wg* RNA (blue) and En protein (brown). In wild-type embryos, *wg* is expressed in a series of single cell wide stripes adjacent to En-expressing cells. In the absence of functional Wg protein, these stripes of expression fade starting at stage 9 before En has faded. The residual *wg* expression seen in (B) is mainly in the nervous system, slightly out of register with and underlying the epidermal stripes (compare the positions of the arrowheads in A and B). Shortly after this time epidermal En expression will also disappear. In this and all following figures, embryos are oriented anterior to the left.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located anterior, but *not* posterior, to these stripes (see Fig. 7C). Thus, unlike what was observed in *hGAL4/UASwg* embryos, cells posterior to the *en* stripes are not directly exposed to exogenous Wg. This localized misexpression of Wg has a profound paracrine effect on *en* expression starting at stage 9 and obvious by stage 11: The odd *en* stripes expand posteriorly up to six cells wide, including cells that have not expressed *UASwg* (Fig. 4C). *en* stripes generally widen in *prdGAL4/UASwg* embryos at least as much as in *hGAL4/UASwg* embryos. However, ectopic *wg* stripes are never induced in *prdGAL4/UASwg* embryos (Fig. 4C). Thus, ectopic *wg* expression appears to be independent of the extent of ectopic *en* and depends instead on the spatial pattern of exogenous Wg. This result suggests that the ectopic induction of endogenous *wg* observed in *hs-wg* embryos (Noordermeer *et al.*, 1992) is not solely a secondary consequence of ectopic *en*.

The larval cuticle provides another assay for the induction of ectopic *wg*. Following heat shock treatment, *hs-wg* embryos ventrally develop only naked cuticle, the opposite of the *wg* null lawn phenotype (Noordermeer *et al.*, 1992). In *hGAL4/UASwg* cuticles, alternate ventral denticle bands, that correspond to the segments where ectopic *wg* is induced, are partially transformed into naked cuticle (Fig. 4E). Since *hGAL4/UASwg* is not able to restore naked cuticle to *wg* mutant larvae (see below; Fig. 6C), the ability to generate ectopic naked cuticle in a wild-type background reflects induction of the endogenous *wg* gene. Despite its significant effect on *en* expression, misexpression of Wg from *prdGAL4* does not result in the deletion of denticle bands, which is consistent with the observation that ectopic *wg* is not induced (Fig. 4F).

En Controls wg Expression by both Positive and Negative Mechanisms

The lack of ectopic endogenous *wg* stripes in *prdGAL4/UASwg* embryos (Fig. 4C) suggests that, although *en* is required for maintenance of *wg* expression (Martinez-Arias *et al.*, 1988; Bejsovec and Martinez-Arias, 1991), ectopic *en* alone is not sufficient to activate *de novo wg* expression. To demonstrate this further, we have used the *GAL4* system to directly misexpress En in the *hGAL4* and *prdGAL4* domains. Although En is present in alternating broad stripes in both *hGAL4/UASen* and *prdGAL4/UASen* embryos (data not shown), ectopic *wg* is never induced in cells neighboring these broadened *en* stripes (Figs. 5A and 5B). In contrast, wild-type even *wg* stripes are repressed by stage 11 in *hGAL4/UASen* and *prdGAL4/UASen* embryos. That En may act as a repressor of *wg* transcription in the *en* cells has been previously suggested (Heemskerk *et al.*, 1991; Bejsovec and Wieschaus, 1993).

If epidermal cells require exposure to Wg as well as signaling from *en* cells in order to maintain *wg* expression, then the combination of exogenous Wg and En might be sufficient to induce endogenous *wg* expression ectopically. To test this possibility, we determined whether exogenous Wg could activate ectopic *wg* expression in cells adjacent to the broad *en* stripes in *prdGAL4/UASen* embryos. In this experiment exogenous Wg was provided by *hs-wg*, but at relatively low levels to avoid the induction of additional ectopic *en* (observed by Noordermeer *et al.*, 1992, after multiple heat shocks with *hs-wg*). Following a single short heat shock treatment (see details in Materials and Methods), ectopic *wg* cells are often induced immediately anterior

FIG. 4. Effects of localized Wg misexpression on endogenous *wg* and *en* and the larval cuticular pattern. *hGAL4/UASwg* (A, B) and *prdGAL4/UASwg* (C) embryos were double labeled for endogenous *wg* RNA (blue, see Materials and Methods) and En protein (brown). (A) A close-up ventral view of a late stage 10 *hGAL4/UASwg* embryo, focused on wild-type *wg* stripes 4–6. The arrows denote spots of ectopic *wg* expression posterior to the even wild-type stripes. Note that the odd *en* stripes anterior to these ectopic *wg* stripes are expanded posteriorly relative to the *wg* stripes. This posterior expansion of the odd *en* stripes was confirmed by double labeling *hGAL4/UASwg* embryos for both Wg and En protein expression (not shown). (B) A stage 12 *hGAL4/UASwg* embryo focused on *wg* stripes 6–10, with ectopic *wg* stripes induced posterior to wild-type stripes 8 and 10. Ectopic *wg* stripes can be found in approximately 50% of *hGAL4/UASwg* embryos. This variable penetrance is probably due to weak expression of *hGAL4* (data not shown). (C) A stage 11 *prdGAL4/UASwg* embryo focused on wild-type *wg* stripes 3–7, with significantly expanded odd *en* stripes (located posterior to the even *wg* stripes). As with *hGAL4/UASwg* embryos, double labeling for Wg and En protein (not shown) confirmed that the *en* stripes expand in a posterior direction. The possibility that the ectopic *wg* detected in *hGAL4/UASwg*, but not in *prdGAL4/UASwg* embryos, reflects different timing or levels of GAL4 expression in the two lines is unlikely: *hGAL4* expression fades prior to that of *prdGAL4* (Fig. 3); furthermore, *prdGAL4/UASwg* appears to have greater activity than *hGAL4/UASwg* in its effects on *en* (compare the odd En stripes in B and C) and in other experiments (Brand and Perrimon, 1993; Figs. 5 and 6). Therefore, the ability of exogenous Wg to activate endogenous *wg* expression is probably dependent on its specific localization in the segment. In the *hGAL4/UASwg* cuticle (E), deletions within alternating denticle bands (that correspond to segments of ectopic *wg* stripes) are observed. Deletions in all six rows of denticles in at least one band occur in approximately 50% of the cuticles scored ($n > 300$). *prdGAL4/UASwg* causes only slight perturbations of the wild-type cuticular pattern (F), including loss of some first row denticles (arrow). Only 1% of the cuticles scored show a deletion in all six rows of any single denticle band ($n > 300$). Although *prdGAL4/UASwg* expression persists until well past stage 10 (Figs. 3D and 3F), when naked cuticle specification by *wg* begins (Bejsovec and Martinez-Arias, 1991), its inability to specify significant ectopic naked cuticle is not surprising since *prdGAL4* is expressed mainly in regions already destined to secrete naked cuticle (see Fig. 7). (D) The wild-type ventral cuticle for comparison. A2, A4, and A6 indicate the second, fourth, and sixth abdominal denticle bands.

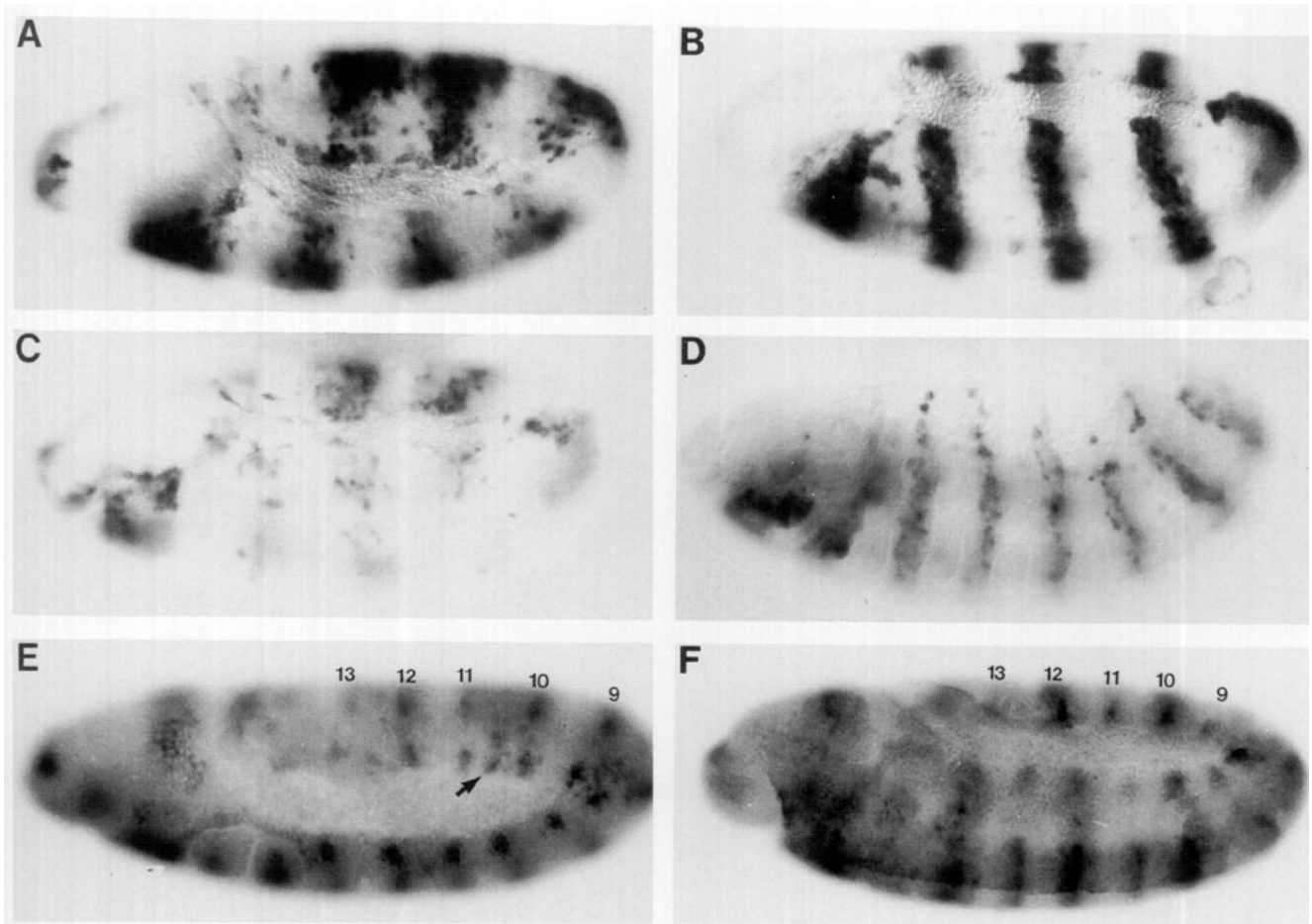

FIG. 3. (Top) Expression patterns of *hGAL4* and *prdGAL4* lines. Expression is shown with *in situs* to *lacZ* RNA (A–D) or Wg antibody staining (E–F) in *UASlacZ;hGAL4/UASwg* (A, C, E) and *UASlacZ;prdGAL4/UASwg* (B, D, F) embryos. Embryo stages shown are 9 (A, B), 10 (C), 13 (D), and 11 (E, F). Double labeling for *lacZ* RNA and Even-skipped protein (which marks odd parasegments; not shown), and also Wg antibody staining (see below) confirmed these expression patterns. These expression patterns are schematized in Fig. 7. The *hGAL4* expression pattern (A, C, E) matches that of the *hairy* gene but is delayed; epidermal expression lasts from stage 8 to late stage 10 in seven segment-wide stripes. Each stripe overlaps the even *wg* stripes and the adjacent odd *en* stripes and extends posteriorly to the next odd *wg* stripe (see Fig. 7). The residual staining at late stage 10 (C) resides mainly in the mesoderm of the most posterior segments (see also Brand and Perrimon, 1993). Wg protein in these embryos (E) is found in seven broad stripes in the epidermis, from stage 8 to early stage 11, in addition to the normal Wg pattern. Endogenous Wg stripes 9–13 are numbered in E and F. Notably, by early stage 11 ectopic stripes of Wg appear in many segments (arrow in E). At this stage exogenous Wg staining (present in between the wild-type Wg stripes in E) is barely visible. The ectopic Wg stripes perdure until late in embryogenesis and are a product of the endogenous *wg* gene (see text). *prdGAL4* (B, D, F) directs *lacZ* expression to part of the wild type *paired* (*prd*) pattern though, as with *hGAL4*, expression is delayed with respect to *prd*. Epidermal expression initiates at stage 8 in seven stripes, each having posterior boundaries coincident with those of odd *en* stripes (see Fig. 7). Expression perdures until after stage 13 (D). The expression patterns of these two *GAL4* lines are the same in the absence of exogenous Wg (not shown).

and/or posterior to the broadened odd *en* stripes in *UASen;prdGAL4/hswg* embryos (Figs. 5C and 5D). Since ectopic *wg* is never induced in *prdGAL4/UASen* embryos (Fig. 5B) and rarely induced adjacent to the wild-type even *en* stripes in *UASen;prdGAL4/hs-wg* embryos (Figs. 5C and 5D), it appears that both exogenous En and Wg are required for activation of ectopic *wg* (Fig. 8). This result, together with the observation that *hGAL4/UASwg* embryos, but not *prdGAL4/UASwg* embryos, display ectopic *wg* (Fig. 4), suggests that En can lead to the activation of *wg* only in neighboring cells that have been exposed directly to Wg.

Exogenous Wg Maintains Endogenous wg Expression Independently of en

Since exogenous Wg, driven by *hGAL4*, can induce ectopic expression of the *wg* gene in wild-type embryos (Fig. 4), we tested whether it could also maintain *wg* expression in *wg* and *en* mutant embryos. In both *wg;hGAL4/UASwg* and *wg;prdGAL4/UASwg* embryos, even epidermal *wg* stripes (which are exposed to exogenous Wg) are maintained, while the odd *wg* stripes fade as in *wg* mutants (Figs. 6A and 6B). In *wg;prdGAL4/UASwg* embryos, seven *wg* stripes usually perdure (Fig.

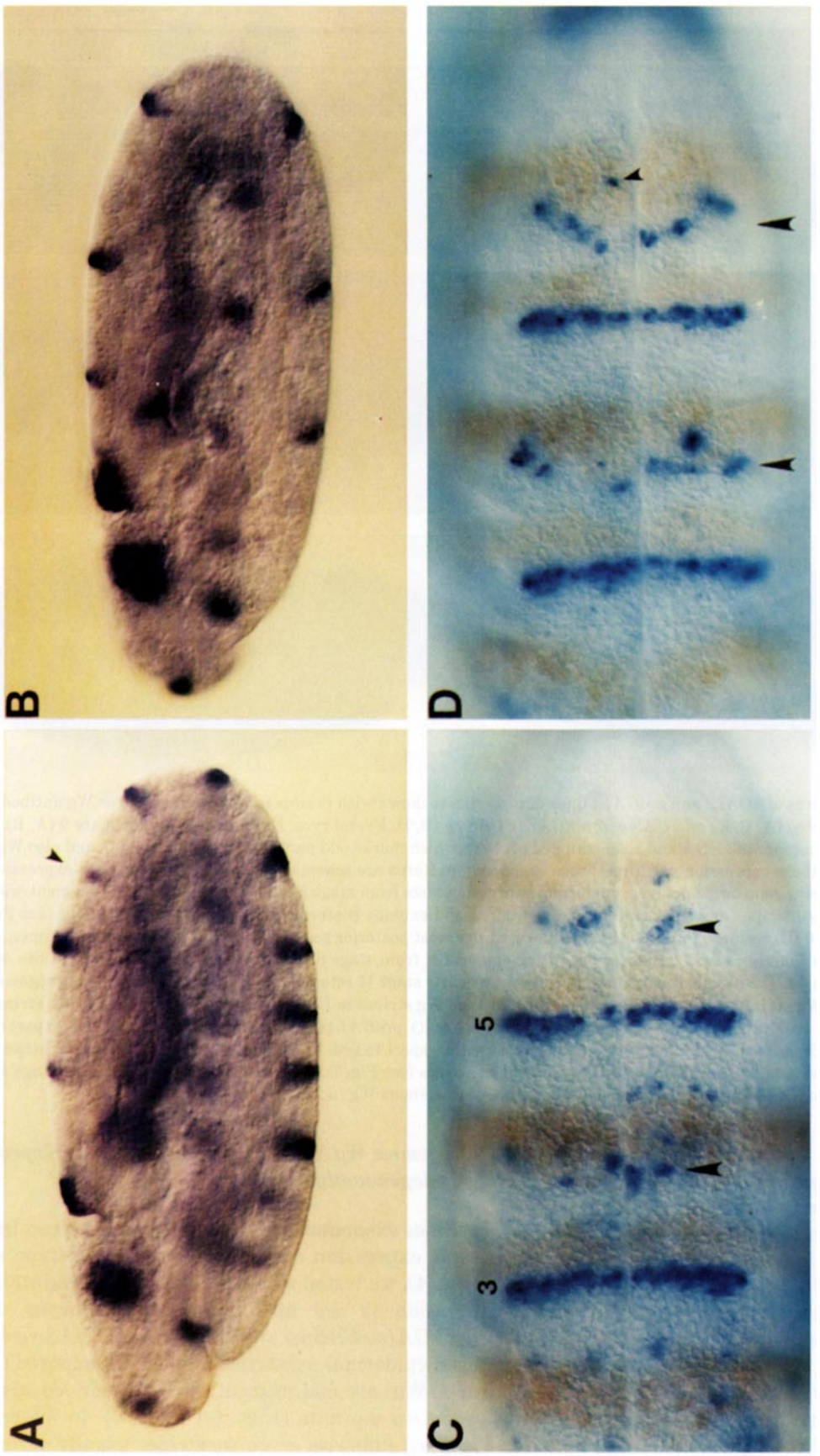

FIG. 5. Effects of localized exogenous En on *wg* expression. Early stage 11 *hGAL4/UASen* (A), *prdGAL4/UASen* (B), and *UASen;prdGAL4/hswg* (C, D) embryos are shown labeled for *wg* RNA (purple; A, B) or *wg* RNA (blue) and En protein (brown; C, D). Odd *wg* stripes are repressed in each embryo, with variable penetrance in *hGAL4/UASen* embryos (small arrowhead in A). No ectopic *wg* is induced as a result of exogenous En in (A) or (B). (C and D) Ventral views of two *UASen;prdGAL4/hswg* embryos, in which ectopic *wg* cells (large arrowheads) are induced anterior (C and D) or anterior and posterior (C) to the broadened odd *en* stripes. The heat shock protocol and identification of these embryos is described under Materials and Methods. The third and fifth wild-type *wg* stripes are indicated by number in (C). The small arrowhead in (D) indicates a remaining *wg*-expressing cell of wild-type *wg* stripe 6. See Fig. 8 for summary of these results.

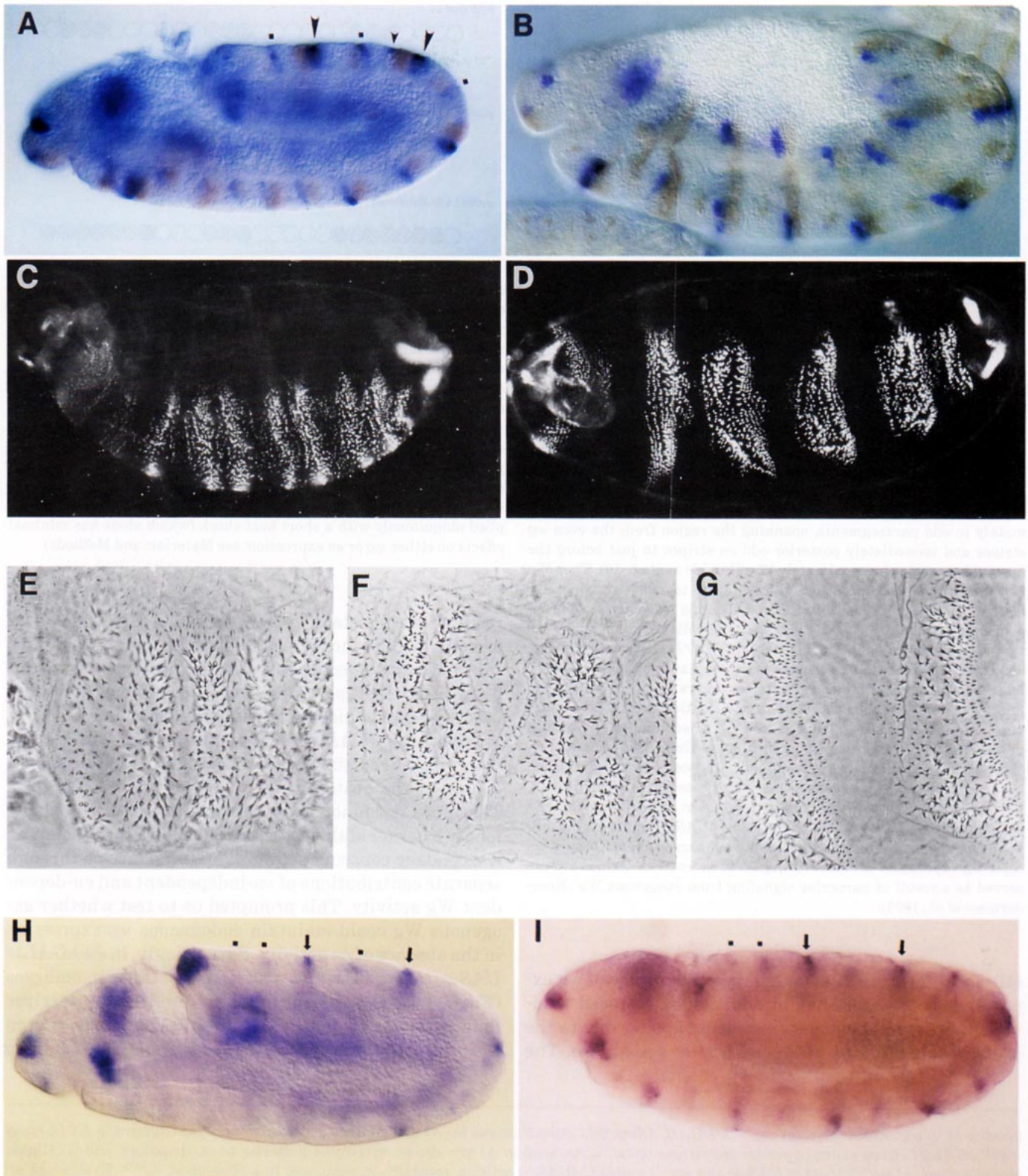

FIG. 6. Localized rescue of *wg* and *en* expression and the resulting phenotypes. wg^{en11};*hGAL4/UASwg* (A, C) and wg^{en11};*prdGAL4/UASwg* (B, D) embryos were either double labeled for *lacZ* RNA (blue) and En protein (brown), shown here at stage 11 (A) and stage 12 (B), or aged and prepared for cuticle examination as first instar larvae (C–G). wg^{en11} is a *lacZ* enhancer trap inserted at the *wg* locus that is a *wg* protein null (Kassis *et al.*, 1992; Siegfried *et al.*, 1992). Large arrowheads in (A) indicate restored *wg* stripes (adjacent to restored *en* stripes) which are most

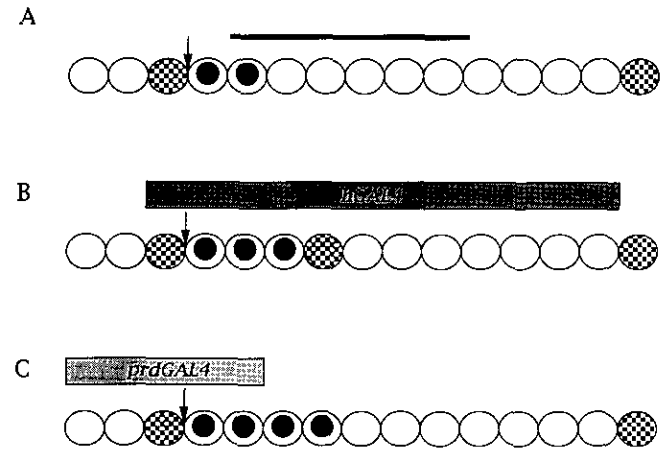

FIG. 7. Summary of *GAL4-UASwg* expression patterns and the effects on *wg* and *en* expression. (A) Representation of one row of a wild-type stage 10 odd parasegment (as well as three anterior even parasegmental cells). The horizontal line indicates the future denticle band, and blank space around this line indicates "naked" cuticle. The arrow indicates the parasegmental border, endogenous *wg*-expressing cells are mottled, and *en*-expressing cells are marked with black nuclei. (B) *hGAL4/UASwg* expression domain and its effects in wild-type or *wg* mutant embryos. As described in the text, *hGAL4* targets Wg mainly to odd parasegments, spanning the region from the even *wg* stripes and immediately posterior odd *en* stripes to just before the next odd *wg* stripe (approximately 12 cells wide at stage 10). Thus Wg is expressed in cells immediately posterior to odd *en* stripes. In *hGAL4/UASwg* embryos (Figs. 4A and 4B), this results in ectopic endogenous *wg* transcription in these posterior cells (as predicted by the second model depicted in Fig. 1). In *wg;hGAL4/UASwg* embryos (Fig. 6A) the even wild-type *wg* stripes are restored and ectopic stripes are induced. A widening of the odd *en* stripes occurs due to exogenous Wg misexpression (Noordermeer *et al.*, 1992). Denticle cell fates are suppressed in these parasegments as a result of ectopic *wg* stripes. (C) *prdGAL4/UASwg* expression domain and its effects. *prdGAL4* expression spans approximately five to six cells, the posterior-most of which are the wild-type odd *en* cells. Since cells posterior to *en* stripes are not directly exposed to Wg, endogenous *wg* is not induced in *prdGAL4/UASwg* embryos (Fig. 4C). In *wg;prdGAL4/UASwg* embryos (Fig. 6B) wild-type *wg* expression is rescued in even parasegments but no ectopic *wg* expression is observed. Again broadened *en* stripes are observed as a result of paracrine signaling from exogenous Wg (Noordermeer *et al.*, 1992).

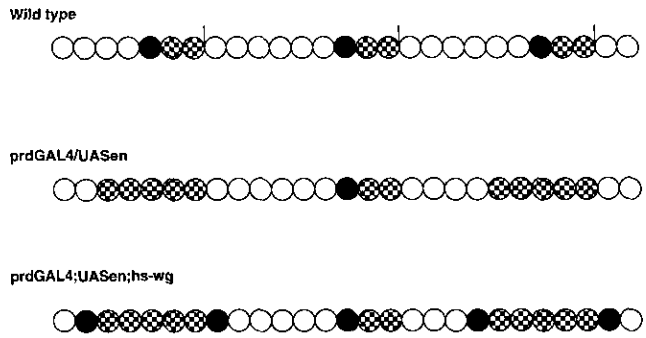

FIG. 8. Summary of *UASen;prdGAL4/hs-wg* results. Each row of circles represents approximately three segments of epidermal cells, each having 9 cells, at stage 10/11. Segmental borders are indicated by vertical lines, *wg*-expressing cells are shaded, and *en* expressing are mottled. In the top row, the wild-type patterns of *wg* and *en* expression are shown. In *prdGAL4/UASen* (middle row) and *UASen;prdGAL4/hs-wg* (bottom row) embryos, alternating broad stripes of *en* expression are driven exogenously. In both of these classes of embryos, the wild-type odd *wg* stripes are repressed (Figs. 5B–5D). No ectopic *wg* stripes are ever observed adjacent to the broadened *en* stripes in *prdGAL4/UASen* embryos (Fig. 5B), but *wg* stripes can be reinduced on either side of these wide *en* stripes in *UASen;prdGAL4/hs-wg* embryos (Figs. 5C and 5D) when cells are exposed to exogenous Wg supplied ubiquitously with a short heat shock (which alone has minimal effects on either *wg* or *en* expression; see Materials and Methods).

6B). In *wg;hGAL4/UASwg* embryos the rescue of wg expression is variable, but in addition to restored wild-type stripes, ectopic *wg* stripes often appear due to the broad localization of *hGAL4* (Fig. 6A; see above). The results are consistent with the spatial localization of exogenous Wg in otherwise wild-type embryos (see Fig. 7). Interestingly, in *wg;hGAL4/UASwg* embryos, the maintenance of wild type *wg* stripes is more efficient than the induction of ectopic *wg* stripes (see Discussion).

In both *wg;hGAL4/UASwg* and *wg;prdGAL4/UASwg* embryos, odd *en* stripes are also maintained in cells adjacent to the restored *wg* stripes (Figs. 6A and 6B). Therefore it cannot be determined from these experiments whether stable *wg* expression is restored solely as a secondary consequence of *en* maintenance or through separate contributions of *en*-independent and *en*-dependent Wg activity. This prompted us to test whether exogenous Wg could maintain endogenous *wg* expression in the absence of *en* activity. Surprisingly, in *en;hGAL4/UASwg* (Fig. 6H) and *en;prdGAL4/UASwg* embryos (Fig. 6I), *wg* expression can be restored. Odd *wg* stripes fade at stage 10 (as in *en* mutants), but many even stripes persist, similar to the case in *wg;hGAL4/UASwg*

prominent in posterior regions of wg^{en11};*hGAL4/UASwg*. Odd *wglacZ* stripes in (A) and (B) fade as in wg^{en11} embryos (squares in A). In many wg^{en11};*hGAL4/UASwg* embryos ectopic *wg* stripes (small arrowhead in A) are also observed just posterior to *en* stripes. (F and G) Higher magnifications of wg^{en11};*hGAL4/UASwg* and wg^{en11};*prdGAL4/UASwg* cuticles, respectively, compared to a close-up of wg^{en11} (E) in which all denticle cell types appear the same and the posterior naked cuticle in each segment is lost. In wg^{en11};*hGAL4/UASwg* larvae (F) some diversity of denticle types is restored, resulting in broad "mirrors," while in wg^{en11};*prdGAL4/UASwg* (G) polarized denticle patches and posterior naked cuticle are restored. (H, I) Rescue of *wg* expression in *en* mutants by *UASwg*. en^{CX1};*hGAL4/UASwg* (H) and en^{CX1};*prdGAL4/UASwg* (I) late stage 10/stage 11 embryos are shown labeled for *wg* RNA. In both of these classes of embryos even *wg* stripes persist (arrows), while odd stripes, whose location is marked by remaining expression in the nervous system (squares), fade.

and *wg;prdGAL4/UASwg*. These results support the proposal that *wg* has an autoregulatory activity distinct from its signaling via *en*. We note that in *en;hGAL4/UASwg* embryos only wild-type *wg* stripes, but not ectopic *wg*-expressing cells, can be observed (Fig. 6H). This is in contrast to *wg;hGAL4/UASwg* embryos, where ectopic as well as wild-type *wg* stripes persist (Fig. 6A).

Rescue of Epidermal Cell Types in wg Mutants

wg null embryos that develop the lawn phenotype are small, have lost their denticle diversity, and are missing naked cuticle within each segment (Nusslein-Volhard and Wieschaus, 1980; Martinez-Arias *et al.*, 1988; Bejsovec and Wieschaus, 1993; Fig. 6E). To test whether different spatial and temporal domains of Wg expression have different abilities to restore pattern to *wg* mutant cuticles, we examined the effects of exogenous Wg, driven from *h-* or *prdGAL4*, on the *wg* mutant cuticular phenotype. In *wg;hGAL4/UASwg* cuticles, the overall size and the diversity of denticles are partially restored in pair rule "mirrors"; however, naked cuticle is not recovered (Figs. 6C and 6F). This result is consistent with experiments demonstrating that prior to stage 11 (when *hGAL4/UASwg* is expressed) *wg* maintains *en* transcription and promotes cell diversity but does not specify naked cuticle (Bejsovec and Martinez-Arias, 1991). Our result also shows that the ectopic naked cuticle seen in *hGAL4/UASwg* larvae (Fig. 4E) is not specified directly by exogenous Wg but requires activity from the endogenous gene, and thus serves as an assay for ectopic activation of *wg*.

Remarkably, *prdGAL4/UASwg* can rescue aspects of all the segmental pattern defects observed in *wg* mutants: size, denticle diversity, and naked cuticle (Figs. 6D and 6G). These cuticles have four instead of the normal eight abdominal denticle bands and each is much wider than in wild type. These wide denticle bands appear to contain many, if not all, of the six different denticle types, in roughly correct order. The regions of naked cuticle between these denticle bands are also much broader than in wild type. This phenotype likely results from the fact that in *wg;prdGAL4/UASwg* embryos, Wg is present past stage 10 in 7 broad stripes instead of the wild-type 14 narrow stripes. Thus, in contrast to previous reports describing *wg;hs-wg* embryos (Sampedro *et al.*, 1993; Noordermeer *et al.*, 1994), exogenous Wg can restore naked cuticle and segmental asymmetry to *wg* mutant embryos when provided by the *GAL4* system, a more persistent and more asymmetric source of exogenous Wg than *hs-wg*.

DISCUSSION

Wg has Distinct Autoregulatory Functions in the Epidermis

This work has examined the respective roles of the putative *en*-dependent and *en*-independent autoregulatory functions of *wg* in the embryonic epidermis (Hooper and Scott, 1992; see Introduction). We provide several lines of evidence in favor of the existence of an *en*-independent autoregulatory function. First, the loss of *wg* expression precedes the loss of En at stage 9 in *wg* mutant embryos (Fig. 2B), indicative of a more direct *wg* autoregulatory role than the *en*-dependent feedback loop. Second, spatially distinct Wg and En misexpression during stages 8–10 has enabled us to uncouple the effects of Wg on endogenous *wg* and *en* expression (Figs. 4A–4C and 5). The induction of ectopic *wg* appears to depend not on the extent of *en* misexpression, but on the specific expression of exogenous Wg in the cells where ectopic *wg* is observed. Third, the observation that endogenous *wg* expression can be maintained in the absence of *en* activity (Figs. 6H and 6I) strongly supports the existence of *en*-independent *wg* autoregulation.

The existence of distinct *wg* autoregulatory pathways in the ventral epidermis of *Drosophila* embryos has been previously reported. The most telling experiments involved *ptc* mutant embryos, in which ectopic *wg* expression is observed in cells away from the borders of the *en* stripes, presumably due to the uncoupling of *wg* from its requirement for signaling from the *en* cells (Ingham *et al.*, 1991). In *ptc* mutants, *wg* activity is required to maintain this ectopic *wg* expression, suggestive of the existence of an *en*-independent *wg* autoregulatory pathway (Ingham and Hidalgo, 1993; Bejsovec and Wieschaus, 1993; Hooper, 1994). However, in *ptc;wg* embryos the ectopic *wg* does not disappear until stage 11, at which time significant *wg* expression is retained at the borders of the *en* stripes. This leaves in question a direct requirement for Wg in its normal expression domain at the parasegment border. Further, another level of complexity may arise from the use of double mutant backgrounds, in which novel regulatory pathways, that do not operate in wild-type embryos, may be activated. In our analysis, we provide evidence for an *en*-independent *wg* autoregulatory function required for both wild-type and ectopic *wg* expression adjacent to the *en* stripes, and show that this autoregulation occurs prior to stage 10 (Figs. 2, 4, 5, 6).

Additional Factors Involved in wg Autoregulation

Results from localized exogenous Wg misexpression (Figs. 4, 5, 6) support the model that cells require direct exposure to Wg in order to maintain the ability to ex-

press *wg* (Hooper and Scott, 1992). An alternative explanation has been offered, suggesting that exogenous Wg induces ectopic *wg* simply by expanding the *en* expression domain to the edge of a "*wg* competence domain," defined by *slp* activity (Ingham *et al.*, 1991; Cadigan *et al.*, 1994). In this scenario, Wg would have required no autoregulatory role in the wild-type embryo other than paracrine signaling via *en* cells. Against this argument, we have shown that ectopic *wg* expression is likely not a sole consequence of input from *en* cells: ectopic *en*, whether provided directly by *UASen* (Figs. 5A and 5B) or induced indirectly by *UASwg* (Fig. 4C), cannot activate ectopic *wg* transcription. Instead, it appears that the presence or absence of ectopic *wg*-expressing cells depends on the direct exposure of these cells to exogenous Wg in addition to their close proximity to *en* cells (Figs. 4A, 4B, 5C, 5D, 6A, and 6B). The observation that in *wg* mutant embryos, *wg* expression disappears before the time that En protein begins to fade (Fig. 2B) indicates that *en*-independent *wg* autoregulation indeed takes place at stage 9 of embryogenesis.

But Wg is clearly not the only factor determining the cells that will maintain *wg* expression. For example, in *wg;hGAL4/UASwg* embryos cells both immediately anterior and posterior to the *en* stripes express Wg (Fig. 7B). Despite this, the wild-type *wg* stripes (anterior to the *en* stripes) are restored in these embryos with a much higher efficiency than posterior ectopic *wg* stripes are induced (Fig. 6A). Thus, consistent with the competence domains model (Ingham *et al.*, 1991; Cadigan *et al.*, 1994), certain cells seem more capable of expressing *wg* than others. To integrate the models of competence domains and *wg* autoregulation (Hooper and Scott, 1992), we propose that, in the wild-type embryo, *en*-independent and *en*-dependent *wg* autoregulatory activities define the precise stripe of cells within the domains of *slp* expression that will maintain *wg* expression.

Our data thus support the model that exposure of a cell to Wg activity is a prerequisite for its ability to maintain *wg* expression (Hooper and Scott, 1992). Interestingly, however, in mutants for some segment polarity genes, such as *ptc*, ectopic *wg* expression is induced late in gastrulation in cells where *wg* expression was not initiated at blastoderm (Martinez-Arias *et al.*, 1988; Siegfried *et al.*, 1992). It has been postulated that in the absence of Ptc, Wg spreads from the wild-type *wg* cells to inappropriate cells, activating ectopic *wg* transcription (Ingham *et al.*, 1991; Bejsovec and Wieschaus, 1993). This is supported by the observation that in *ptc* mutants, *wg* activity is absolutely required for the maintenance of ectopic *wg* expression (Ingham and Hidalgo, 1993; Hooper, 1994).

This analysis has focused on the autoregulatory properties of the Wg secreted protein, and on the involvement of *en* in these processes. What transcription factors in the *wg* cells might be involved in *wg* autoregulation? Two candidates are the putative transcription factors encoded by the segment polarity genes *gooseberry* (*gsb*) and *cubitus-interruptus* (*ci*) (Baumgartner *et al.*, 1986; Orenic *et al.*, 1990). At stage 11, *wg* expression is maintained through an autoregulatory loop involving the homeodomain-containing Gsb protein (Hidalgo, 1991; Li and Noll, 1993). However, since *wg* transcription fades earlier in *wg* mutants (stage 9; Fig. 2B) than it does in *gsb* mutants (stage 11; Hidalgo and Ingham, 1990), transcription factors acting earlier than Gsb must also be involved in *wg* autoregulation (see also Hooper, 1994). One such factor may be the Ci zinc-finger protein. In *ci* mutant embryos, *wg* transcription ceases beginning at stage 9 (Forbes *et al.*, 1993); furthermore, *ci* appears to be required downstream of *wg* activity with regard to *wg* transcription (Hooper, 1994; A.S.M., K.Y., E.L.W., and N.P., in prep.).

The Roles of en in wg Regulation

The En protein has been shown to be required for maintenance of *wg* transcription in neighboring cells at stage 10 (Martinez-Arias *et al.*, 1988; Bejsovec and Martinez-Arias, 1991). Remarkably, the requirement for *en* in *wg* maintenance can be bypassed with exogenous Wg (Figs. 6H and 6I). To explain this observation, we propose that in the wild-type embryo one of the functions of the *en*-dependent signal is to stimulate the *en*-independent autoregulatory activity of *wg*. In *en;hGAL4/UASwg* and *en;prdGALU/UASwg* embryos, the increased amount of Wg protein in the wild-type *wg* cells appears sufficient to substitute for the *en*-dependent signal in the regulation of *wg*. If input from *en* cells stimulates *en*-independent *wg* autoregulation, this could explain the observation that *wg* expression ceases later in *en* mutant embryos (stage 10; Martinez-Arias *et al.*, 1988; Bejsovec and Martinez-Arias, 1991; Fig. 6I) than in *wg* mutants (stage 9; Fig. 2B). In *en;hGAL4/UASwg* embryos, ventral *wg* expression is maintained long after expression of exogenous Wg from *hGAL4* has ceased (late stage 10; Fig. 3C). Therefore, after stage 10 ventral *wg* expression appears to no longer require *en* activity. This is similar to what has been previously observed in the dorsal epidermis (Heemskerk and DiNardo, 1994).

The misexpression of En in the *wg* cells results in the eventual repression of *wg* stripes (Figs. 5A and 5B). This result supports the model that *en* may not only regulate *wg* in a positive manner through paracrine signaling, but also in a negative manner through transcriptional repression in the cells where En is present (Heemskerk *et al.*, 1991, Bejsovec and Wieschaus, 1993). Such a model could possibly account for the mutual exclusion of *wg* and *en* expression in the wild-type embryo.

Spatial Restriction of Wg Is Important for Its Function

The expression of *wg* in very restricted regions of each metamere is crucial for the generation of the correct intrasegmental pattern: ubiquitous Wg misexpression directed by *hs-wg* results in the respecification of all epidermal cell fates to the naked state (Noordermeer *et al.*, 1992). This finding is consistent with a model in which Wg, secreted from a local source, provides positional information to cells either by acting as a graded morphogen or by initiating a series of sequential cell-cell interactions (Baker, 1987; Martinez-Arias *et al.*, 1988; van den Heuvel *et al.*, 1989; Bejsovec and Martinez-Arias, 1991). Another model states that early restricted Wg is important in specifying correctly localized parasegment borders, which then act as "seals" to prevent the inappropriate spread of other pattern regulating factors (Sampedro *et al.*, 1993). In this latter model Wg itself does not directly provide positional information and is permissive rather than instructive.

Although Wg must presumably be restricted to one- to two-cell wide stripes for wild type segments to be generated, stable localized sources of Wg can result in polarized arrays of diverse epidermal cell types, including naked cuticle (*wg;prdGAL4/UASwg;* Fig. 6G). This is in contrast to the symmetric mirror phenotypes seen in *wg;hs-wg* (Sampedro *et al.*, 1993; Noordermeer *et al.*, 1994) or *wg;hGAL4/UASwg* cuticles (Fig. 6F), in which the transient presence of Wg is sufficient to replace some of the cell-type diversity, but not complete polarity (e.g., naked cuticle) to the segment. Thus the stable presence of striped Wg expression seems paramount for the generation of asymmetry and naked cuticle in the epidermis. Whether this requirement is met with gradients of Wg, sequential induction events, or the proper formation of sealed borders remains to be determined.

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