Ectopic expression of either the *Drosophila* gooseberry-distal or proximal gene causes alterations of cell fate in the epidermis and central nervous system

Yu Zhang, Anne Ungar*, Catalina Fresquez† and Robert Holmgren

Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois 60208, USA

*Present address: Department of Pharmacology, University of Washington, Seattle, WA 98195, USA
†Present address: Department of Biology, Barry University, Miami Shores, FL 33161, USA

SUMMARY

Previous studies have shown that the segment polarity locus *gooseberry*, which contains two closely related transcripts gooseberry-proximal and gooseberry-distal, is required for proper development in both the epidermis and the central nervous system of *Drosophila*. In this study, the roles of the gooseberry proteins in the process of cell fate specification have been examined by generating two fly lines in which either gooseberry-distal or gooseberry-proximal expression is under the control of an hsp70 promoter. We have found that ectopic expression of either gooseberry protein causes cell fate transformations that are reciprocal to those of a gooseberry deletion mutant. Our results suggest that the gooseberry-distal protein is required for the specification of naked cuticle in the epidermis and specific neuroblasts in the central nervous system. These roles may reflect independent functions in neuroblasts and epidermal cells or a single function in the common ectodermal precursor cells. The gooseberry-proximal protein is also found in the same neuroblasts as gooseberry-distal and in the descendants of these cells.

Key words: gooseberry, cell fate, segmentation, neurogenesis, *Drosophila*, ectopic expression, central nervous system

INTRODUCTION

In *Drosophila*, the anteroposterior body axis is divided into a series of repeating units called segments. The process of segmentation is initiated at the beginning of embryogenesis by maternally deposited morphogens in the fertilized egg. Subsequently, a hierarchy of three groups of zygotic segmentation genes are sequentially activated to refine the maternal field of positional information (for review see Ingham and Martinez Arias, 1992).

In addition to patterning the epidermis, the segmentation genes are also required for the development of the *Drosophila* central nervous system (CNS). In gap and pair-rule gene mutants, lost epidermal segments are associated with elimination of the corresponding segmental ganglia. While these segmentation defects were expected, a second role for gap and pair-rule genes in the specification of particular neurons was not. A number of gap and pair-rule genes are reexpressed in segmentally repeating patterns in the developing CNS. From the analysis of conditional mutants, the expression of the pair-rule genes, *fushi tarazu* (*ftz*) and *even-skipped* (*eve*) (Doe et al., 1988a,b), has been shown to be critical to the proper specification of certain neuronal identities.

The segment polarity genes occupy an intriguing position within the segmentation hierarchy. They are required in the epidermis to specify cell fate within each segment and are active both before and during the time that neuroblasts (NBs) are delaminating to generate the CNS. In a systematic study of CNS alterations in different segment polarity mutant embryos, Patel et al. (1989) have shown that five of the segment polarity genes (*gooseberry*, *patched*, *Cell*, *wingless* and *hedgehog*) cause specific defects in neurogenesis, while two other segment polarity mutants (*fused* and *armadillo*) have relatively normal CNS patterns. Only mutations in the *patched* and *gooseberry* genes cause CNS defects that appear to mirror the defects observed in the epidermis. These results suggest that segmentation of the CNS is not controlled by epidermal segmentation per se. Some of the segment polarity genes (*patched* and *gooseberry*) may play homologous roles in epidermis and CNS segmentation, perhaps by functioning in common ectodermal precursor cells, while others (*wingless* and *Cell*) function during neurogenesis in a second more restricted fashion. This suggestion is further supported by a more recent report showing that the repeated expression pattern of *achaete* (ac) in NBs is affected only in *naked* and *gooseberry* mutants and not in the other segment polarity mutants (Skeath et al., 1992).

Here we report our studies on the segment polarity gene, *gooseberry* (*gsb*), which plays a critical role in specifying cell fates in the epidermis as well as in NBs and their descendants. The *gsb* locus has been found to contain two highly homologous transcripts, *gooseberry-proximal* (*gspb*) and *gooseberry-
distal (gsbd) (Baugnartner et al., 1987; Côté et al., 1987). Both gsbd and gsbp encode putative transcription factors, each containing a homeodomain and a paired domain. In mutants deficient for both gsb genes, the naked cuticle of each epidermal segment is eliminated and replaced with a mirror-image duplication of the denticle belt (Fig. 8B; Nüsslein-Volhard and Wieschaus, 1980). The alterations in the CNS include the deletion of the posterior commissure and the CQ neurons as well as the duplication of aCC, pCC and RP2 neurons (Fig. 8H; Patel et al., 1989). The regions expressing the gsb genes closely correspond to the positions where structures are eliminated in gsb mutants. In the epidermis, gsbd has been localized to the region bracketing the parasegmental boundary of each segment and, in the CNS, gsbd and gsbp are expressed in a subset of NBs and neurons in the same region (Baugnartner et al., 1987; Ouellette et al., 1992; Gutiárriz et al., 1993b).

In this study, we generated hs-gsbd and hs-gsbp fly lines to misexpress the gsb genes throughout the embryo. We have found that ectopic expression of either gsb gene causes cuticle and CNS alterations that are nearly reciprocal to that of a gsb mutant. Our results suggest that, in the epidermis, gsbd organizes pattern elements around the parasegmental boundary by regulating the expression of wingless (wg) and engrailed (en). In the CNS, gsbd may function as a selector gene to specify the cell fate of a subset of NBs. gsbp is expressed in the descendants of the gsbd-positive NBs and may provide continued gsb function in these cells.

MATERIALS AND METHODS

Fly strains

The OrR, rosy506, Df(2R)SB1(gsbd, Kr), Df(2R)IIIX62(zip, gsb) are described in Lindsay and Zimm (1992). The A31 enhancer trap line was obtained from A. Gyhagen and the wg enhancer trap line CyO/wg17640 was obtained from N. Perrimon. gsbplacZ is a P element transformant in which lacZ coding sequences have been fused in frame to gsbp genomic sequences at an EcoRI site in the third exon. The expression of β-galactosidase in gsbplacZ embryos precisely coincides with gsbp expression in the CNS (Fresquez, unpublished data).

Production of gsb proteins and generation of antibodies

To make gsb protein, a 1 kb HindIII fragment, which contains most of the gsb coding region, was cloned into the T7 expression vector pET8c at the BamHI site. The protein was induced in pBl21(DE3) bacteria cells with IPTG and partially purified from inclusion bodies (Studer et al., 1990).

A full-length gsbp cDNA clone was isolated by screening a 0- to 16-hour cDNA library (a gift from B. Hovemann) with an appropriate probe and confirmed by partial sequence analysis. An NcoI-NeI fragment, which contains the entire gsbp coding region, was cloned into the T7 expression vector pET8c between the NcoI and Xbal sites. The gsbp protein was subsequently induced and partially purified from inclusion bodies.

Standard techniques for generating antibodies (Harlow and Lane, 1988) were used to make both rat polyclonal and monoclonal antibodies specific to gsbd and gsbp, respectively. In both cases, the obtained antisera also cross react with the other gsb protein. To remove the cross reactivity, the antisera were passed through a sepharose column coupled with the other gsb protein (for example, the anti-gsbd antisera was passed through a gsbp coupled column and vice versa). The elimination of the cross reactivity in preabsorbed antisera was confirmed by western blots and embryo stainings. For monoclonal antibodies, immunized rat spleen cells were fused to mouse NSO-1 cells. Two monoclonal lines, 10E10 and 16F12, were isolated which specifically recognize gsbd. One monoclonal line, 9A1, was found to be specific to gsbp. Preabsorbed antisera and monoclonal antibodies give the same results in all the embryo stainings described in this paper; we refer to them generally as gsbp and gsbp stainings.

Antibody stainings

The antibody staining patterns were visualized using indirect immunofluorescence or horseradish peroxidase (HRP) techniques as previously described (Patel et al., 1989). All the fluorescent stainings were examined by confocal microscopy using a BioRad MRC-600 confocal microscope. For double labeling experiments with HRP coupled secondary antibodies, NiSO4 and CoCl2 were added to one of the reactions to give a blue-gray color (Lawrence et al., 1987). The rabbit anti-β-gal antiserum was generated in our laboratory (Holmgren, unpublished), the rabbit anti-eve antiserum was a gift from M. Frasch, the rabbit anti-wg antiserum was a gift from R. Nusse and the anti-ac antibody was a gift from S. Carroll.

Cuticle preparations

Cuticles were mounted in a 1:1 mixture of Hoyer’s solution and lactic acid (van der Meer, 1977) and photographed under phase contrast.

hs-gsbd and hs-gsbp fly lines

A genomic clone containing the entire gsb coding sequence and a cDNA clone containing the entire gsb coding sequence were independently inserted into modified Carnegie 20 P element vectors (Rubin and Spradling, 1983) containing a 0.4 kb hsp70 promoter (from S. Lindquist). Each construct was then coinjected with the wings clipped plasmid into rosy506 embryos as described in Roberts (1986). Multiple insertion sites for each line were examined and gave similar phenotypes after heat shock.

Heat-shock treatment

Embryos were collected for 30 minutes and allowed to develop at room temperature for a specified time period as described in the text. The heat-shock treatment was performed by placing embryos onto prewarmed molasses-agar egg collection plates floating in a water bath at 36.5°C for 30 minutes. Multiple heat shocks were performed with a 1 hour recovery period following every 30 minute heat shock. To generate mutant phenotypes in the CNS and cuticles, the optimal time for heat shocking is 2.5-5 hours. Both heat shocked hs-gsbd and hs-gsb embryos give similar phenotypes, though the hs-gsbp lines respond better than the hs-gsb lines to late heat shocks. Whether this reflects differences in the intrinsic activity of the gsbp and gsb proteins or more efficient expression from the intronless gsbp construct is not known. Heat shocks before 2.5 hours disrupt the segmentation of embryos, presumably by interfering with the normal function of the pair-rule gene, paired, since the gsbp protein and the paired protein can bind to the same DNA target sites (Treisman et al., 1991). Later heat shocks (up to 7 hours) can cause small regions within denticle belts to be transformed into naked cuticle.

In experiments correlating patterns of gene expression with cuticle phenotypes, each collection of heat shocked embryos was split: one half was used for antibody staining and the second half was used for cuticle preparation. In these experiments, at least 50% of the embryos showed cuticle transformations.

RESULTS

Ectopic expression of either gsb or gsbp causes the respecification of the denticle belt to naked cuticle

In order to express gsb or gsbp ectopically in fly embryos, we generated hs-gsbd and hs-gsbp fly lines each of which has a
gsb coding sequence under the control of an hsp70 promoter. Following the heat-shock treatment, there is a ubiquitous expression of gsb in hs-gsbd embryos and gsbp in hs-gsbp embryos, which fades away within the first 60 minutes after heat shock (data not shown). In our study, the effect of this ubiquitous expression of gsb on cell fate specifications was

Fig. 1. Cuticle phenotypes of mutant and heat shocked embryos. Anterior is up and the view is ventral. In contrast to the wild-type pattern (A), gsb mutants (B) have the naked cuticle in each segment replaced by denticle belts. In heat shocked hs-gsbd (C,D) and hs-gsbp (E,F) embryos, denticles are replaced by naked cuticles. Single heat shocks (given at 2.75-3.25 hours for hs-gsbd and at 4-4.5 hours for hs-gsbp) (C,E) eliminate entire denticle belts or sections of denticles, while triple heat shocks (D,F) are able to transform the entire ventral epidermis to nearly naked cuticle. When hs-gsbp (G) and hs-gsbd (H) were crossed into a gsb mutant background (Df(2R)IIX62), triple heat shocks restore a near wild-type cuticle pattern. Note the characteristic holes of the zipper mutation in Df(2R)IIX62 in the anterior end of the embryos (G,H).
assessed in the development of both the ventral epidermis and the CNS.

In gsb mutants, the naked cuticle of each segment is eliminated and replaced by a mirror-image duplication of the denticle belts (Figs 1B, 8B; Nüsslein-Volhard and Wieschaus, 1980). In the cuticles of heat shocked hs-gsbd and hs-gsbp embryos, groups of denticles or entire denticle belts are replaced with naked cuticle (Fig. 1C-F). The severity of the phenotype correlates with the timing of the heat shock. Heat shocks from 2.75 to 3.25 hours transform complete segments while those after 4 hours can transform sections of segments. Multiple heat shocks generate embryos with completely naked cuticles. To verify that cell fates are being transformed, we examined the Keilin’s organs, which are located at the parasegmental border in each thoracic segment. In gsb mutants Keilin’s organs are deleted, while in heat shocked hs-gsbd and hs-gsbp embryos, ectopic Keilin’s organs occasionally develop (data not shown). These results indicate that the cells in the anterior portion of the segment have been respecified and that the cuticle phenotype of heat shocked hs-gsbd and hs-gsbp embryos is nearly reciprocal to that of gsb mutants (Fig. 8C).

It was somewhat surprising that heat shocked hs-gsbp was able to give pattern transformations similar to heat shocked hs-gsbd since gsbp is not normally expressed in the epidermis at the times that the heat shocks were administered (Ouellette et al., 1992; Gutjahr et al., 1993b). Therefore, we examined the ability of heat shocked hs-gsbd and hs-gsbp to rescue the phenotype of Df(2R)HX62 embryos which are deleted for the gsb locus. With a series of three heat shocks both hs-gsbd and hs-gsbp are able to rescue the formation of naked cuticle (Fig. 1G-H).

Heat shocked hs-gsbd or hs-gsbp induces ectopic expression of the endogenous gsb gene and activates expression of wg and en

To determine whether changes at the molecular level correlate with the cuticle phenotype of heat shocked hs-gsb (hs-gsbd or hs-gsbp) embryos, we examined the expression of gsb and two other segment polarity genes wg and en. We have found that hs-gsbd embryos given heat shocks between 2.5 and 4 hours after egg-laying, activate ectopic expression of the endogenous gsb gene (Fig. 2A,C). The ectopic stripe of gsb expression is maintained 2 hours after heat shock and is dependent on the presence of a wild-type copy of the gsb locus (data not shown). Thus the ectopic gsb expression must be due to the activation of the endogenous gsb gene. The activation of ectopic gsb is accompanied by the formation of an ectopic parasegmental groove (data not shown) flanked by inverted ectopic stripes of wg and en (Fig. 2A,C). Thus, it appears that the ubiquitous expression of gsb induces a mirror-image duplication of the region bracketing the parasegmental boundary. Heat shocks after 4 hours also transform the denticle belts into naked

Fig. 2. Expression of gsb, wg and en in heat shocked hs-gsbd embryos. Late stage 10 embryos; anterior is to the left and view is ventral. (A,B) Double labelings of gsb (red) and wg (green); (C,D) double labelings of gsb (red) and en (green). Heat shocks were given at 2.75 to 3.25 hours for A and C, and at 4-4.5 hours for B and D. Early heat shocks induce ectopic stripes of gsb and wg (A), and of gsb and en (C) (marked by \(\wedge\)). Note that the ectopic en stripes are located anterior to the ectopic gsb stripes (C). Late heat shocks do not induce ectopic stripes of gsb, wg or en (B,D), though there is a low level of wg expression throughout the embryo (B). Similar results are observed using the hs-gsbd line. Bar, 20 µm.
Ectopic gooseberry changes cell fate

NB cell fates are respecified in heat shocked hs-gsbd embryos

To study the effects of heat-shock-induced ectopic gsbd expression on NB cell fate, we used the anti-ac antibody. In stage 9 wild-type embryos, four rows of NBs are present within each CNS hemisegment and the anti-ac antibody recognizes NBs in row 3 and 7 (Figs 4A, 8D; Skeath and Carroll, 1992). In gsbd mutants, Skeath et al. (1992) have shown that the ac protein is additionally expressed in the two NBs at the position of row 5 (Fig. 8E). In heat shocked hs-gsbd embryos, ac-positive NBs are restricted to the NBs of row 7 in each CNS hemisegment and the staining of the row 3 NBs is eliminated (Figs 4B, 8F). These reciprocal transformations suggest that, in gsbd mutants, row 5 NBs are transformed into row 3 and that, in heat shocked hs-gsbd embryos, row 3 NBs are transformed into row 5.

This conclusion was strengthened by analyzing wg gene expression in heat shocked hs-gsbd embryos. To monitor wg expression, we used a wg enhancer trap line CyO/wg17en40 in which β-gal expression is regulated by the wg promoter (Perrimon et al., 1991). In wild-type embryos, β-gal from the wg enhancer trap is expressed only in row 5 NBs (Fig. 4C; Doe, 1992). In heat shocked hs-gsbd embryos, additional NBs anterior to row 5 NBs express β-gal (Fig. 4D), suggesting the transformation of row 3 NBs into row 5 NBs.

Ectopic expression of the gsbd genes causes CNS pattern defects reciprocal to that of a gsbd mutant

The effect of ectopic gsbd or gsbp on axonal pattern in the mature embryonic CNS was first examined by anti-HRP antibody staining. We have found that heat shocks at 2.75 to 3.25 hours of development for hs-gsbd embryos and 4.5-5.0 hours for hs-gsbd embryos give the most consistent alterations

Fig. 3. Expression of gsbd and gsbp in the developing CNS. (A,B) Anterior is up and the view is ventral. (A) gsbd is expressed in four NBs of each hemisegment at early stage 9, including NB7-1. (B) By early stage 11, gsbd is expressed in the NBs of row 5 and 6 and NB7-1, where gsbd is also expressed (Gutjahr et al., 1993b). (C-E) Lateral views of a section of a gsbd/lacZ embryo (stage 11) double labeled with anti-gsbd and anti-β-gal (representing gsbd) antibodies. gsbd (green) is expressed in the epidermis, the NBs and their progeny and the mesoderm while β-gal is expressed at high levels only in the gsbd-positive NBs and their progeny. At this particular focal plane, the normally strong epidermal expression of gsbd is out of focus. (N, NB; n, neural cells including GMCs and neurons; m, mesoderm; e, epidermis) Bar, 20 µm.
The defects in heat shocked hs-\textit{gsbd}\ and hs-\textit{gsbp}\ embryos are very similar, typified by the fusion of the two commissures within each segment and the elimination of the longitudinal connectives.

In order to follow cell fate changes in heat shocked hs-\textit{gsb}\ embryos, we chose to examine anti-eve antibody staining, because anti-eve antibodies recognize subsets of neurons with different behaviors in \textit{gsb}\ mutants: CQ neurons are deleted, aCC, pCC and RP2 neurons are duplicated, and EL neurons appear to be unaffected (Fig. 8H; Patel et al., 1989). Anti-eve antibody staining of heat shocked hs-\textit{gsbp}\ embryos reveals pattern alterations nearly reciprocal to that of \textit{gsb}\ mutants. Fig. 6B and 6D show that the eve expression characteristic of the RP2 and EL neurons is eliminated in most segments. In Fig. 6D, the eve expression expected in aCC and pCC neurons also appears to be eliminated in some segments, and there are putative duplications of the CQ neurons.

Using anti-eve antibody stainings, it is difficult to distinguish unambiguously the aCC and pCC neurons from the adjacent CQ neurons in heat shocked hs-\textit{gsbp} or \textit{gsb} mutant embryos. To resolve this problem, we used the A31 enhancer trap line. The A31 enhancer trap line has a P\textit{\lucZ}\ element inserted into the fasciclin II gene (Ghysen and O’Kane, 1989; Grenningloh et al., 1991). In the CNS of A31 embryos, \textbeta-gal\ is only expressed in the aCC and pCC neurons at early stage 12. The A31 enhancer trap line was crossed into the hs-\textit{gsbp}, hs-\textit{gsbd}\ and \textit{gsb}\ mutant backgrounds and the behavior of aCC and pCC neurons was examined by anti-\textbeta-gal\ antibody stainings. Two pairs of aCC and pCC neurons are in each CNS segment of wild-type embryos (Fig. 6E), and apparent duplication of aCC and pCC neurons occurs in \textit{gsb}\ mutant embryos.

![Fig. 4. NB patterns in heat shocked hs-\textit{gsbd} embryos. Anterior is up and the view is ventral. Anti-ac antibody stainings in a wild-type embryo (A) and a heat shocked hs-\textit{gsbd} embryo (B), both at stage 9. In the wild-type embryo, anti-ac antibody recognizes two rows of NBs in every segment, row 3 and row 7 (as indicated). In heat shocked hs-\textit{gsbd} embryos, only the row 7 NBs are recognized by anti-ac antibody and row 3 NBs cease to express the ac protein. \textit{wg}\ expression is monitored by the expression of \textbeta-gal\ from the \textit{wg}\ enhancer trap \textit{wg}\textit{\textit{\luc40}}. At late stage 9 in a wild-type embryo (C), the \textbeta-gal\ of the \textit{wg}\ enhancer trap is expressed only in the three NBs of row 5 in each hemisegment (arrowheads). In a heat shocked hs-\textit{gsbd} embryo (D), besides the three row 5 NBs (arrowheads), \textit{wg}\ enhancer trap expression is also detected in NBs anterior to row 5 (arrows). In both B and D, heat shocks were performed at 2.75-3.25 hours after egg laying. Bar, 20 \textmu m.](image1)

![Fig. 5. The organization of axons in the CNS (stage 15) visualized by anti-HRP antibody labeling. Wild-type CNS (A) shows the ladder-like arrangement of axons; longitudinal connectives run the length of the CNS and are linked in each segment by pairs of commissures. In heat shocked hs-\textit{gsbd} (B) and heat shocked hs-\textit{gsbp} (C) embryos, the two commissures of each segment are fused in most of the segments and the longitudinal connectives between adjacent segments are eliminated. Bar, 50 \textmu m.](image2)
Ectopic gooseberry changes cell fate 1157

(Fig. 6F). Elimination of β-gal-expressing aCC and pCC neurons occurs occasionally in heat shocked hs-gsbp embryos (heat shocking at 4.5±0.25 hours) (Fig. 6G) and occurs much more frequently in heat shocked hs-gsbd embryos (heat shocking at 3.0±0.25 hours) (Fig. 6H). Since the aCC and pCC neurons are never duplicated in heat shocked hs-gsbp embryos, the duplicated neurons (Fig. 6D) are most likely CQ neurons.

Neurons deleted in gsb mutants normally express the gsbp protein, whereas neurons duplicated in gsb mutants do not express the gsbp protein

The neuronal phenotypes observed in gsb mutant and heat shocked hs-gsbp embryos suggest that gsb function is both necessary and sufficient for the cell fate specification of a subset of posterior neurons, like the CQ neurons. It is important to examine whether gsbp is indeed expressed in these neurons but not in neurons which are duplicated in gsb mutants and deleted in heat shocked hs-gsbp embryos. Again, we used eve expression as our neuronal marker.

Double stainings of anti-gsbp and anti-eve antibodies in wild-type embryos show that anti-eve-stained CQ neurons also stain with anti-gsbp antibody, while the EL neuron cluster is outside the gsbp staining region (Fig. 7A). At a more dorsal focal plane of the same embryo (Fig. 7B), the aCC and pCC

---

Fig. 6. Changes in patterns of protein expression in heat shocked hs-gsbp and heat shocked hs-gsbd embryos. Anterior is up and the view is ventral. Heat shocks were performed at 4.25 hours after embryo collections for B, D and G, at 2.75 h for H. A wild-type stage 11 embryo (A) labeled with anti-eve antibodies shows two clusters of neurons composed of aCC, pCC and CQ neurons (arrow), and a pair of RP2 neuron (arrowhead) in every segment. In a stage 11 heat shocked hs-gsbp embryo (B), the RP2 neurons are eliminated in most of the segments (arrowhead) while the aCC/pCC/CQ neuron cluster persists. A wild-type stage 13 embryo (C) stained with anti-eve antibody shows four groups of neurons: the EL neuron cluster (arrowhead), the aCC and pCC neurons (double arrow), the CQ neurons (<) and the RP2 neurons (visible in some segments). In a stage 13 heat shocked hs-gsbp embryo (D), the EL neuron cluster is usually deleted (arrowhead), the aCC and pCC neurons are occasionally eliminated (double arrow), and extra CQ-like neurons are detected (<). Anti-β-gal antibody staining of an early stage 12 A31 embryo (E) shows labeling of the aCC and pCC neurons (double arrow). In an early stage 12 gsb mutant embryo carrying the A31 enhancer trap (F), the aCC and pCC neurons are duplicated in some of the segments (double arrow). In an early stage 12 heat shocked hs-gsbp embryo carrying the A31 enhancer trap (G), the aCC and pCC neurons are occasionally deleted (double arrow). In a similarly staged heat shocked hs-gsbd embryo carrying the A31 enhancer trap (H), the deletion of the aCC and pCC neurons occurs at a much higher frequency (double arrow). Bar, 50 µm.
neurons are stained by the anti-eve antibody and appear to be above the gsbp-expressing neurons, while the RP2 neurons lie just anterior to the gsbp-expressing region.

It is known that the aCC and pCC neurons are progeny from NB1-1 (Goodman et al., 1984; Udolph et al., 1993). During neurogenesis, they migrate anteriorly to the region just dorsal to the gsbp-expressing neurons. To confirm that the aCC and pCC neurons are not labeled by anti-gsbp antibodies, a double labeling experiment was done with the A31 enhancer trap line. In the lateral view of an A31 embryo double labeled by anti-gsbp and anti-β-gal antibodies (Fig. 7C-E), it is clear that the aCC and pCC neurons, recognized by the anti-β-gal antibody, are shown in C and the gsbp expression regions in D. In a merged image (E), it is clear that the aCC and pCC neurons are outside the gsbp expression region. Bar, 50 µm.

DISCUSSION

It is known that the lack of the gsb (gsbd and gsbp) activity results in the cell fate specification of both epidermal and neural cells in gsb mutants. In this study, we set out to ask the converse question: does the ectopic expression of gsb genes commit cells to a particular cell fate during embryonic development? To this end, we have generated the hs-gsbd and hs-gsbp fly lines in which ubiquitous gsb (gsbd or gsbp) expression can be induced by heat-shock treatment. Our results show that ectopic expression of either gsbd or gsbp causes pattern alterations which are approximately reciprocal to that of gsb mutants. In the epidermis, this results in the duplication of structures surrounding the parasegmental boundary and, in the CNS, neuroblasts which do not normally express gsb appear to be respecified to fates associated with the gsb-expressing NBs.

gsbd regulates cell fates in a region bracketing the parasegmental boundary

Previous results have shown that within each segment, gsbd is expressed in a region of the ventral ectoderm bracketing the parasegmental boundary (Gutjahr et al., 1993b). Eventually all wg-expressing cells express gsbd and a subset of the anterior en-expressing cells express gsbd. Epidermal cells in this region normally give rise to naked cuticle, but develop into denticle belts in gsb mutants. This transformation is correlated with the loss of wg expression during stage 11 (Hidalgo, 1991).

With heat shocks from 2.5 to 4.0 hours after egg laying, both hs-gsbd and hs-gsbp cause a transformation of the denticle belts into naked cuticle. Associated with this transformation is the generation of a new mirror-image duplicated parasegmental boundary with ectopic expression of wg, en and the endogenous gsb genes. Our explanation of this result is that the gsb expression can activate inappropriate wg transcription that establishes the feedback loop normally found between the wg- and en-expressing cells (DiNardo et al., 1988; Bejsovec and Martinez Arias, 1991). Establishing this ectopic feedback loop is dependent upon the endogenous gsb gene since heat shocks with hs-gsbd or hs-gsbp in a gsb mutant background do not lead to ectopic expression of either wg or en and the anterior denticle belts are never transformed into naked cuticle.

Heat shocks after 4 hours of development are ineffective in establishing the wg-en cell feedback loop but still cause transformation of the denticle belt into naked cuticle. In these embryos, high level ectopic expression of wg, en and the
Fig. 8. Summary diagram. The upper panels illustrate the cuticle phenotypes in wild-type (A), gsb (B) and hs-gsb embryos (C). The trapezoidal box represents the wild-type abdominal type denticle belt (the first row of denticles at the posterior margin of the segment is not shown) and the three-hair structure depicts the thoracic Keilin’s organ. In the gsb embryo, naked cuticle is replaced by a mirror-image duplication of the denticle belts while, in hs-gsb embryo, denticle belts are replaced by naked cuticle, including an occasional duplication of the Keilin’s organ. The middle panels (D-F) depict the NB pattern in hemisegments at early stage 9. In wild-type embryos (D), the anteroposterior axis has 4 rows of NBs: row 2, 3, 5 and 7 (Doe, 1992). In the dorsoventral axis, there are three columns: medial (m), intermediate (i) and lateral (l) (Hartenstein and Campos-Ortega, 1984). Each NB can be given a row and column designation in this grid. The correspondence of the grid designations with NB identities described in Doe (1992) is: 2m, 2-2; 2i, 3-2; 2l, 2-5; 3m, 5-2; 3i, 5-3; 3l, 5-6; 5m, 7-1 and 7l, 7-4. The L-shaped outline encloses the NBs that express gsb. A key at the bottom of the figure shows the code used to depict the expression patterns of the markers used in this study. Small black symbols show the sites of NB formation at later stages: diamond, NB 4-2 (gives rise to RP2 neurons); triangle, NB 6-2 (gives rise to CQ neurons) and square, NB 1-1 (gives rise to aCC and pCC neurons). In gsb mutants (E), ac is also expressed in what would be row 5. This may represent a transformation of the row 5 NBs into row 3 NBs, though the row 5 NBs continue to express wg. In heat shocked hs-gsb embryos (F), gsb and gsb expression expands throughout the segment and usually has the appearance of a rough mirror-image duplication. This is shown by the dotted outline. ac expression is eliminated in row 3 NBs and is replaced by the expression of wg. This may represent a transformation of row 3 into row 5. (The deletions and duplications of NB 4-2, NB 6-2 and NB 1-1 are inferred from the alterations to their progeny.) (G-I) The neuronal patterns in mature CNS segments. The axon bundles are shown as hatched areas. The gsb-expressing neurons are positioned in the boxed L region, partially overlapping the en-expressing neurons. The labels for the EL, CQ, aCC, pCC and en neurons are shown at the bottom of the figure. In gsb mutants (H) the posterior commissure is eliminated along with the CQ neurons, while there are duplications of the RP2, aCC and pCC neurons. In heat shocked hs-gsb embryos (I), the longitudinal connectives are eliminated and the commissures are fused, the EL, RP2, aCC and pCC neurons are deleted, and the CQ neurons are duplicated.
endogenous gsb is not observed but low level wg expression is seen throughout the segment. Experiments with wg 

and Carroll, 1992; Doe, 1992). In protein in row 3 and row 7 NBs but not in row 2 and 5 (Skeath prediction is approximated in the initial patterning of NBs. is eliminated in row 3, suggesting a reciprocal transformation gsbd mutants do not express pattern transformations reciprocal to those in gsb mutants. This result again suggests the possibility that there are NBs and neuronal lineages that differ from one another only in their expression of the gsb genes. This correlation is not perfect, as the EL neurons do not fit this pattern. The EL neurons are present in gsb mutants, but do not appear to be duplicated. In heat shocked hs-gsbd embryos these neurons are consistently eliminated.

gsb as a selector gene in the CNS

In the CNS analysis, we focused on the cell fate transformations of a subset of NBs and neurons. Our assignment of neuronal and NB transformations is based on changes in the expression patterns of various proteins. It is possible that these changes do not reflect complete transformations of one cell type into another. This will be known only after neuronal phenotypes have been carefully analyzed. We believe that we are following cell fate transformations, as two neuronal markers (eve and A31 β-gal expressions) and two NB markers (ac and wg expressions) used in this study all behave appropriately.

At both neuronal and NB levels, we have found that gsb (both gsbd and gsbp) is normally expressed in those cells that are deleted in gsb mutants but not expressed in cells that are duplicated in gsb mutants. Ectopic expression of gsb leads to cell fate transformations that are nearly reciprocal to those of gsb mutants. These results suggest that the gsb locus may function as a classical selector gene, similar to the homeotic genes, and choose between different neuronal fates (Garcia-Bellido, 1975). The simplest model would be one in which there was a one-to-one correspondence between gsb-expressing and non-expressing NBs and their neurons. If this were the case, inappropriate expression of the gsb genes would lead to pattern transformations reciprocal to those in gsb mutants. This prediction is approximated in the initial patterning of NBs.

It is known that wild-type stage 9 embryos express the ac protein in row 3 and row 7 NBs but not in row 2 and 5 (Skeath and Carroll, 1992; Doe, 1992). In gsb mutants, ac expression is also found in row 5 (Skeath et al., 1992) and may represent the transformation of row 5 into row 3. (It cannot be a transformation of row 5 into row 7 since the NBs in row 5 of gsb mutants do not express en, while row 7 NBs normally do express en.) In heat shocked hs-gsbd embryos, ac expression is eliminated in row 3, suggesting a reciprocal transformation of row 3 into row 5. This conclusion is supported by examining the expression of β-gal from a wg enhancer trap line in hs-gsbd background. wg is normally expressed only in row 5 NBs (Doe, 1992). In heat shocked hs-gsbd embryos, the wg enhancer trap is also detected in some row 3 NBs (Fig. 5B). The role of gsbd in the specification of NB 7–1 is less clear. This NB also expresses en and it continues to express ac in gsb mutants.

Later in nervous system development, additional NBs are formed and a complex pattern of neurons is generated. With the anti-eve antibody, we can follow the progeny from four different NBs (Doe, 1992). The aCC and pCC neurons are derived from NB 1–1, an en-expressing NB located just posterior to row 7 (Fig. 7A). The RP2 neuron is derived from NB 4–2, which does not express gsb and is located just anterior to the gsbd expression region (Fig. 7A). The lineage of the EL neurons is not known, but is outside the region of gsbd expression. The CQ neurons are derived from NB 6–2 which expresses gsb (Fig. 7A). The effects of heat shock hs-gsbd on aCC, pCC, RP2 (deletion) and CQ (duplication) are the reciprocal to that in gsb mutants. This result again suggests the possibility that there are NBs and neuronal lineages that differ from one another only in their expression of the gsb genes. This correlation is not perfect, as the EL neurons do not fit this pattern. The EL neurons are present in gsb mutants, but do not appear to be duplicated. In heat shocked hs-gsbd embryos these neurons are consistently eliminated.

gsb is a link between epidermal and CNS patterning

The roles of several segmentation genes in CNS development have been studied in detail. Some pair-rule genes, such as ftz and eve, have two independent roles, one in epidermal segmentation and a second in neurogenesis (Doe et al., 1988a,b). gsb is distinct from this paradigm since the pattern of gsbd expression in the CNS is always found in segmentally repeating stripes which begin in the neuroectoderm and are maintained as NBs delaminate. This may reflect similar independent functions in the epidermis and NBs or a single function in the common ectodermal precursor cells.

We thank Cindy Motzny, Darlene Buenzow, Jennifer Kennedy and Andrea Brand for stimulating discussions, and Linda Orlofsky for generating the hs-gsdp line. We are grateful to Lin Gu for her expertise and assistance in producing the monoclonals. Jim Skeath for providing his anti-eve antibody, we can follow the progeny from four different NBs (Doe, 1992). The aCC and pCC neurons are derived from NB 1–1, an en-expressing NB located just posterior to row 7 (Fig. 7A). The RP2 neuron is derived from NB 4–2, which does not express gsb and is located just anterior to the gsbd expression region (Fig. 7A). The lineage of the EL neurons is not known, but is outside the region of gsbd expression. The CQ neurons are derived from NB 6–2 which expresses gsb (Fig. 7A). The effects of heat shock hs-gsbd on aCC, pCC, RP2 (deletion) and CQ (duplication) are the reciprocal to that in gsb mutants. This result again suggests the possibility that there are NBs and neuronal lineages that differ from one another only in their expression of the gsb genes. This correlation is not perfect, as the EL neurons do not fit this pattern. The EL neurons are present in gsb mutants, but do not appear to be duplicated. In heat shocked hs-gsbd embryos these neurons are consistently eliminated.

REFERENCES


different spatially restricted transcripts in the embryo. EMBO J. 6, 2793-2802.


(Accepted 28 January 1994)