

Influence of *Drosophila* ventral epidermal development by the CNS midline cells and *spitz* class genes

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SUMMARY

The ventral epidermis of *Drosophila melanogaster* is derived from longitudinal rows of ectodermal precursor cells that divide and expand to form the ventral embryonic surface. The *spitz* class genes are required for the proper formation of the larval ventral cuticle. Using a group of enhancer trap lines that stain subsets of epidermal cells, it is shown here that *spitz* class gene

function is necessary for ventral epidermal development and gene expression. Analysis of *single-minded* mutant embryos implies that ventral epidermal cell fate is influenced by the CNS midline cells.

Key words: *Drosophila*, development, CNS, epidermis, cell fate

INTRODUCTION

How tissue and cell identity are established is a fundamental problem of animal development. The molecular and cellular basis of how cellular identity is established has been productively studied in the *Drosophila* embryo (Lawrence, 1992). One active area of research has investigated how embryonic cells attain their identities along both the anterior/posterior (A/P) and dorsal/ventral (D/V) axes, and at the termini. Genetic and molecular studies have shown that identity along the A/P axis requires a genetic hierarchy that includes maternal, gap, pair-rule, segment polarity and homeotic genes. A number of genes have also been identified that play a role in establishing cell identity along the D/V axis. These maternal and zygotic genes subdivide the embryo into domains including the ectoderm, which gives rise to both epidermis and nervous system. Currently, there is little known about how the epidermis is subdivided into cells with distinct identities along the D/V axis. The subject of this paper concerns how the ventral epidermal cells acquire their fate.

The epidermis can be subdivided into three regions along the D/V axis (Fig. 1; Ferguson and Anderson, 1991). The ventral epidermis (VE) lies at the ventral midline of the embryo and secretes the centralmost section of the ventral denticles. The lateral epidermis (LE) gives rise to the rest of the ventral denticles. The ectodermal precursors of the LE, VE and mesectodermal (MEC) cells are referred to as the 'neurogenic ectoderm' since they give rise to the central nervous system (CNS) as well as the epidermis. The MEC cells should be distinguished from the other neurogenic ectodermal cells because they give rise only to CNS midline cells and not epidermis (Thomas et al., 1988; Nambu et al., 1990). The dorsal epidermis (DE) gives rise to the hairs that form on the dorsal cuticle. The VE can be distinguished from the

LE and DE in a nearly mature stage 16 embryo due to the large, stretched appearance of its cells and nuclei (Fig. 2A).

The genetic work of Nüsslein-Volhard, Wieschaus and co-workers identified a large number of genes involved in formation of the larval cuticle and corresponding epidermis (Nüsslein-Volhard et al., 1984; Jürgens et al., 1984; Wieschaus et al., 1984). Included in this collection were a group of mutants missing the centralmost ventral denticles and presumed to have VE defects (Mayer and Nüsslein-Volhard, 1988). These genes included *pointed* (*pnt*), *sichel* (*sic*), *single-minded* (*sim*), *spitz* (*spi*), *Star* (*S*) and *veinlet* (*ve*; previously called *rhomboid*), and were referred to as the '*spitz* class' of genes. The *ocelliless* (*oc*; previously called *ortho-denticle*) gene can also be included in this group based on its ventral midline cuticular defect (Wieschaus et al., 1984, 1992), as can the *Epidermal growth factor receptor* (*Egfr*, previously called *DER*), gene (Clifford and Schüpbach, 1992). More recently, cellular and genetic analysis of salivary gland placode formation has confirmed that the *spitz* class mutants are defective in formation of the ventralmost ectoderm (Panzer et al., 1992). In this paper, we describe specific cytological markers for VE cells that allow cellular analysis of mutations that influence VE cell fate. It is shown that *spitz* class gene function is required for gene expression and formation of VE precursors. Based on genetic studies and the expression pattern of the *sim* gene, it is proposed that cell fate in the VE is influenced by the CNS midline cells.

MATERIALS AND METHODS

Drosophila strains

The *Drosophila* strains used in this work were generally the most severe alleles available unless noted otherwise and are identified in Lindsley and Zimm (1992). The *sim* strains used were *sim*²

(EMS), *sim*⁵ (EMS) and *Dff(3R)^{γ619}* (X-ray). They are protein nulls and exhibit amorphic CNS phenotypes (Nambu et al., 1991). Alleles of the other genes are as follows: *Egfr^{Δ6}* (severe), *Egfr^{Δ7}* (weak), *oc*², *pnt*¹, *ve*⁴, *spi*¹ and *S^{HN}*. The *oc* allele was provided by R. Finkelstein and N. Perrimon, and the other mutant strains were obtained from the Bowling Green Stock Center.

Epidermal cell markers

The enhancer trap lines AA69, BL97, BP28 and BP48 were identified in our laboratory during a large-scale screen (Crews et al., 1992). All lines localize β -galactosidase to cell nuclei.

AA69

The P-element transposon inserted adjacent to the *ve* gene at 62A on the third chromosome (Nambu et al., 1990). Expression of β -galactosidase accurately reflects the transcription of the *ve* gene (Bier et al., 1990). After germband retraction, AA69 is expressed in one to two rows of epidermal cells that lie in the posterior of the segment (Table 1). β -galactosidase is also expressed in the MEC cells from stages 8-17 and the *ve* gene is expressed in the ventral ectoderm before gastrulation. This likely includes the precursors to both the VE and LE.

BL97

This insertion lies at 32E on the second chromosome and accurately reflects expression of an adjacent gene (S. Hu and S. T. Crews, unpublished data). During stage 10, all epidermal cells express β -galactosidase and continue to do so through stage 17. There is BL97 β -galactosidase expression in all of the MEC cells from stage 9 to stage 17. BL97 is also expressed in the midgut and stomagastric nervous system.

BP28

The BP28 insertion lies at 24DE and accurately reflects expression of an adjacent gene (S. H. Kim and S. T. Crews, unpublished data). It is expressed prominently in the VE beginning at stage 10. The CNS midline cells also show prominent β -galactosidase expression beginning at stage 12. Additional expression is observed in the proventriculus, a small set of lateral CNS cells, PNS, stomagastric nervous system and antennal-maxillary complex.

BP48

The BP48 insertion is on the second chromosome and reflects the expression of an adjacent gene (S. H. Kim and S. T. Crews, unpublished data). This gene is expressed in a subset of VE cells that form the ventral denticles and in a corresponding subset of LE cells (Table 1). BP48 is expressed in a subset of CNS midline cells including the midline glia late in embryogenesis and in numerous other cell types including the hindgut and midgut.

oc

Expression of *oc* was analyzed using a full-length cDNA probe generously provided by R. Finkelstein and N. Perrimon. *oc* is transcribed in the VE cells beginning at stage 9 and continues to be expressed in these cells through stage 15. It is also expressed in the CNS midline precursors beginning at stage 8 and is restricted to a subset of CNS midline cells after stage 12 (Finkelstein et al., 1990).

Immunohistochemistry and in situ hybridization

Antibody staining and in situ hybridization of embryonic whole mounts and sections were performed and observed as previously described (Nambu et al., 1991). Balancer chromosomes included either a P[ftz/lacZ] or P[elav/lacZ] marker that allowed identification of homozygous mutant embryos by staining with an antibody against β -galactosidase (Nambu et al., 1990). The illustration in

Fig. 5E is a computer-assisted representation of stained cell nuclei. Initially, nuclei were traced from a video scan of the embryo. The traced image was entered into a Macintosh computer by digital scanning and the scanned image was used as a template for the stylized Adobe Illustrator representation shown in the figure. Nuclear counts are expressed as the mean \pm standard deviation (s.d.).

RESULTS

The ventral epidermis

Study of VE formation in wild-type and mutant embryos has been made possible by the identification and use of several enhancer trap lines (O'Kane and Gehring, 1987) that express β -galactosidase within the epidermis. These lines and the epidermal cells they stain are listed in Table 1. The definitive line is BP28, which prominently stains the VE cell nuclei but not the adjacent epidermis; it also is expressed in the MEC. In this paper, the VE comprises those cells that show BP28 *lacZ* expression. BP28 is a useful lineage marker for the development of these cells from germband extension (embryonic stage 10) to formation of first instar larva.

BP28 staining during embryonic development is shown in Fig. 2B-F. Initial expression begins during the germband-extended stage 10 of embryogenesis in adjacent columns of cells that lie at the ventral midline of the presumptive epidermis (Fig. 2B). Many of the cells remain on the surface and will form the VE, others delaminate internally from the ventral ectoderm and resemble neuroblasts. This observation is similar to those made previously by Foe (1989) who observed cell division and internal movement of ventral ectodermal cells. Stage 11 embryos also show narrow rows of cell nuclei along the ventral midline with segmentally repeated groups of cells extending laterally (Fig. 2C, note arrows). During stages 12-13 the VE cells spread out as dorsal closure begins (Fig. 2D).

Dorsal closure is complete by stage 16 and the VE cells are well spread out (Fig. 2E). Nuclei of the VE cells are larger and have an elongated morphology in comparison to the smaller, more rounded morphology of more lateral epidermal cells (Fig. 2A). There are approximately 50 nuclei/segment at stage 16 that stain with BP28. Fig. 2F shows a stage 17 embryo that has secreted cuticle and shows the relationship between the VE cells and the ventral denticles. At this stage, the nuclei are more rounded and closer together than at stage 16. There are approximately 25 BP28-staining nuclei that lie beneath the central 30% of the denticles.

spitz class gene function is required for early ventral ectodermal gene expression

Previous experiments by Mayer and Nüsslein-Volhard (1988) had shown that mutations in the *spitz* class genes had ventral cuticle defects consistent with alterations in VE cell fate. These genes include *pnt*, *sic*, *sim*, *spi*, *S* and *ve*. We also include the *oc* and *Egfr* genes. These mutants all appear to be missing a number of ventral denticles, although other cuticular pattern defects are often present.

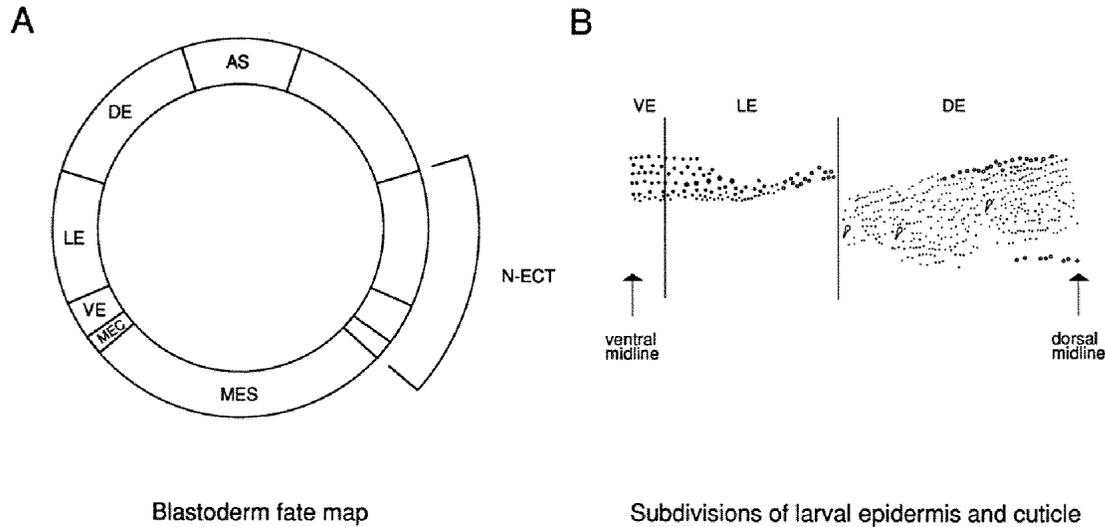


Fig. 1. Embryonic and larval sub-divisions. (A) This schematic cross-section represents the cellular blastoderm fate map. Dorsal is at the top of the drawing. AS, amnioserosa; DE, dorsal epidermis; LE, lateral epidermis; VE, ventral epidermis; MEC, mesectoderm; MES, mesoderm. The neurogenic ectoderm (N-ECT) gives rise to both the ventral-lateral epidermis and CNS. It includes the blastoderm precursors that give rise to the LE, VE and MEC. The MEC gives rise to the midline cells of the CNS. (B) This schematic shows one half-segment of a mature larval cuticle splayed out illustrating the correspondence between epidermal sub-divisions and cuticular structures. The ventral midline of the larva is on the left side of the figure and the dorsal midline is to the right.

We have directly examined the fate of the VE cells in *spitz* class mutants (n.b. the maternal-effect *sic* gene was not analyzed) by using genes and markers expressed specifically in these cells.

We first examined the requirement for *spitz* class gene function on the expression of BP28 and the *oc* gene. The BP28 enhancer trap gene was crossed into mutant strains and embryos were stained with a monoclonal antibody against β -galactosidase. Fig. 3 shows the results of staining embryos at stages 12-13; similar results are observed throughout the time period of BP28 expression (stages 10-17). There is strong VE β -galactosidase expression in wild type (Fig. 3A). However, in *Egfr*, *oc*, *pnt*, *sim*, *spi*, *S* and *ve*, there is a complete or partial absence of BP28 VE expression depending on the mutant strain (Fig. 3B-G). There were less than 10% of the number of wild-type BP28-positive VE cells in *Egfr*, *sim*, *spi*, *S* and *ve* mutant embryos. The number of BP28-positive cells in both *oc* and *pnt* was reduced (10-50% of wild type), but this effect was less dramatic than in the other *spitz* class mutants. These results indicate that *spitz* class gene function is required for BP28 VE expression.

The *oc* gene is expressed in most or all of the VE cells beginning around stage 9 of embryogenesis (Fig. 4A) and continuing to stage 15 or later. Its VE expression is similar to BP28. Figure 4C shows that *oc* expression is greatly reduced along the ventral midline in a *sim* mutant embryo. Similar results were obtained for *Egfr* (Fig. 4B), *pnt*, *spi*, *S* and *ve* (data not shown), although small numbers of staining cells were sometimes observed. These results are similar to those observed for BP28 expression in *spitz* class mutant strains. No *oc* expression was observed in *spitz* class mutants even in stage 9 embryos when the gene is first expressed in the presumptive VE.

Ventral epidermal cells are absent in older *spitz* class mutant embryos

The results with BP28 and *oc* indicate that *spitz* class mutants can influence gene expression within the presumptive VE. To investigate further the role of the *spitz* class genes on VE development, we examined the expression of two other genes that are normally expressed within these cells in mutant strains.

The *ve* gene is expressed in one to two circumferential single-cell rows in the posterior of each segment at the time of germband retraction and later (Fig. 5A,E; Bier et al., 1990). *ve* expression (as detected using an enhancer trap insertion at the locus, AA69) includes the VE cells in the posterior region of the segment as well as the LE and DE. It is an excellent marker to determine whether the VE cells are absent in *spitz* class mutant embryos or present but lacking in VE-specific gene expression. There are 16 ± 1 (\pm s. d.) ($n=5$) VE cells and 43 ± 2 ($n=5$) LE and DE cells that express AA69 in A2 segments of wild-type stage 15-16

Table 1. Epidermal cell markers

Strain	Epidermal cells stained	Embryonic stage when expressed in VE
BP28	All VE	10-17
BL97	All epidermis	10-17
AA69 (<i>ve</i>)	Posterior 1-2 rows of epi (VE, LE, DE)	12-17
BP48	Posterior 4 rows of epi (VE, LE)	11-17
<i>oc</i>	All VE	9-15

Included are four enhancer trap lines and an *oc* cDNA probe. The enhancer trap lines express β -galactosidase in the cells listed. Only relevant epidermal cell staining is mentioned, although all of the lines have other sites of expression. VE, ventral epidermis; LE, lateral epidermis; DE, dorsal epidermis.

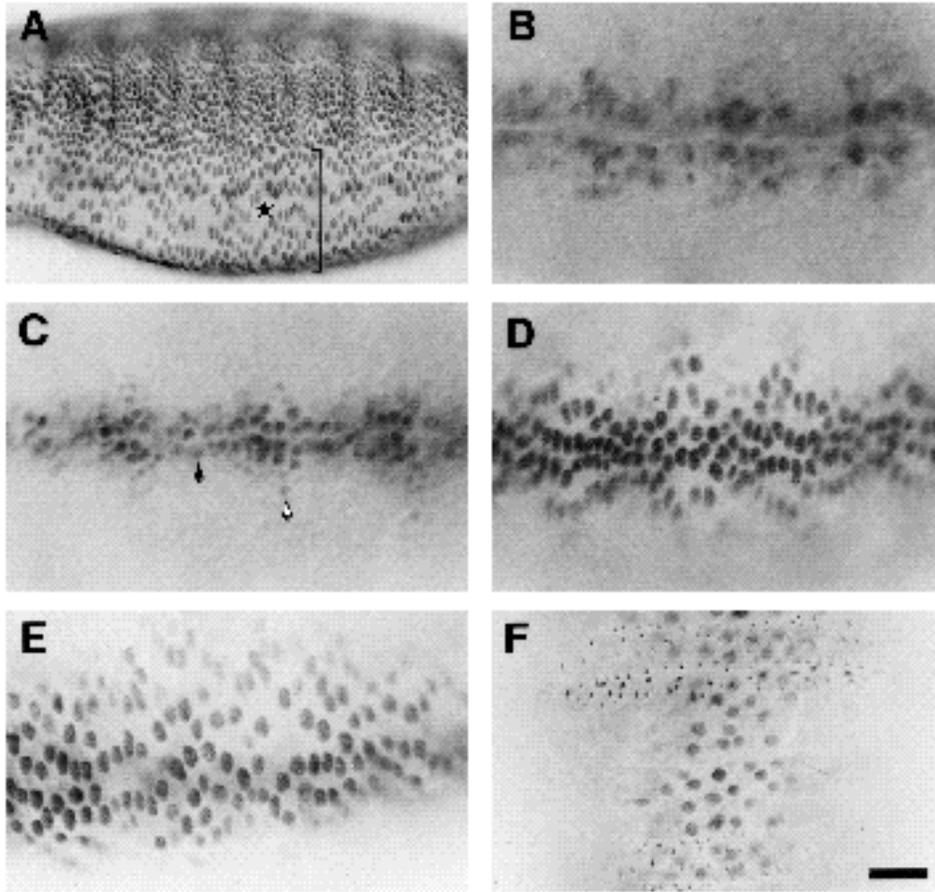


Fig. 2. Development of VE cells in normal development. All embryos are wild-type and stained with a monoclonal antibody against β -galactosidase as visualized with HRP histochemistry. Embryonic stages use the convention of Campos-Ortega and Hartenstein (1985). All embryos show anterior to the left except (F) which has anterior pointing to the top of the page. (A) Stage 15 embryo containing the BL97 enhancer trap insertion, which stains all epidermal cell nuclei. The embryo is tilted so that the lateral and ventral surfaces are shown. The bracket shows the limits of the VE, which is easily visualized at this stage of development, and position of the star indicates the ventral midline. Notice the stretched appearance of the VE cell nuclei compared to the LE and DE cell nuclei. (B) Ventral view of a stage 10 BP28 embryo showing initial expression of β -galactosidase along the ventral midline. There are additional stained cells beneath the focal plane shown in this panel that are probably neuroblasts. This photograph covers about three parasegments. (C) Ventral view of a stage 11 BP28 embryo showing β -galactosidase expression along

the ventral midline. The cells that extend laterally (white arrow) may correspond to those derived from the 14^{25} mitotic domain and the cells arranged in longitudinal rows (black arrow) are likely derived from the 14^M mitotic domain (Foe, 1989). This photograph covers about three parasegments. (D) Ventral view of a stage 13 BP28 embryo as the VE cells begin their lateral spread. Four segments are shown. (E) Ventral view of a stage 16 BP28 embryo after dorsal closure showing the extended VE cell nuclei. These cells correspond to the bracketed cells in panel A. Four segments are shown. (F) Ventral view of a stage 17 BP28 embryo showing the relationship between the VE cells and the ventral denticles of the cuticle. There are about 25 BP28-positive cells that give rise to the centralmost denticles. Segments A6 and A7 are shown. Scale bar, (A) 50 μ m, (B-F) 16 μ m.

embryos based on position and morphology. The expression of AA69 is clearly affected in *spitz* class mutant embryos. All or nearly all of the VE-like cells are absent in *sim* mutant embryos (Fig. 5C,E). This is indicated by nuclear counts that indicate fewer nuclei in the mutants. There are only 44 ± 3 ($n=6$) stained cells in *sim* mutant embryos as opposed to the 59 ± 1 in wild-type, consistent with the loss of the 16 VE cells. Figure 5E provides an interpretative drawing showing that the cells along the ventral midline in *sim* mutant embryos are most likely derived from the LE and DE that are now joined together.

Similar results were observed in homozygous mutants of *Egfr*, *spi* and *S*. The weak *Egfr* allele, *Egfr⁷*, was examined since epidermis of the severe allele, *Egfr⁶*, was too defective for meaningful interpretation. Mutants of *pnt* were less extreme than the other *spitz* class mutants with about half of the wild-type number of VE-like cells present.

Another marker used is an enhancer trap gene (BP48) that corresponds to the subset of VE cells that underlie the denticles (Fig. 5B,E). Expression also extends to a subset of LE cells. Similar to AA69, the BP48 marker provides a

convenient way to determine if VE cells are either present and not expressing VE genes in *spitz* class mutants or if the cells are absent. The experiments examining BP48 staining in *spitz* class mutant embryos were consistent with those of AA69 (Fig. 5D,E). Homozygous *sim* mutant embryos showed an absence of BP48-staining cells along the ventral midline from stage 12 until the end of embryogenesis (stage 15 is shown in Fig. 5D,E). Wild-type embryos have 23 ± 2 ($n=6$) VE cell nuclei and 49 ± 3 ($n=6$) LE cell nuclei that express β -galactosidase in each segment, whereas the *sim* mutant had 43 ± 3 ($n=4$) cell nuclei stained, consistent with an absence of VE cells. Embryos mutant for *Egfr⁷*, *pnt*, *spi* and *S* also showed a reduced number of VE cells.

These experiments show that normal VE development does not occur in *spitz* class mutants as assayed by loss of VE cells and corresponding gene expression. Reduction in the number of VE cells in mutant embryos explains the *spitz* class cuticle defects that are missing the centralmost ventral denticles. There are several explanations for the loss of VE cells. The VE precursors may fail to carry-out

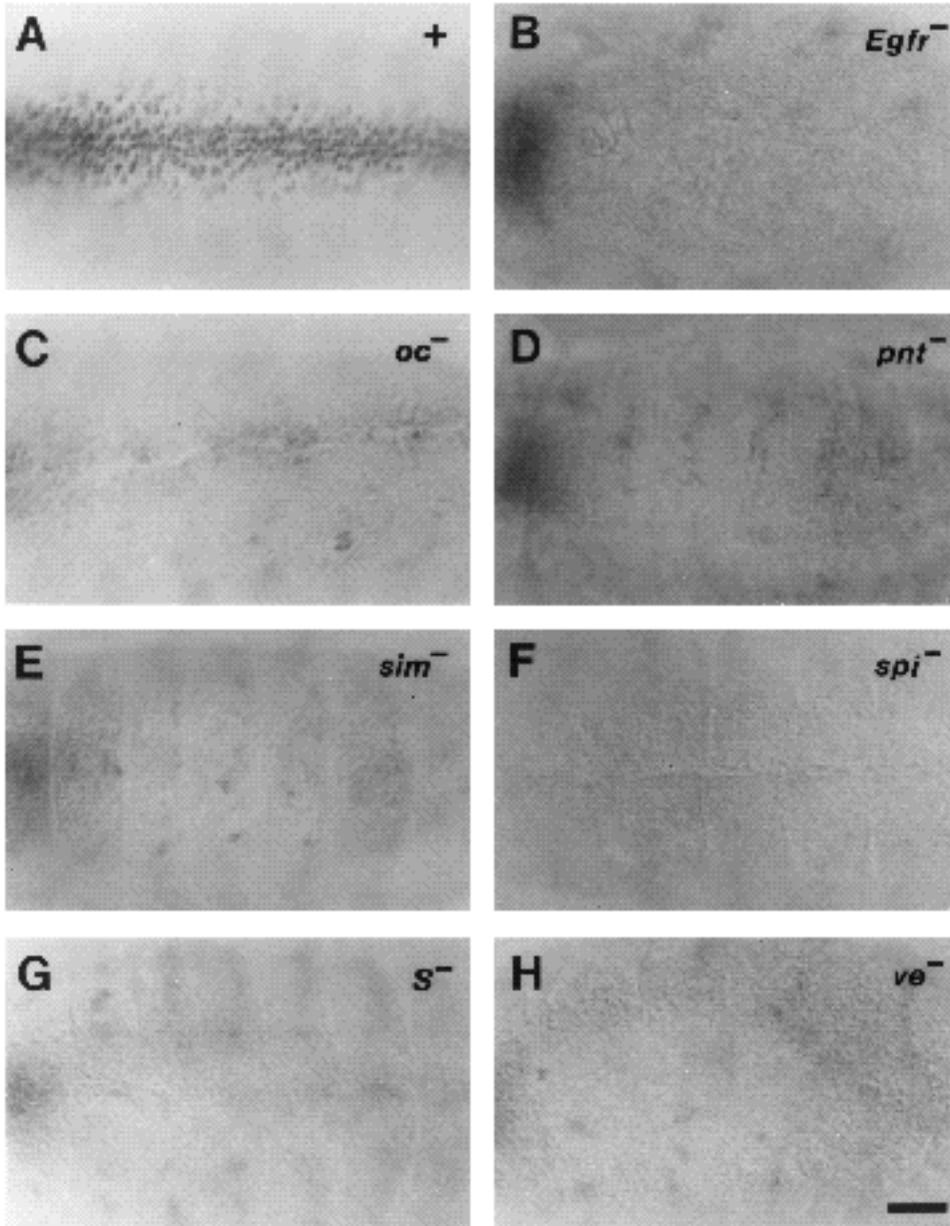


Fig. 3. *spitz* class gene function is required for BP28 VE expression. Embryos all contained the BP28 P[*lacZ*] gene and were stained for β -galactosidase activity. All panels show ventral views of stage 12-13 embryos; results are similar for embryos at other stages of development. Generally, there is a complete absence or loss of >90% of BP28-positive VE cells in all *spitz* class mutants except *oc* and *pnt*, which are often less severe. This may reflect a lesser role of *oc* in VE formation since the *oc*² allele is thought to be null (Finklestein et al., 1990). The few stained cells in the *sim* mutant embryo are due to non-midline BP28 *lacZ*-expressing cells. (A) wild-type (+), (B) *Egfr*⁻, (C) *oc*⁻, (D) *pnt*⁻, (E) *sim*⁻, (F) *spi*⁻, (G) *S*⁻, (H) *ve*⁻. Scale bar, 25 μ m.

their characteristic cell divisions (Foe, 1989). Alternatively, the precursors divide and then either die or take a non-VE cell identity and fail to behave as ventral epithelium. Since the different genes may not influence VE cell formation in a uniform fashion, the requirement of each *spitz* class mutant on cell division will have to be directly examined similar to experiments with *Egfr* mutants, which showed no effect on cycle 14 cell divisions (Clifford and Schüpbach, 1992).

DISCUSSION

Model for commitment of ventral epidermal cell fate

This paper shows that the *spitz* class genes influence VE gene expression and development. This expands previous

observations that these genes were important for the formation of the medial denticles of the cuticle (Mayer and Nüsslein-Volhard, 1988). Furthermore, it was observed that these genes function early in embryogenesis around stage 9 as indicated by the loss of *oc* expression in the mutant embryos. Of particular interest is the observation that function of the *sim* gene is required for VE formation, since *sim* is not expressed in the VE precursor cells, but its expression is restricted to the adjacent MEC (Thomas et al., 1988; Nambu et al., 1990). Although it was previously suggested that the MEC cells are precursors to both the CNS midline cells and VE (Mayer and Nüsslein-Volhard, 1988), this is not the case; the MEC gives rise only to the CNS midline cells (Nambu et al., 1991). It is concluded that VE cell fate is dependent upon CNS midline cell function. How the CNS midline cells influence the VE is unknown. They may send an inductive signal that establishes VE identity,

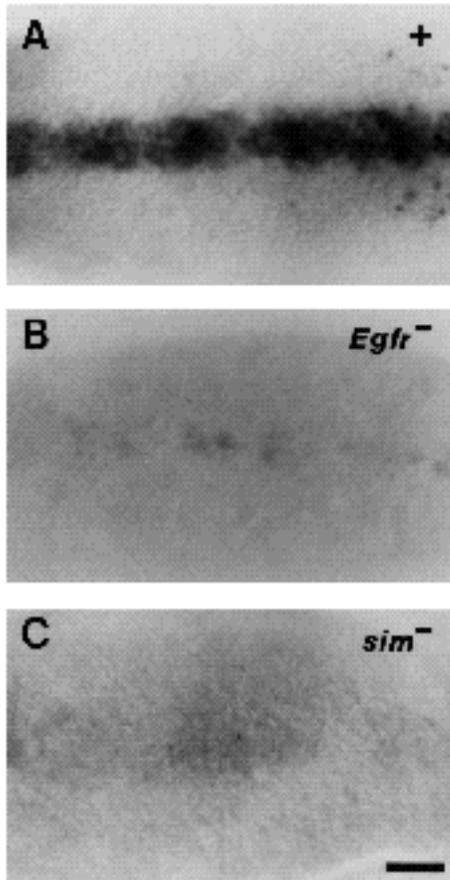


Fig. 4. VE expression of *oc* requires *spitz* class gene function. (A) Ventral view of a stage 10 wild-type (+) embryo hybridized in situ to an *oc* cDNA probe showing prominent VE *oc* expression. (B) Ventral view of a stage 10 *Egfr*⁻ embryo hybridized to an *oc* probe showing absence of expression. Similar results were also observed in *pnt*, *spi*, *S* and *ve* mutants. (C) Ventral view of a stage 10 *sim*⁻ embryo hybridized to an *oc* probe showing absence of expression. Scale bar: 25 μ m.

or be required for the VE precursors to respond to D/V patterning gene activity or a signal emanating from elsewhere.

The following model provides a framework for understanding the commitment of VE cell fate (Fig. 6). In the late blastoderm embryo, D/V patterning genes commit a group of cells on either side of the mesoderm to become lateral neurogenic ectoderm (Fig. 6A). This is reflected in the blastoderm expression of the pro-neural *achaete-scute* complex genes and *ve* gene in cells of the neurogenic ectoderm (Romani et al., 1987; Kosman et al., 1991; Rao et al., 1991). At approximately the same time, D/V patterning genes act to uniquely commit two strips of cells that lie on either side of the presumptive mesoderm to enter the MEC lineage (Fig. 6B). This coincides with the initial transcription of the *sim* gene in these cells. As gastrulation takes place, the MEC cells migrate to the ventral midline of the ectoderm (Fig. 6C), *sim* protein is expressed and the development of the CNS midline lineage begins. The MEC nuclei migrate inwards during germband extension, although they maintain a cytoplasmic connection close to the underlying ectodermal cells (Nambu

et al., 1991). During the time period after gastrulation, the MEC cells influence the development of the adjacent VE precursors as reflected in VE-specific gene expression and formation (Fig. 6D). The VE precursor cells then go on to differentiate resulting in the mature VE (Fig. 6E). It is also possible that cell fate of some medial neuroblasts is influenced by the CNS midline and *spitz* class genes, since they emerge from the same precursor cells as the VE.

The view put forth in this paper that VE identity is influenced by the MEC parallels recent work indicating that establishment of cell identity along both the A/P axis and the DE requires cell signalling (DiNardo and Heemskerk, 1990; Ferguson and Anderson, 1991). With respect to the DE, it has been proposed that the *decapentaplegic* gene product, which is related to the secreted vertebrate TGF- β protein (Padgett et al., 1987), may form a gradient along the dorsal regions of the embryo and specify cell identity along the dorsal ectoderm (Ferguson and Anderson, 1991, 1992). Additionally, the genes *shortened gastrulation*, *shrew* and *tolloid* play a role in this process. The *tolloid* gene product also encodes a secreted protein of the bone morphogenesis protein family (Shimell et al., 1991) as does *decapentaplegic*. The implication is that much of epidermal cell identity is derived via cellular interactions.

Role of the *spitz* class genes in VE development

The evidence presented in this paper indicates that the *spitz* class genes influence VE cell fate and gene expression. Several issues arise with respect to the function of the *spitz* class genes in VE development. Are these genes directly involved in the interaction between MEC and VE precursors or in later steps of VE differentiation? If a *spitz* class gene is involved directly in MEC-VE interactions, which cell type does it function in?

sim

The *sim* gene encodes a nuclear protein that belongs to the basic-helix-loop-helix (bHLH) family of transcription factors and is likely to be a sequence-specific DNA-binding transcriptional activator (Nambu et al., 1991). It is first expressed as nuclear protein at the completion of gastrulation in the MEC before any overt differentiation of the CNS midline cells. It has been shown that *sim* gene function is required either directly or indirectly for the transcription of many and probably all genes expressed in the CNS midline cells including the *spitz* class gene, *ve* (Nambu et al., 1990). As a consequence, CNS midline precursor cells do not form, divide or differentiate. Similarly, it is likely that *sim* is required for the transcription of genes in the MEC that influence VE cell fate.

Egfr, *spi*, *S* and *ve*

As shown in this paper, these genes play a role in VE formation and there is also evidence that they may interact with each other in a number of developmental decisions. For example, they all show defects in midline glial formation, and two or more of the genes have been implicated in oogenesis, wing venation, PNS formation and retinal development (Mayer and Nüsslein-Volhard, 1988; Klämbt et al., 1991; Lindsley and Zimm, 1992; Rutledge et al., 1992). The

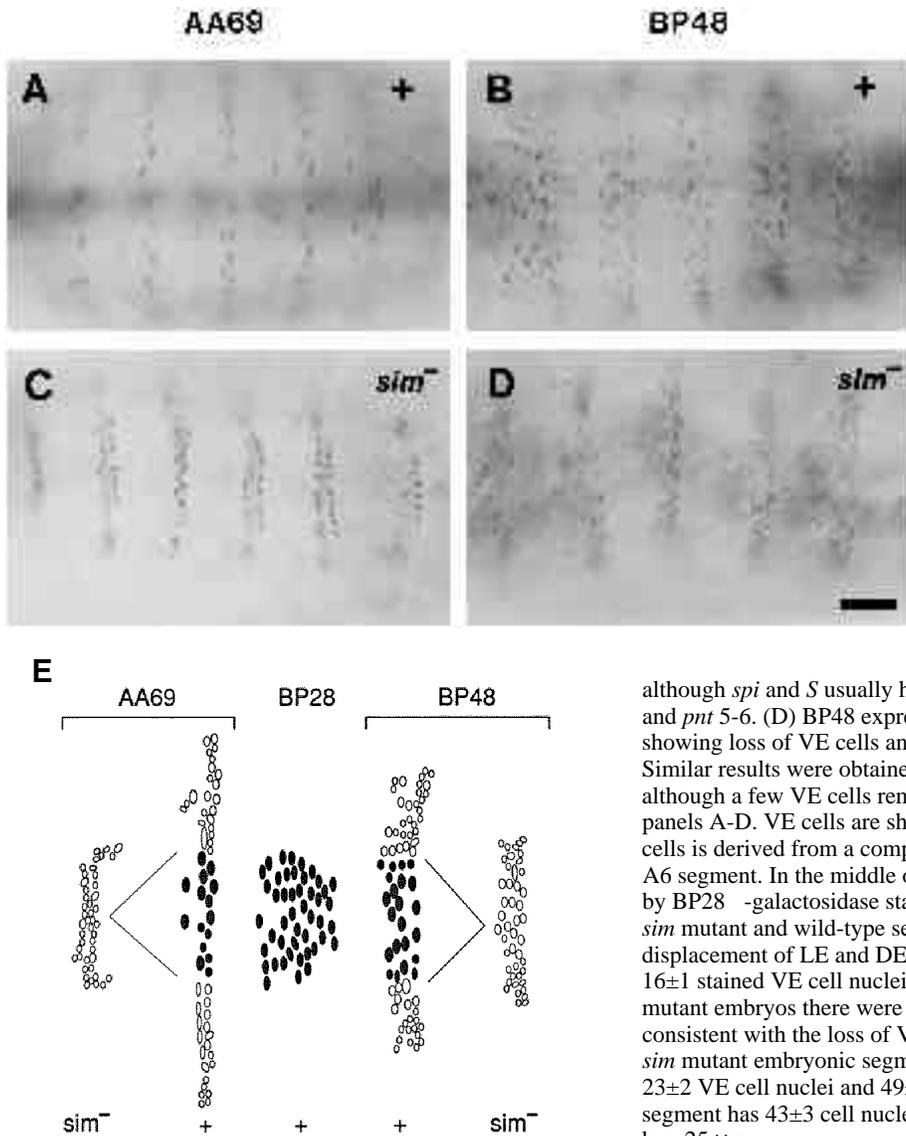


Fig. 5. VE gene expression and cell formation requires *spitz* class gene function. All panels show ventral views of stage 15-16 embryos. Schematic interpretation of A-D is illustrated in panel E. (A) Wild-type embryo (+) showing VE AA69 (*ve*) expression in one to two rows of epidermal cells. Five segments are shown. (B) Wild-type (+) embryo showing BP48 expression in rows of VE. Five segments are shown. (C) AA69 expression in a *sim* homozygous mutant embryo (*Df(3R)⁷⁶¹⁹*) showing absence of VE cells and joining together of LE and DE cells at the ventral midline. The *pnt*, *spi* and *S* mutant embryos also showed a reduction of VE-like cells

although *spi* and *S* usually had one to four VE-like cells still remaining and *pnt* 5-6. (D) BP48 expression in a *sim* homozygous mutant embryo showing loss of VE cells and joining together of LE at ventral midline. Similar results were obtained for *pnt*, *spi* and *S* mutant embryos although a few VE cells remained. (E) Schematic interpretation of panels A-D. VE cells are shaded; LE and DE are not. Each section of cells is derived from a computer-assisted representation of a stage 16 A6 segment. In the middle of the drawing are the VE cells represented by BP28-galactosidase staining. To the left is shown AA69-stained *sim* mutant and wild-type segments showing the loss of VE and ventral displacement of LE and DE. Overall, the wild-type (+) embryos had 16±1 stained VE cell nuclei and 43±2 LE and DE nuclei. In the *sim* mutant embryos there were a total of 44±3 total cell nuclei stained, consistent with the loss of VE. To the right, BP48 wild-type (+) and *sim* mutant embryonic segments are shown. The wild-type segment has 23±2 VE cell nuclei and 49±3 LE nuclei stained. The *sim* mutant segment has 43±3 cell nuclei stained consistent with loss of VE. Scale bar, 25 µm.

CNS midline precursor cells of these mutants appear normal early in the germband-extended embryo when MEC influence of VE formation is first required. This reinforces the idea that these genes play a direct role in VE formation and their VE defect is not an indirect consequence of their midline glial defects.

Sequence analysis of several *spitz* class genes suggest they are involved in cell signalling. The *Egfr* gene encodes the *Drosophila* homologue of the EGF receptor. During stages 9 and 10, when the earliest VE effects of the *spitz* class genes are observed, the *Egfr* gene is expressed in the ventral ectoderm, but not in the MEC (Zak and Shilo, 1990). Inclusion of *Egfr* in this developmental pathway is more tenuous than *spi*, *S* and *ve* because it has a widespread ventral-lateral ectodermal defect (Clifford and Schüpbach, 1992; Raz and Shilo, 1992). However, weak alleles do show a relatively specific ventral midline cuticular effect (Clifford and Schüpbach, 1992), suggesting that the wide-spread *Egfr* mutant defect, which results in loss of all denticles may result from more than one developmental event.

The *spi* gene encodes a protein related to transforming growth factor- (TGF-) (Rutledge et al., 1992), which itself is related to epidermal growth factor (EGF) (Marquardt et al., 1983; Massagué, 1983). It has been shown that vertebrate TGF- binds to and activates the EGF receptor (Todaro et al., 1980), suggesting that *spi* could encode a ligand for the *Egfr* gene product (Rutledge et al., 1992). The *spi* gene is widely expressed, although there is enhanced expression along the ventral midline (Rutledge et al., 1992). The *ve* gene encodes a transmembrane protein expressed in the blastoderm and germband-extended embryo in the LE and VE precursor cells, and also has strong expression in the MEC cells (Bier et al., 1990). The sequence and expression pattern of *S* has not been reported.

OC

The *oc* gene is also clearly required for proper VE formation. It is expressed in the VE precursor cells beginning at stage 9 and continues expression in these cells throughout embryogenesis (Wieschaus et al., 1992). Its function is required for

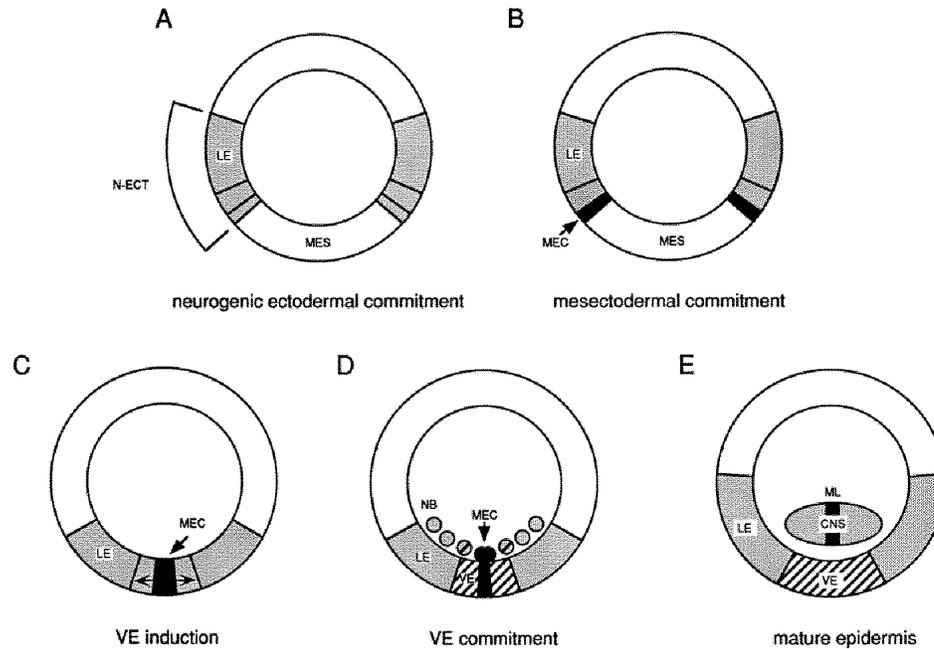


Fig. 6. Model of VE cell commitment. This series of schematic embryonic cross-sections illustrates the formation of the VE. (A) D/V patterning genes function to commit a group of cells to become lateral neurogenic ectoderm (N-ECT). This includes the precursors of the LE, VE and MEC (stippled). These cells give rise to both CNS and epidermis. (B) The MEC lineage (filled) becomes committed around the cellular blastoderm and gastrulation stages coincident with the expression of the *sim* gene. The VE and LE precursors (stippled) may be equivalent at this time. (C) After gastrulation, the MEC influences the formation of the adjacent VE precursors (arrow). The *spitz* class genes, including *sim*, influence VE cell fate during this time of development. (D) The VE precursors become committed to the VE lineage (dashed). At this time, neuroblasts (NB) delaminate from the neurogenic ectoderm and form a neuronal precursor cell layer (stippled and dashed to indicate common origin with LE and VE precursors). The MEC cells undergo nuclear migration inwards, but remain in close cytoplasmic proximity to the adjacent VE. (E) The VE cells spread out during dorsal closure and cover the ventral surface of the mature embryo. The NBs and MEC cells differentiate into the CNS with a distinctive set of midline cells (ML).

proper CNS midline development (Finkelstein et al., 1990; Klämbt et al., 1991) and is transcribed in the CNS midline cells beginning around stage 8 of development (Finkelstein et al., 1990). Thus, *oc* is expressed in both the VE and CNS midline precursor cells during the time of VE formation. Since *oc* is a homeobox-containing protein (Finkelstein et al., 1990), it is likely involved in controlling the transcription of genes involved in VE formation.

pnt

Mutations in *pnt* generally showed a less severe defect than the other *spitz* class genes and may indicate a more limited role in VE development since the allele used behaves like a null pointed allele with respect to other embryonic defects (Klämbt, 1993). The *pnt* gene encodes an *ets*-like transcription factor and is expressed early in the ventral ectoderm (Klämbt, 1993). It is expressed in a subset of CNS midline cells, but not until germband retraction.

Existing data do not allow placing the *spitz* group genes in a developmental sequence, although *sim* functions within the MEC and *Egfr* and *pnt* initially in the ventral ectoderm. The other genes could function in either or both types of precursor cells. Additional analysis of the expression patterns of the *spitz* class genes during VE formation, as well as mosaic studies or additional experiments that can direct expression of *spitz* class genes to either the MEC or

VE cells should confirm which of the *spitz* class genes are directly involved in epidermal cell development and indicate in which cell type they function.

Multiple roles of CNS midline cells in embryogenesis

The mature insect CNS midline cells consist of around 30 functional neurons and glia (Crews et al., 1988; Klämbt et al., 1991). Additional developmental roles for the CNS midline cells have been proposed including interactions with commissural axons (Klämbt et al., 1991; Bastiani et al., 1987), separation of the anterior and posterior commissures (Klämbt et al., 1991), and formation of VE (as shown in this paper). The insect CNS midline cells have a counterpart in the vertebrate embryo at the ventral base of the spinal cord (Jessell et al., 1989). These cells are important for attracting and influencing commissural growth cones (Tessier-Lavigne et al., 1988; Dodd et al., 1988). Additionally, it was shown that the ventral cells are required for proper formation of adjacent neurons (Yamada et al., 1990). This is conceptually similar to the role of the MEC on cell fate commitment of the adjacent ventral neuroectoderm, as described in this paper. It will be of interest to determine if the same genes that are involved in CNS midline formation and VE formation in *Drosophila* are utilized for neural formation in vertebrates.

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