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puckered, a gene involved in position-specific cell differentiation in the dorsal epidermis of the *Drosophila* larva

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SUMMARY

The final pattern of the cuticle of the *Drosophila* larva depends on the position-specific behaviour of the epidermal cells during their differentiation. This behaviour is dictated, in part, by the relative position of the cells during embryogenesis which allows them to receive and integrate signals from their neighbours. The translation of this 'positional information' into pattern might depend on the activity of genes that are able to integrate the outcome of cell interactions and tranfer it to the genes responsible for cell differentiation. Mutations in the gene *puckered* cause spatially restricted defects during the differentiation of the larval epidermal cells. We present data that suggests *puckered* may be involved in linking positional information to cell differentiation.

Key words: *Drosophila*, patterning, differentiation, epidermis

INTRODUCTION

The patterning of the larval epidermis during *Drosophila* embryogenesis can be viewed as a sequence of three well defined periods. The first one takes place during the cleavage of the zygote and results in its regionalization from centers of activity located at each end of the egg (St. Johnston and Nüsslein Volhard, 1992). This phase culminates in the cellular blastoderm with the activation of some segment polarity genes in stripes which outline the metameric units of the larva (Ingham, 1988). A second phase covers the period of postblastoderm divisions and occupies most of what is known as the extended germ band stage. During this period the information laid down in the blastoderm is used to generate region-specific patterns of gene expression within each metameric unit (Ingham, 1991; Ingham and Martinez Arias, 1992; Martinez Arias, 1993). Finally, during the third phase cell division ceases and cells differentiate in a segment and position-specific manner (Martinez Arias, 1993). This view of epidermal patterning during embryogenesis has developed, in part, through the identification and functional characterization of genes involved in the first two phases (Ingham and Martinez Arias, 1992). These two phases are characterized by a progressive increase in the number of cell identities which are organised in distinctive patterns. The progress of these processes is indicated by the patterns of expression of, and requirements for, the segment polarity genes (reviewed by Ingham and Martinez Arias, 1992; Peifer and Bejsovec, 1992; Hooper and Scott, 1992).

The third phase is characterized by two processes: the cessation of cell proliferation and the emergence of position-specific cell shapes as a prelude to their overt differentiation. For example, ventrally in the anterior region of each segment the cells that will secrete denticles become elongated along the dorsoventral axis and acquire a shape that contrasts with that of cells in the posterior region of the segment which remain more isodiametric and will differentiate smooth cuticle (Martinez Arias, 1993). This final phase in the patterning of the epidermal sheet reveals differences between cells that have been generated during the first two phases. The cellular and molecular mechanisms that underlie these late developmental events remain to be investigated.

In the larval epidermis, patterns of cellular differentiation cannot be directly correlated with known patterns of gene expression in cells prior to overt differentiation. Thus, although it is clear that segment polarity genes determine the pattern of the larval cuticle, it is not clear whether they do it directly or indirectly. Specifically, there is no known segment polarity gene whose expression is associated with the prospective denticle belt region or with regions of the cuticle with patterns of hairs. Instead, cells in these regions express overlapping patterns of segment polarity genes (see e.g. Peifer and Bejsovec, 1992) suggesting that the information generated by these genes is integrated by the cells and somehow passed on to the 'differentiation genes'.

Transformation of 'positional' to 'differentiation' information is exemplified by the evolution of Fasciclin III expression during germ band shortening. Fasciclin III is a cell adhesion molecule and although it is eventually found in all epidermal cells, this global distribution is achieved through onset of expression in a sequence of position-

specific patterns initiated after proliferation (Patel et al., 1987; Martinez Arias, 1993). Thus the patterning information present in the form of overlapping patterns of, amongst others, segment polarity gene products results in position-specific downstream gene expression. This transfer of information could occur in one of two ways. Either the information, which undoubtedly exists in the spatial and temporal overlaps of the expression of segment polarity genes, is read directly by the differentiation genes or there are intermediaries that integrate the activity of the former and act as links to the latter. In the first case, the activity of segment polarity genes creates a code that would be interpreted by the promoters of the differentiation genes directly while in the latter, the integration would be performed by a class of intermediate genes. In either case, we believe the key moment for this transfer of information must be the end of proliferation, when cells stop dividing and begin to differentiate according to their position in the embryo.

In order to initiate the study of this process of 'information transfer' we describe here the pattern of expression of a β-galactosidase enhancer trap line in a small and conspicuous set of epidermal cells as they stop proliferating. The P element insertion responsible for this expression causes a mutation in a gene that appears to be required for the normal differentiation of the cells in which β-galactosidase is expressed. We describe the phenotype of mutations in this gene and discuss the implications of the expression pattern of the β-galactosidase insertion, and of the mutation it produces, for the onset of position-specific cell differentiation.

MATERIALS AND METHODS

Fly strains

P[ry^+*lacZ*]E69 was generated in an enhancer trap screen (M. Bate, E. Rushton and A. Martinez Arias) using the starter strain carrying the ry^{506}P[ry^+*lacZ*]C49 chromosome provided by Cahir O'Kane. $l(3)84Eh^{K19}$ was supplied by B. Baker. All other stocks were obtained from the *Drosophila* stock centre at the Department of Biology, Bloomington, Indiana. The following chromosomes were used in this study and are referred to by shortened names in this manuscript (in parentheses); $Df(3R)dsx^{M+R3}$ (*Df(3R)dsx3*), $Df(3R)dsx^{M+R10}$ (*Df(3R)dsx10*), $Df(3R)dsx^{M+R15}$ (*Df(3R)dsx15*) and $Df(3R)dsx^{M+R21}$ (*Df(3R)dsx21*). Balancer chromosomes are described in Lindsley and Zimm (1992).

Cuticle preparations

Cuticle phenotypes examined were of embryos generated by adults heterozygous for a particular mutation and an Oregon R wild-type chromosome. These outcrossed flies were set to lay eggs overnight at 25°C on standard apple juice-agar plates supplemented with live yeast. For examination of unhatched embryos, development was allowed to proceed for a further 24 hours and unhatched embryos were collected. Cuticle preparations were done according to Wieschaus and Nüsslein-Volhard (1986; protocol based on the original technique described by van der Meer, 1977) except that embryos were not fixed before mounting.

Immumocytochemistry

Antibody stains were performed following previously described protocols (Ashburner, 1989) and after dehydration embryos were mounted in DPX. The simultaneous detection of β-galactosidase activity and the expression of spectrin, as revealed by antibodies, also followed published protocols (Couso et al., 1993). The expression of β-galactosidase was detected either as an enzymatic activity or with a monoclonal antibody that was a gift from Dr C. Doe. Antibodies against spectrin (Pesacreta et al., 1989) were kindly provided by D. Branton and antibodies against Fasciclin III (Patel et al., 1987) were a gift from M. Wilcox.

RESULTS AND DISCUSSION

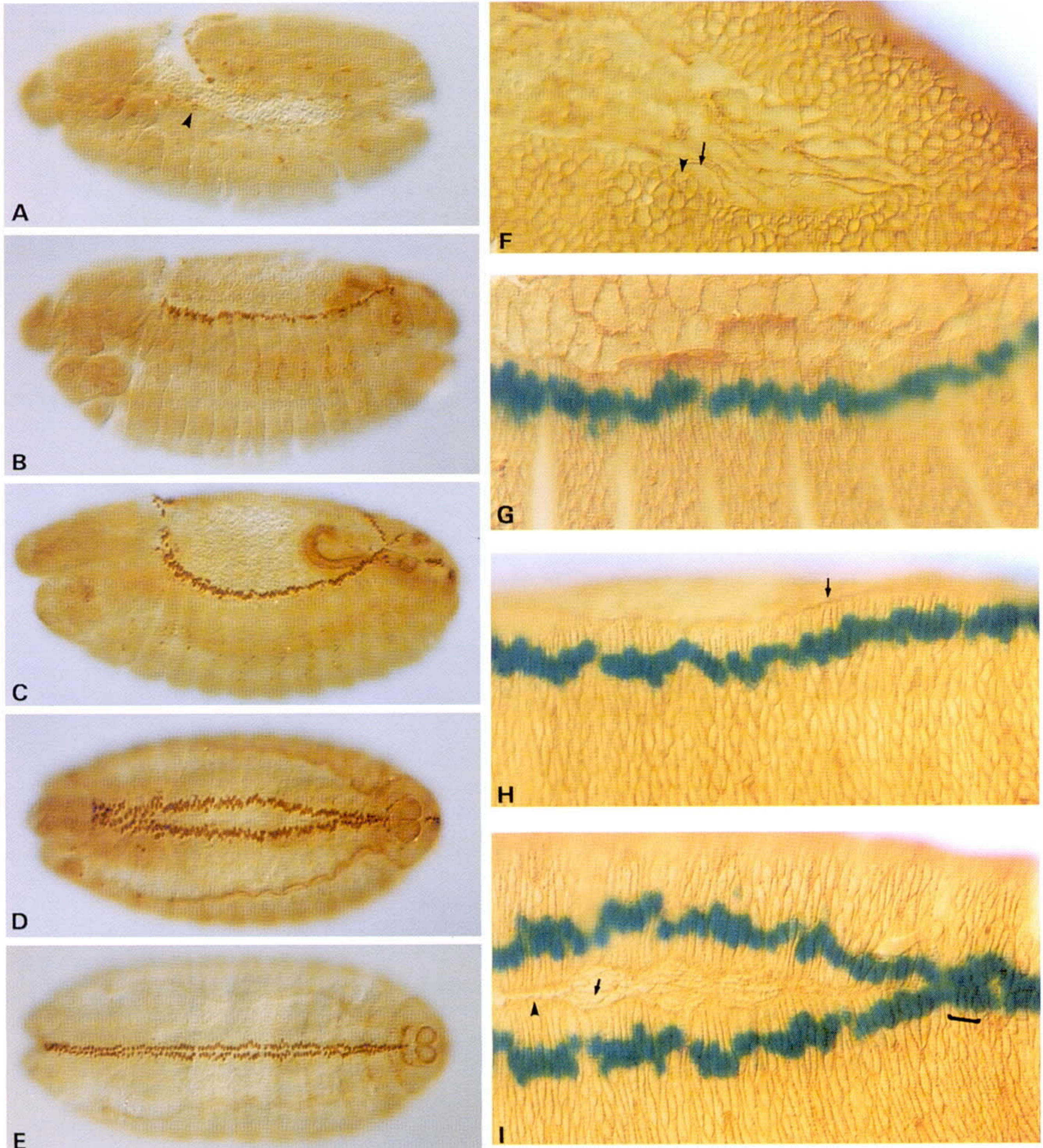
The dorsal midline and dorsal closure

After germ band shortening (end of stage 12) a sheet of epidermal cells covers the ventral and ventrolateral sides of the embryo while its dorsal surface is covered by the amnioserosa, a layer of large cells that do not divide after the blastoderm stage. Amnioserosa cells do not contribute to the larval cuticle since they become internalised and are eventually lysed in the final stages of embryogenesis (Hartenstein and Jan, 1992). We have identified a *lacZ* enhancer trap line P[ry^+*lacZ*]E69 which marks the dorsalmost cells of the epidermis from just before germ band retraction to hatching (Fig. 1A-E). During stages 13-15, the epidermis from the two lateral sides of the embryo is joined into a single sheet of cells when the cells expressing *lacZ* in

Fig. 1. *lacZ* expression and shape changes in the dorsalmost cells of the epidermis during dorsal closure. (A-E) Heterozygous P[ry^+*lacZ*]E69 embryos stained with anti-β-galactosidase. (A) Late stage 11 embryo; initiation of *lacZ* expression in small clusters of cells at the epidermis/amnioserosa junction (arrowhead). (B) Nuclei staining for *lacZ* line the dorsal edge of the epidermis in the retracted germ band embryo (stage 13). (C) Stage 14 and (D) stage 15; *lacZ*-expressing cells continue to line the dorsal epidermal edge throughout dorsal closure, and abut at the dorsal midline when the two sides of the epidermis meet. (E) Stage 16 embryo; dorsal closure is complete and the *lacZ*-expressing nuclei form two parallel rows on either side of the dorsal midline. (F) Wild-type embryo and (G-I) heterozygous P[ry^+*lacZ*]E69 embryos stained with anti-spectrin and for β-galactosidase activity. (F) Late stage 11 embryo; at this stage *lacZ* expression in P[ry^+*lacZ*]E69 embryos is initiated in the dorsalmost epidermal cells which, like neighbouring epidermal cells, are cuboidal (arrowhead). At this stage the amnioserosa cells are anteroposteriorly elongated (arrow). (G) Late stage 13 embryo; cells expressing *lacZ* have elongated dorsoventrally, in the plane of the epidermis. The nuclei in these cells (blue) are restricted to ventral domains of these cells. (H) Late stage 14 embryo; the dorsal extreme of the *lacZ*-expressing cells forms a straight edge to the leading front of the epidermis as it moves dorsally (arrow). The nuclei remain in ventral regions of the cells. (I) Late stage 15 embryo; the dorsal extremes of the highly elongated *lacZ*-expressing cells (arrowhead) extend over the amnioserosa (arrow) towards the dorsal midline where they abut cells from the other side to form a single sheet of epidermal cells. The nuclei relocate to a central position after the two sides meet (bracket) and these cells become more like other cells of the epidermis in appearance and arrangement. Anterior is to the left, except in F where anterior is upper left. (A,B) Lateral view; (C) dorsolateral view; (D-E) dorsal view; (F-H) lateral view; (I) dorsal view.

P[*ry*$^+$*lacZ*]E69 meet at the dorsal midline. This event is known as dorsal closure (Campos-Ortega and Hartenstein, 1985) and can be divided into three processes; (a) the dorsal expansion of the epidermal sheet, (b) the concomitant expansion of the dorsal epidermis anteroposteriorly, and (c) the joining of the two sides of the epidermis at the dorsal midline.

A most conspicuous feature of the dorsal epidermis during dorsal closure is a row of elongated cells that define the dorsal limit of the epidermal sheet (Young et al., 1993; Fig. 1). These cell correspond to the *lacZ*-expressing cells in P[*ry*$^+$*lacZ*]E69 and lead the dorsalward movement of the epidermal sheet during dorsal closure (Fig. 1F-I). *lacZ* expression at the dorsal epidermal edge begins at stage 11 in clusters of cells (Fig. 1A) at about the time they stop proliferating (unpublished observations). They are initially



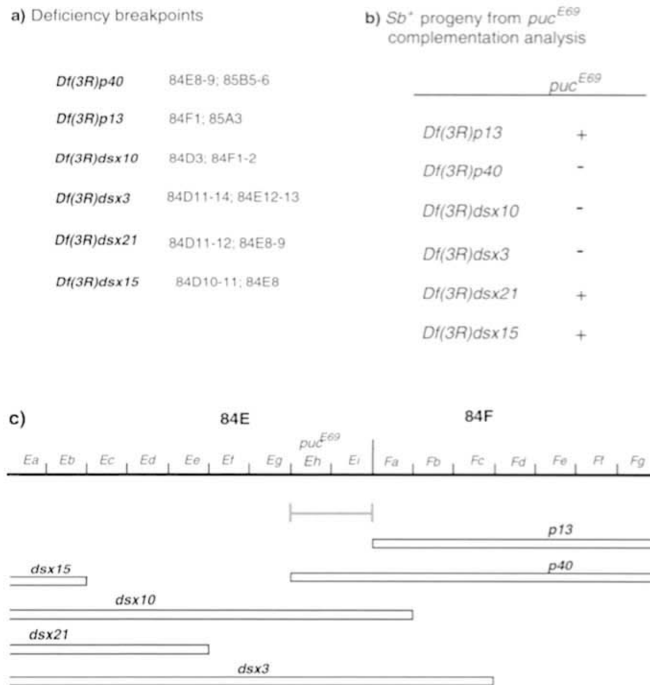


Fig. 2. (A) Deficiencies used in complementation analysis with puc^{E69} showing published breakpoints (Baker et al., 1991). (B) Complementation analysis of puc^{E69}: all stocks were balanced over TM3*Sb*, and complementation was assayed by the presence of Sb^+ progeny. (C) Top line: DNA at distal 84E and proximal 84F showing complementation groups identified by Baker et al. (1991). Open boxes underneath represent the DNA deleted in deficiencies used in the complementation analysis. *puckered* is delimited by breakpoints in $Df(3R)p^{13}$ and $Df(3R)p^{40}$. *l(3)84Eh* (*Eh*) and *l(3)84Ei* (*Ei*) are the two complementation groups placed within this interval by Baker et al. (1991). The order of *Eh* and *Ei* within this interval is not known. The following chromosomes are referred to by shortened names (in parentheses); $Df(3R)dsx^{M+R3}$ (*Df(3R)dsx3*), $Df(3R)dsx^{M+R10}$ (*Df(3R)dsx10*), $Df(3R)dsx^{M+R15}$ (*Df(3R)dsx15*) and $Df(3R)dsx^{M+R21}$ (*Df(3R)dsx21*).

polygonal, as is typical of epidermal cells at this stage (Fig. 1F), but by the time the germ band retracts they are seen to undergo changes specific to these cells: they align in a single, or sometimes double, row (Fig. 1B) and elongate along the dorsoventral axis (Fig. 1G). The dorsal side of these cells, adjacent to the amnioserosa, forms a straight edge to the moving front of the epidermal sheet (see Fig. 1H). All *lacZ*-expressing cells contribute to this leading edge as it moves dorsally. Nuclei are restricted to the ventral side of these cells from stage 12 (Fig. 1G-I) highlighting their planar polarity. When the two sides of the epidermis meet at the dorsal midline the leading edges formed by these cells abut (Fig. 1I), and shortly afterwards these cells change shape again to become more like lateral epidermal cells. At this time the *lacZ*-expressing cells intercalate with each other over the dorsal midline (bracket in Fig. 1I). Nuclei relocate to the centre of the cells after joining of the two sides takes place (bracket in Fig. 1I). At the end of dorsal closure the *lacZ*-stained nuclei form two parallel rows along the length of the dorsal midline.

Another example of the planar polarity of the dorsal-most cells is the accumulation of nonmuscle myosin at their leading edge (Young et al., 1993). Furthermore, Fasciclin III is found on all surfaces of epidermal cells, but in the dorsal-most cells it is excluded from the side that is adjacent to the amnioserosa, during dorsal closure (Fig. 5A). Once the two sides of the epidermis have met at the dorsal midline Fasciclin III is visible on all lateral surfaces of these cells (Fig. 5B). This distribution of Fasciclin III in the dorsal-most cells during dorsal closure represents another aspect of their polarity in the plane of the epidermis.

Genetic characterisation of the *puckered* gene

The P[ry^+*lacZ*]E69 enhancer trap chromosome is homozygous embryonic lethal and produces a mutant cuticle phenotype in the dorsal epidermis (Fig. 3). We have named the mutated gene in this chromosome *puckered* and the *puckered* mutation in the P[ry^+*lacZ*]E69 enhancer trap chromosome is referred to as puc^{E69}. In situ hybridisation of P element DNA to polytene chromosomes from the P[ry^+*lacZ*]E69 line (not shown) indicates that it contains a unique P element at distal 84E(3R), a region that has been extensively characterised genetically (Baker et al., 1991; see Fig. 2). When in trans to deficiencies for this region puc^{E69} is also lethal and the resulting phenotype is similar to, but more severe than, that of homozygous puc^{E69} embryos (not shown). Thus puc^{E69} is not a null mutation. Furthermore, loss of the P element by dysgenesis results in reversion of the *puckered* mutant phenotype to wild type (unpublished observations) indicating that the P-*lacZ* element is the cause of the *puckered* mutation. Deficiency mapping indicated that *puckered* is located between the proximal breakpoints of $Df(3R)p^{13}$ and $Df(3R)p^{40}$ (Fig. 2). Two lethal complementation groups have been identified in this region, one of which, *l(3)84E*, is allelic to *puckered* (Fig. 2). The single existing EMS allele of *l(3)84E*, Eh^{K19} (Baker et al., 1991), is embryonic lethal and displays a mild *puckered* phenotype (Fig. 3). We have renamed the Eh^{K19} allele of *puckered*, puc^{K19}.

Mutations in the *puckered* gene impair the final pattern of the dorsal epidermis

Analysis of the *puckered* mutant phenotype in embryos homozygous for puc^{E69} reveals that the development of the puckered cells is aberrant (Fig. 4). In mutant embryos after germ band shortening the *lacZ*-expressing cells are not restricted to a straight line at the edge of the epidermis, but are seen to extend several cell diameters away from the edge

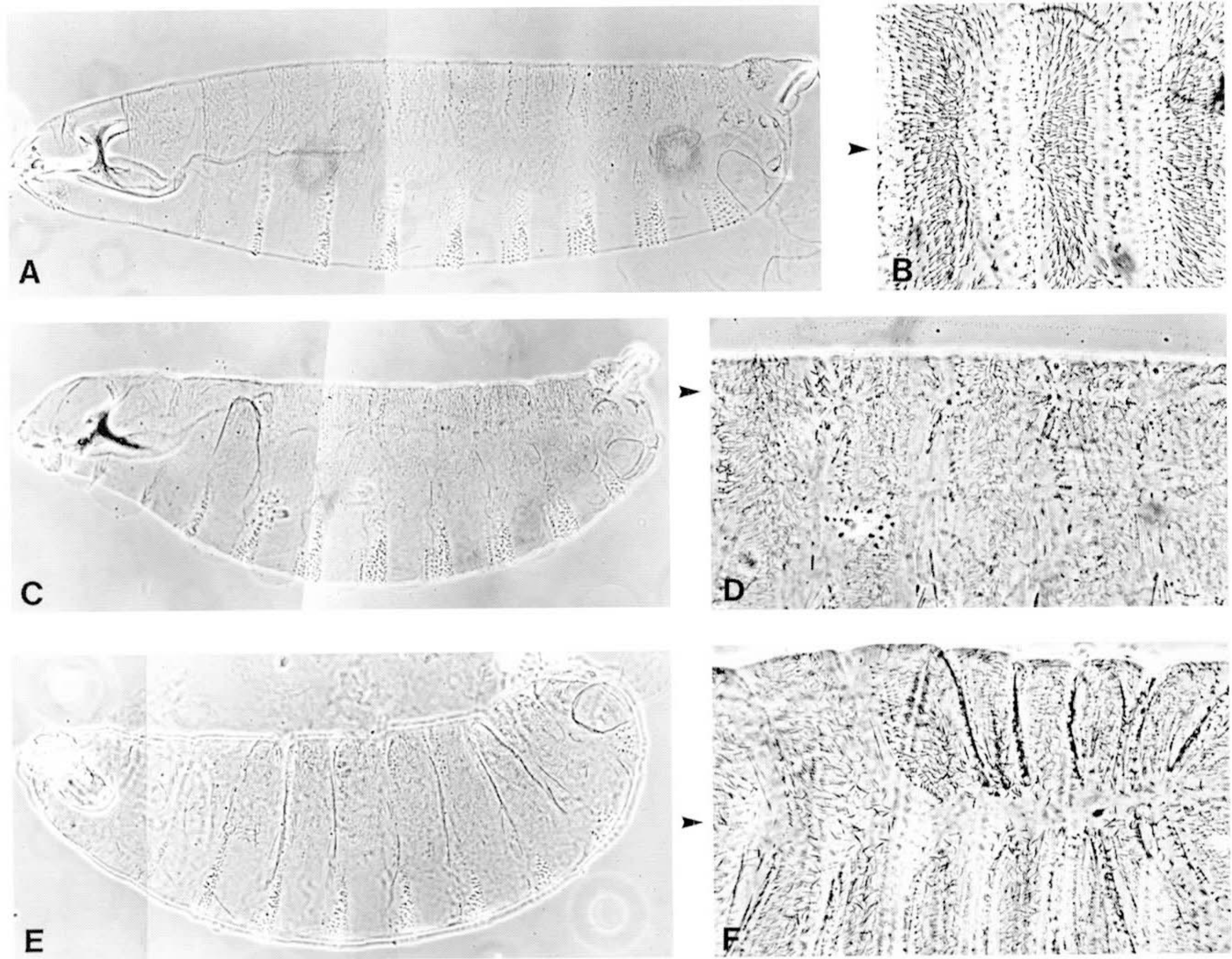


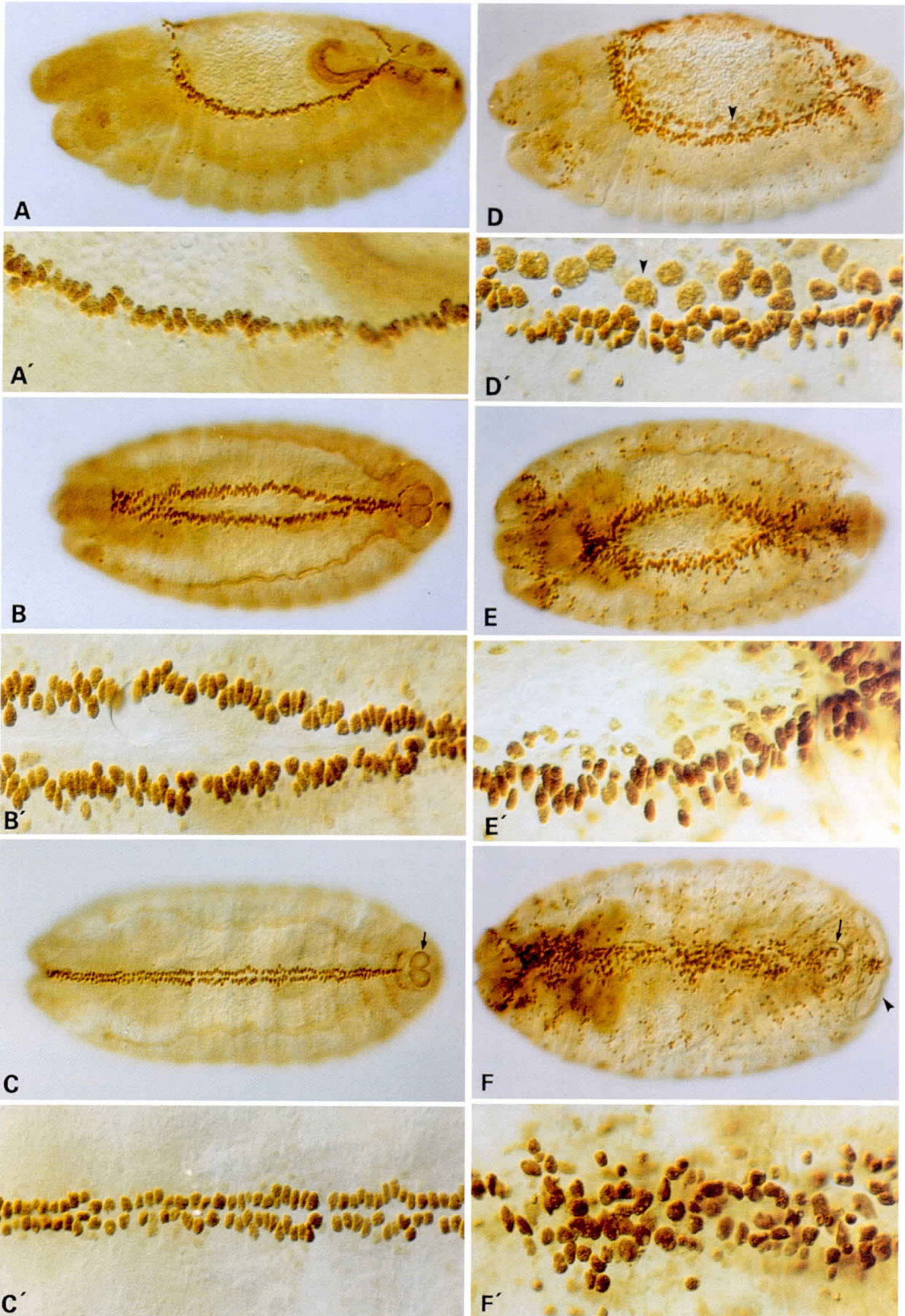
Fig. 3. Cuticle phenotype of wild-type and *puckered* mutant embryos. Anterior is to the left. (A,C,E) Lateral views; (B,D,F) dorsal views. Arrowheads mark the dorsal midline. A and B show the cuticle of mature wild-type embryos; (A) the mature embryo, as viewed laterally, is straight from anterior to posterior, and (B) the dorsal hairs are oriented in an ordered array, either anteriorly or posteriorly depending on the segment and on the level within the segment (see Campos-Ortega and Hartenstein, 1985 for a more detailed description). C and D show the cuticle of homozygous puc^{K19} *(l(3)84Eh)* embryos; (C) the ventral side of the *puckered* mutant embryo is curved, as a consequence of the dorsal surface being shortened in the anterior-posterior axis, (D) the pattern of dorsal hairs is mildly disrupted along the dorsal midline (arrowhead) in these embryos. E and F show the cuticle of homozygous puc^{E69} embryos; (E) the curvature is more exaggerated in puc^{E69} mutant embryos, and the posterior end is clearly kinked dorsalwards. (F) The pattern of dorsal hairs is interrupted at the dorsal midline where hairs are absent or randomly oriented. The dorsal cuticle is gathered at the dorsal midline, buckling the dorsolateral cuticle.

(compare Fig. 4A and 4D). As dorsal closure proceeds these cells become increasingly disorganised (Fig. 4E) so that by completion of dorsal closure they are haphazardly arranged in clusters at the dorsal midline (Fig. 4F) rather than forming a single row on either side of the midline as in wild type (Fig. 4C).

The shape of the dorsal-most cells is also abnormal in *puckered* mutants. During germ band shortening these cells do not elongate as in wild type, but retain the polygonal shape observed in the extended germ band phase (Fig. 5C). Consequently, they do not generate a straight edge to the moving front of the epidermis during dorsal closure. When the two sides of the epidermis meet at the dorsal midline they do not form two parallel rows (Figs 5D, 4F). The pattern of the cuticle secreted by these cells is also abnormal (Fig. 3D,F).

The subcellular distribution of Fasciclin III shows that in mutant embryos the dorsal-most cells do not show the planar polarity normally seen in these cells (Fig. 5). In the majority of the dorsal-most cells. Fasciclin III protein is not excluded from the dorsal side, adjacent to the amnioserosa (Fig. 5C). Moreover, nuclei are not restricted to ventral areas of the cell (Fig. 5C,D). Indeed, none of the specialisations that we have described as characteristic of the dorsal-most cells is adopted in embryos mutant for *puckered*.

Despite the abnormalities of the dorsalmost epidermal cells in *puckered* mutant embryos, dorsal closure does occur to completion. This demonstrates that the shape and


A
A´
B
B´
C
C´
D
D´
E
E´
F
F´

arrangement of the dorsal-most cells is not required for dorsal closure to occur. It is possible that rather than depending on forces generated by shape changes in these cells (Young et al., 1993), dorsal closure draws its mechanical force from a coordinated change in cell shape of all epidermal cells, especially in the lateral regions of the epidermis.

Pattern formation and cell differentiation

The final step during pattern formation within cell assemblies is the activation of the process of cell differentiation which leads to the final arrangement and appearance of the different cell types specified during embryogenesis and, through their specializations, to the shaping and final appearance of organs and tissues and hence the organism (see e.g. Edelman, 1988). For the larval epidermis of *Drosophila* this phase is initiated between the cesation of cell division in stage 11 and the onset of cuticle secretion in stage 15, a period during which cells neither cycle nor divide and undergo position-specific changes in shape which prelude their specializations during cuticle secretion (Martinez Arias, 1993).

By the end of stage 11, epidermal cells have acquired position- and segment-specific identities through the concerted activities of the segment polarity and the homeotic genes. The segment polarity genes encode a class of molecules that are involved in the generation of positional information within every segment (Ingham, 1991; Hooper and Scott, 1992; Martinez Arias, 1989; Peifer and Bejsovec, 1992) whereas the homeotic genes encode a family of trancriptional regulators which allow the interpretation of this information in a segment-specific manner (Akam et al., 1988). While mutations in either homeotic or segment polarity genes result in embryos with dramatic changes of pattern, in each case all cells undergo normal differentiation but differentiate in a manner appropriate to positions elsewhere in the embryo. This means that while the activities of these genes are involved in the assignation of the type of differentiation a cell will ultimately undergo, they do not directly bring about the differentiation of cells. It has been accepted that the homeotic genes act through

Fig. 4. P[ry^+*lacZ*]E69 embryos labelled with anti-β-galactosidase, (A-C) heterozygous (as in Fig.1) and (D-F) homozygous for the puc^{E69} chromosome. Higher magnification of the embryos in A-F are shown in A′-F′. In heterozygous embryos the *lacZ*-expressing nuclei are tightly compacted into a single or double row at the dorsal edge of the epidermis after germ band retraction (A and A′) whereas in homozygous *puckered* mutant embryos these *lacZ*-expressing cells fail to form such an ordered structure and are found roughly grouped at the dorsal epidermal edge (D and D′). Amnioserosa cells adjacent to the epidermis also express *lacZ* in mutant embryos of this line (arrowheads). As dorsal closure proceeds the *lacZ*-expressing nuclei in heterozygous embryos continue to align at the dorsal epidermal edge (B and B′) while in *puckered* mutants these nuclei can be found over a much wider distance, and they do not form a line at the dorsal epidermal edge (E and E′). Upon completion of dorsal closure in heterozygous embryos the *lacZ*-expressing nuclei form two parallel rows on either side of the dorsal midline (C and C′) while in *puckered* mutant embryos the *lacZ*-expressing nuclei are haphazardly arranged at the dorsal midline, and tend to form clusters (F and F′). The dorsal epidermis normally expands in the anterior-posterior axis during dorsal closure. However, in *puckered* mutant embryos this clustering of dorsal epidermal cells appears to contract the dorsal epidermis, thus pulling the posterior epidermis further anterior; the posterior spiracles come to rest at a more anterior position than normal (arrows in C and F) and the anal pads are visible from a dorsal aspect (arrowhead in F). This leads to the curvature of *puckered* mutant embryos as viewed laterally (see Fig. 3).

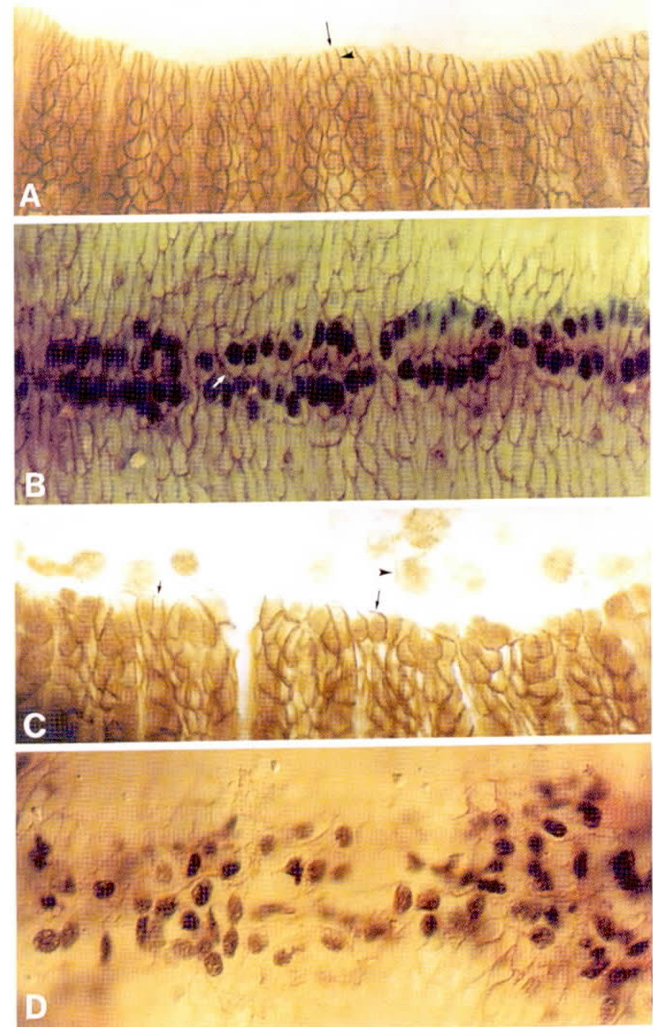


Fig. 5. Subcellular localisation of Fasciclin III in puc^{E69} heterozygotes (A,B) and homozygotes (C,D). Embryos in B-D are also stained with anti-β-galactosidase. (A) Fasciclin III is normally expressed in all epidermal cells but not in the amnioserosa. Fasciclin III is present on the ventral and lateral sides of the dorsalmost epidermal cells (arrowhead) but not on the dorsal side (arrow) before dorsal closure. (B) After dorsal closure Fasciclin III is not excluded from the dorsal side of these cells (arrow). (C) puc^{E69} homozygous embryo; before dorsal closure in these mutant embryos Fasciclin III is not completely excluded from the dorsal sides of dorsal most cells (arrows). Fasciclin III is also found on some amnioserosa cells (arrowhead), which sometimes are seen to express *lacZ* in these mutant embryos. (D) Upon dorsal closure, *lacZ*-expressing cells arrive at the dorsal midline with Fasciclin III distributed over all lateral surfaces.

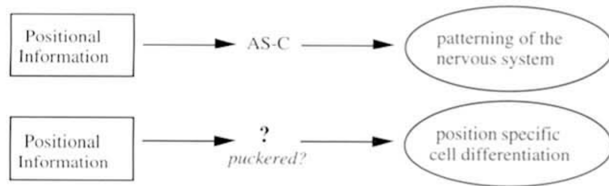


Fig. 6. Positional information as generated during development is transferred to specific processes, for example the patterning of the nervous system or patterns of cell differentiation, through the activity of intermediary genes. In the case of the nervous system, genes of the AS-C play the role of intermediary or downstream genes. The gene *puckered* might perform a similar role in the linking of positional information to cell differentiation.

an intermediary set of genes that in turn control the expression of genes involved directly in differentiation. Although this has always been accepted for the homeotic genes (reviewed by Andrew and Scott, 1992), little is known about the genes that are downstream of the segment polarity genes and which are related to the process of cell differentiation.

One way of thinking about the control of epidermal differentiation is to imagine that information encoded by the combined activities of segment polarity and homeotic genes is read by intermediate genes whose products would thus integrate and transduce positional information into position-specific differentiation. By way of example, genes of the *achaete-scute* complex (AS-C) can be regarded as performing an analogous role to the genes we propose link patterning information to differentiation, but in the linking of patterning information to subsequent patterning processes in the nervous system (Fig. 6). The emergence of neuronal precursors in the central and peripheral nervous systems is closely associated with the patterning of the ectoderm since neuroblasts and sensory mother cells arise at specific places in the ectoderm with identities that reflect the molecular information that exists at that position. The expression of transcripts from the AS-C is linked to the segregation of these neuronal precursors (Cabrera et al., 1987; Romani et al., 1987), closely follows the generation of cell diversity in the ectoderm, and is under the control of genes that establish positional information (Martin Bermudo et al., 1991; Skeath et al., 1992). In addition, this expression is required for the correct pattern of the nervous system (reviewed by Cabrera, 1992). In this sense the AS-C performs a role similar to that of the genes that we are proposing exist between the segment polarity genes and the process of cell differentiation. Such genes would perform a role of integration of information and transfer to a particular process: the patterning of the nervous system in the case of the AS-C, and cell differentiation in the case we have discussed here (Fig. 6).

Here we have described a P-*lacZ* enhancer trap line in which the *puckered* gene is mutated. In this line *lacZ* expression is initiated in the most dorsal epidermal cells as they finish proliferating, a pattern that reflects the expression of the *puckered* transcript (unpublished observations). In embryos mutant for *puckered*, the cells that express *lacZ* fail to differentiate properly: they do not adopt shape changes normally seen in these cells during dorsal closure and secrete cuticle with an abnormal pattern. These observations suggest that *puckered* might be involved in integrating positional information prior to cell differentiation. However, it is also possible that *puckered* acts at a later point to implement the differentiation of these cells. Determination of the precise role of *puckered* in the differentiation of the dorsalmost epidermal cells will require further genetic and molecular studies which are currently in progress.

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REFERENCES

Akam, M. E., Dawson, I. and Tear, G. (1988). Homeotic genes and the control of segment diversity. *Development* **Supplement 104**, 123-134.

Andrew, D. and Scott, M. P. (1992). Downstream of the homeotic genes. *New Biol.* **4**, 5-15.

Ashburner, M. (1989). *Drosophila. A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Baker, S. B., Hoff, G., Kaufman, T. C., Wolfner, M. F. and Hazelrigg, T. (1991). The *doublesex* locus of *Drosophila melanogaster* and its flanking regions: A cytogenetic analysis. *Genetics* **127**, 125-138.

Cabrera, C., Martinez Arias, A. and Bate, M. (1987). The expression of three members of the achaete scute complex correlates with neuroblast segregation in *Drosophila*. *Cell* **50**, 425-433.

Cabrera, C. (1992). The generation of cell diversity during early neurogenesis in *Drosophila*. *Development* **115**, 893-901.

Campos-Ortega, J. A. and Hartenstein, V. (1985). *The Embryonic Development of* Drosophila melanogaster. Berlin: Springer-Verlag.

Couso, J. P., Bate, M. and Martinez Arias, A. (1993). A *wingless* dependent polar coordinate system in *Drosophila* imaginal discs. *Science* **259**, 484-489.

Edelman, G. (1988). *Topobiology. An Introduction to Molecular Embryology*. New York: Basic Books Inc., Pubs.

Hooper, J. and Scott, M. P. (1992). The molecular genetic basis of positional information in insect segments. In *Early Embryonic Development of Animals* (ed. W. Hennig), pp. 1-48. Berlin: Springer Verlag.

Hartenstein, V. and Jan, Y. N. (1992). Studying *Drosophila* embryogenesis with P-*lacZ* enhancer trap lines. *Wilhelm Roux's Arch. Dev. Biol.* **201**, 194-220.

Ingham, P. W. (1988). The molecular genetics of embryonic pattern formation in *Drosophila*. *Nature* **355**, 25-34.

Ingham, P. W. (1991). Segment polarity genes and cell patterning within the *Drosophila* body segment. *Curr. Op. Genet. Dev.* **1**, 261-267.

Ingham, P. W. and Martinez Arias, A. (1992). Boundaries and fields in early embryos. *Cell* **68**, 221-235.

Lindsley, D. L. and Zimm, G. G. (1992). *The Genome of* Drosophila melanogaster. Academic Press Inc.

Martin Bermudo, D., Martinez, C., Rodriguez, A. and Jimenez, F. (1991). Distribution and function of the lethal of scute gene during early neurogenesis in *Drosophila*. *Development* **113**, 445-454.

Martinez Arias, A. (1989). A cellular basis for pattern formation in the insect epidermis. *Trends Genet.* **5**, 262-267.

Martinez Arias, A. (1993). Development and patterning of the larval epidermis of Drosophila. In *The Development of Drosophila* (ed. M. Bate and A. Martinez Arias), pp. 517-608. Cold Spring Harbor Press.

Patel, N. H., Snow, P. M. and Goodman, C. S. (1987). Characterisation and cloning of fasciclin III: a glycoprotein expressed on a subset of neurons and axon pathways in *Drosophila*. *Cell* **48**, 975-988.

Pesacreta, T., Byers, T., Dubreuil; Kiehart, D. and Branton, D. (1989). *Drosophila* spectrin: the membrane skeleton during embryogenesis. *J. Cell Biol.* **108**, 1697-1709.

Peifer, M. and Bejsovec, A. (1992). Knowing your neighbors: cell interactions determine intrasegmental patterning in *Drosophila*. *Trends Genet.* **8**, 243-249.

Romani, S., Campuzano, S. and Modolell, J. (1987). The *achaete scute* complex is expressed in neurogenic regions of *Drosophila* embryos. *EMBO J.* **6,** 2085-2092.

Skeath, J., Panganiban, G., Selegue, J. and Carroll, S. (1992). Gene regulation in two dimensions: the proneural *achaete* and *scute* genes are controlled by combination of axis patterning genes through common intergenic control region. *Genes Dev.* **6**, 2606-2619.

St. Johnston D. and Nüsslein Volhard, C. (1992). The origin of pattern and polarity in the *Drosophila* embryo. *Cell* **68**, 201-219.

van der Meer, J. (1977). Optical clean and permanent whole mount preparations for phase contrast microscopy of cuticular structures of insect larvae. *Drosophila Inf. Service* **52**, 160.

Wieschaus, E. and Nüsslein Volhard, C. (1986). Looking at embryos. In Drosophila, *A Practical Approach* (ed. D. Roberts), pp 199-228. Oxford: IRL Press.

Young, P. E., Richman, A. M., Ketchum, A. S. and Kiehart, D. P. (1993). Morphogenesis in *Drosophila* requires nonmuscle myosin heavy chain function. *Genes Dev* **7**, 29-41.