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Generation of medial and lateral dorsal body domains by the *pannier* gene of *Drosophila*

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SUMMARY

The *pannier (pnr)* gene encodes a GATA transcription factor and acts in several developmental processes in *Drosophila*, including embryonic dorsal closure, specification of cardiac cells and bristle determination. We show that *pnr* is expressed in the mediodorsal parts of thoracic and abdominal segments of embryos, larvae and adult flies. Its activity confers cells with specific adhesion properties that make them immiscible with non-expressing cells. Thus there are two genetic domains in the dorsal region of each segment: a medial (MED) region where *pnr* is expressed and a lateral (LAT) region where it is not. The homeobox gene *iroquois (iro)* is expressed in the LAT region. These regions are not formed by separate polyclones of cells, but are defined topographically. We show that ectopic *pnr* in the wing induces MED thoracic development, indicating that *pnr* specifies the identity of the MED regions. Correspondingly, when *pnr* is removed from clones of cells in the MED domain, they sort out and apparently adopt the LAT fate. We propose that (1) the subdivision into MED and LAT regions is a general feature of the *Drosophila* body plan and (2) *pnr* is the principal gene responsible for this subdivision. We argue that *pnr* acts like a classical selector gene but differs in that its expression is not propagated through cell divisions.

Key words: *pnr*, *iro*, Dorsal subdomain, Selector gene, Compartment, *Drosophila*

INTRODUCTION

A principal feature of the body of *Drosophila* is that it is subdivided into a chain of anterior (A) and posterior (P) compartments. This key subdivision is common to all segments and affects embryonic, larval and adult tissues. Compartments are units of cell lineage (García-Bellido et al., 1973; Morata and Lawrence, 1977) and are the realms of action of selector genes such as *engrailed (en)* and many homeotic genes such as *Ultrabithorax* (Lawrence and Morata, 1994).

The process of compartmentation has been studied in detail in the adult cuticle. The derivatives of the imaginal discs differentiate as cephalic, thoracic and genital structures, and the abdominal histoblasts differentiate as adult abdomen. In the abdominal histoblasts, only an anteroposterior (AP) subdivision has been found (Kornberg, 1981; Struhl et al., 1997) but, in imaginal wing or leg discs (García-Bellido et al., 1973, 1976; Steiner, 1976), compartmentation is reiterative and results in further subdivisions of pre-existing compartments. This results in morphological diversity, as the segregation of new polyclones (the groups of cells that form a compartment, Crick and Lawrence, 1975) during development is associated with the permanent activation or inactivation of selector genes (García-Bellido, 1975; Morata and Lawrence, 1977), specifying the developmental program of each polyclone.

Compartmentation of the wing imaginal disc is the best known; it is composed of eight distinct compartments (García-Bellido et al., 1976), four in the wing blade and four in the thoracic region. Morphogenetic processes in the wing compartments are well understood (see Lawrence and Struhl, 1996, for a review). The activity of the selector gene *engrailed (en)* in the P compartment defines the A and P compartments and also specifies posterior identity (Morata and Lawrence, 1975). The gene *apterous (ap)* plays a similar role for the D and V compartments (Blair et al., 1994; Díaz-Benjumea and Cohen, 1993). Both the AP and the DV compartment borders act as source of long-range morphogens that pattern the wing blade. A number of response genes encoding transcription factors, e.g. *optomotor-blind (omb), spalt (sal), vestigial (vg), Distal-less (Dll),* are activated at different morphogen concentrations (Díaz-Benjumea and Cohen, 1995; Lecuit et al., 1996; Nellen et al., 1996; Zecca et al., 1996) and pattern different subdomains within the wing blade.

In the mesothoracic compartments, patterning processes are less well understood, although the AP segregation is maintained. The anterodorsal mesothorax (notum) is the

largest; it is composed of about 15,000 cells and presents a complex and stereotyped bristle pattern, but no compartments have been detected within this region. The posterior-dorsal mesothorax (postnotum) is much smaller and consists of a band of featureless cuticle with no bristles. The deployment of the signalling molecules Hedgehog (Hh) and Decapentaplegic (Dpp) is similar to that in the wing blade but their roles have not been clarified. In addition, there is a longitudinal stripe of *wg* expression (Phillips and Whittle, 1993) that bears no resemblance to the expression of *wg* in the wing blade.

We have developed a screen that allows gene domains to be visualised directly on the adult patterns (Calleja et al., 1996). In the notum, some of these gene domains define common borders (Calleja et al., 1996, and unpublished observations), suggesting the existence of genetic subdivisions that are not based on lineage segregations. The border of expression of *pannier (pnr)*, is a straight line along the AP axis. *pnr* encodes a transcription factor of the GATA family known to be involved in dorsal closure during embryogenesis, the specification of cardiac cells of the embryo and bristle patterning on the thorax (Gajewski et al., 1999; Heitzler et al., 1996; Ramain et al., 1993; Winick et al., 1993). Its expression is not restricted to the notum, but runs from the head capsule throughout the thorax and the entire abdomen (Calleja et al., 1996), defining a general dorsal subdomain in the adult body.

In the notum, the lateral border of *pnr* expression is parallel to, and overlaps with, the stripe of *wg* expression, and there are at least two other genes whose expression defines the same border (Calleja et al., 1996, and unpublished). Furthermore, the medial limit of expression of *iroquois (iro)*, a homeobox-containing gene necessary for the development of the lateral region of the notum (Diez del Corral et al., 1999; Gómez-Skarmeta et al., 1996; Leyns et al., 1996), appears to be adjacent to the borders of expression of *pnr* and *wg*.

Here we study the expression and function of *pnr* during the development of the embryo, and the adult mesothorax and abdomen. We provide evidence that *pnr* has two functions: it is involved in the partitioning of each dorsal segmental domain into two subdomains, medial (MED) and lateral (LAT), and also specifies the identity of the MED subdomain. These results uncover the existence of a general subdivision of the *Drosophila* body: the entire dorsal region, from the thorax to the end of the abdomen, is composed of homologous segmental medial and lateral subdomains. We show that these regions are not generated by cell lineage, but possibly by antagonistic interactions between the two sets of cells.

MATERIALS AND METHODS

Drosophila stocks

The pnr^{VX6} allele has been described in Heitzler et al. (1996), and can be regarded as a null allele as most of the coding sequence is lacking. The $Df(3)iro^{DFM3}$ lacks all the genes of the iro-C and is described in Diez del Corral et al. (1999). The *wg-lacZ, hh-lacZ, dpp-lacZ, mrr-lacZ* and *iro-lacZ* stocks are all reporter lines of the corresponding genes and are described in FlyBase (http://gin.ebi.ac.ak:7081). *The DC-lacZ* line is described in García-García et al. (1999), and reports the expression of the cluster of cells that differentiate the dorsocentral bristles.

Cell-lineage experiments

To study the lineage of the adult thorax and abdomen, we induced clones marked with y^+ and $forked^{36a}$ during the third larval period (72-96 hours after egg laying). Larvae of the genotype *y hs-FLP122* f^{36a}*; abx>*f^+*-stop>Gal4-lacZ UAS-y/+* were heat shocked. Flipase activity produces recombination that eliminates the f^+ allele and allows the production of Gal4 that induces activity of the *UAS-y* construct. These clones of cells will therefore be mutant for f^{36a} and will contain y^+ activity (conferring dark colour) in the regions where the *abx* promoter is active. A large number of y^+ f^{36a} clones were found in the thorax and abdomen.

To examine whether marked clones can cross the *iro* expression domain in the wing disc, larvae of genotype *y w hsFLP122; FRT82B arm-lacZ/FRT82B Ki Sb* were heat shocked during the third larval period (72-96 hours of development). Clones are marked by the loss of β-gal activity. These clones can also be scored in the adult tissue as they are marked with the loss of the dominant markers *Ki* and *Sb*. The *iro* domain was revealed with the anti-Iro antibody. To score marked clones on a background of *pnr* expression, larvae of genotype *y w hsFLP122; act<stop<lacZ/UAS-GFP; pnr-Gal4/+* were also heat shocked at 72-96 hours of development. The *pnr* domain was visualised with GFP and the induced clones by the gain of *lacZ* activity.

Induction of clones of marked mutant cells

Clones of cells homozygous for pnr^{VX6} were heat induced (1 hour at 37°C) on flies of larvae of genotype *y w hsFLP122; FRT82B* pnr^{VX6}*/FRT82B arm-lacZ Sb Dp* y^+. These clones are marked with y^- Sb^+ in the adult cuticle and for the loss of *arm-lacZ* activity in imaginal discs. For *iro* mutant clones, *y w hsFLP122; mwh* $Df(3L)iro^{DFM3}$ *FRT80B/hsCD2 ri FRT80B* larvae were heat shocked (1 hour at 37°C). These clones were marked with *mwh* in the adult cuticle and with loss of CD2 in discs.

Clones of *pnr*- and *mrr*-expressing cells

The method used to induce these clones is similar to that described for the cell lineage of the adult, but either the *UAS-pnr* or the *UAS-mrr* construct were used. The heat shocked (20 minutes at 34°C) larvae were of genotype *y w hsFLP122*; *abx>*f^+*-stop>Gal4-lacZ UAS-y/+;UAS-pnr/+* for *pnr*-expressing clones and *yw hsFLP*; *abx>*f^+*-stop>Gal4-lacZ /+;UAS-mrr/+* for *mrr*-expressing clones. In the first genotype, *pnr*-expressing clones were marked with f^{36} and y^+ whereas, in the second genotype, *mrr*-expressing clones were marked with f^{36} only.

GAL4/UAS experiments

The *UAS-pnr* chromosome is a gift of Mariann Bienz and is described in Haenlin et al. (1997). The *UAS-y* is described in Calleja et al. (1996) and the *UAS-mrr* in McNeil et al. (1997).

The Gal4 lines, *ap-Gal4, C765, MS1096, omb-Gal4*, have already been reported (Brand and Perrimon, 1993; Calleja et al., 1996; Gorfinkiel et al., 1997). The MD743 line, used to study the abdominal transformations, drives expression in most of the sternite and the lateral part of the tergite. The *vg (quadrant)-Gal4* and *nub-Gal4* are insertions in vestigial and nubbin that induce expression patterns like those of the *vg*-quadrant enhancer (Kim et al., 1996) and the *nub* gene (Ng et al., 1996).

Inmunostaining

Embryos and imaginal discs were stained using standard procedures for confocal microscopy (González-Crespo et al., 1998). Antibody and in situ hybridisation double staining were performed as in Azpiazu and Frasch (1993), and embryos were mounted in Permount (Fisher Scientific). The antibodies were: rat or rabbit anti-β-Gal, rat anti-Iro, mouse anti-Nub and rabbit anti-Vg.

X-gal staining and preparation of adult flies

For X-gal staining of adult patterns, pharates were removed from the puparium, treated as described in Calleja et al. (1996), and mounted

in GMM (Roberts, 1986). Adult flies containing mutant clones were prepared for microscopic examination after digesting soft parts with KOH, washed with alcohol and mounted in Euparal.

RESULTS

The domains of expression of *pnr* and *iro* in the thorax and abdomen are not defined by cell lineage

The domain of expression of *pnr* in adult flies is seen directly in *pnr-Gal4/UAS-y* flies or in flies or imaginal discs of the genotype *pnr-Gal4/UAS-lacZ* (Fig. 1A). It is restricted to a dorsal region of the head, thorax and abdomen. In the head, *pnr* is expressed in the dorsal region of the eye and in the head capsule (Heitzler et al., 1996) and will not be considered further.

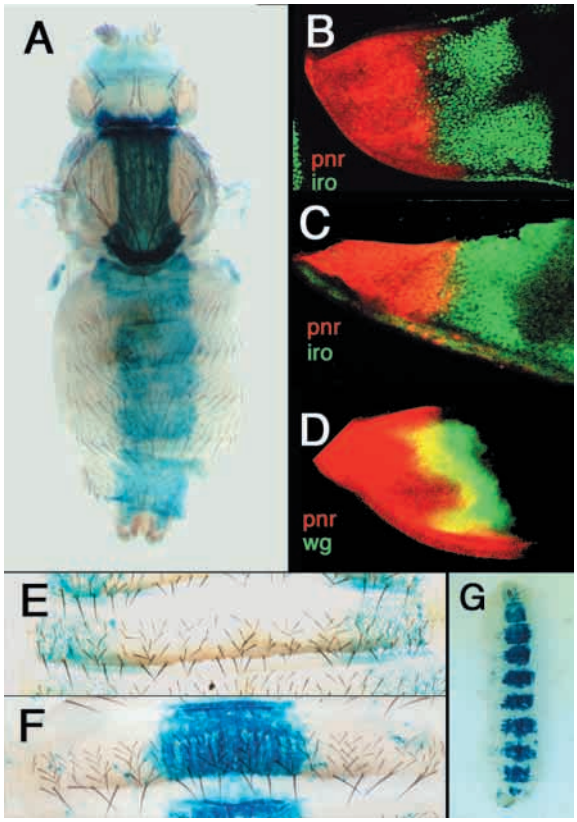

Fig. 1. Expression domains of *pnr* and *iro*. (A) Dorsal view of a fly of genotype *pnr-Gal4/UAS-lacZ* stained with X-gal to reveal the entire adult *pnr* domain. There is *pnr* activity in the back of the head and a medial domain of the thorax and abdomen. (B) Thoracic region of a mature wing imaginal disc of genotype *pnr-Gal4/UAS-lacZ* doubly stained with anti-β-Gal (red) and anti-Iro (green). There is no detectable overlap between the *pnr* and *iro* domains, which together cover the prospective region corresponding to the entire mesothorax. (C) Similar region of the haltere disc of the same genotype as in B also staining with anti-β-Gal and anti-Iro. (D) Thoracic region of a wing disc showing double staining for *pnr* and *wg* (green). The *pnr* and *wg* expression domains overlap (yellow). (E) X-gal staining of an abdominal segment of a fly of *iro-lacZ/+* genotype. The expression is restricted to the lateral region of the segment. (F) X-gal staining in an abdominal segment of a *pnr-Gal4/UAS-lacZ* fly. (G) X-gal-stained larvae of genotype *pnr-Gal4/UAS-lacZ* larvae to show that *pnr* is expressed in a dorsal medial domain, resembling the adult pattern.

In the dorsal mesothorax, *pnr* expression has already been described (Calleja et al., 1996; Heitzler et al., 1996; Ramain et al., 1993): it covers a longitudinal band that occupies about 40% of the notum and extends from the dorsal midline to the medial zone. It is delineated by a straight border that is aligned with, and just lateral to, the dorsocentral bristles (Fig. 1A). In the wing disc, *pnr* is expressed in the region that contains the progenitor cells of the medial region of the adult notum (Fig. 1B).

iro is expressed in both the wing and the thorax (Diez del Corral et al., 1999; Gómez-Skarmeta et al., 1996; McNeil et al 1997), but only the thoracic expression is relevant here. In the second larval instar *iro* is expressed in all prospective thoracic cells but, in third instar discs, it is restricted to the lateral region (Gómez-Skarmeta et al., 1996), indicating that there is a retraction of the *iro* thoracic domain during disc development.

We have compared the expression of *pnr* and *iro* in the thorax of late third instar discs: they are expressed in distinct and complementary subdomains (Fig. 1B) that together cover the entire mesothorax. Their expression extends to both the A (notum) and the P (postnotum) mesothoracic compartments as indicated by double staining for *pnr* and *en* or *iro* and *en* (not shown). The domain of *wg* in the mesothorax overlaps with that of *pnr* and *iro* but is restricted to the A compartment (Fig. 1D). The expression of *pnr*, *iro*, (Fig. 1C), *wg* and *en* in the haltere disc is the same as in the wing disc, indicating that the two discs share the same genetic organisation.

In the abdominal segments, *pnr* defines a medial subdomain that occupies about 35% of each tergite (Fig. 1A,F). Unlike the notum, there is no morphological landmark close to the *pnr* expression boundary and the bristle and pigment patterns are similar on either side.

There are several different stocks that express *lacZ* under promoters from the *iro-C* genes (Leyns et al., 1996). All show some expression in the abdomen, with the strongest expression at the extreme lateral part of the tergites and no staining in the medial regions (Fig. 1E). Generally, the more posterior segments stain strongest, and here there is expression in the P compartments. Within the P compartments, the stain is strongest at the back coming to an end abruptly at the PA (segment) border. A comparison between the Pnr and Iro domains in the abdomen is shown in Fig. 1E,F. Unlike the wing or haltere discs, the areas of expression do not appear to be complementary. It is possible that the *lacZ* lines do not reveal the entire *iro* expression domain.

We have analysed the notum and abdomen to see if there is a lineage restriction separating the *pnr* and *iro* expression domains. Clones of cells were initiated during the third larval instar (72-96 hours) of development. Plotting more than 50 of these clones in the notum, we were unable to find any lineage restriction (Fig. 2A). We also induced marked clones in the wing discs of third instar larvae in which either the *pnr* or the *iro* domains were labelled with the corresponding antibody (see Materials and Methods.). The result is illustrated in Fig. 2B; the clones can cross over the boundary between the two domains. Thus the expression of *pnr* and of *iro* is not clonally inherited (see also Diez del Corral et al., 1999). A similar conclusion was reached from observations in the abdomen: we

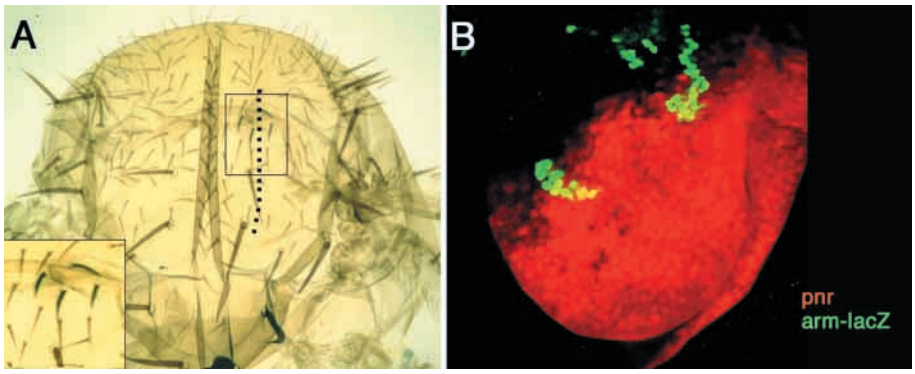

Fig. 2. The Pnr/Iro border is not a lineage border. (A) A clone of cells marked with y^+ and f^{36a} induced during the third larval period. The clone (magnified in the lower part of the panel) extends to both sides of the *pnr*/*iro* border, whose approximate location is indicated by the dotted line. (B) The thoracic region of a wing disc containing two *lac-Z*-expressing clones (green) that extend to both sides of the boundary defined by *pnr* activity (red).

scored a large number of marked clones and did not find any sign of restriction near the medial limit of *pnr* expression.

Taken together, these experiments indicate a genetic subdivision of the dorsal component of the adult thoracic and abdominal segments into a MED region, formed by cells expressing *pnr*, and a LAT region, lacking *pnr* expression. In the mesothorax, the MED region extends from the dorsal midline to just beyond the line defined by the alignment of the dorsocentral bristles, whereas the LAT region extends from just outside the dorsocentral line to the wing hinge. In the abdomen, the MED and LAT regions display similar bristle and pigmentation patterns.

Function of *pnr* in the notum and abdomen: cells expressing *pnr* sort out from non-expressing cells

Since the thorax and abdomen are subdivided into two regions, one with and one without *pnr* activity, we have induced clones of cells that either lack *pnr* function or express the gene ectopically, and studied the consequences in the MED and LAT regions.

(a) Clones of cells lacking *pnr* function

Clones homozygous for the allele pnr^{VX6}, a lethal mutation in which most of the Pnr protein is lacking (Ramain et al., 1993), were induced by the flip-out method at different times during larval development. In the LAT notal region, where *pnr* is not expressed, pnr^{VX6} cells proliferate and differentiate normally, and therefore serve as control clones. In the MED region, after early heat shock (24-48 and 48-72 hours after egg laying) few clones are recovered. Out of a total of 139 flies inspected under the compound microscope, 70 clones were found restricted to the LAT domain and showed a normal pattern whereas only five clones were found in the MED domain and all these were in the process of invaginating from surrounding tissue. Six clones clearly extended to both the MED and LAT regions. In these cases, pnr^{VX6} cells differentiate normally in the LAT region, but form invaginations and necrotic tissue in the MED region (Fig. 3A); in some cases, the invaginated tissue can be seen to differentiate notum structures (Fig. 3B). In addition, 14 clones were found associated with the LAT region, usually near the MED/LAT border, that either formed invaginating vesicles or outgrowths. In these cases, the normal LAT pattern is not altered. We believe that the latter clones probably originated in the MED territory and migrated into the LAT one. These clones may reach a large size and, though they form a characteristic notum pattern, it is not possible to discern the spatial arrangement of the bristles.

Mutant clones initiated late in development (72-96 and 96-120 hours after egg laying) behave similarly to those induced earlier, but the number of clones found in the MED compared to the LAT region is higher than for clones induced early. In a sample of 42 flies heat shocked at 72-96 hours, we scored 29 mutant clones in the MED region, 18 of which formed segregating vesicles containing bristles and 11 were approximately normal although their bristles tended to cluster. Only one clone was found to cross the MED/LAT border and produced malformations in the MED region. 25 clones were restricted to the LAT region and were normal. Three vesicles of mutant cells were also found.

The observation that pnr^{VX6} clones differentiate notum structures that segregate from the MED region and may

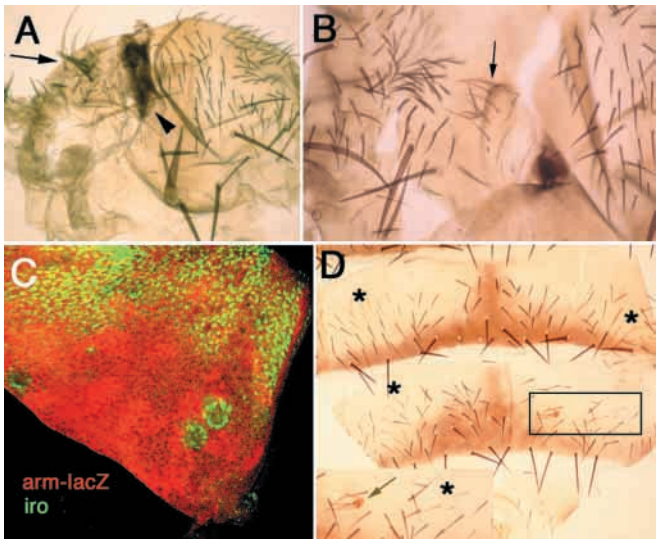

Fig. 3. Behaviour of clones lacking *pnr* activity. (A) Large y^- pnr^{VX6} Sb^+ clone extending from the lateral to the medial region of the thorax. The mutant cells differentiate normally in the lateral region (arrow) but invaginate and become necrotic in the medial region (arrowhead). (B) Another y^- pnr^{VX6} Sb^+ clone (arrow) that penetrates into the medial territory. It was originally hidden by a folding in the medial region but the surrounding wild-type tissue was separated to expose the clone. (C) pnr^{VX6} clones marked by the loss of *arm-lacZ* activity (red) showing derepression of *iro* (green) in the *pnr* domain. Note that the clones are roundish in shape indicating they are sorting out from surrounding cells. (D) Abdominal segments containing several y^- pnr^{VX6} Sb^+ clones in the lateral region (marked *) that are normal. In contrast, only one clone is found in the medial region. This clone, magnified in the inset, is abnormal, all the bristles are clustered (arrow) and the clone is beginning to form an invagination.

sometimes join the LAT region, suggests that these clones differentiate LAT pattern. As the formation of the lateral pattern requires *iro* activity (Diez del Corral et al., 1999), we examined *iro* expression in pnr^{VX6} clones in the MED domain. These clones proliferate well, although they are round in shape, presumably because they are sorting out from the surrounding cells. In nearly all the cases, they acquire *iro* expression, as illustrated in Fig. 3C, showing that *pnr* negatively regulates *iro* activity and suggesting that the Pnr/Iro borderline may result from antagonistic interactions between cells expressing *pnr* or *iro*.

We have also examined the behaviour of *pnr* mutant clones in the abdomen. In the sternites (ventral abdomen) and the lateral region of the dorsal abdomen (tergite), these clones develop normally. In the central region of the tergite, where *pnr* is normally expressed, the great majority of *pnr* mutant clones disappear (Fig. 3D). This can be demonstrated by comparing the ratio of clones recovered in the MED and LAT regions in control (pnr^{+}) and pnr^{-} clones. In 28 control abdomens containing y^{+} f^{36a} clones, 25 were undoubtedly restricted to the MED and 44 to the LAT region. (In addition, there were a number of clones possibly crossing the MED/LAT border; these were discarded.) By contrast, in a sample of 51 abdomens containing $y^{-}pnr^{VX6}$ Sb^{+} clones, 95 were found in the LAT region and only seven in the MED region; the latter were always abnormal, do not integrate with the wild-type cells and form vesicles that segregate from the surround. Nevertheless, they differentiate bristles and cuticle of abdominal character (Fig. 3D). The low frequency of clones in the MED region clearly indicates that the majority of *pnr* mutant clones are lost. The different behaviour of *pnr* mutant clones at either side of MED/LAT border is intriguing because the bristle and pigmentation patterns are uniform along the mediolateral axis of the tergite and yet the genetic requirements appear to be different.

(b) Ectopic expression of *pnr* in clones of cells

The consequences of *pnr* expression in the LAT thoracic region were assayed by inducing expression of *pnr* in clones of cells. Clones were induced during the different larval periods, although no qualitative difference in phenotype was detected with respect to the time of clone initiation. They may either overlap the MED and LAT regions or be confined to one or the other. The general result is that *pnr*-expressing clones are eliminated or grow abnormally in the LAT region, whereas they are virtually normal in the MED one. For example, in one experiment, 30 *pnr*-expressing clones were found in the notum (for comparison, 46 clones were found in the wing blade in the same batch of flies). Of these, 19 were confined to the MED region; they differentiated normally except that they often possessed one extra scutellar bristle. Ten overlapping clones were found extending to both MED and LAT regions; the cells situated in the MED region differentiated normally but, in the LAT region, they tended to sort out from surrounding cells to form vesicles with sharp boundaries. A single clone of *pnr*-expressing cells confined to the LAT region was recovered. This exceptional case was situated in a very anterior position and differentiated only microchaetes. These results suggest that, while increasing the amount of the Pnr product has little effect in the MED domain, *pnr* activity is incompatible with LAT development and *pnr*-expressing cells sort out from non-expressing LAT cells.

Together, experiments inducing either loss or gain of *pnr* activity demonstrate that cells expressing *pnr* tend to segregate from non-expressing cells; in the MED region clones of cells lacking *pnr* activity sort out from surrounding cells, whereas in the LAT region, the *pnr*-expressing cells sort out. However, the presence or absence of *pnr* activity does not compromise the ability of the cells to proliferate and differentiate notal or abdominal structures.

(c) Inducing *pnr* activity in large areas of the notum

The effect of ectopic *pnr* expression has also been studied using Gal4 lines that drive expression in different parts of the notum. Firstly, the *pnr-Gal4* line was used to increase the amount of *pnr* activity in the MED region. The excess of *pnr* function here has no effect except in the scutellum, where a supernumerary bristle often appears. The EM462 line directs activity in a longitudinal stripe of the LAT domain close to the MED region (Calleja et al., 1996); in *EM462/UAS-pnr* flies,

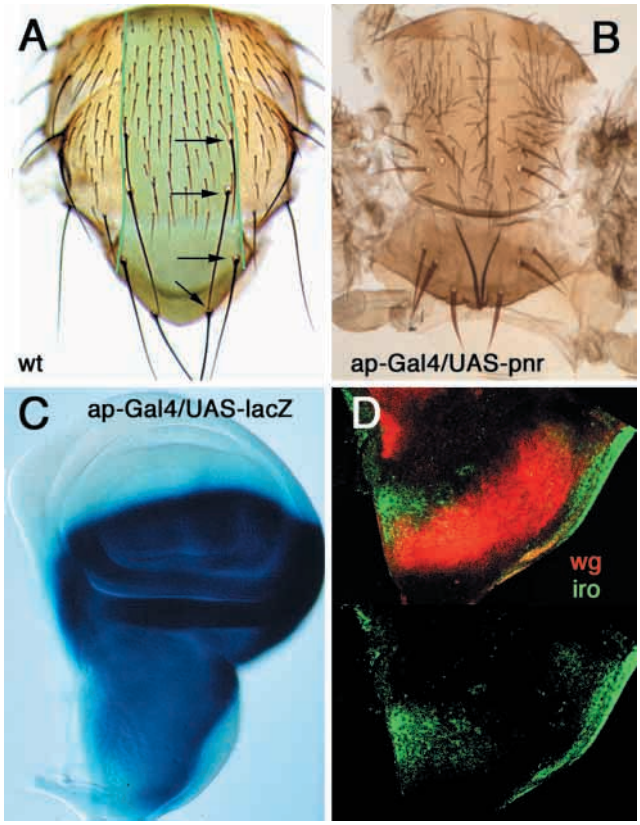

Fig. 4. Effect of uniform *pnr* expression in the mesothorax. (A) Normal mesothoracic structures. The medial region is labelled in green. Note that it includes the dorsocentral and scutellar bristles (arrows). (B) Mesothoracic derivatives of a fly of genotype *ap-Gal4/UAS-pnr*. The entire mesothorax develops only the medial region, although it is somewhat enlarged. All the pattern elements corresponding to the lateral region are missing. (C) Wing disc of genotype *ap-Gal4/UAS-lacZ* stained with X-gal to show that there is Gal4 activity in the region corresponding to the mesothorax, except possibly in the posterior compartment. (D) Thoracic region of a wing disc of *ap-Gal4/wg-lacZ; UAS-pnr* genotype stained with anti-β-Gal (red) and anti-Iro (green). The expression of *wg* is expanded but that of *iro* is diminished (lower panel, compare with Fig. 1).

there is an enlargement of the MED region as indicated by the increased number of dorsocentral bristles. Similar results were obtained with other lines conferring expression in the LAT domain.

A significant result is obtained with the Gal4 lines *ap-Gal4* or C765, that drive uniform and general expression in all notal cells (Fig. 4A-C). The notum of an *ap-Gal4/UAS-pnr* fly is shown in Fig. 4B. Only structures corresponding to those of the MED region are formed; the LAT region does not develop. This suggests that ubiquitous *pnr* expression over the entire primordium prevents the subdivision of the notum and specifies a single MED region that is likely to include the cells originally destined to form the LAT region. The MED pattern is normal except for an increase in the number of dorsocentral bristles and a higher density of microchaetes in the dorsocentral region. The excess of dorsocentral bristles is reflected in an expansion of the *wg* domain in the notum, and the loss of the LAT domain in a decrease of *iro* expression (Fig. 4D).

Function of *iro-C* in the abdomen

The expression of *iro* and the requirements for *iro-C* activity during notum development have already been described (Diez del Corral et al., 1999; Gómez-Skarmeta et al., 1996); *iro-C* genes are expressed and required by all notal cells in early disc development but, during the third instar, they are necessary only in the LAT region.

We have examined the consequences in the abdomen of loss of *iro-C* function and also of ectopic expression of *mirror*, one of the elements of the complex. Over most of the abdomen *iro-C*$^{-}$ clones were normal, but laterally, they were not. The clones found in the lateral domains of the tergite were often depigmented; usually, not all parts of the clone were affected to the same extent. This combination of clear cuticle with hairs and normally distributed bristles is not found anywhere on the normal tergites. Again only in the lateral parts, the clones tended to be roundish, as if they differed in affinity from the cells near them. These lateral clones could not be described as a transforming the cuticle type from lateral to medial, as they did not gain appropriate characters (Fig. 5A). For example, in segment A6, there are hairs laterally but not medially, yet laterally situated *iro-C*$^{-}$ clones carry hairs – even though they are depigmented.

As might be expected, clones of cells that express *iro* genes ectopically are abnormal largely in medial parts of the tergite – where the proteins are normally lacking. In the medial area, the clones tend to be rounded and they produce depigmentation. This depigmentation is exactly coextensive with the clone, showing that this effect of ectopic *mirror* is cell autonomous. In addition, most medial clones are associated with reversal of polarity of the hairs and bristles, this is entirely at the back of the clone and behind it (Fig. 5B). This effect appears to be stronger in those clones situated in the anterior part of the A compartment.

These results suggest that, although the *iro-C* genes are required for normal development of the lateral abdomen their function is not comparable to that of *pnr* in the medial region.

Ectopic expression of *pnr* induces the formation of MED structures in the wing and ventral abdomen

The consequences of ectopic *pnr* activity were also studied in the wing blade and the abdominal segments, either inducing clones of *pnr*-expressing cells or forcing *pnr* expression in large regions of the wing or abdomen.

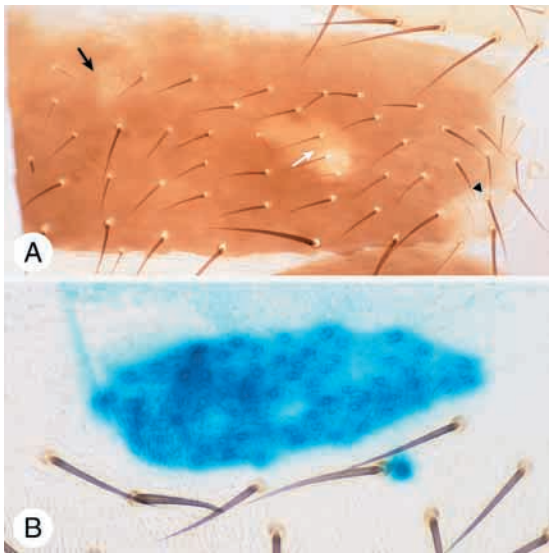

Fig. 5. Effects of loss or gain of *iro* function. (A) Segment A5 of a male with three separate iro^{-} clones. A medial clone (white arrow) develops normally, a more lateral clone has normal and abnormal patches (black arrow) and the most lateral clone is entirely depigmented with abnormally fine bristles (arrowhead). (B) A medial clone expressing Mirror ectopically; the marked cells stain blue. Note the clone is smooth-bordered and, behind it, hairs and bristles have altered polarity.

In the wing, more than one hundred clones of *pnr*-expressing cells were scored. These clones develop structures typical of the notum with the characteristic trichome density and pigmentation as well as notal bristles (Fig. 6A,B). Notal structures are formed in a cell-autonomous manner, since the transformed territory is always associated with the genetic markers used. The wing-to-notum transformations may appear anywhere in the wing except at the distal end, but are found preferentially in the more proximal regions. Clones induced during the first larval instar can build large portions of the notum pattern, although it is very hard to ascertain by morphological criteria that they form MED pattern elements (but see below). Clones differentiating notal structures including macrochaetes and microchaetes appear exclusively in the A wing compartment. In the P compartment, *pnr*-expressing clones differentiate patches of pigmented cuticle devoid of bristles (Fig. 6D) that we interpret to be postnotum. This indicates that A or P compartmental identity influences the thoracic pattern specified by *pnr*. However, dorsal and ventral clones differentiate the same notum structures suggesting that dorsal or ventral identity does not contribute to the type of pattern specified by *pnr*. The wing-to-notum transformation induced by *pnr*-expressing cells is reflected in the suppression of genes such as *vestigial (vg)* (Fig. 6E,F) or *nubbin (nub)* (not shown) involved in wing development (Kim et al., 1996; Williams et al., 1991).

We find that the pattern of the wild-type wing tissue in the vicinity of *pnr*-expressing clones in the A compartment is nearly always altered (Fig. 6C). This consists of local duplications and modifications of the bristle and vein pattern –

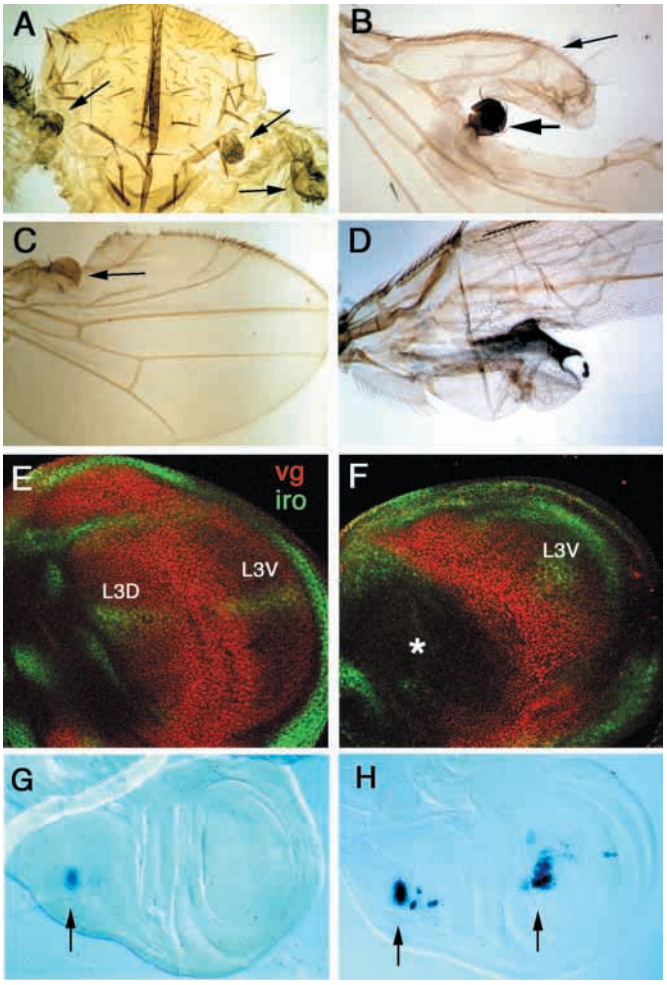

Fig. 6. Developmental effects of ectopic *pnr* expression. (A) Fly containing several clones (arrows) of *pnr*-expressing cells (marked with y^+ and $f^{36a)}$ in the base of the wing. The clones differentiate notum structures. (B) Single y^+ f^{36a} clone (thick arrow) of *pnr*-expressing cells differentiating notum. Note that the wing pattern in the proximity of the clone is affected; there is a vein duplication and also the bristles in the margin (thin arrow) differentiate as if they were in a more distal position. (C) A y^+ f^{36a} clone (arrow) that has a profound effect on the wing pattern. Note the extra veins and the abnormal patterning of the bristles in the wing margin. (D) A *pnr*-expressing clone (the darker pigmentation conferred by y^+ activity is evident) in the posterior wing compartment. (E) Wing pouch region of a wild-type disc stained for *iro* (green) and *vg* (red) activity. The DV boundary goes between the L3V and L3D domains of *iro* expression. (F) The same region of a disc of genotype *1096/UAS-pnr* equally stained for *iro* and *vg*. Note the *vg* expression in the dorsal compartment (*) is much reduced. (G) The expression of the DC enhancer of the A-SC complex (arrow) marks the position of the dorsocentral bristles in the notum. These bristles are included within the MED region. (H) Expression of the DC enhancer (arrows) in a disc of genotype *1096/UAS-pnr,* indicating a transformation of the D wing compartment into a notal region with MED features.

resembling the effects of ectopic expression of genes encoding signalling molecules such as Hh or Dpp (Basler and Struhl, 1994). This non-autonomous effect suggests that *pnr* may induce ectopic activity of some of the signalling genes (M. C. and G. M., unpublished data).

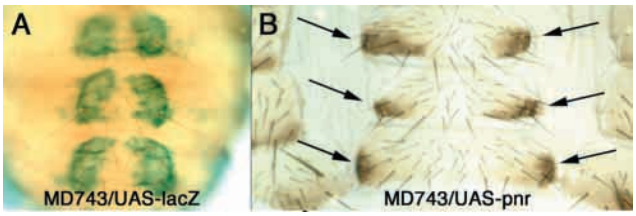

Fig. 7. Effect of ectopic *pnr* expression in the ventral abdomen. (A) X-gal staining of the ventral abdomen of a fly of genotype *MD743/UAS-lacZ* to indicate Gal4 activity in the sides of the sternites. (B) A similar view of the abdomen of an adult of genotype *MD743UAS-pnr*. The lateral region of the sternites acquire pigmentation and bristle pattern similar to those of the tergites (arrows).

The capacity of Pnr to induce wing-to-notum transformation was confirmed by using several Gal4 lines that drive *pnr* expression in different areas of the wing blade. The *nub-Gal4*, *ap-Gal4*, *MS1096*, *C765*, *omb-Gal4* and *vg (quadrant)-Gal4* lines (see Materials and Methods) were utilised and all show the same transformation, although with variable strength. These lines allowed us to test some of the suggestions from the behavior of clones of *pnr*-expressing cells. For example, the MED identity of the ectopic notal tissue can be demonstrated using the construct containing the dorsocentral (DC) enhancer of the *achaete-scute* complex (García-García et al., 1999). This enhancer drives expression in the proneural cluster from which the dorsocentral bristles develop and so can be considered to be a marker for the MED region. As shown in Fig. 6G,H, wing discs of the genotype *MS1096/UAS-pnr/DC-lacZ* show *lacZ* activity in the region corresponding to the wing pouch, suggesting that the notal territory in the wing is of MED identity.

We have also examined the effects of ectopic *pnr* expression in the haltere derivatives, although the lack of morphological markers precludes a detailed study. Nevertheless, *pnr* expression in the capitellum induces transformations into metanotum-like structures and all the effects recorded are consistent those seen in the wing disc derivatives. In the abdomen, we find that the presence of Pnr in the ventral region (sternites) produces a transformation into dorsal abdomen (tergites, Fig. 7). In some cases, the entire set of sternites is transformed into tergites, resulting in flies with an almost complete ventrodorsal transformation in the abdomen.

Dorsal subdomains in embryos

As *pnr* and *iro* define complementary domains in the adult thorax, we have compared their expression in embryos. In early embryogenesis, *pnr* is expressed in a broad zone of the dorsal region and, by germband extension, becomes restricted to a thin stripe that extends from the cephalic suture to the end of the abdomen abutting the border of the amnioserosa (Winick et al., 1993). Double staining for *pnr* and *en* indicates that *pnr* is expressed in A and P compartments (not shown).

During germband retraction, *pnr* expression widens towards the ventral side defining a longitudinal band (Fig. 8A) of about 10 cells wide. *iro* is not active in the early embryos but, from germband extension, it is expressed in a longitudinal stripe just ventral to that of *pnr*: *iro* and *pnr* define two abutting dorsal

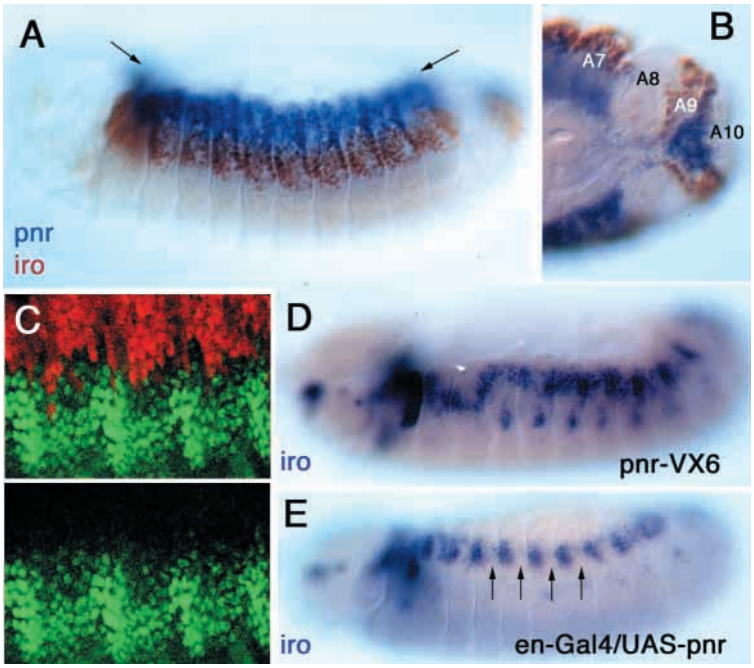

Fig. 8. Embryonic expression of *pnr* and *iro*. (A) *iro-lacZ*/+ embryo after germband retraction doubly stained for *pnr* RNA (blue) and β-Gal (brown). Together, the expression of the two genes covers approximately the dorsal part of the embryo, from the border of the amnioserosa (arrows) to the mid-ventral region. Note that the anterior and posterior borders of expression are the same for the two genes. There is a gap in the posterior abdomen where neither *pnr* nor *iro* are expressed. (B) Posterior region of an embryo stained as in A to illustrate that common spatial control of *pnr* and *iro* expression. The two genes are expressed in A7, absent in A8, expressed in A9, and absent on A10. (C) Confocal image of four embryonic segments stained for *pnr* (red) and *iro* (green) activity to illustrate that their domains do not overlap much if at all. (D) *iro* expression in a pnr^{VX6} embryo. There is *iro* activity in the proximity of the amnioserosa border, where *iro* is normally not expressed. (E) *iro* expression in an *en-Gal4/UAS-pnr* embryo in which *pnr* is active in the P compartment of the normal *iro* domain. Note that *iro* activity is eliminated in the P compartments (arrows).

subdomains (Fig. 8A,C) in thoracic and abdominal embryonic segments. The anterior and posterior limits of their domains are the same. In the posterior region, they are both specifically repressed in the A8 segment (Fig. 8B). The comparison of their expression patterns clearly suggests that they are spatially regulated by common control mechanisms.

We have also examined whether *pnr* and *iro* are involved in embryos in regulatory interactions similar to those described for adult cells and, in particular, if *pnr* represses *iro* activity. The result is illustrated in Fig. 8D. In pnr^{VX6} mutant embryos, *iro* expression is altered, extending more dorsally than in the wild type, and penetrating into the *pnr* domain. Ectopic *pnr* expression in *en-Gal4/UAS-pnr* embryos represses *iro* activity in the P compartments (Fig. 8E).

DISCUSSION

All *Drosophila* segments are subdivided into A and P compartments that have separate lineages and different sets of active selector genes within them (García-Bellido et al., 1973; Lawrence and Morata, 1994). When the imaginal discs form at the end of embryogenesis, they already contain an A and a P compartment. During subsequent development, some discs such as the wing, haltere and leg discs, undergo other compartmentation events, whereas no other lineage restrictions have been found within the dorsal or ventral abdominal histoblasts. Even in the discs in which there are several compartmentation steps such as the wing disc (Blair, 1993; Díaz-Benjumea and Cohen, 1993; García-Bellido et al., 1973), there are large regions that are single lineage units. Therefore any subsequent genetic subdivisions would not be expected to be accompanied by lineage restrictions.

We report here a novel subdivision common to thoracic and abdominal segments that is not based on lineage segregations. A principal role appears to be performed by the *pnr* gene whose mode of action is very similar to classical selector genes, except that its activity is not propagated through cell divisions.

pnr subdivides the dorsal component of each segment into two discrete regions

Our results show that the subdivision into MED and LAT subdomains is a general feature of the thoracic and abdominal segments and that these subdomains correspond to the expression domains of *pnr* and *iro*. In the thorax, the MED and LAT subdomains meet along a longitudinal line close to that defined by the dorsocentral bristles, appear to be complementary and together cover the entire structure. In the haltere disc, *pnr* and *iro* are expressed as in the wing disc, indicating a similar or identical genetic organisation. In the abdomen, *pnr* activity is restricted to the MED region of the tergite (Fig. 1F) in every segment, whereas *iro* expression, just as in the thorax, is restricted to the LAT region. We note that, unlike the thorax, the *iro* and *pnr* domains do not appear to be complementary, but there appears to be a region with neither *pnr* or *iro* activity. However, since these two domains are complementary in the adult mesothorax and metathorax, and in embryos, we believe that the *iro-lacZ* lines that we have used may not reveal the full *iro* expression domain in the abdomen.

Our results also indicate that *pnr* is instrumental in partitioning the dorsal component of segments into MED and LAT regions. The first argument is based on the behaviour of clones of cells either lacking *pnr* activity or expressing *pnr* ectopically. In both types of experiments, the common observation is that cells expressing *pnr* sort out from non-expressing cells. It appears that *pnr* confers cell-specific adhesion properties (Steinberg, 1963; Lawrence et al., 1999), which are likely to be responsible for segregating the MED and LAT regions. The second argument derives from the observation that, in early disc development, *iro* is expressed in all notal cells but, in the third instar, it is restricted to the LAT subdomain (Diez del Corral et al., 1999; Gómez-Skarmeta et

al., 1996). Our finding that *pnr* negatively regulates *iro* suggests that the restriction of *iro* expression to the LAT subdomain, and hence the appearance of two distinct subdomains, is a result of *pnr* activity in the MED region. In cases such as *ap-gal4/UAS-pnr* flies in which the restricted activity of *pnr* is replaced by uniform expression in all presumptive notal cells, the subdivision of the notum does not occur, instead only the MED pattern develops (Fig. 6B). Thus restriction of *pnr* expression to the MED region is a prerequisite for the partitioning of the notum.

pnr acts as a selector gene to specify the identity of MED region

Our experiments indicate that, in addition to its function in partitioning, *pnr* also specifies the pattern of the MED region. It participates together with selector genes in a "genetic address" (García-Bellido et al., 1979) that determines the identity of MED regions of the thoracic and abdominal segments. In the notum, the activity of *pnr* is necessary for the development of the characteristic pattern of the MED region, whereas its absence allows expression of *iro* and consequently formation of the LAT pattern. In the abdomen, *pnr* is also required for the development of the MED region. The developmental capacity of *pnr* and the binary mechanism in which it participates are clearly demonstrated by the ectopic expression experiments that show that Pnr induces different patterns depending on the local genetic context. In the wing blade, Pnr induces the formation of the MED notum in the anterior wing and MED postnotum in the posterior wing. This result suggests that *en* is contributing together with *pnr* to the genetic address of the MED regions. The DV compartmental segregation is not recognised by *pnr*; clones of *pnr*-expressing cells in the dorsal and ventral wing compartments differentiate the same MED-like pattern. But this is not so surprising: no DV segregation takes place within the normal domain of *pnr* expression.

In the haltere disc, the response to *pnr* activity may be modulated by the function of *Ultrabithorax (Ubx)*, the selector gene that discriminates between haltere and wing development (Sánchez-Herrero et al., 1985). In the abdomen, ectopic *pnr* expression in the ventral region (sternites) induces the development of a dorsal pattern (tergite). Here the response to *pnr* function is probably modulated by the corresponding genes of the BX-C (Sánchez-Herrero et al., 1985). This transformation appears to be similar to that described for ectopic *wg* activity in the ventral abdomen (Kopp et al., 1999; Shirras and Couso, 1996). It is possible that Pnr may derepress *wg* or expand the domain of *wg* in the sternites and pleurae. Alternatively, the transformations induced by *wg* may be mediated by activation of *pnr*. Other features of *pnr* are reminiscent of classical selector genes. For example, the effect of *pnr* on cell affinities is a property shared by selector genes and is used to keep groups of cells from mixing during growth (Blair and Ralston, 1997; Díaz-Benjumea and Cohen, 1993; Morata and García-Bellido, 1976; Morata and Lawrence, 1975; Rodríguez and Basler, 1997).

Thus the mode of action of *pnr* is very similar to that of selector genes like *Ubx*, *en* or *ap*. The only difference between the segregation of the MED and LAT subdomains and the AP or the DV compartment segregations is the manner whereby activity of the genes is maintained. Expression of *en* or *ap* is inherited unchanged by the cellular progeny, but this is not so in the case of *pnr* or *iro*. We believe that the really critical outcome is the partitioning of groups of cells into distinct genetic subdomains. The manner in which the genes responsible for segregating the genetic subdomains, be these *en*, *ap* or *pnr*, maintain their activity may be of secondary importance. If *pnr* expression is not inherited by the cell progeny, there has to be some other mechanism to maintain activity in the appropriate region. *pnr* is likely to respond to threshold levels of some specific signal(s) which would probably emanate from either the midline or from the lateral margin of the dorsal field.

pnr and *iro* are not the only developmental genes that define the identity of body regions by a mechanism not based on cell lineage. In the leg disc, the distinction between proximal and distal regions results from the genetic interface between *Dll* and *hth-exd*, which determines the development of the appendage in the proximodistal axis (Abu-Shaar et al., 1999; González-Crespo et al., 1998). This genetic border is not based on lineage (Gorfinkiel et al., 1997) and, as in the case of *pnr* in the notum, *Dll* activity confers specific adhesion properties to cells causing them to sort out from non-expressing cells (Gorfinkiel et al., 1997; Wu and Cohen, 1999). More recently, it has been shown that the distinction between external and internal analia is based on the differential activity of *Dll* within the domain of the Hox gene *caudal*, and that this too is lineage independent (Moreno and Morata, 1999). Recent work on the *omb* domain in the wing (Weigmann and Cohen, 1999) indicates that *omb* activity is not inherited by cells. As in the case of *pnr*, it has been shown that *Dll* and *omb* can induce appendage structures if expressed ectopically (Gorfinkiel et al., 1997; Grimm and Pflugfelder, 1996), suggesting that their products determine the identity of different body regions. In the abdomen, the A compartment is subdivided into anterior and posterior domains. These domains respond to Hedgehog in very different ways and yet the border between them is not colinear with a lineage boundary (Lawrence et al., 1999; Struhl et al., 1997).

The process of compartmentation is an epigenetic mechanism by which groups of cells become geographically divided into subgroups that acquire characteristic and distinct genetic identities. Although compartmentation is normally associated with cell lineage segregations, our results indicate that such segregations are not the defining feature of the process. The MED and LAT subdomains that we report here are not segregated by cell lineage and yet they possess all other features associated with compartments: they originate by subdivision of groups of presumptive cells, are delimited by sharp boundaries, and are genetically specified by the combinatorial activity of a set of selector genes. The subdivision of the leg into distinct genetic domains (González-Crespo et al., 1998; Lecuit and Cohen, 1997) or that of the analia (Moreno and Morata, 1999) may be other examples of genetic partitions without the concourse of lineage segregations. The principal difference with the MED/LAT subdivision is that the latter affects embryonic, larval as well as adult segments and is therefore a basic feature of the body plan.

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