# A Genetic Analysis of *pannier*, a Gene Necessary for Viability of Dorsal Tissues and Bristle Positioning in Drosophila

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ABSTRACT

A genetic and phenotypic analysis of the gene *pannier* is described. Animals mutant for strong alleles die as embryos in which the cells of the amnioserosa are prematurely lost. This leads to a dorsal cuticular hole. The dorsal-most cells of the imagos are also affected: viable mutants exhibit a cleft along the dorsal midline. *pannier* mRNA accumulates specifically in the dorsal-most regions of the embryo and the imaginal discs. Viable mutants and mutant combinations also affect the thoracic and head bristle patterns in a complex fashion. Only those bristles within the area of expression of *pannier* are affected. A large number of alleles have been studied and reveal that *pannier* may have opposing effects on the expression of *achaete* and *scute* leading to a loss or a gain of bristles.

THE precise positioning of the sensory bristles of the peripheral nervous system of adult Drosophila depends upon a complex spatial regulation of the *achaete (ac)* and *scute (sc)* genes. These genes are thought to provide cells with neural potential [see GHYSEN and DAMBLY-CHAUDIÈRE (1988) and CAMPUZANO and MODOLELL (1992) for review]. During development of the imaginal discs they are expressed in small clusters of cells at the sites where bristles subsequently develop (CUBAS *et al.* 1991; SKEATH and CARROLL 1991). Studies of mutant alleles associated with rearrangements suggest the existence of *cis*-regulatory elements necessary for site-specific expression (RUIZ-GOMEZ and MODOLELL 1987). The two genes *hairy (h)* and *extramacrochaetae (emc)* act as repressors of *ac* and *sc*, *h* is a direct transcriptional repressor of *ac* (OHSAKO *et al.* 1994; VAN DOREN *et al.* 1994) and *emc* acts by binding to the *ac-sc* proteins and interfering with the cross- and autoregulation of these genes (MOSCOSO DEL PRADO 1984a,b; ELLIS *et al.* 1990; GARRELL and MODOLELL 1990; VAN DOREN *et al.* 1991).

We have initiated genetic screens designed to recover upstream regulators of *ac-sc* expression and have identified the gene *pannier (pnr)*. Null alleles of *pnr* are lethal (JÜRGENS *et al.* 1984), but a number of viable alleles with altered bristle patterns have been recovered. A parallel study (RAMAIN *et al.* 1993) has demonstrated that in the thoracic discs *pnr* alters the expression patterns of *ac* and *sc* and that the locus encodes a protein showing homology to the vertebrate transcription factor GATA-1 (EVANS *et al.* 1988; EVANS and FELSENFELD 1989). Two classes of dominant mutants with opposing phenotypes with respect to dorsocentral bristles were previously analyzed (RAMAIN *et al.* 1993). Animals mutant for one class ($pnr^{D1}$, $pnr^{D2}$, $pnr^{D3}$ and $pnr^{D4}$) of alleles display additional ectopic bristles, whereas those mutant for the other class of alleles ($pnr^{VX1}$ and $pnr^{VX4}$) are lacking bristles. Here we present a genetic study of 28 mutant alleles of *pnr*, including recessive viable as well as dominant alleles, that cause either a loss of bristles at wild-type positions or a gain of bristles at ectopic sites. It is the bristles at dorsal locations that are affected in the mutants. In addition, allele-specific interactions between *pnr* and *emc* are presented, raising the possibility that these two genes function together in the regulation of *ac* and *sc*.

*pannier* mutants display pleiotropy and two other phenotypes have been studied. We show that *pannier* is expressed in a wide band of cells running the whole length of the dorsal midline of the imaginal tissues (see also RAMAIN *et al.* 1993). Furthermore, *pnr* is probably required for the viability of cells situated along the dorsal midline. In a number of viable allelic combinations, as well as in mitotic clones of lethal alleles, a pronounced cleft forms along the dorsal midline of the thorax that may result from a failure of fusion between the two hemi-thoraces each derived from a separate imaginal disc. Clefts were also observed on the head and abdomen of extreme mutant escaper flies.

Interestingly *pnr* is also required for viability of the most dorsally situated cells of the embryo. In embryos mutant for null alleles of *pnr*, premature loss of the amnioserosa results in a failure of dorsal closure and a dorsal hole in the embryonic cuticle. In wild-type embryos the cells of the amnioseriosa invaginate at dorsal closure and only later undergo apoptotic cell death (RUGENDORFF *et al.* 1994). Specification of dorsal cell

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fates of the Drosophila embryo result from the activity of the *decapentaplegic (dpp)* gene. In *dpp* null mutants nearly all the dorsal pattern elements are missing and there is an expansion of the lateral and ventral structures (IRISH and GELBART 1987; RAY *et al.* 1991; ARORA and NÜSSLEIN-VOLHARD 1992; FERGUSON and ANDERSON 1992). The *dpp* product shows homology to BMP4, a TGFβ-related ligand (PADGETT *et al.* 1987, 1993) and a gradient of its activity may arise posttranslationally. High levels of *dpp* product specify amnioserosa, intermediate levels dorsal ectoderm and low levels ventral ectoderm (FERGUSON and ANDERSON 1992; WHARTON *et al.* 1993). Subsequently the gene *zerknüllt (zen)* is expressed in the amnioserosa and specifies the fate of this tissue (WAKIMOTO *et al.* 1984; IRISH and GELBART 1987; RUSHLOW *et al.* 1987a,b; ROTH *et al.* 1989; RAY *et al.* 1991). Unlike *zen* and *dpp*, in *pnr* mutants dorsal cell fates are not replaced by an expansion of more ventral fates, rather, the cells of the amnioserosa and dorsal epidermis die prematurely. This suggests that *pnr* acts after *zen* and that it is required for the integrity of the cells of the amnioserosa. Disintegration of the cells occurs late, after germ band retraction, but stems from early defects that are detectable shortly after gastrulation. In the embryo, *pnr* transcripts are restricted to the amnioserosa and the most dorsally situated ectodermal cells (see also WINICK *et al.* 1993).

## MATERIALS AND METHODS

**Fly strains:** Flies were raised on standard Drosophila medium at 25° unless otherwise indicated. Descriptions of balancers and mutations that are not described in the text can be found in LINDSLEY and ZIMM (1992). New mutations and abberrations recovered during this work will be described below and listed in Tables 1 and 2. The new lethal loci are named according to their cytological position.

Fly stocks used in this study were provided as follows: *ru h th st cu $pnr^{7G111}$ sr $e^s$ ca* from Tübingen stock center; *Df(3R)$sbd^{26}$* from Laurel Appel; *Dp(3;3)BK43* and *Dp(3;3)BK44* from J. BONNER; *Df(3R)$sbd^{45}$* and *T(2;3)$Sb^V$* from Umeä stock center; *Tp(3;3)$sbd^{104}$* and *T(2;3)$sbd^{106}$* from Bloomington stock center; *T(2;3)GT11* from JOHN ROOTE. *Dp(3;3)BK43* and *Dp(3;3)BK44* were first described in LEICHT and BONNER (1988). The original stocks *$ru^g$ jv Ly Dp(3;3)BK43$d^2$ red cv-c $sbd^2$ sr e/TM3 Sb Ser* (new order: 61A-72D11/89E2-89A6,7/72D-100F) and *ru $Hn^{r3}$ T(2;3)BK44 Dp(3;3)BK44$d^2$ $bx^3$ cu kar/TM3 Sb Ser* (new order: 21A-31A/66C-65E/33F-31A/66C-72D/89E2-89A3,5/72D-100F; 61A-65E/33F-60F) were cleaned after several meïotic recombination rounds, and both duplications were shown to carry a fully functional $pnr^+$ allele since *Dp(3;3)BK43 Df(3R)$sbd^{45}$/$pnr^{VX6}$* and *Dp(3.3)BK44 Df(3R)$sbd^{45}$/$pnr^{VX6}$* give wild-type flies ($pnr^{VX6}$ is a null allele, see RAMAIN *et al.* 1993). We finally used the following $pnr^+$ disomic chromosomes that have reduced duplicated material: *Dp(3;3)BK43 $kar^2$ $ry^{506}$ Df(3R)$sbd^{104}$/TM3 Sb Ser* (*Dp(3;3)BK43* is probably homozygous lethal due to the 72D insertion) and *Dp(3;3)BK44 Df(3R)$sbd^{26}$ e/TM3 Sb Ser* (this stock no longer carries the original translocation and is homozygous viable).

*In(3R)$pnr^{Camel}$* (89B;89E3-4) was obtained from FRANÇOIS KARCH and GINES MORATA. The 89E3-4 break of *In(3R)Camel* occurs between *abd-A* and *Abd-B* in the *bithorax* complex and is associated with the mutant *$iab6^G$* (KARCH *et al.* 1985; LINDSLEY and ZIMM 1992). *In(3R)Camel/+* flies show a dominant partial *iab5* phenotype (transformation of the abdominal segment A5 toward A4) and a pronounced thoracic cleft, which is not affected by increased doses of $pnr^+$. *In(3R)Camel* is allelic to *pnr*: *In(3R)$pnr^{Camel}$/$pnr^{V1}$* flies show a severe cleft, head defects and split tergites, and *In(3R)$pnr^{Camel}$/Df(3R)$sbd^{45}$* or *$pnr^{VX6}$* flies are lethal as pupae with strong imaginal defects (the head is missing). During the course of this study a spontaneous revertant of *Camel* was discovered. Both the cleft and the segmentation traits were lost in *$Camel^{R+1}$/+*, but the lethal phase and the phenotype of hemizygotes were unchanged (same as *Camel/Df(3R)$sbd^{45}$*, not shown).

Five mutant alleles of *emc* were used: *$emc^1$* changes a valine into an aspartic acid that may interfere with the ability of the protein to form dimers (GARRELL and MODOLELL 1990). *$emc^{PX119}$* is a strong X-ray-induced allele. *$emc^{AP6}$* is a null allele carrying a 400-bp coding region deletion (ELLIS 1994). *$emc^{prl}$* and *$emc^{P5c}$[lac,$ry^+$]* have a *P* insertion near the transcriptional start site (GARELL and MODOLELL 1990) that significantly reduces *emc* expression (CUBAS and MODOLELL 1992). *Dp(3;Y;1)M2, $emc^+$* was used as a duplication of *emc*.

The alleles *$dorsal^1$ ($dl^1$)* and *$cactus^1$ ($cact^1$)* were used.

*Crosses:* Crosses were set up in vials cultured at 25°. Complementation crosses were between mutant chromosomes balanced over *TM3 Sb Ser* or *TM2*. Mutations were classified as lethal if <1% of homozygotes survive to the adult stage and noncomplementing if *trans*-heterozygous flies expressed a mutant phenotype or no nonbalancer carrying progeny emerged among 150 or more progeny.

**Mutagenesis:** New mutations were induced in males with EMS according to the protocol of LEWIS and BACHER (1968), by X-rays at a dose of 4000 rad (100 kv, 10 mA for 5 min, 1.5 mm aluminium filter, Philips MG102 constant potential X-ray system, beryllium window) or by *P*-induced mutagenesis.

*Screens for new alleles of pnr:* *$pnr^{V1}$* is a spontaneous viable allele of *pnr* that was found in a *Sb* chromosome from our collection and was separated from *Sb* for these experiments. *$pnr^{VX1}$*, *In(3R)$pnr^{VX2}$*, *In(3LR)$pnr^{VX3}$*, *$pnr^{VX4}$*, *In(3R)$pnr^{VX5}$*, *T(1;3)89Bo$^2$*, and *Tp(3;3)$pnr^{VX9}$* were X-ray-induced in a *st e* isogenic chromosome and were recovered in an $F_1$ screen using *$pnr^{V1}$* (114,000 chromosomes). *$pnr^{VX6}$* and *T(2;3)$pnr^{VX7}$* were X-ray-induced in a *$kar^2$ $ry^{506}$* isogenic chromosome and were recovered in an $F_1$ screen using *In(3R)$pnr^{VX5}$* (12,500 chromosomes). *$pnr^{D1}$* was found in an $F_1$ screen for EMS-induced mutations showing a strong synergism (extra dorsocentral bristles) with *$emc^1$* ($n$ = 9012 flies). *$pnr^{D2}$*, *$pnr^{D3}$*, and *$pnr^{D4}$* were found as dominant EMS-induced mutations in various $F_1$ or $F_2$ screens for extra dorso-central bristles or a strong thoracic cleft (P. HEITZLER, unpublished results).

To isolate revertants of the class 4 alleles *$pnr^{D1}$*, *$pnr^{D2}$*, *$pnr^{D3}$* and *$pnr^{D4}$*, $F_1$ screens were performed in bottles (D1, EMS, 17,500 chromosomes; D2, EMS, 22,500; D3, EMS, 15,000; D3, X-ray, 15,000; D4, EMS, 5000; D4, X-ray, 32,000). Revertants were screened in the progeny as flies with a perfect wild-type thorax (no extra-macrochaetae and no thoracic cleft).

To induce a *P*-element insertional mutation at the *pnr* locus, two stocks were used as a *P* source, one with one nonautonomous *P*[*lac*,$ry^+$] element on the *X* chromosome and a stock containing five nonautonomous *P*[*lac*,$w^+$] elements also on the *X* chromosome. *P*[$ry^+$,Δ*2-3*](*99B*) was used as the source of the *P* transposase (ROBERTSON *et al.* 1988). In the first experiment, *P*[*lac*,$ry^+$]; *$kar^2$ $ry^{506}$/$ry^{506}$ Sb e P*[$ry^+$,Δ*2-3*](*99B*) males were crossed to *$kar^2$ $ry^{506}$ $pnr^{V1}$ Sb/TM2, $emc^2$* females: *pnr* mutants were screened in bottles in the $F_1$ progeny among the *$kar^2$ Sb* flies for a thoracic cleft, and *emc* mutants were screened among the *$Sb^+$* flies for additional macrochaetes. No *pnr* alleles, but three *emc* alleles, were recovered among 83,000 flies. Among the *emc* alleles, two were viable (*$emc^{jp11}$* and

*emc*$^{jp12}$) and carry a *P[lac,ry*$^{+}$] insert. The last allele, *emc*$^{jp15}$, was induced in the *P[ry*$^{+}$,Δ*2-3](99B)* chromosome, and a *emc*$^{jp15}$ *kar*$^{2}$ *ry*$^{506}$ isogenic stock was made after recombination, this chromosome is lethal and *ry*$^{-}$. In the second experiment, *y w P[lac,w*$^{+}$]$_{5x}$; *kar*$^{2}$ *ry*$^{506}$/*ry*$^{506}$ *Sb e P[ry*$^{+}$,Δ*2-3](99B)* males were crossed to *st In(3R)pnr*$^{VX5}$ *e/TM3 Sb e Ser* females: *pnr* mutants were screened in bottles in the $F_1$ progeny for a thoracic cleft. One allele of *pnr*, *pnr*$^{VP8}$, was scored among 90,000 flies as a cluster. Unfortunately the mutation was found in the *P[ry*$^{+}$,Δ*2-3](99B)* chromosome and was immediately separated from the transposase source. A *kar*$^{2}$ *ry*$^{506}$ *pnr*$^{VP8}$ *Sb/TM2* stock was made. It is not *w*$^{+}$ and does not express β-galactosidase, but nevertheless contains an insertion in the *pnr* gene (RAMAIN *et al.* 1993).

A new *P*-induced viable allele, *pnr*$^{MD237}$, was recovered from a GAL4 enhancer trap screen (M. CALLEJA and G. MORATA, personal communication). For this strain, the enhancer detector reveals the wild-type pattern of *pnr*, suggesting that GAL4 is under the control of the *pnr* promoter.

**Exchange mapping:** Several lethal mutations of the new complementation groups surrounding *pnr* were ordered by recombination. Females of the *ru h th st cu pnr*$^{7G111}$ *sr e*$^{s}$ *ca/st l(3)89B e* genotype were crossed to *st Df(3R)sbd*$^{45}$ *e Pr/TM2* males. The very rare *st Pr* progeny were then crossed to *ru h th st cu sr e*$^{s}$ *ca* flies to test them for *cu* or *sr*, which then gives the order relative to *pnr*.

A viable allele of *pnr*, *pnr*$^{V1}$, and a lethal one, *pnr*$^{VP8}$, were mapped relative to *Sb: kar*$^{2}$ *ry*$^{506}$ *pnr*$^{V1(VP8)}$ *Sb/+* females were crossed to *st In(3R)pnr*$^{VX5}$ *e/TM3 Sb Ser* males. Respectively, five and four *Sb*$^{+}$ recombinants were scored among 3611 and 2920 $F_1$ *pnr* flies (*pnr*$^{V1}$/*pnr*$^{VX5}$ and *pnr*$^{VP8}$/*pnr*$^{VX5}$ are viable, fertile and exhibit a thoracic cleft). Further crosses reveal that the recombinant chromosomes contain the *kar*$^{2}$ *ry*$^{506}$ markers.

**Cytology:** Chromosome aberrations were analyzed cytologically with temporary propionic-orcein-carmine squash preparations of larval salivary gland chromosomes. The abberations were interpreted with the aid of the revised polytene chromosome maps of BRIDGES (see LEFEVRE 1976).

**Production of mosaic animals:** Mutant clones were produced by mitotic recombination induced by either by X-rays or hs-FLP/FRT (GOLIC and LINDQUIST 1989; GOLIC 1991). Twenty-four hour egg collections were made and larvae were irradiated (1000 rads) or heat-shocked (1 hr, 37°) between 48 and 72 hr after egg laying. Clones were marked with *pawn* (*pwn*), which labels both sensory bristles and epidermal hairs, *mutiple wing hairs* (*mwh*), which labels hairs, and *javelin* (*jv*), which labels sensory bristles. For a description of these gratuitous mutant cell markers see LINDSLEY and ZIMM (1992). Thoraces were mounted between coverslips in Struhl's medium. Clones were induced in flies of the following genotypes.

hs-FLP/FRT system:
(1) *y FLP*$^{1}$/+; *pr pwn; FRT*$^{75A}$ *pnr*$^{VX6}$/*FRT*$^{75A}$ *Dp(2;3)P32, pwn*$^{+}$
(2) *pr pwn FLP*$^{38}$/*pr pwn; FRT*$^{82B}$ *kar*$^{2}$ *ry*$^{506}$ *pnr*$^{VX6}$/*FRT*$^{82B}$ *kar*$^{2}$ *ry*$^{506}$ *Dp(2;3)P32, pwn*$^{+}$
(3) *y FLP*$^{1}$/+; *pr pwn; FRT*$^{75A}$ *pnr*$^{VX1}$/*FRT*$^{75A}$ *Dp(2;3)P32, pwn*$^{+}$
(4) *y FLP*$^{1}$/+; *pr pwn; FRT*$^{75A}$ *pnr*$^{D1}$/*FRT*$^{75A}$ *Dp(2;3)P32, pwn*$^{+}$
X-ray method:
(5) *pr pwn; st pnr*$^{7G111}$ *e/Dp(2;3)P32, pwn*$^{+}$
(6) *emc*$^{1}$ *mwh jv pnr*$^{VX6}$/*Dp(3;3)BK43 Df(3R)sbd*$^{45}$
(7) *emc*$^{1}$ *mwh jv pnr*$^{7G111}$/*Dp(3;3)BK43 Df(3R)sbd*$^{45}$

The *FLP*$^{1}$ and *FRT*$^{75A}$ inserts are described in GOLIC (1991).

**Staining for β-galactosidase activity:** Embryos were stained as described by GHYSEN and O'KANE (1989). Mutant embryos were identified using a new *TM6C cu*$^{+}$*Sb Tb* "blue" balancer carrying a *P[twi-β-gal,w*$^{+}$] insert (P. HEITZLER, unpublished results). Imaginal discs were stained as described by standard methods.

**Fixation of embryos for Nomarski optics:** Embryos were prefixed with heptane and glutaraldehyde, then fixed with glutaraldehyde after hand removal of the vitelline membranes. They were mounted in Euparal.

**Expression of *pannier* transcripts *in situ*:** Digoxygenin-labeled DNA was synthesized according to the Boehringer-Mannheim protocol and hybridized to whole mount embryos according to TAUTZ and PFEIFLE (1989).

**Staining with acridine orange:** Embryos were stained as described by ABRAMS *et al.* (1993), and wing discs were stained as described by MASUCCI *et al.* (1990). Samples were viewed either with a conventional fluorescence microscope (Zeiss Axiophot) or with a TCS confocal scanning laser microscope (LEICA, DMRE microscope, Argon Krypton laser type). Confocal image processing was performed either with logicial elements and macrocommands provided by the manufacturer or by programs concieved and developed by J. L. VONESCH at the Institut de Génétique et de Biologie Moléculaire et Cellulaire (I.G.B.M.C.). Photographs were made from a focus graphics flasher.

## RESULTS

**Genetics of *pannier*:** The locus *pnr* was first described by JÜRGENS *et al.* (1984) in a large-scale screen for zygotic mutations affecting the pattern of the larval cuticle. Two lethal alleles were identified in this screen and the mutant embryos display a hole in the dorsal cuticle. The gene has been located cytologically to the interval 89B4–9 by means of chromosomal rearrangements: strong alleles of *pnr* are lethal over *Df(3R)sbd*$^{105}$ (88F9–89A1; 89B9–10) and *Df(3R)sbd*$^{45}$ (89B4; 89B10–13) and display a similar embryonic phenotype as embryos homozygous for *Df(3R)sbd*$^{45}$. *pannier* maps meiotically to 3–58.05. One viable allele, *pnr*$^{V1}$, and one lethal allele, *pnr*$^{VP8}$, were mapped 0.15 map unit to the left of *Sb* (see MATERIALS AND METHODS). A preliminary cytogenetic analysis of the proximal 89B region of chromosome 3R is presented in Figure 1. EMS-treated isogenic *st e* chromosomes (1600) were tested for visible and lethal mutations when heterozygous with the deletion *Df(3R)sbd*$^{45}$. Six new complementation groups have been ordered with regards to *pnr* and to two previously known loci, *serpent (srp)* and *stubbloid (sbd)* (ASHBURNER 1991) either by deletion mapping (Figure 1) or exchange mapping (Table 1).

We have isolated (see MATERIALS AND METHODS for screens) a number of dominant and recessive alleles of *pnr*. A summary of the phenotypes of these alleles is presented in Table 2. Most of the recessive lethal alleles, whether or not they behave as dominants in the adult, display the same embryonic phenotype when homozygous, hemizygous or in transheterozygotes of a dominant over a recessive allele. Revertants of the dominant alleles behave like the recessive alleles. Viable alleles were also recovered. They display a cleft in the notum and an altered bristle pattern. These imaginal defects are also seen in some transheterozygotes between viable and lethal alleles and even in some viable allelic combinations between alleles that are homozygous lethal. Pairwise combi-

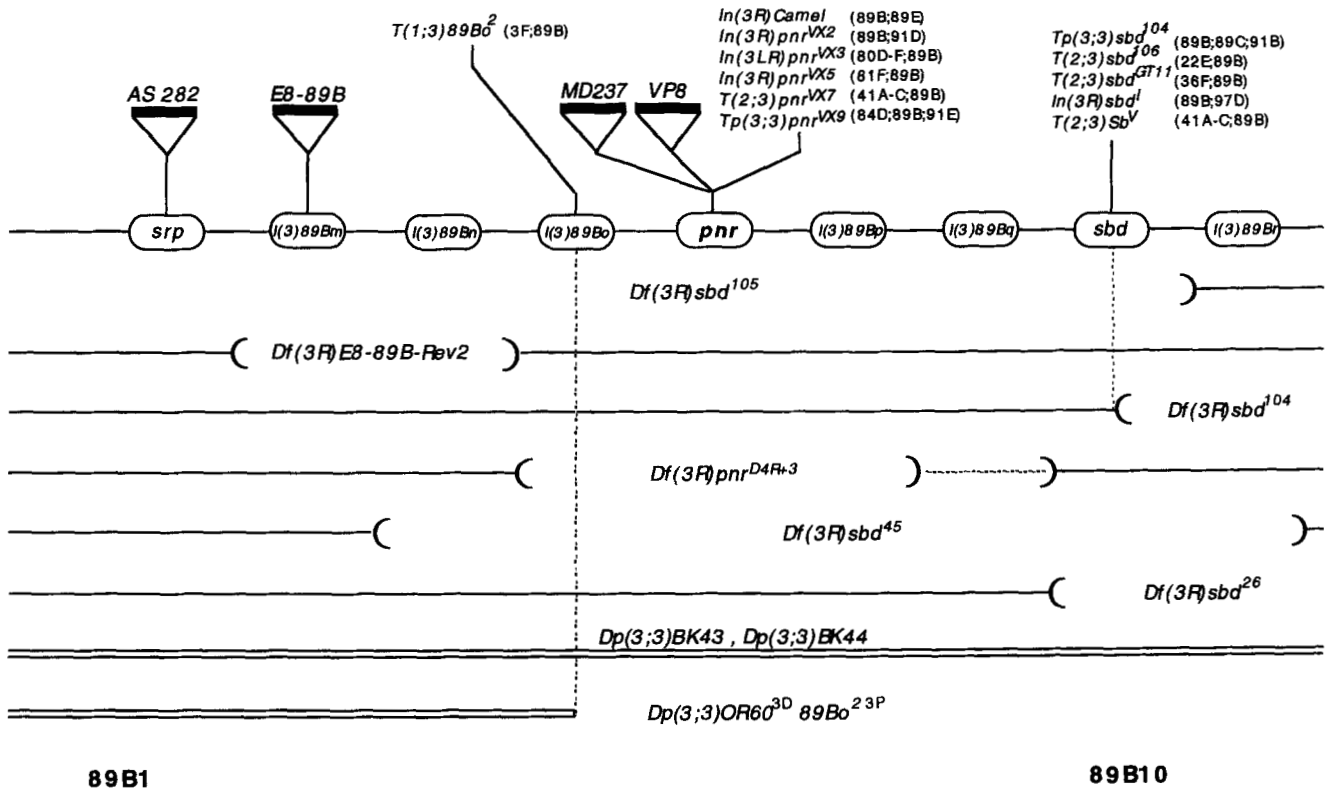


FIGURE 1.—Cytogenetic map of the proximal half of section 89B. *pannier* and the surrounding complementation groups are shown, together with the genetic limits of deletions and rearrangements in this region. The *P[gro⁺,ry⁺]E8(89B)* insert was first described in PREISS *et al.* (1988) and was shown to map in 89B1-7. A single lethal revertant that failed to complement *Df(3R)sbd*$^{45}$ was obtained (*Df(3R)E8–89B-Rev2*) among 270 independently occurring jump events from the excision of this transposon (this study). The dominant *pnr*$^{D4}$ allele is associated with *l(3)Bq*$^{2}$, so it is not known whether *Df(3R)pnr*$^{D4R+3}$, a revertant of *pnr*$^{D4}$, is mutant or deleted for *l(3)89Bq*.

nations were performed for all alleles and suggest a complexity within the structure or the regulatory control of the gene. The three mutant phenotypes will be described separately: the dorsal hole in the embryo, the cleft in the thorax and the altered bristle patterns.

**Distribution of *pannier* transcripts in embryos:** The distribution of *pnr* mRNA in embryos was determined by means of *in situ* hybridization with a digoxigenin-labeled *pnr* probe (Figure 2). At cellular blastoderm *pnr* is expressed in a broad band of dorsally situated cells between 35 and 60% egg length. These cells are the precursors of the amnioserosa and the dorsal ectoderm. At germ band elongation expression remains in the same cells but now extends as far back as the posterior midgut primordium, which is itself not labeled. Up until stage 8, staining in the amnioserosa remains intense but fades from stage 9 onward. Expression in a band of dorsal epidermal cells remains strong, however, right through germ band retraction and dorsal closure. The band of labeled ectodermal cells covers the thoracic and abdominal segments, but the head and the termini do not exhibit staining.

***pannier* is required for viability of the cells of the amnioserosa:** The cuticular phenotypes of embryos homozygous for a number of mutant alleles were examined. In the case of strong alleles, such as *pnr*$^{VX6}$ or *pnr*$^{7G111}$, the dorsal hole is quite extensive (Figure 3c). The pattern of denticles is, however, fairly normal and we were able to identify the ventral and lateral sensory organs of the larval peripheral nervous system (PNS), although due to a contraction of the epidermis that is not held together dorsally, the spacing between them is reduced. In the trunk region, dorsal hairs, Keilin's organs, and kölbchen are present. In the head, both antennal and maxillary sense organs are formed. The cephalopharyngeal skeleton is disrupted: the dorsal arms and dorsal bridges are absent while the mouth hooks and the cirri are present. As a consequence of the lack of dorsal structures, the ventral arms and the unpigmented wall are rotated anteriorly and protrude at the tip of the embryo.

For weaker alleles, the extent of the dorsal hole is reduced and mouthparts are intact. For example, *pnr*$^{D3}$ embryos are open dorsally at the anterior end but the cuticle forms a tube at the posterior end. The different lethal alleles can be ordered into a series according to the extent of the dorsal hole. For example, *pnr*$^{7G111}$ > *Df(3R)sbd*$^{45}$ = *pnr*$^{VX6}$ = *pnr*$^{VP8}$ = *pnr*$^{D1}$ > *pnr*$^{D3}$. *pannier*$^{VX6}$ can be considered as a reference null allele, since it is associated with a small deletion within the gene that

**TABLE 1**

**Description of the loci in the *pannier stubbloid* region**

| Locus | No. of alleles | Phenotype |
|---|---|---|
| *srp* | 4 | Recessive lethal; germ band shortening incomplete; $srp^4$ is semi-viable and associated with a *P[hs-neo$^R$]AS282(89B)* insertion (CATHY MATTHEWS, personal communication) |
| *l(3)89Bm* | 6 | Recessive lethal; weak transient escapers in viable combinations; $l(3)89Bm^1$ is semi-viable and associated with a *P[gro$^+$,ry$^+$]E8(89B)* insertion. The other alleles were obtained from the imprecise excision of this insert |
| *l(3)89Bn* | 0 | Recessive lethal; this complementation group is defined solely by the failure of complentation between *Df(3R)E8-89B-Rev2* and $Df(3R)sbd^{45}$ |
| *l(3)89Bo* | 2 | Recessive lethal; $l(3)89Bo^1$ is semi-viable and fertile as homozygotes but lethal as hemizygotes; $l(3)89Bo^2$ is associated with a *T(1;3)3F;89B* rearrangement. $l(3)89Bo^1$ maps 0,03% proximal to $pnr^{7G111}$ |
| *pnr* | 28[a] | Recessive embryonic lethal; dorsal hole; for gain-of-function and viable phenotypes, see Table 2; $pnr^{V1}$ and $pnr^{VP8}$ map 0,15% proximal to *Sb* |
| *l(3)89Bp* | 6 | Recessive lethal; $l(3)89Bp^5$ is semi-viable and escapers have a distended abdomen and heldout wings; $l(3)89Bp^1$ maps 0,008% distal to $pnr^{7G111}$ |
| *l(3)89Bq* | 2 | Recessive lethal; $l(3)89Bq^2$ was induced together with $pnr^{D4}$; $l(3)89Bq^1$ maps 0,15% distal to $pnr^{7G111}$ |
| *sbd* | 15[b] | Recessive lethal; hypomorphic alleles show good viability, short bristles, shortened wings and legs; $sbd^{GT11}$ associated with *T(2;3)GT11* |
| *I(3)89Br* | 1 | Recessive semilethal; weak transient escapers |

[a] $pnr^{7G111}$ was described by JÜRGENS *et al.* (1984) and *ln(3R)Camel* by KARCH *et al.* (1985).

[b] Seven, previously known, recessive and dominant alleles of *sbd* (LINDSLEY and ZIMM 1992) and *T(2;3)GT11* (GUBB *et al.* 1990), which has been shown to be mutant for *sbd* (this study).

would remove all but nine amino acids of the *pnr* protein (RAMAIN *et al.* 1993). Though the chromosome $pnr^{7G111}$ has been cleaned by crossing over, a closely linked altered genetic function may explain the stronger phenotype. The molecular nature of the allele $pnr^{7G111}$ is unknown.

To study the origin of the dorsal hole, embryos derived from a cross between flies heterozygous for $pnr^{7G111}$ were fixed at different stages with glutaraldehyde and examined with Nomarski optics. One-quarter of the embryos are mutant. Before stage 12, the mutant embryos are indistinguishable from their wild-type siblings and at this level of observation development appears to proceed normally. Throughout germ band retraction, however, in a quarter of the embryos the cells of the amnioserosa can be seen to disintegrate and then disappear (Figure 3b). The cell death does not extend very far ventrally and it is unclear how much dorsal epidermis is lost. However, although the dorsal arms of the mouthparts are later found to be missing, all cells of the larval peripheral nervous system PNS can be identified so it is probable that the loss of epidermal cells is restricted to the most dorsal ones.

Cell death was studied in more detail by means of acridine orange staining of embryos derived from a cross between $pnr^{VX6}$ heterozygotes. The cells of the amnioserosa took up the stain and staining was the most intense at around stage 11 (Figure 3e). At this stage no staining could be seen in wild-type control embryos (Figure 3f). In wild-type embryos the amnioserosa persists until dorsal closure, when it changes to a columnar epithelium and invaginates: only at stage 17 do the cells undergo apoptotic cell death (RUGENDORFF *et al.* 1994). In addition, in *pnr* mutant embryos a band one to two cells deep of the dorsal-most epidermis could also be seen to be labeled, although less intensely.

Although the dorsal cells are physically lost only late in development, they are morphologically abnormal shortly after the onset of gastrulation. This was apparent in studies of $pnr^{VX6}$ embryos derived from a strain carrying an enhancer-trap P-*lacZ* fusion gene inserted 99 nucleotides upstream of the origin of transcription of the *emc* locus (GARRELL and MODOLELL 1990). This insert, $emc^{P5c}$[*lac,ry$^+$*], causes the expression of *lacZ* in the cells of the amnioserosa and some cells of the dorsal epidermis. The large nuclei of cells of the amnioserosa are very visible in such stained animals. In the mutant embryos at ~4 hr after egg laying, the nuclei of the marked cells degenerate into densely staining fragments of various sizes (not shown). Similar observations were made using marked *anti-ush* antibodies that also stain the nuclei of the amnioserosa (M. HAENLIN, unpublished results).

Following the loss of the cells of the amnioserosa, dorsal closure fails to occur and the yolk sac can be seen to protrude from the embryo. Fusion of the anterior and posterior midgut takes place however. Subsequently the embryo changes shape slightly; not being held together dorsally the gut protrudes and the epidermis contracts ventrally before cuticle deposition. Nevertheless, much of the remainder of development proceeds normally and a study of 24-hr-old embryos stained with the anti-HRP antibody reveals a normal structure for the central nervous system (not shown).

**TABLE 2**

**Description of mutant *pannier* alleles**

| Alleles | Mutagen | Chromosome | Phenotype | Comments |
|---|---|---|---|---|
| *Camel* | X-ray | ? | *Camel*/+: show a strong thoracic cleft and an *iab5* phenotype; lack IV and PV<br>*Camel*/*DF(3R)sbd*$^{45}$: die as headless pupae; lack DC and Sc | In(3R)89B;89E; hypomorphic + neomorphic effects |
| *Camel*$^{R+1}$ | Spontaneous | *st Camel e* | *Camel*$^{R+1}$/+: no thoracic cleft but lack IV and PV<br>*Camel*$^{R+1}$/*Df(3R)sbd*$^{45}$: die as head-less pupae; lack DC and Sc. | — |
| *V1* | Spontaneous | *Sb* | *V1*/*V1*: viable<br>*V1*/*Df(3R)sbd*$^{45}$: semi-viable, lack bristles and occasionally show head defects | Hypomorph |
| *VX1* | X-ray | *st e* | *VX1*/+: occasionally lack one DC<br>*VX1*/*VX1*: die as larvae or early pupae<br>*VX1*/*V1*: viable and show a much stronger loss of bristle phenotype than *Df(3R)sbd*$^{45}$/*V1* | Gain of function<br>Antimorphic; normal cytology<br>Deletion in coding sequence-truncated protein |
| *VX2* | X-ray | *st e* | *VX2*/*VX2* and *VX2*/*Df(3R)sbd*$^{45}$: semi-viable with cleft loss of bristles and occasionally head defects | In(3R)89B;91D; hypomorph |
| *VX3* | X-ray | *st e* | *VX3*/*VX3* and *VX3*/*Df(3R)sbd*$^{45}$: viable with cleft and loss of bristles<br>*VX3*/+: females die; however after a long selection of escapers a normal balanced stock has been established (when outcrossed, all *VX3*/+ females die) | In(3LR)80D-F;89B; special allele because unlike the other viable alleles, *VX3* suppresses the bristle phenotype of *D1*, *D2* and *D3* |
| *VX4* | X-ray | *st e* | Same as *VX1* | Same as *VX1* |
| *VX5* | X-ray | *st e* | *VX5*/*VX5*: viable with cleft and extra DC but occasionally lack DC<br>*VX5*/*Df(3R)sbd*$^{45}$: viable with stronger cleft and loss of bristles | In(3R)81F;89B; hypomorph; *VX5* viable over all other alleles except *D4*; shows variegating position effect |
| *VX6* | X-ray | *kar*$^{2}$ *ry*$^{506}$ | Embryonic lethal, same as deficiency | Amorph; normal cytology; deletion of most of the coding sequence |
| *VX7* | X-ray | *kar*$^{2}$ *ry*$^{506}$ | Embryonic lethal; same as deficiency; *VX7*/+ lack IV bristles | T(2;3)41A-C;89B; amorph + dominant trans-sensing effects; hypomorph insertion within the gene |
| *VP8* | *P*[*ry*$^{+}$,Δ*2-3*] *(99B)* | *ry*$^{506}$ *Sb e P*[*ry*$^{+}$,*D2-3*] *(99B)* | Embryonic lethal; dorsal hole; *VP8*/*D3* give escapers whereas *Df(3R)sbd*$^{45}$/*D3* do not | |
| *VX9* | X-ray | *st e* | *VX9*/+ show a dominant extra-wing-veing phenotype, lack PS bristles<br>*VX9*/*Df(3R)sbd*$^{45}$: viable with weak cleft, loss of bristles and extra wing veins<br>*VX9*/*VX9* die (perhaps this is due to other lethals on same chromosome) | Tp(3;3)84D;89B;91E; new order: 61A-84D/89B-91E/84E-89B/91F-100F;<br>Neomorph? |
| *MD237* (from Madrid) | *P*[*ry*$^{+}$,Δ*2-3*] *(99B)* | ? | *MD237*/+: show a thoracic cleft; occasionally lack IV and PV<br>*MD237*/*MD237*: viable<br>*MD237*/*Df(3R)sbd*$^{45}$: semi-viable, lack bristles and occasionally show head defects | Hypomorph + neomorphic effects ? |
| *7G111* (from Tübingen) | EMS | *ru h th st cu sr e*$^{s}$ *ca* | Embryonic lethal: dorsal hole broader than for *VX6*/*VX6* or *Df(3R)sbd*$^{45}$/*Df(3R)sbd*$^{45}$<br>*7G111*/*D3* give escapers whereas *Df(3R)sbd*$^{45}$/*D3* do not<br>*7G111*/*VX5* show extra DC unlike *Df(3R)sbd*$^{45}$/*VX5* | Special allele |
| *D1* | EMS | ? | *D1*/*D1*: embryonic lethal; dorsal hole<br>*D1*/+ show a slight cleft and extra DC and Sc (PV occasionally absent) | Gain of function; antimorphic; point mutation in first zinc finger domain |
| *D1*$^{R+1}$ | EMS | *st D1 e* | Embryonic lethal; dorsal hole | Amorph |
| *D1*$^{R+2}$ | EMS | *st D1 e* | Embryonic lethal; dorsal hole | Amorph |

**TABLE 2**

**Continued**

| Alleles | Mutagen | Chromosome | Phenotype | Comments |
|---|---|---|---|---|
| *D2* | EMS | ? | *D2/D2*: embryonic lethal; dorsal hole<br>*D2/+*: show some extra DC & Sc | Same as D1 |
| $D2^{R+1}$ | EMS | *st D2 e* | Embryonic lethal; dorsal hole | Amorph |
| $D2^{R+2}$ | EMS | *st D2 e* | Embryonic lethal; dorsal hole<br>*VX4/*$D2^{R+2}$ die as pupae whereas *VX4/ Df(3R)sbd*$^{45}$ die as embryos or larvae (first instar) | Hypomorph?<br>The *VX4* cross shows that this allele has retained some *D2* activity since *D2/VX4* is viable |
| $D2^{R+3}$ | EMS | *st D2 e* | Wild type | Precise revertant? |
| *D3* | EMS | ? | *D3/D3*: embryonic lethal; dorsal hole less extreme<br>*D3/+*: show a slightly stronger cleft than *D1* and occasionally extra DC | Same as *D1* |
| *D4* | EMS | *TM3 Sb Ser* | *D4/D4*: embryonic lethal; dorsal hole<br>*D4/+*: show a strong cleft but less extreme that Camel; lack PV, IV and sometime AO; have more DC and Sc than *D1/+* | Gain of function; antimorphic; Coinduced with *l(3)89Bq*$^2$; all *D4* derivatives carry *l(3)89Bq*$^2$ point mutation in first zinc finger domain |
| $D4^{R+1}$ | EMS | *TM3 D4 Sb Ser* | Embryonic lethal; dorsal hole<br>$D4^{R+1}$*/VX5*: show extra DC unlike *Df(3R)sbd*$^{45}$*/VX5* | Hypomorph?<br>The *VX5* cross shows that $D4^{R+1}$ is not an amorph |
| $D4^{R+2}$ | X-ray | *TM3 D4 Sb Ser* | Embryonic lethal; dorsal hole | Amorph |
| $D4^{R+4}$ | X-ray | *TM3 D4 Sb Ser* | Embryonic lethal; dorsal hole | Amorph |
| $D4^{R+5}$ | X-ray | *TM3 D4 Sb Ser* | Embryonic lethal; dorsal hole<br>$D4^{R+5}$*/VX5*: is semi-lethal whereas *Df(3R)sbd*$^{45}$*/VX5* is viable (*D4/VX5* is lethal) | The *VX5* cross shows that $D4^{R+5}$ has retained some *D4* antimorphic activity. |
| $D4^{R+6}$ | X-ray | *TM3 D4 Sb Ser* | Embryonic lethal; dorsal hole<br>$D4^{R+6}$*/+* show often one drooping wing | Amorph |

Bristle nomenclature: IV, inner vertical; PV, postvertical; DC, dorsocentral; Sc, scutellar; PS, presutural; AO, anterior orbital.

To investigate whether the dorsal cells die as a result of their dorsal position in the embryo or because of their tissue type, embryos doubly mutant for $pnr^{7G111}$ and *dorsal (dl)* or *cactus (cact)* were constructed. Females homozygous for *dl* produce embryos in which all cells develop as dorsal epidermis (NÜSSLEIN-VOLHARD 1979), whereas females homozygous for *cact* produce partially ventralised embryos in which the denticles characteristic of the ventral cuticle extend around the entire circumference of the embryo (ROTH *et al.* 1991). *pannier* embryos derived from *dl* females differentiated virtually no cuticle, occasionally small vesicles of cuticle are found (not shown). In contrast *pnr* embryos derived from *cact* females are identical to their $pnr^+$ siblings and display a continuous tube of cuticle and no dorsal hole (not shown). We conclude that cells bearing the characteristics of the amnioserosa or dorsal-most hypoderm, whatever their position in the embryo, fail to differentiate in the absence of the *pnr* product. They either die or fail to secrete cuticle.

Embryos mutant for *emc* are lethal and display mild cuticular abnormalities (CUBAS *et al.* 1994). The genes *pnr* and *emc* have been found to interact for the formation of the thoracic sensory bristles (see below). Therefore we have examined the phenotype of embryos doubly mutant for *emc* and *pnr*. Two alleles, $pnr^{7G111}$ and $pnr^{D1}$, were combined with either $emc^1$, $emc^{FX119}$ or $emc^{AP6}$ (MOSCOSO DEL PRADO and GARCIA-BELLIDO 1984a; GARCIA-ALONSO and GARCIA-BELLIDO 1988; ELLIS 1994). Remarkably, these double-mutant embryos, while lethal, do not display a dorsal hole, the cuticle forms a continuous tube (Figure 3d). Dorsally the cuticle is lacking in dorsal hairs, although a few sparse ones remain. The ventral denticle bands are present.

The expression pattern of *lacZ* (amnioserosa and dorsal epidermis) characteristic of *emc* flies is unchanged in $emc^{P5c}$[*lac,ry*$^+$] $pnr^{7G111}/pnr^{7G111}$, $emc^{P5c}$[*lac,ry*$^+$] $pnr^{D1}/pnr^{D1}$ and $emc^{P5c}$[*lac,ry*$^+$] $pnr^{VX6}/pnr^{VX6}$ embryos (not shown). Two other inserts of a *P-lacZ* fusion gene into the *emc* locus (see MATERIALS AND METHODS), $emc^{ip11}$ and $emc^{ip12}$, show identical *lacZ* patterns to that of $emc^{P5c}$.

Since the bristle phenotype of viable *pnr* mutants is strongly modified by varying the dosage of *ac* and *sc* (RAMAIN *et al.* 1993), we examined lethal embryonic *pnr* mutants in the presence of one or three copies of $ac^+$ and $sc^+$. The embryonic phenotype was unchanged (not shown). The pattern of *ac* expression (by *in situ* antibodies) in $pnr^-$ embryos is essentially indistinguishable from wild type (M. HAENLIN, unpublished results).

**The pattern of expression in imagos:** The allele $pnr^{MD237}$ is homozygous viable and is associated with a slight thoracic cleft. It carries a GAL4 insertion. When

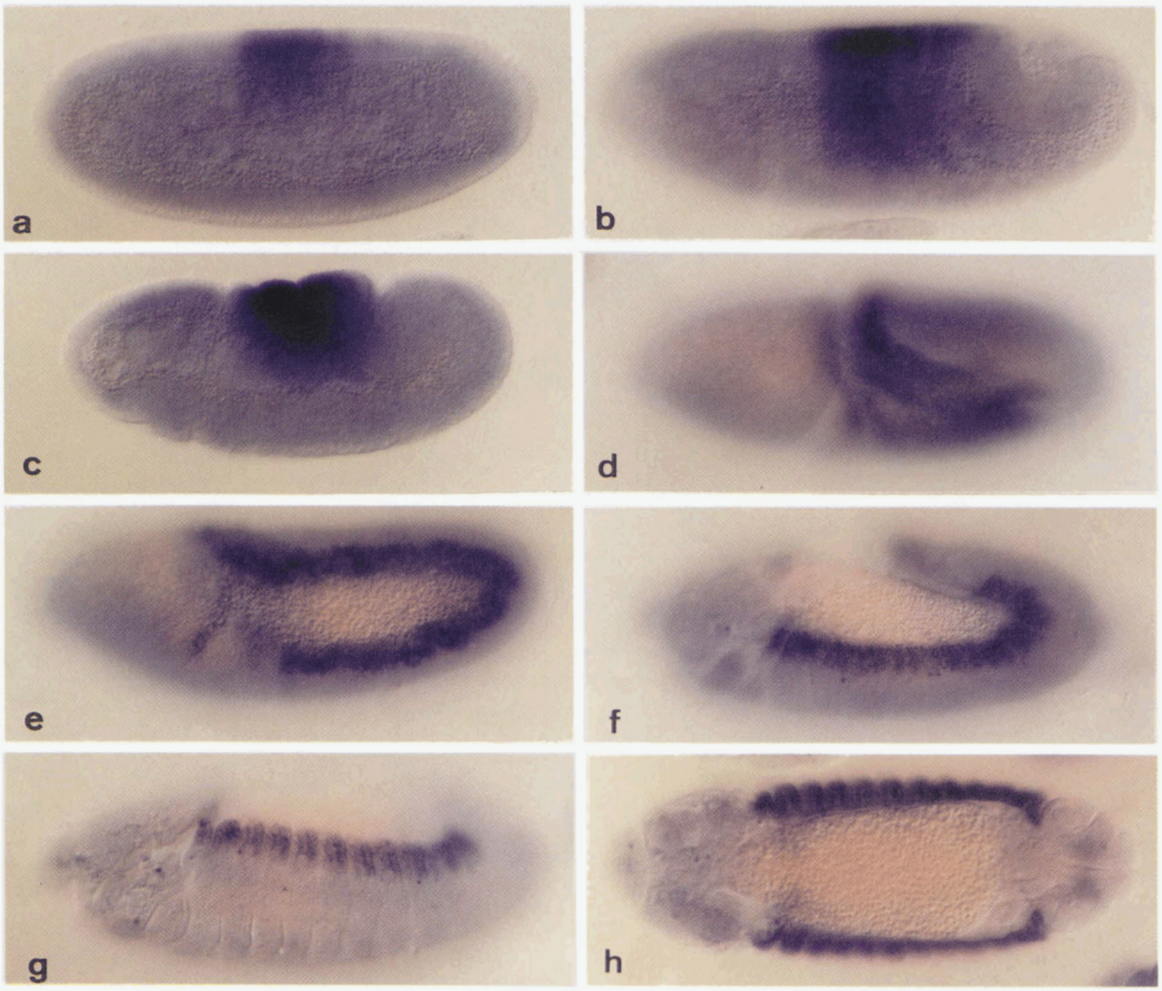


FIGURE 2.—The pattern of expression of *pnr* mRNA in whole mount embryos. At cellular blastoderm (a), *pnr* is expressed in a broad band of cells at the position of the future amnioserosa. These cells contract during gastrulation and germ band elongation (b and c), and staining is apparent in the amnioserosa and part of the dorsal epidermis at the end of germ band elongation (d and e). At germ band retraction staining is no longer apparent in the cells of the amnioserosa but remains strong in the dorsal epidermis (f–h).

flies carrying this insertion are crossed to flies carrying UAS $y^+$, the *y* gene is expressed under the control of the *pnr* promoter (M. CALLEJA and G. MORATA, unpublished results). Flies (that are otherwise mutant for *y*) display a broad stripe of $y^+$ tissue along the dorsal midline (Figure 4a). The edges of the stripe are remarkably straight. This band extends along the full length of the thorax and abdomen. In the head $y^+$ tissue is restricted to the occipital region between the eyes. On the thorax the band includes the dorsocentral bristles. When crossed to flies carrying UAS *lacZ*, the $pnr^{MD237}$ strain expresses *lacZ* in the imaginal discs. A similar stripe can be seen along the dorsal midline in adult flies due to the accumulation of persisting galactosidase (Figure 4b). The pattern of *lacZ* staining in the thoracic discs is very similar to that of *pnr* mRNA as described by RAMAIN *et al.* (1993; Figure 4d). Expression is more extensive than previously observed in the eye-antennal disc, however, and staining extends over most of the dorsal half of the disc (Figure 4e).

**The thoracic cleft:** Viable mutant *pnr* flies display a cleft in the center of the thorax (Figure 4c). This is seen in flies homozygous for recessive viable alleles, such as $pnr^{V1}$, $In(3R)pnr^{VX2}$, $In(3LR)pnr^{VX3}$ and $In(3R)pnr^{VX5}$, that display a stronger phenotype over deletions and over most recessive lethal alleles and are therefore hypomorphic for this trait. Flies heterozygous for the dominant alleles $pnr^{D1}$, $pnr^{D2}$, $pnr^{D3}$, $nr^{D4}$, $pnr^{Camel}$ and $pnr^{MD237}$ also form a cleft. Finally, occasional adult escapers of transheterozygotes of otherwise homozygous lethal alleles, such as $pnr^{D3}/pnr^{7G111}$ and $pnr^{D3}/pnr^{VP8}$, are recovered and they display a very extreme thoracic cleft.

Mutant alleles can be placed in a series according to the severity of the cleft. In flies displaying the mildest phenotype, the cleft is only distinguishable by the slight spacing on the center of the notum between the median rows of microchaetae. Interestingly, this phenotype is also observed in flies carrying four doses of $pnr^+$. In animals with more extensive clefts, the two halves of the scutum are reduced, and in $pnr^-$ clones ($pnr^{VX6}$),

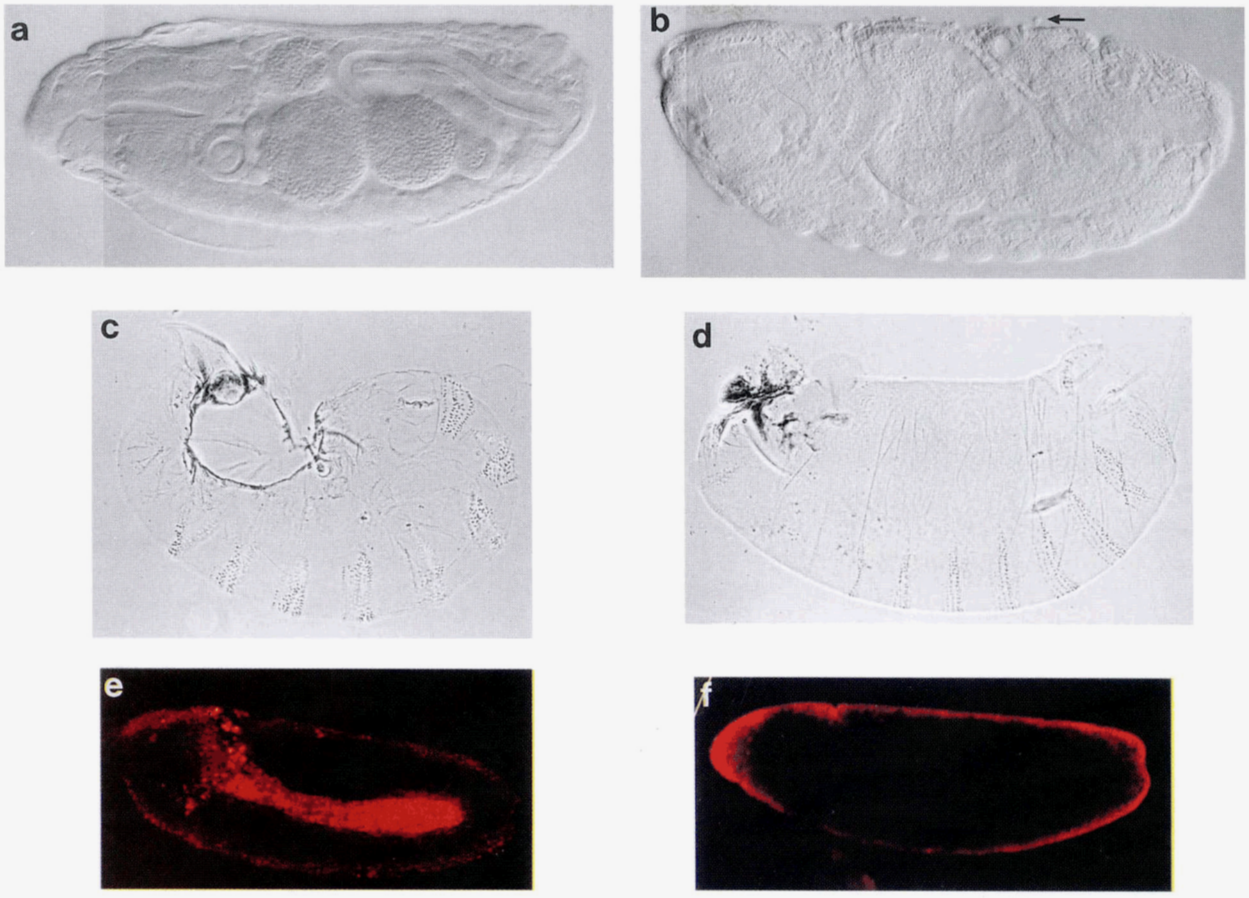


FIGURE 3.—Mutant *pnr* phenotypes. Anterior is to the left. Embryos in a and b were fixed in glutaraldehyde and photographed under Nomarski optics. (a) A late wild-type embryo after germ band retraction and dorsal closure. (b) A $pnr^{7G111}$ mutant embryo in which germ band retraction, but not dorsal closure, has occurred. Dissociated dying cells of the amnioserosa can be seen along the dorsal edge of the embryo (arrow). c and d show the cuticles of $pnr^{7G111}$ and $pnr^{7G111}$ $emc^{1}$ embryos, respectively. The dorsal hole characteristic of *pnr* mutants (c) is suppressed in the double mutant (d). The head remains abnormal however. (e) A stage 11 $pnr^{VX6}$ embryo stained with acridine orange. Staining is very intense in the amnioserosa and weaker in a few cells of the dorsal epidermis. (f) A wild-type embryo, also at stage 11, shows no staining.

the entire scutellum disappears. In the strong viable or pupal lethal allelic combinations, the defects are not restricted to the thorax. The head is often deformed or split in the regions of the postvertical and the vertical bristles. The eyes are sometimes smaller or even missing. In several pupal lethal allelic combinations, the head is entirely missing. The dorsal abdomen exhibits a regular antero-posterior cleft formed by split tergites. Finally, in certain specific viable transallelic combinations, males have a rotated genitalia.

The cleft presumably results from a failure of the two halves of the thorax to fuse correctly at the dorsal midline during metamorphosis. It is possible that the midline stripe of *pnr*-expressing cells are lost. Late third instar larval and early pupal thoracic discs were stained with acridine orange but no cell death was observed (not shown). This does not exclude the possibility of cell death during pupal stages. The abnormalities, however, can extend further laterally than the *pnr*-expressing band of cells and thus include cells that express *pnr* and also non-*pnr*-expressing cells. To investigate this phenotype further, homozygous thoracic clones of $pnr^{VX6}$, $pnr^{7G111}$ and $pnr^{D1}$ were examined. Remarkably, when adjacent to the thoracic midline, these can cause a sizeable bilateral cleft involving most of the scutum in spite of a relatively small number of mutant cells that often only abuts the midline for a short distance (Figure 4c). Of 51 clones, 22 touched the thoracic midline and were all associated with a cleft thorax. Clones homozygous for $pnr^{D1}$ behaved similarly: of 24 clones, six touched the midline and each of these was associated with a cleft. The extensive loss of thoracic tissues in clones touching the midline involves both cells of the clone that are mutant and adjacent cells of wild-type genotype. Therefore the mutant cells can cause a non-autonomous loss of adjacent wild-type cells.

In contrast quite large clones of cells that run close to, but do not abut, the thoracic midline develop normally (albeit with altered bristle patterns) and do not induce a cleft (Figure 4g). None of the 29 nonmidline clones of $pnr^{VX6}$ and $pnr^{7G111}$ (and 18 $pnr^{D1}$ clones) were associated with clefts. Many of these clones include cells

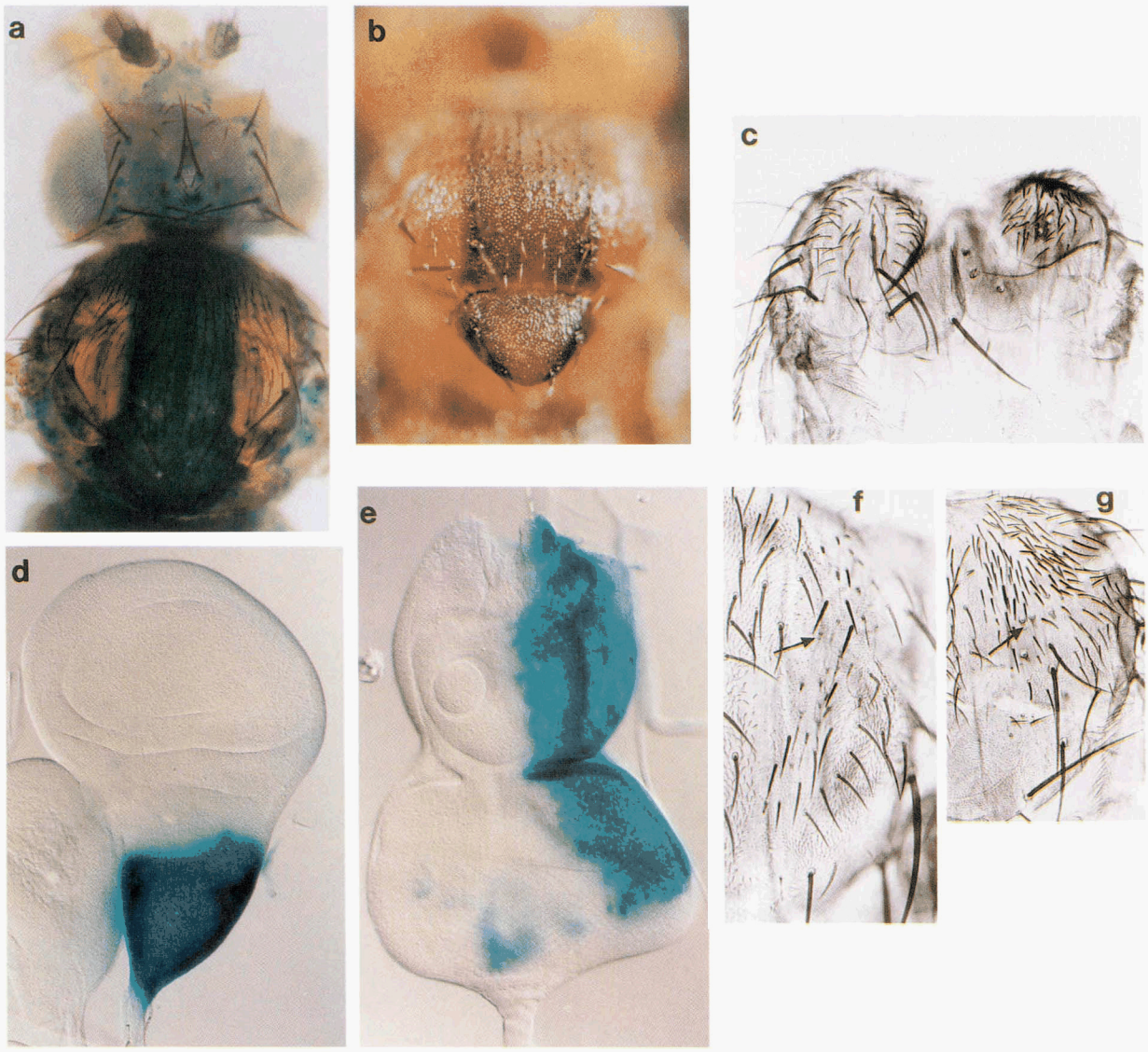


FIGURE 4.—The pattern of expression of *pnr* in flies and in larval imaginal discs. (a) Flies carrying a GAL4 insertion in *pnr,* in the presence of UAS *lacZ,* display a dorsal stripe of *lacZ* expression, as seen here on the thorax. (b) Flies carrying a GAL4 insertion in *pnr,* in the presence of UAS $y^+$, reveal a stripe of $y^+$ tissue along the dorsal midline, seen here on the thorax. (c) The thorax of an adult fly bearing a clone of cells homozygous mutant for $pnr^{7G111}$. This clone touched the midline and caused the cleft appearance of the thorax. (d) The pattern of *lacZ* staining seen in the thoracic disc of flies of the same genotype as in a. Staining covers a broad dorsal area of the disc. (e) The pattern of *lacZ* staining seen in the eye-antennal disc of flies of the same genotype as in a. Staining is seen in the dorsal half of the disc. f shows the hemithorax of a wild-type fly bearing a clone of cells marked with *pawn.* The *pawn* marker causes transparent bristles often with the tip missing and with a small spur at the base that is not visible in the photograph (arrows). g shows the hemithorax of a fly containing a clone of cells homozygous for *pawn* and $pnr^{D1}$ that did not touch the thoracic midline and was not associated with a cleft thorax. Note the development of additional thoracic macrochaetae at the dorsocentral site.

that lie within the *pnr*-expressing stripe. In these cases the mutant cells are perfectly viable. We conclude that tissue loss and formation of a cleft only occurs when cells at or very close to the midline are mutant.

Clones of cells doubly mutant for *emc* and *pnr* ($emc^1$ $pnr^{VX6}$ and $emc^1$ $pnr^{7G111}$) were also examined and these too can induce the formation of a cleft when adjacent to the midline (not shown).

**The pattern of sensory bristles:** Alleles of *pnr* display missing and/or extra bristles. The phenotypes have been examined in viable alleles and allelic combinations and somatic clones of lethal alleles. The macrochaetes of the head and thorax are affected; there is no effect on microchaetes nor on the wing bristle pattern. Some alleles cause the development of additional macrochaetes at precise locations together with a loss of bristles at others; other alleles cause only either a loss of macrochaetes or a gain of new ones. Each transallelic combination has its own constant and precise bristle pattern that may differ from the two homozygotes.

There is no apparent relationship between the severity of the thoracic cleft and the presence or absence of specific bristles. Thus the deformation of the thorax does not determine the bristle pattern. Varying the number of wild-type doses of $pnr^+$ has no effect on the distribution of bristles: monosomic, trisomic and tetrasomic $pnr^+$ flies have a normal bristle pattern.

In an attempt to understand the role of this gene on the positioning of bristles, we have examined a large number of alleles. From an extensive study of the complementation patterns and phenotypes we have grouped the *pnr* alleles into four classes (see Table 2 for the salient points relative to each allele).

**Loss of function alleles:** *Class 1: Viable recessive alleles:* There are four viable recessive alleles. $pannier^{V1}$ and $In(3R)pnr^{VX2}$ generally cause a loss of two or three macrochaetes: one or two dorsocentrals, the inner vertical bristles, and sometimes the postvertical, whereas $In(3LR)pnr^{VX3}$ flies lack one dorsocentral bristle and the inner vertical. $In(3R)pnr^{VX5}$ is the only allele for which flies may display either a loss of the dorso-central bristles or a tuft of supernumerary dorso-central bristles. Furthermore, some animals may display a loss of dorsocentral bristles on one hemithorax and a cluster of these bristles on the other. It is likely that this variability results from position effect variegation, which can spread linearly along a chromosome from a heterochromatic breakpoint (see HENIKOFF 1994 for a recent review), since $In(3R)pnr^{VX5}$ has breakpoints that juxtapose the 89B region to the centric 3R heterochromatin (Figure 5). The four viable alleles are also viable over deletions and in such hemizygous flies more bristles are missing, suggesting that they are hypomorphic in their effects on the bristle pattern.

*Class 2: Lethal recessive alleles:* There are 12 alleles in this group and nine of these were obtained as revertants of dominant alleles. They are all lethal over one another (except $pnr^{7G111}/pnr^{VP8}$, which gives some adult escapers with missing dorsocentral and scutellar bristles) but are all viable over $In(3R)pnr^{VX5}$ and occasionally over $pnr^{V1}$. Such viable transallelic combinations with $In(3R)pnr^{VX5}$ display a loss of bristles (a subset is shown in Figure 6). Flies show a loss of up to four macrochaetes (dorsocentrals, inner verticals and sometimes one scutellar). Several of these combinations exhibit an additional posterior postalar bristle. Some show patterns similar to that of $pnr^{VX5}$ over a deficiency and may thus represent null alleles. This is the case for the known null $pnr^{VX6}$. The phenotype of $pnr^{7G111}/In(3R)pnr^{VX5}$ and also $pnr^{D4R+1}/In(3R)pnr^{VX5}$ differs from the others in that these flies exhibit three dorsocentral bristles per hemithorax, suggesting that neither $pnr^{7G111}$ nor $pnr^{D4R+1}$ are amorphic alleles.

**Gain of function alleles:** *Class 3: Dominant alleles causing a loss of bristles:* Two distinct groups of mutants form class 3. Flies heterozygous for either of the two alleles $pnr^{VX1}$ and $pnr^{VX4}$, lack occasionally one dorsocentral bristle (Figure 7). This phenotype is never observed in flies from stocks heterozygous for a deletion of the gene. The cytology of these two X-ray-induced alleles is normal; they were mapped meiotically near *Sb*. A molecular analysis has shown that they are associated with small deletions in the coding sequence of the gene (RAMAIN *et al.* 1993). The dominant effect is enhanced over viable alleles and so $pnr^{VX1}/pnr^{V1}$ and $pnr^{VX4}/pnr^{V1}$ flies are viable and are missing up to six macrochaetae. This is the strongest mutant bristle phenotype that we have observed in a viable combination. The loss of bristles is greater than that seen in $pnr^{V1}/Df(3R)sbd^{45}$, $pnr^-$ flies. In a gene dosage analysis these alleles were found to behave as antimorphs (RAMAIN *et al.* 1993). Clones homozygous for either mutant also have missing bristles on the thorax; the head bristles are all present. Three other mutants, *In(3R)Camel*, $Camel^{R+1}$ and $pnr^{MD237}$, show a dominant loss of postverticals and innerverticals from the head. They are associated with rearrangements that probably disrupt a critical element of the promoter.

*Class 4: Dominant alleles causing a gain of bristles:* Four alleles, $pnr^{D1}$, $pnr^{D2}$, $pnr^{D3}$ and $pnr^{D4}$, when heterozygous, cause, simultaneously, a loss of bristles (postvertical) on the head and a gain of bristles (dorsocentral) on the notum (Figure 7). Flies mutant for $pnr^{D4}$, the strongest allele, display up to five or six dorsocentrals per hemithorax and sometimes lack the inner-vertical and anterior orbital bristles. They are all EMS-induced alleles and have been meiotically mapped (except for $pnr^{D4}$, which was found on a TM3 balancer chromosome) near *Sb*. A molecular analysis has shown that these alleles, too, are associated with lesions in the coding sequence of the gene (RAMAIN *et al.* 1993). They can be ordered in an allelic series according to the severity of the altered bristle pattern in heterozygous flies. Like class 3 alleles, many of the phenotypes of class 4 mutants are enhanced by the viable alleles. Thus transheterozygous flies of dominant alleles over $pnr^{V1}$ may display up to 12 dorso-central bristles per hemithorax but lack all postvertical and also scutellar bristles (Figure 7). Clones homozygous for $pnr^{D1}$ show a phenotype similar to the heterozygotes and have additional bristles. Like class 3 mutants, and in spite of the opposite phenotype, they behave as antimorphs in a gene dosage analysis (RAMAIN *et al.* 1993). The class 3 alleles complement the lethality of class 4 alleles: for example, $pnr^{VX1}/pnr^{D1}$ give fertile flies with eight dorsocentrals and no posterior postalars.

**Alleles of class 4 are sensitive to the dosage of *extramacrochaetae*:** Animals mutant for *emc* display additional bristles, and mutant alleles of this gene can be arranged into a clear allelic series (GARCIA-ALONSO and GARCIA-BELLIDO 1988). The mutant phenotype of animals heterozygous for class 4 alleles changes dramatically when the number of copies of the *emc* gene is varied. Data for $pnr^{D1}$ are shown in Figure 8, but all four alleles behave similarly. With decreasing amounts of $emc^+$ the number of dorsocentral (and other) bristles increases. The effects can be spectacular: $pnr^{D1}/+$ animals simultaneously mu-

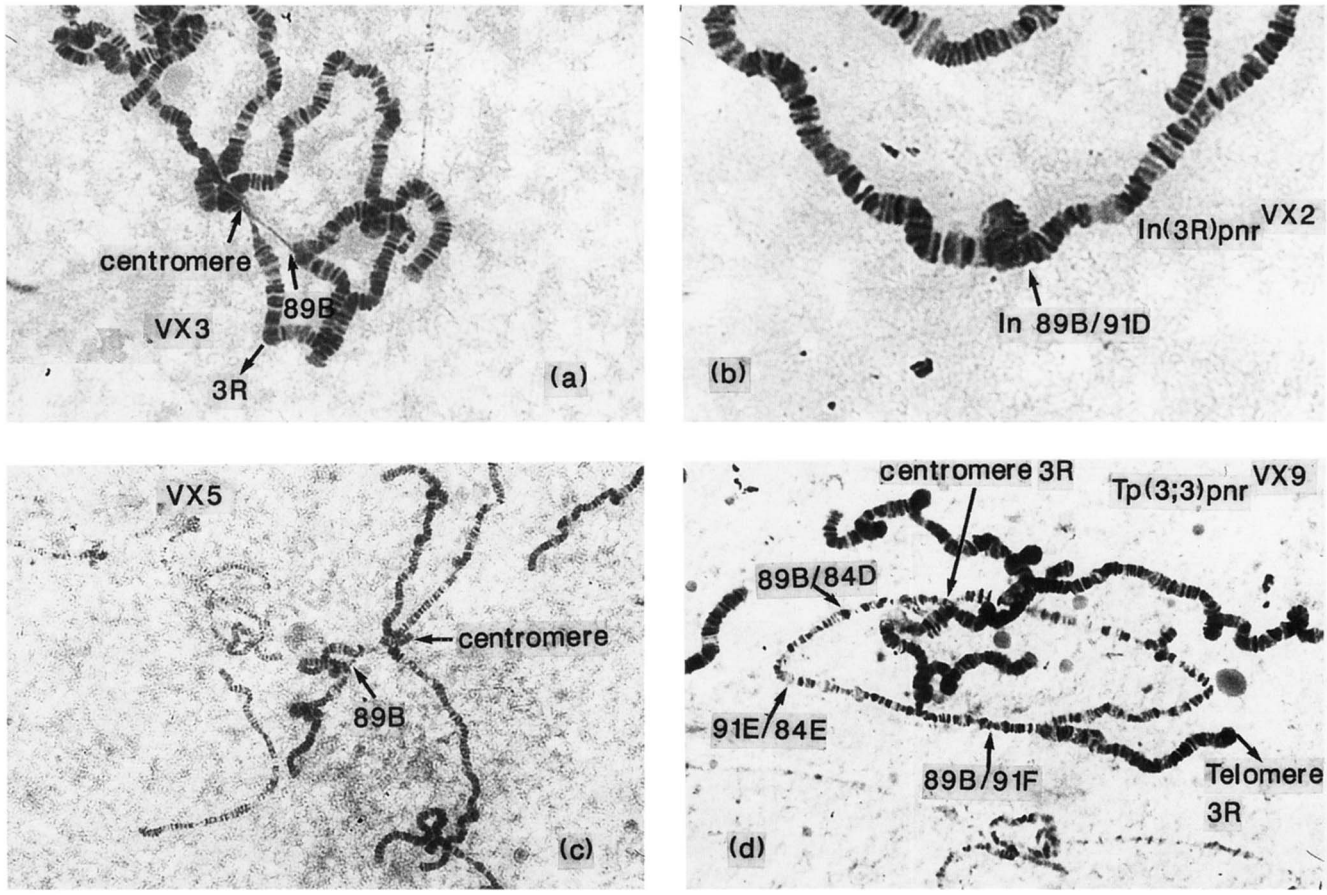


FIGURE 5.—Cytology of the chromosomes bearing rearrangements mutant for *pnr*. a shows *In(3LR)pnr*$^{VX3}$, which breaks at 89B and 80D-F. b shows *In(3R)pnr*$^{VX2}$, which breaks at 89B and 91D. c shows *In(3R)pnr*$^{VX5}$, which breaks at 89B and 81F. d shows *Tp(3;3)pnr*$^{VX9}$, the new order of which is 61A-84D/89B-91E/84E-89B/91F-100F.

tant for a strong viable *emc* combination display up to 55 macrochaetae on the thorax, compared to the 11 seen in the wild-type fly. Increasing the *emc*$^{+}$ copy number partially suppresses the dominant phenotype of extra dorso-central bristles. This suggests that the *pnr* mutants of class 4 and *emc* act synergistically to induce extra bristles. In contrast, the phenotype of animals mutant for all other classes of *pnr* alleles does not change when the dose of *emc*$^{+}$ is varied (not shown).

One possibility is that *pnr* and *emc* function together in the same pathway of *ac-sc* repression in those parts of the epithelium where both are present. Therefore animals doubly mutant for *emc* and *pnr* mutants causing a loss of bristles were examined. Two viable double mutant genotypes are shown in Figure 8. In *emc*$^{1}$ *pnr*$^{V1}$/ *emc*$^{bel}$ *pnr*$^{VX1}$ flies, characteristics of both *emc* and *pnr*$^{V1}$/ *pnr*$^{VX1}$ were observed. However, the *emc*-specific pattern of additional bristles is found outside the domain of *pnr* expression. Within this domain, loss of bristles is the rule and so *pnr* appears to be epistatic over *emc*.

## DISCUSSION

***pannier* is required for viability of cells situated dorsally in embryos and imaginal discs:** Lethal alleles of *pnr* (including the null allele, *pnr*$^{VX6}$) die as embryos with a dorsal hole in the larval cuticle. Our results show that this is due to cell death. In wild-type embryos the cells of the amnioserosa undergo apoptosis at stage 17 after the completion of dorsal closure (RUGENDORFF *et al.* 1994). Acridine orange staining for *pnr*$^{-}$ embryos reveals apoptosis of the amnioserosa as early as stage 11. It is likely that the dorsal-most cells of the ectoderm are also lost since they stain positively with acridine orange, albeit rather weakly. Furthermore, the dorsal-most structures of the mouthparts are missing and double mutant *dl pnr* embryos do not differentiate cuticle (*dl* embryos are composed of cells with characteristics of both the amnioserosa and the dorsal ectoderm, RAY *et al.* 1991). Alternatively, the dorsal epidermis may fail to secrete cuticle. Perhaps the fate of the dorsal epidermal cells is wrongly specified; the cells do not die because of the lack of some basic metabolic feature, since there is no dorsal hole in double mutant *pnr emc* embryos. Transcripts of *pnr* are restricted to the amnioserosa and dorsal ectoderm (see also WINICK *et al.* 1993) presumably through the prior action of genes such as *zen*, whose activity is critically required for the amnioserosa. However, *pnr* is expressed in a larger domain than that of *zen* and so its regulation must also rely on other

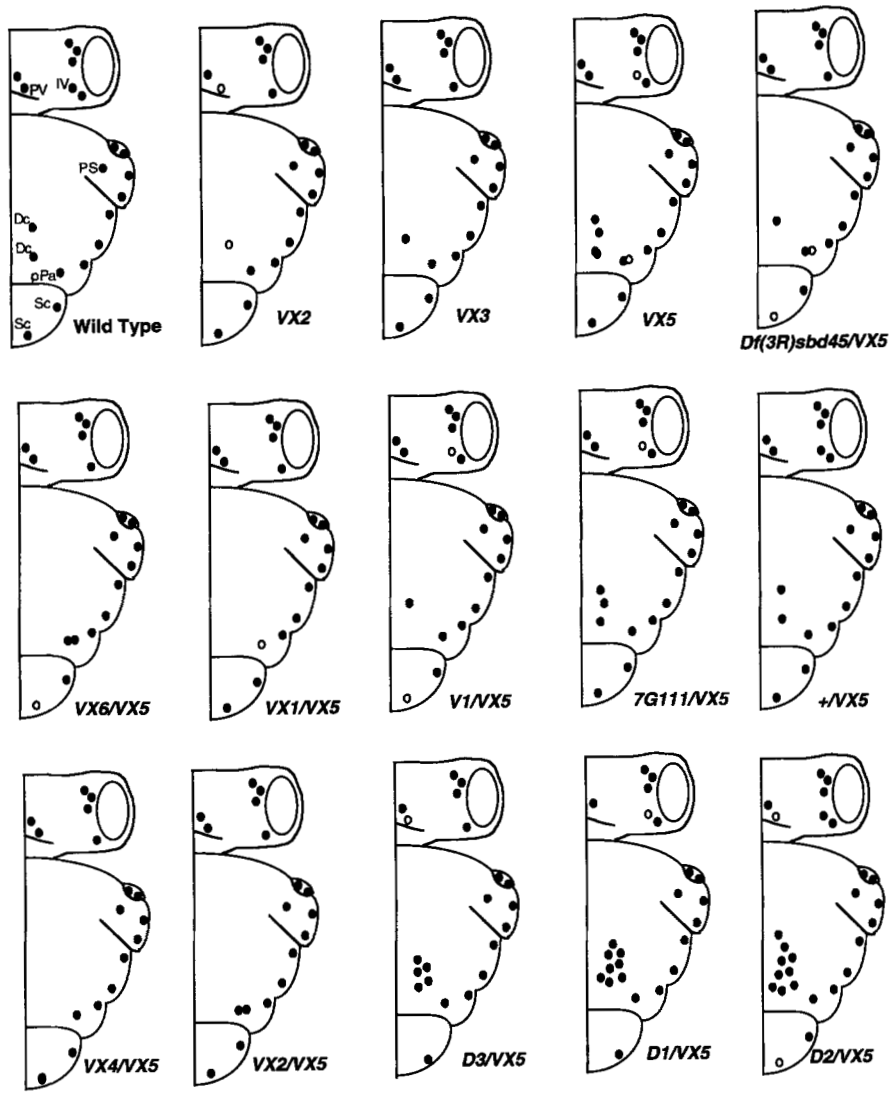


FIGURE 6.—Schematic representation of the bristle patterns on the head and thorax of a number of viable mutant *pnr* alleles and allelic combinations. In *pnr* mutants the dorso-central bristles (DC) of the thorax and the inner vertical bristle (IV) on the head are the most frequently affected, followed by the thoracic scutellar (Scu) and posterior postalar bristles (PPA) and the postvertical (PV) on the head. The rest of the bristle pattern is rarely altered. For each genotype >27 hemithoraces were examined. •, bristles present >50% of the time; ○, those present 10–50% of the time; and those present <10% of the time are not drawn. Hypomorphic alleles, such as $pnr^{VX2}$, are associated with a loss of bristles. $pnr^{VX5}$ is a viable allele that may show position effect variegation and displays either a loss or a gain of bristles. This allele is hypomorphic and is viable of many lethal alleles. Such combinations of recessive lethal alleles over $pnr^{VX5}$ always display a loss of bristles. When in trans to the dominant alleles of class 3 ($pnr^{VX1}$ or $pnr^{VX4}$), $pnr^{VX5}$ accentuates the loss of bristles characteristic of these mutants. When in trans to dominant alleles of class 4 ($pnr^{D1}$, $pnr^{D2}$, $pnr^{D3}$) $pnr^{VX5}$ accentuates the gain of bristles characteristic of these mutants.

genes expressed in this area. *pannier* mutants do not cause changes in the dorsoventral fate map and so their effects are presumably limited to the *pnr*-expressing cells themselves.

*extramacrochaetae* is expressed in a dynamic changing pattern in many tissues throughout embryogenesis: transcripts are found in the amnioserosa but are absent from the neuro-ectoderm (CUBAS *et al.* 1994). Mutant *emc* embryos display a slight dorsal bend and dorsal closure is often abnormal (CUBAS *et al.* 1994). The suppression of the *pnr* phenotype in embryos simultaneously mutant for *emc* suggests that both of these genes contribute to the determination of cell fate in the dorsal part of the embryo. There is no evidence that this involves *ac* and *sc*, *pnr* and *emc* may act upon different regulatory proteins in the embryo.

*pannier* is also required in the most dorsally situated cells of the head, the thorax and the abdomen. The adult expression pattern, seen with the GAL4 insertion in $pnr^{MD237}$, reveals a broad stripe along the entire dorsal midline of the fly. This corresponds to a domain of expression at the dorsal edge of the thoracic disc (RAMAIN *et al.* 1993), where the two hemithoraces, each derived from a separate imaginal disc, will fuse together at metamorphosis. Cells at the midline fail to differentiate since the mutants display a pronounced cleft along the dorsal midline. There seems to be no cell death along the dorsal edge during larval stages, but we were unable to determine whether cells at this site die during metamorphosis. The cells either die, or else after a failure of fusion of the two sides the epidermis may curl up and the cells be unable to secrete cuticle. In the most extreme mutants there is a substantial loss of tissue including cells that do not express *pnr*. A study of mosaic thoraces showed that if the midline cells are mutant there is an extensive loss of adjacent wild-type cells in addition to the mutant ones. On the other hand, if the midline cells are wild type, then adjacent mutant cells are quite viable. This suggests that the midline cells have special properties that depend, at least in part, upon expression of *pnr*. In a similar fashion to the dorsal midline cells of the embryo, the dorsalmost cells of the imaginal discs on either side of the

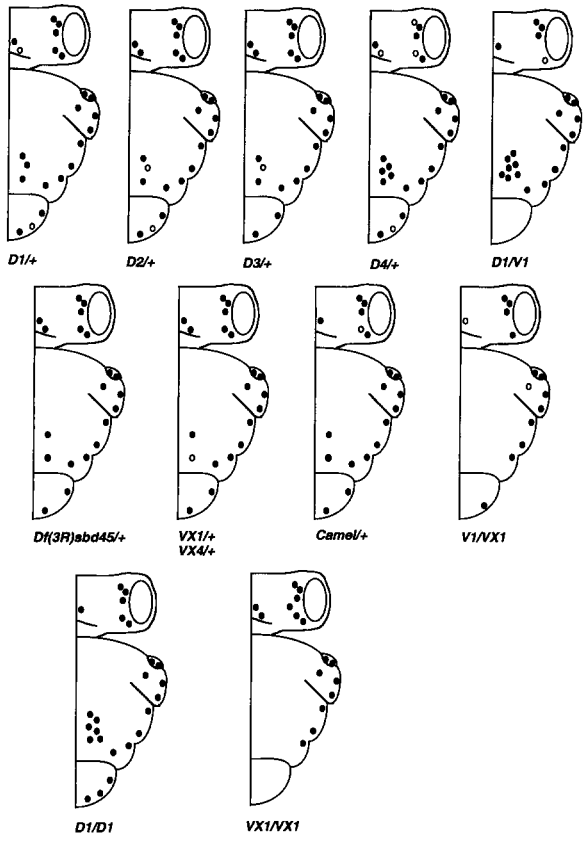


FIGURE 7.—Schematic representation of the bristle patterns on the head and thorax of flies carrying dominant *pnr* mutants. The alleles $pnr^{D1}$, $pnr^{D2}$, $pnr^{D3}$ and $pnr^{D4}$ form an allelic series for the presence of additional dorsocentral and scutellar bristles. At the same time the postvertical and sometimes the inner vertical bristles are missing in these flies. This phenotype is modified in $pnr^{D1}/pnr^{V1}$ flies where the gain of dorsocentral bristles is accentuated, but the scutellar, postvertical and inner vertical bristles are missing. Flies heterozygous for $pnr^{VX1}$, $pnr^{VX4}$ and *In(3R)Camel* display a loss of bristles that is accentuated when these mutants are in trans with $pnr^{V1}$. In clones of cells homozygous for $pnr^{D1}$, only additional bristles are found, whereas in clones of cells homozygous for $pnr^{VX1}$, only missing bristles are seen (RAMAIN *et al.* 1993).

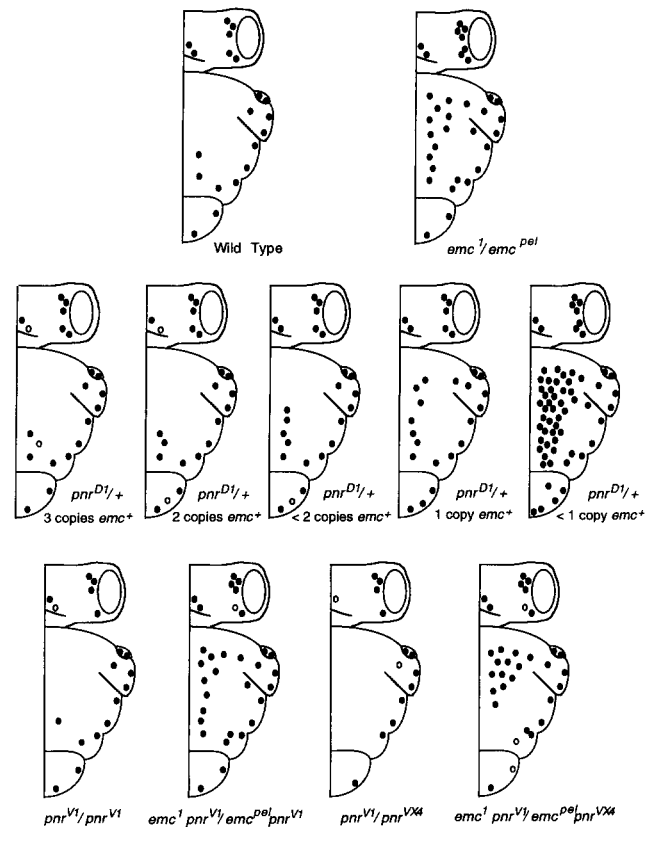


FIGURE 8.—Schematic representation of the bristle patterns of flies bearing different mutant combinations of *pnr* and *emc*. The number of dorsocentral and scutellar bristles present on $pnr^{D1}/+$ flies increases dramatically with decreasing doses of *emc*. The bristle patterns of flies doubly mutant for different allelic combinations of *pnr* and different mutant alleles of *emc* are shown. They display phenotypes that are different from either of the two mutants alone. In $emc^{1}$ $pnr^{V1}/emc^{pel}$ $pnr^{VX1}$ flies, characteristics of both *emc* and $pnr^{V1}/pnr^{VX1}$ were observed: the *emc*-specific pattern of additional bristles is found outside the domain of *pnr* expression. Within this domain of expression loss of bristle is seen, so that *pnr* is epistatic over *emc*.

imago must meet, line up and fuse together. It is likely that this requires an exchange of signals between the two epithelia. Signaling between the midline cells would then be required for maintenance of the integrity of the neighboring epithelial cells.

Animals mutant for viable *emc* alleles and clones of lethal *emc* mutants are never associated with thoracic clefts (GARCIA-ALONSO and GARCIA-BELLIDO 1988; our unpublished observations). However, double mutant *emc pnr* clones that reach the thoracic midline are associated with a cleft thorax. Although part of the area of *pnr* expression in the thoracic epithelium overlaps with that of *emc*, the most dorsally situated cells that later form the midline do not express appreciable levels of *emc* (CUBAS and MODOLELL 1992). Therefore the two genes act synergistically in the dorsal cells of the embryo where their expression coincides, but some mutant phenotypes of *pnr*, such as the thoracic cleft, do not involve the *emc* gene product.

**The bristles are differentially sensitive:** The bristle phenotypes of *pnr* are restricted to the dorsal part of the head and thorax. When all viable combinations are taken into account, it can be seen that the inner vertical and the dorso-central bristles are the most frequently affected, followed by the scutellar, postvertical and the posterior postalar bristles. Other bristles are never altered. *pannier* is expressed most strongly in the dorsal part of the thoracic disc, and there is thus a fairly good correlation between the site of origin of the affected bristles and the area of gene expression. With a single exception ($pnr^{VX5}$), for any one mutant allele the bristle pattern is the same for all flies. A given bristle, however, can be missing in one strain or be duplicated in another. Both recessive and dominant alleles can cause either a loss or a gain of macrochaetes and in some mutants a loss of bristles at some sites is accompanied by the development of extra bristles at other sites.

The inner vertical bristle on the head is missing in viable mutants of *pnr*. All the viable alleles, except $pnr^{V1}$, which is of spontaneous origin, are associated with visible rearrangements that are unlikely to break within the coding region. They could therefore affect either the quantity or the spatial distribution of Pnr. Genetically, however, these mutants behave as hypomorphs. $ac^{-}$ $sc^{-}$/+; $pnr^{-}$/+ double heterozygotes also lack the inner vertical bristles in 100% of the cases. Thus, hypomorphic mutants, or a reduction in the number of copies of $pnr^{+}$, seem to result in a failure of *ac-sc* expression at this site. These results suggest that the lack of the inner-verticals results from a reduction of $pnr^{+}$ activity. It is therefore surprising that this bristle is unaffected in clones of the null allele $pnr^{VX6}$ that are, furthermore, associated with extra bristles at other sites.

The two classes of dominant alleles have opposite effects on the bristle pattern and this is particularly obvious for the dorsocentral bristles. Alleles of class 4 that are associated with single base substitutions in the first zinc finger of the Pnr GATA domain (RAMAIN *et al.* 1993) are associated with additional dorsocentral bristles. This was shown to result from an overexpression of *ac* and *sc* (RAMAIN *et al.* 1993). The dominant alleles $pnr^{VX1}$ and $pnr^{VX4}$ of class 3 cause the opposite phenotype of a loss of dorsocentral bristles resulting from an underexpression of *ac* and *sc* (RAMAIN *et al.* 1993). These alleles are associated with deletions in a second domain of the protein bearing two amphipathic a helices (RAMAIN *et al.* 1993).

Different mutant proteins therefore seem to either repress or activate *ac-sc*. $pannier^{VX5}$ mutants, which may be subject to position effect variegation, display either a loss or a gain of dorsocentral bristles that could depend on temporal or quantitative variations in the levels of protein. Indeed, preliminary experiments in which the wild-type *pnr* product is expressed under the control of a heat shock promoter, allowed the recovery of flies bearing either additional or missing bristles (at the same sites) depending upon the time of heat shock (P. RAMAIN, unpublished data).

It is not yet known whether Pnr acts directly on the *ac* and *sc* genes. If this does prove to be the case, then perhaps Pnr acts as both a repressor and an activator depending upon either association with another factor(s), or perhaps on a different role for monomer and dimer forms of Pnr (SAUER and JÄCKLE 1995).

**Allele-specific interactions with *extramacrochaetae*:** The gene *emc* is expressed everywhere but at greatly varying levels throughout the thoracic epithelium and is known to act as a repressor of *ac/sc* (GARCIA-ALONSO and GARCIA-BELLIDO 1988; CUBAS and MODOLELL 1992). The *emc* protein bears an HLH domain but no basic domain and is thought to bind to the *ac* and *sc* proteins and prevent them from binding to DNA (ELLIS *et al.* 1990; GARRELL and MODOLELL 1990; VAN DOREN *et al.* 1991, 1992). This would interfere with the autoregulatory loops and result in a decrease of *ac* and *sc* transcripts and indeed, in the thoracic epithelium, sites of high *emc* expression are generally associated with low levels of *ac/sc* (CUBAS and MODOLELL 1992).

Allele-specific interactions resulting in altered bristle patterns have been found between *pnr* and *emc*. The extra bristle phenotype of animals heterozygous for class 4 alleles is accentuated when the amount of *emc* is reduced and suppressed when the amount is increased. This is consistent with a role of both of these genes in the repression of *ac* and *sc*. In these mutants the first zinc finger domain is mutated, but the helix-bearing domain of the protein does not carry any mutations and is potentially functional. In contrast, the two class 3 alleles are not sensitive to the dosage of *emc* and they carry deletions that would result in the absence of the two $\alpha$ helices in the second domain of the protein. Thus the presence of the helical structures seems to be a prerequisite for the interactions observed between *pnr* and *emc*. This suggests the possibility that the *pnr* and *emc* proteins may interact via the helix-bearing domain of *pnr* and so perhaps act together in a common pathway.

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