Cell patterning in the *Drosophila* segment: spatial regulation of the segment polarity gene *patched*

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Summary

Intrasegmental patterning in the *Drosophila* embryo requires the activity of the segment polarity genes. The acquisition of positional information by cells during embryogenesis is reflected in the dynamic patterns of expression of several of these genes. In the case of *patched*, early ubiquitous expression is followed by its repression in the anterior portion of each parasegment; subsequently each broad band of expression splits into two narrow stripes. In this study we analyse the contribution of other segment polarity gene functions to the evolution of this pattern; we find that the first step in *patched* regulation is under the control of *engrailed* whereas the second requires the activity of both *cubitus interruptus* and *patched* itself. Furthermore, the products of *engrailed*, *wingless* and *hedgehog* are essential for maintaining the normal pattern of expression of *patched*.

Key words: *Drosophila*, segment polarity genes, cell interactions, *patched*.

Introduction

The epidermis of the *Drosophila* segment presents an attractive system for investigating the mechanism of cellular positional specification. Mutational analysis has identified a class of genes, the segment polarity class, that play a major role in this process. Lesions in these loci produce localised segmentally repeated defects (Nüsslein-Volhard and Wieschaus, 1980; Nüsslein-Volhard *et al.* 1984; Jurgens *et al.* 1984; Wieschaus *et al.* 1984) suggesting that they are specifically required for the generation of particular elements of the segmental pattern. Moreover, molecular analysis has revealed that the products of some segment polarity genes are similarly expressed in spatially restricted patterns (Fjose *et al.* 1985; Kornberg *et al.* 1985; DiNardo *et al.* 1985; Baker, 1987; Baumgartner *et al.* 1987; Coté *et al.* 1987; Nakano *et al.* 1989; Hooper and Scott, 1989; van den Heuvel *et al.* 1989). Elucidating the mechanisms that control these spatial patterns of expression is therefore an important step in understanding the cellular basis of patterning. Segmentation of the *Drosophila* embryo is accomplished by a cascade of gene regulation, starting with maternal products deposited in the developing oocyte and culminating in the activation of the pair-rule genes, which subdivide the embryo into fourteen periodic domains of gene activity (reviewed in Akam, 1987; Ingham, 1988). At least two of the segment polarity genes have been shown to respond directly to positional cues generated by the pair-rule genes (Howard and Ingham, 1986; DiNardo and O'Farrell, 1987; Ingham *et al.* 1988). For instance, *fushi-tarazu* (*fit*) and *even-skipped* (*eve*) act as positive regulators of *engrailed* (*en*) and negative regulators of *wingless* (*wg*). As a result of this regulation, *en* expression is initiated in a series of single-cell-wide stripes at the cellular blastoderm stage (stage 5), which mark the anterior boundaries of the primordia of each parasegment (Lawrence *et al.* 1987) and persists in this location throughout embryogenesis. Similarly, *wg* expression appears in fourteen stripes at the same stage (Baker, 1987), a pattern maintained in the ventral epidermis, to occupy a narrow domain immediately anterior to that of *en*, within each parasegment.

Several lines of evidence suggest that local cell interactions play an important role in the positional specification process after the activity of the pair-rule genes has ceased. Observations of the altered patterns of *wg* and *en* expression in embryos mutant for these and other segment polarity genes provided the first indications that such interactions occur (Martinez-Arias *et al.* 1988). Although *en* and *wg* are expressed in adjacent groups of ectodermal cells, the continued expression of either gene is dependent upon the activity of the other, implying that they mediate inductive interactions between neighbouring cells. This interpretation is supported by the finding that *wg* shows significant sequence similarity to the mouse proto-oncogene *int-1* (Rijswijk *et al.* 1987), which encodes a secreted glycoprotein found associated with the extracellular matrix of cultured cells (Bradley and Brown, 1990; Papkoff and Schryver, 1990). Moreover, studies of the distribution of the *wg* protein in *Drosophila* embryos show that it is closely associated with the membrane of
cells transcribing the wg gene and is apparently endocy-
tosed by neighbouring cells (van den Heuvel et al.
1989). The response of cells to the wg protein is thought
to depend crucially upon the activity of the patched
(ptc) gene, which encodes a putative transmembrane
protein of unknown biochemical function (Nakano et al.
1989; Hooper and Scott, 1989). In this study, we
analyse the regulation of ptc transcription during em-
byogenesis; we present evidence that supports the
proposed role of ptc in modulating cell competence with
respect to the wg signal and provides further indications
of cell interactions during the elaboration of pattern in
the body segment.

Materials and methods

Drosophila stocks

Embryos were collected from flies heterozygous for the
appropriate mutations, generated by crossing the balanced
stocks to the wild-type laboratory stock Oregon R; this
procedure eliminates balancer chromosomes from the paren-
tal flies (and, therefore, balancer homzygotes from the
progeny) and ensures maximum fecundity. The following
stocks were used: Df(2R)en B, Df(2R)SBl,gsb~ Kr~
naked
Df(2R)IIX62,gsb-bzip, ptc~ 08 wg™ 4, ptcIB34, ptc-022,
pic*51, wg™ 4, ptc™ 59, ptc™ 1C34, ptc™ 1^08, ptc™ 099, ptc™
1, ptc™ R87, ptc™ xM, ptc™ wm, ptc™ 012, ptc™ B28, ptc™

served in some preparations, notably with a pair-rule
effect that is essentially ubiquitous, a weak modulation can be ob-
erved for 20 min as previously described (Ingham
et al. 1989). Although expression at this stage is
broadly expressed in the embryo with the exception of the pole cells and an
dorsal boundary (Nakano
et al. 1989; Hooper and
Scott, 1989), occurring throughout most of the
blastoderm stage (stage 5, of Campos-Ortega and
Hartenstein, 1985), occurring throughout most of the
germ band. The posterior stripe coincides with the domain of expression of wg whereas the anterior
one is immediately posterior to the en domain (data not
shown).

ptc transcription by

Because the initial segmental pattern of ptc appears

The expression domain of ptc is reduced in naked

embryos

In a stage 10 embryo, the bands of ptc transcript
appear reciprocal to that of en, we monitored the effects of
mutations on ptc expression. As shown in Fig. 2B, ptc expression remains ubiquitous in the ectoderm of a
stage 10 en~ embryo, suggesting that the normal
pattern of expression is generated by the repression of
ptc transcription by en. Although the ectodermal stripes
are not formed, some modulation of expression is still
apparent (Fig. 4E). This is due to the periodic
expression in the underlying mesoderm which is out of
phase with the normal ectodermal stripes (Nakano et al.
1989) and therefore presumably independent of en
function.

Results

Evolution of the wild type pattern of ptc

Expression of ptc is first clearly detectable at the end of
the blastoderm stage (stage 5, of Campos-Ortega and
Hartenstein, 1985), occurring throughout most of the
embryo with the exception of the pole cells and an
anterodorsal region (Nakano et al. 1989; Hooper and
Scott, 1989). Although expression at this stage is
essentially ubiquitous, a weak modulation can be ob-
erved in some preparations, notably with a pair-rule
periodicity (data not shown). After gastrulation (stage
8), ptc transcript becomes restricted to segmentally

repeated domains in the presumptive epidermis. These
occupy about three fourths of the width of each
parasegment and are reciprocal to the narrower
domains of en (Fig. 1A). By stage 11 (Fig. 1E), tran-
scription accumulation is further restricted to two narrow
stripes of cells in each segment, which arise in a
stereotyped manner: at late stage 10, ptc transcripts
show a heterogeneously distributed within each parase-
ment, the highest level of expression being in the most
posterior cells (Fig. 1B–C). Subsequently, transcript
appears first from the cells located most dorsally in
the middle of each parasegment (Fig. 1D and 3A) and
then from the more ventrally located cells. Concomi-
tantly, first a distinct anterior stripe and then a posterior
stripe starts to appear; the anterior stripe reaches a
higher level of transcription than the posterior one, first
dorsally (Fig. 1E) and then continuously along the
dorsalventral axis. The posterior stripe coincides with
the domain of expression of wg whereas the anterior
one is immediately posterior to the en domain (data not
shown).

in situ hybridisation

Embryos were decarboxylated in sodium hydrochlorite and
fixed for 20 min as previously described (Ingham et al. 1985).
Transcripts were detected by in situ hybridisation on whole
embryos with digoxigenin (Boehringer)-labelled DNA
probes, following a modification of the protocol of Tautz and
Pfeifle (1989) (C. Oh and B. Edgar, personal communi-
cation). Probes were prepared from purified plasmid inserts
obtained from the following digests: ptc: 1.0+0.7 kb BamHI–
EcoRI fragments from pc2 (Nakano et al. 1989); wg, 1.3+0.7 kb EcoRI–HindIII fragments from D-intl (Rijsewijk
et al. 1987); en; 2.4 kb HindIII–EcoRI fragment from pE2C
(Poole et al. 1985). Hybridisation was in 50 jg final volume at a
probe concentration of 0.2ng/ml. Samples were incubated
for 1 h at room temperature in anti-digoxigenin antibody
(1:2000) which had been preabsorbed against 50 v volume of
fixed embryos for 1 h. Stained embryos were mounted in JB4
(Polysciences) A+C: 25:1, after dehydrating in 95 % EtOH
for 1–2 min and infiltrating in JB4 A+C for 5–10 min.
Cell patterning in the Drosophila segment

Fig. 1. Detail of the evolution of the normal transcriptional pattern of ptc; each panel shows three adjacent parasegments from embryos between stages 9 and 11, (staging according to Campos-Ortega and Hartenstein (1985)). Orientation in this and all subsequent figures is anterior to the left, dorsal surface uppermost, unless stated otherwise. (A) Stage 9, ptc transcripts are essentially uniformly distributed anteroposteriorly in the non-en-expressing cells of each parasegment. (B) Stage 10 to early 11; expression is highest in the most ventral posterior cells of each ptc domain (arrowhead indicates the ventral midline). (C) Ventral and (D) lateral aspects of stage 11 embryos. High levels of expression are seen in a row of ventral posterior cells (C) (arrowhead indicates the ventral midline); simultaneously, high level expression is seen dorsally at the anterior margin of each parasegment (D) and transcripts disappear gradually from the central region. (E) By late stage 11, ptc bands have completely split, but still show strongest signal anterodorsally (arrow).

Fig. 2. Expression of ptc in wild-type (A), en (B) and nkd (C) embryos. (A and B) Stage 10, (C) early stage 11, just before the splitting of the ptc domains takes place. Note the continuous ectodermal expression in B (dots indicate where ptc is normally repressed) and the narrower stripes in each parasegment in C. The parasegment boundary shifts in nkd embryos (C) (arrow).
Fig. 3. Gene activities required for the maintenance of the late phase of ptc expression. The panels on the left show stage 11 embryos, those on the right stage 12. (A,D) ptc expression in wild-type embryos. (B,E) In wg embryos, the narrow stripes form but rapidly decay except in the maxillary, labial and prothoracic segments (arrows); this reflects the expression of en which also persists in the gnathal segments in embryos of the same genotype (Martinez-Arias et al. 1988; DiNardo et al. 1988) (C) In hh, ptc expression is confined most ventrally and posteriorly at stage 11 and has completely disappeared by stage 12 (F). (G) Most expression similarly disappears from en homozygotes by stage 12 except in the head, anal pads and mesoderm (arrows).

In late stage 11 the only periodicity is manifested in ellipses of cells located most ventrally (Fig. 3B and 4D) and circles of cells in the approximate location of the tracheal placodes (not shown), while levels of transcript decrease in the remaining regions. After stage 11, expression begins to disappear, and by the end of germ band retraction is absent from most cells, with the exception of irregularly repeated lateral circles of cells and a dorsoventral stripe in the first thoracic segment and two patches in the maxillary and labial segments (Fig. 3E).

Mutations of hedgehog (hh) have similar effects on the late expression of ptc. Expression is normal up to the early extended germ band stage, but subsequently ptc transcripts start to disappear, being detectable only most ventrally and posteriorly, within each parasegment at stage 11 (Fig. 3C and 4C). By stage 12, there is no evidence of ptc expression anywhere in the embryo (Fig. 3F).

In en mutant embryos, transcripts also begin to disappear from the ectoderm as the germ band starts to retract, (though persisting in the mesoderm for some time); by stage 14 ptc expression is confined to a small region in the head around the stomodaeeum and pos-
We also analysed the expression of ptc in embryos mutant for gooseberry (gsb) but found no significant differences from the wild-type pattern (data not shown).

Establishment of the late pattern of ptc transcription depends upon selective repression by cubitus interruptus Dominant and ptc

In embryos mutant for the cubitus interruptus Dominant (ciD) allele (which is equivalent to a null mutation of the locus (Orenic et al. 1987)), alterations in ptc expression are first evident at stage 11; the broad bands of transcript persist, though with highest intensity in their posterior half ventrally, and in the anterior cells, dorsally (Fig. 5C). Simultaneously, expression disappears completely from the lateral region of each parasegment (Fig. 6C), creating a discontinuous pattern of expression similar to that typical of wg in normal embryos at the same stage (compare Fig. 5F and K). Slightly later, a narrow stripe of non-expressing cells appears ventrally, splitting each domain into a narrow anterior stripe and a broad posterior stripe (Fig. 5F and I).

All of the EMS-induced alleles of ptc (with one exception) express ptc transcript at elevated levels and in a similarly aberrant pattern, the transition to the two narrow stripes of expression in each parasegment failing to occur normally at stage 11 (Fig. 5B,E,H and 6B). At stage 10, prior to the splitting, an ectopic ectodermal groove appears within each band of ptc expression. Subsequently, these initial domains do split, but into two stripes of unequal width, both of which are broader than in wild type, the posterior member of each pair being broader than the anterior one. The gap between the stripes is quite narrow and they frequently fuse at several points in stage 13–14 embryos.

In the one exceptional case, ptc<sup>Jun12</sup>, the alterations in expression are initially similar, two broad bands forming instead of the normal narrow stripes typical of stage 11–12. The levels of transcript, however, are not as high as in the other ptc mutations and, after stage 11, the bands narrow somewhat resembling more the wild-type pattern; these, however, seem to fuse across segments and look sloppier than wild type.

In some ptc mutant embryos we observed a differential intensity of the ptc stripes along the dorsoventral axis: highest signal intensities are localised to the most ventral area of both stripes within each parasegment, while the most anterior of these stripes, that is, the one that does not overlap the wg domain, is more intense dorsally.
To determine whether the derepression of ptc in ptc mutants is dependent upon wg activity, we analysed ptc expression in wg-pts double mutant embryos. In such embryos, transcript is distributed uniformly and at higher levels than in wg or wild-type embryos. After stage 12, when ptc starts to disappear in wg embryos, expression of ptc is maintained in wg-pts, persisting through all the late stages to the fully retracted germ band (Fig. 5J).

Expression of wg in ciD, gsb and hh mutant embryos
Because ptc and wg are coexpressed in some cells in each parasegment, we analysed the expression of wg in the same mutant embryos to investigate the relationship between the regulation of the two genes.

In ciD and gsb embryos wg expression is initiated normally, but by stage 11 diverges significantly from the wild-type pattern. In ciD mutants, the stripes of expression appear slightly broader and less regular than in wild type. From stage 12 onwards, expression becomes sporadic, and is frequently absent from some segments (Fig. 5L). In stage 11 gsb mutant embryos, transcripts disappear from the ventral region, whilst persisting dorsally (Fig. 7D,G). At this stage in wild-type embryos, wg expression normally splits dorsoventrally into two distinct domains (Baker, 1988; van den Heuvel et al. 1989) whilst gsb evolves from expanding dorsoventrally to occupying only the most ventral cells (Baumgartner et al. 1987).

In embryos homozygous for a strong hh allele, wg expression also begins normally, but starts to decay at stage 10 and by stage 11 is completely absent from the ectoderm of the trunk region of the embryo, persisting only in the terminal domains (Fig. 7B).

Discussion
The emergence of pattern in the Drosophila body segments requires the activity of the segment polarity genes and is reflected in their dynamic patterns of transcription during embryogenesis. Although cell patterning is a continuous process, it is helpful in thinking about the problem to distinguish three distinct phases as revealed by segment polarity gene expression: an initiation phase, when transcription begins, just prior to gastrulation; a consolidation phase during the extension of the germ band, when the initial anteroposterior asymmetries are reinforced uniformly around the dor-
sOventral axis; and an elaboration phase, beginning towards the end of the proliferative phase of embryogenesis, when more complicated patterns arise along both the anteroposterior and dorsoventral axes.

Whereas control of the first phase depends upon the pair-rule genes, and, through them, the well-characterised segmentation gene hierarchy, the subsequent expression patterns are regulated by interactions mediated by the segment polarity genes themselves. In contrast to the simple transcriptional cascade that characterises the segmentation gene hierarchy, these interactions involve the complex processes of intercellular signalling and signal transduction.

The emergence of spatial differences within each developing parasegment is particularly well illustrated by the dynamic pattern of ptc expression (Fig. 1) and accordingly it is the transcriptional regulation of this gene upon which we have focused our attention in this study. The principle conclusions of our analysis are depicted schematically in Fig. 8 and can be summarised as follows. During the early phase of germ band extension, ptc is down regulated by en in all en expressing cells, an event which is crucial for the maintenance of en expression by their wg neighbours. Towards the end of the extended germ band stage there is a switch in the requirement for en, the maintenance of ptc expression in cells flanking en-expressing cells depending upon en activity. This later non-autonomous role of en implies a signalling mechanism between the two cell types, a mechanism which we suggest is mediated by the hh gene. At the same time, cells in the middle of each parasegment cease transcribing ptc, a process which requires the function of ptc itself, implying a response of cells to some other signal of unknown identity. In what follows, we discuss the data supporting each of these conclusions in some detail, and consider their implications for the cell patterning process.

In contrast to en, wg and gsb, each of which is activated in response to pair-rule genes in precisely defined domains in the blastoderm, transcription of ptc at this stage is almost ubiquitous, showing only a transient modulation. This modulation may reflect a fortuitous interaction between some pair-rule gene products and the ptc promoter, but does not appear to have any consequence for the later function or expression of the gene. The first significant spatial regulation of ptc occurs after the onset of gastrulation, with the repression of transcription in the anterior-most quarter of each presumptive parasegment (Nakano et al. 1989; Hooper and Scott, 1989). This domain corresponds to those cells that express en, and two lines of evidence suggest that en itself is responsible for this repression. First, in en embryos, the repression does not occur and ptc continues to be expressed ubiquitously throughout most of the ectoderm. Second, in nkd embryos, the expansion of the en domain of expression described by Martinez-Arias et al. (1988) is accompanied by a corresponding decrease in the size of the ptc expression domain. Although both effects could be independent consequences of the absence of nkd function, the simplest interpretation seems to be that the ectopic expression of en results in the ectopic repression of ptc.

The functional significance of this repression is revealed by the changes in en expression in ptc mutant embryos. At around stage 11, an additional domain of en is initiated anterior to the wg domain in each parasegment, apparently in direct response to the expression of wg (DiNardo et al. 1988; Martinez-Arias et al. 1988). Since such induction is blocked in wild-type

**Fig. 6.** Expression of ptc in the lateral region of three adjacent parasegments of wild-type (A), ptc (B) and ciD (C and D) late stage 11 embryos. Note the narrow stripes of repression and the ectopic segment boundaries (arrow) associated with the ectopic expression of en in the ptc mutant (B). A lateral band (arrows) lacking ptc transcripts appears in ciD embryos (C). Note the broad posterior stripe in D and the domain lacking ptc transcripts (arrow), which is not associated with ectopic en expression.
embryos, it follows that the expression of ptc influences the response of cells to wg. If reception of the wg signal is necessary for maintaining the expression of en, it is clearly essential that the en cells repress ptc transcription if they are to continue to express en. Thus, the negative regulation of ptc by en can be regarded as part of a feedback loop which ensures the continued expression of en only in the cells posterior to the wg domain, and hence maintains the polarity of the parasegment (see Fig. 9). Whereas simple transcriptional autoregulation of en would allow the en cell population to maintain itself independently of size and position, this somewhat more complex mechanism imposes specific constraints on the en cells, ensuring that their initial position and proportion is maintained during the major cell rearrangements that occur during germ band extension (see also discussion in Mohler, 1988).

A reciprocal dependence of the wg-expressing cells on their en neighbours has also been described by Martinez-Arias et al. (1988). Given the predominantly nuclear localisation of the homeodomain protein encoded by en (DiNardo et al. 1985), it seems unlikely that this interaction is directly mediated by en itself. Instead, en probably controls the expression of some other signalling molecule that acts in a manner analogous to wg. A good candidate for such a molecule is the product of the hedgehog gene. Mutations of hh have been shown to act non-autonomously in genetic mosaics (Mohler, 1988) and our results show that the expression

Fig. 8. Schematic representation of the interactions influencing the evolution of the ptc expression pattern during embryogenesis. Each circle represents one quarter the width of a parasegment (indicated by square brackets): at the blastoderm stage (A), this is equivalent to a single cell, but at subsequent stages (B and C) a circle represents several cell diameters. W and E denote domains of wg and en expression, respectively; note however, that the wg domain does not increase in size proportionately so that the designation of an entire circle as a wg domain in B and C is a simplification. (A) At blastoderm, all cells express ptc (shading) whereas expression of wg and en is established in single cell wide domains. W and E denote domains of wg and en expression, respectively; note however, that the wg domain does not increase in size proportionately so that the designation of an entire circle as a wg domain in B and C is a simplification. 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of wg at the posterior margin of each parasegment disappears in hh embryos by stage 11. Taken with the observation that hh is probably expressed in the en-expressing cells of each parasegment (J. Mohler, personal communication) these findings strongly suggest that hh is involved in a signalling process between the en and wg cells. Whatever the identity of the signal, such a process raises a similar question to that posed by the putative wg signalling function, namely what determines the polarised response of cells to the signal? In this context, the effects of nkd mutations may be significant. Whereas in ptc mutant embryos, expansion of the wg domain is accompanied by the ectopic induction of en expression, in nkd embryos, there is an expansion of the en domain accompanied by the ectopic induction of wg expression in adjacent cells (Martinez-Arias et al. 1988). These reciprocal effects of the two mutations imply that the wild-type genes may perform complementary functions, expression of nkd blocking or modifying the response of cells to hh in a manner analogous to that proposed above for the relationship between wg and ptc (see also DiNardo and Heemskerk, 1990).

We can thus identify at least five genes involved in consolidating the polarity of each parasegment established at the blastoderm by the pair-rule dependent activation of wg and en, with ptc, nkd and hh being involved in signalling processes that maintain wg and en expression in the appropriate spatial domains. These interactions apparently occur between neighbouring cells and are responsible for the maintenance of the boundaries of parasegments. We now consider the factors involved in the subsequent elaboration of pattern within each parasegment.

At the end of stage 10, shortly after the interactions between wg- and en-expressing cells take place, the pattern of ptc transcription undergoes a further refinement; cells immediately anterior to the wg domain, which we postulate require ptc activity to prevent them inducing en expression in response to wg, now cease transcribing ptc. Expression of the gene thus becomes restricted to two narrow stripes in each parasegment, one marking the anterior border of the future segment, the other coinciding with the wg domain (see Fig. 9).

This pattern of expression is never established in embryos mutant for en, hh or wg, implying that the maintenance of ptc transcription after stage 10 requires the expression of all three genes. Such a positive requirement for en at this stage contrasts with its postulated negative regulatory role prior to stage 11. Moreover, whilst the repressing activity of en is a cell autonomous function, the subsequent maintenance of ptc expression is limited to two narrow stripes of cells which flank the en expression domain of each parasegment. This apparently non-autonomous effect of en on ptc expression therefore implies an indirect interaction between the two genes, en presumably regulating the expression of some signalling molecule which in turn stimulates ptc transcription. Although the evidence that wg encodes a signalling molecule is compelling, it seems unlikely that it plays such a direct role in the maintenance of ptc expression. While ptc expression more or less ceases by stage 11 in wg mutant embryos, we find it to be normal in gsb~ embryos despite the fact that such embryos lack wg expression ventrally. This suggests that the cessation of ptc expression in wg mutants is due to some earlier effect of wg, presumably the maintenance of en expression in neighbouring cells described above. Once this function is complete, however, wg seems to have no further role in the maintenance of ptc.

The possibility that hh encodes a signalling molecule has already been discussed above; our finding that ptc expression also disappears in hh mutants is thus consistent with a role for hh in signalling to cells neighbouring the en domain to maintain ptc expression. Unlike the postulated interaction between hh and wg, this response of cells to hh would not be polarised and should therefore be independent of nkd activity, consistent with our finding that the narrow stripes of ptc expression are indeed established and maintained in nkd embryos.

These considerations might suggest that the evolution of the ptc expression pattern depends on its negative regulation by en followed by positive regulation in cells adjacent to the en domain. Our analysis indicates however, that ptc expression is also actively repressed in cells in the middle region of each of the initial broad domains; the elimination of this repression results in constitutive expression of the gene which is independent of its putative positive regulators.

In all of the ptc mutations we analysed, ptc transcript is initially normally expressed, but after stage 10 persists at much higher levels than in wild type in a broad domain, interrupted only by a thin line of non-expressing cells which probably correspond to those in which en expression is ectopically induced. In ptc mutants which also lack wg expression, transcription is virtually ubiquitous, suggesting that this release from repression is independent of the positive regulatory mechanism outlined above.
This persistent expression of ptc in the cells anterior to the normal wg domain can be interpreted in a number of ways. One possibility is that expression of ptc in the two narrow stripes of cells at the edges of the initial broad expression domains is required for those cells to produce a further signal that represses ptc expression in the central cells of each domain. This model, while seemingly unnecessarily complex, has the virtue of providing a function for the continued expression of ptc in cells whose differentiation appears unaffected in its absence (see Hooper and Scott, 1989). Moreover, the results of mosaic analysis of ptc mutants in imaginal disc cells provide a precedent for such non-autonomous effects of ptc, ptc− cells affecting the differentiation of their genetically wild-type neighbours (Phillips et al. 1990).

A simpler alternative, however, is to suppose that ptc expression is autonomously required for its own repression. According to this interpretation, presence of the ptc protein in the cell membrane would enable cells to respond to a signal, one aspect of this response being to repress further ptc transcription, an effect in some ways analogous to the down regulation of growth factor receptors elicited by ligand binding (e.g. Russell et al. 1987). Whether or not ptc itself functions as the receptor for such a signal or instead modulates its transduction, for instance, by coupling the receptor to a particular second messenger pathway or modulating the internal physiology of the cell, remains to be determined.

Discrimination between these models requires the analysis of genetic mosaics which we are now undertaking; in either case, the identity of the postulated signal to which the ptc-repressing cells respond remains obscure. Clearly it could emanate from the wg-expressing cells; however, it is unlikely to be wg itself since ptc transcription, rather than persisting, disappears from wg− embryos by stage 11.

Embryos homozygous for the ciD mutation, by contrast, show an alteration in the distribution of ptc transcripts which is in some ways reminiscent of the effect of ptc mutations. Dorally and ventrally, the broad domains of ptc expression persist, although ventrally, a narrow strip of cells does cease expression; laterally, however, the ptc transcript completely disappears. This latter effect is intriguing not least because it mirrors the lateral repression of wg expression which normally occurs at the same stage in wild-type embryos. It is difficult to interpret these changes in terms of indirect effects of the ciD mutation on the expression of other segment polarity genes: en expression is essentially unaffected by the mutation (unpublished observations) whereas the expression domains of wg decay prematurely after broadening slightly. While our data do not allow us to determine whether ciD acts upstream or downstream of ptc to control its transcription, the finding that the ciD gene encodes a zinc finger gene (Orenic et al. 1990) is consistent with a direct interaction between the genes. Why ciD mutations should have apparently opposite effects on ptc expression at different positions around the dorsoventral axis remains unclear; however, these effects provide a further indication of the extent of the heterogeneity around this axis at the end of the extended germ band stage, most clearly exemplified by the wild-type patterns of expression of wg and gsb.

**Conclusion**

Our results suggest that the evolution of the pattern of ptc transcription during embryogenesis depends upon three distinct control mechanisms. Whilst the first of these appears to be a cell-autonomous function, the latter two seem to involve intercellular communication. Although these conclusions require testing by mosaic analysis, our data provide strong support for the notion that local cell interactions play a major role in the specification of positional information in the developing embryo.

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**References**


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