Development 117, 283-291 (1993)
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Regulation of *wingless* transcription in the *Drosophila* embryo

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SUMMARY

The segment polarity gene *wingless* (*wg*) is expressed in a complex pattern during embryogenesis suggesting that it plays multiple roles in the development of the embryo. The best characterized of these is its role in cell pattening in each parasegment, a process that requires the activity of other segment polarity genes including *patched* (*ptc*) and *hedgehog* (*hh*). Here we present further evidence that *ptc* and *hh* encode components of a signal transduction pathway that regulate the expression of *wg* transcription following its activation by pair-rule genes. We also show that most other aspects of *wg* expression are independent of this regulatory network.

Key words: *wingless*, *Drosophila*, segment polarity gene, *patched*, *hedgehog*, regulation

INTRODUCTION

The specification of positional identity within each developing parasegment of the *Drosophila* embryo is a highly complex process requiring the activity of a number of genes, identified by the segment polarity mutations (reviewed in DiNardo and Heemskerk, 1990; Ingham and Nakano, 1990; Ingham, 1991; Peifer and Bejsovec, 1992). The development of each parasegment is initiated at the blastoderm stage of embryogenesis when transcription of the *engrailed* (*en*) and *wingless* (*wg*) genes is activated at the presumptive boundaries of each parasegment. Thereafter, the expression of these two genes plays a crucial role in the elaboration of positional identity, the two cell populations defined by their expression domains acting as signalling centres that instruct neighbouring cells (Ingham and Martinez-Arias, 1992). The role of the *wg* gene product in this process has been particularly well characterised: *wg* protein is secreted by cells that express the gene and is taken up by neighbouring cells either side of its expression domain (van den Heuvel et al., 1989; Gonzalez et al., 1991). Posteriorly, the activity of *wg* is important for the maintenance of *en* expression since, in its absence, *en* transcript and protein rapidly disappear after gastrulation (DiNardo et al., 1988; Martinez-Arias et al., 1988; Bejsovec and Martinez-Arias, 1991). Anteriorly, the secreted *wg* protein seems to have a number of consequences at different developmental stages: during gastrulation, cells respond to *wg* protein by accumulating Armadillo protein at their membranes (Riggleman et al., 1990); later, *wg* activity seems to be required to suppress the differentiation of denticles by epidermal cells in the ventral part of each segment. (Baker, 1987; Bejsovec and Martinez-Arias, 1991). Since the response of cells to *wg* protein differs according to their location along the anteroposterior axis, it has been suggested that different cells have differing competences to respond to *wg* (Ingham et al., 1991). Thus only cells in the anterior half of each parasegment appear predisposed to express *en* in response to *wg*, a property that is revealed when *wg* is ectopically expressed through mutation of the regulatory gene *patched* (*ptc*) (DiNardo et al., 1988; Martinez-Arias et al., 1988; Ingham et al., 1991).

There are a number of striking similarities between the activity of *wg* and that of its vertebrate orthologue *Wnt-1*. Mutation of the latter results in the loss of *engrailed* expression in the mesencephalon of the mouse (McMahon et al., 1992), suggesting a functional relationship between the two genes paralleling that in *Drosophila*. Moreover, the results of grafting experiments in the chick provide circumstantial evidence that different regions of the vertebrate brain have differing competences to respond to the *Wnt-1* signal (Martinez et al., 1991; Bally-Cuif et al., 1992). In addition, RNA injection experiments in *Xenopus* have shown that murine *Wnt-1* and *wg* can have identical biological effects, their ectopic expression in the early embryo resulting in the duplication of the main body axis (McMahon and Moon, 1989; Chakrabarti et al., 1992), apparently through the induction of a second Spemann's organiser (see also Smith and Harland, 1991; Sokol et al., 1991). The penetrance of this effect varies with the location of the injected mRNAs, implying that their protein products act over only a limited distance, consistent with the known properties of both proteins in tissue culture assays (Jue et al., 1992; N. Ramarkrishna and A. Brown, personal communication).

Thus, in both invertebrates and vertebrates, the Wg protein and its mammalian counterpart appear to act as short-range signalling molecules, the effects of which depend upon two parameters: the differing competences of respond-

ing cells and the restriction of the source of the signal (see Ingham, 1991). In this study, we analyse a number of the factors that control the spatial regulation of *wg* transcription in the *Drosophila* embryo.

MATERIALS AND METHODS

Drosophila strains

$prd^{2.45}$, ptc^{IN}, $eve^{1.27}$, wg^{IIS} and hh^{IJ} are all null or near amorphic alleles of their respective loci; *Df(3R)Scb* deletes the *ftz* locus (Jürgens et al., 1984). All of these strains were obtained from C. Nüsslein-Volhard in Tübingen, Germany. ptc^{S2} is an amorphic allele and ptc^{G12} is a deletion of the *ptc* coding region (Nakano et al., 1989). The recombinant chromosomes: $prd^{2.45}$ ptc^{S2}, ptc^{S2} $eve^{1.27}$, wg^{IIS} ptc^{G12} and ftz^{9H34} hh^{IJ} were constructed using conventional genetic techniques. The triple mutant combination ptc^{G12}; ftz^{9H34} hh^{IJ} was generated by crossing parents of the genotype: ptc^{G12}/+; ftz^{9H34} hh^{IJ}/*TM3*. Triple mutants were recognised by their failure to express *ptc* and their reduced number of parasegments, due to the *ftz* mutation; since the ftz^{9H34} hh^{IJ} chromosome was balanced, all *ftz* embryos were necessarily also homozygous for hh^{IJ}.

In situ hybridization

Embryos were collected at the appropriate times, and fixed and processed essentially as previously described (Tautz and Pfeifle, 1989) except that probes were single-stranded RNA. DIG-labelled antisense *wg* RNA was prepared from a full-length *wg* cDNA (Rijesiwik et al., 1987) subcloned in *Bluescript*. DIG-labelled antisense *hh* RNA was prepared from a partial *hh* cDNA (Mohler and Vani, 1992) subcloned in Bluescript. Staging of embryos was according to Campos-Ortega and Hartenstein (1985). Embryos were mounted in glycerol and photographed using differential interference contrast optics on a Zeiss Axioplan microscope.

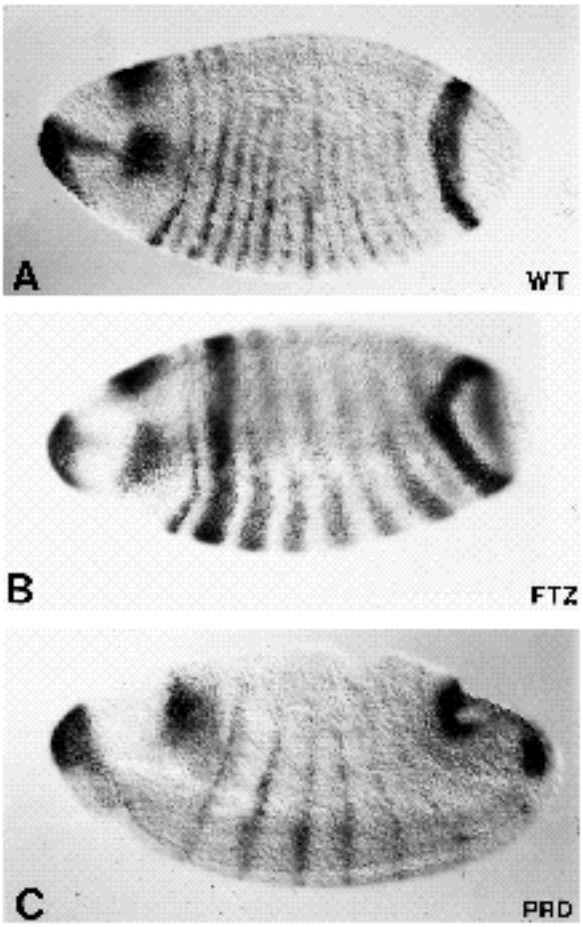

Fig. 1. Aberrant initiation of *wg* transcription in *ftz* (*Df (3R) Scb*) (B) and $prd^{2.45}$, (C) embryos; the normal pattern of activation is shown in A.

RESULTS

Aberrant activation and regulation of *wingless* expression in pair-rule mutant embryos

Transcription of *wg* is activated at the blastoderm stage of *Drosophila* embryogenesis in three broad domains at either pole of the embryo and then in single-cell-wide stripes (Baker, 1987) that define the posterior margins of each parasegmental primordium (Fig. 1A); these correspond to the cells that accumulate little or no Ftz and Eve protein as the blastoderm cellularises (Lawrence and Johnston, 1989). The parasegmental activation of *wg* is controlled by both positive and negative regulatory effects of various pair-rule gene products. In the odd-numbered parasegments, *wg* is under the negative control of *eve*, since in the absence of *eve* activity, all the cells of each primordium express *wg* (Ingham et al., 1988). Similarly, in *ftz* mutants, *wg* is activated in all the cells of the even-numbered parasegments (Fig. 1B), indicating that the *ftz* product normally acts to repress *wg* expression (Ingham et al., 1988). By contrast, in the absence of *prd* activity, there is no activation of *wg* in the cells at the margins of each even-numbered parasegment (Fig. 1C) implying that *prd* acts as a positive regulator of *wg* expression.

Maintenance of *wg* expression depends upon activity of the *hedgehog* gene

During normal development, cells at the posterior margin of each parasegment continue to express *wg* as the germ band extends; this maintenance of expression is not an autonomous property of these cells but rather is a response to a signal(s) from their posterior *en*-expressing neighbours (Martinez-Arias et al., 1988) and depends upon the activity of the *hedgehog* (*hh*) gene (Hidalgo and Ingham, 1990) that is expressed in *en*-expressing cells (Mohler and Vani, 1992; see also Fig. 3C). To define more precisely the relationship between *hh* function and *wg* expression, we analysed the pattern of *wg* transcription from the onset of gastrulation in embryos homozygous for the strong loss of function allele, hh^{IJ}. At blastoderm, no differences in the activation of *wg* transcription can be detected between wild-type and hh^{IJ} homozygotes (data not shown). Reductions in the level of transcript can first be unequivocally distinguished at stage 8 in *hh* mutant embryos; by the end of stage 9, expression is normal in the so-called 'head blob' and labrum and in the foregut and hindgut primoria (Fig. 2A,B), but has disappeared from all other ectodermal cells with the exception of six neuroblasts in each parasegment (Fig. 2C-F). As

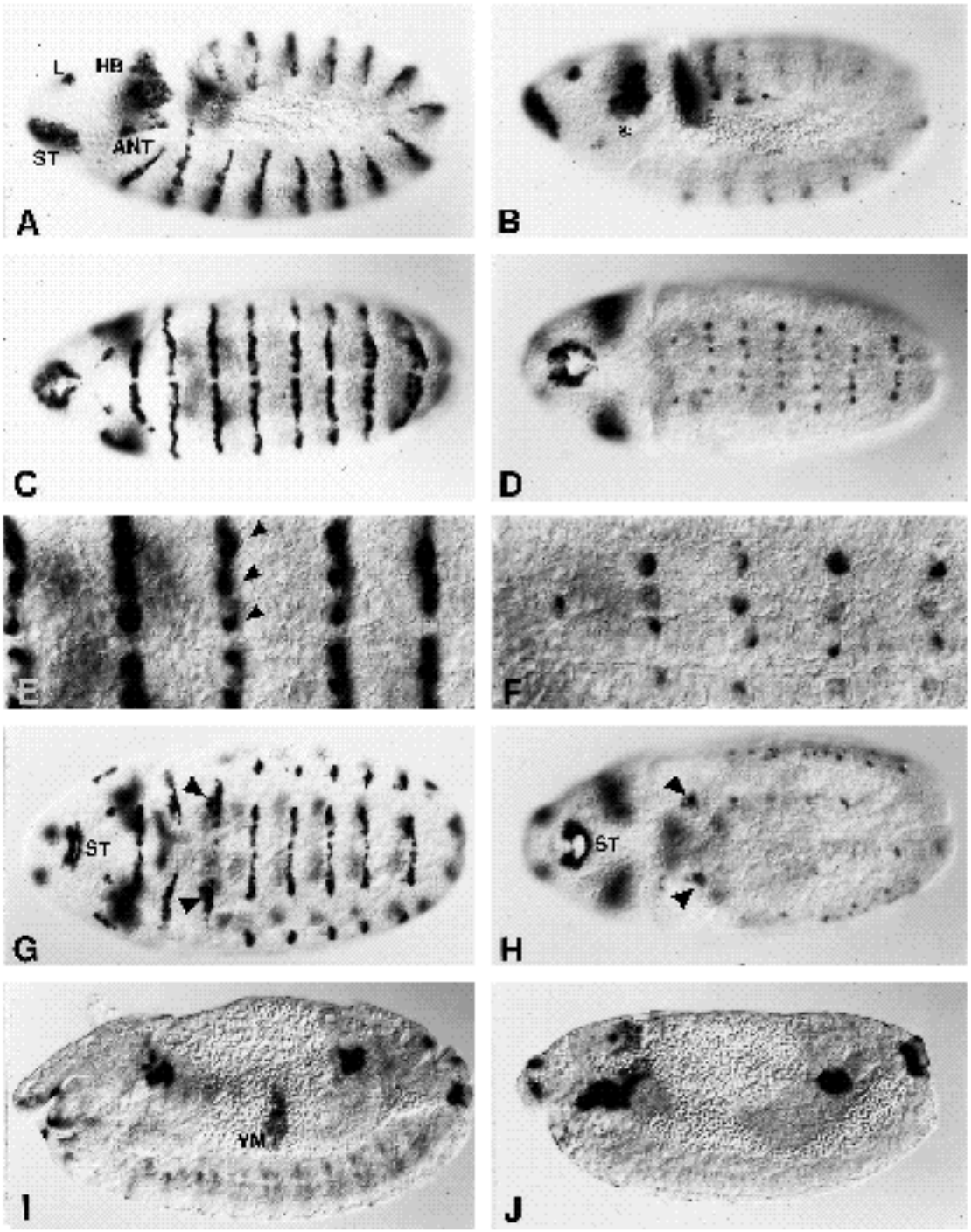

Fig. 2. Transcription of *wingless* in wild-type (left-hand panels) and hh^{IJ} (right-hand panels) embryos. At the end of stage 9 (A), transcript is present in fifteen stripes of cells marking the posterior boundaries of each parasegment. In addition, *wg* is expressed in the primordia of the labrum (L) and antenna (ANT) as well as in a 'head blob' (HB), the stomodeum (ST) and proctodeum (P). In hh^{IJ} mutant embryos at the same stage (B), expression is normal in the proctodeum, stomodeum labrum and head blob but disappears from the antennal primordium (*) and from all except the most posterior parasegmental stripes; transcript persists in the underlying neuroblasts in each parasegments through stage 10 (D; detail shown in F). Three neuroblasts in each hemisegment express *wg* at this stage as in wild type (C; indicated by arrowheads in E). As embryogenesis proceeds, expression in these neuroblasts ceases; at stage 11, two new sites of expression appear in the labial segments, marking the primordia of the salivary glands (G; arrowheads). This de novo activation also occurs in hh^{IJ} embryos (H; arrowheads). Following germ band shortening (panel I), *wg* is activated in a discrete band in the visceral mesoderm (VM). This activation does not occur in hh^{IJ} embryos (panel J); note, however, the persistent expression in the labrum, foregut, hindgut and anus.

embryogenesis proceeds, this neuroblast expression fades as in wild type. At stage 11, *wg* transcript appears in two patches in the labial segment of *hh* mutants (Fig. 2H); these correspond to the salivary gland primordia that also initiate *wg* expression in wild-type embryos (Fig. 2G). Expression of *wg* in the visceral mesoderm, that is initiated in wild-type embryos at stage 13 (Fig. 2I; van den Heuvel et al., 1989) does not, however, occur in *hh* mutants (Fig. 2J).

hh acts only on neighbouring cells to maintain *wg* expression

The ectopic expression of *wg* seen in *ftz* and *eve* mutants is not stable but fades away rapidly after gastrulation. In the case of *ftz* mutants, expression of *wg* persists only in a single-cell-wide stripe of cells at the posterior boundary of each of the abnormally large parasegments that becomes visible in the extended germ band (Martinez-Arias et al., 1988; Fig. 3H). In *ftz hh* double mutants, expression of *wg* is initiated throughout each even-numbered parasegment at blastoderm but disappears completely from the presumptive epidermis after gastrulation (Fig. 3I). Thus the persistence of *wg* in narrow stripes of cells at the posterior margins of the enlarged parasegments that form in *ftz* embryos depends upon the activity of *hh*; that only these cells maintain *wg* expression suggests that *hh* can act over only very short distances, i.e. on adjacent cells, to maintain *wg* transcription activated at blastoderm.

In the absence of *eve* activity, expression of *wg* completely disappears from the epidermal cells following gastrulation (Fig. 3E). Since *hh* is required to maintain *wg*, one explanation for this disappearance may be that *hh* is not activated in *eve* mutants; activation of *en* in the same domains at the anterior of each parasegment is known to depend absolutely upon *eve* activity (Macdonald et al., 1986). To test this possibility, embryos lacking all *eve* activity were hybridised with *hh* probe; as predicted, there is no parasegmental expression of *hh* in these embryos (Fig. 3F).

The suppression of ectopic *wg* transcription depends upon *patched* activity

In embryos lacking the activity of *ptc*, the domain of *wg* expression becomes expanded anteriorly so that by the beginning of stage 10 it occupies about one third of each parasegment (Fig. 4A,B). Prior to gastrulation, the distribution of *wg* transcripts is the same as in wild type, but subsequently broadening of the *wg* domains is detected with the beginning of germ band extension (stage 8/9). The broad *wg* domains are maintained throughout embryogenesis (Fig. 4C,D), though expression is still subject to the same selective repression along the dorsoventral axis as in wild type (data not shown; but see Fig. 4E,F).

To determine whether this repressive activity of *ptc* is involved in the suppression of ectopic *wg* expression that we observe in *eve* and *ftz* mutant embryos following gastrulation, we constructed *ptc; ftz* and *ptc eve* double mutant embryos. In both cases, expression of *wg* is activated throughout the even-numbered or odd-numbered parasegments respectively at blastoderm but instead of fading from most or all of these cells, it persists throughout the extended germ band stage and during germ band retraction (Fig. 4E,F). Thus absence of *ptc* activity results in the maintenance of *wg* transcription in the progeny of all cells in which it is initiated at blastoderm, with the exception of lateral cells in which transcription is repressed, as in wild type, during mid stage 10.

Expression of *wg* in the absence of *ptc* activity is independent of the putative *hh* signal

Since *hh* is not expressed in *eve* mutants (see above), the persistence of *wg* expression in *ptc eve* double mutants suggests that the derepression of *wg* by removing *ptc* activity is independent of *hh* activity. To test this proposal directly, we investigated whether the persistent expression of *wg* observed in *ptc*; *ftz* double mutants is *hh*-independent by constructing embryos mutant for *ptc, ftz* and *hh* (see Materials and Methods for experimental details). Expression of *wg* persists throughout germ band extension in these embryos, the transcription pattern being indistinguishable from that of *ptc; ftz* double mutants (data not shown); thus derepression of *wg* transcription in the absence of *ptc* activity is independent of *hh* function.

Absence of *ptc* activity can result in de novo activation of *wg* after gastrulation

While *ptc* activity appears to repress *wg* transcription in cells in which it is aberrantly activated at blastoderm, we wondered whether it might also act to suppress *wg* expression in other cells. In particular, we wished to investigate the possibility that some cells become 'competent' to express *wg* at blastoderm, even if they do not activate transcription at this stage. We therefore constructed a recombinant chromosome carrying null alleles of both *prd* and *ptc* and analysed *wg* expression in embryos homozygous for this chromosome. At blastoderm, *wg* is activated in these embryos only in the odd-numbered parasegments, as in embryos homozygous for the *prd* allele alone (see Fig. 1C). At stage 9/10, these odd *wg* domains broaden as in *ptc* mutants; in addition, new stripes of *wg*-expressing cells appear in the even-numbered parsegments (Fig. 4G,H). These stripes are initially narrower than their odd-numbered counterparts but subsequently become broader. The position of the stripes, located mid way between the odd-numbered ones, implies that they are composed of cells that would normally have activated *wg* transcription in response to *prd* activity.

wg activity is required only indirectly for *wg* expression at the parasegment boundary

In tissue-culture systems, the murine orthologue of *wg*, *Wnt-1*, can act in both a paracrine and an autocrine fashion to transform cells morphologically (Jue et al., 1992). We therefore wondered whether *wg* might itself be required for the maintenance of its own expression Embryos homozygous for the allele wg^{IIS} exhibit a typical *wg* null cuticular phenotype but still produce a transcript. Transcription of *wg* in these embryos is initiated normally at blastoderm, but as in *hh* embryos disappears from the ectoderm during germ band extension. By stage 10, the ectodermal stripes of *wg* transcript that characterise the trunk region of the embryo are missing (Fig. 5A) though

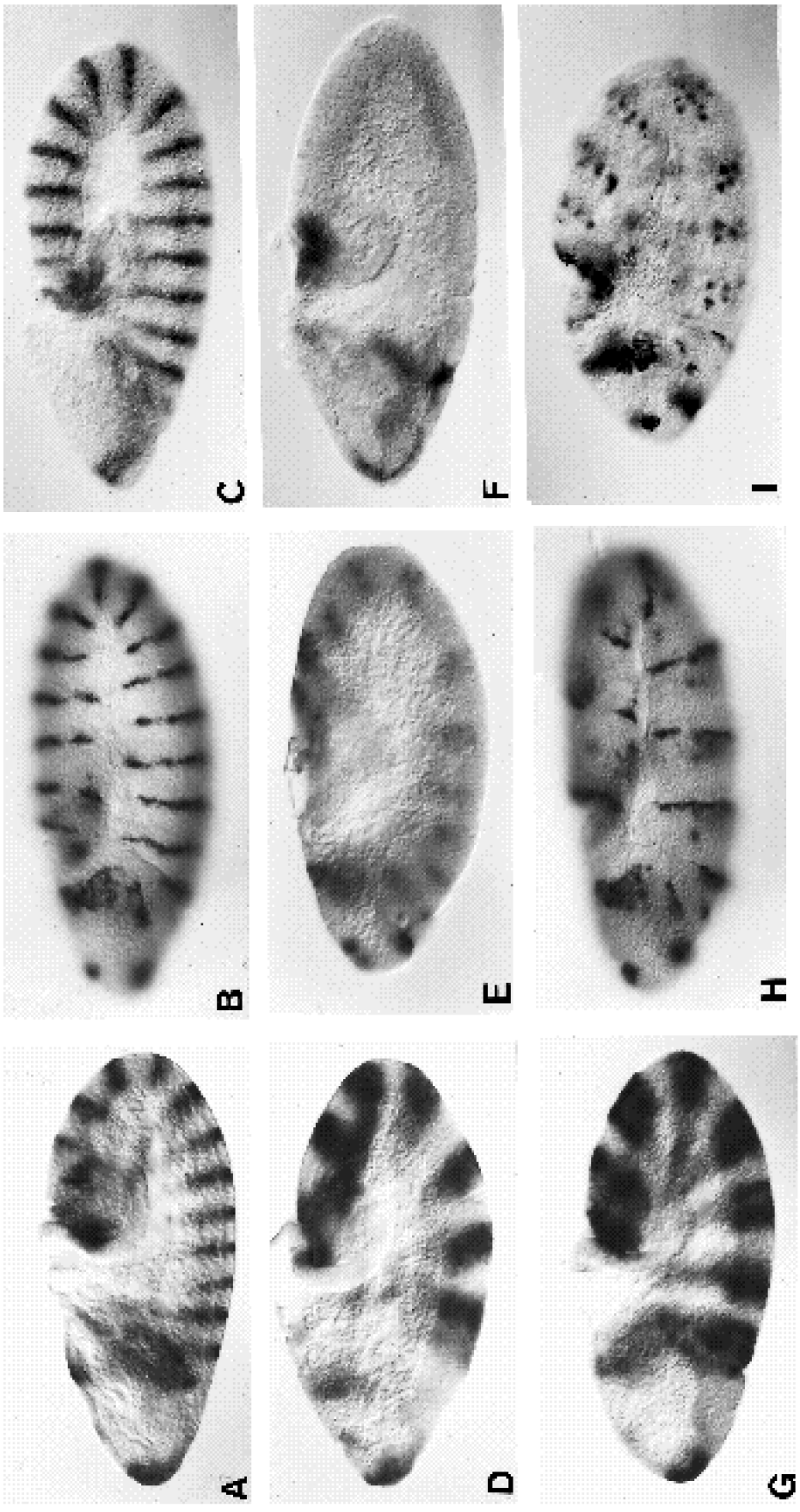

Fig. 3. Regulation of ectopic *wg* expression in *ftz* (*Df(3R) Scb*) and *eve*$^{1.27}$. Panels A and B show the wild-type patterns of *wg* transcription at stage 8 and stage 10; panels D and E and G and H show *eve* and *ftz* embryos, respectively, at equivalent stages. Panels C and F show the expression of *hh* in stage 9 wild-type and *eve* mutant embryos, respectively. Expression of *hh* disappears from all parasegments in *eve* mutants (F); similarly, *wg* transcripts disappear from the epidermal cells of each parasegment (E) but persist in groups of neuroblasts (out of focus). In *ftz* embryos (H), *wg* expression persists in the epidermis at the posterior boundaries of the enlarged parasegments as well as in groups of neuroblasts; in *ftz*^{9H34} *hh*^{J1} embryos (I), there is no expression of *wg* in the epidermal cells after gastrulation; however, transcript is present in seven clusters of neuroblasts, as in the *ftz* mutant shown in H.

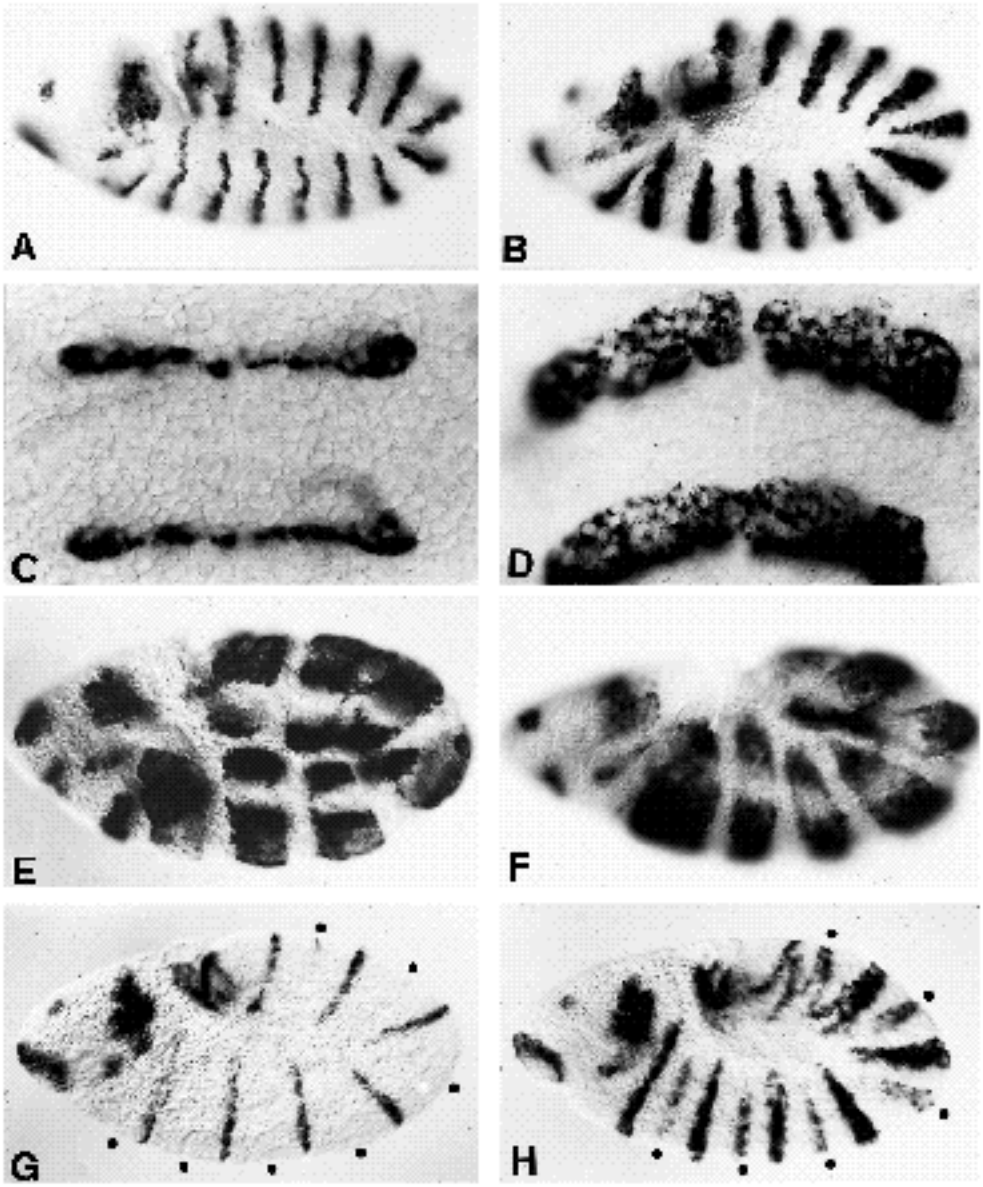

Fig. 4. At the end of stage 9, the parasegmental domains of *wg* expression are significantly broader in embryos lacking *ptc* activity (B) than in wild type (A). Panel C shows a high magnification image of the ventral region of a wild-type embryo at the end of stage 10; by this time, the *wg* domain has narrowed further, whereas in ptc^{G12} mutants, it continues to occupy about one third of each parasegment (panel D). The ectopic expression of *wg* established at blastoderm persists through stage 11 in ptc^{IN}; *Df(3R) Scb,ftz*$^{-}$ (E) and ptc^{S2} $eve^{1.27}$ (F) mutant embryos; note, however, the lateral repression of transcription in both cases. Expression of *wg* in $prd^{2.45}$ (G) and $prd^{2.45}$ ptc^{S2} (H) late stage 9 embryos. Dots indicate the positions of the even-numbered parasegments.

expression persists in the same groups of neuroblasts seen in wild-type and *hh* mutant embryos (Fig. 5B; compare with Fig. 2C,D). In contrast to *hh* mutants, however, the transcript also disappears from the stomodeum and hindgut primordium (Fig. 5B,C; compare with Fig. 2H) and is much reduced in the head blob by stage 11 (Fig. 5C); at the same time, new expression of *wg* is initiated in ectodermal cells at the dorsal margins of each segment (Fig. 5C). The similarity between the patterns of *wg* transcript in *hh* and *wg* mutants suggests that *wg* may exert its effects indirectly, maintenance of *hh* expression depending upon *wg* activity (Mohler and Vani, 1992). To test this possibility, we constructed the double mutant *wg ptc* in which any effect of the loss of *hh* activity (due to the *wg* mutation) on *wg* transcription should be overriden by the removal of *ptc*. In these double mutant embryos, *wg* transcript persists in the most ventral part of the ectoderm throughout germ band extension (Fig. 5D); this indicates that *wg* function is not essential for *wg* transcription at the parasegment boundaries and suggests that the effect of the *wg* mutation is indeed mediated through its control of *hh* expression. By contrast, expression of *wg* still disappears from the stomodeum and hindgut (Fig. 5D) indicating that this requirement for *wg* activity cannot be compensated for by removal of *ptc* activity.

DISCUSSION

At the blastoderm stage of embryogenesis the initiation of *wg* transcription in single-cell-wide stripes at the posterior boundary of each parasegmental primordium depends upon opposing activities of various pair-rule gene products. The expression of *prd* is required in alternate primordia for the activation of *wg* transcription whereas both the *ftz* and *eve* proteins act to repress this activation.

Our analysis of *wg* in *hh* embryos shows that *hh* activity is required for the maintenance of *wg* transcription within

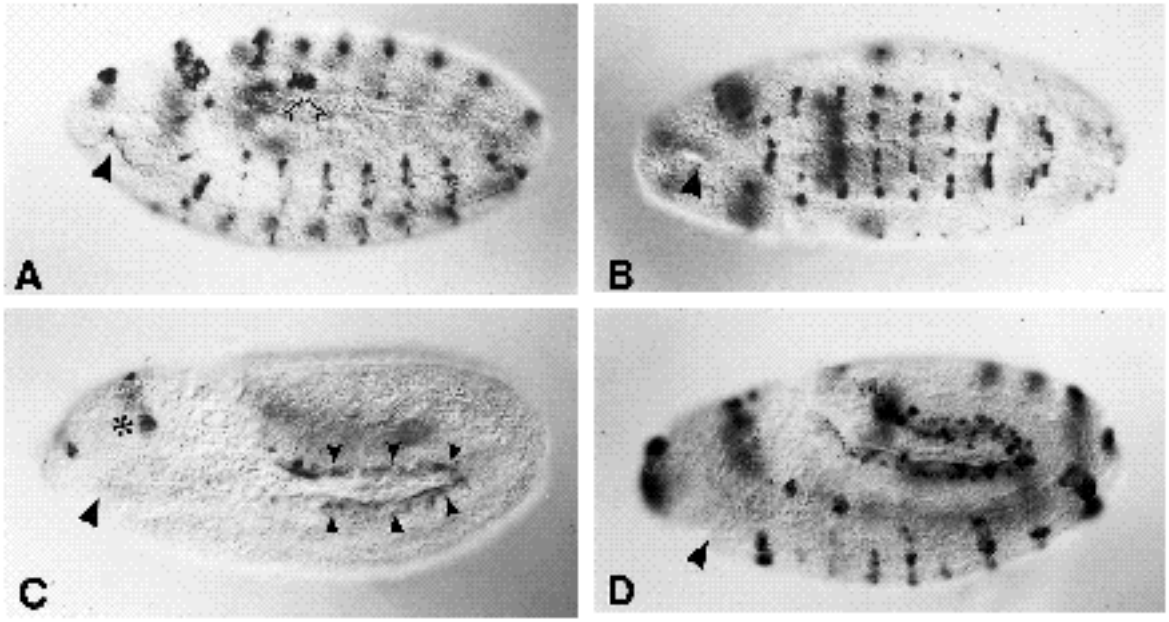

Fig. 5. Transcription of *wg* in the absence of *wg* activity. Although embryos homozygous for the mutation wg^{IIS} exhibit a null *wg* phenotype, they still make a *wg* transcript detectable by in situ hybridisation. By stage 10 (panels A and B), the *wg* transcript has disappeared from the ectoderm of all the parasegments with the exception of the most posterior; in addition, expression persists in parasegment 14 in a lateral patch (open arrowhead). As in hh^{IJ} embryos, expression persists in neuroblasts in each parasegment and is still detectable in the labrum and head blob; however, it disappears from the stomodeum (large arrowhead) and proctodeum (compare with Fig. 2 panels A-D). By late stage 11 (C), expression in the head blob (*) has almost disappeared; new expression appears at the dorsal margins of each parasegment (small arrowheads). (D) wg^{IIS} ptc^{G12} embryo at early stage 11. *wg* transcription persists ventrally at each parasegmental boundary, though the width and intensity of each of these domains is variable; note the absence of expression in the stomodeum (arrowhead).

one hour of its activation in response to these pair-rule cues. Interestingly, this requirement is limited to those regions of the embryo where *wg* is expressed in narrow bands of cells; thus, in the head, the stripe of cells in the antennal primordium requires *hh* activity whereas expression in the adjacent broad patch of cells, the 'head blob' is independent of *hh* function. This implies a separate mechanism for the maintenance of *wg* expression in large patches of cells such as those of the head blob and of the foregut and hindgut primordia that are also independent of *hh* function; the initiation of expression in these regions is known to be under different control, the head blob expression depending upon activity of the head gap gene *orthodenticle* (Cohen and Jurgens, 1990). Within each parasegment, six neuroblasts also continue to express *wg* in *hh* mutant embryos after the expression in the overlying ectoderm has disappeared. Presumably transcription in these cells becomes fixed as they delaminate from the ectoderm and is maintained autonomously until the neuroblasts divide, when expression ceases both in the mutant and in wild-type embryos.

The misexpression of *wg* that ensues in the absence of either *ftz* or *eve*, is rapidly compensated for by a second mechanism that is able to repress *wg* transcription following its aberrant activation. Here we have shown that this repressive mechanism depends upon the activity of *ptc*; in the *ptc eve* and *ptc; ftz* double mutant combinations that we analysed, the ectopic expression of *wg* along the anteroposterior axis in each parasegment persists throughout embryogenesis, although the gene is still subject to regulation along the dorsovental axis. Thus *ptc* activity is capable of repressing *wg* expression in any cell in which it has been activated, irrespective of its location within the parasegment, but this negative regulatory activity of *ptc* is not the only mechanism by which *wg* can be repressed.

In normal development, however, the activation of *wg* is restricted from the outset to cells at the posterior margin of each parasegment; thus the ability of *ptc* to repress *wg* in other regions of the parasegment might seem gratuitous. However, when *ptc* is eliminated from an otherwise genetically wild-type embryo, there is a broadening of the *wg* expression domain anteriorly. This ectopic expression of *wg* is limited to cells in the posterior part of each parasegment, even though *ptc* is itself expressed (and potentially functional) in a much broader domain (Nakano et al., 1989; Hooper and Scott, 1989). One way of interpreting these findings is to suppose that the normal function of *ptc* is to repress *wg* transcription in a subset of the polyclone that initiates *wg* expression at blastoderm. It has previously been proposed that *ptc* acts constitutvely to repress *wg* transcription and that *hh* antagonises this repressive effect of *ptc*, thereby positively regulating *wg* (Ingham et al., 1991; Hidalgo, 1991). Accordingly, following the cellular rearrangements and divisions that occur during germ band extension, *wg* expression would be maintained in just those cells that are within range of the putative *hh* signal, a range that from our observations seems to be limited to adjacent cells. Recent cell lineage studies have suggested that an analogous mechanism acts to restrict the *en* domain fol-

lowing gastrulation, some cells that initially express *en* at blastoderm subsequently switching off expression if they become displaced more than a certain distance from the neighbouring *wg*-expressing cells (Vincent and O'Farrell, 1992). The difference between the respective widths of the *en* and *wg* domains presumably reflects the differing ranges of the putative signals: *wg*, like its mammalian orthologue, *Wnt-1*, seems able to act across more than one cell diameter (Gonzalez et al., 1991; Bejsovec and Martinez-Arias, 1991; Jue et al., 1992) whereas *hh* appears to be restricted to acting only on its immediate neighbours. This latter finding fits well with the molecular structure of the Hh protein which, from sequence analysis is predicted to contain a single *trans*-membrane domain (Mohler and Vani, 1992); thus the activity of the protein might be expected to be limited to cells that come into direct contact with those expressing *hh*.

Whilst our data are consistent with such a mechanism, we consider it likely that the activity of *ptc* is not exclusively restricted to repressing *wg* expression within the polyclone in which it is activated at blastoderm, but also to suppressing its expression in other cells that have the *potential* to activate *wg* transcription. This proposal is based partly upon consideration of the extent to which the *wg* expression domain is expanded in *ptc* mutant embryos; *wg*-expressing cells occupy about one third of each parasegment, a rather larger proportion than would be expected to be contributed by the polyclone that initiates *wg* expression at blastoderm. Thus, in the absence of *ptc*, it seems that cells anterior to the *wg* polyclone have ectopically activated *wg* expression following gastrulation.

The expression of *wg* that we observe in *prd ptc* mutant embryos tends to support the idea that *ptc* suppresses a latency to express *wg*. In these embryos, expression of *wg* in the even-numbered parasegments is not initiated at blastoderm, due to the absence of *prd* activity, yet following gastrulation, these expression domains appear in the appropriate locations. This implies that, although *wg* transcription was not initiated, the cells in the posterior part of each parasegment are selectively specified to be competent to express *wg*, a competence that is revealed if the repressive activity of *ptc* is removed. In embryos mutant for *prd* alone, this competence to express *wg* is not manifest, presumably because the corresponding domains of *en/hh*-expressing cells are not established; thus even though the *wg*-competent cells are present, they do not activate transcription because the repressive activity of *ptc* cannot be antagonised by *hh*.

How such transcriptional competence is determined is at present uncertain, but one possibility is that the pair-rule genes themselves are instrumental in establishing the potential of the *wg* promoter to be activated. According to this view, low levels of Eve or Ftz protein at the blastoderm stage would render the *wg* transcription unit 'open for business' (see Peifer et al., 1987); transcription would only be initiated immediately in nuclei of cells expressing *prd* (and in the odd-numbered parasegments *opa*); however, the other nuclei would retain their 'open for business' conformation and hence be receptive to activity of a second level of transcriptional activation that commences as gastrulation proceeds. It is this second level of transcriptional activators (which might be ubiquitously distributed), necessary for the maintenance of *wg* expression after pair-rule activity has ceased, that would be modulated by *ptc* and *hh* activity. According to this interpretation, the levels of *ftz* and *eve* activity at blastoderm would play the key role in specifying the future propensities of the cells of each parasegment, subdividing them into *wg*-competent and *en*-competent equivalence groups. In embryos lacking a functional *prd* gene, alternative *wg* domains fail to be activated at blastoderm; however, the cells in the posterior half of every parasegment primordium should become 'open for business' with respect to *wg*, as in wild type, since the *prd* mutation has no effect on *ftz* or *eve* expression.

The significance of this hypothesis is that it predicts that the specification of cell fate at the blastoderm stage of embryogenesis is not restricted to the cells at the boundaries of each parasegment that activate transcription of *en* or *wg*, but in addition includes the intervening cells. Thus cells adjacent to those expressing *wg* or *en* do not remain developmentally naive, but rather acquire differing potentials to respond to inductive signals by being allocated to different 'equivalence groups' in response to varying levels of expression of *ftz*, *eve* and possibly other pair-rule genes. The notion of equivalence groups helps to explain why cells in different locations can respond to the same signal with different outcomes. For instance, cells anterior to the *wg* domain respond to *wg* activity by accumulating Arm protein at their surface (Riggleman et al., 1990), while those posterior to this domain respond to the same signal by expressing *en*. Similarly, cells anterior and posterior to the *hh* domain respond differentially to *hh* activity (Hidalgo and Ingham, 1990); in our terms, the differential responses reflect the differing competences of the responding cells.

The data that we have presented indicate that the spatial restriction of *wg* expression is of crucial importance to the patterning of each parasegment; although *wg* is initially activated under strict spatial control, subsequent cell-cell interactions are required to maintain its expression in the appropriate cells. We have shown that *hh* and *ptc* play a central role in this process; however, by analogy with its mammmalian orthologue, *Wnt-1*, it is possible that *wg* itself could act in an autocrine manner to maintain its own expression. The finding that *wg* expression depends upon *wg* activity might at first sight seem to support this view; however, our finding that this requirement is suppressed in *wg* mutants that also lack *ptc* activity suggests that the effect of *wg* mutants on its own expression is indirect. In the absence of *ptc* activity, the cells expressing *wg* can be considered physiologically equivalent to those that normally express *wg* in the wild-type embryo, assuming that the latter also effectively lack *ptc* activity due to the antagonistic effect of *hh*. Thus the persistence of *wg* transcription in mutants lacking *ptc* and *wg* activity suggests that there is no direct effect of Wg protein on the cells that transcribe *wg*; rather, the disappearence of *wg* transcript in *wg* mutants is likely to be due to the requirement for *wg* activity to maintain *hh* expression (Mohler and Vani, 1992). Thus we conclude that, at the parasegmental boundaries at least, *wg* acts exclusively in a paracrine fashion to specify cell fate. Although expression of *wg* persists for some time in some of these domains in the absence of *wg* activity, the

expression in the stomodeum and hindgut primorium rapidly fades in the absence of *wg* activity. This may indicate an autocrine control of *wg* in these regions, although we cannot rule out the possibility of regulation by some other paracrine mechanism that is itself dependent upon *wg* activity.

We would like to dedicate this paper to the memory of Carlos Cabrera our friend and one time colleague who shared with us an interest in the role of *wingless* in the *Drosophila* embryo. We thank C. Nüsslein-Volhard and R. Whittle for mutant stocks, J. Mohler for *hh* DNA, A. Forbes, Y. Nakano and A. Taylor for many stimulating discussions, U. Strähle for comments on the manuscript and A. Taylor for technical assistance. This work was supported by the Imperial Cancer Research Fund.

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(Accepted 8 October 1992)