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The *hedgehog* gene family in *Drosophila* and vertebrate development

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SUMMARY

The segment polarity gene *hedgehog* plays a central role in cell patterning during embryonic and post-embryonic development of the dipteran, *Drosophila melanogaster*. Recent studies have identified a family of *hedgehog* related genes in vertebrates; one of these, *Sonic hedgehog* is implicated in positional signalling processes that show interesting similarities with those controlled by its *Drosophila* homologue.

Key words: *hedgehog*, cell-signalling, floor-plate induction, limb patterning, imaginal discs, segment polarity genes

INTRODUCTION

Although the role of signalling factors in organising cell populations in developing embryos has long been recognised, it is only fairly recently that the molecular nature of these signals has begun to be elucidated. Some of the most notable examples to date are the various proteins found to mimic the mesoderm-inducing capacity of cells of the vegetal hemisphere of early *Xenopus* embryos. These include members of the FGF (Slack et al., 1987) and TGFβ (Green et al., 1990; Kimmelman and Kirshner, 1987) growth factor families; in addition, members of the Wnt family of growth factor-like proteins have been implicated in this process (Christian et al., 1992; Smith and Harland, 1991). While the genes encoding these various protein families have been highly conserved at the structural level throughout evolution, few similarities in their deployment during the embryonic development of species from different phyla have been reported. One possible exception is provided by the *Wnt-1* gene and its *Drosophila* orthologue, the segment polarity gene *wingless*. Activity of *Wnt-1* in the mid-brain of vertebrate embryos appears to be required for the expression of the *Engrailed* genes (McMahon et al., 1992), a regulatory relationship that recalls the interaction between *wingless* and *engrailed*-expressing cells in the developing *Drosophila* embryo (discussed below).

The recent molecular characterisation of the segment polarity gene *hedgehog*, (Lee et al., 1992; Mohler and Vani, 1992; Tabata et al., 1992; Tashiro et al., 1993) has led to the discovery of a new family of putative signal-encoding genes in various vertebrate species that are highly homologous to the *Drosophila* gene (Echelard et al., 1993; Krauss et al., 1993; Riddle et al., 1993; Roelink et al., 1994). The deployment of one of these, *Sonic hedgehog* (*Shh*), in embryos of several different species presents some striking parallels with that of its invertebrate homologue. The *hedgehog* gene family thus provides the first clear example of a conserved signalling factor that regulates analogous processes in species of different phyla.

THE *hedgehog* FAMILY: A NEW CLASS OF SIGNALLING MOLECULES

The *Drosophila hedgehog* gene contains a 471 codon open reading frame (ORF) capable of encoding a polypeptide of M_r 52,147 (Lee et al., 1992). Hydropathy analysis identifies a highly hydrophobic region near the N terminus between residues 63 and 83. In vitro translation analysis suggests that this region may act either as a conventional signal sequence, leading to a secreted form of the protein, or as a membrane spanning domain, anchoring the protein in the cells in which it is expressed (Lee et al., 1992). The results of immunolocalisation analysis on fixed *Drosophila* tissues are consistent with both of these possibilities (Tabata and Kornberg, 1994; Taylor et al., 1993). Thus the properties of the Hh protein may implicate it in either short or long range signalling.

Using a combination of reduced stringency hybridisation and polymerase chain reaction, we have identified a number of *hh*-related genes in the genomes of several vertebrate species including mouse (Echelard et al., 1993), chick (Riddle et al., 1993), *Xenopus* (J-P. C. and P.W.I. unpublished results) and zebrafish (Krauss et al., 1993). The proteins encoded by these genes show a high degree of sequence identity both within and between species which is reflected at the functional level by the ability of the zebrafish *Shh* gene to activate the *Drosophila hh* signal transduction pathway (Krauss et al., 1993; M.J.F. and P.W.I., in preparation).

Alignment of the predicted amino acid sequences of the *Drosophila* Hh protein with those of the mouse Dhh, Ihh and Shh proteins and the chick and zebrafish Shh proteins reveals several interesting features of the *hh* family (see Fig. 1). Like the *Drosophila* protein, all the vertebrate proteins possess an amino-terminal hydrophobic region of approximately 20 residues; however, the initiation codon is located immediately upstream of this region, in contrast to *Drosophila* Hh which initiates some 60 residues upstream of this region. Thus it is likely that in vertebrates this sequence acts exclusively as a signal peptide sequence giving rise to secreted and not membrane spanning proteins. Interestingly, the *Drosophila* gene has a second ATG at a similar position, raising the possibility that it generates different forms of the protein via the control of translational initiation (Lee et al., 1992).

Sequence conservation between the proteins is highest in their amino-terminal ends; indeed, from position 85 (immediately after the predicted shared cleavage site) to position 249, 62% of the residues are completely invariant among the *Drosophila* and vertebrate proteins. Comparison of the different mouse proteins in this more conserved region, indicates that Ihh and Shh are more closely related to each other (90% amino acid identity) than to Dhh (80% identity). Comparison of Shh between species reveals a 99% identity between mouse and chick and 94% identity between mouse and fish in the same region. Conservation falls off rapidly after residue 266, apart from a short stretch at the C terminus.

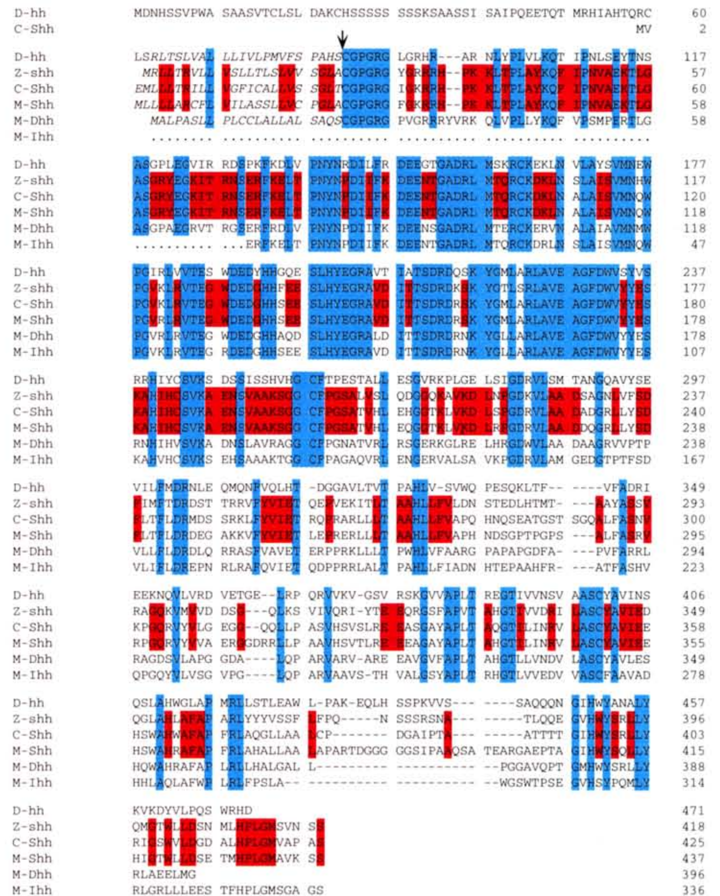

Fig. 1. Alignment of the *Drosophila* and vertebrate *hh*-family amino acid sequences. The predicted hydrophobic transmembrane/signal sequences are indicated in italics; the arrowhead indicates the predicted signal sequence processing site. Amino acids shared by all six proteins are shown in blue; identities between the mouse, chicken and zebrafish Shh proteins are shown in red. The amino acid sequence shown for the zebrafish Shh protein differs slightly from that previously published by Krauss et al., (1993); the corrected nucleic acid sequence from which this is derived is deposited in the EMBL data base under accession number Z35669.

SIGNALLING CENTRES AND *hh* FUNCTION IN THE *DROSOPHILA* EMBRYO

During the early stages of its development, the *Drosophila* embryo is subdivided into a series of repeating units, the parasegments. This subdivision is marked by the activation of the segment polarity genes *wingless* and *engrailed* in a series of discrete bands of cells along the anteroposterior axis of the embryo. Each *wg* domain abuts an adjacent *en* domain and these interfaces define the parasegment boundaries. Genetic studies have shown that parasegment boundaries have special properties, acting as sources of signals that organise the patterning and polarity of the cellular fields which they define (reviewed by Ingham and Martinez Arias, 1992). One of these signals is encoded by *wg* itself: in the absence of *wg* activity, *en* expression is lost from neighbouring cells (Di Nardo et al., 1988; Martinez Arias et al., 1988) and the positional specification of all the cells in each parasegment is disrupted, each cell now adopting a similar fate; this effect is clearly manifested at the end of embryogenesis in the cuticular pattern secreted by the epidermal cells.

Several lines of evidence indicate that the signal produced by *en*-expressing cells is encoded by *hedgehog*. Like *wg*, *hh* activity influences the development of the entire parasegment and embryos homozygous for loss of function *hh* alleles display a phenotype very similar to that seen in *wg* mutants. In the absence of *hh* activity, *wg* transcription is activated normally, but disappears rapidly after gastrulation (Ingham and Hidalgo, 1993). Thus one of the principal functions of *hh* is to maintain the transcription of *wg* in the cells of neighbouring parasegments. Notably, the maintenance of *wg* is restricted to a single row of cells immediately apposed to those expressing *hh*. This characteristic suggests that the range of *hh* activity is extremely limited, perhaps even contact dependent; alternatively, it could be that only these cells are able to respond to the *hh*-encoded signal. This latter possibility can however, be ruled out since in transgenic embryos in which *hh* is expressed ubiquitously, transcription of *wg* is activated ectopically (Ingham, 1993; Tabata and Kornberg, 1994; see Fig. 2). Significantly, this ectopic activation is limited to a subset of cells in each parasegment, immediately anterior to those that normally express *wg*. The capacity of cells to express *wg* in response to the *hh* signal depends upon the activity of the *sloppy paired* (*slp*) gene, a transcription factor belonging to the *forkhead* related family. Activity of *slp* is necessary but not sufficient for *wg* transcription, the *slp* expression domain defining an equivalence group of "*wg*-competent" cells (Cadigan et al., 1994). Thus in normal development, *hh* acts to trigger expression of *wg* in a subset of the cells of this equivalence group, thereby restricting its expression to the parasegment boundary.

The importance of the restricted range of *hh* activity is illus-

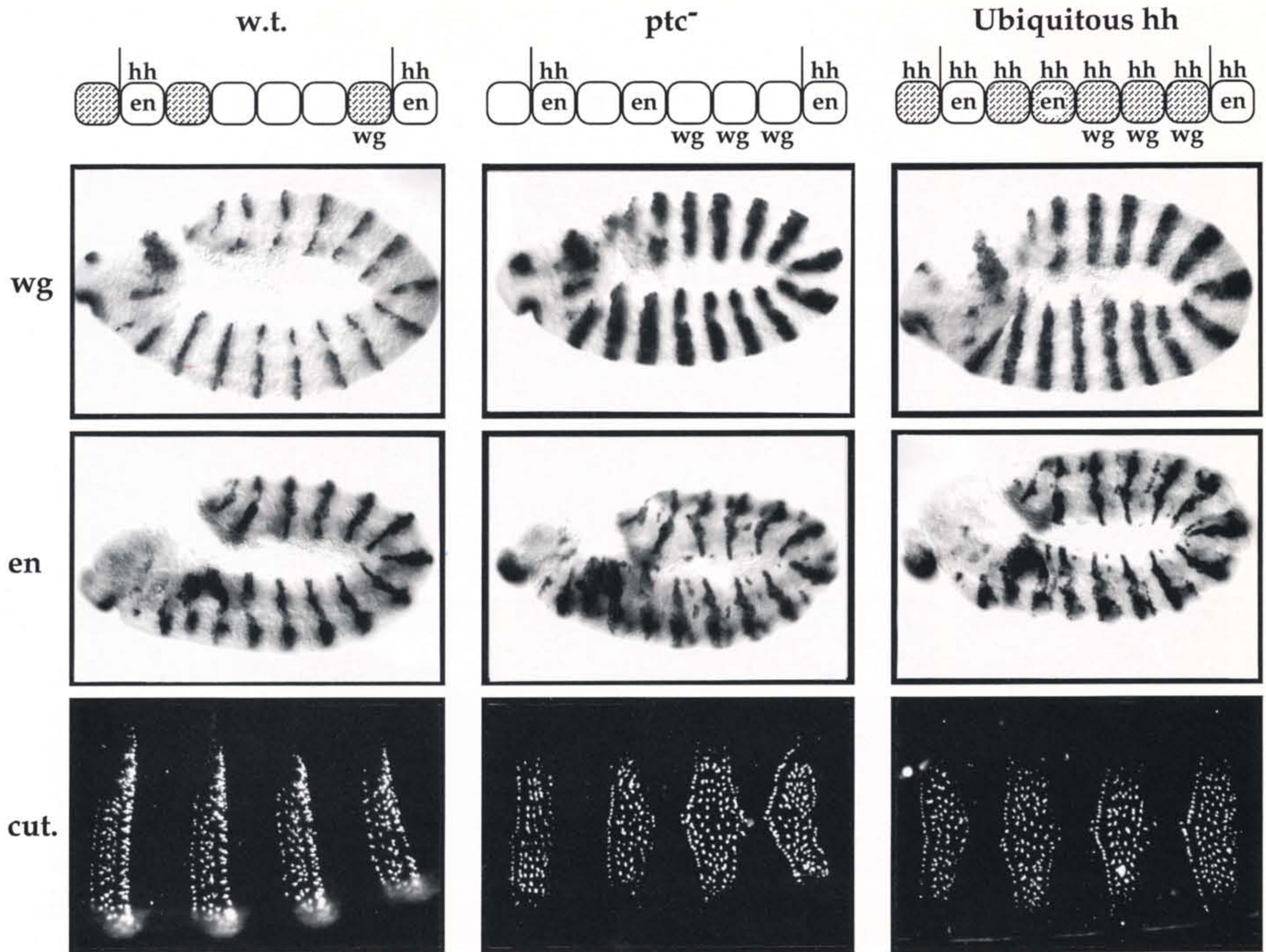

Fig. 2. Patterns of *wingless* (*wg*) and *engrailed* (*en*) expression and ventral cuticular (cut.) differentiation in wild type (w.t.) (left) and *patched* (*ptc*) mutant (centre) embryos and in embryos in which *hedgehog* (*hh*) is ubiquitously expressed (right). The expression domains of each gene in a single parasegment are represented schematically at the top of the figure. Ubiquitous expression of *hh* or absence of *ptc* activity leads to the expansion of the *wg* domain relative to wild-type and the ectopic induction of *en* expression in the centre of each parasegment. These changes in gene activity result in the duplication and deletion of specific pattern elements as manifested in the ventral cuticle.

trated by the pattern defects that ensue when it is overexpressed. Expansion of the *wg* domain results in the ectopic induction of *en* (Tabata and Kornberg, 1994) (Fig. 2). The interface between these ectopically located *en*-expressing cells and their anterior neighbours in turn induces the formation of an additional segment border in each parasegment and this is accompanied by the elimination of certain denticle types and their replacement by others with reversed polarity. These effects mimic precisely the phenotype of mutations of the another segment polarity gene named *patched* (*ptc*) (Martinez Arias et al., 1988; Fig. 2). This finding could suggest a role for *ptc* in restricting the range of the Hh protein and indeed, Hh is much more widely distributed in *ptc* mutant embryos than in wild type (Tabata and Kornberg, 1994; Taylor et al., 1993). Notably, however, activation of *wg* is rendered independent of *hh* activity in the absence of *ptc* function (Ingham and Hidalgo, 1993; Ingham et al., 1991) suggesting instead that the normal role of *ptc* is to suppress the *hh* signalling pathway, leaving it constitutively active in the absence of *ptc*. Since *ptc* encodes an integral membrane protein (Hooper and Scott, 1989; Nakano et al., 1989), one possibility is that the Ptc and Hh proteins interact at the cell surface, the latter inactivating the former and hence triggering the pathway that controls *wg* transcription. Despite this close functional relationship, no homologue of the *ptc* gene has yet been identified in any vertebrate species.

MIDLINE SIGNALLING AND *Sonic hedgehog* EXPRESSION IN VERTEBRATE EMBRYOS

One of the best characterised sources of signalling activity in developing vertebrate embryos is the notochord, the derivative of the axial mesoderm. Several processes have been associated with the inductive properties of this tissue including the induction of specialised ventral neural cells that form the floorplate (Placzek et al., 1990; van Straaten et al., 1989), the specification of neuronal differentiation (Placzek et al., 1991;

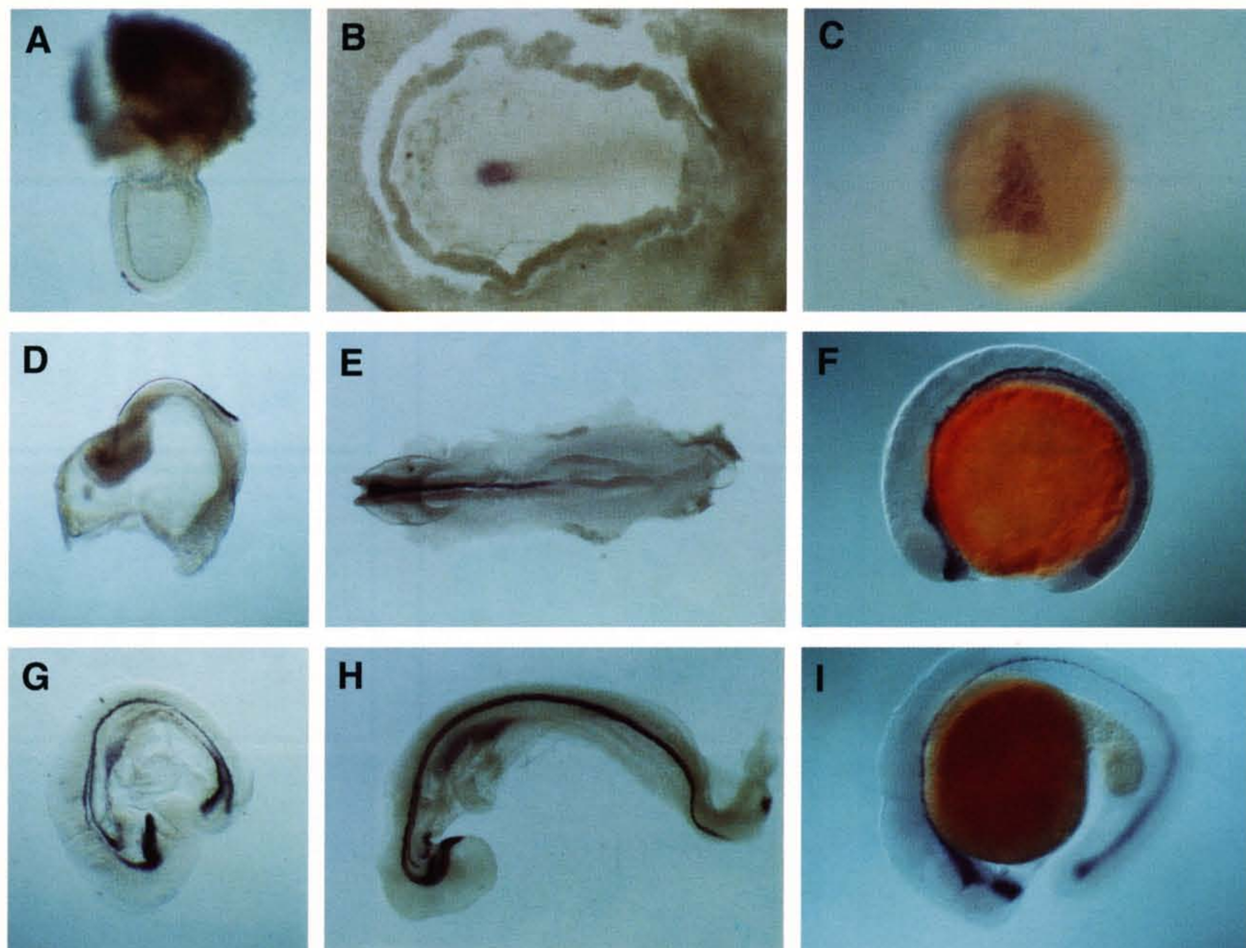

Fig. 3. A comparison of the expression of *Shh* in developing mouse (left), chicken (centre) and zebrafish (right) embryos. (A-C) Onset of *Shh* expression in gastrulation stages; (A) expression in the mouse is restricted to the head process; (B) in the chick, expression is limited to Hensen's node and in the fish (C) to the embryonic shield. (D-F) Early somitogenesis (~8-10 somites); expression is seen throughout the axial mesoderm (presumptive notochord) in all three species and is already detectable in the presumptive floorplate of the fish. (G-I) At later stages of somitogenesis, expression is detectable throughout the ventral floor of the central nervous system; note that in the fish embryo expression has already disappeared from most of the notochord.

Yamada et al., 1991) and the induction of paraxial mesoderm to form scleretome (Dietrich et al., 1993; Koseki et al., 1993; Pourquie et al., 1993).

Evidence for these interactions comes principally from experimental manipulations of developing mouse and chick embryos; in embryos of both species, ablation of the notochord results in a failure of floor plate and motor neuron differentiation, whereas grafting of notochord to ectopic locations in chick embryos results in the induction of ectopic floor plate and motor neurons in close proximity to the graft. Since notochord is closely apposed to floor plate cells both in normal development and in the experimentally manipulated embryos, it has been suggested that the inductive signal must be contact dependent (Placzek et al., 1990), a conclusion supported by the results of in vitro studies (Placzek et al., 1993). Motor neuron differentiation, by contrast, depends upon diffusible factors that act in a contact independent manner (Yamada et al., 1993) and which emanate both from the notochord and the floorplate cells induced by the notochord. Thus the patterning of the neural tube in amniotes can be seen in terms of a sequence of inductive interactions, in which one signalling centre, the notochord, induces another, the floorplate, the activity of which alone can pattern the ventral half of the neural tube. We have found that the putative signal encoding *hh* family gene, *Sonic hedgehog*, is expressed in both the axial mesoderm and the floorplate of mouse and chick embryos (Echelard et al., 1993; Riddle et al., 1993), thus implicating it in at least some of the signalling activities associated with these tissues. Moreover, the spatiotemporal expression pattern of *Shh* is remarkably similar in zebrafish embryos (Krauss et al., 1993) suggesting that the molecular basis of mid-line signalling may be conserved between fishes and amniotes.

Expression of *Shh* is first detectable during gastrulation stages of each species: in the fish embryo, transcripts are restricted to the inner cell layer of the embryonic shield, the equivalent of the amphibian organiser, while in chick, expression is detectable in the homologous structure, Hensen's node (Figure 3B,C). A slight difference is apparent in the mouse at this stage, where expression can first be detected in the midline mesoderm of the head process that arises from the

node, though not in the node itself (Figure 3A); however, expression is detectable in the node soon thereafter.

Extension of the body axis of embryos of each species is accompanied by an extension of the *Shh* expression domain. In the zebrafish, by 9.5 hours of development, the *Shh* expression domain constitutes a continuous band of cells that extends from the tail into the head, the anterior boundary of expression being positioned in the centre of the animal pole anterior to the presumptive midbrain. In the mouse and chick, expression similarly extends rostrally from the node, although expression appears limited to the level of the midbrain. Whilst the early phase of *Shh* expression is restricted to the midline mesoderm a new phase of expression in the overlying neuroectoderm is initiated during early somitogenesis. In the mouse, neural expression is first seen at around the 8 somite stage when it is initiated at the ventral midline of the midbrain, above the rostral limit of the head process. Expression extends rapidly both rostrally, into the forebrain, and caudally into the hindbrain and spinal cord. In the chick, neural expression of *Shh* is initiated at the 7-8 somite stage and, in contrast to the mouse embryo, appears simultaneously along almost the entire length of the neural fold. In zebrafish, *Shh* expression is apparent in the embryonic CNS at the 5 somite stage extending from the tip of the forebrain caudally through the hindbrain and rapidly extends caudally along the length of the neural keel. Expression in each species is restricted in the hindbrain and spinal cord to the ventral midline, whilst in midbrain and forebrain , it extends more laterally. Up to the mid-brain forebrain boundary the expressing cells correspond to the morphologically identifiable floorplate; the rostral extension of the *Shh* domain suggests that the ventral forebrain may be functionally homologous to the floorplate in all vertebrates.

The spatiotemporal expression pattern of *Shh* together with the strong conservation of this pattern during vertebrate evolution provides good circumstantial evidence implicating *Shh* in the induction of floorplate and/or motor-neuron differentiation. In line with this possibility, overexpression of *Shh* in fish, frog or mouse embryos is sufficient to induce ectopic expression of the floorplate markers *axial/HNF3*β, *F-spondin* as well as *Shh* itself (Echelard et al., 1993; Krauss et al., 1993; Roelink et al., 1994; J.-P.C. and P.W.I., unpublished data). Furthermore, in vitro assays have shown that the rat *Shh* orthologue, *vhh1* is capable of inducing floorplate and motorneuron differentiation in neural tube explants (Roelink et al., 1994).

Despite the strong similarities between the initial phases of *Shh* expression an interesting difference arises after its induction in the ventral CNS. Whereas in chick and mouse, expression persists in the notochord at least until the end of somitogenesis, in fish, mesodermal expression begins to fade away soon after transcription is activated in the floor plate (Fig. 3G-I). This down-regulation proceeds, like the CNS induction, in a rostral to caudal sequence, coinciding with the changes in cell shape that accompany notochord differentiation. Thus by the 22 somite stage, while *Shh* expression is maintained at high levels throughout the ventral CNS, expression in the mesoderm is restricted to the caudal region of the notochord and to a bulge of undifferentiated cells in the tail bud. Although the significance of this difference is unclear it could reflect a divergence in the mechanisms of CNS patterning between fish and amniotes. One possibility is that floorplate induction represents the original function of *Shh* in vertebrates and that subsequently it has been recruited to an additional midline signalling role, including secondary motorneuron induction, in amniotes. Certainly, the presence of a floorplate in the nerve cord of cephalochordates (Lacalli et al., 1994) implies an ancient origin for floorplate induction, predating the vertebrate radiation. By contrast, whereas signals from both the floorplate and notochord have been implicated in motorneuron differentiation in chick and mouse embryos, the differentiation of primary and secondary motorneurons appears to be independent of any floorplate-derived signal in zebrafish. This conclusion is based upon studies of the *cyclops* mutation, in which floorplate differentiation is blocked but motorneuron differentiation is unaffected (Hatta, 1992); because of the rapid decay of *Shh* transcripts in the notochord, such embryos are devoid of all midline *Shh* expression at the time of motorneuron differentiation, a situation that contrasts with the persistent expression of *Shh* in both floorplate and notochord in amniotes at the equivalent developmental stage. Thus, whereas *Shh* is capable of inducing motorneurons and is expressed at the appropriate time and place in amniote embryos, it appears dispensable for their differentiation in the fish. The persistent expression of *Shh* in the floorplate of fish embryos may reflect some other function in this tissue or it may simply be redundant. Clearly, mutations of *Shh* in fish and mouse will be required to resolve these issues.

Shh AND LIMB PATTERNING IN VERTEBRATE EMBRYOS

In addition to its expression in axial midline structures, *Shh* is transcribed in a cluster of posterior mesenchymal cells in the limb buds of mouse and chick embryos (Echelard et al., 1993; Riddle et al., 1993; Fig. 4). The temporal and spatial pattern of *Shh* expression in these structures suggests a close association between the gene and the organising activity possessed by posterior mesenchymal cells that constitute the so-called zone of polarising activity or ZPA. Transplantation of cells from the *Shh*-expressing region of the limb bud to its anterior margin has long been known to result in the duplication of digits with reversed polarity. This phenomenon has been interpreted in terms of the ZPA acting as a source of a morphogen, a diffusible signal, different levels of whose activity would act to instruct cells to differentiate appropriate to their position within the developing limb field. The pattern duplicating activity of the ZPA can be reproduced by overexpression of *Shh* in cells at the anterior limb margin (Riddle et al., 1993; Fig. 5) strongly suggesting that *Shh* represents the molecular basis of the ZPA. Notably, *Shh* is similarly expressed in the posterior mesenchyme of the pectoral fin buds in fish embryos (Krauss et al., 1993; Fig. 4C), suggesting that the same patterning mechanism operates in these homologous structures.

Since the number and character of duplicated structures caused by ectopic *Shh* expression seems to vary as a function of the level of its activity, one possibility is that Shh protein itself acts as a morphogen. Alternatively, like its postulated floorplate inducing activity in the notochord, Shh may act at short range in the limb, inducing the expression of another sig-

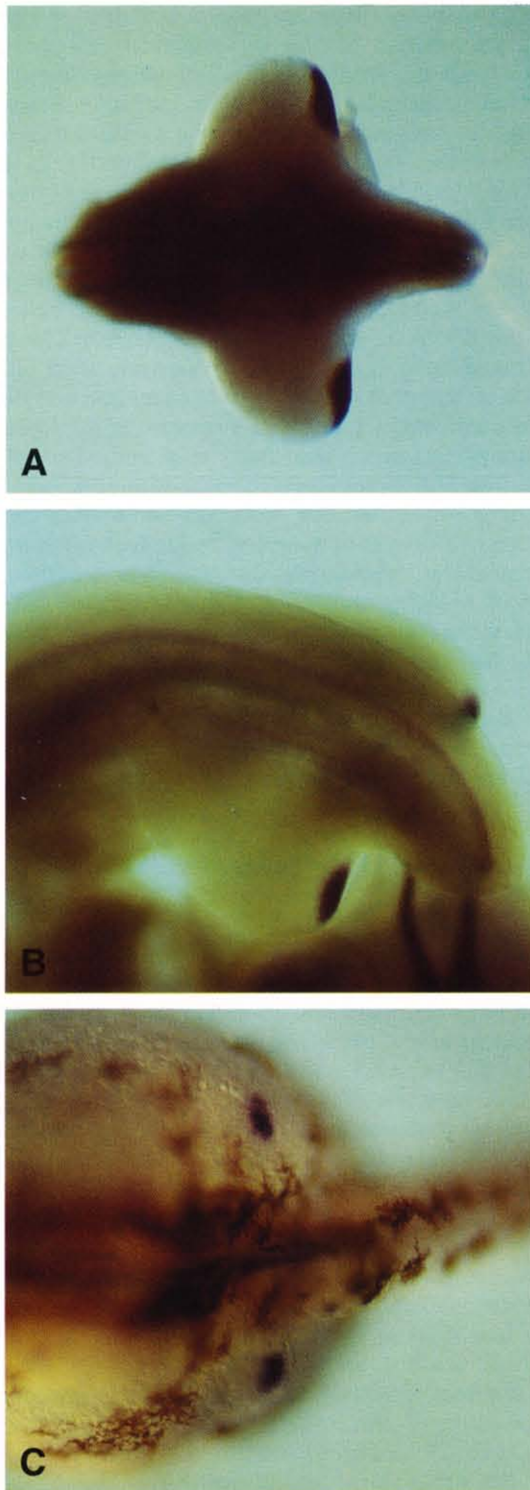

Fig. 4 *Shh* expression in mouse (A) and chick (B) limb buds and in the pectoral fin buds of the zebrafish (C). In all three species, expression is restricted to the posterior mesenchyme.

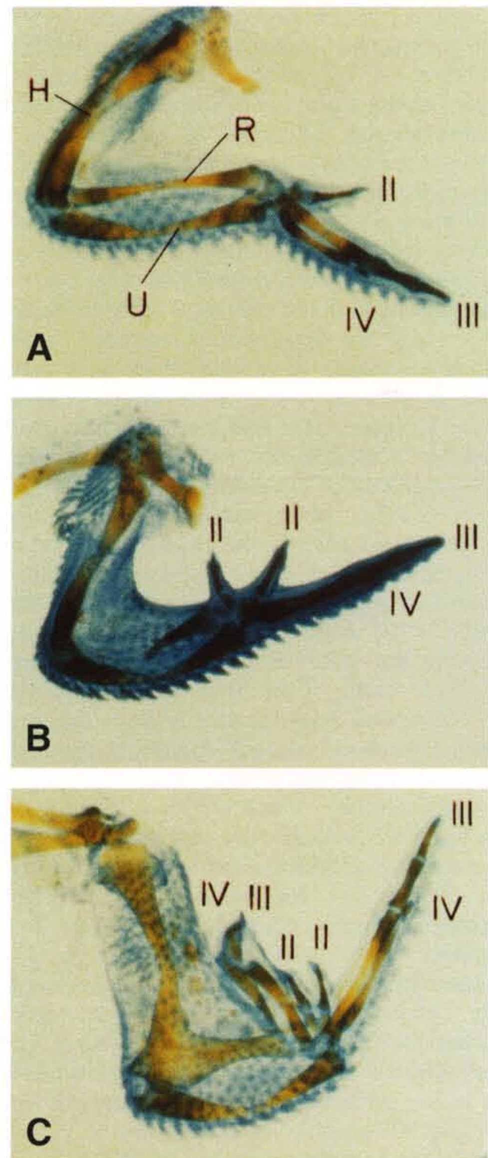

Fig. 5. Digit duplications induced by ectopic *Shh* expression in chick limb buds. (A) Normal limb. (B,C) Examples of the variable pattern duplication induced by grafting of *Shh*-expressing cells into the anterior margin of the limb bud.

nalling molecule or molecules in neighbouring cells. One possible candidate for such a molecule is the TGFβ family member BMP2; the gene encoding this protein is initially transcribed in a restricted domain in the posterior limb mesenchyme (Francis et al., 1994) that overlaps and surrounds the *Shh*-expressing cells (Fig. 6). Moreover *BMP2* transcription is first detectable just after the onset of *Shh* expression (R.J and C.T., unpublished results) and can be induced ectopically in the anterior half of the limb bud both by ZPA grafts (Francis et al., 1994) and by ectopic *Shh* expression (R.J., E. Laufer and C.T. unpublished results). While these observations are consistent with a role for *Shh* in inducing *BMP2* expression, presenting BMP2 as a possible effector of *Shh* activity in limb patterning, functional studies have so far failed to establish such a role for *BMP2* (Francis et al., 1994). Remarkably, however, a similar relationship between *hh* and the *Drosophila BMP2* homologue *decapentaplegic* (*dpp*) appears to underlie the patterning of imaginal discs, the fly equivalent of limb buds.

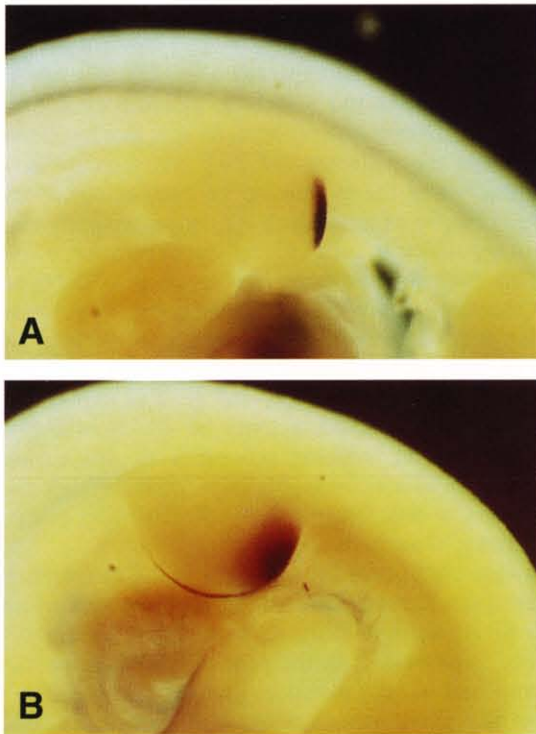

Fig. 6. Overlapping expression domains of *Shh* (A) and *BMP2* (B) in the forelimb of a stage 23 chicken embryo.

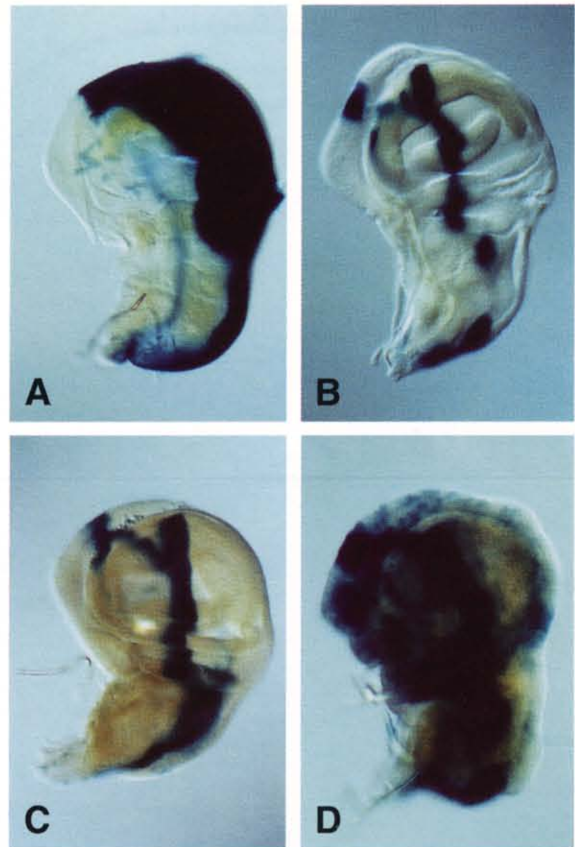

Fig. 7. Expression domains of *hh*, *decapentaplegic* (*dpp*) and *patched* (*ptc*) in wing imaginal discs of third instar *Drosophila* larvae. The expression of *hh* is restricted to the posterior compartment of the wing imaginal disc, revealed here (A) by β-galactosidase staining of an animal carrying an *en-lacZ* reporter gene. *dpp* (B) and *ptc* (C) by contrast are expressed in the anterior compartment, in a stripe of cells that runs along the compartment boundary. Transient ubiquitous expression of *hh* results in the ectopic expression of *dpp* throughout most of the anterior compartment (D).

hh AND THE PATTERNING OF *DROSOPHILA* LIMBS

The limbs or appendages of holometabolous insects develop from imaginal discs, simple epithelial cell sheets whose primordia arise at the parasegment borders of the developing embryo (Bate and Marinez Arias, 1991). This origin means that each disc incorporates and propagates portions of the cell populations that define the parasegmental borders, their progeny forming distinct polyclonal lineages that subdivide the appendages into developmental compartments. The posterior compartment of each disc is thus characterised by the expression of *hh* (Lee et al., 1992; Tabata et al., 1992), whereas *ptc* is expressed in cells of the anterior compartment (Phillips et al., 1990; see Fig. 7).

The function of *hh* in imaginal disc development was first analysed by Mohler (1988) using genetic mosaic techniques to remove the activity of *hh* from cells in different regions of the discs. These experiments demonstrated a requirement for *hh* activity in posterior compartment cells for the correct development of genetically wild-type cells in the neighbouring anterior compartment. We have investigated further this aspect of *hh* function using transgenic animals carrying an *HS-hh* construct to induce transient ectopic expression of *hh* in the anterior compartments of the wing discs. Such ectopic expression results in the duplication of anterior wing structures with mirror image symmetry (see Fig. 8) an effect that shows a striking analogy to the digit duplications induced by ZPA grafts or ectopic *Shh* expression in vertebrate limbs (compare with Fig. 5). The same kinds of duplications have also recently been reported by Basler and Struhl (1994), who used the "flip-out" technique to generate clones of cells expressing *hh* constitutively.

In some cases, ectopic *hh* activity results in duplication of only the most anterior structures, such as the wing margin and veins I and II, (Fig. 8B), whereas in other instances, differentiation of the anterior margin is almost completely suppressed, being replaced by veins II and III (Fig. 8C). As in the case of the chick limb, these variable effects could be indicative of a role for *hh* as a morphogen, different pattern elements being specified by different thresholds of *hh* activity. Several lines of evidence suggest, however, that in the imaginal disc, as in the embryo, *hh* acts in the wing to regulate the transcription of another signal-encoding gene.

Expression of *dpp*, which is absolutely required for normal wing morphogenesis (Posakony et al., 1991; Spencer et al., 1982), is restricted to a narrow band of cells that runs along the antero-posterior compartment boundary of the wing disc (Blackman et al., 1991; Masucci et al., 1990; see Fig. 7), closely apposed to the *hh*-expressing cells of the posterior compartment. In discs in which *hh* has been ectopically activated, *dpp* is similarly inappropriately expressed (Basler and Struhl, 1994; M.J.F. and P.W.I. in preparation; see Fig. 7), implying the latter to be a target of *hh* activity. Ectopic expression of

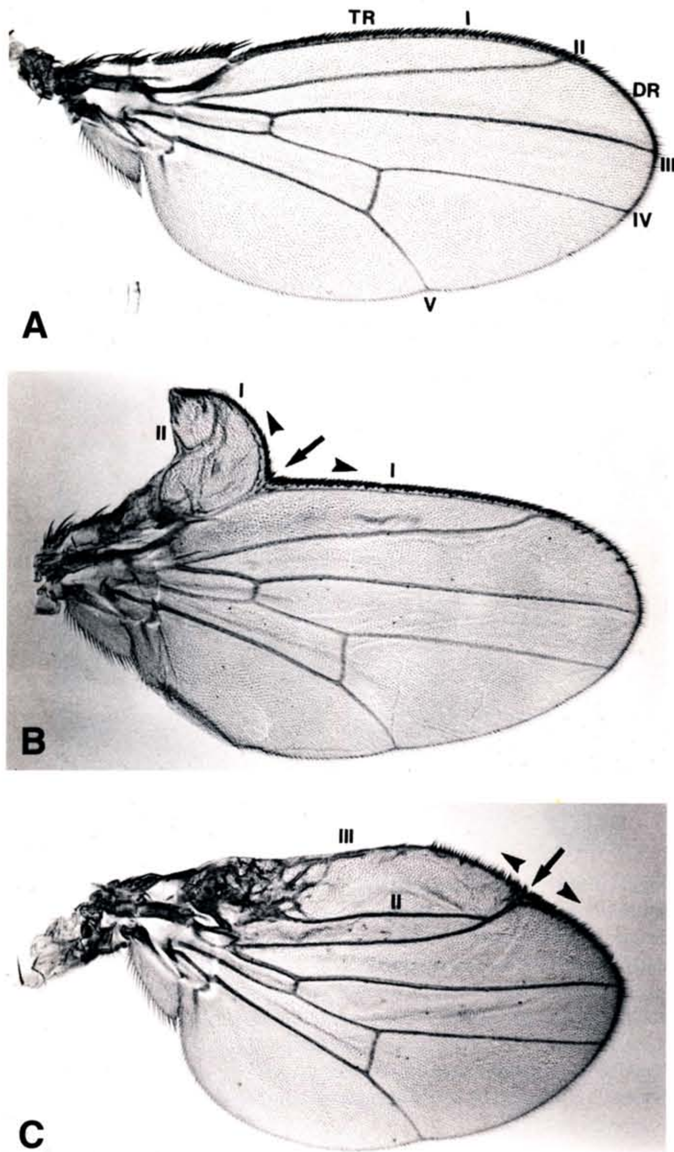

Fig. 8. Duplication and deletion of anterior compartment structures in the wing following transient ubiquitous expression of *hh*. (A) normal wild-type wing showing the characteristic venation pattern. The anterior margin is distinguished by the triple row (TR) and double row (DR) bristles. Veins I, II and III reside in the anterior compartment, veins IV and V in the posterior. (B,C) Examples of the variable mirror image duplications of anterior compartment structures induced by ectopic *hh* activity. The arrowheads indicate the proximodistal polarity of the normal and duplicated structures. the arrow indicates the boundary between normal and duplicated structures.

dpp is similarly induced in imaginal discs from animals with reduced activity of *ptc* (Capdevila et al., 1994; M.J.F and P.W.I. in preparation); thus as in the embryo, over-expression of *hh* has the same effect as the reduction or removal of *ptc* activity, suggesting that the same signalling mechanism acts to regulate *dpp* and *wg* at different stages of development. Thus in both cases, *hh* appears to act to regulate the source of other signalling molecules.

CONCLUSIONS

The parallels between the expression and function of *hh* family genes in *Drosophila* and vertebrate development are indeed striking. In the *Drosophila* embryo, *hh* acts as a localised signal that organises the patterning of each parasegment at least in part by regulating the expression of another signal-encoding gene *wg*. Ectopic expression of *hh* causes inappropriate activation of *wg* which in turn induces the expression of *en* in the middle of each parasegment; the result is a duplication of pattern elements and reversal of polarity that is reminiscent of the polarity reversals and ectopic differentiation induced by notochord grafts in chick embryos. Intriguingly, we have found that a close relative of *hh*, the *Shh* gene is expressed in the developing notochord, the activity of which is likely to be responsible for the inducing properties of this tissue. Thus molecules that have been highly conserved through evolution are deployed in different phyla to effect similar processes in the patterning of secondary fields.

The expression of *hh* family genes in the developing limbs of vertebrates and insects provides a yet more striking example of such functional similarity. In both cases, a member of the *hh* family is expressed in the posterior half of the limb primordium - and in each instance, its ectopic expression results in the duplication of pattern elements. Moreover, in both cases, activity of *Shh* and *hh* appears intimately associated with the expression of closely related members of the TGFβ family, namely *BMP2* and *dpp*. Whereas functional analysis of *dpp* has clearly implicated it in appendage morphogenesis, no such role for *BMP2* has yet been established. Nevertheless, it is difficult to escape the conclusion that, despite their apparently independent evolutionary origin, the limbs of vertebrate and invertebrates may be patterned by very similar mechanisms. Whether the remarkable similarities in the deployment of *hh* genes in the development of deuterostome and protostome embryos reflects a common origin for these various patterning processes or an example of evolutionary convergence remains to be seen. The isolation of *hh* family genes and analysis of their expression in organisms of other phyla should provide important new insights into the origin of the signalling mechanisms that underlie pattern formation in all metazoa.

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