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# The Long and Short of *hedgehog* Signaling

**Minireview**

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The past decade has seen a revolution in our understanding of the molecular basis of embryonic development in higher organisms. As our understanding of vertebrate development has grown, a number of completely unanticipated and truly remarkable parallels between mechanisms of patterning in vertebrates and Drosophila have been revealed (Scott, 1994). These findings suggest that the wealth of genetic and molecular information available concerning fly development will continue to provide an enormous resource for gaining further insight into vertebrate development. Indeed, many significant genes known to control various aspects of fly development have vertebrate homologs. Although their developmental roles may not be specifically conserved, analysis of their function will provide clues to the general processes they control and mechanisms by which they act. The role of *hedgehog* (*hh*) genes as intercellular signals in establishing embryonic pattern provides a dramatic example of this transfer of developmental insight from Drosophila to vertebrates and shows how studies in both organisms can synergistically lead to rapid elucidation of the molecular mechanisms underlying embryological processes.

One mechanism by which developing embryos attain proper position-specific cell differentiation is to organize cell fates relative to a discrete inducing tissue. In principle, such induction could be achieved by a single long-range secreted signal instructing cell fate in a concentration-dependent manner (Figure 1a). Molecules acting via this mechanism have been termed morphogens. Alternatively, the primary inductive response could be quite local, initiating a cascade of short-range signals that are then propagated through responding tissues (Figure 1b). Finally, the inductive trigger could act locally to initiate long-range and graded secondary signals (Figure 1c). The identification of *hh* genes as key signals in a variety of embryonic inductive processes provides an opportunity to determine which of these theoretical mechanisms are actually used in regulating pattern.

### *Short- and Long-Range Signaling by* hh

*hh* was identified by Nüsslein-Volhard and Wieschaus (1980) in a saturation screen for mutants that affect larval cuticular patterning in Drosophila. Subsequent studies have shown that *hh* encodes a secreted protein that plays multiple inductive roles during fly development (reviewed by Perrimon, 1995). Via short-range action, over 1- or 2-cell diameters, *hh* regulates aspects of embryonic segmentation and patterning of adult appendages. In establishing early segmental borders, the inductive targets of *hh* signaling cells are directly adjacent cells. A cascade of short-range interactions is thereby initiated that programs cell fate at different positions within the segment, corresponding to the model diagrammed in Figure 1b. In the case of appendages, *hh* again acts locally to pattern cells within the larval appendage anlage, the imaginal discs. In this instance, however, cells respond locally by secreting decapentaplegic (dpp), which then may serve to pattern the disc in a graded manner over considerable distances, as shown in Figure 1c. Besides these short-range activities, *hh* also is responsible for long-range specification of cell types in the dorsal epidermis. While at times cited as evidence of long-range *hh* induction, this latter process could result either from a direct action of *hh* on both adjacent and distant cells, as shown in Figure 1a, or it could depend upon the secretion of a second (as yet unidentified) long-range factor, as shown in Figure 1c.

Vertebrate homologs of *hh* have been isolated in screens utilizing the cloned Drosophila gene. One homolog in particular, *Sonic hedgehog* (*Shh*), displays a surprisingly wide range of activities in vertebrate embryos (Smith, 1994). SHH regulates dorsal–ventral patterning of the neural tube, the somites, and the anterior–posterior axis of the limb bud. As with its Drosophila homolog, the *Shh* gene product appears to act locally in some circumstances (floor

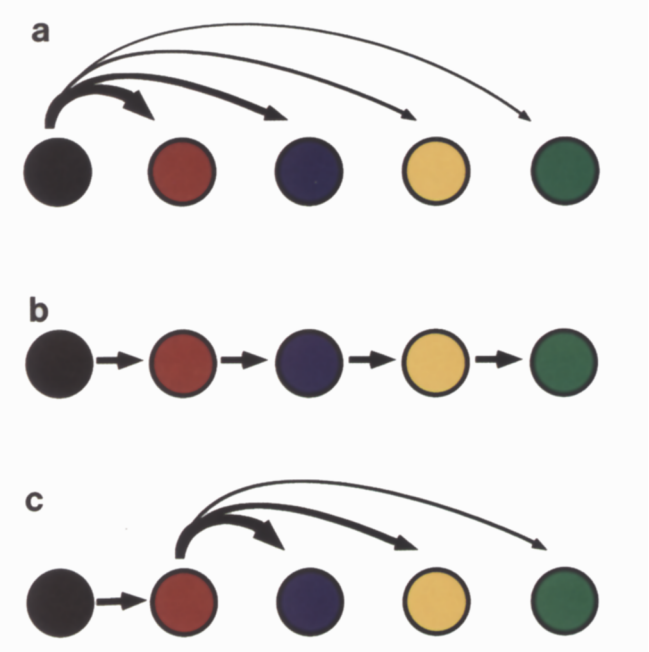


Figure 1. Mechanisms for Achieving Position-Specific Patterning

Colored dots represent cells that adopt different fates, in each case in response to an inductive signal emanating from the cell at far left (black). Arrows represent intercellular signals, with the thickness of the arrow indicating its relative strength.

(a) Differential cell fates are induced by the secretion of a long-range signal acting in a concentration-dependent manner (a morphogen).

(b) Differential fates resulting from a cascade of multiple short-range interactions, initiated by a signal produced by the inducing cell.

(c) Differential cell fates are achieved in response to a long-range graded secondary signal, produced as a consequence of a local primary inductive signal.

plate formation within the neural tube) and at a distance in others (motor neuron formation in the neural tube, sclerotome induction and proliferation in the somites, and limb patterning).

***Biochemistry of Short- and Long-Range Signaling***

Biochemical and molecular analyses of Drosophila and vertebrate *hh* genes have provided insight as to how the same molecule might function both locally and distantly. All *hh* genes encode processed, secreted polypeptides, indicative of their noncell-autonomous function. Following signal sequence cleavage, a second processing event, occurring through a novel self-cleaving mechanism, generates N-terminal and C-terminal fragments. Initial experiments by Lee et al. (1994) indicated that the N-terminal fragment remained tightly associated with the extracellular matrix, while the C-terminal fragment was released into the culture medium. One intriguing interpretation of these studies was that the N-terminal fragment might encode for short-range activities of hh and the C-terminal fragment might act as a long-range signal.

To test for the activities of the N- and C-terminal fragments of hh in Drosophila, Fietz et al. (1995) and Porter et al. (1995) generated expression constructs containing only the N-terminal or C-terminal domains. In assays measuring short-range activities, the N-terminal portion of hh is sufficient for signaling, as expected. However, the N-terminal fragment also displayed long-range activities, implicating the same domain of hh in both short- and long-range signaling (ventral and dorsal ectoderm). The C-terminal region of hh showed no activity in these assays. Moreover, increasing wild-type or N-terminal expression broadened the range of hh activity, while increasing the levels of the C-terminal form had no effect. Extending these studies to vertebrate embryos, Fan et al. (1995 [this issue of *Cell*]) and Roelink et al. (1995 [this issue of *Cell*]) likewise demonstrate that the N-terminal processed form of SHH is sufficient to direct long-range effects (sclerotome differentiation and proliferation and motor neuron induction) as well as short-range effects (floor plate induction). In retrospect, these results are not altogether unexpected since the N-terminal regions of HH proteins are highly conserved, while the C-terminal portions are divergent.

What then is the function of the C-terminal domain? First, it contains the proteolytic activity necessary for processing of the HH precursor protein (Lee et al., 1994). However, since the N-terminal fragment is sufficient for all known activities of HH, it is not readily apparent why this processing event has been conserved from flies to vertebrates. Studies of the vertebrate protein by Roelink et al. (1995) provide a suggestion as to why this feature of HH proteins has been maintained. When expressed in COS cells, the full-length SHH precursor is cleaved into N- and C-terminal fragments, each of which can be found secreted into the culture medium. However, while the C-terminal polypeptide is readily detectable in the medium, the N-terminal region is found primarily associated with cells producing SHH. In contrast, when the N-terminal form of SHH is expressed in COS cells independently, it is found to be abundantly secreted into the medium. This would indicate that the function of the C-terminus is to restrict the diffusion of SHH by an unknown mechanism, tethering the majority of secreted N-terminal SHH to the cell surface.

The potential importance of such a mechanism is illustrated by the dorsal–ventral differentiation of the neural tube. The notochord, a source of SHH, has the ability to induce both floor plate and motor neurons in lateral neural tube explants. Floor plate induction requires contact between the notochord and responding tissue, while motor neuron induction does not (reviewed by Smith, 1993). Bacterially produced soluble SHH N-terminus is effective in inducing both floor plate and motor neurons; however, the concentration required to induce floor plate is 5-fold higher than that required to induce motor neurons (Roelink et al., 1995). Therefore, the apparent requirement for contact to induce floor plate would seem to reflect a requirement for a higher local concentration of SHH. The C-terminal portion of SHH ensures that a majority of SHH N-terminus remains associated with the notochord and thus provides a high local concentration required to induce floor plate. Lower concentration of SHH diffusing away from the notochord could then be responsible for long-range effects on sclerotome and motor neuron induction. In support of this latter possibility, the concentration of SHH that is effective in motor neuron induction is within the range required to induce sclerotome.

***Concentration Dependence and Secondary Signals***

If SHH is directly responsible for the differentiation of floor plate locally at a high concentration and motor neurons distantly at lower concentrations, it must be classified as a morphogen. Although available data for direct action of SHH in induction of the floor plate are compelling in that induction of floor plate markers in lateral neural tube explants does not require protein synthesis and hence probably does not arise from the de novo production of a second signal, the effect on motor neurons is less clear (Roelink et al., 1995). Several rounds of cell division occur prior to induction of the motor neuron marker *Islet-1*, leaving ample time for the production of secondary signals. If such signals are present, however, they must be induced in the absence of an obvious floor plate, as lower concentrations of SHH can induce motor neurons without inducing molecular markers characteristic of floor plate tissue. Thus, in neural tube explants, SHH can elicit clear concentration-dependent responses, whether or not the lower concentration response operates over a short or long range.

For SHH to be functioning at long range, it must be present at a distance from its site of synthesis to affect cell-type decisions. However, SHH protein cannot be detected by immunostaining methods at sites distant from the notochord or floor plate, either in the neural tube or in the developing somites (Roelink et al., 1995). This may simply reflect a technical limitation in the sensitivity of detection of secreted SHH. In the somites, induction of the sclerotome marker *Pax-1* is found at a considerable distance from the notochord in vivo as well as in vitro (Johnson et al., 1994; Fan and Tessier-Lavigne, 1994). If SHH is directly inducing *Pax-1* expression, then it must be able to move considerable distances within somitic mesoderm. However, if SHH-mediated induction of *Pax-1* is indirect,

somitic tissue must be the sole source of a secondary signal since sclerotome can be induced in somite explant cultures by purified SHH protein (Fan et al., 1995). Moreover, such a secondary signal cannot be stable in the absence of SHH, as explanted sclerotome tissue is unable to induce the expression of *Pax-1* in responsive presomitic mesoderm (Fan and Tessier-Lavigne, 1994).

Analysis of the signaling pathway from *hh* to induction of new gene expression should help to define which cells are in fact directly responding to SHH. Although a complete pathway is lacking, recent work has implicated protein kinase A (PKA) as a possible component of *hh* signaling in Drosophila imaginal discs, since mutant clones of cells lacking PKA function generate similar phenotypes to clones ectopically expressing *hh*, and high PKA activity can antagonize hh function. However, the point at which hh and PKA activities become competitive is unclear: both signals may repress the downstream consequences of each other while maintaining distinct signaling pathways (reviewed by Perrimon, 1995). Fan et al. (1995) have extended these findings to vertebrate embryos. SHH-mediated induction of *Pax-1*, repression of *Pax-3*, and proliferation of sclerotome can all be completely abolished by pharmacological reagents that activate PKA. One possible explanation for these findings is that *hh* signals via inhibiting PKA activity. Alternatively, SHH and PKA may have opposing influences on a common downstream target. In either case, these results reinforce the validity of considering Drosophila and vertebrate *hh* genes together in analyzing mechanisms of *hh* signaling and may eventually lead to a means by which direct gene targets of HH can be identified.

### *Morphogens in the Limb?*

Many initial insights into the existence and mechanism of developmental signaling centers have been derived from studies on limb bud development. Indeed, one of the first applications of the morphogen model (Figure 1a) was to account for specification of digit identity along the anterior–posterior limb bud axis (Wolpert, 1969). When posterior limb bud tissue is transplanted to the anterior margin of a second limb bud, signaling from the graft induces a mirror-image symmetrical set of extra digits (a property called polarizing activity; Saunders and Gasseling, 1968). This effect is both long range (Honig, 1981) and proportional to the number of grafted inducing cells (Tickle, 1981), properties that can be modeled either in terms of a long-range graded signal or a cascade of local interactions.

SHH's ability to mimic the activity of the zone of polarizing activity (ZPA) (Riddle et al., 1993) parallels the effects of SHH on somite and neural tube development. First, SHH induces gene expression both locally and distantly. Second, SHH induces proliferation within limb bud mesoderm. In the case of the limb bud, there is direct evidence that SHH can induce the production of secondary signals that account in part for the observed activities of SHH. Almost certainly, the effect of SHH on limb bud proliferation is indirect, mediated through the action of a member of the fibroblast growth factor family derived from the apical ectodermal ridge (Niswander et al., 1993, 1994; Laufer et al., 1994). Another probable secondary signal induced by *Shh* is the secreted protein BMP2 (a relative of transforming growth factor β). This is particularly intriguing because dpp, the homolog of BMP2, is secreted in response to *hh* in imaginal discs and is responsible for the long-range effects of *hh* on disc patterning. BMP2 could likewise be a true morphogenetic signal in the limb mesenchyme, acting at a greater range than SHH. However, application of BMP2 alone to developing limb buds produces no effect on digit patterns (Francis et al., 1994), perhaps indicating that multiple secondary factors are required in concert to transduce the initial SHH signal. Alternatively, SHH itself could act as a long-range concentration-dependent signal in the limb bud. Of note in this regard is that the range of ZPA signaling in the limb bud is approximately 200 μm (Honig, 1981), roughly equivalent to the distance that sclerotome is induced in presomitic mesoderm by the notochord (Fan and Tessier-Lavigne, 1994), suggesting that if SHH is acting at long range within these tissues, the distances by which it would have to act are comparable. The availability of reagents to supply defined amounts of SHH protein to limb bud tissue coupled with progress in our understanding of *hh* signaling in other systems should eventually provide molecular explanations for the classical observations of dose dependency and long-range action of the ZPA in regulating limb bud pattern.

### *Conclusions*

An outstanding question in both invertebrate and vertebrate embryology concerns our understanding of mechanisms by which inductive systems pattern the embryo. Current studies in both flies and vertebrates have directly implicated the N-terminal portion of HH proteins in initiating both short- and long-range inductions. In addition, evidence is accumulating that HH can affect cell fate decisions in a concentration-dependent manner. However, it is still uncertain to what extent the long-range effects of HH proteins are direct. In Drosophila limb formation, hh clearly depends on intermediate signals. Nevertheless, purified SHH protein signals over a distance. The degree to which hh acts alone or in concert with other signals is a key question. Future studies are clearly required to define the direct cellular and genetic targets of *hh* signaling in both flies and vertebrates. One outcome of these investigations will to be determine whether *hh* signaling occurs via the same mechanism in all responding tissues or whether each tissue has adopted unique means of receiving and interpreting the *hh* signal. Analysis of HH function in vertebrates and Drosophila should lead to a rapid resolution of these questions.

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