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Review

# Structure and Function of the Developmental Signaling Molecule Hedgehog

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## Introduction

In this review, I wish to provide a brief introduction to the structure and function of the Hedgehog (Hh) signaling molecule, which is the product of the *hedgehog* (*hh*) gene. Given the explosion of interest in Hh in the last several years — a recent search of the MEDLINE database using the keyword 'hedgehog' identified over 1380 articles — no review could be exhaustive. The aim here will be to introduce some of the special features of Hedgehog chemistry and biology to the general reader.

*hh* was first identified in a screen for genes involved in *Drosophila* development and was so named because the uniform pattern of thick bristles observed in *hh* mutant embryos is reminiscent of the appearance of the bristled coat of hedgehogs (Nüsslein-Volhard and Wieschaus, 1980) (see Fig. 1). Early in development, normal *Drosophila* embryos become compartmentalized along the antero-posterior axis into distinctive segments that possess stereotypical patterns of gene expression. These segments presage an external pattern of repeated bristle stripes (see Fig. 1B) and represent precursors of the body segments observed in the adult animal. Proper formation of these segments is thus vital to normal development, and disruption of normal segmentation in *hh* mutant embryos implicated *hh* in early processes involved in establishing the body plan of adult flies. Subsequent genetic experiments suggested that the specific role of Hh was likely to be that of a secreted signaling molecule (Mohler, 1988).

A fruitful and perhaps surprising consequence of the identification of genes involved in *Drosophila* development has been the discovery that homologues of many of these genes participate in related processes in vertebrates. Cloning of the *hh* gene from *Drosophila* (Lee *et al.*, 1992; Mohler and Vani, 1992) led quickly both to confirmation of Hh as a secreted signaling molecule and to identification of vertebrate *hh* genes (Echelard *et al.*, 1993; Krauss *et al.*, 1993; Riddle *et al.*, 1993; Chang *et al.*, 1994; Roelink *et al.*, 1994). Vertebrate *hh* homologues also constitute signaling molecules involved in proper development and patterning of embryonic tissues. Sonic Hedgehog (Shh) — named for a video game character — is one of at least three Hh homologues found in all vertebrates examined to date. Among Hh molecules, Shh has attracted much attention because of its role as the signaling agent in several classically defined tissues involved in induction of pattern formation during embryonic development. Processes in which Shh has been implicated as an important component include induction of floor plate and motor neurons by the notochord (Roelink *et al.*, 1994), induction of asymmetry in the limb digits by the Zone of Polarizing Activity (ZPA) (Riddle *et al.*, 1993), induction of dopaminergic neurons (Hynes *et al.*, 1995), induction of ventral forebrain structures and subdivision of the eye field (Chiang *et al.*, 1996), and production of left-right asymmetry during development of the heart (Levin *et al.*, 1995).

Signals that initiate tissue patterning during animal development must in many cases be narrowly restricted in time and place to generate the complexity observed in the adult organism. Much of this restriction is achieved through regulation of the sites and times of gene expression. In addition, however, the extent of Hh

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Abbreviations: BCC, basal cell carcinoma; BCNS, basal cell nevus syndrome; Ci, Cubitus interruptus; Hh, Hedgehog protein; *hh*, *hedgehog* gene; Hh-C, carboxy-terminal domain of Hh; Hh-C17, 17 kDa fragment of Hh-C; Hh-N, amino-terminal domain of Hh; HPE, holoprosencephaly; Shh-N, Sonic Hedgehog N-terminal; SSD, sterol-sensing domain; SRR, sterol-recognition region.

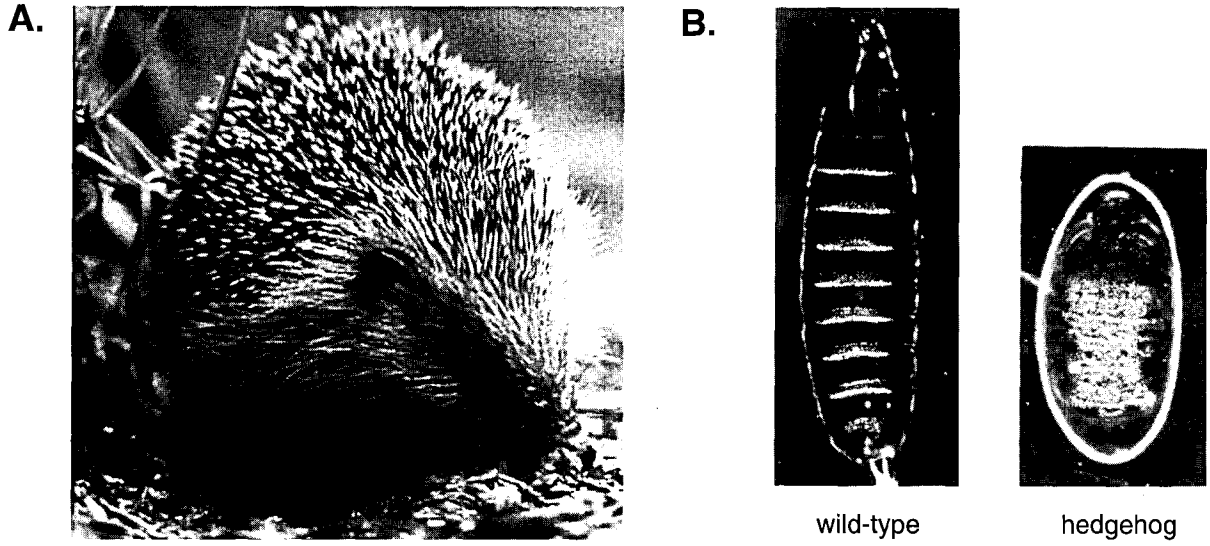


**Fig. 1.** *hh* mutant *Drosophila* embryos resemble hedgehogs. A. Hedgehogs, of which a picture is shown, possess a porcupine-like coat of bristles. B. A normal *Drosophila* embryo, shown on the left, exhibits a stereotypical segmented pattern of large and small bristle stripes while an *hh* mutant embryo, shown on the right, displays a uniform pattern of large bristles. The bristle pattern of *hh* mutant embryos recalled the bristle pattern of hedgehogs and led to the name chosen for this gene (Nüsslein-Volhard and Wieschaus, 1980). Photographs courtesy of P. A. Beachy.

signaling is also restricted through covalent modifications of Hh that prevent free diffusion of the Hh signal. Hh proteins are synthesized as precursors that undergo an autoprocessing reaction in which the C-terminal region of the Hh protein (Hh-C) cleaves the full-length Hh protein and covalently attaches a cholesterol molecule to the remaining N-terminal portion of the molecule (Hh-N) (Chang *et al.*, 1994; Lee *et al.*, 1994; Bumcrot *et al.*, 1995; Porter *et al.*, 1995; 1996a; 1996b). All of the signaling activities of Hh proteins map to Hh-N (Fan *et al.*, 1995; Fietz *et al.*, 1995; Lopez-Martinez *et al.*, 1995; Marti *et al.*, 1995; Porter *et al.*, 1995; Roelink *et al.*, 1995), and the attached cholesterol serves to anchor Hh-N in the plasma membrane and restrict the range of Hh signaling (Bumcrot *et al.*, 1995; Porter *et al.*, 1995; 1996a; 1996b). This restriction in the range of Hh signaling is essential for normal embryonic development. Although Hh proteins remain the only proteins known to undergo cholesterol modification, several as yet unidentified proteins become labeled when cells are incubated in the presence of radioactive cholesterol (Porter *et al.*, 1996b). Whether these unknown proteins become modified by cholesterol by the same mechanism as Hh proteins is unknown. Hh-C modules do not appear to work in trans and mediate no known functions beyond the intramolecular Hh autoprocessing event.

## The Hedgehog Protein

Hh proteins are synthesized as ~45 kDa precursor proteins with an N-terminal signal sequence that is removed early in biogenesis (see Fig. 2). Hh homologues have been found

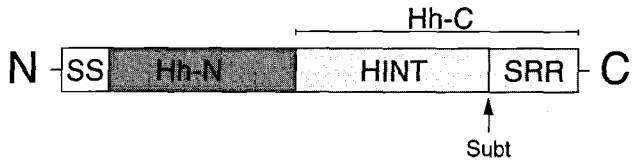


**Fig. 2.** Schematic of Hh protein. A schematic diagram of an Hh protein is shown with an N-terminal signal sequence (SS), the N-terminal signaling domain (Hh-N), the C-terminal autoprocessing domain (Hh-C), the HINT module that possesses homology to the self-splicing region of inteins, and the sterol-recognition region (SRR) that is necessary for cholesterol transfer indicated. The site of subtilisin cleavage of Hh-C is indicated.

in species ranging from *Drosophila* to human, but an Hh homologue has yet to be found in *C. elegans* despite completion of 99% of its genome sequence (*C. elegans* Consortium, 1998; Ruvkun and Hobert, 1998). Alignment of the amino-acid sequences of Hh proteins from many species revealed that the N-terminal ~170 amino-acids are highly conserved between diverse species (67% sequence identity between human and *Drosophila*) while the C-terminal ~210 amino acids are less well conserved (30% between human and *Drosophila*). Several lines of evidence indicated early on that the N- and C-terminal regions of Hh proteins represent discrete structural and functional domains. As noted, full-length Hh processes itself into two fragments, Hh-N and Hh-C, and these fragments correspond precisely with the regions identified by the differing levels of sequence conservation. The modularity of Hh proteins is also indicated by the presence of 11 proteins in the *C. elegans* genome with sequence

homology to Hh-C but with N-terminal sequences that are unmistakably different from Hh-N (Porter *et al.*, 1996; *C. elegans* Consortium, 1998).

## Structure and Function of Hh-N

A major question in Hh biology is the mechanism by which Hh-N conveys signals to target tissues. Sequence homology searches failed to identify homologues for Hh-N suggesting that Hh-N proteins might represent a new signaling paradigm. Determination of a high-resolution crystal structure of the N-terminal region of murine Sonic Hedgehog (Shh-N) revealed a bound zinc ion coordinated in a fashion similar to zinc hydrolases such as carboxypeptidase A and thermolysin (Hall *et al.*, 1995). The overall structure of Shh-N is not homologous to either carboxypeptidase A or thermolysin but is homologous to the D-ala-D-ala carboxypeptidase from *Streptomyces albus* and other zinc hydrolases involved in bacterial cell wall synthesis including VanX (Dideberg *et al.*, 1982; Murzin, 1996; Bussiere *et al.*, 1998).

This homology suggested that a hydrolase activity might be an important component of Hh-N signaling. For example, Shh-N could activate its receptor by a specific cleavage event as in the case of thrombin (Vu *et al.*, 1991), or some secondary signaling component could be released by hydrolysis. While conserved in all other known Hh sequences, several of the residues involved in zinc coordination in Shh-N are not conserved in *Drosophila* Hh. This lack of conservation casts some doubt as to whether hydrolytic activity is indeed important for Hh-N function. Differences in the mechanism of Hh action in invertebrates and vertebrates seems unlikely to underlie the anomalous *Drosophila* sequences as the zinc coordinating residues are retained in mosquito Hh. Preliminary *in vitro* studies suggest that the Shh-N zinc site is not essential for Shh-N activity *in vitro* (Beachy, P. A. and Fuse, N. unpublished observations), although these results may not be completely indicative of Hh function *in vivo*. Attempts to identify a hydrolase activity for Shh-N utilizing various potential natural substrates and substrate analogs have also so far proven unsuccessful (Hall, T. M. T., Porter, J. A., Beachy, P. A. and Leahy, D. J. unpublished material; Stone *et al.*, 1996). Thus, while Hh-N is almost certainly evolutionarily derived from a zinc hydrolase, no evidence currently implicates a hydrolase activity in Hh-N signaling.

The potential roles of post-translational modification in Hh-N mediated signaling have also generated much interest. In addition to the cholesteryl moiety that becomes covalently attached to the C-terminus of Hh-N during Hh autoprocessing, the N-terminus of Shh-N has also been found to be palmitoylated in cultured cells (Pepinsky *et al.*, 1998). Palmitoylated Shh-N is more potent than unmodified protein in a cell-based assay, and membrane attachment of Hh-N and restriction of the range of Hh signaling are likely mediated at least in part by this modification (Pepinsky *et al.*, 1998). The cholesterol attachment has been shown to restrict the range of Hh-N signaling to adjacent cell layers and may also be necessary to increase the local concentration of Hh-N above a threshold for signaling (Bumcrot *et al.*, 1995; Porter *et al.*, 1995; 1996b). More recent work suggests that cholesterol may also play an important role in the reception of the Hh signal. Target tissues treated with cholesterol biosynthesis inhibitors are unable to respond to Hh-N (Cooper *et al.*, 1998; Incardona *et al.*, 1998), and the likely Hh receptor, Patched, contains a region homologous to the sterol-sensing domain of proteins such as HMG CoA reductase and the Niemann-Pick C disease protein, which modulate their activity in response to cholesterol levels (Beachy *et al.*, 1997; Carstea *et al.*, 1997). It remains unclear whether the Patched receptor interacts directly with the cholesterol attached to Hh-N or, alternatively, that disruption of normal cholesterol homeostasis has indirect effects on Hh signaling.

## Structure and Function of Hh-C

Hh-C regions comprise ~210 amino acids and mediate both the cleavage and cholesterol transfer components of Hh autoprocessing. Hh proteins from which virtually the entire Hh-N region has been deleted are fully capable of autoprocessing *in vitro* (Lee *et al.*, 1994; Porter *et al.*, 1996a), and proteins with C-terminal regions homologous to Hh-C but otherwise unrelated N-terminal regions have been identified in the *C. elegans* genome. At least one of these *C. elegans* protein has been shown to undergo an autoprocessing reaction similar to Hh proteins (Porter *et al.*, 1996a). These observations indicate that Hh-C and its homologues are likely to be autonomous units whose function is largely independent of N-terminal sequences.

Conserved residues within Hh-C domains provided early clues to the mechanism of Hh autoprocessing. Cleavage always occurs N-terminal to an absolutely conserved cysteine, and mutation of this cysteine to alanine reduces autoprocessing activity to undetectable levels (Porter *et al.*, 1995). The positioning of this residue adjacent to the site of cleavage suggested that the sulfhydryl group of this cysteine might function as a nucleophile and attack the carbonyl carbon of the preceding residue resulting in a thioester in place of the peptide bond. Consistent with the presence of this thioester, small nucleophiles such as dithiothreitol (DTT), hydroxylamine, and even cysteine-containing peptides are able to stimulate cleavage of Hh at this site *in vitro* (Porter *et al.*, 1996a). Coupled with mutagenesis data, these results have led to wide acceptance of a two step mechanism for Hh autoprocessing in which the first step is the N to S shift that results in the formation of the thioester (see Fig. 3A). The second step involves a nucleophilic attack on this thioester by the hydroxyl oxygen of cholesterol that results in cleavage of Hh with the covalent attachment of

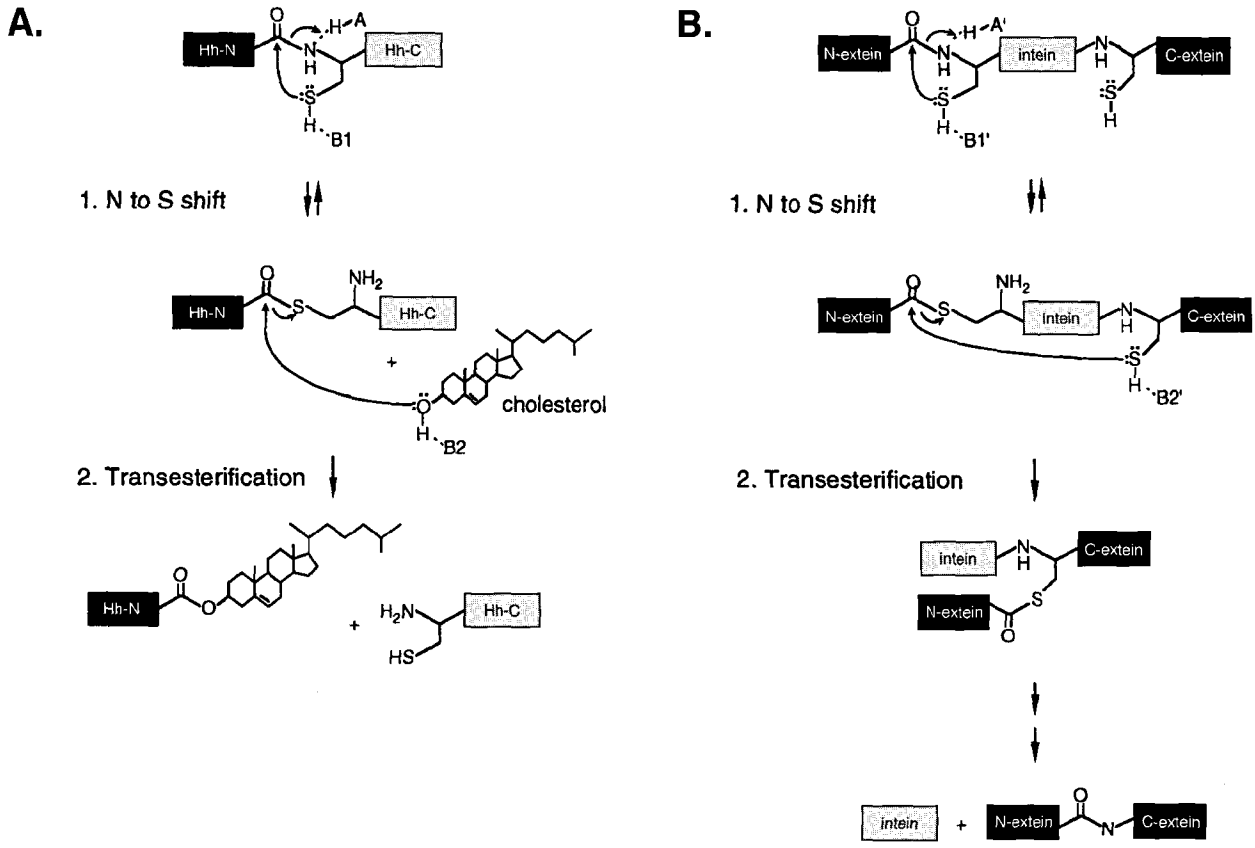


**Fig. 3.** Mechanism of autoprocessing by Hh and self-splicing proteins. A. A two-step mechanism for autoprocessing by Hh proteins is shown. In the first step, a conserved cysteine side chain attacks the carbonyl carbon of the preceding residue to result in the substitution of a thioester for the peptide bond. In the second step, this thioester undergoes nucleophilic attack from a cholesterol molecule resulting in attachment of the cholesterol to Hh-N and release of Hh-C. B. The first two steps of the mechanism of self-splicing by inteins is shown. As for Hh proteins, the first step involves the shift from a peptide bond to an ester or thioester. The second step involves a nucleophilic attack of this ester/thioester by the side chain of a conserved serine or cysteine at the N-terminus of the C-terminal extein. For a discussion of subsequent steps in self-splicing see Xu and Perler (1996).

cholesterol to the C-terminus of the newly generated Hh-N fragment (see Fig. 3A). Hh-C mediated processing may also be stimulated *in vitro* by addition of cholesterol, which then becomes attached to the resulting N-terminal Hh fragment (Porter *et al.*, 1996b).

Attempts to delineate further the mechanism of Hh autoprocessing through determination of the crystal structure of Hh-C were hampered by poor solubility of this fragment. Limited proteolysis of *Drosophila* Hh-C, however, resulted in identification of a soluble N-terminal fragment of Hh-C with a molecular weight of 17 kDa (Hh-$C_{17}$). This fragment of Hh-C proved capable of mediating thioester formation but not cholesterol transfer (Hall *et al.*, 1997). Determination of the crystal structure of Hh-$C_{17}$ revealed three residues in the vicinity of the nucleophilic cysteine with side chains capable of participating directly in the autoprocessing reaction (Hall *et al.*, 1997). These residues included a histidine, a threonine, and an aspartic acid (His 329, Thr 326, and Asp 303 in *Drosophila* Hh). Both the histidine and threonine are conserved in all known Hh sequences, and mutation of the histidine to alanine had previously been shown to reduce Hh autoprocessing activity to undetectable levels (Lee *et al.*, 1994). The aspartic acid residue was conserved in all Hh proteins as either aspartic acid or histidine, consistent with a role as a proton donor or acceptor. As with the histidine, mutation of the threonine disrupted thioester formation in the context of full-length Hh-C. Mutation of the aspartic acid did not hinder thioester formation, but it did reduce cholesterol transfer activity to below detectable levels leading to the suggestion that this residue may function as a general base to deprotonate the cholesterol hydroxyl prior to nucleophilic attack of the thioester (Hall *et al.*, 1997).

## Homology of Hh-C to Intein Regions of Self-splicing Proteins

The crystal structure of Hh-$C_{17}$ demonstrated an unexpected homology between Hh-C domains and the

intein regions of self-splicing proteins (Duan *et al.*, 1997; Hall *et al.*, 1997; Klabunde *et al.*, 1998). This homology was also detected independently by sequence comparison methods (Dalgaard *et al.*, 1997a; Pietrokovski, 1998). Self-splicing proteins are synthesized as single polypeptide chains that undergo an intramolecular autoprocessing reaction in which an internal segment, termed an intein, is excised from the protein and the N- and C-terminal regions, termed exteins, are religated to form the mature protein products. Inteins have been found within several prokaryotic and eukaryotic proteins including polymerases and ATPases and do not appear to interfere with the function of the host protein. The intein region itself frequently possesses an active endonuclease domain as well as the self-splicing activity (Dalgaard *et al.*, 1997b).

Protein self-splicing occurs post-translationally, requires no additional proteins or co-factors, and shares many features with Hh autoprocessing (Xu and Perler, 1996; Perler, 1998). Both reactions involve an intramolecular rearrangement to form an ester or thioester, followed by a nucleophilic attack on this ester/thioester. As diagrammed in Fig. 3B, during protein self-splicing the side chain of a conserved cysteine, threonine, or serine residue at the boundary between the N-terminal extein and the intein attacks the carbonyl carbon of the preceding residue resulting in the substitution of an ester or thioester for the peptide bond. This ester/thioester then undergoes a second nucleophilic attack from the side chain of a serine or cysteine positioned at the C-terminal intein/extein boundary that results in a branched intermediate. The intein region is then hydrolyzed away via a succinamide intermediate that is formed with the side chain of a conserved asparagine, and the extein regions rearrange such that a new peptide bond is formed between them (see Fig. 3B) (Xu and Perler, 1996).

The region conserved between Hh-C and the self-splicing portion of inteins encompasses only the N-terminal 17 kDa proteolytic fragment (144/207 amino acids in the case of *Drosophila* Hh-C) for which a crystal structure is available. In *in vitro* experiments, this fragment of Hh-C (termed the HINT module for *H*edgehog-*Int*ein) is capable of thioester formation but not cholesterol transfer (Hall *et al.*, 1997). Thus, the HINT modules of both Hh and inteins are responsible for thioester (or ester) formation and utilize a conserved nucleophilic residue (cysteine, serine, or threonine) and a conserved histidine and threonine in this process. The ultimate product of the autoprocessing reaction for both Hh and self-splicing proteins is determined by the nucleophile in the second step (cholesterol in the case of Hh proteins and the C-terminal extein in the case of self-splicing proteins), and this second nucleophile is selected or contributed by sequences C-terminal to the HINT module (see Fig. 4).

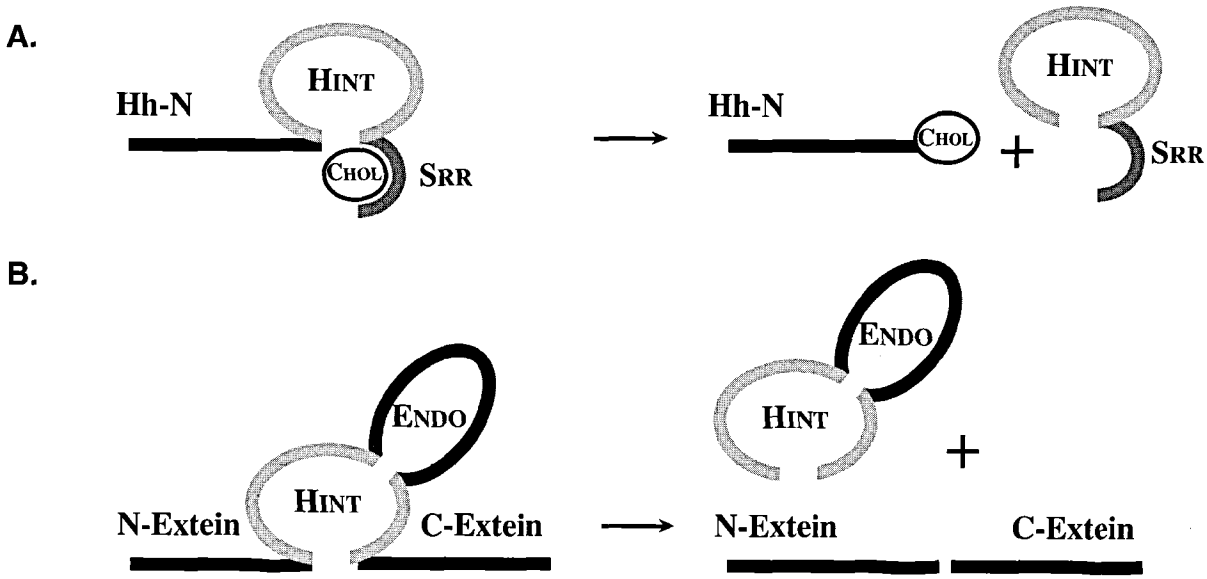


**Fig. 4.** The HINT module in different contexts. A. The HINT module is shown in the context of Hh proteins in which the thioester is attacked by a cholesterol molecule that is selected by the SRR sequences C-terminal to the HINT module. This cholesterol becomes covalently attached to sequences N-terminal to the HINT module (Hh-N). B. The HINT module is shown in the context of self-splicing proteins in which the thioester is attacked by a side chain at the N-terminus of the C-extein. The C-extein then become covalently attached to the N-extein. Conservation of structure, function, and active site residues between HINT modules in both contexts clearly suggests a common ancestor for the HINT modules in Hh and self-splicing proteins. The order in which additional sequences have been added or lost by HINT modules is not clear, but it is interesting to speculate that HINT modules, by virtue of associated endonuclease domains that cleave genes at hospitable sites, have inserted into preexisting proteins. In the case of Hh proteins, an initially neutral insertion (as is self-splicing proteins) may have then evolved to result in cholesterol transfer.

### Downstream Effectors of Hedgehog Signaling

Several downstream components of the Hh signaling pathway have been identified, and work is currently underway to elucidate the precise role of each of these proteins (Johnson and Scott, 1998) (See Fig. 5). Two integral membrane proteins, Patched and Smoothened, are genetically downstream of the Hh signal, and a direct interaction between Hh and Patched has been reported (Marigo *et al.*, 1996; Stone *et al.*, 1996). Unlike most ligand-receptor interactions, the function of Hh and Patched appear opposed to one another (Ingham *et al.*, 1991; Ingham, 1993). The presence of Hh appears to release an inhibitory function of Patched and lead to activation of Smoothened and other downstream signaling events (Alcedo *et al.*, 1996; Chen and Struhl, 1996; van den Heuvel and Ingham, 1996). How the status of Patched and Smoothened is communicated to the cytoplasm is not known, but several cytoplasmic proteins are genetically implicated in the Hh signaling pathway. These proteins include a kinase (Fused), a kinesin-like molecule (Costal-2), a protein of unknown function (Suppressor of Fused), and a zinc-finger containing transcription factor (Cubitus Interruptus (CI)) (Forbes *et al.*, 1993). CI is homologous to vertebrate *gli* genes that are amplified in glioblastomas and appears to directly regulate the transcription of several Hh responsive genes (Dominguez *et al.*, 1996). These proteins are not discussed in detail here, and the reader is instead referred to a recent review by Johnson and Scott (1998).

### Hedgehog and Human Disease

Shh knockout mice display a striking phenotype that includes cyclopia and other defects of midline features of the brain and face (Chiang *et al.*, 1996). This phenotype strongly resembles the severest form of holoprosencephaly (HPE), a syndrome that encompasses a spectrum of human developmental malformations involving defects in midline structures (Cohen and Sulik, 1992). Embryological experiments carried out more than half a century ago had shown cyclopia to result from disruption of prechordal plate mesoderm and its influence upon the neuroepithelium (Adelman, 1936a; 1936b). Shh expression in the prechordal plate mesoderm coincides with, or just precedes, the requirement for prechordal plate signaling (Chang *et al.*, 1994; Echelard *et al.*, 1993), consistent with a role for Shh in this signaling function. Indeed, a subtype of HPE in humans has recently been shown to result from mutations in the Shh gene (Belloni *et al.*, 1996; Roessler *et al.*, 1996). Curiously, while mutation of Shh is entirely recessive in the mouse, Shh appears to be haploinsufficient in human, and the HPE-causing mutations in human are autosomal dominant. The severity of defects observed in humans heterozygous for Shh mutations is, however, somewhat variable and typically less severe than observed in the homozygous mouse knockout (Chiang *et al.*, 1996).

The association of certain forms of HPE with mutations in Shh suggested the possibility that other instances of HPE-like phenotypes may result from defects at different steps in the Hh signaling pathway. Of particular interest were observations that feeding pregnant rats inhibitors of cholesterol biosynthesis led to malformations resembling HPE (Roux *et al.*, 1979; Dehart *et al.*, 1997). Given the importance of cholesterol for Hh function, it was thought that these compounds might exert their effects through disruption of normal Hh signaling. The most obvious mechanism for such a disruption would be for the altered cholesterol metabolism to reduce Hh signaling by affecting normal Hh autoprocessing, but this does not seem to be the case. Studies of tissue explants indicate that the signaling properties of cellular Hh are unaffected by the presence of these drugs. Treatment of target tissues with these drugs, however, does render them unable to receive the Hh signal

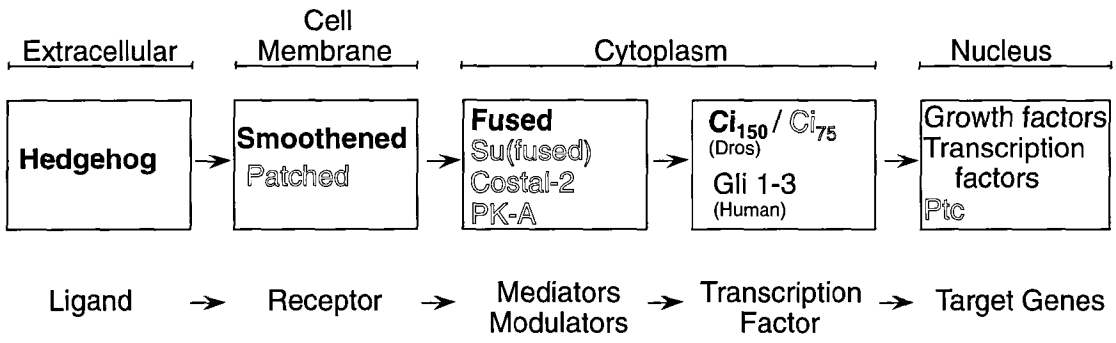


**Fig. 5.** Downstream effectors of Hh function. Proteins that have been shown to mediate or modulate Hh signaling are shown. Proteins that appear to activate Hh signaling are shown in bold while proteins that appear to inhibit Hh signaling are shown in outline. Patched and Smoothened are 12- and 7-pass integral membrane proteins, respectively, Fused and protein kinase A (PK-A) are kinases, Costal-2 is a kinesin-like molecule, Cubitus interruptus (Ci) is a zinc finger containing transcription factor that is homologous to vertebrate Gli proteins and undergoes a cleavage from a 150 kDa to a 75 kDa form.

(Cooper *et al.*, 1998). That is, normal cholesterol metabolism is required for a particular tissue to respond to an exogenous Hh signal. A molecular rationale for this requirement is not yet apparent, but it is curious to note that the likely Hh receptor, the integral membrane protein Patched, contains a region of five transmembrane spanning helices that is homologous to the sterol-sensing domain (SSD) of proteins such as HMG CoA reductase and SCAP (Beachy *et al.*, 1997). The SSDs of these proteins are responsible for regulating their activity in response to cholesterol levels. The role of the SSD region in Patched remains unknown, but it may provide the link between normal cholesterol homeostasis and the ability of target tissues to respond to Hh.

The absence of appropriate Patched function underlies the basal cell nevus syndrome (BCNS), which is characterized by developmental abnormalities and postnatal occurrence of cancers, especially basal cell carcinoma (BCC) (Johnson *et al.*, 1996). In addition to the heritable mutations in *patched* that lead to BCNS, sporadic *patched* mutations have been found in BCCs, the most common human cancer. BCNS presumably arises because of deregulation of the Hh signaling pathway. Consistent with this conclusion, overexpression of Shh in mouse skin results in many features of BCNS, including BCCs (Oro *et al.*, 1997). The connection of Hh and its downstream targets to both developmental malformations and cancer highlights the delicate balance between cell growth and differentiation required during animal development to reliably produce a viable organism.

## Summary

Hh proteins represent a new signaling paradigm in metazoan development. In species ranging from fruit flies to humans, Hh proteins mediate multiple processes vital to appropriate pattern formation in the developing embryo. Hh proteins undergo an autoprocessing event in which the full-length protein is cleaved into N-terminal and C-terminal domains (Hh-N and Hh-C, respectively), and a cholesterol moiety becomes covalently attached to Hh-N. All known signaling activities of Hh proteins are mediated by Hh-N while both the cleavage and cholesterol transfer reactions are mediated by Hh-C. The cholesterol attached to Hh-N is required to retrict the range of Hh signaling and may be involved in ensuring appropriate reception of the Hh signal in target tissues. Disruptions of Hh signaling pathways lead to severe developmental defects in newborns and cancers in adults. While studies of Hh proteins have yielded a wealth of new insight into the molecular mechanisms of metazoan development, many outstanding questions concerning Hh signaling mechanisms ensure that unraveling the secrets of this molecule will keep scientists well entertained for the foreseeable future.

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## References

Adelman, H. B. (1936a) The problem of cyclopia (part I). *Q. Rev. Biol.* **11**, 116.

Adelman, H. B. (1936b) The problem of cyclopia (part II). *Q. Rev. Biol.* **11**, 284.

Alcedo, J., Ayzenzon, M., Von Ohlen, T., Noll, M. and Hooper, J. E. (1996) The *Drosophila* smoothened gene encodes a seven-pass membrane protein, a putative receptor for the hedgehog signal. *Cell* **86**, 221–232.

Beachy, P. A., Cooper, M. K., Young, K. E., von Kessler, D. P., Park, W.-J., Hall, T. M. T., Leahy, D. J. and Porter, J. A. (1997) Multiple roles of cholesterol in hedgehog protein biogenesis and signaling. *Cold Spring Harbor Symposia on Quantitative Biology* **LXII**, 191–204.

Belloni, E., Muenke, M., Roessler, E., Traverso, G., Siegel-Bartelt, J., Frumkin, A., Mitchell, H. F., Donis-Keller, H., Helms, C., Hing, A. V., Heng, H. H., Koop, B., Martindale, D., Rommens, J. M., Tsui, L. C. and Scherer, S. W. (1996) Identification of Sonic hedgehog as a candidate gene responsible for holoprosencephaly. *Nat. Genet.* **14**, 353–356.

Bumcrot, D. A., Takada, R. and McMahon, A. P. (1995) Proteolytic processing yields two secreted forms of *sonic hedgehog*. *Mol. Cell. Biol.* **15**, 2294–2303.

Bussiere, D. E., Pratt, S. D., Katz, L., Severin, J. M., Holzman, T. and Park, C. H. (1998) The structure of VanX reveals a novel amino-dipeptidase involved in mediating transposon-based vancomycin resistance. *Molecular Cell* **2**, 75–84.

*C. elegans* Consortium (1998) Genome sequence of the Nematode *C. elegans*: a platform for investigating biology. *Science* **282**, 2012–2018.

Carstea, E. D., Morris, J. A., Coleman, K. G., Loftus, S. K., Zhang, D., Cummings, C., Gu, J., Rosenfeld, M. A., Pavan, W. J., Drizman, D. B., Nagle, J., Polymeropoulos, M. G., Sturley, S. L., Ioannou, Y. A., Higgins, M. E., Comly, M., Cooney, A., Brown, A., Kaneski, C. R., Blanchette-Mackie, E. J., Dwyer, N. K., Neufeld, E. B., Chang, T.-Y., Liscum, L., Strauss, J. F. I., Ohno, K., Zeigler, M., Carmi, R., Sokol, J., Markie, D., O'Neill, R. R., van Diggelen, O. P., Elleder, M., Patterson, M. C., Brady, R. O., Vanier, M. T., Pentchev, P. G. and Tagle, D. A. (1997) Niemann-Pick C1 disease gene: homology to mediators of cholesterol homeostasis. *Science* **277**, 228–231.

Chang, D. T., Lopez, A., von Kessler, D. P., Chiang, C., Simandl, B. K., Zhao, R., Seldin, M. F., Fallon, J. F. and Beachy, P. A. (1994). Products, genetic linkage and limb patterning activity of a murine *hedgehog* gene. *Development* **120**, 3339–3353.

Chen, Y. and Struhl, G. (1996) Dual roles for patched in sequestering and transducing Hedgehog. *Cell* **87**, 553–563.

Chiang, C., Ying, L., Lee, E., Young, K. E., Corden, J. L., Westphal, H. and Beachy, P. A. (1996) Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function. *Nature* **383**, 407–413.

Cohen, M. M. and Sulik, K. K. (1992) Perspectives on holoprosencephaly. II. Central nervous system, craniofacial

anatomy, syndrome commentary, diagnostic approach, and experimental studies. *J. Craniofacial Genet. Dev. Biol.* **12**, 196–244.

Cooper, M. K., Porter, J. A., Young, K. E. and Beachy, P. A. (1998) Teratogen-mediated inhibition of target tissue response to Shh signaling. *Science* **280**, 1603–1607.

Dalgaard, J. Z., Moser, M. J., Hughey, R. and Mian, I. W. (1997a) Statistical modeling, phylogenetic analysis and structure prediction of a protein splicing domain common to inteins and hedgehog proteins. *J. Comput. Biol.* **4**, 193–214.

Dalgaard, J. Z., Klar, A. J., Moser, M. J., Holley, W. R., Chatterjee, A. and Mian, I. S. (1997b) Statistical modeling and analysis of the LAGLIDADG family of site-specific endonucleases and identification of an intein that encodes a site-specific endonuclese of the HNH family. *Nuc. Acids Res.* **25**, 4626–4638.

Dehart, D. B., Lanoue, L., Tint, G. S. and Sulik, K. K. (1997) Pathogenesis of malformations in a rodent model for Smith-Lemli-Opitz syndrome. *Am. J. Med. Genet.* **68**, 328–337.

Dideberg, O., Charlier, P., Dive, G., Joris, B., Frere, J. M. and Ghuysen, J. M. (1982) Structure of a $Zn^{2+}$-containing D-alanyl-D-alanine-cleaving carboxypeptidase at 2.5 Å resolution. *Nature* **299**, 469–470.

DiNardo, S., Sher, E., Heemskerk-Jongens, J., Kassis, J. A. and O'Farrell, P. H. (1988) Two-tiered regulation of spatially patterned *engrailed* gene expression during *Drosophila* embryogenesis. *Nature* **363**, 549–552.

Dominguez, M., Brunner, M., Hafen, E. and Basler, K. (1996) Sending and receiving the hedgehog signal: control by the *Drosophila* Gli protein Cubitus interruptus. *Science* **272**, 1621–1625.

Duan, X., Gimble, F. S. and Quiocho, F. A. (1997) Crystal structure of PI-SceI, a homing endonuclease with protein splicing activity. *Cell* **89**, 555–564.

Echelard, Y., Epstein, D. J., St.-Jacques, B., Shen, L., Mohler, J., McMahon, J. A. and McMahon, A. P. (1993) Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell* **75**, 1417–1430.

Fan, C.-M., Porter, J. A., Chiang, C., Chang, D. T., Beachy, P. A. and Tessier-Lavigne, M. (1995) Long-range sclerotome induction by *sonic hedgehog*: Direct role of the amino-terminal cleavage product and modulation by the cyclic AMP signaling pathway. *Cell* **81**, 457–465.

Fietz, M. J., Jacinto, A., Taylor, A. M., Alexandre, C. and Ingham, P. W. (1995) Secretion of the amino-terminal fragment of the Hedgehog protein is necessary and sufficient for *hedgehog* signalling in *Drosophila*. *Current Biology* **5**, 643–650.

Forbes, A. J., Nakano, Y., Taylor, A. M. and Ingham, P. W. (1993) Genetic analysis of hedgehog signalling in the *Drosophila* embryo. *Dev Suppl*, 115–124.

Hall, T. M. T., Porter, J. A., Beachy, P. A. and Leahy, D. J. (1995) A potential catalytic site revealed by the 1.7 Å crystal structure of the amino-terminal signalling domain of Sonic hedgehog. *Nature* **378**, 212–216.

Hall, T. M. T., Porter, J. A., Young, K. E., Koonin, E. V., Beachy, P. A. and Leahy, D. J. (1997) Crystal structure of a hedgehog autoprocessing domain: homology between hedgehog and self-splicing proteins. *Cell* **91**, 85–97.

Hidalgo, A. and Ingham, P. (1990) Cell patterning in the *Drosophila* segment: spatial regulation of the segment polarity gene *patched*. *Development* **110**, 291–301.

Hynes, M., Porter, J. A., Chiang, C., Chang, D., Tessier-Lavigne, M., Beachy, P. A. and Rosenthal, A. (1995) Induction of midbrain dopaminergic neurons by Sonic hedgehog. *Neuron* **15**, 35–44.

Incardona, J. P., Gaffield, W., Kapur, R. P. and Roelink, H. (1998) The teratogenic Veratrum alkaloid cyclopamine inhibits sonic hedgehog signal transduction. *Development* **125**, 3553–3562.

Ingham, P. W., Taylor, A. and Nakano, Y. (1991) Role of the *Drosophila patched* gene in positional signalling. *Nature* **353**, 184–187.

Ingham, P. W. (1993) Localized *hedgehog* activity controls spatial limits of *wingless* transcription in the *Drosophila* embryo. *Nature* **366**, 560–562.

Johnson, R. L., Rothman, A. L., Xie, J., Goodrich, L. V., Bare, J. W., Bonifas, J. M., Quinn, A. G., Myers, R. M., Cox, D. R., Epstein, E. H. J. and Scott, M. P. (1996) Human homolog of *patched*, a candidate gene for the basal cell nevus syndrome. *Science* **272**, 1668–1671.

Johnson, R. L. and Scott, M. P. (1998) New players and puzzles in the Hedgehog signaling pathway. *Curr. Opin. Genet. Dev.* **8**, 450–456.

Klabunde, T., Sharma, S., Telenti, A., Jacobs, W. R. J. and Sacchettini, J. C. (1998) Crystal structure of GyrA intein from *Mycobacterium xenopi* reveals structural basis of protein splicing. *Nat. Struct. Biol.* **5**, 31–36.

Krauss, S., Concordet, J.-P. and Ingham, P. W. (1993) A functionally conserved homolog of the *Drosophila* segment polarity gene *hh* is expressed in tissues with polarizing activity in zebrafish embryos. *Cell* **75**, 1431–1444.

Lee, J. J., Ekker, S. C., von Kessler, D. P., Porter, J. A., Sun, B. I. and Beachy, P. A. (1994) Auto-proteolysis in *hedgehog* protein biogenesis. *Science* **266**, 1528–1537.

Lee, J. J., von Kessler, D. P., Parks, S. and Beachy, P. A. (1992) Secretion and localized transcription suggest a role in positional signaling for products of the segmentation gene *hedgehog*. *Cell* **71**, 33–50.

Levin, M., Johnson, R. L., Stern, C. D., Kuehn, M. and Tabin, C. (1995) A molecular pathway determining left-right asymmetry in chick embryogenesis. *Cell* **82**, 803–814.

Lopez-Martinez, A., Chang, D. T., Chiang, C., Porter, J. A., Ros, M. A., Simandl, B. K., Beachy, P. A. and Fallon, J. F. (1995) Limb-patterning activity and restricted posterior localization of the amino-terminal product of Sonic hedghog cleavage. *Current Biology* **5**, 791–796.

Marigo, V., Davey, R. A., Zuo, Y., Cunningham, J. M. and Tabin, C. J. (1996) Biochemical evidence that patched is the Hedgehog receptor. *Nature* **384**, 176–179.

Marti, E., Bumcrot, D. A., Takada, R. and McMahon, A. P. (1995) Requirement of 19K form of sonic hedgehog for induction of distinct ventral cell types in CNS explants. *Nature* **375**, 322–325.

Martinez-Arias, A., Baker, N. E. and Ingham, P. W. (1988) Role of segment polarity genes in the definition and maintenance of cell states in the *Drosophila* embryo. *Development* **103**, 157–170.

Mohler, J. (1988). Requirements for Hedgehog, a segmental polarity gene, in patterning larval and adult cuticle of *Drosophila*. *Genetics* **120**, 1061–1072.

Mohler, J. and Vani, K. (1992) Molecular organization and embryonic expression of the hedgehog gene involved in cell-cell communication in segmental patterning of *Drosophila*. *Development* **115**, 957–971.

Murzin, A. G. (1996). Structural classification of proteins: new superfamilies. *Curr. Opin. Struct. Biol.* **6**, 386–394.

Nüsslein-Volhard, C. and Wieschaus, E. (1980) Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**, 795–801.

Oro, A. E., Higgins, K. M., Hu, Z., Bonifas, J. M., Epstein, E. H. J. and Scott, M. P. (1997) Basal cell carcinomas in mice overexpressing sonic hedgehog. *Science* **276**, 817–821.

Pepinsky, R. B., Zeng, C., Wen, D., Rayhorn, P., Baker, D. P., Williams, K. P., Bixler, S. A., Ambrose, C. M., Garber, E. A., Miatkowski, K., Taylor, F. R., Wang, E. A. and Galdes, A. (1998) Identification of a palmitic acid-modified form of human Sonic hedgehog. *J. Biol. Chem.* **273**, 14037–14045.

Perler, F. B. (1998) Protein splicing of inteins and hedgehog autoproteolysis: structure, function, and evolution. *Cell* **92**, 1–4.

Pietrokovski, S. (1998) Modular organization of inteins and C-terminal autocatalytic domains. *Protein Sci.* **7**, 64–71.

Porter, J. A., von Kessler, D. P., Ekker, S. C., Young, K. E., Lee, J. J., Moses, K. and Beachy, P. A. (1995) The product of *hedgehog* autoproteolytic cleavage active in local and long-range signalling. *Nature* **374**, 363–366.

Porter, J. A., Ekker, S. C., Park, W.-J., von Kessler, D. P., Young, K. E., Chen, C.-H., Ma, Y., Woods, A. S., Cotter, R. J., Koonin, E. V. and Beachy, P. A. (1996a) Hedgehog patterning activity: role of a lipophilic modification mediated by the carboxy-terminal autoprocessing domain. *Cell* **86**, 21–34.

Porter, J. A., Young, K. E. and Beachy, P. A. (1996b) Cholesterol modification of hedgehog signaling proteins in animal development. *Science* **274**, 407–413.

Riddle, R. D., Johnson, R. L., Laufer, E. and Tabin, C. (1993) *Sonic hedgehog* mediates the polarizing activity of the ZPA. *Cell* **75**, 1401–1416.

Roelink, H., Augsburger, A., Heemskerk, J., Korzh, V., Norlin, S., Ruiz i Altaba, A., Tanabe, Y., Placzek, M., Edlund, T., Jessell, T. M. and Dodd, J. (1994) Floor plate and motor neuron induction by *vhh-1*, a vertebrate homolog of *hedgehog* expressed by the notochord. *Cell* **76**, 761–775.

Roelink, H., Porter, J. A., Chiang, C., Tanabe, Y., Chang, D. T., Beachy, P. A. and Jessell, T. M. (1995) Floor plate and motor neuron induction by different concentrations of the amino-terminal cleavage product of *sonic hedgehog* autoproteolysis. *Cell* **81**, 445–455.

Roessler, E., Belloni, E., Gaudenz, K., Jay, P., Berta, P., Scherer, S. W., Tsui, L. C. and Muenke, M. (1996) Mutations in the human Sonic Hedgehog gene cause holoprosencephaly. *Nat. Genet.* **14**, 357–360.

Roux, C., Horvath, C. and Dupuis, R. (1979) Teratogenic action and embryo lethality of AY $9944^{R}$: Prevention by a hypercholesterolemia-provoking diet. *Teratology* **19**, 35–38.

Ruvkun, G. and Hobert, O. (1998) The taxonomy of developmental control in *Caenorhabditis elegans*. *Science* **282**, 2033–2041.

Stone, D. M., Hynes, M., Armanimi, M., Swanson, T. A., Gu, Q., Johnson, R. L., Scott, M. P., Pennica, D., Goddard, A., Phillips, H., Noll, M., Hooper, J. E., de Sauvage, F. and Rosenthal, A. (1996) The tumour-suppressor gene *patched* encodes a candidate receptor for Sonic hedgehog. *Nature* **384**, 129–134.

van den Heuvel, M. and Ingham, P. W. (1996) Smoothened encodes a receptor-like serpentine protein required for hedgehog signalling. *Nature* **382**, 547–551.

Vu, T.-K. H., Hung, D. T., Wheaton, V. I. and Coughlin, S. R. (1991) Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell* **64**, 1057–1068.

Xu, M.-Q. and Perler, F. B. (1996) The mechanism of protein splicing and its modulation by mutation. *EMBO J.* **15**, 5146–5153.