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Hedgehog and Patched in Neural Development and Disease

Review

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The vertebrate central nervous system is organized into functional units dedicated to specific tasks such as the detection of form and of movement. Understanding the embryogenesis of these patterns is a daunting task, but it has been aided greatly by genetic studies in model organisms such as the fruit fly *Drosophila*. The strength of this approach is exemplified by the Hedgehog (Hh) signaling system, initially identified in flies and subsequently found to play an essential role in human neural development and disease. Misregulation of Hh signaling in humans is associated with mental retardation and gross neural tube defects such as spina bifida, which is a malformation of the spinal column, and holoprosencephaly, a loss of midline structures in the brain and face (Belloni et al., 1996; Hahn et al., 1996b; Johnson et al., 1996; Roessler et al., 1996). In adults, the Hh pathway is essential for restraining growth in the nervous system and other tissues; mutations in pathway components lead to a variety of tumors, including basal cell carcinoma, the most common human cancer, and medulloblastoma, a childhood brain tumor (Gailani et al., 1996; Hahn et al., 1996b; Johnson et al., 1996; Oro et al., 1997; Pietsch et al., 1997; Raffel et al., 1997; Unden et al., 1997; Wolter et al., 1997; Xie et al., 1997). Understanding Hh signaling may lead to improved treatments for these human disorders.

Although "the Hh pathway" is now diagrammed in many textbooks, many questions remain, not least of which is how the Hh signal is sent and received. In this review, we focus on what has been learned about Hh signaling in the nervous system, how regulation of cell fate and cell division are interwoven, and what we now know about the intracellular steps in Hh signal transduction. We begin with an introduction to two key members of the pathway: Hh itself and its proposed receptor, Patched (Ptc).

Hh and Ptc Have Opposite Effects on Target Gene Transcription and Cell Fates

As was initially described in the *Drosophila* embryonic epidermis, the segment polarity genes *hh* and *ptc* oppose each other's activity (Ingham et al., 1991). This mutual restraint leads to precisely coordinated patterns of gene transcription, differentiation, and growth. *hh* encodes a secreted protein (Lee et al., 1992; Mohler and Vani, 1992; Tabata et al., 1992) that regulates cell fate determination and, in some tissues, induces proliferation. Ptc is a transmembrane protein (Hooper and Scott, 1989; Nakano et al., 1989) that dictates alternative developmental decisions or inhibits growth unless opposed by Hh activity. For example, in *ptc* mutant fly embryos, the central pattern elements of the ventral epidermis are deleted and replaced with mirror-image duplications of the segment borders (Nüsslein-Volhard and Wieschaus, 1980; Martinez Arias et al., 1988); nearly the same phenotype is seen when *hh* is ubiquitously expressed (Ingham and Hidalgo, 1993). Either loss of Ptc or too much Hh leads to ectopic transcription of the *wingless* (*wg*) gene, which encodes a secreted Wnt-class signal that controls cell fates. Thus, *hh* normally maintains *wg* transcription in a limited region, and *ptc* normally represses *wg* outside that region. Despite the gross patterning defects observed in *ptc* mutant embryos, no change in cell division frequency or pattern has been noted (Martinez Arias et al., 1988).

In contrast, during imaginal disc development, Hh signaling strongly affects both cell fate and growth. Cells that lack Ptc or are exposed to high levels of Hh assume fates of cells usually found in the central wing region (Figure 1) (Phillips et al., 1990; Ingham and Fietz, 1995). In addition, either ectopic expression of Hh or loss of Ptc function causes dramatic mirror-image duplications or triplications in the legs and wings (Phillips et al., 1990; Basler and Struhl, 1994). In contrast, excess Ptc inhibits growth, leading to small wings (Johnson et al., 1995). Some of these effects are due to Hh induction of *decapentaplegic* (*dpp*), a TGFβ-class signal, in a stripe along the border between the anterior and posterior compartments (Figure 1) (Basler and Struhl, 1994; Tabata and Kornberg, 1994). Again, Ptc acts in opposition to Hh and inhibits *dpp* transcription (Capdevila et al., 1994b; Johnson et al., 1995). Dpp appears to be responsible for much of Hh's long-range activity in discs (Capdevila and Guerrero, 1994; Ingham and Fietz, 1995). Both Dpp and Wg act as long-range signals and can cause extensive pattern rearrangements when misexpressed (Nellen et al., 1996; Zecca et al., 1996). Normal development therefore depends on the ability of Hh and Ptc to restrict production of secondary signals to the right places.

The mutual antagonism between Hh and Ptc also controls the transcription of *ptc* itself (Ingham et al., 1991). In many tissues, cells that receive Hh signal increase transcription of *ptc* (Figure 2). Out of range of a Hh source, Ptc represses its own transcription and therefore *ptc* RNA is present at low levels. *ptc* expression remains at low levels in *hh* mutants or when Ptc is overexpressed (Hidalgo and Ingham, 1990; Ingham et al., 1991; Sampedro and Guerrero, 1991; Capdevila et al., 1994b; Johnson et al., 1995). Conversely, *ptc* transcription is elevated in *ptc* mutant embryos and discs (Hidalgo and Ingham, 1990; Capdevila et al., 1994b). This relationship is evolutionarily conserved in vertebrates, where three different Hh proteins—Sonic hedgehog (Shh), Desert hedgehog (Dhh), and Indian hedgehog (Ihh)—each induce high-level *ptc* transcription (Bitgood et al., 1996; Concordet et al., 1996; Goodrich et al., 1996; Marigo et al., 1996c; Vortkamp et al., 1996). In addition, Ptc is both necessary and sufficient for its own transcriptional repression in most murine tissues (Goodrich et

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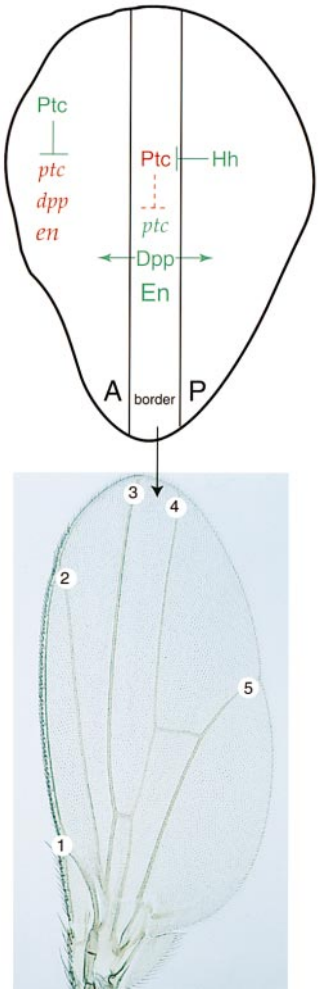

Figure 1. Hh and Ptc Signaling in the Fly Imaginal Disc

In the leg imaginal disc, Hh is expressed in the posterior (P) compartment (top). At the anterior–posterior border, Hh protein inactivates Ptc function, relieving Ptc-mediated repression of *ptc* itself, of *dpp*, and of *en* (dashed lines). En determines cell fates at the border, and Dpp acts at long range to induce patterning and proliferation in the anterior and posterior compartments (bottom). In the anterior (A) compartment, Ptc protein remains active and inhibits target gene transcription. These events lead to the formation of wing veins (numbered) with stereotyped morphologies and positions along the AP axis. Active proteins and genes that are induced are in green; inactive proteins and genes that are inhibited are in red.

al., 1997; L. V. G. and M. P. S., unpublished data). Thus, in both flies and mice, *ptc* transcription is an excellent indicator of Hh and Ptc activity (Figure 2).

Hh and Ptc Organize the Vertebrate Neural Tube

Studies of the Hh pathway in vertebrates have pushed forward our understanding of a number of developmental processes, especially in the nervous system. A striking feature of vertebrate CNS organization is the spatial organization of neurons according to their functions. For example, sensory neurons are in the dorsal horn of the spinal cord and motor neurons are ventral (reviewed by Tanabe and Jessell, 1996). How does this pattern arise during development? Embryological manipulations and molecular analysis have brought developmental neurobiologists much closer to an answer to this classic question. The dorsal–ventral polarization is apparently established in the developing neural tube by signals from the overlying surface ectoderm and from an underlying rod of axial mesoderm, the notochord (Figure 3). The dorsal-most and ventral-most cells of the neural tube—the roof plate and floor plate, respectively—acquire the organizing properties of the ectoderm and notochord and subsequently help pattern the rest of the neural tube. The roof plate and floor plate consist of specialized, nonneuronal cells that are histologically distinct from the neuroepithelium. While dorsal cell types can be induced by bone morphogenetic protein (BMP)–like signals from the ectoderm and roof plate (Liem et al., 1995, 1997), ventral cell types can be induced by the Hh family member Sonic hedgehog (Shh) produced by the notochord and floor plate.

Shh Signaling and Floor Plate Development

Much evidence supports the hypothesis that Shh secreted from the axial mesoderm induces formation of the floor plate by preventing Ptc function. Both *Shh* and *ptc* are expressed at the right time and place, *Shh* in the notochord (Echelard et al., 1993) and *ptc* in the presumptive floor plate though not in the differentiated floor plate (Figure 2) (Goodrich et al., 1996). Since Shh induces *ptc* transcription (Goodrich et al., 1996; Marigo et al., 1996c), a Shh signal from the mesoderm is apparently received by cells in the ventral neural tube. Moreover, Shh has floor plate–inducing activity: ectopic Shh induces neural cells to assume both molecular and histological properties of the floor plate (reviewed by Ericson et al., 1997b). High concentrations of Shh are needed for this activity, consistent with the fact that notochord induction of floor plate is contact dependent (Placzek et al., 1990, 1993; Roelink et al., 1994, 1995). *Shh* is also necessary for floor plate formation; the floor plate fails to develop in mice lacking *Shh* function and when Shh activity is inhibited by function-blocking antibodies (Chiang et al., 1996; Ericson et al., 1996). As in flies, mutations in *ptc* have the opposite effect: floor plate–like cells develop throughout much of the neuroepithelium of mouse *ptc* mutants (Goodrich et al., 1997).

Transplant experiments and mutant phenotypes indicate that Shh inactivation of Ptc is important for floor plate determination, but they do not settle the question of *when* the Shh pathway works during normal development. Does Shh signaling normally occur between the notochord and ventral neural tube, or does Shh direct floor plate development earlier, during gastrulation? The node is a morphologically distinct region anterior to the primitive streak of the gastrula and can induce formation of an additional body axis when transplanted to an ectopic location (reviewed by Tam et al., 1997). It therefore has the properties of the Spemann organizer first described in *Xenopus*. Several observations support the idea that Shh acts first in the node and then in the neural tube. First, unlike the rest of the neural tube, the floor plate, like the notochord, is derived in part from the node and not from the ectoderm (Selleck and Stern, 1991; Catala et al., 1996). Thus, floor plate and notochord precursor cells are closely associated in the gastrula.

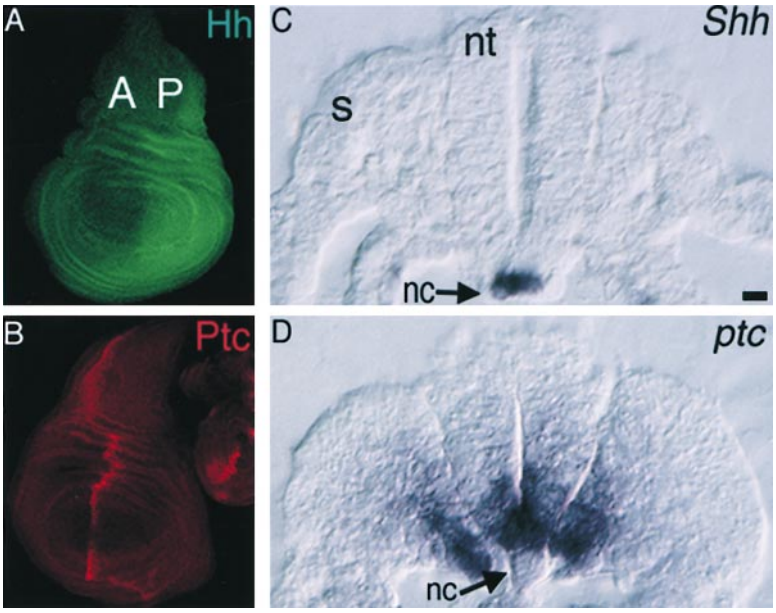

Figure 2. Hh and Ptc Expression in Flies and Mice

Due to the opposing activities of Hh and Ptc, *ptc* is expressed at high levels in cells that respond to Hh and at low levels away from the Hh source. This is a conserved feature of the pathway.

(A and B) In the fly leg disc, Hh (green) from the posterior compartment (P) induces Ptc (red) along the compartment border. Ptc is expressed at very low levels throughout the rest of the anterior compartment (A).

(C and D) Similarly, in the mouse neural tube, Shh from the notochord (nc) induces high-level *ptc* transcription in the ventral neural tube (nt) and somite (s). *ptc* is expressed at progressively lower levels in cells farther away from the Shh source.

Second, both *Shh* and *ptc* are expressed in the node (Echelard et al., 1993; Riddle et al., 1993; Goodrich et al., 1996; Marigo et al., 1996c). Third, a differentiated notochord does not appear to be required for floor plate formation, since a *Shh*-expressing floor plate develops in *no tail* zebrafish mutants, which have notochord precursor cells but no notochord (Halpern et al., 1993; Odenthal et al., 1996). Taken together, the results suggest that floor plate induction by the axial mesoderm might commence within the node, not after the notochord and neural tube are fully formed.

Another unresolved issue is whether the Shh signal is instructive, permissive, or both. In the fly epidermis, Hh signaling does not initiate the *wg* expression pattern but instead maintains preexisting *wg* transcription (Ingham and Hidalgo, 1993). By analogy, in the vertebrate nervous system, preexisting floor plate precursors could be preserved, or their growth stimulated, by a Shh signal. Indeed, Shh can act as a trophic factor for some midbrain progenitors (Miao et al., 1997) and as a mitogen in the spinal cord and retina (Echelard et al., 1993; Jensen and Wallace, 1997; Levine et al., 1997). There is noticeable overgrowth in the neural tube of *ptc* mutant embryos (Goodrich et al., 1997). In support of an instructive role, different concentrations of Shh can direct different cell fates in the ventral neural tube (Ericson et al., 1996, 1997a), and ectopic Shh can reprogram dorsal cells to assume a floor plate identity. Thus, in different biological contexts, Shh may control cell fate, cell survival, or cell growth. Since proliferation of a committed set of precursors also generates cell fate changes, defining the primary role of Shh is challenging.

Shh Signaling in the Ventral Neural Tube

In addition to its proposed role in floor plate development, Shh signaling also appears to control the fate and position of motor neurons and interneurons in the ventral spinal cord (Figure 3). Shh is proposed to ventralize neural tube progenitors by repressing expression of dorsal determinants such as *Pax3* (Ericson et al., 1996, 1997a). This requires very low concentrations of Shh, in keeping with the repression of dorsal markers within ten cell diameters of the floor plate (Ericson et al., 1996). In vitro experiments suggest that 2-fold changes in the concentration of Shh subsequently induce specific types of cells at different dorsal–ventral positions in the spinal cord (Ericson et al., 1997a). The position and number of motor neurons is also sensitive to the amount of Shh provided (Roelink et al., 1995; Tanabe et al., 1995; Ericson et al., 1996).

Despite evidence that Shh is a dose-dependent signal, it has been difficult to prove that Shh acts directly, not via induction of secondary signaling molecules. In favor of direct action, low concentrations of Shh can induce motor neurons in the absence of floor plate (Martí et al., 1995a; Roelink et al., 1995; Tanabe et al., 1995), and the tissue adjacent to the floor plate cannot induce motor neuron development (Placzek et al., 1991). Moreover, antibody blocking experiments reveal a continued need for Shh protein for motor neuron development in ventrolateral explants that do not produce Shh themselves (Ericson et al., 1996), suggesting that Shh protein from the notochord or floor plate reaches these neighboring cells. Whether or not Shh induces secondary signals, it is increasingly clear that Shh can act in combination with other signaling proteins, such as the BMPs and fibroblast growth factors (FGFs) (Dale et al., 1997; Ye et al., 1998).

Shh protein is hard to detect outside the floor plate, so a gradient has not been directly observed (Martí et al., 1995b; Roelink et al., 1995). The detectable signaling portion of Shh is tightly associated with the cell surface (Bumcrot et al., 1995; Roelink et al., 1995), making it difficult to envision how a Shh gradient might be established. However, Shh activity outside the floor plate has been demonstrated with function blocking antibodies that inhibit development of motor neurons and interneurons in neural tube explants (Ericson et al., 1996, 1997a). Another indirect measure of Shh signaling is the domain

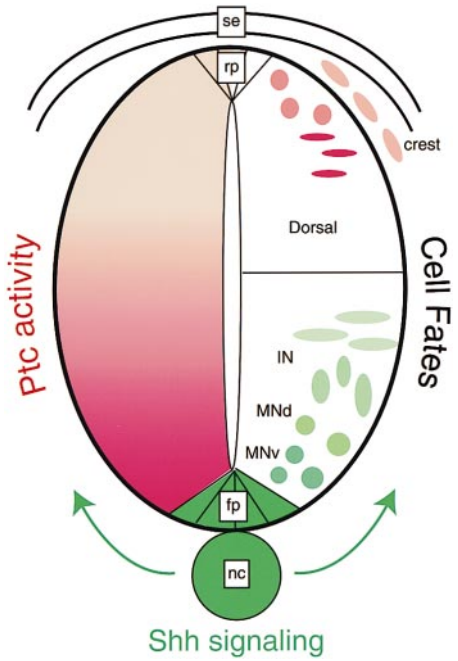

Figure 3. Dorsal–Ventral Patterning in the Spinal Cord

The vertebrate neural tube is polarized along the dorsal–ventral axis by cues from the notochord (nc) and overlying surface ectoderm (se). The most ventral cells are in the floor plate (fp), with motor neurons and interneurons at progressively more dorsal positions. The most dorsal cells are in the roof plate (rp), followed by sensory neurons and dorsal interneurons. Neural crest cells are an early dorsal cell type that are pinched off during neural tube closure and migrate away from the neural tube (crest). Ventral cell types are controlled by graded Shh signaling (Ericson et al., 1997a) from the notochord and floor plate (green). This gradient of activity is reflected in a gradient of *ptc* transcription and function (red). Close to Shh in the floor plate, *ptc* is expressed at high levels but is inactive. In the dorsal neural tube, *ptc* RNA can barely be detected but Ptc function is required (Goodrich et al., 1997).

of expression of Shh target genes, such as *ptc*. *ptc* is transcribed in a broad gradient in the neural tube (Figure 2) (Goodrich et al., 1996; Hahn et al., 1996a; Marigo and Tabin, 1996), suggesting that Shh travels over many cell diameters. The failure to detect a Shh protein gradient may be due to limited sensitivity of available antibodies or to the extreme potency of low-level Shh.

Additional Roles for Shh Signaling in the Nervous System

Roles for Shh and Ptc in the nervous system are by no means limited to the early spinal cord. In fact, Shh and Ptc act throughout the nervous system and at multiple stages of development. For example, Shh induces dopaminergic and serotonergic neurons in the midbrain and hindbrain, respectively (Hynes et al., 1995a, 1995b; Wang et al., 1995; Ye et al., 1998), and patterns the ventral forebrain (Ericson et al., 1995; Chiang et al., 1996; Dale et al., 1997; Goodrich et al., 1997; Shimamura and Rubenstein, 1997). In addition, *Shh* mutant embryos are cyclopic, due to an early failure to divide the eye field (Chiang et al., 1996), and, later, Shh is involved in the organization of the proximodistal axis of the eye (Krauss et al., 1993; Barth and Wilson, 1995; Ekker et al., 1995; Macdonald et al., 1995). *Shh* and *ptc* are expressed in complementary domains of the zona limitans, which divides the dorsal and ventral thalamus (Puelles and Rubenstein, 1993; Goodrich et al., 1996), though their function there has not been determined.

Hh Signal Transduction: Short- and Long-Range Actions of Hh Protein

The diverse and interesting functions for Hh in neural development raise the obvious question of how Hh signaling works. Although we are far from understanding the events leading from Hh secretion from one cell to changes in target gene transcription in another cell, much progress has been made in elucidating the biochemistry of the Hh protein. Hh ligands seem to act both locally and over a distance, and their range of action is tightly controlled by components of the Hh pathway itself.

In some cases, Hh acts directly over multiple cell diameters, while at other times the long-range effects are mediated by secondary signaling molecules (Heemskerk and DiNardo, 1994; Struhl et al., 1997). The short- and long-range modes of action are particularly distinct in the wing imaginal disc. At first glance, Hh appears to act over a distance to regulate growth and pattern. Diffusion of Hh ligand is in fact essential for proper determination of central wing cell fates, but a tethered form of Hh is sufficient for normal *dpp* expression in a narrow stripe of cells (Strigini and Cohen, 1997). Dpp, a TGFβ-class secreted signal, in turn regulates pattern and growth across the whole wing (Sanicola et al., 1995; Strigini and Cohen, 1997). Hh acts locally to control *dpp* expression but over a distance to control central cell fates. Similarly, in the vertebrate nervous system, Shh induction of floor plate seems to require direct contact with the notochord, but patterning of the ventral neural tube occurs over many cell diameters. Evidence for long-range Shh signaling has also been reported in the somites (Fan and Tessier-Lavigne, 1994; Fan et al., 1995). In contrast, in the limb bud, a tethered form of Shh is able to mimic the activities of a diffusible form, suggesting that here signaling is local (Yang et al., 1997).

A major factor affecting the range of Hh action is the biochemical nature of the Hh ligand itself, which appears to be tethered to the producer cells by a cholesterol moiety. Autocatalytic cleavage of the full-length Hh protein (Lee et al., 1994) results in the addition of cholesterol to the N-terminal fragment, Hh-N, which constitutes the signaling part (Porter et al., 1996a, 1996b). The C-terminal fragment (Hh-C), which is related to serine proteases and the self-splicing inteins, catalyzes the processing event (Lee et al., 1994; Hall et al., 1997). Hh-N is responsible for all of Hh's known biological activities in flies (Porter et al., 1995) and vertebrates (Ekker et al., 1995; Fan et al., 1995; Lai et al., 1995; Lopez-Martinez et al., 1995; Martí et al., 1995a; Roelink et al., 1995). The cholesterol moiety may tie Hh-N to the cell membrane and thus limit its range of action (Porter et al., 1996a, 1996b). Without cholesterol modification, Hh-N is a more effective inducer of target gene transcription and cuticle pattern defects than the wild-type Hh-N signal and acts at a greater range (Porter et al., 1996a). In addition, a

palmitoylated form of Hh-N has recently been identified and found to be extremely potent (Pepinsky et al., 1998). Hh-N protein has been crystallized and resembles a zinc hydrolase or peptidase (Hall et al., 1995) but has not been shown to have any enzymatic activity. How sterol modification of Hh-N affects its movement and/or activity is unknown. While it is possible that Hh protein diffuses, it is also possible that controlled transport systems exist. We are currently left with a major mystery about how Hh acts at a distance in tissues such as the vertebrate neural tube.

The range of action of the Hh ligand is also controlled by Ptc. An apparently universal consequence of Hh signaling is induction of *ptc* transcription. Hh regulation of Ptc activity and *ptc* transcription leads to a paradoxical situation: *ptc* is expressed at high levels in the very cells where its repressive activity is blocked in some way by Hh. This curious relationship is perhaps best envisioned as a mechanism for buffering against erroneous or excessively prolonged target gene transcription. High levels of Hh overcome Ptc function and cause transcription of target genes close to the Hh source. At the same time, Hh binding to Ptc limits the range of Hh action (Chen and Struhl, 1996), so that further away Ptc remains active and represses target gene expression.

Transduction of the Hh Signal: Is Ptc a Hh Receptor?

The current model of the Hh pathway (Figure 4) portrays the binding of Hh to Ptc. The Ptc protein can bind Hh protein with high affinity and specificity in cell overexpression assays (Marigo et al., 1996a; Stone et al., 1996). Whether the binding of Hh to Ptc is the way in which Hh prevents Ptc from repressing target gene transcription is unknown, and the possibility of other modes of Hh reception in target cells remains. Ptc evidently has two separable biochemical functions: Hh binding and target gene repression. For example, the ptc^{S2} allele of *Drosophila* makes a mutant Ptc protein that can restrict Hh diffusion but cannot inhibit transcription (Chen and Struhl, 1996). Molecular analysis of this allele may reveal a region of the Ptc protein responsible for its repressive activities. At present, hints of functional domains in the Ptc protein come only from comparisons among *ptc* and *ptc*-related genes. Most of the studies of vertebrates described above involve the originally identified *ptc* gene, now called *ptc1*. Two *ptc* genes, *ptc1* and *ptc2*, have been identified in multiple vertebrate species (Concordet et al., 1996; Goodrich et al., 1996; Hahn et al., 1996a; Johnson et al., 1996; Marigo et al., 1996c; Takabatake et al., 1997; Motoyama et al., 1998), and a more distantly related gene, *TRC8*, has been found as a tumor suppressor gene (Gemmill et al., 1998). The biological functions of *ptc2* remain unclear, but *ptc2* does not appear to substitute for *ptc1* function (Hahn et al., 1996b; Johnson et al., 1996; Goodrich et al., 1997). Comparisons of Ptc proteins reveal 12 conserved putative transmembrane domains arranged in a 6 + 6 array (Goodrich et al., 1996; Johnson and Scott, 1997). Though this arrangement is common to a family of channels and transporters (Saier, 1994), no recognizable primary sequence is shared between Ptc and this family. The possibility remains that Ptc acts as a channel or transporter, although attempts to detect Ptc-dependent ion movement have not been successful (Marigo et al., 1996a).

Whatever Ptc does, its functions appear not to be restricted to Hh-related events. In the fly embryonic nervous system, specific kinds of neurons arise in a stereotypic series of delaminations and cell divisions (reviewed by Doe and Skeath, 1996). In *ptc* mutants, some neurons are duplicated, while others, such as the RP2 neurons, are lost, consistent with a role for Ptc in controlling cell fate in the CNS (Patel et al., 1989; Bhat, 1996). As in the epidermis, Ptc repression of target genes in RP2 neurons appears to be inhibited by a secreted signal from adjacent cells (Bhat and Schedl, 1997). However, this signal cannot be Hh, since both Ptc target gene expression and the RP2 lineage are unaffected in *hh* mutant embryos (Bhat and Schedl, 1997).

Hh does play an important role in other aspects of fly neural development. Many neuroblasts and glia are missing in *hh* mutants, and the CNS is severely disorganized (Patel et al., 1989; Matsuzaki and Saigo, 1996). How Hh regulates the proliferation or survival of neurons remains uncertain, but in the embryonic nervous system Hh operates independently of *wg* (Matsuzaki and Saigo, 1996). In the developing eye, Hh regulates cell proliferation and progression of the morphogenetic furrow by antagonizing Ptc and thereby inducing *dpp* expression, as in wing and leg imaginal discs (Ma et al., 1993; Heberlein et al., 1993, 1995).

Other tissues also provide hints of Ptc-independent Hh function. In the dorsal embryonic epidermis of the fly, double $hh^{-}ptc^{-}$ homozygotes have a phenotype distinct from the *ptc* phenotype (Bejsovec and Wieschaus, 1993). If all of the activities of Hh were mediated by Ptc, the double mutant would be expected to look like the *ptc* mutant. In the dorsal cuticle, another segment polarity gene, *lines*, modifies how cells respond to Hh independently of *ptc* (Bokor and DiNardo, 1996). *ptc* and *hh* also appear to act independently in some segments of the fly head (Gallitano-Mendel and Finkelstein, 1997) and in the embryonic mesoderm (Forbes et al., 1993). Hh and Ptc may interact with additional ligands and receptors that have not yet been identified.

Transduction of the Hh Signal: Downstream Effectors

Before tackling possible branches and variations in the Hh pathway, researchers have focused on unveiling the intracellular events that take place in "standard" Hh signaling. Most of our insight into the transduction of the Hh and Ptc signals comes from genetic experiments in flies. Although several components of the pathway have been identified, how the proteins accomplish signal transduction is scarcely understood.

Hh or Ptc may affect transcription by regulating the activity of another transmembrane protein, Smoothened (Smo). *smo* function is necessary for Hh induction of target gene transcription (Alcedo et al., 1996; van den Heuvel and Ingham, 1996). Genetic studies suggests that the normal function of Ptc is to prevent Smo activity, perhaps directly (Alcedo et al., 1996; Chen and Struhl, 1996; Stone et al., 1996). Smo is a seven-pass protein

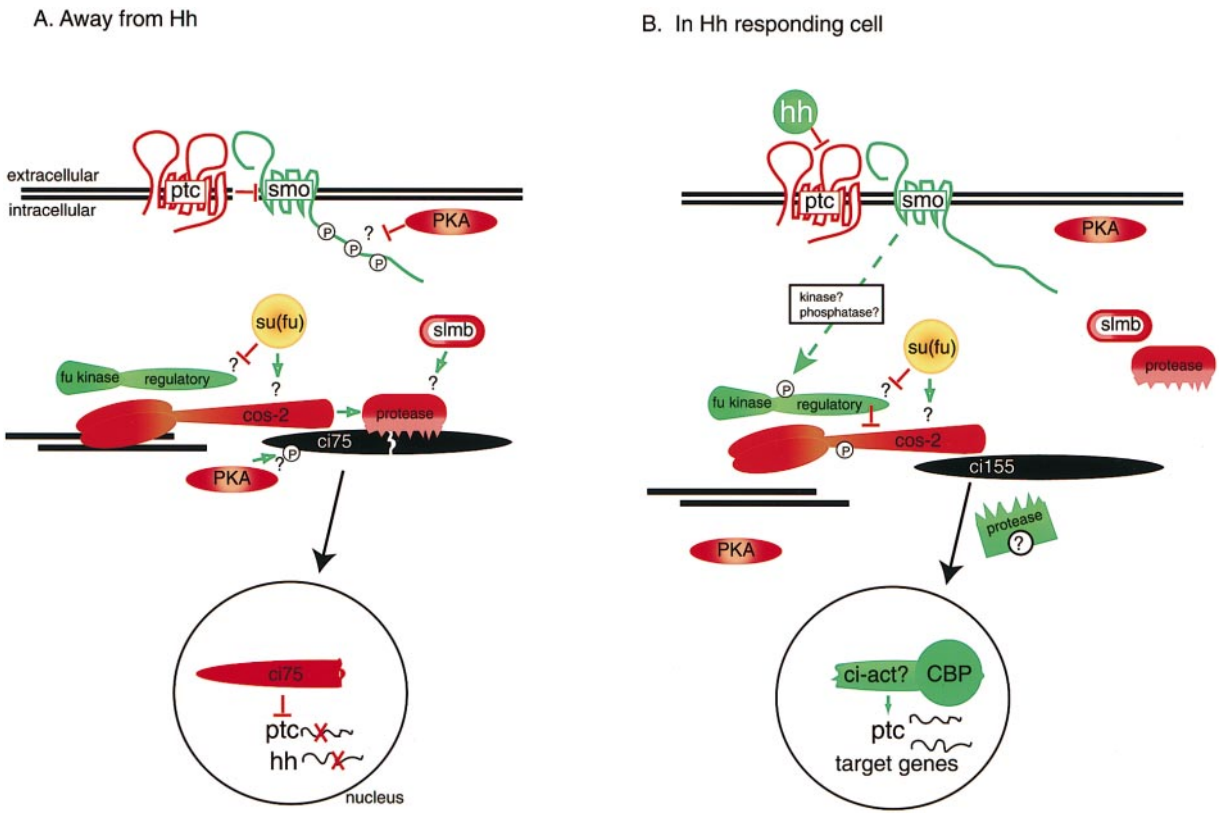

Figure 4. The Hh–Ptc Signaling Pathway

Components with a repressing role genetically are red. Components with an activating role genetically are green. Full-length Ci might have both repressive and activating roles (black), and Su(fu) is nonessential (yellow). Hypothetical interactions are indicated with question marks.
(A) In the absence of Hh signal, Ptc represses Smo function. Inside the cell, Fu, Cos-2, and Ci form a complex that binds to microtubules. Cos-2 somehow causes activation of a protease, perhaps in conjunction with Slmb. The protease, which has not been identified, cleaves Ci, releasing the repressor form, Ci75, which goes to the nucleus and inhibits transcription. PKA is a negative regulator of the pathway that might desensitize Smo or activate Cos-2 by phosphorylation (P). PKA phosphorylation also appears to promote processing of Ci. Su(fu) is a nonessential component that genetically activates Cos-2 activity, perhaps by inhibiting Fu activity. No target genes are induced.
(B) In the presence of Hh, Ptc can no longer inhibit Smo. Fu and Cos-2 are phosphorylated by an unidentified kinase(s). FuP, Cos-2, and Ci155 may remain associated, but the complex is no longer attached to microtubules. In the complex, Fu inhibits Cos-2, thereby preventing protease and/or Slmb activity. Full-length Ci therefore accumulates in the cytoplasm. A protease may generate an activating form of Ci (Ci-act) that goes to the nucleus. Acting with a CBP cofactor, Ci-act induces transcription of *ptc* and of other Hh target genes. PKA is no longer able to inhibit the pathway, perhaps due to the presence of an activated phosphatase. The net effect is transcription of *ptc* and tissue-specific target genes.

of the serpentine receptor family related to the putative Wg receptor dFrizzled2 (Alcedo et al., 1996; van den Heuvel and Ingham, 1996). Despite its similarity to receptors, vertebrate Smo has not been found to bind Hh (Stone et al., 1996). Hh might signal by binding to Ptc and thereby relieving inhibition of Smo activity by Ptc.

How Hh, Ptc, and Smo interactions at the membrane affect events in the cytoplasm is completely unknown, but several candidate signaling effectors have been identified. Hh signaling causes a stable phosphorylation of the serine–threonine kinase Fused (Fu) and of the kinesin-related protein Costal-2 (Cos-2) (Thérond et al., 1996b; Robbins et al., 1997). Fu positively regulates target gene transcription and is required for Hh function (Préat et al., 1990; Limbourg-Bouchon et al., 1991; Forbes et al., 1993; Ingham, 1993; Thérond et al., 1993). In contrast, loss of *cos-2* leads to derepression of Hh target genes and a phenotype much like that seen in *ptc* mutants (Forbes et al., 1993; Capdevila et al., 1994b; Sánchez-Herrero et al., 1996; Sisson et al., 1997). At least one more kinase is likely to participate in the pathway, since Fu is not solely responsible for phosphorylation of Cos-2 in response to Hh (Robbins et al., 1997). The similarity between Cos-2 and kinesins suggests that Hh signaling involves the cytoskeleton. Kinesins are molecular motors that carry vesicles or chromosomes along microtubules (Goldstein, 1993). It is not known whether Cos-2 has motor activity, but it binds tightly to microtubules (Sisson et al., 1997). Cos-2 may regulate the subcellular localization of other proteins in the pathway.

The inadequately understood events in the cytoplasm eventually affect the nucleus via the transcription factor Cubitus interruptus (Ci). *ci* is related to the human oncogene *GLI* (sometimes called *GLI1*), which encodes a transcription factor (Orenic et al., 1990). Ci can activate transcription in vitro and is likely to bind DNA in vivo (Alexandre et al., 1996; Hepker et al., 1997; Von Ohlen

and Hooper, 1997; Von Ohlen et al., 1997). Interestingly, Ci can both activate and repress transcription (Hooper, 1994; Motzny and Holmgren, 1995; Alexandre et al., 1996; Domínguez et al., 1996; Hepker et al., 1997). As an activator, Ci works with a general activating cofactor, dCBP, the *Drosophila* homolog of CREB binding protein (Akimaru et al., 1997). Despite genetic evidence that Ci activates transcription, the molecular form of Ci responsible for this function has not been identified.

The repressor form of Ci is generated by Hh-regulated proteolysis (Aza-Blanc et al., 1997). Two stable forms of Ci have been described: a cytoplasmic full-length protein (Ci155) and a nuclear N-terminal fragment (Ci75). An unidentified protease cleaves Ci155, removing a cytoplasmic tether in the C terminus and releasing Ci75. Ci75 directly represses transcription of *hh* (Domínguez et al., 1996; Aza-Blanc et al., 1997), providing another point of feedback in the pathway. Hh signaling prevents proteolysis of Ci, while Ptc seems to drive proteolysis and therefore production of Ci75 (Chen and Struhl, 1996; Domínguez et al., 1996; Hepker et al., 1997). Full-length Ci accumulates close to Hh sources, while the repressor form is produced where Ptc protein is at low levels but active. In flies, Hh and Ptc have no effect on *ci* transcription (Schuske et al., 1994; Johnson et al., 1995; Aza-Blanc et al., 1997), apparently exerting their effects purely at the posttranscriptional level. One possibility is that the WD40-repeat protein Slimb (Slmb) (Jiang and Struhl, 1998) targets Ci for ubiquitin-mediated processing.

Although most features of the Hh pathway are conserved, *Gli* genes are regulated differently than their homolog *ci*. There are at least three *Gli* family members: *Gli1*, *Gli2*, and *Gli3* (Kinzler et al., 1987, 1988; Ruppert et al., 1988, 1990; Walterhouse et al., 1993; Hui et al., 1994). Like *ci*, *Gli1* is expressed in Hh-responsive cells and activates Hh target gene transcription, probably directly (Marigo et al., 1996b; Hynes et al., 1997; Lee et al., 1997; Platt et al., 1997; Sasaki et al., 1997; Yoon et al., 1998). Vertebrate Hh signals induce *Gli* expression in contrast to fly *ci*, which is not transcriptionally regulated by Hh signals (Vortkamp et al., 1996; Hynes et al., 1997; Lee et al., 1997; Sasaki et al., 1997; Ruiz i Altaba, 1998). An intriguing question is whether Gli proteins, like Ci, are processed posttranslationally. One possibility is that the activating and repressing functions of Ci are served by distinct Gli proteins (Marigo et al., 1996b). Indeed, *Gli3* appears to act as a repressor of *Shh* itself and of Shh target gene transcription (Buscher et al., 1997; Sasaki et al., 1997; Buscher and Ruther, 1998; Ruiz i Altaba, 1998), just as Ci75 prevents transcription of *hh* and *ptc* in flies.

Studies in the vertebrate neural tube suggest that Shh signaling determines diverse cell fates by regulating the combination of *Gli* genes expressed in a cell. Shh induces *Gli1* expression in the floor plate, probably by preventing Ptc activity since *Gli1* is ectopically expressed in *ptc* mutants (Goodrich et al., 1997; Hynes et al., 1997). In addition, *Gli1* but not *Gli3* induces transcription of floor plate marker genes (Hynes et al., 1997; Lee et al., 1997). Gli3 inhibits floor plate formation and *Shh* expression (Sasaki et al., 1997; Ruiz i Altaba, 1998). Conversely, Shh inhibits *Gli3* expression (Ruiz i Altaba, 1998). Shh regulation of *Gli* genes might establish negative feedback that limits where floor plate cells differentiate (Ruiz i Altaba, 1998). However, since Gli proteins appear to regulate expression of *Shh* itself, it is difficult to interpret results from experiments manipulating the expression of either Gli proteins or Shh. For example, *Gli2* can induce motor neurons in frogs, but this effect could be direct or due to induction of *Shh* (Ruiz i Altaba, 1998). Although *Gli2* is required for floor plate formation in mice, ectopic expression of *Gli2* inhibits floor plate development in frogs (Ding et al., 1998; Matise et al., 1998; Ruiz i Altaba, 1998).

Current data are consistent with a model in which Shh acts through a Gli "code" of cell fates, but both fly and vertebrate Hh proteins appear to act through transcription factors in addition to Ci/Gli. In a cell culture assay, Hh induces binding of unidentified nuclear proteins to a vertebrate Hh response element (Krishnan et al., 1997); these proteins do not appear to be Gli proteins. Similarly, in flies, *ptc*-mediated repression of *wg* can be mediated through a regulatory element that does not contain Ci binding sites (Lessing and Nusse, 1998).

Fused, Costal-2, and Ci Form a Complex that Binds to Microtubules

In flies, regulation of Ci proteolysis and nuclear import probably occurs in or near a 500–700 kDa protein complex that also includes Costal-2 and Fused. Both Ci155 and Ci75 associate with Fu and Cos-2 (Aza-Blanc et al., 1997; Robbins et al., 1997; Sisson et al., 1997). Consistent with the presence of Cos-2, the complex binds to microtubules and is released from microtubules in response to Hh signaling (Robbins et al., 1997). However, Cos-2 does not appear to be directly responsible for retention of Ci since Ci stays primarily cytoplasmic in *cos-2* mutant cells (Sisson et al., 1997). Cos-2 probably binds to the C-terminal regulatory domain of Fu (Thérond et al., 1996a; Robbins et al., 1997; Monnier et al., 1998). It is not yet known if a similar complex exists in vertebrates; homologs of Fu and Cos-2 have not been described.

Protein Kinase A Affects Hh Signaling at Multiple Levels

One potential regulator of the complex is the cAMP-activated protein kinase (PKA). PKA is a serine–threonine kinase that is activated by increased levels of cAMP (Taylor et al., 1990). Like Ptc and Cos-2, PKA is a negative regulator of Hh target gene transcription (Jiang and Struhl, 1995; Lepage et al., 1995; Li et al., 1995; Pan and Rubin, 1995; Ohlmeyer and Kalderon, 1997). PKA is also involved in vertebrate Hh signaling (Fan et al., 1995; Hynes et al., 1995a, 1995b; Concordet et al., 1996; Epstein et al., 1996; Hammerschmidt et al., 1996; Ungar and Moon, 1996).

Although it is tempting to place PKA directly downstream of the seven-transmembrane receptor Smo, genetic analysis suggests instead that PKA acts in a parallel pathway. Ptc does not regulate PKA activity, and constitutively active PKA cannot rescue *ptc* mutations, as might be expected in a linear pathway (Jiang and Struhl, 1995; Li et al., 1995). Surprisingly, constitutively

active PKA is able to rescue *PKA* mutants, indirectly suggesting that regulation by cAMP is not important for PKA function in this pathway (Li et al., 1995; Ohlmeyer and Kalderon, 1997). PKA appears to be a basal inhibitor of Hh target gene transcription. In vertebrates, Hh signaling correlates with activation of a PP2A-like serine–threonine phosphatase, so Hh might counteract basal PKA activity indirectly by increasing phosphatase activity (Krishnan et al., 1997). Cos-2, Smo, and Ci all have consensus sites for PKA phosphorylation (Alcedo et al., 1996; Robbins et al., 1997; Sisson et al., 1997; Chen et al., 1998). When the sites in Ci are mutated, Ci is no longer proteolytically cleaved and has increased transcriptional activity, suggesting that, in vivo, PKA phosphorylation leads to proteolysis of Ci (Chen et al., 1998). It is not known whether Cos-2 or Smo is also regulated by PKA. Analysis of PKA function in flies and in tissue culture has revealed at least two genetically independent roles for PKA, in keeping with multiple roles for PKA in the Hh–Ptc pathway (Ohlmeyer and Kalderon, 1997; Chen et al., 1998).

Probable Signaling Components, with Unknown Functions

Two additional genes appear to be involved in Hh signaling in flies, but their functions in the pathway are incompletely understood. One such gene is *oroshigane* (*oro*) (Epps et al., 1997). Ten to fifteen percent of *oro* mutant embryos have *hh*-like cuticle phenotypes. Genetic epistasis experiments place *oro* upstream of *ptc*, raising the possibility that *oro* encodes an additional ligand for Ptc or Smo. The molecular basis of *oro* mutations is unknown. Another gene found in flies, *Suppressor of fused* or *Su(fu)*, interacts strongly with mutant forms of Fused but is not required for normal Hh signaling. *Su(fu)* encodes a novel protein (Pham et al., 1995). Mutations in *Su(fu)* suppress some types of *fu* mutations while interacting with others to create novel phenotypes (Préat et al., 1993; Pham et al., 1995; Thérond et al., 1996a). A biochemical function for Su(fu) may be to sequester Ci in the Fu–Ci–Cos-2 complex (Monnier et al., 1998). Null alleles of *Su(fu)* are completely viable and exhibit no obvious phenotype, suggesting that *Su(fu)* is not an essential component of the complex.

Transduction of the Hh Signal: Clues from NPC1?

A long standing puzzle in Hh signal transduction is the molecular mechanism of Ptc protein function, and the recent discovery of the relationship between *ptc* and the Niemann-Pick type C disease gene *NPC1* has provided some unexpected clues (Carstea et al., 1997). Overall, mouse Ptc is 23% identical and 50% similar to mouse NPC1 (Loftus et al., 1997). One particularly well-conserved region (32% identical, 63% similar) is also related to the sterol-sensing domains that are found in the cholesterol homeostasis enzymes SREBP cleavage–activating protein (SCAP) and HMG CoA reductase (Chin et al., 1984; Hua et al., 1996). In addition to the proposed sterol-sensing domain, Ptc and NPC1 share extensive homology through putative transmembrane domains eight to twelve (35% identical, 62% similar), but this region does not correspond to any known functional domain. The sequence similarities suggest that NPC1 may have a biochemical function related to that of Ptc. Another Ptc-related protein, TRC8, has similarity both to the second putative extracellular loop of Ptc and to the proposed sterol-sensing domain (Gemmill et al., 1998). TRC8 has been implicated in renal cell carcinoma; its normal function is unknown. The cellular pathology of Niemann-Pick type C disease, in contrast, is well described.

Niemann-Pick type C disease is a progressive neurological disease caused by defects in lipid transport. Beginning in childhood, patients display ataxia and other psychological and motor defects that worsen with time, culminating in death in the teenage years (Pentchev et al., 1995). The neurologic phenotype is caused by abnormal trafficking of cholesterol and other lipids that leads to a dangerous accumulation of lipids in neurons and other cells (Blanchette-Mackie et al., 1988; Coxey et al., 1993; Neufeld et al., 1996). Normally, esterified cholesterol is carried in low-density lipoprotein particles that are endocytosed and brought to the lysosome for deesterification. Free cholesterol is either reesterified in the cytoplasm for storage or sent to other cellular compartments. These transport events are not fully understood, but some steps require *NPC1* function. In *NPC1* mutant fibroblasts, unesterified cholesterol accumulates in lysosomes (Neufeld et al., 1996). Since the enzymes for esterification remain functional in *NPC1* mutant cells (Liscum and Faust, 1987), the buildup of free cholesterol instead seems to be due to an inability to move it from the lysosome to the esterification machinery in the cytoplasm.

If Ptc and NPC1 have any similar biochemical activities, then Ptc might also have a role in vesicle transport. This hypothesis is supported not only by the sequence similarity with NPC1 but also by two other features of the Hh pathway. First, most Ptc protein resides not at the plasma membrane but instead in intracellular vesicles (Forbes et al., 1993; Taylor et al., 1993; Capdevila et al., 1994a; Tabata and Kornberg, 1994). In Hh responding cells, Hh colocalizes with Ptc here (Tabata and Kornberg, 1994). Second, Cos-2 is related to kinesins and binds microtubules, consistent with the idea that vesicles could be important in the pathway (Sisson et al., 1997). Taken together, these characteristics suggest that Ptc-bearing vesicles might play a role in transducing the Hh signal. Conversely, the similarities between NPC1 and Ptc also suggest that NPC1 vesicle movement may be regulated by an unidentified ligand. Discoveries in one pathway may stimulate important experiments in the other.

The presence of a putative sterol-sensing domain in Ptc raises the possibility that detection of sterols serves an important purpose in Hh signaling. There is considerable evidence that cholesterol is important in the pathway. First, Hh-N has a cholesterol moiety (Porter et al., 1996a, 1996b). Second, insufficient amounts of cholesterol during embryogenesis cause phenotypes similar to what is caused by *Shh* mutations. For example, some patients affected by Smith–Lemli–Opitz (SLO) syndrome, which is caused by a defect in cholesterol synthesis, have symptoms similar to holoprosencephaly (HPE), a malformation of the nervous system that is

caused by *Shh* mutations (Belloni et al., 1996; Roessler et al., 1996). HPE and SLO-like phenotypes also arise in rats treated with cholesterol synthesis inhibitors (Kolf-Clauw et al., 1997). Cyclopia and other neural tube defects appear in sheep and cow fetuses whose mothers ingest certain plants during gestation (Keeler, 1975). The teratogens produced by these plants, cyclopamine and jervine, are cholesterol-related compounds (Keeler, 1970) that block Shh induction of target genes in neural tube explants (Cooper et al., 1998; Incardona et al., 1998). The inhibitory action of the teratogens is not due to any alteration to the cholesterol link formed during Shh biosynthesis. Given their structural similarity to cholesterol, cyclopamine and jervine might occupy a cholesterol binding site and stimulate Ptc activity (Cooper et al., 1998; Incardona et al., 1998).

The Hh Pathway and Neural Diseases

One of the most gratifying discoveries of the past decade is that many of the genes originally identified in model organisms are involved in human development and disease. Ptc and Shh are central players in two inherited human developmental disorders and in cancer. Each of these human phenotypes further confirms what has been learned about Hh signaling in invertebrate and vertebrate model systems.

Holoprosencephaly

Confirmation of the importance of *Shh* in human development came with the discovery that mutations in *Shh* are one cause of holoprosencephaly (HPE) (Belloni et al., 1996; Roessler et al., 1996). HPE is a developmental disorder that affects the midline of the nervous system and face, with a wide range of defects such as a single central incisor or nostril, mild fusion of the ventricles of the brain, or complete cyclopia. The incidence of HPE in live births is about 1 in 16,000 (Fitz, 1994; Rasmussen et al., 1996; Olsen et al., 1997; Ming and Muenke, 1998). Survival varies depending on the severity of the defects and whether other organ systems are also affected (Ming and Muenke, 1998). The CNS defects appear to arise due to an incomplete division of the initial neural field during development, but it is not known why cleavage is impaired. Similar defects occur in mice lacking *Shh* function, confirming that Shh is essential for this process (Chiang et al., 1996). Based on what is known about Shh activity, it is likely that HPE is caused by insufficient floor plate or ventral neuron development, but it remains unclear whether these tissues are missing due to insufficient proliferation of progenitors, increased cell death, or respecification to more dorsal cell fates. Trisomy 13 and several other genetic loci (21q, 2p21, and 18p) are also associated with HPE in humans and could lead to the identification of additional components of the Hh pathway or of other pathways important for neural development. HPE does not arise in mice heterozygous for *Shh*, possibly due to the presence of suppressors in that inbred line.

Basal Cell Nevus Syndrome

Mutations in the human *PTC* gene are associated with the dominant disorder basal cell nevus syndrome (BCNS; also Gorlin's syndrome or nevoid basal cell carcinoma syndrome) (Hahn et al., 1996b; Johnson et al., 1996). BCNS patients display a wide range of phenotypes including general overgrowth, polydactyly, and fused or bifid ribs (Gorlin, 1987; Kimonis et al., 1997). These phenotypes appear to be due to haploinsufficiency of *PTC*, emphasizing the importance of a balance between Hh and Ptc activities. *PTC* mutations have been identified in 30%–40% of BCNS patients examined (Gailani et al., 1996); the difficulty of mutation detection means the real number is probably higher. Most *PTC* mutations result in truncation of the protein and are probably null, but nevertheless phenotypes vary in severity among BCNS patients. Even patients with identical molecular lesions can have very different manifestations of the disease, suggesting that other genes modify the BCNS phenotype (Wicking et al., 1997). Identification of these putative genetic enhancers and suppressors might reveal novel components of the proposed pathway. Neural defects arise occasionally in BCNS patients and include agenesis of the corpus callosum, asymmetry of the ventricles, and mental retardation (Gorlin, 1987; Kimonis et al., 1997), but the developmental basis of these abnormalities is not known. In mice homozygous for *ptc* mutations, the forebrain is severely deformed, the hindbrain is overgrown, and the neural tube fails to close completely (Goodrich et al., 1997). Mice heterozygous for *ptc*, and therefore providing a parallel to human BCNS, display several phenotypes reminiscent of BCNS, including large body size and polydactyly (Goodrich et al., 1997; Hahn et al., 1998). Both BCNS patients and *ptc* heterozygous mice have a greatly increased incidence of the brain tumor medulloblastoma (Gorlin, 1987; Evans et al., 1991; Goodrich et al., 1997; Kimonis et al., 1997).

Medulloblastoma

Medulloblastoma is a deadly tumor of the cerebellum that is responsible for about 20% of all childhood brain tumors (Novakovic, 1994; reviewed by Peringa et al., 1995; Rorke et al., 1997). Medulloblastomas are rare in the normal population, but are found in about 3% of people with BCNS (Lacombe et al., 1990; Evans et al., 1991; Kimonis et al., 1997). Medulloblastoma is dangerous both because of its location near the brain stem and because it may metastasize (Molenaar and Trojanowski, 1994). Classic medulloblastomas are generally found along the midline, deep in the cerebellum, while desmoplastic medulloblastomas are lateral and superficial (reviewed by Katsetos and Burger, 1994). Although many of the tumor cells resemble undifferentiated neuroepithelium, others show varying signs of differentiation and express neuronal antigens. Aggressive treatment of medulloblastomas with combined surgery, radiation, and chemotherapy allows 67%–83% of patients to survive at least 5 years (Packer et al., 1994). Patients with recurrent cases of medulloblastoma fare much worse, with a median survival time of only 5 months (Bouffet et al., 1998). Treatments based upon more complete understanding of the biology of these tumors might improve the prognosis.

A number of mutations in the human homolog of *PTC* have been found in both classic and desmoplastic medulloblastoma, and one mutation was detected in a cerebral tumor (Pietsch et al., 1997; Raffel et al., 1997; Wolter et al., 1997; Xie et al., 1997). About 15% of all primitive neuroectodermal tumors examined have *PTC* mutations, often accompanied by the deletion of the second

allele, suggesting that a loss of Ptc function contributes to the formation of tumors. Consistent with this interpretation, both the *ptc* and *Gli1* genes are consistently highly transcribed in medulloblastomas (Wolter et al., 1997; Reifenberger et al., 1998).

Studies of *ptc* mutant mice have provided useful information about medulloblastoma. About 30% of *ptc* heterozygous mice develop medulloblastoma (Goodrich et al., 1997); other kinds of neuroectodermal tumors have not yet been investigated. As in human medulloblastoma, both *ptc* and *Gli* are ectopically expressed in the tumor cells. *ptc* is also derepressed in possible precancerous cells, before an overt tumor forms, on the surface of the cerebellum in about 50% of heterozygotes. These cells may be remnants of the external germinal layer (EGL), a transient population of rapidly dividing cells on the surface of the cerebellum (Hatten and Heintz, 1995). Normally, EGL cells migrate into the cerebellum and differentiate as granule cells. Medulloblastoma tumor cells resemble granule cells and express several genes normally transcribed in the EGL (Kozmik et al., 1995). Medulloblastomas may therefore arise from misregulation of Hh–Ptc signaling in cells of the EGL.

The involvement of *ptc* in medulloblastoma suggests a possible link between Hedgehog signaling and normal cerebellar development. During the main growth phase of cerebellar development, both *ptc* and *Gli1* are transcribed in the EGL, and *Shh* is transcribed in Purkinje cells, deeper in the cerebellum. Cell ablations have demonstrated the importance of Purkinje cells for proliferation of EGL cells (Smeyne et al., 1995). Addition of Shh protein to quiescent EGL cells in vitro causes sustained proliferation and blocks differentiation, suggesting that growth of the granule cell precursors of the cerebellum is governed by a Shh signal from the Purkinje cells (Wechsler-Reya and Scott, 1999, submitted; N. Dahmane and A. Ruiz i Altaba, submitted). Conversely, cells producing an antibody that inactivates Shh function, injected into the brain, reduce the size of the EGL. The stimulation of growth by Shh is consistent with the loss of growth control in medulloblastoma due to reduced *ptc* function. Regulation of proliferation by Shh in the cerebellum contrasts with the well-characterized cell fate control in the neural tube at earlier stages.

Other Tumor Types

In addition to medulloblastoma, *PTC* appears to be involved in a number of other, more common types of cancer. One of the defining features of BCNS is an unusually high number of basal cell carcinomas (BCC) of the skin, the tumors for which the syndrome was named (Gorlin, 1987; Kimonis et al., 1997). *PTC* mutations have also been reported in sporadic BCC, the most common kind of human cancer (Gailani et al., 1996; Hahn et al., 1996b; Johnson et al., 1996). Consistent with the current model for Hh signaling, ectopic expression of *Shh* or *Gli1* causes tumors resembling BCCs in animal models, apparently mimicking a loss of Ptc function (Dahmane et al., 1997; Fan et al., 1997; Oro et al., 1997). Similarly, activating mutations in a vertebrate *Smo* gene have been described in BCCs (Xie et al., 1998), supporting the idea that Ptc and Smo have opposing activities in humans as in flies. People with BCNS and mouse *ptc* heterozygotes are also at increased risk for the muscle tumor rhabdomyosarcoma (Gorlin, 1987; Hahn et al., 1998). As in medulloblastoma, loss of Ptc function leads to a derepression of both *PTC* and *GLI* transcription in BCCs (Gailani et al., 1996; Dahmane et al., 1997; Unden et al., 1997) and rhabdomyosarcoma (Hahn et al., 1998). *GLI* was originally described as an oncogene that is amplified in human gliomas (Kinzler et al., 1987), but a role for Hh–Ptc signaling in this tumor type has not been described. The involvement of the Hh pathway in multiple kinds of cancer raises a new set of questions, including how the pathway ultimately affects cell division and whether different kinds of tumor cells are transformed via a common series of events.

The Hh pathway exemplifies the growing realization that basic research into the developmental biology of model organisms such as the fly directly enrich our understanding of human development and disease. We expect many more surprises and insights as studies of this important signal transduction pathway go forward.

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Note Added in Proof

The data referred to throughout as ''Wechsler-Reya and Scott, 1999, submitted'' are now in press: Wechsler-Reya, R., and Scott, M.P. (1999). Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. Neuron, in press.