Control of Cell Growth and Fate by patched Genes

R.L. JOHNSON AND M.P. SCOTT

Howard Hughes Medical Institute, Departments of Developmental Biology and Genetics, Stanford University
School of Medicine, Stanford, California 94305-6427

When the Hedgehog (Hh) protein signals to a cell bearing the Patched (Ptc) receptor, events are triggered which can drastically affect the cell's fate and growth properties. Ptc exerts its profound effects by inhibiting the transcription of a number of different genes, such as members of the Wnt and transforming growth factor-β (TGF-β) families and the ptc gene itself. Hh opposes Ptc activity to induce gene transcription; cells receiving the Hh signal respond by inducing high levels of ptc (Fig. 1) and other transcriptional targets. The Hh/Ptc signaling system, originally identified in Drosophila, is now known to be broadly conserved in the animal kingdom (for review, see Hammerschmidt et al. 1997). The involvement of ptc mutations in different human tumor types demonstrates an important additional role for Hh and Ptc in the control of cell proliferation in adult tissues. Understanding how Hh and Ptc regulate gene expression will have substantial implications for comprehending animal development and tumorigenesis.

Ptc TRANSCRIPTION AS AN INDICATOR OF Hh SIGNALING DURING DEVELOPMENT

The outcome of Hh signaling is the transcriptional activation of specific target genes, and the target genes can vary with tissue and developmental stage. For example, in Drosophila, wingless (wg), a homolog of the vertebrate Wnt family (Rijsewijk et al. 1987), is activated by Hh in embryos and some imaginal discs (Hidalgo and Ingham 1990; Basler and Struhl 1994), whereas the TGF-β class gene decapentaplegic (dpp) (Padgett et al. 1987) is activated by Hh only in imaginal discs (Basler and Struhl 1994; Kojima et al. 1994; Tabata and Kornberg 1994; Felsenfeld and Kemison 1995; Ingham and Fietz 1995). Although the genes controlled by Hh vary from tissue to tissue, one common target of Hh regulation is the ptc gene itself (Hidalgo and Ingham 1990). When Ptc is inhibiting target gene transcription, it keeps its own transcript levels low. In the presence of Hh ligand, ptc transcripts are elevated (Hidalgo and Ingham 1990; Ingham et al. 1991). Expression of Hh in areas where it is normally not present results in increases in ptc levels (Basler and Struhl 1994; Kojima et al. 1994; Tabata and Kornberg 1994; Ingham and Fietz 1995). Hence, cells that receive the Hh signal respond by raising ptc expression.

Why should a ligand induce higher-level expression of a receptor while at the same time opposing the receptor's function? One possibility is that the buildup of Ptc leads to feedback damping of Hh signaling, limiting the duration of the effective signal. In keeping with this idea, increased production of Ptc overcomes normal Hh functions and turns off target genes where they should be activated (Schuske et al. 1994; Johnson et al. 1995). Another possibility is that the range of action of the Hh signal is restricted by binding and sequestration of Hh by Ptc, an idea supported by the increased range of Hh protein (Taylor et al. 1993) and Hh response (Chen and Struhl 1996) when cells lack Ptc. Whatever the function of Ptc induction, it is perhaps the most reliable reporter to date of Hh signal received.

High-level ptc transcription may provide a measure of how far Hh moves from its source. ptc induction appears to be due uniquely to Hh and is detected adjacent to all sources of Hh proteins reported to date. ptc expression patterns in Drosophila and vertebrates suggest that Hh can act over a broad range. In the fly embryo, Hh emanates from the posterior cells of each segment primordium and acts upon more anterior cells to maintain the expression of wg (Ingham et al. 1991). The range of normal Hh action in fly embryos appears to be about two cells, but expanded Hh expression readily induces wg transcription in additional anterior cells (Ingham 1993; Chang et al. 1994). In vertebrates, the Hh homolog Sonic Hedgehog (Shh), appears to have both short- and long-range actions. In the developing neural tube, Shh is first produced in the notochord to induce ptc in the adjacent floor plate. Here, Shh range is short, as indicated by ptc transcription (Goodrich et al. 1996; Hahn et al. 1996a; Marigo and Tabin 1996) and by the contact dependence of the notochord-derived signal (Placzek et al. 1999). Later, the floor plate itself becomes a source of Shh to induce motor neurons further away in the ventral lateral regions (Echelard et al. 1993; Roelink et al. 1994). At this time, ptc expression is absent from the floor plate but is highly elevated in the ventral neural tube where it appears as a gradient reaching far toward the dorsal neural tube (Goodrich et al. 1996; Hahn et al. 1996a; Marigo and Tabin 1996). The induction of ptc over a broad area of the neural tube suggests that Shh is acting over many cell diameters, a hypothesis supported by tissue culture experiments (Roelink et al. 1995; Ericson et al. 1996).
known as Gorlin or nevoid basal cell carcinoma syndrome) carry mutations in PATCHED (PTC), a human ptc homolog (Hahn et al. 1996b; Johnson et al. 1996). The developmental defects in these individuals correlate well with the developing tissues known from mouse studies to employ Hh/Ptc signaling. For example, mouse ptc is expressed prominently in the developing limb, neural tube, and branchial arches (Goodrich et al. 1996; Hahn et al. 1996a), and BCNS patients can manifest polydactyly, spinal bifida, and abnormal craniofacial development (Gorlin 1995; Bale 1997). BCNS patients also have a high incidence of certain tumors, most notably basal cell carcinoma (BCC) of the skin and medulloblastoma, a dangerous tumor most commonly observed in the cerebellum. Most BCNS patients develop large numbers of BCCs by age 30 (Kimonis et al. 1997), whereas about 3% acquire medulloblastoma as young children (Lacombe et al. 1990; Evans et al. 1991).

More often, BCCs and medulloblastomas occur sporadically in normal individuals. BCC is a very common cancer in people of northern European descent where the tumors arise in small numbers late in life (Miller 1991; Miller and Weinstock 1994). Both inherited and sporadic forms of BCC arise from mutations in a tumor suppressor locus on chromosome 9q (Gaillani et al. 1992). BCNS individuals were predicted to inherit one normal and one defective copy of this gene, and BCCs were proposed to arise by mutation of both copies (Gaillani et al. 1992; Chevreux-Trench et al. 1993; Bonifas et al. 1994; Compton et al. 1994; Goldstein et al. 1994a; Wicking et al. 1994). These predictions were borne out upon the identification of PTC mutations in BCNS patients and in sporadic BCCs (Hahn et al. 1996b; Johnson et al. 1996). In many sporadic and inherited BCCs, both alleles of PTC are mutated, suggesting that tumors arise from homozygous loss of PTC function (Gaillani et al. 1996; Hahn et al. 1996b; Unden et al. 1996). In Drosophila, one indicator of Ptc activity is the negative regulation of its own transcription—the loss of Ptc function leads to the accumulation of high levels of ptc mRNA (Hidalgo and Ingham 1990). In BCNS BCCs as well, transcripts of PTC accumulate to high levels, implying that Ptc function is greatly reduced in the tumor cells and that Ptc autoregulation is conserved between flies and vertebrates (Gaillani et al. 1996). PTC mutations have been found in sporadic medulloblastomas, and, as in BCCs, both alleles appear to be damaged (Piotsh et al. 1997; Raffel et al. 1997; Xie et al. 1997). This suggests that loss of PTC function leads to the formation of several different types of tumors.

The tumor-suppressing properties of Ptc correlate with its role in Drosophila wing development and its inferred role in vertebrate limb formation. In both of these tissues, ectopic Hh (Basler and Struhl 1994; Kojima et al. 1994; Tabata and Kornberg 1994; Felsenfeld and Kennison 1995; Ingham and Fietz 1995) or Shh (Riddle et al. 1993; Chung et al. 1994) signaling can induce rapid and dramatic proliferation of cells. Correspondingly, the loss of Ptc function in the wing imaginal disc also leads to overgrowth (Capdevila et al. 1994). However, the absence of

**Figure 1.** ptc expression in Drosophila and mouse. (Top panel) ptc LacZ expression in a Drosophila embryo. During late embryogenesis, ptc is expressed in all cells that lack the transcription factor, Engrailed. The bright pin stripes of ptc staining indicate cells where Ptc activity has been blocked by the adjacent sources of Hh expression. Low ptc staining in each segment indicates cells outside the range of Hh where Ptc is actively inhibiting its own transcription. (Lower panel) ptc mRNA expression in a mouse at about 13.5 days of development. High levels of ptc expression are detected in the developing limb bud and hair follicles adjacent to sources of Hh and Shh, respectively. Elevated levels of ptc suggest that Ptc-negative autoregulation is inhibited by Hh family members.

**ROLE OF PTC IN TUMOR SUPPRESSION**

The first evidence implicating Ptc function in tumorigenesis was the discovery that individuals with the inherited disorder, basal cell nevus syndrome (BCNS; also...
Ptc activity does not always result in cell proliferation. In homozygous Drosophila ptc-embryos, cell fates and wg transcription are altered, but there is no excess growth (Hidalgo and Ingham 1990). Why cells respond differently to the Hh signal is not well understood. Possibly the cell's history or its neighbors influence the ways it interprets the signal. Understanding the link between cell differentiation and division, and how Hh influences these processes, will be critical for understanding how BCCs and medulloblastomas form.

**SURPRISING COMPONENTS IN THE Hh SIGNALING PATHWAY**

The membrane proteins, Ptc and Smoothened (Smo), are important in regulating the detection of Hh at the plasma membrane (Fig. 2). Ptc contains multiple transmembrane domains (Hooper and Scott 1989; Nakano et al. 1989) and constitutively inhibits activation of the pathway (Ingham et al. 1991). Ptc is thought to mediate his repressive effects by blocking the activity of Smo. Smo has seven potential transmembrane domains, is genetically downstream from Ptc, and is required to activate the pathway (Alcedo et al. 1996; van den Heuvel and Ingham 1996). Biochemical studies in vertebrate systems have shown that Shh binds to Ptc but not Smo (Marigo et al. 1996b; Stone et al. 1996). In addition, Ptc and Smo associate with one another in the membrane (Stone et al. 1996). These results suggest that in the absence of Hh, Ptc associates with and prevents Smo from activating the pathway. The binding of Hh to Ptc somehow blocks Ptc inhibition of Smo (although Ptc and Smo appear to remain associated) and permits activation of gene transcription.

Ptc, Hh, and Smo might have activities other than signaling in this pathway. In the embryonic neuroectoderm of Drosophila, Ptc inhibition of gene expression does not appear to be blocked by Hh but by an unidentified factor (Bhat 1996; Bhat and Scheld 1997). In the Drosophila epidermis, Hh has Ptc-independent influences on pattern (Hejsovec and Wieschaus 1993; Bokor and DrNdaro 1996). Hence, Ptc may bind other ligands and Hh may have other receptors. Smo has an extracellular cysteine-rich domain that shares sequence similarity to the Wnt-binding region of the serpine Frizzled receptors (Alcedo et al. 1996; van den Heuvel and Ingham 1996). This raises the possibility that Smo may bind a Wnt ligand and integrate Wnt and Hh signaling. Although Smo has some sequence hallmarks of a G-protein-coupled receptor (Alcedo et al. 1996; van den Heuvel and Ingham 1996), there is yet no biochemical or genetic evidence implicating heterotrimeric G-proteins in this pathway.

In Drosophila, a large cytoplasmic complex has been implicated in regulating the Hh signal to the nucleus (Azia-Blanc et al. 1997; Robbins et al. 1997; Sisson et al. 1997). Three proteins in this complex have been identified: the serine/threonine kinase, Fused (Fu) (Preat et al. 1990); the zinc finger protein, Cubitus interruptus (Ci) (Orenic et al. 1990); and the kinesin-related protein, Costal2 (Cos2) (Sisson et al. 1997). Fu and Ci are required for transmitting the Hh signal, whereas Cos2, like Ptc, negatively regulates the pathway (Forbes et al. 1993). Ci has homology with the Gli family of transcription factors in vertebrates (Orenic et al. 1990) and is proposed to control directly the transcription of Hh target genes (Alexandre et al. 1996; Dominguez et al. 1995; Aza-Blanc et al. 1997; Hecker et al. 1997; von Ohlen et al. 1997). Cos2, which binds to microtubules in vitro, may inhibit Hh signaling by tethering the complex to the cytoskeleton and controlling the entry of Ci into the nucleus (Axia-Blanc et al. 1997; Robbins et al. 1996; Sisson et al. 1997). How this complex is regulated is not known, but phosphorylation may be important since Cos2 and Fu become phosphorylated in response to Hh (Pinter et al. 1996; Robbins et al. 1997).

Protein kinase A (PKA) (Jiang and Stuhl 1995; Li et al. 1995; Pan and Rubin 1995) and Suppressor of fused (SuFu) (Preat et al. 1993; Pham et al. 1995) act in a manner similar to that of Ptc by being negative regulators of Hh signaling. Whereas PKA functions downstream from smo (van den Heuvel and Ingham 1996), it has not been placed in a linear pathway with Ptc because activated PKA cannot replace Ptc function. Identifying the relevant targets of PKA phosphorylation will help determine if PKA is in a parallel pathway or different.

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**Figure 2.** The Hh signaling pathway. A view of the pathway assembled from Drosophila and vertebrate studies. Hh is proposed to bind to Ptc and block its activity, thereby relieving Ptc inhibition of Smo. Smo transmits the signal to the cytosol in an unknown manner to either activate Fu or inhibit Cos2. Cos2, Fu, and Ci are part of a complex that regulates the entry of Ci into the nucleus. In the nucleus, Ci binds to upstream sequences of select genes to activate or repress transcription. PKA and SuFu block Hh signaling in an unknown manner.
ent branch of the pathway, SuFu appears to be a nonessential novel protein, yet reduction of SuFu function in the absence of Fu can restore normal development (Preat et al. 1993; Pham et al. 1995). The loss of Fu kinase activity is apparently tolerable if SuFu function is also reduced. The effects of some Fu alleles are dramatically altered, and are in fact worsened, by reduced SuFu function. These alleles generally map to the potential regulatory domain of Fu, leaving the kinase domain possibly still active. Similarly, reduction Cos2 function suppresses or enhances Fu alleles with the same allele specificity as that of SuFu (Preat et al. 1993; Therond et al. 1996b). The molecular events underlying these remarkable genetic interactions are probably related to the Cos2/Fu/Ci protein complex but are not understood.

Most of the information about Hh signal transduction has come from studies in Drosophila, but the expected parallels in vertebrate development are emerging quickly. In mice, the Hh family members are Shh, Desert hedgehog, and Indian hedgehog (Ihh) (Echelard et al. 1993). The extent to which each protein sequence confers functional differences is unclear, but each is produced in different cells and has distinct developmental functions. Cells adjacent to the sources of all three Hh proteins activate ptc transcription, which suggests that Ptc may be used by all three Hh members. However, the identification of a second ptc gene in vertebrates (Concordet et al. 1996; Takabayake et al. 1997) could imply the specialization of different Ptc proteins for different Hh members or the existence of different signal transduction events. As in Drosophila, vertebrate PkA has been implicated in the repression of Shh target genes (Concordet et al. 1996; Epstein et al. 1996; Hammerschmidt et al. 1996) and Gli appears to be an activator of Shh targets (Marigo et al. 1996a; Sasaki et al. 1997). However, in contrast to flies, where Ci is not transcriptionally regulated by Hh (Motzny and Holmgren 1995; Slusarski et al. 1995), Gli transcription is induced by Shh (Marigo et al. 1996a; Sasaki et al. 1997). Vertebrate relatives of Fu, Cos2, and SuFu have not yet been identified.

Aside from PTC, can mutations in other genes in the Hh pathway also cause BCC? When Shh is overexpressed under the control of a keratin promoter in the epidermis of developing mice, tumors similar to BCCs are seen (Oro et al. 1997). Presumably, excess production of Shh binds to and inactivates Ptc, mimicking genetic loss of PTC in the skin. In addition, potential gain-of-function mutations in human Shh have been found in three types of sporadic tumors which raises the possibility that Shh is a oncogene (Oro et al. 1997). This evidence argues that activation of Shh target genes, whether by increasing Shh activity or decreasing Ptc function, causes BCC formation. Other components of the pathway, like smo or Gli, might also become oncoogenic in some BCCs or medulloblastomas. Although Gli has not yet been implicated in these tumors, it has been found to be amplified in another brain tumor, glioblastoma (Kinzler et al. 1987).

**CONSERVATION OF THE Ptc PROTEIN**

Homologs of ptc have been identified in mice (Goodrich et al. 1996; Hahn et al. 1996a), humans (Hahn et al. 1996a; Johnson et al. 1996), chicks (Marigo et al. 1996c), zebrfish (Concorde et al. 1996), butterflies (L. Goodrich et al., unpubl.), and worms (Wilson et al. 1994). Hydropathy analysis of these homologs suggests that the topological structure of Ptc is well conserved, and Ptc may contain 12 potential transmembrane domains (Fig. 3)(Hooper and Scott 1989; Nakano et al. 1989; Goodrich et al. 1996). The transmembrane regions are arranged as a tandem array that is composed of one membrane-spanning domain separated from a cluster of five domains by a large hydrophilic loop. The large number of membrane-spanning regions and topologically duplicated structure suggest that Ptc may have transporter or channel function in addition to its proposed role as an Hh receptor and regulator of Smo. The highly conserved transmembrane domains may form an aqueous pore through which small molecules or ions pass or they may form a surface that contacts Smo. Unlike many transporters and channels, Ptc appears to lack sequence similarity between its first and second sets of six transmembrane domains. No sequence similarity has been detected between Ptc homologs and any member of the transporter or channel families, and an attempt to detect channel activity in Xenopus oocytes expressing Ptc had negative results (Marigo et al. 1996b).

An alignment of all seven Ptc protein sequences shows that about 30% of the residues are conserved over a span of about 1100 amino acids (Fig. 3). This conservation is rather evenly dispersed throughout the putative extracellular and transmembrane regions. In each set of six transmembrane domains, the first two domains have little conservation (15%), whereas the third, fourth, and sixth domains are much more conserved (47%). The first extracellular loop is composed of about 350 residues of which 25% are conserved including six invariant cysteines. The cysteine residues could potentially form disulfide linkages within the loop to form part of a interaction domain for binding to Hh proteins. In addition, an N-linked glycosylation site is also present in the first extracellular loop of five of the seven homologs. The second extracellular loop is smaller, consisting of about 250 amino acids of which 35% are conserved. This loop has no conserved cysteines but does contain an invariant N-linked glycosylation site. Ptc appears to be a glycoprotein since tunicamycin treatment of cells, which blocks N-linked glycosylation, results in reduced mobility of Ptc in polyacrylamide gels (Marigo et al. 1996b). The two conserved glycosylation sites present in the loops are the likely targets of this modification and suggest that the two hydrophilic loops are extracellular.

The amino-terminal, carboxy-terminal, and central hydrophilic regions of the Ptc homologs are highly divergent in both length and amino acid composition and are proposed to be intracellular. For instance, the carboxy terminus varies from 53 (zebrafish Ptc 1) to 256 amino acids in length (mouse Ptc), whereas the central cytosolic loop ranges from 70 (worm Ptc) to 159 amino acids (zebrafish Ptc 1). The conserved residues in these regions are located mostly within 30-30 amino acids of the putative transmembrane regions (Fig. 3).
**HUMAN ptc MUTATIONS IN BCNS INDIVIDUALS AND BCCs**

More than 70 mutations in *PTC* have been reported either in sporadic BCCs or in individuals with the BCNS (Chidambaram et al. 1996; Gaitani et al. 1996; Hahn et al. 1996b; Johnson et al. 1996; Unden et al. 1996; Pietzsch et al. 1997; Raffel et al. 1997; Wicking et al. 1997; Xie et al. 1997). *PTC* mutations map throughout the predicted protein sequence (Fig. 4) and do not appear to cluster in specific regions, in contrast to the *APC* tumor suppressor gene (Polakis 1995). Of the human *PTC* mutations, about three quarters are nonsense, frameshift, or splice site mutations predicted to result in truncation of *PTC* at positions from amino acid 85 to amino acid 1121. Two thirds of these truncation mutations are predicted to delete at least half of the protein. The most carboxy-terminal truncation mutation yet found involves two independently identified deletions that are predicted to truncate the protein just before transmembrane 11 at amino acid 1121 (Wicking et al. 1997; M. Asztterbaum and E. Epstein, pers. comm.). This mutation suggests that more than 10 of the 12 potential transmembrane domains are required for Ptc to function.

Missense mutations that result in amino acid changes, insertions, or in-frame deletions in *PTC* might indicate residues that are important for function. About half of these mutations map to the two putative extracellular loops of Ptc and may denote residues involved in contacting Hh or Smo. In addition, the loops might also be important in maintaining a stable protein conformation since a three-amino-acid duplication (PNI) at residue 815 in the second extracellular loop results in greatly reduced expression of the protein (Stone et al. 1996). Missense mutations have also been found in some of the putative transmembrane domains and cysteolic loops.

Only 9 of the 23 missense mutations alter highly conserved amino acids, so changes at less conserved positions in Ptc appear to be important. For instance, in two independent sporadic BCCs, an amino acid change and an in-frame deletion have been detected in the highly variable regions of the major cysteolic loop and carboxyl terminus, respectively. BCC formation appears to require complete loss of Ptc function, and in both of the BCCs, the second allele of *PTC* is deleted, so these mutations are unlikely to be benign polymorphisms (Gaitani et al. 1996). Therefore, although much of the major cysteolic loop and of the carboxyl terminus is highly variable between species, their composition may be functionally important within a species.
Figure 4. Locations of mutations in human PTC. The positions of mutations predicted to result in truncation (black), amino acid change (gray), and in-frame deletion (Δ) are shown for the human Ptc sequence. 9aa indicates a nine-amino-acid insertion.

The type and severity of developmental defects vary widely within and between BCNS families (Goldstein et al. 1994b; Gorlin 1995). The severity of BCNS phenotypes does not appear to correlate with the extent of predicted truncation of PTC, in contrast to findings for the APC gene (Polakis 1995). One study of 24 BCNS families found no correlation between the position of truncation mutations in PTC and the age of onset of BCNs or the number of defective features. Additionally, individuals with missense mutations did not appear to have milder symptoms (Wicking et al. 1997). This suggests that most mutations in PTC that give rise to identifiable BCNS may result in complete loss of function, whereas mutations that partially reduce PTC activity may be phenotypically normal. As yet, no correlation has been found between distinct developmental defects or tumor types in BCNS families and particular types of PTC mutations. On the contrary, two families that independently carry the same PTC mutation have distinct inherited developmental abnormalities; only one family carries heritable palate defects, whereas the second family does not (Wicking et al. 1997). These data and others (Chidambaram et al. 1996; Unden et al. 1996) suggest the existence of other genetic and environmental factors that influence the types and severity of phenotypes in BCNS individuals.

NPC, A GENE RELATED TO ptc, SUGGESTS NEW FUNCTIONS IN HL SIGNALING

The recent identification of a ptc-related gene involved in a cholesterol trafficking disease suggests that Ptc and other components of the pathway may function in vesicle loading, movement, or targeting. Niemann-Pick type C (NPC) disease results from a defect in cholesterol homeostasis in which cells accumulate abnormal amounts of deesterified cholesterol in the lysosomes and other intracellular compartments (Liscum and Faust 1987; Pentechev et al. 1987). The NPC gene encodes a large multiple transmembrane protein that has homology with Ptc in the hydrophobic regions. One region conserved between NPC and Ptc appears to be a sterol-sensing domain, based on similarity to the sterol-regulated proteins, HMG-CoA reductase and SREBP cleavage-activating protein (Carstea et al. 1997; Loftus et al. 1997). This similarity implies that Ptc and NPC may detect and be regulated by sterols. This idea is strengthened by the finding that several conserved residues within the NPC homology regions of Ptc (including the putative sterol-sensing domain) are the locations of missense mutations in BCNS individuals (Fig. 5) (Chidambaram et al. 1996; Wicking et al. 1997). The NPC protein also contains a presumed extracellular domain that is rich in cysteine residues and may, like Ptc, bind a ligand (Carstea et al. 1997; Loftus et al. 1997).

What new functions for Hh signaling does the NPC/Ptc homology imply? The presence of a sterol-sensing motif in Ptc suggests that Ptc may be regulated by sterol levels within the cell. Cholesterol or a derivative may regulate Ptc protein stability, subcellular localization, or its ability to signal. Moreover, the NPC homology with Ptc suggests that Ptc may function intracellularly in vesicular compartments. This suggests an elegant function for the kinesin-related protein, Cos2, for moving Ptc-containing vesicles within the cell (Sisson et al. 1997).

The unexpected linkage between Ptc and cholesterol is
intriguing in light of the fact that the amino-terminal half of Hh, a ligand for Ptc, is itself covalently attached to cholesterol and rendered lipophilic (Porter et al. 1996a,b). Perhaps Ptc binds both the Hh protein and its cholesterol moiety to sequester Hh within the receiving cell. This would require moving membrane-bound Hh between the lipid bilayers of the secreting and receiving cells, perhaps by exocytosis. Alternatively, the two connections between Hh/Ptc signaling and cholesterol may indicate separate aspects of the pathways. In this case, there would not be any transfer of Hh-cholesterol between cells, and sterol sensing by Ptc could involve cholesterol that is not linked to Hh.

NPC homologs exist in yeast and worms (Figs. 5 and 6) (Casteu et al. 1997; Lofthus et al. 1997) and are similar to Ptc in the hydrophobic regions, including the sterol-sensing domain. A unique situation occurs in the worm, where at least 14 NPC/Ptc homologs have been identified and

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**Figure 5.** Sequence alignment of Ptc and 13 NPC homologs. An alignment of mouse and fly Ptc and the predicted NPC proteins from mouse, yeast, (YPL006), and worm (F028.1, F44F4.4, R09H10.4, K07Cl0.1, C53Cl1.3, F31F6.5, C2AB5.3, C2DE8.8, F58F8.1, C54A21.1, C54B2.7) was made using the GCG program, Pileup. Sequence similarity is detected in two separate regions of Ptc: putative transmembrane domains 3–6 (including the putative sterol sensing domain) and 8–12. Bars over sequence indicate potential membrane spanning segments of Ptc. Consensus sequence indicates similar or identical residues shared between 11 of the 15 sequences. Asterisks (*) indicate positions where missense mutations have been identified in PTC in BCNS patients or in BCCs.
Figure 6. Conservation between Ptc and 13 NPC homologs. A model of the mouse Ptc sequence showing conserved residues with a family of NPC homologs. (Closed circle) Residues that are similar for at least 11 of 15 sequences.

appear to be transcribed since expressed sequence tags have been identified for most members (Wilson et al. 1994). The NPC/Ptc homologs might represent a family of receptors with functions, like Ptc, in cell-cell signaling. The conserved hydrophobic regions may form a common functional domain, whereas the unconserved hydrophilic loops might bind different ligands. Ligands for this group of proteins may be encoded by a family of putative secreted proteins in C. elegans that are related in sequence to the carboxy-terminal half of Hh but have divergent amino-terminal domains (Burglin 1996; Porter et al. 1996). One family member contains autocatalytic cleavage activity that releases the amino-terminal half in a manner like Hh (Porter et al. 1996b). These Hh-related genes might function as a group of novel signaling molecules that interact with the NPC/Ptc proteins to control diverse cell-cell signaling events. Alternatively, the worm NPC/Ptc homologs might have specialized functions in different cell types or within a cell for moving vesicles to different organelles.

PERSPECTIVES

The recent findings about the fundamental role of Hh signaling in vertebrate development and tumorigenesis have created much excitement and interest. However, many questions remain to be answered. How do two cells respond differently to the same Hh signal to induce the transcription of distinct sets of genes? How is the Hh signal transduced from the plasma membrane to the nucleus? The entire repertoire of pathway components has yet to be identified and the interactions among them understood. The similarities between NPC and Ptc suggest a function common to these proteins. Do the parallels between these two proteins extend to interacting components as well? For instance, is there a ligand and a kinesin motor involved in the NPC pathway? What role does cholesterol have in regulating Hh, Ptc, and other components of this pathway? Answering these questions is certain to reveal more surprises about this extraordinary pathway.

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