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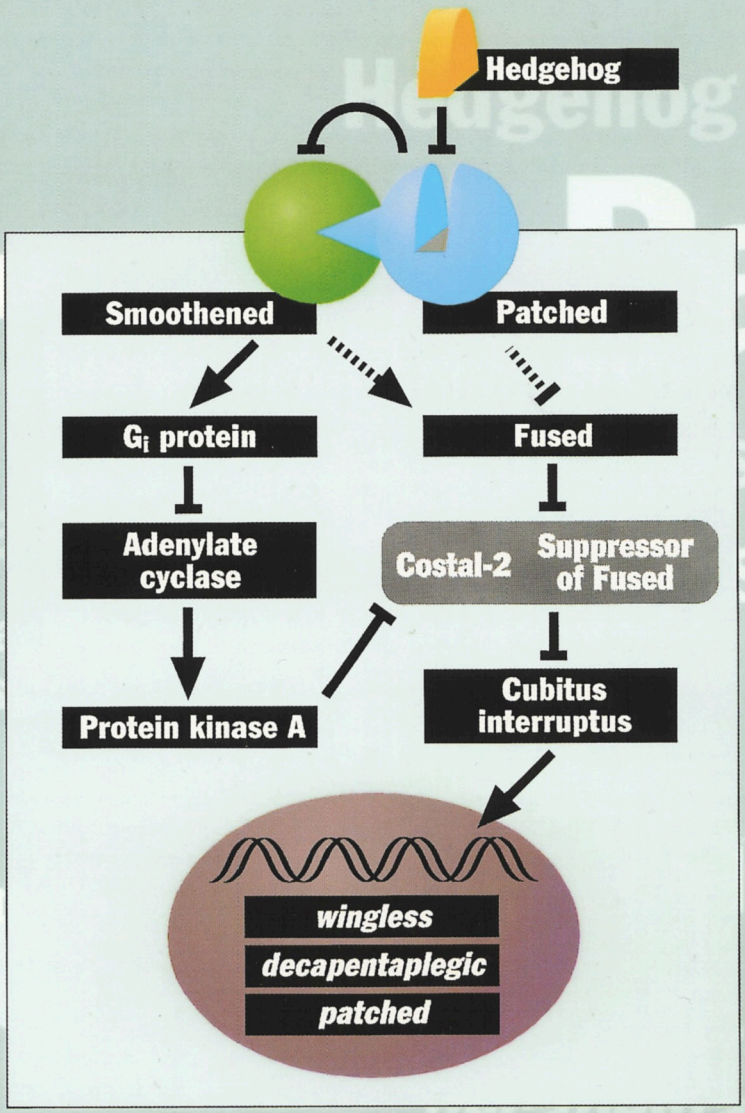

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Review

Hedgehog and Its Patched-Smoothened Receptor Complex: A Novel Signalling Mechanism at the Cell Surface

Joy Alcedo and Markus Noll*

Institut für Molekularbiologie der Universität Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland

* Corresponding author

Pattern formation and morphogenesis depend on the careful execution of complex genetic programs, which are conserved in multicellular organisms. An important signal in some of these programs in *Drosophila* and vertebrates is the secreted Hedgehog (Hh) protein, which primarily functions as an inducer of morphogenetic signals. The Hh signal plays a decisive role in such critical developmental processes as neurulation and somite and limb formation. The Hh signalling pathway exhibits a novel mechanism of signal reception and transduction. In the absence of the Hh signal, the membrane protein Patched (Ptc) represses the constitutive signalling activity of a second membrane protein, Smoothened (Smo), by virtue of its ability to form a Ptc-Smo complex. Hence, mutations within the *ptc* gene that result in the failure of Ptc to inhibit Smo lead to constitutive activity of the Hh signalling pathway and to cancer, such as basal cell carcinoma. For activation of Hh-target genes, the N-terminal signalling domain of Hh binds to the Ptc-Smo receptor complex to activate two parallel signalling pathways. Furthermore, Hh limits its own range of action by impeding its diffusion through (i) covalent linkage of its N-terminal signalling moiety to cholesterol, mediated by the cholesterol transferase activity of its C-terminal moiety, and (ii) induction of, and sequestration by, its antagonist, Ptc.

Key words: Constitutive signalling / Ligand-regulated receptor repressor.

The Signalling Activities of the Hh Protein

The development of multicellular organisms is determined by mechanisms that specify pattern formation, of which signal transduction pathways are integral components. One of these signalling pathways, which is conserved from flies to man, involves the secreted gene products of the *hedgehog (hh)* gene family. Mutations in these genes not only disrupt *Drosophila* and mouse development but also lead to disease in humans, such as holoprosencephaly, which is characterised by poorly developed brain vesicles and severe facial abnormalities (Belloni *et al.*, 1996; Roessler *et al.*, 1996).

In *Drosophila*, Hh is crucial in the establishment of embryonic segments and their parasegmental boundaries. It is expressed in posterior compartments and exerts its function as a short-range signal by maintaining *wingless (wg)* expression in adjacent anterior stripes of cells (Hidalgo and Ingham, 1990; Ingham *et al.*, 1991; Ingham and Hidalgo, 1993). In imaginal discs, the anlagen of the adult epidermal structures, Hh also locally induces the expression of secondary signals, Wg and the TGF-β family member Decapentaplegic (Dpp) (Basler and Struhl, 1994; Tabata and Kornberg, 1994; Heberlein *et al.*, 1995; Zecca *et al.*, 1995), which function as morphogens required in the pattern formation of these structures (Nellen *et al.*, 1996; Zecca *et al.*, 1996). In addition to its short-range function, Hh is believed to have a long-range activity as observed within the dorsal epidermis of the *Drosophila* embryo (Heemskerk and DiNardo, 1994). Similarly, vertebrate Hh homologues exhibit both short-range and long-range activities in the patterning of tissues neighbouring the cells that express these signals. For example, Sonic hedgehog (Shh), a member of the vertebrate Hh protein family expressed in the chordamesodermal notochord, induces floor-plate formation within the ectodermal neural tube apparently in a contact-dependent manner (Martí *et al.*, 1995; Roelink *et al.*, 1994; Tanabe *et al.*, 1995). Yet, in other cases, induction does not require cell contact. Thus, when neural plate or presomitic mesoderm explants were grown at a distance of many cell diameters from the Shh-expressing cells (Fan and Tessier-Lavigne, 1994; Martí *et al.*, 1995; Roelink *et al.*, 1995; Tanabe *et al.*, 1995) or when contact was prevented by a nucleopore filter between explants and Shh-expressing cells (Fan and Tessier-Lavigne, 1994; Tanabe *et al.*, 1995), induction of motor neurons or the sclerotome did still occur. Although Shh and different members of the vertebrate Hh protein family have also been implicated in the patterning of other vertebrate structures, *e.g.*, the limb (Riddle *et al.*, 1993; Chang *et al.*, 1994) and the eye (Ekker *et al.*, 1995b), it remains unknown if formation of these tissues are mediated by the Hh proteins in a contact-dependent or -independent manner. However, short- and long-range activities of Hh probably do not reflect fundamental differences between contact-dependent and -independent signalling mechanisms but simply differences in the response elicited by high and low concentrations of the Hh signal (Roelink *et al.*, 1995; see below).

Hh Signalling Acts through Two Parallel Pathways

Since the correct reception or transmission of the Hh signal is a recurrent theme in animal development, molecular dissection of the Hh pathway, which has been done extensively in *Drosophila*, would allow insight into this process. Transmission of the Hh signal depends on the *smoothened (smo)* gene product, which encodes a putative membrane protein (Alcedo *et al.*, 1996). Moreover, the possibility that Smo has characteristics of G protein-coupled receptors (Alcedo *et al.*, 1996) is further supported by the observation that the cyclic AMP-dependent protein kinase (PKA) acts antagonistically downstream of the Hh signal (Jiang and Struhl, 1995; Johnson *et al.*, 1995; Lepage *et al.*, 1995; Li *et al.*, 1995; Pan and Rubin, 1995; Strutt *et al.*, 1995). The *smo* gene has also been shown to act upstream of PKA by genetic analysis (Chen and Struhl, 1996; van den Heuvel and Ingham, 1996a), a result suggesting that Smo may activate an inhibitory G protein which inhibits adenylate cyclase and consequently PKA (Figure 1; Alcedo *et al.*, 1996).

Hh function is antagonised not only by PKA but also by Patched (Ptc) (Ingham *et al.*, 1991), a multiple-spanning membrane protein (Hooper and Scott, 1989; Nakano *et al.*, 1989). Since Hh-target genes, such as *dpp*, *wg*, or *ptc*, are expressed in *pka*$^-$ cells in the absence of functional Hh despite the presence of Ptc, PKA is epistatic to Ptc and thus must act either downstream of and/or in parallel to Ptc (Jiang and Struhl, 1995; Li *et al.*, 1995). Evidence for the second possibility has been obtained in elegant experiments employing a PKA transgene whose product is constitutively active (Jiang and Struhl, 1995; Li *et al.*, 1995). If Ptc acted exclusively through PKA, the level of constitutively active PKA needed to inhibit the expression of Hh-target genes in *pka*$^-$ cells should be the same as that in *ptc*$^-$ cells. However, compared to the levels required to suppress the expression of Hh-target genes in *pka*$^-$ cells, only very high levels of constitutively active PKA can inhibit the expression of the same genes in *ptc*$^-$ cells (Li *et al.*, 1995). Therefore, Ptc can antagonise the Hh signal in a PKA-independent pathway although it may also act through PKA, as illustrated by the model in Figure 1. Ptc seems to exert its inhibitory activity on the serine/threonine kinase Fused (Fu) (Préat *et al.*, 1990; Thérond *et al.*, 1996), whose function is required to activate the expression of Hh-target genes (Limbourg-Bouchon *et al.*, 1991; Forbes *et al.*, 1993). In contrast, PKA does not appear to act on Fu (Thérond *et al.*, 1996) and, thus, the Ptc- and PKA-dependent pathways of Hh signalling seem to converge downstream of Fu. Moreover, the observation that loss of PKA in the absence of the Hh signal activates Hh-target genes regardless of Ptc concentration suggests that the Ptc inhibition through Fu is not sufficient to inhibit the Hh signalling pathway (Li *et al.*, 1995). Conversely, only unphysiologically high concentrations of constitutively active PKA are able to repress Hh-target genes in the absence of Ptc (Li *et al.*, 1995). This indicates that the inhibitory action of PKA alone is also insufficient for repression of the Hh signalling pathway. Hence, both the Ptc-dependent and PKA-dependent pathways are required to repress Hh target genes (Figure 1). Since it is unclear if Ptc action on Fu is independent of Smo, the possibility also exists that both pathways depend on Smo, i.e., Hh signalling bifurcates downstream rather than upstream of Smo (Figure 1).

It is not known on which downstream component PKA acts. Through phosphorylation, it may destabilise factors essential in the activation of Hh-target genes, such as the zinc-finger transcription factor *Cubitus interruptus* (Ci) (Hidalgo and Ingham, 1990; Orenic *et al.*, 1990; Forbes

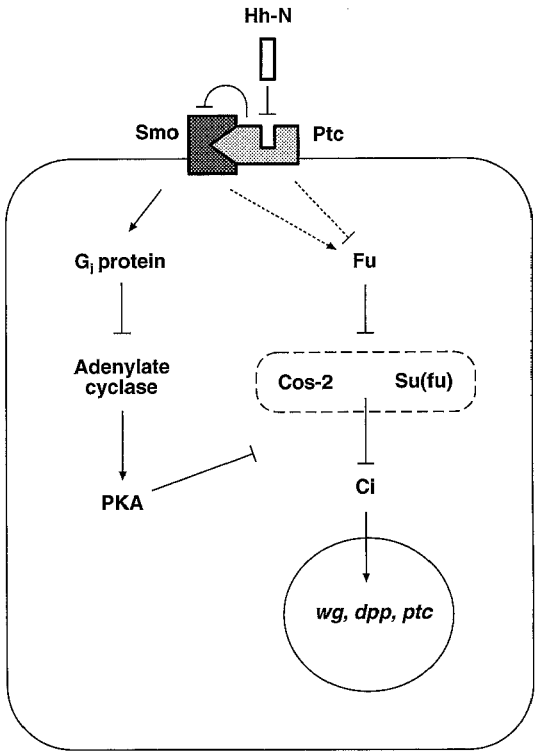

Fig. 1 A Model of the Hh Signalling Pathway.
To activate its target genes, *wg*, *dpp* and *ptc*, the N-terminal signalling domain of Hh, Hh-N, binds to its antagonist Ptc, which is complexed with Smo, to counteract the inhibition by Ptc of the constitutive signalling activity of Smo and of the Fu-branch of the signalling pathway. However, it remains unclear if the Ptc action on Fu is independent of or dependent on Smo as illustrated by the dotted lines. The left branch of the signalling pathway, namely that Smo inhibits PKA through activation of a G_i protein, which would lead to a decrease in cAMP levels, is inferred from the structural homology of Smo to G protein-coupled receptors. Thus far, no experimental evidence has been obtained for the proposed regulation of PKA activity. PKA may inhibit the Hh signal through stimulation of other antagonists of the pathway, Cos-2 or Su(fu), or through direct or indirect repression of the function of the zinc-finger protein Ci. Ptc antagonises the Hh signal not only in a PKA-dependent but also in a parallel, PKA-independent pathway through Fu. The two parallel pathways converge downstream of Fu, whose activity is required to relieve the repression mediated by Cos-2 and Su(fu) on Ci. Subsequently, Ci translocates into the nucleus to exert its action on Hh-target genes.

et al., 1993; Alexandre *et al.*, 1996; van den Heuvel and Ingham, 1996b). It is possible that the PKA-mediated inhibition of Ci is alleviated by the activity of a phosphatase that is activated by Smo (van den Heuvel and Ingham, 1996b). However, the mechanism proposed in Figure 1, namely inhibition of PKA by Smo through its modulation of cAMP levels via a G protein, is more economical because it is the simplest model to account for the predicted structural characteristics of Smo (Alcedo *et al.*, 1996). Recently, it has also been proposed that PKA phosphorylates the *Drosophila* homologue of the vertebrate transcription factor CREB2, which then binds to the *Drosophila* homologue of the coactivator CBP (dCBP) and thereby limits the amount of dCBP available for interaction with Ci to enhance its function (Akimaru *et al.*, 1997). On the other hand, instead of inhibiting Ci, PKA function may stimulate other antagonists of the Hh signal, such as Suppressor of fused [Su(fu)] (Préat, 1992) and Costal-2 (Cos-2) (Forbes *et al.*, 1993; Préat *et al.*, 1993).

The role of *Su(fu)* has been derived from the following observations. The absence of a functional *Su(fu)* gene product, a protein with a PEST sequence, has been shown to result in the suppression of the *fu* phenotype, *i.e.*, *Su(fu)* mutants can rescue the lethality of *fu* mutations (Préat, 1992; Pham *et al.*, 1995). Furthermore, homozygous *Su(fu)* mutants are viable and display a wild-type phenotype (Préat, 1992). Hence, the Su(fu) protein must inhibit a component downstream of Fu and the alleviation of such an inhibition must require the kinase activity of Fu, thereby allowing expression of Hh-target genes (Figure 1). Another component downstream of the Hh signal which is inhibited by Fu is the still unidentified gene product of *cos-2*, which also represses the expression of Hh-target genes (Forbes *et al.*, 1993; Préat *et al.*, 1993). Both Su(fu) and Cos-2 may block the action of Ci (Forbes *et al.*, 1993), while activation of Fu by the Hh signal might stimulate Ci function directly or indirectly (Figure 1; Forbes *et al.*, 1993; Motzny and Holmgren, 1995; Domínguez *et al.*, 1996). Whereas most of the components downstream of the Hh signal transduction pathway are found conserved in vertebrates (Concordet *et al.*, 1996; Goodrich *et al.*, 1996; Hammerschmidt *et al.*, 1996; Marigo *et al.*, 1996a, c; Stone *et al.*, 1996; Vortkamp *et al.*, 1996), vertebrate homologues of Fu or Su(fu) have not yet been identified.

The Hedgehog Signal Restricts Its Own Range of Action

The Smo protein was postulated to be a possible receptor for the Hh signal (Alcedo *et al.*, 1996) because of its homology to the *Drosophila* Frizzled (Fz) receptor protein (Vinson *et al.*, 1989) and its position in the Hh pathway. The demonstration by Bhanot *et al.* (1996) that the Fz protein and some of its close homologues bind the Wg protein and thus activate the Wg signalling pathway raises the intriguing possibility that the Hh and Wg proteins may recognise very similar receptors.

Consistent with the hypothesis that a receptor must be able to sequester its ligand, recent analysis in imaginal discs of the effect of *smo*$^-$ clones on Hh diffusion showed that its range is enhanced in the absence of Smo although this effect appears to be indirect because it is compensated by the simultaneous loss of PKA function (Chen and Struhl, 1996). This led the authors to suggest that loss of PKA would induce, even in the absence of Smo, a Hh-target gene whose product limits Hh diffusion. One of the Hh-target genes shown to be activated upon loss of PKA activity is *ptc* (Li *et al.*, 1995), whose product, a membrane protein, has been postulated previously to be the receptor of the Hh protein (Ingham *et al.*, 1991). Chen and Struhl (1996) demonstrated that the absence of Ptc causes the signal to spread further from its source, whereas upregulating Ptc expression restricts its movement. Thus, it is Ptc that sequesters the Hh signal in the absence of PKA and Smo. Therefore, it appears that Ptc and not Smo is the Hh receptor. However, the Hh signalling pathway is constitutively active in the combined absence of Ptc and Hh (Ingham *et al.*, 1991) yet is completely dependent on Smo (Hooper, 1994; Alcedo *et al.*, 1996). Considering that both Ptc and Smo are integral membrane proteins, the simplest explanation for these observations is that transduction of the Hh signal is mediated by a Ptc-Smo complex, in which the constitutive signalling activity of Smo is inhibited by its association with Ptc (Figures 1 and 2A; Alcedo *et al.*, 1996). Such a hypothesis is consistent with recent biochemical data (Stone *et al.*, 1996). In vertebrates, Ptc and Smo are coexpressed in many tissues and Ptc can form a complex with Smo (Stone *et al.*, 1996). In addition, Ptc (Marigo *et al.*, 1996b; Stone *et al.*, 1996), but not Smo (Stone *et al.*, 1996), binds the N-terminal domain of Hh (Hh-N).

The various Hh proteins, one identified in *Drosophila* (Nüsslein-Volhard and Wieschaus, 1980; Lee *et al.*, 1992; Mohler and Vani, 1992; Tabata *et al.*, 1992) and several in vertebrates (Echelard *et al.*, 1993; Krauss *et al.*, 1993; Riddle *et al.*, 1993; Chang *et al.*, 1994; Roelink *et al.*, 1994; Ekker *et al.*, 1995a, b), undergo not only signal peptide cleavage but also a further autoproteolytic cleavage into a 19 kDa amino-terminal peptide (Hh-N) and a carboxy-terminal peptide (Hh-C) that ranges in size from 26 to 28 kDa (Lee *et al.*, 1992, 1994; Chang *et al.*, 1994; Ekker *et al.*, 1995b; Lai *et al.*, 1995; Porter *et al.*, 1995), the cleavage being dependent on a conserved domain within their C-termini (Lee *et al.*, 1994; Porter *et al.*, 1995). Whereas Hh-C diffuses from the cell in which it was expressed, Hh-N remains associated with the cell surface (Lee *et al.*, 1994; Fan *et al.*, 1995; Porter *et al.*, 1995; Roelink *et al.*, 1995). Based on the spatial distribution patterns of Hh-N and Hh-C, the Hh-N fragment was postulated to mediate the short-range signalling activity of the protein, while the Hh-C fragment was thought to be the long-range signal (Lee *et al.*, 1994).

However, in *Drosophila* and vertebrates, Hh-C had no signalling activity whereas Hh-N was sufficient to exert both short- and long-range activities (Fan *et al.*, 1995; Fietz *et al.*, 1995; Martí *et al.*, 1995; Porter *et al.*, 1995;

Roelink *et al.*, 1995). Surprisingly, ubiquitous expression of a Hh-N or a full-length Hh transgene gave essentially the same dorsal and ventral cuticular defects in *Drosophila* as those produced by localised expression of a Hh-N transgene in cells that expressed the endogenous wild-type full-length protein (Porter *et al.*, 1996b). In contrast, the same localised expression of an exogenous full-length Hh protein generated dorsal and ventral cuticle similar to wild-type (Porter *et al.*, 1996b), a difference that may be explained by the observation that Hh-N, translated from a truncated Hh-coding region, is more freely diffusible than Hh-N derived from a transcript that includes the entire coding region (Roelink *et al.*, 1995; Porter *et al.*, 1996b). Hence, the Hh-C moiety is required in restricting the spatial distribution of the Hh-N signal within the extracellular matrix, a requirement decisive for the proper execution of the signal's patterning function. Indeed, Porter *et al.* (1996a, b) showed that the association of Hh-N with the cell surface requires the covalent attachment of the peptide to a cholesterol moiety in the membrane, a reaction catalysed by an intramolecular cholesterol transferase activity of Hh-C.

Furthermore, only Hh-N has signalling activity whereas mutant Hh that cannot undergo autoproteolytic cleavage is inactive (Porter *et al.*, 1996a). This implies that activation requires cleavage and, as a consequence, the simultaneous transfer of the resulting Hh-N fragment to cholesterol. There are two possibilities of how the signal could reach cells that are not in contact with the cells that display membrane-anchored Hh-N-cholesterol (Hh-N-ch) on their surface: either

(i) the Hh-N-ch fragment must diffuse from the membrane of the cell in which it is synthesised into the intercellular space (Porter *et al.*, 1996b) until captured by Ptc on the surface of adjacent and more distant cells or
(ii) Hh protein that has escaped autoproteolysis is secreted into the intercellular space, as it has been observed in S2 cells (Porter *et al.*, 1995).

In this second case, as Hh encounters its substrate cholesterol on the surface of neighbouring and more distant cells, immediate production of an active, cholesterol-linked fragment will ensue that is capable of binding to Ptc protein present on these cells. The crucial aspect is that this arrangement ensures that the range of signal is limited, which is required for its morphogenetic action, a condition that appears to be satisfied by the density of both Ptc and cholesterol molecules on the surface of the target cells.

The remaining uncertainties concern the form in which Hh is secreted, cleaved or uncleaved, the relative rates of diffusion within the intercellular space of Hh-N-ch and Hh and the affinity of Hh for cholesterol. Clearly, if the secreted form is the lipophilic Hh-N-ch fragment, binding to the membrane of target cells will be efficient and so will be binding to Ptc within the same membrane. However, if primarily hydrophilic Hh is secreted, its diffusion might not be sufficiently restricted to prevent long-range signalling, especially if the rate of reaction with cholesterol on the surface of the target cells is low. Indeed, the observation by Chen and Struhl (1996) that, in the absence of Ptc, the signal diffuses over many cell diameters, would be consistent with both of these assumptions, namely that primarily Hh is secreted and its reaction with target cell cholesterol is inefficient. The question then arises why the long-range signalling described by Chen and Struhl (1996) is prevented in the presence of Ptc despite the inefficiency of activation by cholesterol. The only way out of this dilemma would be to postulate that Hh itself binds to Ptc with an affinity that is much greater than that of its binding to the substrate cholesterol. Once retained in the membrane by Ptc, the probability of Hh colliding with membrane-bound cholesterol is greatly enhanced. This collision results in the activation of Hh that would then trigger the signal transduction.

The novel feature of this model is that binding of Hh to Ptc proceeds in two steps, a first, reversible binding that does not result in signalling, followed by the cleavage-induced allosteric transition of Hh-N-ch to a state that activates the two parallel signalling pathways. These events may be visualised to involve two sites on the extracellular surface of Ptc, one site, the Hh-binding site, that binds Hh until it reacts with cholesterol and a second site, the Hh-N-binding site, that binds the cleaved fragment Hh-N-ch to trigger the signalling pathway.

This model that could explain the observed diffusion of Hh in the absence of Ptc (Chen and Struhl, 1996) might not be compelling if the secretion product of the Hh-expressing cells is exclusively Hh-N-ch. Yet, because of the high affinity of cholesterol for membranes, diffusion over many cell diameters under these conditions seems unlikely.

While the basis for the enhanced range of Hh-N diffusion is the failure of its covalent linkage to the cell surface, the diffusion range of uncleaved Hh would be increased only when it fails to be bound by Ptc. Therefore, we would expect that mutations of Ptc exist that do not impede Hh diffusion but still repress the constitutive signalling of Smo. This contrasts with *ptc* null mutants, which fail to inhibit both Hh diffusion and constitutive Smo signalling, and with apparent null mutations of *ptc* that only impede Hh diffusion (Chen and Struhl, 1996).

In summary, then, we conclude that Hh restricts its own range of action via two novel mechanisms:

(i) Through the cholesterol transferase activity of Hh-C, which covalently links Hh-N to cholesterol and thus increases its affinity to the cell surface membrane (Porter *et al.*, 1996a, b); and
(ii) through the induction of its own antagonist, Ptc.

Furthermore, the antagonism by Ptc of the Hh signal features two distinct aspects:

(i) Sequestration of Hh limiting its diffusion (Chen and Struhl, 1996); and
(ii) inhibition of the constitutive signalling of Smo (Alcedo *et al.*, 1996).

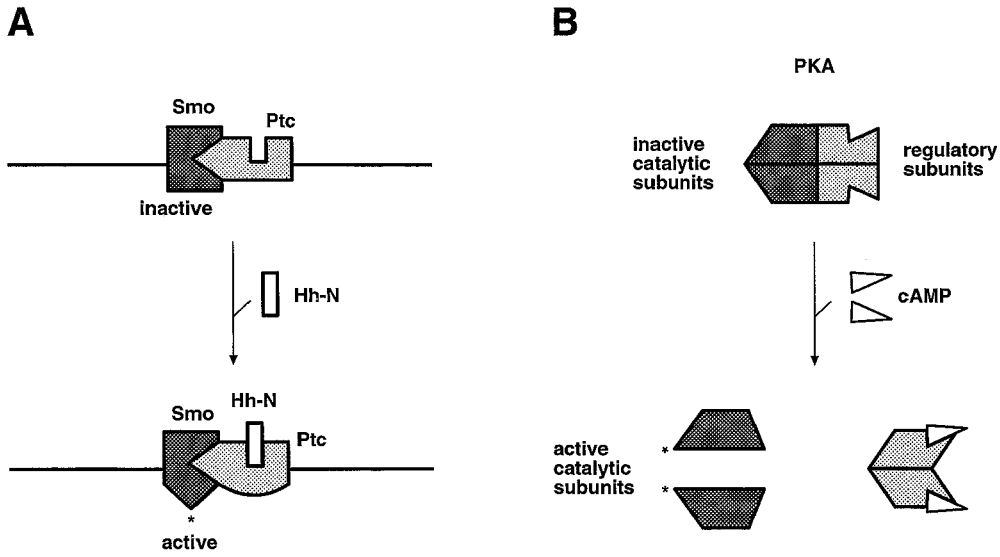

Fig. 2 Analogy between the Mode of Reception and Transmission of the Hh Signal at the Cell Surface and the Mode of cAMP-Mediated Regulation of PKA Activity within the Cytoplasm.
(A) Ptc maintains Smo in an inactive state within a Ptc-Smo complex in the absence of Hh. Upon binding of Hh-N to Ptc, Smo and Ptc undergo conformational changes that alter their state of activity. In this process, Smo may remain associated with, or dissociate from, the Ptc-Hh-N complex to assume an active state of signalling. In favor of the 'dissociation' model is the observation that constitutive signalling occurs in the absence, and hence independently, of Ptc while the model shown here is favored by the observation that Hh-N can be coimmunoprecipitated with a Ptc-Smo complex. (B) In the absence of cAMP, the regulatory subunits of PKA maintain its catalytic subunits in an inactive state. cAMP binding to its regulatory subunits releases the catalytic subunits, which become active to phosphorylate their substrates.

A Novel Mechanism of Signal Reception and Transmission at the Cell Surface

Since the signalling moiety of Hh, Hh-N, binds only to Ptc (Marigo *et al.*, 1996b; Stone *et al.*, 1996) and Ptc can bind Smo, Ptc might be a ligand-regulated repressor of a constitutively active signalling moiety, Smo, in a multi-component receptor complex (Figure 2A; Alcedo *et al.*, 1996; Stone *et al.*, 1996). While such a mechanism is new among cell surface receptors, it is analogous to long-known cytoplasmic signalling complexes (Gill and Garren, 1971; Brostrom *et al.*, 1971), of which indeed an example, the cAMP-dependent PKA, is encountered further downstream in the Hh-signalling pathway. The second messenger cAMP binds to the regulatory subunits of PKA to induce their dissociation from, and activation of, the catalytic subunits (Figure 2B). In contrast to the induction of PKA activity by cAMP, Smo may be activated by an allosteric transition without its dissociation from Ptc upon binding of Hh-N to Ptc (Figure 2A). Although not illustrated in Figure 2A, it is also conceivable that, in analogy to PKA, binding of Hh-N to Ptc shifts the equilibrium between the Ptc-Smo complex and its free components towards the dissociated state. While this second mechanism is favored by the observation that constitutive Smo signalling does not require Ptc (Hooper, 1994; Alcedo *et al.*, 1996), the first mechanism appears to be supported by the fact that Hh-N can be coimmunoprecipitated with a Ptc-Smo complex (Stone *et al.*, 1996). If Ptc does not act through Smo on Fu, a more prominent difference between the regulation of cytoplasmic PKA and that of the Smo signalling activity at the cell surface is that Ptc is more versatile than the regulatory subunit of PKA: Ptc does not act only on Smo but also regulates the signalling pathway parallel to Smo that passes through Fu (Figure 1).

However, this model of Hh signal reception and transmission appears to be inconsistent with the observation that *Drosophila* embryos mutant for both *hh* and *ptc* have a phenotype different from embryos lacking only a functional Ptc protein (Bejsovec and Wieschaus, 1993). This finding implies that Hh does not act through Ptc alone but also through another receptor whose activity it modulates to affect gene expression. For example, Hh might also bind to Smo to further stimulate the signalling function of Smo. Since Hh-N does not bind to Smo (Stone *et al.*, 1996), Hh might bind through its C-terminal portion to Smo. This C-terminal portion of Hh might have signalling properties that are undetectable in the absence of Hh-N (Fietz *et al.*, 1995; Porter *et al.*, 1995). According to this model, inhibition of Ptc by Hh-N is necessary for the upregulation of constitutive Smo activity by the C-terminal portion of Hh. However, it is not necessary to invoke such a model if the *ptc* alleles, used to compare *ptc* mutants with *ptc hh* double mutants (Bejsovec and Wieschaus, 1993), generate Ptc proteins that are not completely null in function. In this case, a strong *ptc* allele producing a weakly functional Ptc protein might still exert some inhibitory activity on both pathways in the absence, but no longer in the presence, of a functional Hh protein, and thus resemble a null allele in a wild-type but not in a *hh* mutant background.

Hh Primarily Functions as Short-Range Inducer of Secondary Long-Range Signals

As both short- and long-range signalling of Hh are attributable to Hh-N, which seems to remain predominantly associated with the cell membrane, it is possible that Hh primarily functions as a short-range inducer of secondary long-range signals, like Dpp and Wg (Nellen *et al.*, 1996; Zecca *et al.*, 1996), as had previously been proposed by Basler and Struhl (1994). The Wg and Dpp signals have been shown to have direct long-range activities because only ectopic expression of the signals, and not the ligand-independent ectopic activation of their downstream transducing components, resulted in nonautonomous repatterning (Nellen *et al.*, 1996; Zecca *et al.*, 1996). Therefore, the long-range patterning activities of both these signals are not mediated by the synthesis of secondary signals. In contrast, nonautonomous repatterning can be induced by either ectopic Hh expression or by the Hh-independent ectopic activation of its downstream transducing components because either event can induce secondary signals (Jiang and Struhl, 1995; Lepage *et al.*, 1995; Li *et al.*, 1995; Pan and Rubin, 1995; Chen and Struhl, 1996). Hence, these observations are consistent with the hypothesis that Hh mediates long-range patterning indirectly by its short-range activation of secondary signals (Zecca *et al.*, 1995; Nellen *et al.*, 1996). The long-range activity associated with Hh in the dorsal patterning of the *Drosophila* epidermis (Heemskerk and DiNardo, 1994) may also be mediated by other long-range signals, like Wg or the gene product of *lines*, which has not yet been identified, but not by Dpp (Bokor and DiNardo, 1996). In addition, the long-range activity of Hh observed in vertebrates during filter barrier experiments does not exclude the possibility that a secondary long-range signal is induced by Hh in an autocrine manner in the Hh-expressing cells.

Since the Hh-induced long-range activities of Dpp and Wg are also concentration-dependent, Dpp and Wg act as morphogens (Nellen *et al.*, 1996; Zecca *et al.*, 1996). Thus, the short-range induction properties of Hh are crucial for the generation of a morphogen source that is limited to a narrow band of cells adjacent to a region of Hh-expressing cells, which necessitated the evolution of the self-limiting properties of Hh that depend on Ptc and Smo. In other words, the strategy to specify a 'one-dimensional' morphogen source relies on a two-step mechanism. First, two adjacent and distinct areas are defined: the posterior compartment expresses a signalling protein, Hh; the anterior compartment another protein, Ptc. Subsequently, the interaction between the two proteins at the border of the two compartments limits the range of the signalling protein and thus defines a precise, 'one-dimensional' source of the morphogen (Lawrence and Struhl, 1996).

Aberrant Hh Signal Transduction in Cancer

Not only mutations in the *hh* gene but also defects in the reception or transmission of its signal can lead to disease in humans. Most notably, mutations in the *ptc* gene have been associated with the basal cell naevus syndrome (BCNS), which is characterised by developmental abnormalities and cancer, such as basal cell carcinoma (BCC; Hahn *et al.*, 1996; Johnson *et al.*, 1996). These mutations may lead to constitutive activation of Smo since a mutant Ptc protein, which is unable to bind to Smo, would fail to block its activity (Stone *et al.*, 1996). According to this view, Ptc acts as a tumour suppressor by suppressing the oncogenic activity of Smo. Furthermore, the transcription factor Ci, which is required for the activation of Hh-target genes, is the *Drosophila* homologue of the vertebrate family of Gli zinc-finger proteins, one of which has been associated with human glioblastomas due to its amplification in these tumours (Kinzler *et al.*, 1988; Hidalgo and Ingham, 1990; Orenic *et al.*, 1990; Forbes *et al.*, 1993; Alexandre *et al.*, 1996; Marigo *et al.*, 1996a; Vortkamp *et al.*, 1996). Thus, further elucidation of the Hh signalling pathway should be of benefit in counteracting diseases arising from its aberrant functions.

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