Hedgehog signal transduction: recent findings
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The Hedgehog (Hh) family of signaling molecules are key agents in patterning numerous types of tissues. Mutations in Hh and its downstream signaling molecules are also associated with numerous oncogenic and disease states. Consequently, understanding the mechanisms by which Hh signals are transduced is important for understanding both development and disease. Recent studies have clarified several aspects of Hh signal transduction. Several new Sonic Hedgehog binding partners have been identified. Cholesterol and palmitic acid modifications of Hh and Sonic hedgehog have been examined in greater detail. Characterization of the trafficking patterns of the Patched and Smoothened proteins has demonstrated that these two proteins function very differently from the previously established models. The Fused kinase has been demonstrated to phosphorylate the kinesin-like protein Costal2 and the sites identified, while Cubitus interruptus has been shown to be phosphorylated in a hierarchical manner by three different kinases. Finally, the interactions, both genetic and physical, between Fused, Costal2, Cubitus interruptus, and Suppressor of Fused have been further elucidated.

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Abbreviations
Ci	Cubitus interruptus
CK1	casein kinase 1
cmn	central missing
CORD	cos2 responsive domain of ci
Cos2	Costal2
Disp	Dispatched
dPKA	*Drosophila* protein kinase A
Fu	Fused
GAS-1	growth arrest specific 1
GPI	glycophosphatidyl-inositol
GSK3	glycogen synthase kinase 3
Hh	Hedgehog
Hip1	Hedgehog interacting protein 1
HSPG	heparan sulphate proteoglycan
opb	open brain
Ptc	Patched
Shh	Sonic hedgehog
sit	Sightless
ski	Skinny hedgehog
Slimb	Supernumerary limbs
Smo	Smoothened
Su(fu)	Suppressor of Fused
zw3/sgg	*zeste white 3/shaggy*

Introduction
Studies in both vertebrates and invertebrates have identified proteins of the Hedgehog (Hh) family of secreted signaling molecules as key organizers of tissue patterning. Initially discovered in *Drosophila*, Hh family members have been discovered in animals with body plans as diverse as mammals, insects, and echinoderms [1]. Hh proteins mature through a novel mechanism involving the addition of two hydrophobic moeities (see below). Hh signal transduction also utilizes an unusual mechanism involving novel cytoplasmic and membrane components. A 'standard' model of the accepted mechanism of Hh signal transduction is presented in Figure 1. In this review, we discuss recent advances in determining the mechanism of Hh signaling, focusing especially on studies done in the past year. We do not discuss the many biological functions of the Hh signal transduction pathway. (For a recent and exhaustive review of the patterning effects of Hh, see McMahon *et al.* [2].)

Hedgehog modification, secretion, and movement
The Hh family of molecules are secreted proteins that undergo several post-translational modifications to gain full activity. Hh molecules undergo a maturation process in which they autocatalytically cleave, generating an N-terminal polypeptide (Hh-Np) containing all the signaling functions, and a C-terminal polypeptide that appears to have no function other than catalyzing the autoproteolytic cleavage. During the autoproteolytic cleavage, a cholesterol moeity is covalently attached to the last amino acid of the N-terminal fragment. This hydrophobic cholesterol moeity is thought to bind Hh to cell membranes. The hydrophobicity of the the the Hh molecule is further increased by the addition of a palmitoyl group to the cysteine immediately following the signal peptide cleavage site (see [1,3] for recent reviews).

Despite the intensive study of Hh, the roles of the cholesterol and palmitic acid adjuncts are still not clear. Forms of *Drosophila* Hh in which the C-terminus has been deleted (refered to as Hh-Nu), have a much more potent and seemingly longer range of signaling activity in the wing imaginal disc of *Drosophila* than the cholesterol-modified forms, which led to the idea that the cholesterol molecule acts as an anchor to cell membranes and limits the spread of Hh [4,5]. However, several recent papers examining the function of this cholesterol adjunct in vertebrate Sonic hedgehog (Shh) indicate that it may actually be required to spread Shh activity rather than anchor it one place. Lewis *et al.* [6••] have used gene targeting to replace the endogenous Shh allele of mice with a Shh-N form which would not be cholesterol modified (Shh-Nu). They then compared the phenotype of mice heterozygous for this allele and either a wild type or a Shh null allele. Given the phenotype of *Drosophila* Hh-Nu expression, one might predict that Shh-Nu would produce longer range Shh signaling than the native cholesterol-modified form. Surprisingly, the Shh-Nu form actually had a more restricted range of signaling when compared with the wild type. Shh has been

Figure 1

The 'Standard' Model of Hh signal transduction. This model, which is not intended to be complete, outlines most of the previously accepted features of Hh signal transduction. The multiple-pass, transmembrane proteins Patched (Ptc) and Smoothened (Smo) interact with one another at the cell surface and Ptc inhibits the positive signaling activity of Smo. The Costal2 (Cos2), Fused (Fu), and Cubitus interruptus (Ci) proteins are bound together in a high molecular weight protein complex which is attached to microtubules. Cos2 is a kinesin-like microtubule motor protein with a N-terminal motor domain; Fused is a serine/threonine kinase; and Ci is a zinc-finger transcription factor. In the absence of Hh stimulation, the complex is bound to microtubules and Ci is cleaved into a smaller N-terminal fragment called Ci75 which moves to the nucleus and represses Hh target genes. Upon secretion, Hh binds to Ptc and relieves the inhibitory effect that Ptc normally has on Smo. Once Smo is freed of the inhibitory effects of Ptc, Smo then signals through unknown mechanisms to the Fu/Cos2/Ci complex, causing hyperphosphorylation of Fu and Cos2 and causing the complex to loosen its hold on microtubules. This leads to the stabilization of full length Ci, which can then travel to the nucleus and function as a transcriptional activator, upregulating transcription of Hh target genes. Also involved
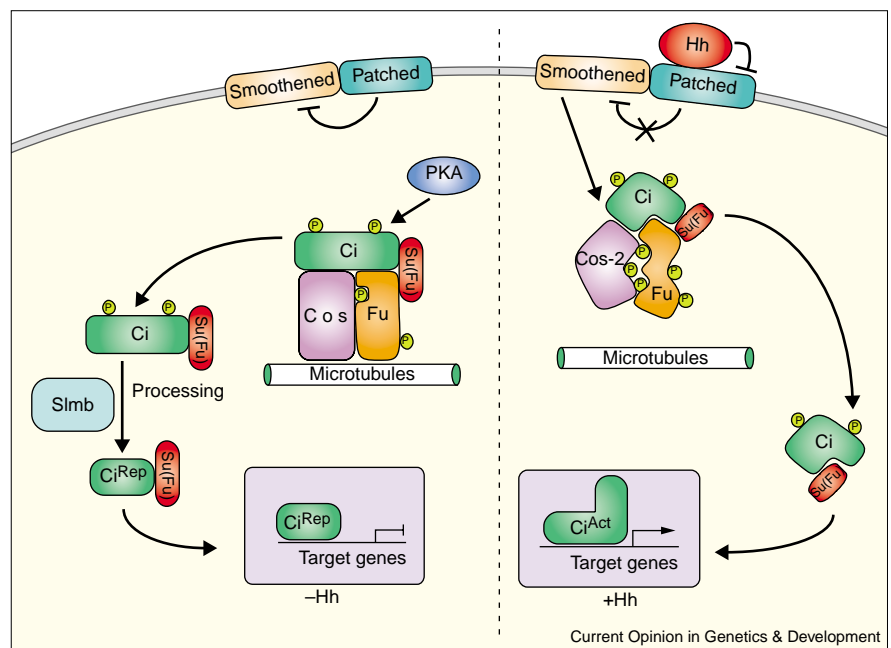
in Hh signaling are Suppressor of Fused (Su[Fu]), a protein which binds Fu and Ci and which appears to be involved in retaining Ci in the cytoplasm, and Supernumerary limbs (Slmb, an F-box protein), an F-box protein involved in proteasomal targetting of Ci.

Finally, Drosophila Protein Kinase A phosphorylates Ci on several sites and these phosphorylation events are required for the cleavage of Ci into Ci75 (reviewed in [1]). Phosphates are represented by yellow circles with a 'P'.

shown to act both at short and long distances in vertebrate tissue, being responsible for the induction of posterior limb identities at short distance in the embryonic limb and for the induction of anterior limb identities at long distance. Shh-Nu/Shh[null] heterozygotes exhibited loss of anterior limb stuctures while retaining posterior limb structures. Furthermore, the anterior extent of expression of numerous Shh target genes was reduced in these heterozygotes. These data indicate that the cholesterol moiety is, in fact, required for long-distance signaling in the vertebrate limb.

A recent study by Zeng *et al*. [7••] has suggested how cholesterol modification of Shh may function in long-range Shh signaling. Using a sensitive alkaline phosphatase induction assay, a diffusible form of Shh that is cholesterol modified, but which seems to occur in relatively small amounts, was identified. The authors demonstrate that this diffusible, cholesterol-modified form of Hh is multimeric and very potent in their cell culture assays. They also demonstrate that cholesterol-modified Shh appears to be required for formation of the secreted Shh multimers (referred to as s-ShhNp); gel filtration chromatography of s-ShhNp and Shh-N revealed that Shh-N ran as a monomer or dimer whereas s-ShhNp (the cholesterol modified form) migrated at roughly six times the molecular weight of ShhNp alone [7••]. Hence, the reduction in Shh signaling in mice heterozygous for the Shh-Nu knock-in and the Shh null allele could be a result of the lack of

generation of this multimeric form. One unanswered question, however, is why this multimeric, secreted form appears to be so rare and difficult to detect when most, if not all, Shh appears to be cholesterol modified. Perhaps only a small portion of Shh is converted to the multimeric, secreted form and this multimeric Shh is highly unstable or is rapidly degraded after binding to Ptc. It is also possible that the multimeric form is only responsible for a subset of Hh signaling activities whereas the non-multimeric form is responsible for the remaining Hh-signaling duties.

The disparity between the results of Hh-Nu expression in flies and in vertebrates could be explained if there were inhibitors of Hh signaling found in vertebrates that are not found in flies. One such inhibitor, Hip1 (Hedgehog interacting protein 1), is already known. Hip1 was isolated as a Hh-interacting protein from a mouse cDNA library and is found in vertebrates but not in flies [8]. Hip1 encodes a membrane-bound glycoprotein that binds Shh and is expressed in regions adjacent to, but not overlapping, regions of Shh expression. Hip1 appears to be a negative regulator of Shh signaling as overexpression of Hip1 in transgenic mice reduces the effective range of Shh signaling [8]. Recently, another protein, called GAS-1 (growth arrest specific 1), was also identified as a Shh-binding protein that inhibits the Shh signaling pathway [9]. GAS-1 is a 45 kDa glycophosphatidyl-inositol (GPI)-linked protein that has no identifiable domains (other than the GPI linkage)

Figure 2

A schematic depiction of some of the roles that newly identified components of the Shh-signaling pathway could play in Shh sequestration and/or reception in vertebrate tissues. Both Hip1 and GAS-1 appear to be involved in sequestering Shh and attenuating its signaling in Shh-responsive cells. This attenuation of Shh signaling is speculatively illustrated as Hip1 and GAS-1 binding of Shh at the surface of the receiving cell which then prevents Shh binding to Ptc or prevents endocytosis of a Shh–Ptc complex thus preventing signaling. The large glycoprotein megalin, which is involved in control of endocytosis, has also been shown to bind Shh and could function as a new Hh coreceptor (not illustrated) or could be involved in bringing Shh into the receiving cell by endocytosis and then presenting Shh to Ptc in intracellular vesicles. Caveolae, and the caveolin 1 protein which coat caveolae, may play a role in Shh uptake as Ptc has been shown to interact with caveolin 1 in transfection studies. HSPGs appear to act as coreceptors for Shh, optimizing the Shh signal in certain contexts, and could act either on the surface of the cell or inside vesicles. Rab23, a member of the Rab family of vesicular transport proteins, may also regulate Shh signaling. As illustrated here, Rab23 could affect Shh signaling by regulating endocytosis of Shh–Ptc complexes from the cell surface, or by regulating vesicles containing the Shh–Ptc or Shh–megalin complexes once they are inside the cell. As most of the interactions depicted here are newly characterized, the roles each may play in Shh signaling are necessarily speculative.
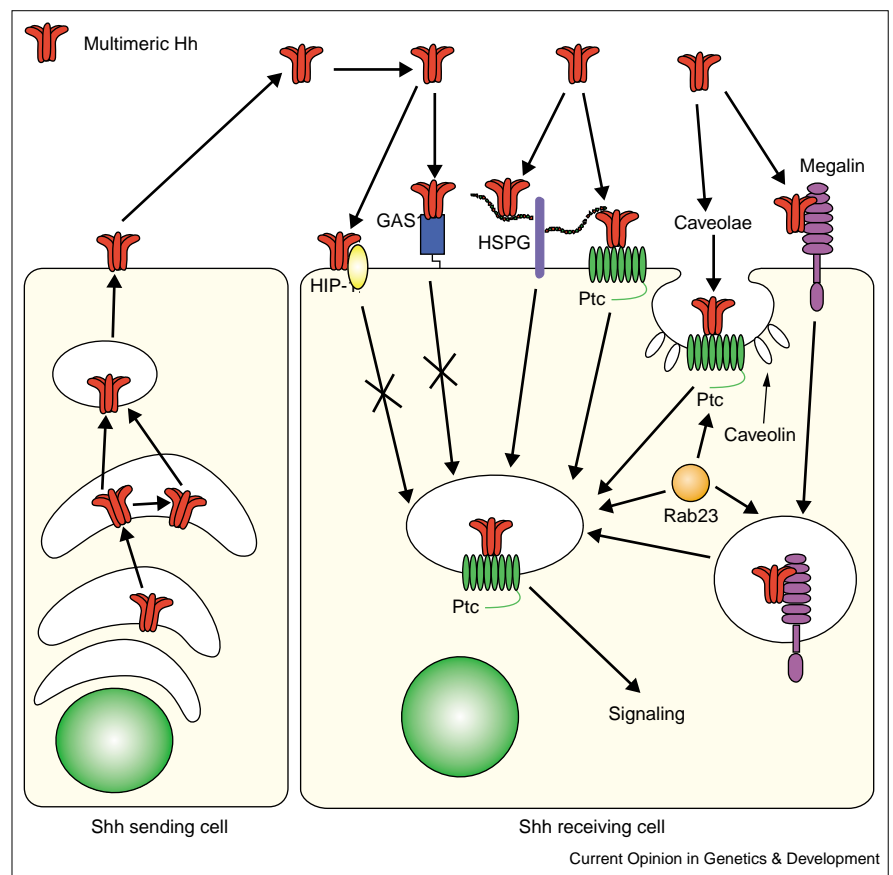
but was originally identified by its ability to arrest the cell cycle when overexpressed [10–13]. GAS-1's interaction with Shh is dependent on GAS-1's N-glycosylation sites, indicating that glycosylation may modulate GAS-1's binding to Hh. Lee *et al.* [9] also show that GAS-1 is a Wnt-inducible gene and is expressed in regions of the developing mouse that do not express Shh, but that are responsive to Shh (see Figure 2). This suggests that GAS-1 is not involved in production or emission of Shh, but rather in attenuating the signal [9]. If Hip1 and GAS-1 are involved in limiting the range of Hh signaling, then Hip1 and GAS-1 null mutant mice should display phenotypes similar to those seen when Shh is ectopically expressed.

To further complicate the issue of the role of Hh-binding proteins in Hh signaling, the large glycoprotein megalin has recently been identified as a Shh-binding protein. Megalin is a multi-ligand-binding protein of the low-density lipoprotein (LDL) receptor superfamily whose function has principally been examined in the context of the regulation of endocytosis [14]. The binding between Shh and megalin appears quite strong and specific as it is inhibited by megalin-blocking antibodies and by RAP

(receptor-associated protein), a specific inhibitor of megalin–ligand interactions [15]. What role megalin's binding to Shh could play in signal transduction is not clear. It is unlikely that megalin is a Shh receptor independent of Patched (Ptc). Megalin could, however, function as a coreceptor for Shh, and so would join the heparan sulphate proteoglycans (HSPGs) in this role [16]. Alternatively, megalin could be required for endocytosis of Shh and delivery of Shh to Ptc in intracellular vesicular pools (Figure 2). More detailed examinations of Shh localization in megalin wild-type and mutant cells will be required to distinguish these posibilities.

Hh-Np peptides are also acylated by palmitic acid on their N terminus and this acylation appears to have different effects in vertebrates versus invertebrates. In flies, mutation of the cysteine to which the acyl group is attached to serine results in a non-functional Hh that acts as a dominant negative, giving a phenotype similar to Ptc overexpression [17•–19•]. In mice, however, the analogous mutant still retains significant patterning activity, again suggesting that the vertebrate and *Drosophila* Hh pathways may be differentially regulated [19•]. The gene that encodes the
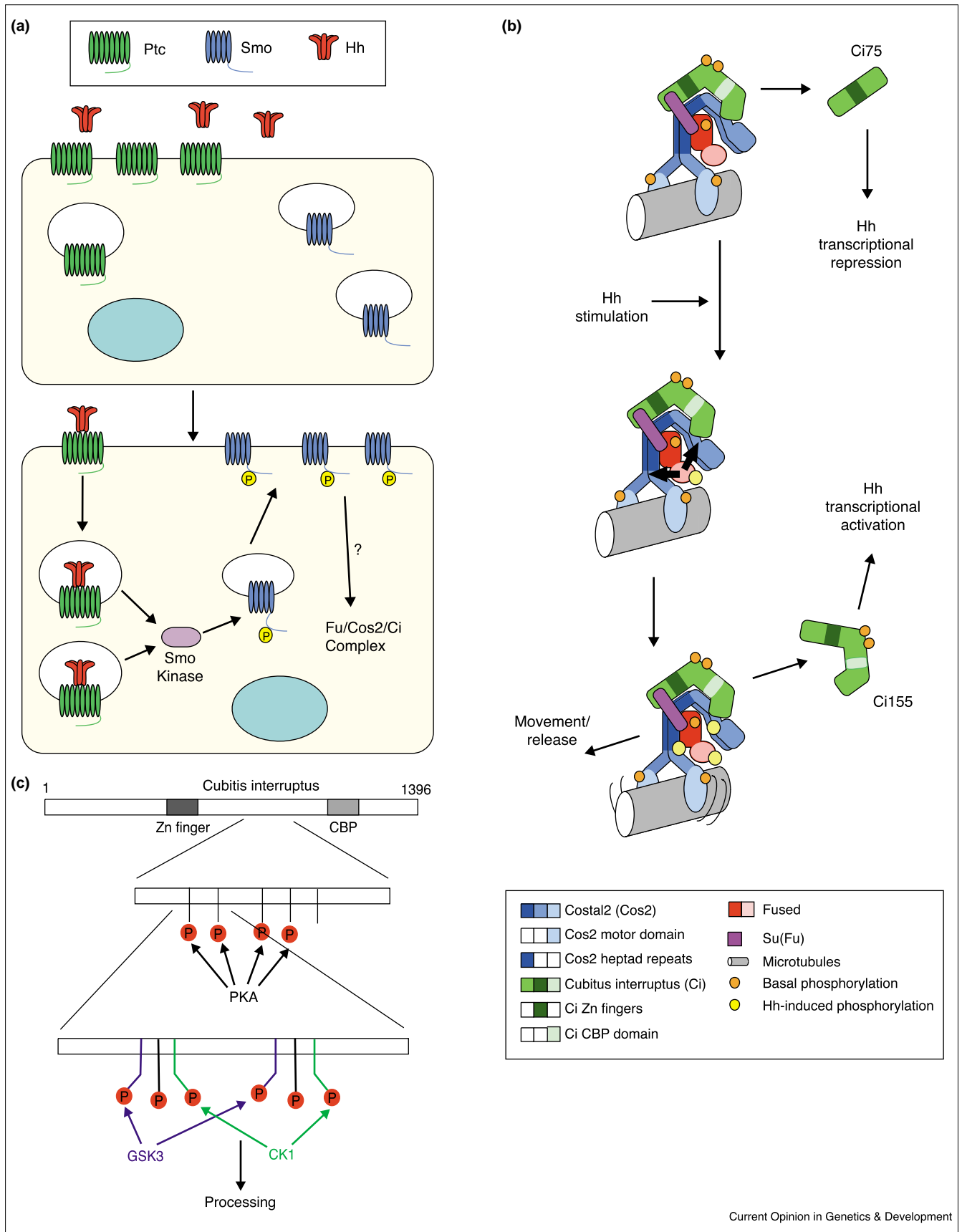
Figure 3

(a) A model of the intracellular actions of Ptc and Smo in Hh signaling. In the absence of Hh signal, Ptc is found principally on the surface of cells, while Smo is mainly found intracellularly in vesicles. In response to Hh stimulation, Ptc is taken into the cells, and this leads, indirectly, to movement of Smo to the surface of the cell and to the hyperphosphorylation of Smo through an unidentified kinase. It is not clear how closely linked the transport of Smo to the cell surface is to Smo phosphorylation. Phosphates are indicated by yellow circles labeled 'P'. (b) A schematic model of the interactions of Fu, Cos2, and Ci. In the absence of Hh signaling, Fu, Cos2, Su(Fu), and Ci are bound together in a large molecular weight protein complex that is attached to microtubules through the Cos2 motor domain. Cos2 is shown as a dimer (although this has yet to be demonstrated) and Cos2 and Fu are basally phosphorylated, as indicated by the orange phosphates. The location of the basal phosphorylation sites is not known. In the absence of Hh stimulation, Ci is cleaved to generate the Ci75 repressive N-terminal fragment of Ci. In response to Hh signaling, Fu is activated by hyperphosphorylation, possibly in its activation loop (represented by the pink region of Fu). Activation of Fu then stimulates Fu to hyperphosphorylate Cos2 at two sites: one phosphorylation site is in the region between the motor domain and the heptad repeats of Cos2 and the second in a region near the C terminus. These Hh-induced phosphorylation sites are indicated by yellow phosphates. The Fu-induced phosphoylation of Cos2 then causes the complex to either release from or move along microtubules. In an as yet undetermined manner, this leads to the prevention of Ci cleavage and the accumulation of full-length Ci which can then activate Hh target gene transcription. (c) An outline of the course of Ci phosphorylation in the absence of Hh stimulation. Ci is normally phosphorylated by PKA on several putative PKA phosphorylation sites found in the middle of the Ci protein. Once these sites have been phosphorylated by PKA, further phosphorylation by GSK3/shaggy and *Drosophila* CKI on serine residues adjacent to the PKA phosphorylation sites can occur. The sum of all these phosphorylation events leads to Ci processing.

Drosophila Hh acyltransferase has now been identified by four different groups and been given four different names: *sightless* (*sit*), *skinny hedgehog* (*ski*), *rasp*, and *central missing* (*cmn*) (references [18•,17•,20•] and [21•], respectively; we will refer to it as *sit*). Mutation of *sit* does not change the levels of Hh expression in the wing and eye imaginal disc but does totally inhibit the ability of Hh to induce Hh target gene transcription in the anterior compartment of the wing disc. Hopefully, identification of these acyltransferase mutants will further elucidate how these palmitic acid adjuncts regulate Hh dispersion.

In another study examining the role of Hh modification [22•], the N terminus of ShhN was modified with moieties of varying hydrophobicity to ascertain what effect these modifications would have on Shh signaling. The potency of Shh signaling in both *in vivo* and *in vitro* assays was found to increase with increasing hydrophobicity of the attached moiety, suggesting that it is the hydrophobicity rather than the chemical nature of the adduct that determines Shh's signaling ability [22•].

Mechanism of Hedgehog reception

The 'standard' model of Hh signal transduction (Figure 1), as derived from both *Drosophila* and vertebrate data, has the Hh receptor Ptc binding to and inhibiting Smoothened (Smo) in the absence of Hh, with removal of this inhibition when Hh binds to Ptc. However, this view has been challenged by several studies that point to a more complex interaction between Hh, Smo, and Ptc. Two groups have examined the localization of the Smo protein and found an inverse relationship between Smo and Ptc levels. Smo protein, although distributed throughout the wing imaginal disc, accumulated in wing compartments and clones of cells lacking Ptc protein but was reduced in cells overexpressing Ptc, even in the absence of Hh signaling [23••,24••]. Similarly, embryos mutant for Ptc or over-expressing Hh greatly upregulate the levels of Smo protein, although levels of ectopic Smo protein expressed via a *Smo* transgene are still regulated by Hh signaling [23••,24••,25•].

In addition, cell-surface levels of Smo increased in response to Hh stimulation, whereas cell-surface levels of Ptc were reduced by Hh stimulation (Figure 3a). Interestingly, Ci (Cubitus interruptus) does not affect Smo levels, whereas dPKA (*Drosophila* protein kinase A) plays a role in downregulating Smo [23••]. As these results indicate that most of Smo is probably not in the same place as Ptc within the cell, it seems unlikely that Ptc and Smo binding, if it even occurs *in vivo*, is an important event in Hh signaling. Smo is also phosphorylated in response to Hh signaling and depletion of Ptc in S2 cells by RNAi (RNA interference) caused the phosphorylation of Smo in the absence of Hh stimulus [24••]. Thus, it will now be important to determine the mechanism by which Ptc controls Smo localization and phosphorylation as it seems that the trafficking of Ptc- and Smo-containing vesicles may play an important role in Hh signaling.

Another clue as to how trafficking of Ptc could be involved in Hh signaling has come from a study that identifies caveolin as a Ptc-binding partner ([26]; Figure 2). Caveolae are non-clathrin-coated plasma membrane invaginations that are important in endocytosis and trafficking; and caveolins are the major coat proteins of caveolae. Whereas Ptc was found to interact with caveolin 1, Smo did not [26]. Although these data were obtained using transfection studies and must be verified using endogenous proteins, these data would also tend to indicate that some populations of Ptc and Smo are found in separate compartments within the cell. Endocytosis had previously been implicated in Hh signaling on the basis that Ptc accumulated on the surface of cells when *Drosophila* dynamin (encoded by the *shibire* gene) function was blocked [27,28]. Recently, dynamin-dependent endocytosis of Shh was visualized in neural plate explants and was also shown to be dependent on Ptc binding to Shh [29].

Vesicle transport was also implicated in Shh signaling when a member of the Rab superfamily of vesicle-transport proteins was found as a downstream negative regulator of

Shh signaling in the mouse [30]. Rab 23 mouse mutants, called *open brain*, partially rescue the phenotype of Shh mutant mice. As Rab 23 is expressed only in tissues fairly distant from the regions of Shh and Ptc expression in the vertebrate neural tube, it may be involved in attenuating the Shh signal by reducing the effective range of Shh, similar to the effect of GAS-1 on Shh. Determining whether Rab23 is involved in transporting Shh itself, or if it might be involved in transporting another negative regulator of Shh, such as GAS-1, will clarify its function in Shh signaling.

Functional characterization of the domains of the Ptc protein and their effects on Hh signaling has revealed differential requirements for the various parts of Ptc. Overexpression of full-length Ptc in the wing blade causes enhanced proteolysis of Ci and reduced Hh target gene expression. Expression of either the N- or C-terminal half of the Ptc protein alone has no effect in the wing blade [31]. But, surprisingly, coexpression of the two separate halves together closely mimics the phenotype caused by overexpression of the full-length Ptc protein, indicating that the two halves of Ptc can associate non-covalently and recapitulate function [32••]. This is an interesting result because several small molecule transporter proteins, including the bacterial lac permease and the yeast a factor transporter, can have their functions recapitulated by coexpressing the two halves of the protein separately [33–35]. Ptc has some homology to transporters, and this result lends further credence to the idea that Ptc could be a transporter. This same study also demonstrated that expression of a form of Ptc lacking its last 156 amino acids ectopically activates the Hh pathway, suggesting that these last amino acids are required to repress Hh signaling. Exactly how these last amino acids repress Hh signaling is not known. Similarly, mutations in the sterol-sensing domain of *Drosophila* Ptc act as dominant negatives (with respect to Ptc function), causing derepression of Hh target genes in the wing imaginal disc in spite of the fact that these mutant forms of Ptc still appear able to bind Hh [36,37]. Analogous mutations in mouse Ptc1, however, did not cause ectopic activation of Shh targets, hinting that the vertebrate and fly Ptc may function in slightly different ways [38].

Lastly, HSPGs have been shown to be required for the transmission of Hh from the posterior compartment of the *Drosophila* wing imaginal disc to the anterior compartment, where Hh acts [16,39]; but the region of the Hh protein required for interaction with HSPGs had not been identified. A heparan binding motif in the N terminus of Shh has now been identified and shown by mutational analysis to be required for binding of Shh to HSPGs in the granule cells of the mouse cerebellum but not for binding of Shh to Ptc. Interaction of Shh with HSPGs in the cerebellum appears to occur only at certain times in the course of cerebellar development, being required for proliferation later but not earlier. The times that Shh interact with HSPGs parallels the expression in the cerebellum of two of the exostosins,

the proteins that polymerize heparan sulphate addition to protein cores [40•]. These data imply that HSPGs synthesized by the exostosins directly bind Shh and control cell proliferation.

Progress in understanding the cytoplasmic transduction of the Hedgehog signal

Several recent studies have shed further light on how Fused (Fu), Costal2 (Cos2), Ci, and Suppressor of Fused (Su[fu]) function in Hh signaling. Although Fu kinase activity is known to be required for Hh signaling, the substrates of Fu have remained uncharacterized [1]. One potential target of Fu is Cos2. Cos2 and Fu have been shown to bind each other directly and a fragment of Fu lacking the kinase domain acts as a dominant inhibitor of Hh signaling when overexpressed in a wild-type background [1,41]. As Cos2 is hyperphosphorylated in response to Hh signaling, Cos2 is an obvious candidate for a Fu kinase substrate. It has now been demonstrated that Fu, in fact, phosphorylates Cos2 at two different positions: one in the region between the motor domain and the heptad repeats and another in the C-terminal domain of Cos2 (Figure 3b) [42•]. These phosphorylation sites are the same as those induced by Hh stimulation, suggesting that Hh stimulation activates Fu which then phosphorylates Cos2 [42•]. What role each of these Cos2 phosphorylations plays in modulating Cos2 function is not clear but it is tempting to speculate that either one or both phosphorylation events regulates the binding of the Fu/Cos2/Ci complex to microtubules or the movement of the complex along microtubules. Phosphorylation of Fu also increases in response to Hh signaling [43], but it is not known if this phosphorylation of Fu stimulates its kinase activity. Mutational analysis of threonine residues in the putative activation loop of Fu has demonstrated that these residues are required for Fu transcriptional activation ability in a cell-based assay but these sites have not been shown to be phosphorylated [44]. Identification of the phosphorylation sites of Fu will help determine the kinase that activates Fu and how Ptc and Smo control this kinase.

Recent studies have also clarified how Ci processing is regulated in *Drosophila*. Drosophila protein kinase A (dPKA) can phosphorylate Ci *in vitro* and is required for processing of Ci155 into Ci75 *in vivo* [45–47]. Two groups have now found that phosphorylation of Ci by dPKA primes Ci for further phosphorylation by two other kinases, glycogen synthase kinase 3 (GSK3) and casein kinase 1 (CK1) [48••,49••]. These kinases can phosphorylate Ci, but only after PKA phosphorylation of Ci, and all of these phosphorylation events appear to be required for the processing of Ci155 to Ci75 to occur (Figure 3c). Furthermore, the *Drosophila* GSK3 encoded by the *zeste white 3/shaggy* (*zw3/sgg*) gene is required for proteolysis of Ci as mutants in *zw3/sgg* result in the accumulation of full length Ci and mild activation of downstream Hh targets [48••,49••]. In *Drosophila*, GSK3 was first identified as a member of the Wg signal transduction cascade were it plays a similarly

vital role in phosphorylating the beta-catenin, Armadillo (Arm), and targeting it for degradation. Hence, these studies suggest that GSK3 plays a role in regulating the proteolysis of transcriptional effectors in several signaling pathways.

The effects of Fu, Cos2, and Su(fu) on Ci processing, localization, and activation have recently been clarified. Loss of *cos2* and *dPKA* had previously been shown to cause accumulation of uncleaved Ci [50,51], and it has now been demonstrated that the uncleaved Ci found in cells mutant for these genes is competent to activate transcription of Hh target genes if normal Fu is present [52•,53•]. Loss of Fu also causes an accumulation of full-length Ci, but this Ci is not competent to activate Hh target genes [50,54]. Interestingly, *cos2*, *fu*, and *dPKA* activity are also required to generate the repressive form of Ci, Ci75 [52•]. Expression of forms of Ci in which the putative PKA phosphorylation sites have been mutated or in which the cleavage site has been deleted result in ectopic activation of Hh target genes. These forms of Ci are not completely independent of Hh signaling for activation of target gene transcription as loss of *smo* and/or *fu* activity inhibits the ectopic activation [52•,53•]. These papers also demonstrate that Su(fu) regulates retention of Ci in the cytoplasm, as loss of Su(fu) causes accumulation of Ci in the nucleus. However, most of these experiments depend on the presence of a compound called Leptomycin B, which prevents nuclear export, to show nuclear localization of Ci. Because it has been very difficult to show nuclear localization of endogenous Ci by either biochemical or immunofluorescence means, it is not clear if these experiments reflect a genuine mechanism of Ci regulation. In the future, it will be interesting to determine if there are any nuclear export mutants that affect Hh signaling.

In constrast to the studies in which Su(fu) is found to play a role in retaining Ci in the cytoplasm, a recent study has suggested that Su(fu) might actually play a more direct role in modulating transcription by Ci. Previous work had shown that vertebrate Su(fu) homologs can enhance the binding of Gli proteins, the vertebrate homologs of Ci, to DNA [55]. Cheng and Bishop now [56] show that vertebrate Su(fu) physically interacts with SAP18, a component of the histone deacetylase complex Sin3, and represses transcription of Gli reporter constructs by recruiting the mouse Sin3 complex to promoters containing Gli-binding elements.

Cos2 has also been shown to bind Ci in addition to Fu [43,51]. A study utilizing glutathione S-transferase (GST) pulldowns and yeast two hybrid assays has been used to map the domains of interaction between Fu, Cos2, and Ci. This study reaffirmed several of the known interactions between these proteins and also identified new sites of Fu interaction with Cos2 and regions of Ci interaction with Fu and Cos2 [57]. More detailed structure–function studies of Cos2 and Ci using transgenic flies have now mapped the Cos2-interacting domain of Ci to a region between amino acids 941 and 1065 which has been termed the CORD domain [53•].

Conclusions

Linking the various intracellular members of the Hh signaling pathway into a coherent circuit has begun to yield a more detailed framework of the mechanisms that transduce the Hh signal. However, there are still many questions that remain. How Hh is secreted and what controls Hh movement within cells and between tissues are issues that require further investigation. Future work should also allow us to bridge the gaps in the pathway that remain between Ptc and Smo and between Smo and the Fu/Cos2/Ci complex. More detailed molecular descriptions of the function and localization of all of the Hh signaling proteins will also aid in clarifying Hh signaling. The advent of more sensitive mass spectrometry techniques will allow characterization of proteins binding to known Hh signaling proteins, whereas techniques such as fluorescence resonance energy transfer will allow subcellular localization of Hh signaling events. Every new discovery in the Hh signaling field reminds us of just how unusual and interesting this pathway is and promises to be.

Acknowledgements

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