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The Hedgehog Signal Transduction Network

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Abstract

Hedgehog (Hh) proteins regulate the development of a wide range of metazoan embryonic and adult structures, and disruption of Hh signaling pathways results in various human diseases. Here, we provide a comprehensive review of the signaling pathways regulated by Hh, consolidating data from a diverse array of organisms in a variety of scientific disciplines. Similar to the elucidation of many other signaling pathways, our knowledge of Hh signaling developed in a sequential manner centered on its earliest discoveries. Thus, our knowledge of Hh signaling has for the most part focused on elucidating the mechanism by which Hh regulates the Gli family of transcription factors, the so-called “canonical” Hh signaling pathway. However, in the past few years, numerous studies have shown that Hh proteins can also signal through Gli-independent mechanisms collectively referred to as “noncanonical” signaling pathways. Noncanonical Hh signaling is itself subdivided into two distinct signaling modules: (i) those not requiring Smoothened (Smo) and (ii) those downstream of Smo that do not require Gli transcription factors. Thus, Hh signaling is now proposed to occur through a variety of distinct context-dependent signaling modules that have the ability to crosstalk with one another to form an interacting, dynamic Hh signaling network.

Introduction

The Hedgehog (Hh) family of proteins plays an evolutionarily conserved, instructional role during the development of many meta-zoans (1, 2). Hh also plays an important role in the maintenance of many adult structures that include proliferating cell populations, perhaps by regulating the various progenitor cells that give rise to these structures (3, 4). The specific roles Hh plays in these processes are diverse because it functions as a morphogen, a mitogen, a survival factor, and even as a guidance molecule, depending on the context (1). Given these important physiological roles, it was of no surprise that disruption of Hh signaling was found to be the underlying cause of numerous human developmental disorders and that perturbations of Hh signaling play a causative or facilitating role in many human cancers (5–7). Driven mostly by the important role Hh plays in cancer, many laboratories

and pharmaceutical companies have focused on identifying and clinically developing small-molecule Hh inhibitors (8, 9). Several such inhibitors are now in clinical trials, with the most advanced compound having been recently approved for use in advanced basal cell carcinoma patients (10). This considerable influence on human health has resulted in an explosion of Hh research over the past decade, which should continue into the foreseeable future.

Hh was originally identified as a gene important for proper segmental identity during embryonic development in the fruit fly *Drosophila melanogaster* (11, 12). Many other genes important for the control of embryonic segmental development were identified in a similar fashion. A subset of these segment polarity genes were later implicated in the Hh signal transduction pathway, in which they were organized into a basic signaling scheme on the basis of their genetic epistatic relationships (13). Additional work in *Drosophila* extended the analyses of this nascent signaling pathway beyond embryonic development, including the development of the imaginal discs that give rise to the various adult *Drosophila* structures (14–19). This work, particularly that involving mosaic analyses of wing, leg, and eye imaginal discs, both confirmed and extended the analyses of this signaling pathway in embryos (20). There are three Hh family members in vertebrates: (i) Sonic hedgehog (Shh), (ii) Desert hedgehog (Dhh), and (iii) Indian hedgehog (Ihh) (1). Whereas Dhh is the closest homolog to *Drosophila* Hh, Shh and Ihh are more closely related to one another than they are to fly Hh.

The discovery of mechanisms governing the processing, delivery, and signal transduction of Hh established many precedents in biology, several of which are probably attributable to the hydrophobic nature of Hh proteins (21, 22). *Hh* transcripts are translated into proteins of ~45 kD. Consistent with being secreted, Hh proteins have a distinct amino-terminal (N-terminal) signal sequence that is removed during its trafficking to the plasma membrane to yield its full-length form. Hh proteins are typically processed to yield a lipid-modified signaling ligand of ~19 kD that exhibits biological activity in a non-cell autonomous manner and can act directly on the cell in which it was made or on cells neighboring or far from its source. Various models exist to explain the ability of these hydrophobic proteins to signal over many cell diameters and have been extensively reviewed elsewhere (23–27). Here, we focus on the mechanism (or mechanisms) of signal transduction initiated upon binding of Hh to its receptor and present a model that consolidates various disparate aspects of this signaling pathway. Similar to the elucidation of many other signaling pathways, our knowledge of Hh signaling developed in a sequential manner centered on its earliest discoveries. Thus, our knowledge of Hh signaling has for the most part been focused on elucidating the mechanism by which Hh regulates the activity of members of the Gli family of transcription factors—the canonical Hh signaling pathway. In the past few years, numerous studies have shown that Hh proteins can also signal through Gli-independent mechanisms, which we collectively refer to as “noncanonical” Hh signaling pathways (28, 29). Noncanonical Hh signaling can itself be subdivided into two distinct signaling modules: those not requiring Smo (Type I) and those downstream of Smo that do not require Gli transcription factors (Type II).

Hh Receptors

Patched (Ptc) functions as the core Hh receptor, binding to Hh ligands with an affinity in the low nanomolar range (30, 31). The identification of Ptc as a Hh receptor was supported by *Drosophila* genetics, which had already implicated *Ptc* as a pivotal regulator of Hh signaling that functioned downstream of Hh but upstream of the other known signaling components (13, 32–34). The predicted membrane topology of Ptc suggests 12 transmembrane domains and two large extracellular loops. On the basis of this topology, and its primary sequence, Ptc is most homologous to the resistance-nodulation-division (RND) family of bacterial

transporters (35). These transporters are found almost exclusively in bacteria, in which they function to pump lipophilic toxins and heavy metals out of the cell in a manner driven by a proton gradient. Although there are few examples of such gradients in eukaryotic cells, mutating highly conserved amino acids in Ptc that are absolutely required for RND transporter activity attenuates Ptc activity (36). Ptc also contains a sterol-sensing domain, an ~180-amino-acid motif found in many cholesterol-binding proteins (37–40). Missense mutations that alter conserved amino acids within the sterol-sensing domain of Ptc affect its ability to regulate the expression of Hh target genes without affecting its ability to bind Hh. Besides binding Hh to initiate signaling, Ptc also modulates the extracellular gradient of Hh observed in a developing field of cells and does so in a manner that is functionally distinct from its ability to modulate Hh signal transduction (41). Mammals have a second distinct *Ptc* gene, *Ptc2*, which encodes a slightly smaller protein with topology and domains similar to Ptc1 (42–44). *Ptc1* and *Ptc2* expression patterns show some overlap but also display many expression domains with minimal overlap. Transcription of *Ptc* genes is directly regulated by Gli transcription factors, and both *Ptc1* and *Ptc2* are up-regulated in response to Hh (45–47).

Several other Hh binding proteins have now been shown to function as Hh coreceptors (48). Furthermore, although many of these co-receptors are highly conserved between mammals and *Drosophila*, the ability of these homologs to bind Hh proteins is regulated in distinct ways in different classes of animals. The first identified Hh co-receptor was isolated in a screen that used a library of small interfering RNAs (siRNAs) to modulate Hh signaling in a *Drosophila* cell line and was called interference hedgehog (Ihog) for this reason (49). Ihog functions as a positive regulator in Hh signaling because decreased Ihog function reduced Hh signaling. A homologous protein, brother of Ihog (Boi), was also identified in *Drosophila*. Ihogs are single-pass transmembrane proteins that contain a small nonconserved intracellular domain and an extracellular domain characterized by four immunoglobulin-like domains and two fibronectin type III (FN3) domains. This topology is reminiscent of other extracellular ligand receptors, providing the initial insight that Ihogs might function as coreceptors for Hh. Ihog associates with both Hh and Ptc through its FN3 domains, with the first FN3 domain binding to Hh and the second FN3 domain associating with Ptc (50, 51). The FN3 domains of Ihog were unable to associate directly with purified Hh in vitro unless heparin was included, suggesting that heparin is necessary for formation of the *Drosophila* Hh receptor complex in vivo. The phenotype of *Drosophila* lacking either *Ihog* or *Boi* is relatively mild, but flies lacking both gene products display a severe reduction of Hh signaling in embryos and defects in Hh-regulated development of numerous adult structures (51). Thus, Boi functions redundantly with Ihog in the fly, forming discrete receptor complexes that bind Hh through one FN3 domain and interact with Ptc through the other FN3 domain, in order to initiate the Hh signaling cascade.

Two vertebrate orthologs of Ihog have been identified, cell adhesion molecule down-regulated by oncogenes (Cdo) and brother of Cdo (Boc), which function as co-receptors for Hh proteins in a manner similar to that of Ihogs (49, 52–54). Mice lacking either *Cdo* or *Boc* are viable but display phenotypes consistent with minor disruptions in Hh signaling (52–58). Mice lacking both *Cdo* and *Boc* exhibit more severe Hh loss-of-function phenotypes, which is consistent with their acting in a redundant fashion. However, the phenotypes of mice lacking both *Cdo* and *Boc* indicated that signaling was not completely abrogated in some Hh-dependent structures. This residual Hh activity was provided by a third, vertebrate-specific, co-receptor, growth arrest-specific gene 1 (Gas1) (55–57, 59). Gas1 has little primary sequence homology to Ihogs and lacks a true transmembrane domain, associating with membranes through a glycosylphosphatidylinositol anchor (48, 60). Similar to what was observed between Ihog and Hh, the purified Hh binding domains of the mammalian co-receptors were unable to bind mammalian Hh proteins. Further, unlike what was observed

with the *Drosophila* proteins, the addition of heparin did not increase the binding affinity between the vertebrate Hh proteins and their co-receptors (61). Instead, it was noted that the direct association between Hh and the vertebrate co-receptors required high concentrations of calcium ions (Ca^{2+}). When Shh was cocrystallized with the various co-receptor Hh-binding domains, it was found that Shh coordinates two Ca^{2+} molecules along the surface that binds to the various co-receptors. The binding affinity of Shh for Cdo or Boc increased dramatically in the presence of Ca^{2+} , and mutation of those amino acids in Shh responsible for Ca^{2+} coordination reduced its affinity for co-receptor binding. Missense mutations of the same amino acids were identified as the underlying cause of two distinct, inherited developmental disorders, highlighting the importance of this Ca^{2+} -binding surface (61). Another mechanistic difference between *Drosophila* and vertebrate Hh co-receptors is the presence of a third FN3 domain in the vertebrate co-receptors (53, 61). Unexpectedly, although the first FN3 domains of Cdo and Boc share extensive homology with the first, Hh-binding FN3 domain of Ihog and Boi, Cdo and Boc associated with Shh through the third FN3 domain rather than through the first FN3 domain. The identification of a functionally inactive Shh mutant that can bind to Ptc1 but is unable to bind to Cdo, Boc, or Gas1 illustrated the importance of co-receptor formation for signaling (57). Missense mutations in *Cdo* or *Gas1* were also identified in patients with holoprosencephaly, a developmental disorder previously shown to result from decreased Hh activity (54, 58, 62). These mutant gene products displayed a reduced association with Ptc1 but still bound Shh similarly to the wild-type co-receptors, providing genetic support for the importance of Hh co-receptors during human development (57, 62, 63).

Smoothed

Drosophila Smoothed (*Smo*) encodes a protein predicted to belong to the seven-transmembrane G protein-coupled receptor (GPCR) superfamily (64). Activation of Smo occurs in two steps: translocation of Smo from intracellular vesicles to the cell surface and subsequent phosphorylation. Translocation of Smo to the cell surface in an inactive conformation can be induced by the Smo inhibitor cyclopamine, but not by other Smo inhibitors, suggesting that translocation alone is not sufficient for activation. Unlike the majority of these family members, Smo contains a distinctively long carboxyl-terminal (C-terminal) domain (C-tail) that is absolutely necessary for Hh-dependent signaling (64–66). This domain contains four conserved clusters of basic amino acids embedded within a region containing six Ser-Thr phosphorylation sites. *Drosophila* Smo exists as a constitutive multimer through homomeric association of its N-terminal regions, and Hh binding to Ptc promotes phosphorylation of the C-tail, which neutralizes the net positive charge of the basic residue clusters, allowing the C-tails to move closer to one another (67, 68). Despite the low overall sequence homology between *Drosophila* and mammalian Smo, these self-interaction domains, phosphoregulatory motifs, and regulated conformational changes are conserved, and phosphorylated Smo translocates to the plasma membrane in *Drosophila* or to the plasma membrane of the primary cilium in mammals (69–72). The majority of *Drosophila* cells do not have primary cilia, but this organelle is absolutely required for Hh signaling in vertebrates. *Drosophila* Smo is phosphorylated by adenosine 3',5'-monophosphate (cAMP)-dependent protein kinase (PKA), casein kinase 1 (CK1), casein kinase 2 (CK2), and G protein-coupled receptor kinase 2 (GRK2) in the presence of Hh (73–76). In the absence of Hh, several distinct protein phosphatases—including protein phosphatase 2A (PP2A), PP1, and PP4—maintain a low steady state of Smo phosphorylation (77, 78). Differential phosphorylation of the Smo C-tail correlates with graded activation of Smo, which is consistent with such a mechanism being required to respond to distinct concentrations of Hh (67, 72, 75, 78). Phosphorylation of the C-tail also reduces the ubiquitin-activating enzyme 1 (UBA-1)-mediated ubiquitination of multiple sites within this domain, thus inhibiting endocytosis and subsequent proteasomal and

lysosomal degradation of Smo (79, 80). CK1 α , GRK2, and another still-unidentified protein kinase phosphorylate the C-tail of mammalian Smo in the presence of Hh proteins (72). Deletion of the basic amino acid motifs of mammalian Smo is sufficient to induce Smo accumulation in the primary cilia (68). GRK2-mediated phosphorylation of vertebrate Smo allows Smo to bind to β -arrestins 1 or 2 (81, 82). β -Arrestin 2 was subsequently shown to bridge Smo to the kinesin motor Kif3a to promote ciliary accumulation of Smo in mammalian cells (82).

Consistent with its predicted topology, Smo couples to a specific family of inhibitory G proteins (G_i s) to regulate Hh signaling (83, 84). Heterotrimeric G proteins are subdivided into four families according to the sequence similarity of their α subunit and the effectors they stimulate. G_i s inhibit adenylate cyclase (AC) and activate some potassium (K^+) channels and phospholipase $C\gamma$ (85). Because PKA negatively regulates the Gli family of transcription factors (86–89), it was proposed that Smo might signal through activation of G_i proteins to reduce PKA activity. Early studies used pertussis toxin (PTX) to discern whether a G_i -dependent mechanism modulated Hh signaling. All mammalian $G\alpha_i$ family members, with the exception of $G\alpha_z$ and the single *Drosophila* $G\alpha_i$ isoform, are sensitive to inhibition by PTX (90). Expression of a transgene encoding PTX in zebrafish embryos results in a phenotype similar to loss-of-function *Hh* mutations (91). This phenotype is rescued by coexpression of a construct encoding a dominant negative form of PKA, but not by coexpression of *Ihh*, suggesting that one or more G_i family members act upstream of PKA and downstream of Hh. Another study showed that expression of *Smo* in frog melanophore cells induces a change in melanosome aggregation consistent with decreased cAMP, and this aggregation is attenuated by PTX. These results suggest that Smo could induce a decrease in cAMP concentration, either directly through coupling to G_i or indirectly by promoting coupling to another GPCR (92). More recent observations have substantiated that Smo acts as a G_i -coupled GPCR in some contexts both in vertebrates and in *Drosophila* (83, 84, 90, 93, 94). To demonstrate coupling, vertebrate Smo and different combinations of transgenes encoding G protein α subunits representing the four G protein families plus the β_1 and γ_2 subunits were expressed in the Sf9 insect cell line. In these cells, which lack Ptc, Smo increased the rate of guanosine tri-phosphate (GTP) exchange of all G_i protein family members in a dose-dependent manner but had no effect on members of other G protein families (83). Moreover, three distinct Smo antagonists blocked G_i activation with a similar potency as that with which they can inhibit activation of a Gli reporter gene. Furthermore, PTX impaired Gli activation downstream of Smo in mouse fibroblast NIH3T3 cells and did so in a manner that could be rescued by a constitutively active $G\alpha_{i1}$ mutant (83). RNA interference (RNAi)-mediated knockdown of the single $G\alpha_i$ isoform produced in a *Drosophila* cell line also reduced the activation of several Hh-induced posttranslational modifications of downstream signaling components, allowing $G\alpha_i$ activity to be placed downstream of Smo but upstream of the *Drosophila* Gli family member Cubitus interruptus (Ci) (84). In addition, a substantial reduction in cAMP abundance was observed in cultured insect S2 cells within minutes of treatment with Hh and was dependent on both Smo and $G\alpha_i$. In flies, loss-of-function $G\alpha_i$ mutants also revealed phenotypes and reduced patterns of Hh target gene expression consistent with a role of $G\alpha_i$ in Hh signaling (84). In support of Hh regulating the steady-state abundance of cAMP through $G\alpha_i$, increasing cAMP production by reducing the gene dosage of the phosphodiesterase *Dunce* or by expressing a transgene encoding a viral AC enhanced *Smo* loss-of-function phenotypes (84, 95).

GPCRs are activated by binding of one or more ligands to their extracellular domain (64, 85). However, the identification of a definitive physiological ligand for Smo has proven to be elusive. An elegant study of Ptc-mediated Smo repression supports a catalytic rather than stoichiometric mechanism of action for Ptc, leading to the speculation that Ptc functions by regulating the intracellular concentration of endogenous small-molecule Smo modulators

(96). The homology of Ptc to RND proteins and the presence of a sterol-sensing domain in Ptc suggested that such a modulator could be a sterol or lipid. Numerous groups have now identified distinct classes of oxysterol Smo modulators, a subset of which activates Smo and a subset of which inhibits it. *Ptc1*-transfected cells secrete 3 β -hydroxysteroid (provitamin D₃), which inhibits Smo function. Inhibiting the synthesis of provitamin D₃ impairs Ptc1-mediated repression of Smo (97). Two independent groups identified oxysterol agonists of Smo (98, 99), showing that inhibition of cholesterol biosynthesis abolishes activation of Gli proteins in medulloblastoma cells and that this could be rescued by the addition of cholesterol or various oxysterol (OHC) metabolites, including 20 α -OHC, 22(*S*)-OHC, 24-OHC, and 25-OHC. Furthermore, these oxysterols were potent Smo-dependent stimulators of maximal Gli-dependent transcription. More recently, 20(*S*)-OHC has been shown to bind directly to Smo and activate Gli-dependent transcription synergistically with the Smo agonist (SAG), suggesting positive allosteric modulation of Smo by oxysterols (100). However, another report proposed that Ptc represses Smo activity by promoting a reduction in the abundance of phosphatidylinositol-4-phosphate (PI4P) (101). That study showed that Smo activation in *Drosophila* is dependent on the concentration of PI4P and that loss of *Ptc* results in increased concentration of PI4P. Consistent with this suggestion, PI4P also regulates the membrane translocation and activation of Smo. Moreover, depletion of PI4P kinase in NIH3T3 cells also impairs activation of a Gli-luciferase reporter gene by Shh (101).

Gli Family of Transcription Factors

Ultimately, *Drosophila* Smo modulates the abundance and activity of the transcription factor Ci to modulate the expression of a specific set of Hh target genes (102) (Fig. 1A). Ci belongs to the Gli family of zinc finger transcription factors (103, 104) and contains an N-terminal transcriptional repressor domain and a C-terminal transcriptional activator domain (102). In the absence of Hh, Ci exists in both a full-length form with low intrinsic activation activity (Ci_{FL}) and a proteolytically processed form, lacking the C-terminal activator domain, which functions solely as a transcriptional repressor (Ci_R) (105). Upon Hh stimulation, the processing of Ci_{FL} into Ci_R is attenuated, thus allowing Ci_{FL} to accumulate and to be further modified into its most active form, Ci_A (106, 107). For the most part, Ci_R and Ci_{FL} bind to the same sites in the promoters of Hh target genes to maintain a low steady-state of Hh target gene regulation, even in the absence of Hh (108–110).

The abundance of these various forms of Ci is regulated by the ubiquitin proteasome system, which targets Ci_{FL} for ubiquitination through a series of self-contained degradation domains known as degrons (111, 112). Ubiquitination of proteins is controlled by a series of enzymatic activities (E1 to E4), with the specificity of ubiquitination dictated by a large family of E3 ubiquitin ligases (113, 114). Ci stability is controlled by two multiprotein E3 ligases: (i) the Skp1-Cullin1 (Cul1)-F-box (SCF) complex and (ii) the Cul3-HIB complex, which includes the scaffolding protein Cul3 and the ubiquitin ligase HIB (Hh-induced MATH and BTB domain-containing protein), which is also known as SPOP (Speckle-type POZ protein) (112, 115). In the absence of Hh, SCF promotes the processing of Ci_{FL} into Ci_R (116). The Cul1 degrons of Ci consist of Ser- and Thr-rich stretches of amino acids that require sequential phosphorylation by PKA, glycogen synthase kinase-3 (GSK3), and CK1 for recognition of Ci_{FL} by Cul1 (86–88, 117–121). Increased phosphorylation of Ci_{FL} renders it a better substrate for the F-box protein Slimb, which acts as a substrate recognition subunit to target Ci_{FL} for ubiquitination (116). This ubiquitination of Ci renders it a substrate for the proteasome. However, unlike most proteasome substrates, Ci is not completely proteolyzed. Instead, only the C-terminal half of Ci_{FL} is proteolyzed, thus yielding the truncated Ci_R form. This unusual partial processing of Ci_{FL} requires its zinc finger region and Lys750 (122). In cells exposed to high concentrations of Hh, Ci_A is quite

labile (106) and is completely proteolyzed through a mechanism linked to its transcriptional activity. This complete degradation of Ci_A is mediated by two distinct N- and C-terminal degrons that are targeted by HIB (115, 123, 124). Further, HIB acts as a feedback regulator of Ci activity, because Hh stimulation up-regulates expression of the gene encoding HIB. Thus, in response to moderate amounts of Hh, the loss of Ci_R and accumulation of Ci_{FL} results in moderate amounts of Ci transcriptional regulatory activity, and in response to large amounts of Hh, the short-lived, highly active Ci_A form is produced.

Mammals possess three Ci homologs—Gli1, Gli2, and Gli3—and the combinatorial activity of these regulate a diverse set of Hh target genes (2). Like Ci, Gli activity is controlled through a combination of regulated expression, protein stability, and modulation of specific activities, much of which is directed by a diverse array of posttranslational modifications. Whereas Gli1 acts only as a transcriptional activator, Gli2 is the principal Hh-regulated transcriptional activator (Gli_A), and Gli3 is the principal Hh-regulated repressor (Gli_R) (89, 125–138). In the absence of Hh signaling, Gli2_{FL} and Gli3_{FL} are partially proteolyzed in a manner that removes and degrades the C-terminal activation domain, yielding proteins that are essentially transcriptional repressors (Gli_R). In the presence of Hh, this proteolytic processing is blocked, allowing Gli2_{FL} and Gli3_{FL} to accumulate and be subsequently converted into potent, nuclear-enriched transcriptional activators (Gli_A). The transcription of *Gli1* increases in response to Hh (127, 139), and increased abundance of Gli1 is a widely used bio-marker for increased canonical Hh signaling activity (140). Thus, the proteolytic processing, stability, and transcriptional potency of the three Gli transcription factors combine to regulate basal and Hh-stimulated expression of Hh-responsive genes and do so in a manner that varies with the amount of Hh to which cells are exposed. Many of the proteins that play pivotal roles in regulating the activity, nuclear localization, or stability of Ci are conserved in mammals (Fig. 1B) (2, 7). Remarkably, though, the relative importance of these orthologous Gli modulators in Hh signaling varies between classes of animals, differing between flies, fish, and mammals (141–144).

Canonical Hh Signaling

Drosophila Smo regulates the activity and amounts of Ci through a series of membrane-associated protein complexes that contain the protein kinase Fused (Fu), the kinesin-related protein Costal2 (Cos2), and a genetic suppressor of Fu called Suppressor of Fused (Sufu) (145). Cos2 serves as a scaffold for Fu, Sufu, Ci, and Smo enrichment (146–150). Cos2 also associates with many of the protein kinases and phosphatases that regulate Ci activity in the absence of Hh (118) and subsequently regulate Smo activity in the presence of Hh (78, 151). Fu regulates Ci activity primarily through phosphorylation-mediated regulation of Cos2 and Sufu activity (150, 152–163). A Cos2-nucleated complex associates with Smo on vesicular membranes in the absence of Hh and releases Ci upon Hh stimulation (147–150). Cos2 associates with Smo in two distinct regions, one of which reflects constitutive binding and one that is highly regulated by Hh, in a manner that may be regulated by Fu kinase activity (146, 150, 161, 162, 164). However, the estimated 12-fold stoichiometric excess of Cos2 over Smo suggests that, at steady state, the bulk of Cos2 in cells is not associated with Smo (165). These other Cos2 complexes associate with the microtubule cytoskeleton in highly motile vesicles whose motility is dependent on microtubules, adenosine 5'-triphosphate (ATP), and the adenosine triphosphatase (ATPase) domain of Cos2 (157). In response to Hh stimulation, the pool of Cos2 associated with Smo moves from intracellular vesicular membranes to the plasma membrane (147, 149). As Smo abundance is stabilized in response to Hh (71), an increase in the Smo-associated Cos2 complex is also observed. Smo can regulate the majority of the Cos2 complexes (166, 167), presumably by interacting with these Cos2 complexes in a dynamic manner that allows the bulk of Cos2 to interact with Smo at the plasma membrane.

The *Fu* gene encodes a protein with an N-terminal serine-threonine protein kinase domain (168) and a large noncatalytic C-terminal domain (the Fu tail) (169) that serves as a scaffold for many protein-protein interactions (155). Fu plays a role in converting Ci_{FL} into both Ci_A and Ci_R. Whereas the kinase activity of Fu is required for Ci_A formation and to attenuate the processing of Ci_{FL} to Ci_R (106, 161–163, 170, 171), the processing of Ci_{FL} to Ci_R requires only the Fu tail (163, 169, 172). Targeting Fu to the plasma membrane renders it constitutively active (173), but the regulation of Fu activity is complex, as is its genetics, which is consistent with its pivotal role in regulating the functionally distinct forms of Ci (14). In the absence of Hh, the two domains of Fu self-associate to generate a form that interacts with Cos2 (172). In response to Hh, Fu forms a constitutively active dimer that is phosphorylated both within the kinase activation loop and the Fu tail domain, and these phosphorylation events are likely mediated by both autophosphorylation and by CK1 (161–163). Furthermore, Fu phosphorylation increases in response to increasing Hh, promoting Fu-mediated phosphorylation of Cos2, Sufu, and perhaps an additional undetermined substrate (161–163). As well as functioning downstream of Smo, Fu may also act in a positive feedback loop to further activate Smo (151, 173). Although Fu is absolutely required in *Drosophila* Hh signaling, its role in mammalian Hh signaling is less clear—possibly because of its redundancy with other Hh-regulated protein kinases (143, 174, 175).

Originally identified as a genetic suppressor of the phenotype resulting from decreased *Fu* function (176), Sufu has proven to be one of the more enigmatic components of the Hh signaling pathway (177). Contributing to our lack of understanding of Sufu is that fact that it contains few identifiable primary sequence motifs, and null mutations in *Sufu* causes only minimal effects on Hh-induced patterning in *Drosophila* (178). However, *Sufu* does play an important role in Hh signaling, albeit one that is genetically redundant with *Cos2* (153). Sufu also associates directly with Fu and Ci in a manner analogous to that of *Cos2* (152, 156). Sufu is an abundant protein that is 10- to 100-fold enriched as compared with other members of this signaling complex (165). However, when Sufu is isolated from a membrane-enriched *Cos2* signaling complex, it is present in approximately equimolar amounts with Fu, *Cos2*, and Ci (165). The reason for this vast stoichiometric excess is not clear, but may reflect its requirement for attenuating the activity of any Ci that is not complexed with *Cos2* (150) because Sufu is capable of physically sequestering Ci in the cytoplasm (170, 179–181). Although the bulk of Sufu is cytoplasmic (156), small amounts have also been observed in the nucleus (181). Thus, Sufu has been proposed to modulate the expression of Ci target genes by attenuating Ci activity. Although *Drosophila* lacking *Sufu* are viable (176), vertebrates lacking *Sufu* exhibit many developmental defects, which are severe in mice (141, 182) because Sufu plays an important functional role in controlling the stability of Gli2_{FL} and Gli3_{FL} in vertebrates (115, 183–187).

Primary Cilia and Hh Signaling

It is now clearly established that maximal vertebrate Hh signaling requires the primary cilium (188), an organelle previously thought to be vestigial (189). Most vertebrate cells contain one nonmotile primary cilium, a membrane-encased protrusion located on the apical side of polarized cells. Primary cilia are composed of nine microtubule pairs anchored to the basal body and a specialized septin-like region at the base that limits access to the body of the primary cilia. They are distinct from motile cilia, lacking both the central pair of microtubules and radial spokes required for motility, and their formation is intimately tied to the cell cycle. Trafficking through primary cilia occurs in a microtubule-dependent manner and is regulated by two large, non-membrane-bound, multiprotein complexes. The trafficking of these multiprotein complexes, from the basal body to the tip of the primary cilium and from the tip of the cilium back to the basal body, is regulated by several anterograde and retrograde molecular motors.

Ptc1 is enriched in primary cilia in the absence of Hh and moves out of cilia upon binding to Hh (190). Several other components of the Hh signaling pathway—including Smo (70, 191–193), Sufu (194), Gli2, and Gli3 (184, 195–198)—are thought to traffic into and back out of primary cilia in the absence of Hh signaling, continuously sampling the environment of this organelle (188, 199). Basal trafficking of Sufu-Gli complexes through primary cilia occurs in a Smo-independent manner (183, 186). The basal trafficking of the Sufu-Gli2_{FL} and Sufu-Gli3_{FL} complexes through cilia allows the Glis to be hyperphosphorylated at their Cul1-dependent degrons. These phosphorylation events may be regulated by the mammalian Cos2 ortholog Kif7, which acts as a scaffolding protein for PKA, GSK3, and CK1, similarly to the role of Cos2 in *Drosophila* Hh signaling. Subsequent to ubiquitination, the Sufu-Gli_{FL} association is reduced by a mechanism that is not well understood, and Gli2_{FL} and Gli3_{FL} are partially cleaved by proteasomes, such as those enriched at the basal body (183). The liberated Gli2_R and Gli3_R then leave the primary cilium and translocate to the nucleus, where they repress Hh target genes. In response to Hh, Smo becomes enriched along the membrane of the primary cilium, whereas Kif7 moves from the basal body to the tip of the cilium, and this latter translocation requires the molecular motor activity of Kif7 (183, 200). Gli2_{FL}, Gli3_{FL}, and Sufu then translocate to the tip of the primary cilium in a manner dependent on the molecular motors Kif7 and Kif3A.

The Sufu-Gli2_{FL} and Sufu-Gli3_{FL} complexes accumulate in primary cilia within minutes of exposure to Hh, and they do so independently of new protein synthesis (183). These observations suggested that the trafficking of Gli2_{FL} and Gli3_{FL} into primary cilia is an immediate and direct response to Hh signaling. Gli2_{FL} and Gli3_{FL} then become differentially phosphorylated and dissociate from Sufu. This differential phosphorylation of Gli proteins requires primary cilia trafficking. These modifications allow Gli2_{FL} and Gli3_{FL} to be converted into Gli2_A and Gli3_A, translocate from the primary cilium into the cytoplasm, then translocate into the nucleus, where they promote expression of Hh target genes. Nuclear-enriched Gli2_A and Gli3_A are quite labile and are quickly degraded by the proteasome (112, 123). Dissociation of Sufu-Gli complexes serves two purposes: (i) attenuating Gli_{FL} processing to Gli_R and (ii) promoting the formation of Gli_A. In the absence of Hh, formation of complexes with Sufu promotes processing of Glis through a Cul1 ubiquitin proteasome system. In the presence of Hh, these complexes dissociate, leading to activation of Gli2_{FL} and Gli3_{FL} and subsequent complete degradation through a Cul3 ubiquitin proteasome system. Gli_A formation occurs independently of Hh in cells lacking Sufu, and this occurs whether the cells have a primary cilium or not (184, 201). This is distinct from the Hh-independent activation observed in *Ptc1*^{-/-} mouse embryonic fibroblasts (MEFs), in which constitutive Gli activity requires the primary cilium (202). These observations suggest that the main function of the primary cilium in Hh signaling is to attenuate Sufu function. It has also been proposed that Sufu might associate with newly translated Gli1, which would attribute to primary cilia an additional level of control over Gli1 activity (183).

Type I Noncanonical Hh Signaling

Ptc1 can function as a dependence receptor (Fig. 2), regulating cell survival in a ligand-dependent manner (203), as illustrated by the finding that overexpression of *Ptc1* induces apoptosis in a variety of ex vivo and in vitro assays (204). Like other dependence receptors, Ptc1 contains a dependence-associated receptor C-terminal motif that is cleaved by caspases at a conserved aspartic acid (Asp¹³⁹²) in the absence of Shh, to expose a proapoptotic domain (204, 205). Mutation of this cleavage site (Ptc1^{D1392N}) abolished Ptc1-induced cell death, and this mutant receptor acted as a dominant negative inhibitor of endogenous Ptc1-induced apoptosis. Furthermore, a C-terminally truncated Ptc1 mutant that mimics caspase-cleaved Ptc1 induced cell death regardless of the presence of Shh, demonstrating the

functional importance of caspase cleavage. Ptc1 associates with a multiprotein complex that is required for induction of cell death, and its association with this proapoptotic complex was disrupted in the presence of Hh (206). All three vertebrate Hh ligands exhibited an antiapoptotic effect in cultured cells, which was not blocked by various Smo antagonists, was not mimicked by *Smo* overexpression or the Smo agonist SAG, and was independent of Gli transcriptional activity (207). Thus, these findings suggest that this signaling mechanism was dependent on both Hh and Ptc1 but independent of Smo.

Canonical Hh signaling plays an essential role in cell proliferation through induction of the genes encoding cyclin D1 and N-Myc (208–210). Interestingly, a Ptc1-dependent noncanonical signaling activity also regulates the cell cycle through modulation of the subcellular localization of cyclin B1. Ptc1 is a binding partner of phosphorylated cyclin B1, and their association is regulated at the G2/M checkpoint (211, 212). *Ptc1* ablation or Shh binding to Ptc1 disrupted this interaction, allowing nuclear translocation of cyclin B1 and subsequent completion of mitosis (Fig. 2). Conversely, in the absence of Shh, overexpression of *Ptc1* in human embryonic kidney 293 T cells resulted in redistribution of cyclin B1 from the nucleus to the cytoplasm and decreased cell proliferation. A truncated mutant form of Ptc1 that is unable to interact with cyclin B1 was identified in a subset of basal cell carcinomas, and epidermal ablation of *Ptc1* in mice increased nuclear cyclin B1, which is consistent with cyclin B1 driving mitosis in these Hh-dependent tumors (213). Interestingly, in response to Shh, GRK2 could displace cyclin B1 in its association with Ptc1 (214). Furthermore, RNAi-mediated knockdown of GRK2 abrogated the Shh-induced redistribution of cyclin B1 to the nucleus, and this effect could be rescued by expressing wild-type *GRK2*. Taken together, these results suggest that Shh stimulation induces a conformational change in Ptc1 that increases its affinity for GRK2 at the expense of its interaction with cyclin B1. The direct association of cyclin B1 and GRK2 with Ptc1 and the modulation of this association by Shh suggest that this mechanism does not require Smo.

Type II Noncanonical Hh Signaling

A large number of studies have implicated a noncanonical Hh signaling pathway in regulating the actin cytoskeleton through activation of the Rho family of small GTPases, such as RhoA and Rac1 (Fig. 3). In cultured endothelial cells, all Hh ligands promote actin stress fiber formation and tubulogenesis in a Smo-dependent manner through activation of RhoA (207, 215). In fibroblasts, stimulation of RhoA and Rac1 by Smo is required for migration in response to Shh (216). Pharmacological analysis has demonstrated that stress fiber formation and tubulogenesis depend on Smo- and G_i-mediated activation of RhoA. Activation of small GTPases by Hh proteins is not limited to endothelial cells and fibroblasts. For example, Shh increases the number of dendritic spines in hippocampal neurons, but apparently through an alternative mechanism (217). This latter work suggested that inactive Smo prevents Rac1 activation by interacting with the Rac guanine nucleotide exchange factor (GEF) T-lymphoma invasion and metastasis 1 (Tiam1). This Smo-Tiam1 complex dissociates upon Shh-mediated activation of Smo, thus allowing Tiam1 to activate Rac1. The rapid time course of these responses, the lack of detectable Gli-dependent transcriptional activity, and the inability of Gli3_R to prevent these responses suggest that this is a Gli-independent Hh signaling pathway. Thus, there is considerable evidence supporting Smo-dependent, Gli-independent, noncanonical Hh signaling mediated by activation of small GTPases.

It is now well established that Shh can also act as an axon guidance cue (218). Shh signaling acts as an attractive cue for commissural axons and a repulsive cue for retinal axons during embryonic development. Commissural axons extend from the dorsal neural tube and project toward and then across the floor plate guided by the concerted effects of several molecular

cues. Shh acts in a Smo-dependent manner as a chemoattractant in this process: Cultured commissural neurons turn their axons toward a source of Shh within minutes of exposure, and this response requires the presence of Smo (220). This rapid response provided the first suggestion that Shh-induced axon turning would not require transcription. Consistent with this signaling mechanism being independent of Gli-mediated transcriptional regulation, expression of *Gli3R* did not attenuate Shh-induced axon turning. Instead, Shh rapidly and locally stimulated phosphorylation of the Src family kinase (SFK) members Src and Fyn in a Smo-dependent fashion. Inhibition of SFK activity, through the use of a pharmacological inhibitor or expression of a negative regulator of Src, completely blocked Shh-induced axon turning. Thus, the control of commissural axon guidance is mediated through a Smo-dependent, Gli-independent, noncanonical Hh pathway. Additional support for this process occurring through a noncanonical signaling pathway include the failure to identify Src and Fyn in a series of cell-based screens for canonical Hh signaling components (49, 219, 220) and observations that *Src* and *Fyn* double-mutant mice do not exhibit phenotypes consistent with deregulated canonical Hh signaling (221). The role of Src and Fyn in Shh-mediated axon guidance also agrees with a large body of evidence that shows other axon guidance molecules mediating actin cytoskeletal changes through SFKs (222–224). In vitro studies showed that in contrast to commissural neurons, the retinal ganglion cells turned away from a source of Shh (225). Shh elicited a decrease in cAMP concentration within these growth cones (226, 227), and this effect required the presence of Smo. Both the reduction in cAMP and the retraction of growth cones occurred transiently 15 to 30 min after the addition of Shh. This rapid response to Shh appears incompatible with a mechanism requiring Gli-mediated transcriptional regulation but is consistent with Shh activating G_i proteins, which in turn inhibit AC to reduce axonal cAMP.

Calcium ions regulate a vast number of processes in neuronal and neuronal precursor cells, including proliferation, differentiation, apoptosis, and migration (228, 229). Recently, Shh was shown to induce a dose-dependent increase in Ca²⁺ spikes in neural tube explants (230). This effect was mimicked by treatment with a Smo agonist and attenuated by cotreatment with a Smo antagonist or pretreatment with PTX, suggesting that Smo activation in this context promoted the activity of a PTX-sensitive G_i protein. Furthermore, this work suggested that the Gβγ subunits released upon G_i activation activated phospholipase C-γ (PLC-γ) to produce inositol 3-phosphate (IP₃), which would subsequently increase intracellular Ca²⁺ abundance. Consistent with this hypothesis, inhibitors of PLC-γ and of IP₃ receptors reduced Ca²⁺ spike activity. In addition, acute stimulation of Smo with SAG resulted in sequential IP₃ and Ca²⁺ transients in the primary cilia of neurons, and Smo antagonists abolished this effect. Although the involvement of Gli-dependent transcription was not formally ruled out in these studies, the rapid activation of these responses is inconsistent with a transcription-based mechanism. This provocative finding suggests that Hh signaling might regulate a cohort of other physiological processes through Ca²⁺-dependent signaling pathways.

Hh Signaling Networks—A Consolidated View

Historically, many of the components initially proposed as constituting the signaling pathway for a newly discovered ligand have depended on how the initial pieces of that puzzle were identified and on the scientific interests of the individuals assembling the puzzle. Thus, whether a researcher is, for example, a geneticist, a pharmacologist, or a biochemist will influence the experimental approaches and therefore what will eventually come to be known as the “canonical” signaling pathway for that ligand. Eventually, however, other researchers with different backgrounds and other model systems will begin to uncover context-specific modifications of this “canonical” signaling pathway. Further, some of these context-specific differences may eventually coalesce into “noncanonical”

signaling pathways. Differences and similarities between distinct experimental models will also contribute to how these signaling modules are viewed, although context-specific signaling variations may also confound such analyses. And as is the case with all scientific disciplines, the difficulty in interpreting new findings as they are reported in any rapidly moving field will distort many of these models—at least temporarily. To date, many of these issues have also played key roles in the genesis of our understanding of Hh signaling. An additional hurdle imposed on these models of Hh signaling has been the discovery that primary cilia are of central importance for Hh signaling in mammals, but they are irrelevant to Hh signaling in *Drosophila* (231).

When Hh signaling is examined from a global perspective, several generalities emerge, many of which also apply to other signaling pathways. Hh signaling should be viewed as a signaling network rather than as a simple linear pathway (Fig. 4). This network has multiple distinct signaling modules that can function independently or in an overlapping manner, depending on cellular context. Signaling through the network may branch into different modules at the level of Ptc, in a manner that may or may not be dependent on various Ptc coreceptors. Another pivotal branch point for these signaling modules is Smo, which can regulate both G protein and non-G protein-mediated signaling modules. Last, regulation of the stability and activity of Gli transcription factors by Hh proteins serves as the dominant transcriptional nexus of Hh activity. These major signaling modules thus combine in different ways to initiate signaling upon Hh binding to Ptc. However, once this signaling network is initiated, a series of additional feedback, feedforward, and crosstalk mechanisms serve to further modulate this network. Because the early elucidation of the core Hh signal transduction components was dominated by geneticists, in many cases these latter signaling events have been difficult to separate from the initial events in Hh signaling because of the involvement of multiple feedback and feedforward regulatory mechanisms. One could also envision cellular contexts in which initial early events induced by Hh signaling are very rapid and, therefore, transcription-independent but are followed by a series of transcription-dependent events that are subject to additional feedback and feedforward regulation. Such temporally regulated signaling modules might also begin to explain the slow rate of Gli target gene expression, which appears slower than one might posit, given the rapid rates of early developmental processes regulated by Hh. This is especially true when comparing Gli target gene expression to the immediate-early genes transcribed in response to serum growth factors (232). Thus, as our knowledge of Hh signaling matures, we anticipate a clearer understanding of the spatial, temporal, and cell-contextual regulation of the various signaling modules that comprise the Hh signaling network.

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References and Notes

1. Ingham PW, McMahon AP. Hedgehog signaling in animal development: Paradigms and principles. *Genes Dev.* 2001; 15:3059–3087. [PubMed: 11731473]
2. Hui CC, Angers S. Gli proteins in development and disease. *Annu. Rev. Cell Dev. Biol.* 2011; 27:513–537. [PubMed: 21801010]
3. Beachy PA, Karhadkar SS, Berman DM. Tissue repair and stem cell renewal in carcinogenesis. *Nature.* 2004; 432:324–331. [PubMed: 15549094]
4. Ruiz i Altaba A. Hedgehog signaling and the Gli code in stem cells, cancer, and metastases. *Sci. Signal.* 2011; 4:pt9. [PubMed: 22114144]

5. Bale AE. Hedgehog signaling and human disease. *Annu. Rev. Genomics Hum. Genet.* 2002; 3:47–65. [PubMed: 12142354]
6. Curran T, Ng JM. Cancer: Hedgehog's other great trick. *Nature.* 2008; 455:293–294. [PubMed: 18800119]
7. Teglund S, Toftgård R. Hedgehog beyond medulloblastoma and basal cell carcinoma. *Biochim. Biophys. Acta.* 2010; 1805:181–208. [PubMed: 20085802]
8. Rubin LL, de Sauvage FJ. Targeting the Hedgehog pathway in cancer. *Nat. Rev. Drug Discov.* 2006; 5:1026–1033. [PubMed: 17139287]
9. Ng JM, Curran T. The Hedgehog's tale: Developing strategies for targeting cancer. *Nat. Rev. Cancer.* 2011; 11:493–501. [PubMed: 21614026]
10. Guha M. Hedgehog inhibitor gets landmark skin cancer approval, but questions remain for wider potential. *Nat. Rev. Drug Discov.* 2012; 11:257–258. [PubMed: 22460111]
11. Nüsslein-Volhard C, Wieschaus E. Mutations affecting segment number and polarity in *Drosophila*. *Nature.* 1980; 287:795–801. [PubMed: 6776413]
12. Martinez-Arias A, Lawrence PA. Parasegments and compartments in the *Drosophila* embryo. *Nature.* 1985; 313:639–642. [PubMed: 3919303]
13. Forbes AJ, Nakano Y, Taylor AM, Ingham PW. Genetic analysis of hedgehog signalling in the *Drosophila* embryo. *Dev. Suppl.* 1993; 1993:115–124. [PubMed: 8049467]
14. Ogden SK, Ascano M Jr, Stegman MA, Robbins DJ. Regulation of Hedgehog signaling: A complex story. *Biochem. Pharmacol.* 2004; 67:805–814. [PubMed: 15104233]
15. Sánchez L, Guerrero I. The development of the *Drosophila* genital disc. *Bioessays.* 2001; 23:698–707. [PubMed: 11494318]
16. Kalderon D. Morphogenetic signalling. Responses to hedgehog. *Curr. Biol.* 1995; 5:580–582. [PubMed: 7552160]
17. Blair SS. Compartments and appendage development in *Drosophila*. *Bioessays.* 1995; 17:299–309. [PubMed: 7741723]
18. Basler K, Struhl G. Compartment boundaries and the control of *Drosophila* limb pattern by hedgehog protein. *Nature.* 1994; 368:208–214. [PubMed: 8145818]
19. Domínguez M, Brunner M, Hafen E, Basler K. Sending and receiving the hedgehog signal: Control by the *Drosophila* Gli protein Cubitus interruptus. *Science.* 1996; 272:1621–1625. [PubMed: 8658135]
20. Hooper JE, Scott MP. Communicating with Hedgehogs. *Nat. Rev. Mol. Cell Biol.* 2005; 6:306–317. [PubMed: 15803137]
21. Farzan SF, Singh S, Schilling NS, Robbins DJ. The adventures of sonic hedgehog in development and repair. III. Hedgehog processing and biological activity. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2008; 294:G844–G849. [PubMed: 18239057]
22. Mann RK, Beachy PA. Novel lipid modifications of secreted protein signals. *Annu. Rev. Biochem.* 2004; 73:891–923. [PubMed: 15189162]
23. Chuang PT, Kornberg TB. On the range of hedgehog signaling. *Curr. Opin. Genet. Dev.* 2000; 10:515–522. [PubMed: 10980429]
24. Goetz JA, Suber LM, Zeng X, Robbins DJ. Sonic Hedgehog as a mediator of long-range signaling. *Bioessays.* 2002; 24:157–165. [PubMed: 11835280]
25. Kornberg TB. Barcoding Hedgehog for intracellular transport. *Sci. Signal.* 2011; 4:pe44. [PubMed: 22114141]
26. Guerrero I, Chiang C. A conserved mechanism of Hedgehog gradient formation by lipid modifications. *Trends Cell Biol.* 2007; 17:1–5. [PubMed: 17126548]
27. Gallet A. Hedgehog morphogen: From secretion to reception. *Trends Cell Biol.* 2011; 21:238–246. [PubMed: 21257310]
28. Jenkins D. Hedgehog signalling: Emerging evidence for non-canonical pathways. *Cell. Signal.* 2009; 21:1023–1034. [PubMed: 19399989]
29. Brennan D, Chen X, Cheng L, Mahoney M, Riobo NA. Noncanonical Hedgehog signaling. *Vitam. Horm.* 2012; 88:55–72. [PubMed: 22391299]

30. Marigo V, Davey RA, Zuo Y, Cunningham JM, Tabin CJ. Biochemical evidence that patched is the Hedgehog receptor. *Nature*. 1996; 384:176–179. [PubMed: 8906794]
31. Stone DM, Hynes M, Armanini M, Swanson TA, Gu Q, Johnson RL, Scott MP, Pennica D, Goddard A, Phillips H, Noll M, Hooper JE, de Sauvage F, Rosenthal A. The tumour-suppressor gene patched encodes a candidate receptor for Sonic hedgehog. *Nature*. 1996; 384:129–134. [PubMed: 8906787]
32. Hooper JE, Scott MP. The *Drosophila* patched gene encodes a putative membrane protein required for segmental patterning. *Cell*. 1989; 59:751–765. [PubMed: 2582494]
33. Nakano Y, Guerrero I, Hidalgo A, Taylor A, Whittle JR, Ingham PW. A protein with several possible membrane-spanning domains encoded by the *Drosophila* segment polarity gene patched. *Nature*. 1989; 341:508–513. [PubMed: 2797178]
34. Ingham PW, Taylor AM, Nakano Y. Role of the *Drosophila* patched gene in positional signalling. *Nature*. 1991; 353:184–187. [PubMed: 1653906]
35. Routh MD, Zalucki Y, Su C-C, Long F, Zhang Q, Shafer WM, Yu EW. Efflux pumps of the resistance-nodulation-division family: A perspective of their structure, function, and regulation in gram-negative bacteria. *Adv. Enzymol. Relat. Areas Mol. Biol.* 2011; 77:109–146. [PubMed: 21692368]
36. Taipale J, Cooper MK, Maiti T, Beachy PA. Patched acts catalytically to suppress the activity of Smoothened. *Nature*. 2002; 418:892–897. [PubMed: 12192414]
37. Johnson RL, Zhou L, Bailey EC. Distinct consequences of sterol sensor mutations in *Drosophila* and mouse patched homologs. *Dev. Biol.* 2002; 242:224–235. [PubMed: 11820817]
38. Kuwabara PE, Labouesse M. The sterol-sensing domain: Multiple families, a unique role? *Trends Genet.* 2002; 18:193–201. [PubMed: 11932020]
39. Martín V, Carrillo G, Torroja C, Guerrero I. The sterol-sensing domain of Patched protein seems to control Smoothened activity through Patched vesicular trafficking. *Curr. Biol.* 2001; 11:601–607. [PubMed: 11369205]
40. Strutt H, Thomas C, Nakano Y, Stark D, Neave B, Taylor AM, Ingham PW. Mutations in the sterol-sensing domain of Patched suggest a role for vesicular trafficking in Smoothened regulation. *Curr. Biol.* 2001; 11:608–613. [PubMed: 11369206]
41. Chen Y, Struhl G. Dual roles for patched in sequestering and transducing Hedgehog. *Cell*. 1996; 87:553–563. [PubMed: 8898207]
42. Motoyama J, Heng H, Crackower MA, Takabatake T, Takeshima K, Tsui LC, Hui C. Overlapping and non-overlapping Ptch2 expression with Shh during mouse embryogenesis. *Mech. Dev.* 1998; 78:81–84. [PubMed: 9858693]
43. Motoyama J, Takabatake T, Takeshima K, Hui C. Ptch2, a second mouse Patched gene is co-expressed with Sonic hedgehog. *Nat. Genet.* 1998; 18:104–106. [PubMed: 9462734]
44. Zaphiropoulos PG, Undén AB, Rahnama F, Hollingsworth RE, Toftgård R. PTCH2, a novel human patched gene, undergoing alternative splicing and up-regulated in basal cell carcinomas. *Cancer Res.* 1999; 59:787–792. [PubMed: 10029063]
45. Marigo V, Scott MP, Johnson RL, Goodrich LV, Tabin CJ. Conservation in hedgehog signaling: Induction of a chicken patched homolog by Sonic hedgehog in the developing limb. *Development*. 1996; 122:1225–1233. [PubMed: 8620849]
46. Marigo V, Tabin CJ. Regulation of patched by sonic hedgehog in the developing neural tube. *Proc. Natl. Acad. Sci. U.S.A.* 1996; 93:9346–9351. [PubMed: 8790332]
47. Goodrich LV, Johnson RL, Milenkovic L, McMahon JA, Scott MP. Conservation of the hedgehog/patched signaling pathway from flies to mice: Induction of a mouse patched gene by Hedgehog. *Genes Dev.* 1996; 10:301–312. [PubMed: 8595881]
48. Beachy PA, Hymowitz SG, Lazarus RA, Leahy DJ, Siebold C. Interactions between Hedgehog proteins and their binding partners come into view. *Genes Dev.* 2010; 24:2001–2012. [PubMed: 20844013]
49. Lum L, Yao S, Mozer B, Rovescalli A, Von Kessler D, Nirenberg M, Beachy PA. Identification of Hedgehog pathway components by RNAi in *Drosophila* cultured cells. *Science*. 2003; 299:2039–2045. [PubMed: 12663920]

50. Yao S, Lum L, Beachy P. The ihog cell-surface proteins bind Hedgehog and mediate pathway activation. *Cell*. 2006; 125:343–357. [PubMed: 16630821]
51. Zheng X, Mann RK, Sever N, Beachy PA. Genetic and biochemical definition of the Hedgehog receptor. *Genes Dev*. 2010; 24:57–71. [PubMed: 20048000]
52. Okada A, Charron F, Morin S, Shin DS, Wong K, Fabre PJ, Tessier-Lavigne M, McConnell SK. Boc is a receptor for sonic hedgehog in the guidance of commissural axons. *Nature*. 2006; 444:369–373. [PubMed: 17086203]
53. Tenzen T, Allen BL, Cole F, Kang JS, Krauss RS, McMahon AP. The cell surface membrane proteins Cdo and Boc are components and targets of the Hedgehog signaling pathway and feedback network in mice. *Dev. Cell*. 2006; 10:647–656. [PubMed: 16647304]
54. Zhang W, Kang JS, Cole F, Yi MJ, Krauss RS. Cdo functions at multiple points in the Sonic Hedgehog pathway, and Cdo-deficient mice accurately model human holoprosencephaly. *Dev. Cell*. 2006; 10:657–665. [PubMed: 16647303]
55. Allen BL, Tenzen T, McMahon AP. The Hedgehog-binding proteins Gas1 and Cdo cooperate to positively regulate Shh signaling during mouse development. *Genes Dev*. 2007; 21:1244–1257. [PubMed: 17504941]
56. Allen BL, Song JY, Izzi L, Althaus IW, Kang JS, Charron F, Krauss RS, McMahon AP. Overlapping roles and collective requirement for the coreceptors GAS1, CDO, and BOC in SHH pathway function. *Dev. Cell*. 2011; 20:775–787. [PubMed: 21664576]
57. Izzi L, Lévesque M, Morin S, Laniel D, Wilkes BC, Mille F, Krauss RS, McMahon AP, Allen BL, Charron F. Boc and Gas1 each form distinct Shh receptor complexes with Ptch1 and are required for Shh-mediated cell proliferation. *Dev. Cell*. 2011; 20:788–801. [PubMed: 21664577]
58. Zhang W, Hong M, Bae GU, Kang JS, Krauss RS. Boc modifies the holoprosencephaly spectrum of Cdo mutant mice. *Dis. Model Mech*. 2011; 4:368–380. [PubMed: 21183473]
59. Kavran JM, Ward MD, Oladosu OO, Mulepati S, Leahy DJ. All mammalian Hedgehog proteins interact with cell adhesion molecule, down-regulated by oncogenes (CDO) and brother of CDO (BOC) in a conserved manner. *J. Biol. Chem*. 2010; 285:24584–24590. [PubMed: 20519495]
60. Lee CS, Buttitta L, Fan CM. Evidence that the WNT-inducible growth arrest-specific gene 1 encodes an antagonist of sonic hedgehog signaling in the somite. *Proc. Natl. Acad. Sci. U.S.A.* 2001; 98:11347–11352. [PubMed: 11572986]
61. McLellan JS, Zheng X, Hauk G, Ghirlando R, Beachy PA, Leahy DJ. The mode of Hedgehog binding to Ihog homologues is not conserved across different phyla. *Nature*. 2008; 455:979–983. [PubMed: 18794898]
62. Bae GU, Domené S, Roessler E, Schachter K, Kang JS, Muenke M, Krauss RS. Mutations in CDON, encoding a hedgehog receptor, result in holoprosencephaly and defective interactions with other hedgehog receptors. *Am. J. Hum. Genet*. 2011; 89:231–240. [PubMed: 21802063]
63. Martinelli DC, Fan CM. A sonic hedgehog missense mutation associated with holoprosencephaly causes defective binding to GAS1. *J. Biol. Chem*. 2009; 284:19169–19172. [PubMed: 19478089]
64. Kristiansen K. Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: Molecular modeling and mutagenesis approaches to receptor structure and function. *Pharmacol. Ther*. 2004; 103:21–80. [PubMed: 15251227]
65. van den Heuvel M, Ingham PW. smoothened encodes a receptor-like serpentine protein required for hedgehog signalling. *Nature*. 1996; 382:547–551. [PubMed: 8700230]
66. Alcedo J, Ayzenzon M, Von Ohlen T, Noll M, Hooper JE. The *Drosophila* smoothened gene encodes a seven-pass membrane protein, a putative receptor for the hedgehog signal. *Cell*. 1996; 86:221–232. [PubMed: 8706127]
67. Fan J, Liu Y, Jia J. Hh-induced Smoothened conformational switch is mediated by differential phosphorylation at its C-terminal tail in a dose- and position-dependent manner. *Dev. Biol*. 2012; 366:172–184. [PubMed: 22537496]
68. Zhao Y, Tong C, Jiang J. Hedgehog regulates smoothened activity by inducing a conformational switch. *Nature*. 2007; 450:252–258. [PubMed: 17960137]
69. Wang Y, Zhou Z, Walsh CT, McMahon AP. Selective translocation of intracellular Smoothened to the primary cilium in response to Hedgehog pathway modulation. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:2623–2628. [PubMed: 19196978]

70. Corbit KC, Aanstad P, Singla V, Norman AR, Stainier DY, Reiter JF. Vertebrate Smoothened functions at the primary cilium. *Nature*. 2005; 437:1018–1021. [PubMed: 16136078]
71. Deneff N, Neubüser D, Perez L, Cohen SM. Hedgehog induces opposite changes in turnover and subcellular localization of patched and smoothened. *Cell*. 2000; 102:521–531. [PubMed: 10966113]
72. Chen Y, Sasai N, Ma G, Yue T, Jia J, Briscoe J, Jiang J. Sonic Hedgehog dependent phosphorylation by CK1a and GRK2 is required for ciliary accumulation and activation of smoothened. *PLoS Biol*. 2011; 9:e1001083. [PubMed: 21695114]
73. Jia H, Liu Y, Xia R, Tong C, Yue T, Jiang J, Jia J. Casein kinase 2 promotes Hedgehog signaling by regulating both smoothened and Cubitus interruptus. *J. Biol. Chem*. 2010; 285:37218–37226. [PubMed: 20876583]
74. Chen Y, Li S, Tong C, Zhao Y, Wang B, Liu Y, Jia J, Jiang J. G protein-coupled receptor kinase 2 promotes high-level Hedgehog signaling by regulating the active state of Smo through kinase-dependent and kinase-independent mechanisms in *Drosophila*. *Genes Dev*. 2010; 24:2054–2067. [PubMed: 20844016]
75. Jia J, Tong C, Wang B, Luo L, Jiang J. Hedgehog signalling activity of Smoothened requires phosphorylation by protein kinase A and casein kinase I. *Nature*. 2004; 432:1045–1050. [PubMed: 15616566]
76. Apionishev S, Katanayeva NM, Marks SA, Kalderon D, Tomlinson A. *Drosophila* Smoothened phosphorylation sites essential for Hedgehog signal transduction. *Nat. Cell Biol*. 2005; 7:86–92. [PubMed: 15592457]
77. Jia H, Liu Y, Yan W, Jia J. PP4 and PP2A regulate Hedgehog signaling by controlling Smo and Ci phosphorylation. *Development*. 2009; 136:307–316. [PubMed: 19088085]
78. Su Y, Ospina JK, Zhang J, Michelson AP, Schoen AM, Zhu AJ. Sequential phosphorylation of smoothened transduces graded hedgehog signaling. *Sci. Signal*. 2011; 4:ra43. [PubMed: 21730325]
79. Li S, Chen Y, Shi Q, Yue T, Wang B, Jiang J. Hedgehog-regulated ubiquitination controls smoothened trafficking and cell surface expression in *Drosophila*. *PLoS Biol*. 2012; 10:e1001239. [PubMed: 22253574]
80. Xia R, Jia H, Fan J, Liu Y, Jia J. USP8 promotes smoothened signaling by preventing its ubiquitination and changing its subcellular localization. *PLoS Biol*. 2012; 10:e1001238. [PubMed: 22253573]
81. Chen W, Ren XR, Nelson CD, Barak LS, Chen JK, Beachy PA, de Sauvage F, Lefkowitz RJ. Activity-dependent internalization of smoothened mediated by beta-arrestin 2 and GRK2. *Science*. 2004; 306:2257–2260. [PubMed: 15618519]
82. Meloni AR, Fralish GB, Kelly P, Salahpour A, Chen JK, Wechsler-Reya RJ, Lefkowitz RJ, Caron MG. Smoothened signal transduction is promoted by G protein-coupled receptor kinase 2. *Mol. Cell. Biol*. 2006; 26:7550–7560. [PubMed: 16908539]
83. Riobo NA, Saucy B, Dilizio C, Manning DR. Activation of heterotrimeric G proteins by Smoothened. *Proc. Natl. Acad. Sci. U.S.A.* 2006; 103:12607–12612. [PubMed: 16885213]
84. Ogden SK, Fei DL, Schilling NS, Ahmed YF, Hwa J, Robbins DJ. G protein Galphai functions immediately downstream of Smoothened in Hedgehog signalling. *Nature*. 2008; 456:967–970. [PubMed: 18987629]
85. Marinissen MJ, Gutkind JS. G-protein-coupled receptors and signaling networks: Emerging paradigms. *Trends Pharmacol. Sci*. 2001; 22:368–376. [PubMed: 11431032]
86. Price MA, Kalderon D. Proteolysis of cubitus interruptus in *Drosophila* requires phosphorylation by protein kinase A. *Development*. 1999; 126:4331–4339. [PubMed: 10477300]
87. Chen Y, Gallaher N, Goodman RH, Smolik SM. Protein kinase A directly regulates the activity and proteolysis of cubitus interruptus. *Proc. Natl. Acad. Sci. U.S.A.* 1998; 95:2349–2354. [PubMed: 9482888]
88. Wang G, Wang B, Jiang J. Protein kinase A antagonizes Hedgehog signaling by regulating both the activator and repressor forms of Cubitus interruptus. *Genes Dev*. 1999; 13:2828–2837. [PubMed: 10557210]

89. Wang B, Fallon JF, Beachy PA. Hedgehog-regulated processing of Gli3 produces an anterior/posterior repressor gradient in the developing vertebrate limb. *Cell*. 2000; 100:423–434. [PubMed: 10693759]
90. Fields TA, Casey PJ. Signalling functions and biochemical properties of pertussis toxin-resistant G-proteins. *Biochem. J*. 1997; 321:561–571. [PubMed: 9032437]
91. Hammerschmidt M, McMahon AP. The effect of pertussis toxin on zebrafish development: A possible role for inhibitory G-proteins in hedgehog signaling. *Dev. Biol*. 1998; 194:166–171. [PubMed: 9501021]
92. DeCamp DL, Thompson TM, de Sauvage FJ, Lerner MR. Smoothed activates Gα_i-mediated signaling in frog melanophores. *J. Biol. Chem*. 2000; 275:26322–26327. [PubMed: 10835429]
93. Barzi M, Kostrz D, Menendez A, Pons S. Sonic Hedgehog-induced proliferation requires specific Gα_i inhibitory proteins. *J. Biol. Chem*. 2011; 286:8067–8074. [PubMed: 21209076]
94. Low WC, Wang C, Pan Y, Huang XY, Chen JK, Wang B. The decoupling of Smoothed from Gα_i proteins has little effect on Gli3 protein processing and Hedgehog-regulated chick neural tube patterning. *Dev. Biol*. 2008; 321:188–196. [PubMed: 18590719]
95. Guichard A, Park JM, Cruz-Moreno B, Karin M, Bier E. Anthrax lethal factor and edema factor act on conserved targets in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* 2006; 103:3244–3249. [PubMed: 16455799]
96. Chen JK, Taipale J, Cooper MK, Beachy PA. Inhibition of Hedgehog signaling by direct binding of cyclopamine to Smoothed. *Genes Dev*. 2002; 16:2743–2748. [PubMed: 12414725]
97. Bijlsma MF, Spek CA, Zivkovic D, van de Water S, Rezaee F, Peppelenbosch MP. Repression of smoothed by patched-dependent (pro-) vitamin D3 secretion. *PLoS Biol*. 2006; 4:e232. [PubMed: 16895439]
98. Corcoran RB, Scott MP. Oxysterols stimulate Sonic hedgehog signal transduction and proliferation of medulloblastoma cells. *Proc. Natl. Acad. Sci. U.S.A.* 2006; 103:8408–8413. [PubMed: 16707575]
99. Dwyer JR, Sever N, Carlson M, Nelson SF, Beachy PA, Parhami F. Oxysterols are novel activators of the hedgehog signaling pathway in pluripotent mesenchymal cells. *J. Biol. Chem*. 2007; 282:8959–8968. [PubMed: 17200122]
100. Nachtergaele S, Mydock LK, Krishnan K, Rammohan J, Schlesinger PH, Covey DF, Rohatgi R. Oxysterols are allosteric activators of the oncoprotein Smoothed. *Nat. Chem. Biol*. 2012; 8:211–220. [PubMed: 22231273]
101. Yavari A, Nagaraj R, Owusu-Ansah E, Folick A, Ngo K, Hillman T, Call G, Rohatgi R, Scott MP, Banerjee U. Role of lipid metabolism in smoothed derepression in hedgehog signaling. *Dev. Cell*. 2010; 19:54–65. [PubMed: 20643350]
102. Aza-Blanc P, Kornberg TB. Ci: A complex transducer of the hedgehog signal. *Trends Genet*. 1999; 15:458–462. [PubMed: 10529809]
103. Orenic TV, Slusarski DC, Kroll KL, Holmgren RA. Cloning and characterization of the segment polarity gene cubitus interruptus Dominant of *Drosophila*. *Genes Dev*. 1990; 4:1053–1067. [PubMed: 2166702]
104. Eaton S, Kornberg TB. Repression of ci-D in posterior compartments of *Drosophila* by engrailed. *Genes Dev*. 1990; 4:1068–1077. [PubMed: 2384212]
105. Aza-Blanc P, Ramírez-Weber FA, Laget MP, Schwartz C, Kornberg TB. Proteolysis that is inhibited by hedgehog targets Cubitus interruptus protein to the nucleus and converts it to a repressor. *Cell*. 1997; 89:1043–1053. [PubMed: 9215627]
106. Ohlmeyer JT, Kalderon D. Hedgehog stimulates maturation of Cubitus interruptus into a labile transcriptional activator. *Nature*. 1998; 396:749–753. [PubMed: 9874371]
107. Méthot N, Basler K. Hedgehog controls limb development by regulating the activities of distinct transcriptional activator and repressor forms of Cubitus interruptus. *Cell*. 1999; 96:819–831. [PubMed: 10102270]
108. Müller B, Basler K. The repressor and activator forms of Cubitus interruptus control Hedgehog target genes through common generic gli-binding sites. *Development*. 2000; 127:2999–3007. [PubMed: 10862738]

109. Biehs B, Kechris K, Liu S, Kornberg TB. Hedgehog targets in the *Drosophila* embryo and the mechanisms that generate tissue-specific outputs of Hedgehog signaling. *Development*. 2010; 137:3887–3898. [PubMed: 20978080]
110. Von Ohlen T, Hooper JE. Hedgehog signaling regulates transcription through Gli/Ci binding sites in the wingless enhancer. *Mech. Dev.* 1997; 68:149–156. [PubMed: 9431812]
111. Kalderon D. Hedgehog signalling: Ci complex cuts and clasps. *Curr. Biol.* 1997; 7:R759–R762. [PubMed: 9382827]
112. Jiang J. Regulation of Hh/Gli signaling by dual ubiquitin pathways. *Cell Cycle*. 2006; 5:2457–2463. [PubMed: 17102630]
113. Bader M, Steller H. Regulation of cell death by the ubiquitin-proteasome system. *Curr. Opin. Cell Biol.* 2009; 21:878–884. [PubMed: 19850458]
114. Ravid T, Hochstrasser M. Diversity of degradation signals in the ubiquitin-proteasome system. *Nat. Rev. Mol. Cell Biol.* 2008; 9:679–690. [PubMed: 18698327]
115. Zhang Q, Zhang L, Wang B, Ou CY, Chien CT, Jiang J. A hedgehog-induced BTB protein modulates hedgehog signaling by degrading Ci/Gli transcription factor. *Dev. Cell*. 2006; 10:719–729. [PubMed: 16740475]
116. Jiang J, Struhl G. Regulation of the Hedgehog and Wingless signalling pathways by the F-box/WD40-repeat protein Slimb. *Nature*. 1998; 391:493–496. [PubMed: 9461217]
117. Jia J, Zhang L, Zhang Q, Tong C, Wang B, Hou F, Amanai K, Jiang J. Phosphorylation by double-time/CKIepsilon and CKIalpha targets cubitus interruptus for Slimb/beta-TRCP-mediated proteolytic processing. *Dev. Cell*. 2005; 9:819–830. [PubMed: 16326393]
118. Zhang W, Zhao Y, Tong C, Wang G, Wang B, Jia J, Jiang J. Hedgehog-regulated Costal2-kinase complexes control phosphorylation and proteolytic processing of Cubitus interruptus. *Dev. Cell*. 2005; 8:267–278. [PubMed: 15691767]
119. Smelkinson MG, Zhou Q, Kalderon D. Regulation of Ci-SCFSlimb binding, Ci proteolysis, and hedgehog pathway activity by Ci phosphorylation. *Dev. Cell*. 2007; 13:481–495. [PubMed: 17925225]
120. Smelkinson MG, Kalderon D. Processing of the *Drosophila* hedgehog signaling effector Ci-155 to the repressor Ci-75 is mediated by direct binding to the SCF component Slimb. *Curr. Biol.* 2006; 16:110–116. [PubMed: 16386907]
121. Price MA, Kalderon D. Proteolysis of the Hedgehog signaling effector Cubitus interruptus requires phosphorylation by Glycogen Synthase Kinase 3 and Casein Kinase 1. *Cell*. 2002; 108:823–835. [PubMed: 11955435]
122. Wang Y, Price MA. A unique protection signal in Cubitus interruptus prevents its complete proteasomal degradation. *Mol. Cell. Biol.* 2008; 28:5555–5568. [PubMed: 18625727]
123. Zhang Q, Shi Q, Chen Y, Yue T, Li S, Wang B, Jiang J. Multiple Ser/Thr-rich degrons mediate the degradation of Ci/Gli by the Cul3-HIB/SPOP E3 ubiquitin ligase. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:21191–21196. [PubMed: 19955409]
124. Kent D, Bush EW, Hooper JE. Roadkill attenuates Hedgehog responses through degradation of Cubitus interruptus. *Development*. 2006; 133:2001–2010. [PubMed: 16651542]
125. Aza-Blanc P, Lin HY, Ruiz i Altaba A, Kornberg TB. Expression of the vertebrate Gli proteins in *Drosophila* reveals a distribution of activator and repressor activities. *Development*. 2000; 127:4293–4301. [PubMed: 10976059]
126. von Mering C, Basler K. Distinct and regulated activities of human Gli proteins in *Drosophila*. *Curr. Biol.* 1999; 9:1319–1322. [PubMed: 10574767]
127. Dai P, Akimaru H, Tanaka Y, Maekawa T, Nakafuku M, Ishii S. Sonic Hedgehog-induced activation of the Gli1 promoter is mediated by GLI3. *J. Biol. Chem.* 1999; 274:8143–8152. [PubMed: 10075717]
128. Sasaki H, Nishizaki Y, Hui C, Nakafuku M, Kondoh H. Regulation of Gli2 and Gli3 activities by an amino-terminal repression domain: Implication of Gli2 and Gli3 as primary mediators of Shh signaling. *Development*. 1999; 126:3915–3924. [PubMed: 10433919]
129. Bai CB, Auerbach W, Lee JS, Stephen D, Joyner AL. Gli2, but not Gli1, is required for initial Shh signaling and ectopic activation of the Shh pathway. *Development*. 2002; 129:4753–4761. [PubMed: 12361967]

130. Park HL, Bai C, Platt KA, Matise MP, Beeghly A, Hui CC, Nakashima M, Joyner AL. Mouse Gli1 mutants are viable but have defects in SHH signaling in combination with a Gli2 mutation. *Development*. 2000; 127:1593–1605. [PubMed: 10725236]
131. Buttitta L, Mo R, Hui CC, Fan CM. Interplays of Gli2 and Gli3 and their requirement in mediating Shh-dependent sclerotome induction. *Development*. 2003; 130:6233–6243. [PubMed: 14602680]
132. Ding Q, Motoyama J, Gasca S, Mo R, Sasaki H, Rossant J, Hui CC. Diminished Sonic hedgehog signaling and lack of floor plate differentiation in Gli2 mutant mice. *Development*. 1998; 125:2533–2543. [PubMed: 9636069]
133. Grachtchouk M, Mo R, Yu S, Zhang X, Sasaki H, Hui CC, Dlugosz AA. Basal cell carcinomas in mice overexpressing Gli2 in skin. *Nat. Genet*. 2000; 24:216–217. [PubMed: 10700170]
134. Hu MC, Mo R, Bhella S, Wilson CW, Chuang PT, Hui CC, Rosenblum ND. GLI3-dependent transcriptional repression of Gli1, Gli2 and kidney patterning genes disrupts renal morphogenesis. *Development*. 2006; 133:569–578. [PubMed: 16396903]
135. Lebel M, Mo R, Shimamura K, Hui CC. Gli2 and Gli3 play distinct roles in the dorsoventral patterning of the mouse hindbrain. *Dev. Biol*. 2007; 302:345–355. [PubMed: 17026983]
136. Mill P, Mo R, Fu H, Grachtchouk M, Kim PC, Dlugosz AA, Hui CC. Sonic hedgehog-dependent activation of Gli2 is essential for embryonic hair follicle development. *Genes Dev*. 2003; 17:282–294. [PubMed: 12533516]
137. Mo R, Freer AM, Zinyk DL, Crackower MA, Michaud J, Heng HH, Chik KW, Shi XM, Tsui LC, Cheng SH, Joyner AL, Hui C. Specific and redundant functions of Gli2 and Gli3 zinc finger genes in skeletal patterning and development. *Development*. 1997; 124:113–123. [PubMed: 9006072]
138. Motoyama J, Liu J, Mo R, Ding Q, Post M, Hui CC. Essential function of Gli2 and Gli3 in the formation of lung, trachea and oesophagus. *Nat. Genet*. 1998; 20:54–57. [PubMed: 9731531]
139. Lee J, Platt KA, Censullo P, Ruiz i Altaba A. Gli1 is a target of Sonic hedgehog that induces ventral neural tube development. *Development*. 1997; 124:2537–2552. [PubMed: 9216996]
140. Scales SJ, de Sauvage FJ. Mechanisms of Hedgehog pathway activation in cancer and implications for therapy. *Trends Pharmacol. Sci*. 2009; 30:303–312. [PubMed: 19443052]
141. Svärd J, Heby-Henricson K, Persson-Lek M, Rozell B, Lauth M, Bergström A, Ericson J, Toftgård R, Teglund S. Genetic elimination of Suppressor of fused reveals an essential repressor function in the mammalian Hedgehog signaling pathway. *Dev. Cell*. 2006; 10:187–197. [PubMed: 16459298]
142. Merchant M, Evangelista M, Luoh SM, Frantz GD, Chalasani S, Carano RA, van Hoy M, Ramirez J, Ogasawara AK, McFarland LM, Filvaroff EH, French DM, de Sauvage FJ. Loss of the serine/threonine kinase fused results in postnatal growth defects and lethality due to progressive hydrocephalus. *Mol. Cell. Biol*. 2005; 25:7054–7068. [PubMed: 16055717]
143. Chen MH, Gao N, Kawakami T, Chuang PT. Mice deficient in the fused homolog do not exhibit phenotypes indicative of perturbed hedgehog signaling during embryonic development. *Mol. Cell. Biol*. 2005; 25:7042–7053. [PubMed: 16055716]
144. Karlstrom RO, Tyurina OV, Kawakami A, Nishioka N, Talbot WS, Sasaki H, Schier AF. Genetic analysis of zebrafish gli1 and gli2 reveals divergent requirements for gli genes in vertebrate development. *Development*. 2003; 130:1549–1564. [PubMed: 12620981]
145. Farzan SF, Ogden SK, Robbins DJ. Quantitative insight into models of Hedgehog signal transduction. *Fly (Austin)*. 2010; 4:141–144. [PubMed: 20081354]
146. Jia J, Tong C, Jiang J. Smoothened transduces Hedgehog signal by physically interacting with Costal2/Fused complex through its C-terminal tail. *Genes Dev*. 2003; 17:2709–2720. [PubMed: 14597665]
147. Ogden SK, Ascano M Jr, Stegman MA, Suber LM, Hooper JE, Robbins DJ. Identification of a functional interaction between the transmembrane protein Smoothened and the kinesin-related protein Costal2. *Curr. Biol*. 2003; 13:1998–2003. [PubMed: 14614827]
148. Stegman MA, Goetz JA, Ascano M Jr, Ogden SK, Nybakken KE, Robbins DJ. The Kinesin-related protein Costal2 associates with membranes in a Hedgehog-sensitive, Smoothened-independent manner. *J. Biol. Chem*. 2004; 279:7064–7071. [PubMed: 14645371]

149. Ruel L, Rodriguez R, Gallet A, Lavanant-Staccini L, Thérond PP. Stability and association of Smoothed, Costal2 and Fused with Cubitus interruptus are regulated by Hedgehog. *Nat. Cell Biol.* 2003; 5:907–913. [PubMed: 14523402]
150. Lum L, Zhang C, Oh S, Mann RK, von Kessler DP, Taipale J, Weis-Garcia F, Gong R, Wang B, Beachy PA. Hedgehog signal transduction via Smoothed association with a cytoplasmic complex scaffolded by the atypical kinesin, Costal-2. *Mol. Cell.* 2003; 12:1261–1274. [PubMed: 14636583]
151. Liu Y, Cao X, Jiang J, Jia J. Fused-Costal2 protein complex regulates Hedgehog-induced Smo phosphorylation and cell-surface accumulation. *Genes Dev.* 2007; 21:1949–1963. [PubMed: 17671093]
152. Monnier V, Dussillol F, Alves G, Lamour-Isnard C, Plessis A. Suppressor of fused links fused and Cubitus interruptus on the hedgehog signalling pathway. *Curr. Biol.* 1998; 8:583–586. [PubMed: 9601642]
153. Prat T, Thrond P, Limbourg-Bouchon B, Pham A, Tricoire H, Busson D, Lamour-Isnard C. Segmental polarity in *Drosophila melanogaster*: Genetic dissection of fused in a Suppressor of fused background reveals interaction with costal-2. *Genetics.* 1993; 135:1047–1062. [PubMed: 8307322]
154. Nybakken KE, Turck CW, Robbins DJ, Bishop JM. Hedgehog-stimulated phosphorylation of the kinesin-related protein Costal2 is mediated by the serine/threonine kinase fused. *J. Biol. Chem.* 2002; 277:24638–24647. [PubMed: 11934882]
155. Robbins DJ, Nybakken KE, Kobayashi R, Sisson JC, Bishop JM, Thrond PP. Hedgehog elicits signal transduction by means of a large complex containing the kinesin-related protein costal2. *Cell.* 1997; 90:225–234. [PubMed: 9244297]
156. Stegman MA, Vallance JE, Elangovan G, Sosinski J, Cheng Y, Robbins DJ. Identification of a tetrameric hedgehog signaling complex. *J. Biol. Chem.* 2000; 275:21809–21812. [PubMed: 10825151]
157. Farzan SF, Ascano M Jr, Ogden SK, Sanial M, Brigui A, Plessis A, Robbins DJ. Costal2 functions as a kinesin-like protein in the hedgehog signal transduction pathway. *Curr. Biol.* 2008; 18:1215–1220. [PubMed: 18691888]
158. Sisson JC, Ho KS, Suyama K, Scott MP. Costal2, a novel kinesin-related protein in the Hedgehog signaling pathway. *Cell.* 1997; 90:235–245. [PubMed: 9244298]
159. Monnier V, Ho KS, Sanial M, Scott MP, Plessis A. Hedgehog signal transduction proteins: contacts of the Fused kinase and Ci transcription factor with the kinesin-related protein Costal2. *BMC Dev. Biol.* 2002; 2:4. [PubMed: 11914126]
160. Ho KS, Suyama K, Fish M, Scott MP. Differential regulation of Hedgehog target gene transcription by Costal2 and Suppressor of Fused. *Development.* 2005; 132:1401–1412. [PubMed: 15750186]
161. Zhou Q, Kalderon D. Hedgehog activates fused through phosphorylation to elicit a full spectrum of pathway responses. *Dev. Cell.* 2011; 20:802–814. [PubMed: 21664578]
162. Zhang Y, Mao F, Lu Y, Wu W, Zhang L, Zhao Y. Transduction of the Hedgehog signal through the dimerization of Fused and the nuclear translocation of Cubitus interruptus. *Cell Res.* 2011; 21:1436–1451. [PubMed: 21844892]
163. Shi Q, Li S, Jia J, Jiang J. The Hedgehog-induced Smoothed conformational switch assembles a signaling complex that activates Fused by promoting its dimerization and phosphorylation. *Development.* 2011; 138:4219–4231. [PubMed: 21852395]
164. Ranieri N, Ruel L, Gallet A, Raisin S, Thrond PP. Distinct phosphorylations on kinesin costal-2 mediate differential hedgehog signaling strength. *Dev. Cell.* 2012; 22:279–294. [PubMed: 22306085]
165. Farzan SF, Stegman MA, Ogden SK, Ascano M Jr, Black KE, Tacchelly O, Robbins DJ. A quantification of pathway components supports a novel model of Hedgehog signal transduction. *J. Biol. Chem.* 2009; 284:28874–28884. [PubMed: 19717563]
166. Ogden SK, Casso DJ, Ascano M Jr, Yore MM, Kornberg TB, Robbins DJ. Smoothed regulates activator and repressor functions of Hedgehog signaling via two distinct mechanisms. *J. Biol. Chem.* 2006; 281:7237–7243. [PubMed: 16423832]

167. Hooper JE. Smoothed translates Hedgehog levels into distinct responses. *Development*. 2003; 130:3951–3963. [PubMed: 12874118]
168. Prat T, Thron P, Lamour-Isnard C, Limbourg-Bouchon B, Tricoire H, Erk I, Mariol M-C, Busson D. A putative serine/threonine protein kinase encoded by the segment-polarity fused gene of *Drosophila*. *Nature*. 1990; 347:87–89. [PubMed: 2168522]
169. Thron P, Alves G, Limbourg-Bouchon B, Tricoire H, Guillemet E, Brissard-Zahraoui J, Lamour-Isnard C, Busson D. Functional domains of fused, a serine-threonine kinase required for signaling in *Drosophila*. *Genetics*. 1996; 142:1181–1198. [PubMed: 8846897]
170. Lefers MA, Wang QT, Holmgren RA. Genetic dissection of the *Drosophila* Cubitus interruptus signaling complex. *Dev. Biol.* 2001; 236:411–420. [PubMed: 11476581]
171. Raisin S, Ruel L, Ranieri N, Staccini-Lavanant L, Thron PP. Dynamic phosphorylation of the kinesin Costal-2 in vivo reveals requirement of fused kinase activity for all levels of hedgehog signalling. *Dev. Biol.* 2010; 344:119–128. [PubMed: 20435030]
172. Ascano M Jr, Robbins DJ. An intramolecular association between two domains of the protein kinase Fused is necessary for Hedgehog signaling. *Mol. Cell. Biol.* 2004; 24:10397–10405. [PubMed: 15542847]
173. Claret S, Sanial M, Plessis A. Evidence for a novel feedback loop in the Hedgehog pathway involving Smoothed and Fused. *Curr. Biol.* 2007; 17:1326–1333. [PubMed: 17658259]
174. Wilson CW, Nguyen CT, Chen MH, Yang JH, Gacayan R, Huang J, Chen JN, Chuang PT. Fused has evolved divergent roles in vertebrate Hedgehog signalling and motile ciliogenesis. *Nature*. 2009; 459:98–102. [PubMed: 19305393]
175. Maloverjan A, Piirsoo M. Mammalian homologues of *Drosophila* fused kinase. *Vitam. Horm.* 2012; 88:91–113. [PubMed: 22391301]
176. Prat T. Characterization of Suppressor of fused, a complete suppressor of the fused segment polarity gene of *Drosophila melanogaster*. *Genetics*. 1992; 132:725–736. [PubMed: 1468628]
177. Cheng SY, Yue S. Role and regulation of human tumor suppressor SUFU in Hedgehog signaling. *Adv. Cancer Res.* 2008; 101:29–43. [PubMed: 19055941]
178. Pham A, Therond P, Alves G, Tournier FB, Busson D, Lamour-Isnard C, Bouchon BL, Prat T, Tricoire H. The Suppressor of fused gene encodes a novel PEST protein involved in *Drosophila* segment polarity establishment. *Genetics*. 1995; 140:587–598. [PubMed: 7498739]
179. Wang G, Amanai K, Wang B, Jiang J. Interactions with Costal2 and suppressor of fused regulate nuclear translocation and activity of cubitus interruptus. *Genes Dev.* 2000; 14:2893–2905. [PubMed: 11090136]
180. Wang QT, Holmgren RA. Nuclear import of cubitus interruptus is regulated by hedgehog via a mechanism distinct from Ci stabilization and Ci activation. *Development*. 2000; 127:3131–3139. [PubMed: 10862750]
181. Mthot N, Basler K. Suppressor of fused opposes hedgehog signal transduction by impeding nuclear accumulation of the activator form of Cubitus interruptus. *Development*. 2000; 127:4001–4010. [PubMed: 10952898]
182. Koudijs MJ, den Broeder MJ, Groot E, van Eeden FJ. Genetic analysis of the two zebrafish patched homologues identifies novel roles for the hedgehog signaling pathway. *BMC Dev. Biol.* 2008; 8:15. [PubMed: 18284698]
183. Humke EW, Dorn KV, Milenkovic L, Scott MP, Rohatgi R. The output of Hedgehog signaling is controlled by the dynamic association between Suppressor of Fused and the Gli proteins. *Genes Dev.* 2010; 24:670–682. [PubMed: 20360384]
184. Chen MH, Wilson CW, Li YJ, Law KK, Lu CS, Gacayan R, Zhang X, Hui CC, Chuang PT. Cilium-independent regulation of Gli protein function by Sufu in Hedgehog signaling is evolutionarily conserved. *Genes Dev.* 2009; 23:1910–1928. [PubMed: 19684112]
185. Barnfield PC, Zhang X, Thanabalasingham V, Yoshida M, Hui CC. Negative regulation of Gli1 and Gli2 activator function by Suppressor of fused through multiple mechanisms. *Differentiation*. 2005; 73:397–405. [PubMed: 16316410]
186. Tukachinsky H, Lopez LV, Salic A. A mechanism for vertebrate Hedgehog signaling: Recruitment to cilia and dissociation of SuFu-Gli protein complexes. *J. Cell Biol.* 2010; 191:415–428. [PubMed: 20956384]

187. Wang C, Pan Y, Wang B. Suppressor of fused and Spop regulate the stability, processing and function of Gli2 and Gli3 full-length activators but not their repressors. *Development*. 2010; 137:2001–2009. [PubMed: 20463034]
188. Oro AE. The primary cilia, a 'Rab-id' transit system for hedgehog signaling. *Curr. Opin. Cell Biol.* 2007; 19:691–696. [PubMed: 18061425]
189. Satir P, Christensen ST. Overview of structure and function of mammalian cilia. *Annu. Rev. Physiol.* 2007; 69:377–400. [PubMed: 17009929]
190. Rohatgi R, Milenkovic L, Scott MP. Patched1 regulates hedgehog signaling at the primary cilium. *Science*. 2007; 317:372–376. [PubMed: 17641202]
191. Kovacs JJ, Whalen EJ, Liu R, Xiao K, Kim J, Chen M, Wang J, Chen W, Lefkowitz RJ. Beta-arrestin-mediated localization of smoothened to the primary cilium. *Science*. 2008; 320:1777–1781. [PubMed: 18497258]
192. Aanstad P, Santos N, Corbit KC, Scherz PJ, Trinh A, Salvenmoser W, Huisken J, Reiter JF, Stainier DY. The extracellular domain of Smoothened regulates ciliary localization and is required for high-level Hh signaling. *Curr. Biol.* 2009; 19:1034–1039. [PubMed: 19464178]
193. Milenkovic L, Scott MP, Rohatgi R. Lateral transport of Smoothened from the plasma membrane to the membrane of the cilium. *J. Cell Biol.* 2009; 187:365–374. [PubMed: 19948480]
194. Chen Y, Yue S, Xie L, Pu XH, Jin T, Cheng SY. Dual Phosphorylation of suppressor of fused (Sufu) by PKA and GSK3 β regulates its stability and localization in the primary cilium. *J. Biol. Chem.* 2011; 286:13502–13511. [PubMed: 21317289]
195. Kim J, Kato M, Beachy PA. Gli2 trafficking links Hedgehog-dependent activation of Smoothened in the primary cilium to transcriptional activation in the nucleus. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:21666–21671. [PubMed: 19996169]
196. Haycraft CJ, Banizs B, Aydin-Son Y, Zhang Q, Michaud EJ, Yoder BK. Gli2 and Gli3 localize to cilia and require the intraflagellar transport protein polaris for processing and function. *PLoS Genet.* 2005; 1:e53. [PubMed: 16254602]
197. May SR, Ashique AM, Karlen M, Wang B, Shen Y, Zarbalis K, Reiter J, Ericson J, Peterson AS. Loss of the retrograde motor for IFT disrupts localization of Smo to cilia and prevents the expression of both activator and repressor functions of Gli. *Dev. Biol.* 2005; 287:378–389. [PubMed: 16229832]
198. Wen X, Lai CK, Evangelista M, Hongo JA, de Sauvage FJ, Scales SJ. Kinetics of hedgehog-dependent full-length Gli3 accumulation in primary cilia and subsequent degradation. *Mol. Cell Biol.* 2010; 30:1910–1922. [PubMed: 20154143]
199. Eggenschwiler JT, Anderson KV. Cilia and developmental signaling. *Annu. Rev. Cell Dev. Biol.* 2007; 23:345–373. [PubMed: 17506691]
200. Liem KF Jr, He M, Ocbina PJ, Anderson KV. Mouse Kif7/Costal2 is a cilia-associated protein that regulates Sonic hedgehog signaling. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:13377–13382. [PubMed: 19666503]
201. Jia J, Kolterud A, Zeng H, Hoover A, Teglund S, Toftgård R, Liu A. Suppressor of Fused inhibits mammalian Hedgehog signaling in the absence of cilia. *Dev. Biol.* 2009; 330:452–460. [PubMed: 19371734]
202. Ocbina PJ, Anderson KV. Intraflagellar transport, cilia, and mammalian Hedgehog signaling: analysis in mouse embryonic fibroblasts. *Dev. Dyn.* 2008; 237:2030–2038. [PubMed: 18488998]
203. Bredesen DE, Mehlen P, Rabizadeh S. Apoptosis and dependence receptors: A molecular basis for cellular addiction. *Physiol. Rev.* 2004; 84:411–430. [PubMed: 15044679]
204. Thibert C, Teillet MA, Lapointe F, Mazelin L, Le Douarin NM, Mehlen P. Inhibition of neuroepithelial patched-induced apoptosis by sonic hedgehog. *Science*. 2003; 301:843–846. [PubMed: 12907805]
205. Kagawa H, Shino Y, Kobayashi D, Demizu S, Shimada M, Ariga H, Kawahara H. A novel signaling pathway mediated by the nuclear targeting of C-terminal fragments of mammalian Patched 1. *PLoS ONE*. 2011; 6:e18638. [PubMed: 21533246]

206. Mille F, Thibert C, Fombonne J, Rama N, Guix C, Hayashi H, Corset V, Reed JC, Mehlen P. The Patched dependence receptor triggers apoptosis through a DRAL-caspase-9 complex. *Nat. Cell Biol.* 2009; 11:739–746. [PubMed: 19465923]
207. Chinchilla P, Xiao L, Kazanietz MG, Riobo NA. Hedgehog proteins activate pro-angiogenic responses in endothelial cells through non-canonical signaling pathways. *Cell Cycle.* 2010; 9:570–579. [PubMed: 20081366]
208. Dahmane N, Ruiz i Altaba A. Sonic hedgehog regulates the growth and patterning of the cerebellum. *Development.* 1999; 126:3089–3100. [PubMed: 10375501]
209. Wallace VA. Purkinje-cell-derived Sonic hedgehog regulates granule neuron precursor cell proliferation in the developing mouse cerebellum. *Curr. Biol.* 1999; 9:445–448. [PubMed: 10226030]
210. Wechsler-Reya RJ, Scott MP. Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. *Neuron.* 1999; 22:103–114. [PubMed: 10027293]
211. Barnes EA, Kong M, Ollendorff V, Donoghue DJ. Patched1 interacts with cyclin B1 to regulate cell cycle progression. *EMBO J.* 2001; 20:2214–2223. [PubMed: 11331587]
212. Takizawa CG, Morgan DO. Control of mitosis by changes in the subcellular location of cyclin-B1-Cdk1 and Cdc25C. *Curr. Opin. Cell Biol.* 2000; 12:658–665. [PubMed: 11063929]
213. Barnes EA, Heidtman KJ, Donoghue DJ. Constitutive activation of the shh-ptc1 pathway by a patched1 mutation identified in BCC. *Oncogene.* 2005; 24:902–915. [PubMed: 15592520]
214. Jiang X, Yang P, Ma L. Kinase activity-independent regulation of cyclin pathway by GRK2 is essential for zebrafish early development. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:10183–10188. [PubMed: 19502428]
215. Renault MA, Roncalli J, Tongers J, Thorne T, Klyachko E, Misener S, Volpert OV, Mehta S, Burg A, Luedemann C, Qin G, Kishore R, Losordo DW. Sonic hedgehog induces angiogenesis via Rho kinase-dependent signaling in endothelial cells. *J. Mol. Cell. Cardiol.* 2010; 49:490–498. [PubMed: 20478312]
216. Polizio AH, Chinchilla P, Chen X, Kim S, Manning DR, Riobo NA. Heterotrimeric Gi proteins link Hedgehog signaling to activation of Rho small GTPases to promote fibroblast migration. *J. Biol. Chem.* 2011; 286:19589–19596. [PubMed: 21474452]
217. Sasaki N, Kurisu J, Kengaku M. Sonic hedgehog signaling regulates actin cytoskeleton via Tiam1-Rac1 cascade during spine formation. *Mol. Cell. Neurosci.* 2010; 45:335–344. [PubMed: 20654717]
218. Charron F, Tessier-Lavigne M. Novel brain wiring functions for classical morphogens: A role as graded positional cues in axon guidance. *Development.* 2005; 132:2251–2262. [PubMed: 15857918]
219. Varjosalo M, Björklund M, Cheng F, Syvänen H, Kivioja T, Kilpinen S, Sun Z, Kallioniemi O, Stunnenberg HG, He WW, Ojala P, Taipale J. Application of active and kinase-deficient kinome collection for identification of kinases regulating hedgehog signaling. *Cell.* 2008; 133:537–548. [PubMed: 18455992]
220. Nybakken K, Vokes SA, Lin TY, McMahon AP, Perrimon N. A genome-wide RNA interference screen in *Drosophila melanogaster* cells for new components of the Hh signaling pathway. *Nat. Genet.* 2005; 37:1323–1332. [PubMed: 16311596]
221. Kuo G, Arnaud L, Kronstad-O'Brien P, Cooper JA. Absence of Fyn and Src causes a reeler-like phenotype. *J. Neurosci.* 2005; 25:8578–8586. [PubMed: 16162939]
222. Liu G, Li W, Gao X, Li X, Jürgensen C, Park HT, Shin NY, Yu J, He ML, Hanks SK, Wu JY, Guan KL, Rao Y. p130CAS is required for netrin signaling and commissural axon guidance. *J. Neurosci.* 2007; 27:957–968. [PubMed: 17251438]
223. Robles E, Woo S, Gomez TM. Src-dependent tyrosine phosphorylation at the tips of growth cone filopodia promotes extension. *J. Neurosci.* 2005; 25:7669–7681. [PubMed: 16107653]
224. Suter DM, Forscher P. Transmission of growth cone traction force through apCAM-cytoskeletal linkages is regulated by Src family tyrosine kinase activity. *J. Cell Biol.* 2001; 155:427–438. [PubMed: 11673478]
225. Torres M, Gómez-Pardo E, Gruss P. Pax2 contributes to inner ear patterning and optic nerve trajectory. *Development.* 1996; 122:3381–3391. [PubMed: 8951055]

226. Trousse F, Martí E, Gruss P, Torres M, Bovolenta P. Control of retinal ganglion cell axon growth: A new role for Sonic hedgehog. *Development*. 2001; 128:3927–3936. [PubMed: 11641217]
227. Song HJ, Poo MM. Signal transduction underlying growth cone guidance by diffusible factors. *Curr. Opin. Neurobiol.* 1999; 9:355–363. [PubMed: 10395576]
228. Berridge MJ. Neuronal calcium signaling. *Neuron*. 1998; 21:13–26. [PubMed: 9697848]
229. Komuro H, Rakic P. Intracellular Ca^{2+} fluctuations modulate the rate of neuronal migration. *Neuron*. 1996; 17:275–285. [PubMed: 8780651]
230. Belgacem YH, Borodinsky LN. Sonic hedgehog signaling is decoded by calcium spike activity in the developing spinal cord. *Proc. Natl. Acad. Sci. U.S.A.* 2011; 108:4482–4487. [PubMed: 21368195]
231. Milenkovic L, Scott MP. Not lost in space: Trafficking in the hedgehog signaling pathway. *Sci. Signal.* 2010; 3:e14.
232. Fowler T, Sen R, Roy AL. Regulation of primary response genes. *Mol. Cell.* 2011; 44:348–360. [PubMed: 22055182]

Fig. 1. The canonical Hh signaling pathway

(A) Hh signaling in *Drosophila*. In the absence of Hh (left), Ptc prevents Smo membrane localization and activation so that Smo is retained on intracellular vesicles. In this context, full-length Ci (yellow, indicating partial activity) is held in a microtubule-associated complex containing the kinesin-like protein Costal2 (Cos2), the kinase Fused (Fu), and Suppressor of Fused (Sufu), which promotes phosphorylation of Ci by PKA, GSK3, and CK1 and its partial proteasomal processing into a transcriptional repressor form (Ci_R, red). Binding of Hh to the receptor complex composed of Ptc and an Ihog co-receptor (Ihog or Boi, right) results in internalization of the ligand-receptor complex and phosphorylation and translocation of Smo to the plasma membrane, where it interacts with Cos2 to partially disrupt the microtubule-associated complex, leading to release of Ci and activation of the heterotrimeric G_i protein. Ci is subsequently converted into a fully active labile transcriptional activator (Ci_A, blue) by an unknown mechanism. (B) Hh signaling in vertebrates. Hh signaling in vertebrates is similar to Hh signaling in *Drosophila*, with the important distinction that signaling takes place on primary cilia. When a Hh ligand binds to the receptor complex formed by Ptc and an Ihog co-receptor (Cdo, Boc, or Gas1), Smo translocates to both the plasma membrane and to the primary cilium, where it regulates Gli processing and activation.

Fig. 2. Type I noncanonical Hh signaling

In the absence of a Hh ligand (left) Ptc interacts with cyclin B1 and a proapoptotic complex that includes caspase-9, the CARD (caspase-associated recruitment domain)-domain containing protein Tucan-1, and the adaptor protein Dral. The interaction with cyclin B1 inhibits proliferation by sequestering cyclin B1 outside the nucleus. In some cell types, Ptc recruitment of this proapoptotic complex depends on a preceding cleavage event in the C-terminal domain of Ptc by caspase-3 and results in nucleation and activation of caspase-9. Active caspase-9 speeds the formation of this complex by promoting activation of caspase-3, leading to apoptosis. Hh binding (right) disrupts the interaction of Ptc with cyclin B1 and the proapoptotic complex, likely through a conformational change in Ptc, leading to increased proliferation and survival. Yellow highlighted and shaded caspase shapes indicate active and inactive caspases, respectively.

Fig. 3. Type II noncanonical Hh signaling

Smo regulates the actin cytoskeleton through the small GTPases RhoA and Rac1. This regulation occurs in a context-specific manner, through G_i proteins and PI3K in fibroblasts, and through Tiam1 or through the Src kinase family (SFK) members Src and Fyn in neurons. In addition, Smo stimulates calcium release from the endoplasmic reticulum (ER) in spinal neurons through G_i and PLC-γ–catalyzed generation of IP₃ and opening of IP₃-dependent channels.

Fig. 4. The Hh signaling network

In vertebrates, Hh binding to Ptc modulates one or more of the following signaling modules: non-canonical type I Hh signaling exclusively through Ptc; canonical Hh signaling through Ptc, Smo, and Glis (mediated in some instances by G_i proteins); and a noncanonical type II signaling through Smo and G_i proteins. The cellular responses to each signaling module are illustrated at the bottom. The red crosses indicate inhibition of the pathway in the presence of Hh proteins.