New players and puzzles in the Hedgehog signaling pathway
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Members of the Hedgehog (Hh) family of signaling proteins control cell fates and proliferation during animal development in part by regulating the transcription of specific genes. Depending on the tissue, Hh can act over long or short distances, to signal directly or by inducing secondary signals. Recent discoveries include new components of the pathway as well as novel regulatory mechanisms involving cholesterol, proteolysis, and the cytoskeleton. The role of Hh in carcinogenesis is underscored by the identification of mutations in several pathway components in skin and brain tumors.

Introduction
In this commentary, we emphasize recent studies that have expanded our knowledge of the molecular mechanisms by which Hedgehog (Hh) signals regulate changes in gene expression. The relevance of this pathway to the study of human cancer has been highlighted by the involvement of some pathway components in several tumors and inherited syndromes (see Table 1). Developmental aspects of Hh signaling will be addressed with an emphasis on the mechanism of signal transduction.

In Drosophila, Hh regulates the transcription of specific genes such as members of the Wnt and TGF-β families of signaling proteins. Hh modulates gene expression by opposing the activity of Patched (Ptc), a multiple transmembrane protein [1,2]. In the absence of Hh, Ptc blocks gene expression but, when present, Hh relieves Ptc inhibition to activate transcription [3]. Biochemical studies with vertebrate homologs show that Ptc can bind Hh proteins [4,5]. However, genetic data from Drosophila have revealed some effects of Hh that are independent of Ptc [6,7], suggesting that other Hh receptors exist. Ptc appears to inhibit signaling by associating with and inactivating Smoothened (Smo) [5], a seven transmembrane domain protein required to relay the Hh signal [8,9]. How signaling is transduced within the cell is not understood but pathway regulation converges on the control of Cubitus interruptus (Ci), a zinc finger transcription factor homologous to the vertebrate family of Gli transcriptional regulators [10]. Ci and Gli bind a similar consensus sequence and mutation of these sites results in loss of Hh activation in vivo [11–13]. Recent evidence has revealed a host of unusual molecules and mechanisms that intricately regulate this pathway.

The range of Hedgehog signaling and its control by Patched
In flies and vertebrates, Hh and Sonic hedgehog (Shh) can act either as long or short range signals. Recent experiments using membrane-bound forms suggest that the mechanism and range of signaling varies between tissues. In the developing wing of Drosophila, membrane-tethered Hh constitutes just part of the patterning activity of the normal protein, suggesting that Hh diffuses over several cell widths to pattern the wing [14•]. In the chick limb bud, however, membrane-tethered Shh is highly localized and yet has long-distance effects similar to those of a freely diffusible form. In this instance, Shh may be inducing other long-range signals [15•]. The complexity of Hh patterning is revealed in studies of the developing Drosophila abdomen, where Hh appears to act directly to specify different cell fates but indirectly to establish polarity by inducing secondary signals [16,17].

A reliable indicator of Hh signaling (Figure 1) is thought to be high-level ptc transcription as ptc is induced in most tissues in response to Hh proteins [18,19]. The increased Ptc protein made in response to Hh constitutes an important feedback mechanism that limits the range of Hh signaling. In cells where Ptc is absent, Hh protein diffuses further [20] and induces target gene expression at a longer distance [21]. In the mouse, Hh signaling is widely operative throughout early development because, in embryos lacking Ptc1 function, ptc1 transcription is derepressed in all tissues except the endoderm [22••]. A second ptc gene, ptc2, has been identified in mice and, in the epidermis, it is regulated differently than ptc1 [23,24•]. In the developing tooth and follicles, ptc2 is expressed not in cells that receive the Shh signal, but in cells which produce Shh. Although Ptc1 and Ptc2 share ~60% amino acid identity, they are not well conserved in some regions, such as the carboxy-terminal
domain [24•]. Whether these proteins function differently in regulating Hh signaling is not yet known.

Control of Hedgehog-regulated transcription by cytoplasmic and nuclear proteins

Recent work has shown that Ci can repress and activate target gene expression. Ci has at least three domains of function: an amino-terminal region involved in transcriptional repression [11,25], a zinc finger domain that binds DNA [26]; and a carboxy-terminal region that mediates transcriptional activation [11,25]. In Gli1, the activator domain has been resolved to 18 amino acids and is proposed to form a negatively charged helix similar to that of viral protein 16 [27].

How can Ci behave both as an activator and repressor? Initial studies in Drosophila suggested that Hh activates gene expression by elevating Ci protein levels post-translationally [28] but recent work suggests that Hh stabilizes full-length Ci by preventing its cleavage into a repressor form [29••]. In the absence of Hh, full-length Ci (155 kDa) is proteolytically cleaved into a 75 kDa fragment containing the amino-terminal half including the zinc finger domain.

The fate of the carboxy-terminal half of Ci is not known. The 75 kDa cleavage product is found in the nucleus and can repress target gene expression. In the presence of Hh, proteolysis is blocked and the full-length form is stabilized [29••]. The activator form of Ci has not been identified but it may be the full-length protein or an alternatively processed product.

Ci cleavage may be linked to the ubiquitin/proteosome pathway. In cells mutant for slimb, Ci cleavage is blocked and Hh-mediated gene expression is induced [30••]. Slimb is related to Cdc4p, a component of the yeast ubiquitination pathway, and appears to oppose Hh action by facilitating Ci degradation [30••]. It is not known whether the vertebrate Gli family is also regulated by proteolysis but some evidence suggests that the activator and repressor functions have been delegated to different Gli members [12,27,31,32].

In the cytosol, Hh signaling appears to regulate a multi-component complex that controls Ci localization and/or processing. Biochemical studies in Drosophila have identified two proteins associated with Ci: the serine/threonine kinase...
Fused (Fu) and the kinesin-related protein, Costal2 (Cos2) [33**,34**]. Fu is required for the transmission of Hh signaling whereas Cos2 opposes Hh function [35]. Ci, Fu, and Cos2 are associated in a soluble complex, or set of complexes, of ~400–800 kDa in size. Cos2 binds microtubules and appears to tether the complex to the cytoskeleton [33**,34**]. Hh signaling apparently releases the complex as in cultured cells, Fused and Cos2 dissociate from microtubules after Hh stimulation. This dissociation may be mediated in part by phosphorylation as both Fu and Cos2 phosphorylation levels increase in the presence of Hh [33**,36]. The kinase(s) involved has not been identified but do not appear to be either Fu or PKA. How these findings apply to vertebrates is not yet known but Shh has been found to regulate a serine/threonine phosphatase to induce Gli-dependent and independent gene expression [37*].

The convergence of cholesterol metabolism and Hedgehog signaling

The involvement of cholesterol in Hh signaling was first noted in studies of Hh biosynthesis. Within the secreting cell, Hh undergoes an autocatalytic cleavage to attach a cholesterol moiety to the amino-terminal half which anchors it to the cell membrane [41]. Signaling is mediated by the amino-terminal half alone and does not require the cholesterol attachment. The carboxy-terminal half catalyses the cleavage reaction [42] and its structure is similar to self-splicing proteins [43*]. Mutations in the human hh gene, Shh are responsible for some cases of holoprosencephaly, a developmental disorder that results in craniofacial defects [44–46]. Severe holoprosencephaly resembles the phenotype of Shh null mice which have cyclopia and a rudimentary nasal structure [47].

<table>
<thead>
<tr>
<th>Drosophila gene</th>
<th>Vertebrate homologs</th>
<th>Function of product</th>
<th>Effect on Hh Target genes</th>
<th>Human disease</th>
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<tr>
<td>hedgehog</td>
<td>Sonic hedgehog</td>
<td>Secreted protein</td>
<td>+</td>
<td>Holoprosencephaly</td>
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<td>Desert hedgehog</td>
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<td>patched1</td>
<td>Hh reception</td>
<td>−</td>
<td>Basal cell nevus syndrome, basal cell carcinoma, medulloblastoma, trichoepithelioma [72], meningioma [70], breast carcinoma [70].</td>
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<td>patched2</td>
<td>Hh reception?</td>
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<td>costal 2</td>
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<td>Kinesin related protein</td>
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<td>Gli1</td>
<td>Transcription factor</td>
<td>+</td>
<td>Glioma [74]</td>
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<td>Gli2</td>
<td>Transcription factor</td>
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<td></td>
<td>Gli3</td>
<td>Transcription factor</td>
<td>−/+</td>
<td>Greig cephalopolysyndactyly [75,76], Pallister-Hall syndrome [77]</td>
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<td>CBP</td>
<td>Transcriptional coactivator</td>
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<td>slimb</td>
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<td>Ubiquitination facilitator</td>
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Note: references are added only to subjects which are not referred to in the text.
Cholesterol may also regulate Ptc function. Ptc contains domains similar to those of NPC1, a candidate gene for Niemann-Pick Type C disease (NP-C) [48•,49•]. NP–C is an neurovisceral degenerative disease caused by a defect in cholesterol homeostasis (reviewed in [50]). Cultured NP-C fibroblasts are defective in cholesterol trafficking as seen by the accumulation of exogenously provided cholesterol in lysosomes and the delay in movement to the endoplasmic reticulum [51,52]. NPC1 and Ptc are similar in their hydrophobic regions, including a putative sterol-sensing domain, a motif found in several sterol-regulated proteins such as HMG CoA reductase, a key enzyme in cholesterol biosynthesis [48•,49•]. Although NPC1 and Ptc have not been shown to be modulated by sterols, the NPC1 homology implies that Ptc may respond to sterols and be involved in intracellular trafficking. Whether kinesin-related proteins like Cos2 have a role in vesicle trafficking to regulate Hh signaling or cholesterol movement is unknown. Investigation of the cellular localization and molecular function of both NPC1 and Ptc may yield additional insights.

Roles of Hedgehog signaling in carcinogenesis

The Hh signaling pathway figures prominently in the skin tumor, basal cell carcinoma (BCC). Loss-of-function mutations have been identified in a human homolog of ptc1 in sporadic BCCs and in the basal cell nevus syndrome (BCNS) [53–55]. BCNS (also known as Gorlin’s syndrome) is an inherited disorder characterized by large numbers of BCCs and a variety of developmental defects (reviewed in [56]). The mutations identified in BCC and BCNS do not localize to specific regions of the Ptc1 coding sequence but instead are rather dispersed. About three quarters of ptc1 mutations are predicted to prematurely truncate the protein. In addition, there does not appear to be a correlation between phenotypes of BCNS with specific types of ptc1 mutations [57,58•].

In Drosophila, Hh-regulated genes are derepressed in ptc mutant cells [18]. Similarly, in BCCs, genes controlled by Shh (such as ptc1 and Gli1) are transcriptionally derepressed [59•,60,61]. Overexpression of Shh would be expected to overwhelm Ptc activity and mimic a ptc mutant phenotype. Consistent with this notion, immune-deficient mice with skin grafts of human or mouse keratinocytes overproducing Shh develop a condition that resembles human BCC [62•,63•]. Mutations in a human homolog of smo have been found in several sporadic BCCs and these mutant forms are capable of cellular transformation in conjunction with E1A [64••]. Presumably, mutated Smo is constitutively active and is free from Ptc inhibition. Collectively, these studies suggest that mutations which cause unregulated activation of Hh signaling in the skin can give rise to BCCs.

Mutations in ptc1 have also been detected in the brain tumor, medulloblastoma. Although most medulloblastomas occur sporadically, ~3% are found in BCNS individuals [65]. ptc1 mutations have been found in sporadic medulloblastomas and, in most cases, both alleles of ptc1 appear to be mutated [66–70]. In one study, ~20% of 37 independent medulloblastomas contained ptc1 mutations or were missing one ptc1 allele [68]. Mice that are heterozygous for ptc1 have a 30% incidence of medulloblastoma after six months of age, providing a promising mouse model of the disease. In these tumors, ptc1 transcription is derepressed, suggesting that Ptc1 function is compromised [22••].

How do mutations in the Hh signaling pathway lead to skin and brain tumors? At present, there is little information to address this critical question. In the skin, the cells which are the target of Shh signaling and which give rise to BCC are not known. Whereas little Shh or ptc1 expression is detected in normal human skin [53], high levels of expression are seen in the presumptive follicles of the developing mouse [63•]. Perhaps BCCs arise from cell types involved in follicular growth or regeneration. Medulloblastomas have been proposed to arise from precursor granule cells in the cerebellum [71]. In ptc1 heterozygous mice, cells that express high ptc1 levels are frequently detected in the cerebellar region where granule cells arise during development [22••]. It is possible that these cell populations later become medulloblastomas. Studies of BCC and medulloblastoma have been hampered by the inability to culture these tumors in vitro and to identify the stem cell population. The involvement of Hh signaling in the formation of these tumors now provides a molecular means for tackling these problems.

Conclusions

Recent investigations of Hh signaling have revealed a complexity and novelty in how these signals are transmitted. The identification of additional components and regulatory mechanisms provides a better understanding of how Hh is transduced. Future work will be directed at identifying and linking the components of this pathway and in discovering how Hh signaling is integrated with other signaling cascades. A particularly fruitful area will be understanding the cell biology of Hh signal transduction; where do the proteins reside in the cell and where does signal transmission occur? Resolving the many perplexing and fascinating aspects of Hh signaling may bring new ideas for cancer therapy and prevention. The continuing rapid progress in this field has bright prospects. If present trends continue, many more surprises await us.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


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59. Dahmane N, Lee J, Robins P, Heller P, Ruiz-Albasta A: Activation of the transcription factor Gl1 and the Sonic hedgehog signalling pathway in skin tumours. Nature 1997, 389:876-881. Gl1 is found to be transcriptionally induced in most BCCs and may be an important mediator of the formation of these tumors. This and other studies have shown that Gl1 induction is mediated by Hedgehog and repressed by Patched. The current view is that Gl1 is a mediator of Hedgehog signaling, active both when Hedgehog is present (to functionally oppose Ptc protein) and when ptc is inactivated as it is known to be in many tumors. Thus Hedgehog induces higher level production of its own mediator.


63. Oro AE, Higgins KM, Hu Z, Bonifas JM, Epstein E Jr, Scott MP: Basal cell carcinomas in mice overexpressing sonic hedgehog. Science 1997, 276:817-821. Overexpression of Shh in the skin of transgenic mice is shown to cause developmental defects including spina bifida and polydactyly, and a BCC-like condition. This study demonstrates the role of the Hedgehog pathway in BCC formation, and demonstrates the similarity between flies and mammalian skin in that too much Hedgehog has the same effect as too little patched in both cases.


