EMBO MEMBER'S REVIEW

Transducing Hedgehog: the story so far

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The secreted proteins of the Hedgehog family have been implicated in many different processes in vertebrate development including cartilage differentiation, myotome and sclerotome specification, hair follicle development, limb morphogenesis and the specification of different neuronal cell types. In addition, the aberrant activation of the Hedgehog pathway has been identified as the likely cause of a number of tumours in humans including basal cell carcinomas (BCCs) and primitive neuroectodermal tumours (PNETs). Elucidating the mechanisms by which Hedgehog signals are transduced will thus have widespread implications for our understanding of both normal development and disease.

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Introduction

Members of the *hedgehog* (*hh*) gene family encode a novel class of secreted proteins that act as intercellular signals. The active signalling form of the protein (HH-N) (Lee *et al.*, 1994; Bumcrot *et al.*, 1995; Porter *et al.*, 1995, 1996b) is covalently coupled to cholesterol during autoproteolysis of full-length Hh (Porter *et al.*, 1996a), a modification that appears to influence the range of its signalling activity. Now widely recognized as being of major importance in vertebrate development (Hammerschmidt *et al.*, 1997), Hh signalling was originally described in *Drosophila*. Unlike vertebrates, where multiple Hh family members have been identified, flies have only a single *hh* gene, but this nevertheless controls a number of key developmental processes. Though most recent studies have focused on its role in regulating the growth and patterning of the wing and other appendages in the adult fly (Basler and Struhl, 1994; Diaz-Benjumea and Cohen, 1994; Zecca *et al.*, 1995; Blair and Ralston, 1997; Rodriguez and Basler, 1997), *hh* was first identified as one of the *Drosophila* segment polarity genes, so called because their mutations disrupt the pattern and polarity of the larval segments (Nüsslein-Volhard and Wieschaus, 1980). In *hh* homozygotes, the larval cuticle becomes covered in spiky processes called denticles (Mohler, 1988), hence the name 'hedgehog'. This phenotype is shared by many members of the segment polarity class, suggesting that all of the genes identified by these mutations may function in a common pathway. Genetic analysis has revealed that most segment polarity genes are in fact

involved in the transduction of either the Hh signal (Forbes *et al.*, 1993) or of the signal encoded by the *wingless* (*wg*) gene, a member of the Wnt family of signalling proteins (van den Heuvel *et al.*, 1993). The segment polarity genes have thus provided a key resource in the dissection of these two signalling pathways, and their molecular characterization is now shedding light on the detailed biochemical mechanisms that underlie transduction of Hh signals.

A major function of *hh* in the *Drosophila* embryo is the maintenance of *wg* transcription at the boundary of each segmental unit (Hidalgo and Ingham, 1990; Ingham, 1993; Ingham and Hidalgo, 1993); from here, Wg protein diffuses across the segment to specify the character of the ectodermal cells that secrete the larval cuticle (Lawrence *et al.*, 1996). Like *hh*, mutations in three other segment polarity genes *smoothed* (*smo*), *fused* (*fu*) and *cubitus interruptus* (*ci*) eliminate *wg* transcription at parasegmental borders (Forbes *et al.*, 1993; Ingham, 1993; Prat *et al.*, 1993; van den Heuvel *et al.*, 1993; Motzny and Holmgren, 1995; Alcedo *et al.*, 1996; van den Heuvel and Ingham, 1996); by contrast, mutation of a fourth gene, *patched* (*ptc*), leads to the derepression of *wg* (Ingham *et al.*, 1991; Martinez Arias *et al.*, 1988). By making double mutant combinations between *ptc* and the other genes, it was established that *smo*, *fu* and *ci* all act downstream of *ptc* to activate *wg* transcription (Forbes *et al.*, 1993; Hooper, 1994) whilst, on the other hand, transcription of *wg* becomes independent of *hh* in the absence of *ptc* (Ingham and Hidalgo, 1993). These findings suggest a simple pathway whereby *hh* acts to antagonize the activity of *ptc* which in turn antagonizes the activity of *smo*, *fu* and *ci*. The universality of this pathway subsequently has been established both in *Drosophila*, where *ptc*, *smo*, *fu* and *ci* mediate the activity of Hh in all processes studied to date (Ma *et al.*, 1993; Chen and Struhl, 1996; Forbes *et al.*, 1996; Sanchez-Herrero *et al.*, 1996; van den Heuvel and Ingham, 1996; Strutt and Mlodzik, 1997), and in vertebrates, where homologues of *ptc*, *smo* and *ci* (but not as yet *fu*) have been identified and implicated in processes mediated by one or other of the Hh family proteins (Concordet *et al.*, 1996; Goodrich *et al.*, 1996, 1997; Marigo *et al.*, 1996b,c; Stone *et al.*, 1996; Hynes *et al.*, 1997; Lee *et al.*, 1997; Quirk *et al.*, 1997).

Patched and Smoothed: opposing partners in the Hedgehog receptor

Since *ptc* encodes a transmembrane protein (Hooper and Scott, 1989; Nakano *et al.*, 1989), the simplest interpretation of the genetic data from *Drosophila* is that it acts as a receptor for Hh (Ingham *et al.*, 1991). The predicted topology of the Ptc protein—two large extracellular loops and 12 transmembrane domains—is not, however, typical of receptors for secreted polypeptides and its proposed

Fig. 1. The Hh signalling pathway: a view from the fly. Negatively acting components are shown in red, positively acting components in green. (A) In the absence of Hh induction, the activity of Smo is inhibited by Ptc probably through their physical association. Full-length Ci forms a complex with Fu, Cos-2 and Su(fu), via which it associates with microtubules. This association leads to targeting of Ci to the proteasome where it is cleaved to release the transcriptional repressing form Ci⁷⁵. The phosphorylation of Ci¹⁵⁵ promotes its cleavage, either by promoting association with the Cos-2-Fu or by promoting ubiquitination (or both). Although Ci⁷⁵ translocates to the nucleus, it is also found in association with Fu and Cos-2 (Aza-Blanc *et al.*, 1997). It is not clear whether it dissociates from the complex prior to translocation; there is, however, no evidence that Cos-2 enters the nucleus (Robbins *et al.*, 1997). The intracellular distribution of Fu protein has not been determined. (B) When Hh binds to Ptc, the inhibitory effect on Smo is suppressed. The resulting activation of Smo leads, by an unknown mechanism (but possibly via a heterotrimeric G protein signal), to the dissociation of the Fu-Cos-2-Ci complex from microtubules. Cleavage of Ci¹⁵⁵ is blocked; this or a related form of Ci then presumably enters the nucleus to activate transcription of *ptc*, *wg*, *dpp* and other unidentified target genes in association with CREB binding protein (CBP).

role accordingly remained controversial until relatively recently. In the meantime, an alternative candidate for a receptor emerged with the discovery that *smo* also encodes a transmembrane protein that bears at least a passing resemblance to the large family of G protein-coupled receptors (Alcedo *et al.*, 1996; van den Heuvel and Ingham, 1996). Moreover, Smo exhibits sequence similarity to members of the Frizzled family of serpentine proteins, some of which have been shown to act as receptors for Wnt proteins (Bhanot *et al.*, 1996), making the case for Smo as the Hh receptor seem more compelling. *In vitro* binding assays have, however, failed to detect any physical interaction between vertebrate Smo and Hh proteins (Stone *et al.*, 1996) whereas, under the same conditions, vertebrate Ptc binds the Sonic hedgehog (Shh) protein with relatively high affinity (Marigo *et al.*, 1996a; Stone *et al.*, 1996). As might be expected, the two large extracellular domains of Ptc are required for this binding (Marigo *et al.*, 1996a), though the binding domains have yet to be mapped precisely. These biochemical properties are supported by genetic data from *Drosophila* implicating Ptc in binding Hh. In the appendage primordia of the adult fly, the diffusion of Hh away from expressing cells is strictly limited, but can be significantly increased by eliminating Ptc expression from the Hh-receiving cells. The Hh protein diffuses across clones of cells lacking Ptc expression until it reaches genetically wild-type Ptc-expressing cells at the edge of the clone. The implication of this behaviour

is that Hh is normally sequestered by Ptc (Chen and Struhl, 1996).

Taken together, these findings suggest that Hh acts by binding to Ptc, thereby releasing an inhibitory effect of Ptc on Smo (Figure 1). Since Ptc and Smo are both transmembrane proteins, a likely scenario is that they physically associate to form a receptor complex. This interpretation is supported by the fact that Ptc can be co-immunoprecipitated with Smo from cultured cells expressing both proteins (Stone *et al.*, 1996). The release of Smo from Ptc inhibition most likely involves a conformational change rather than the dissociation of the complex, because the association of the two proteins, as assayed by co-immunoprecipitation, is unaffected by the presence or absence of Hh (Stone *et al.*, 1996). It is, however, important to remember that binding of Smo to Hh-bound Ptc is not essential for its activity, since Smo becomes constitutively activated in the complete absence of Ptc protein (Alcedo *et al.*, 1996; Quirk *et al.*, 1997). This does of course beg the question as to whether some as yet unidentified protein may act as a ligand for Smo, a possibility suggested by the long extracellular N-terminal domain in the Smo protein. Indeed, although the *in vitro* binding assays cannot detect an interaction between Hh and Smo, a direct interaction between the two proteins cannot be ruled out entirely; in this regard, it is notable that the phenotype of an animal lacking both *hh* and *ptc* activity is not identical to that of an animal mutant for

ptc alone, implying that Hh can signal even in the absence of Ptc (Bejsovec and Wieschaus, 1993). Whether or not this residual activity is mediated by Smo remains to be established.

It seems clear, however, that the principal regulation of Smo is via its interaction with Ptc, and it follows from the model that at least some loss-of-function mutations in *ptc* should act by disrupting binding to Smo. The discovery that mutations in the human *ptc* homologue, *Ptch*, are widespread in basal cell carcinomas (BCCs) (Hahn *et al.*, 1996; Johnson *et al.*, 1996) has provided a major stimulation for the analysis of Ptc/Smo function as well as an abundant source of loss-of-function mutations. Many tumour-derived alleles of *Ptch* have now been sequenced, but the results of these studies to date have been relatively unrevealing, the majority of the mutations characterized being due to premature termination of the coding region (Chidambaram *et al.*, 1996; Wicking *et al.*, 1997).

Disruption of Smo–Ptc binding could also be caused by mutations in *smo*; in contrast to *ptc* mutations, these should be dominantly acting (since they would lead to constitutive activity of the mutant protein) and would not, therefore, be recovered in conventional *Drosophila* screens (since they would be dominant lethal). Recent studies of human BCCs however, have identified two such activating mutations in Smo, both of which appear to be responsible for the transformation of basal keratinocytes (Xie *et al.*, 1998). Despite the predictions, neither of these appear to act by disrupting the association of the protein with Ptc, since both mutant forms can still be co-immunoprecipitated with Ptc. While it is possible that these mutations reduce the affinity of Smo for Ptc, an alternative explanation is that they uncouple the intracellular signalling activity of Smo from regulatory domains within the protein. Intriguingly, one of these alleles has been identified as a Trp to Leu substitution at a position in the seventh transmembrane domain which, when mutated in the G-protein coupled α -1B adrenergic receptor, causes its constitutive activation (Xie *et al.*, 1998). This raises the possibility that Smo may itself couple to heterotrimeric G proteins, a possibility supported by recent evidence suggesting that related Frizzled family members stimulate the phosphatidylinositol signalling pathway via G proteins (Slusarski *et al.*, 1997).

Inside the cell: a complex process

If events at the cell surface seem unconventional, the manner in which the signal is transduced inside the cell is no less unusual. Although genetic analysis identified Fu and Ci as downstream components of the pathway, their epistatic relationships with Ptc could not determine precisely where in the signalling pathway each protein acts. A high degree of sequence similarity with members of the vertebrate Gli family of transcription factors (Orenic *et al.*, 1990), however, gave a strong indication that *ci* encodes the ultimate component of the pathway. A considerable body of evidence suggesting that Ci does indeed directly mediate the transcriptional activation of target genes in response to Hh signalling has now been adduced both from *in vitro* and *in vivo* studies: binding sites for Ci have been identified upstream of the promoters both of *wg* and of *ptc*, which is itself transcriptionally regulated by Hh, and these sites have been shown to be

sufficient to mediate activation of reporter genes by Ci in tissue culture assays (Von Ohlen and Hooper, 1997; Von Ohlen *et al.*, 1997) and, in the case of the *ptc* promoter, to mediate Hh-dependent transcription in transgenic flies (Alexandre *et al.*, 1996).

So how does Hh signalling regulate the activity of *ci*? In vertebrates, there is good evidence that the transcription of Gli genes is under the control of Hh proteins (Marigo *et al.*, 1996b; Borycki *et al.*, 1998), the consequent increase in levels of Gli proteins induced by Hh signalling resulting in the activation of different targets. However, in *Drosophila*, transcription of *ci* is clearly independent of Hh (Slusarski *et al.*, 1995); in both the embryonic segments and the adult appendage primordia (the imaginal discs), *ci* is transcribed uniformly in all cells that are responsive to Hh. Thus, if Hh does control *ci* activity, it must do so post-transcriptionally. Since overexpression of *ci* is sufficient to activate target genes independently of Hh activity (Alexandre *et al.*, 1996; Dominguez *et al.*, 1996; Hepker *et al.*, 1997), a simple model is that Hh acts by increasing the levels of Ci protein, perhaps by regulating its rate of turnover. A key insight into this process came last year with the discovery by Aza-Blanc *et al.* (1997) that Ci is subject to proteolytic cleavage. In cells not exposed to the Hh signal, the predominant form of Ci is a 75 kDa species that comprises the N-terminus and the central DNA-binding zinc finger domain, but lacks the C-terminal half of the full-length protein (Aza-Blanc *et al.*, 1997). The missing portion contains not only an acidic region identified as a transcriptional activation domain (Alexandre *et al.*, 1996) but also a binding site for the CBP, which itself is essential for the activation of Hh target genes (Akimaru *et al.*, 1997). Thus, in the absence of Hh induction, it seems that the form of Ci that can activate transcription is specifically eliminated from cells by this proteolytic cleavage. When cells receive the Hh signal, however, the cleavage of Ci is blocked, resulting in the accumulation of the full-length 155 kDa form of the protein. While the simplest interpretation of these data would seem to be that Hh acts by promoting the accumulation of the active form of Ci, the picture is complicated by the fact that the smaller 75 kDa form retains DNA-binding activity and can itself act as a transcriptional repressor (Aza-Blanc *et al.*, 1997). This property, together with its accumulation in non-Hh-induced cells, correlates well with the finding that some genes—including another Hh target decapentaplegic (*dpp*), as well as *hh* itself—are ectopically transcribed in cells that lack expression of Ci. This paradoxical finding suggests the possibility that activation of *dpp* by *hh* might actually be achieved by the elimination of the 75 kDa repressor form of the protein from *hh*-responding cells rather than by accumulation of the activating form of the protein (Aza-Blanc *et al.*, 1997). Against this, however, genetic analysis has shown that *ci* is absolutely required for the activation of other Hh targets such as *ptc* and *wg* (Forbes *et al.*, 1993); moreover, ectopic expression of *ci* is sufficient to activate *dpp* as well as *ptc* and *wg* transcription (Alexandre *et al.*, 1996; Dominguez *et al.*, 1996; Hepker *et al.*, 1997). Thus, the principal role of Ci seems to be to activate transcription of Hh targets.

A picture of how Ci processing is controlled has emerged recently, based to a large extent on the characterization

of three genes identified by mutations that cause wing duplications (Jiang and Struhl, 1995, 1998; Lepage *et al.*, 1995; Li *et al.*, 1995; Robbins *et al.*, 1997; Sisson *et al.*, 1997). This phenotype is characteristic of the inappropriate activation of the Hh pathway in the developing wing primordium and, consistent with this, high levels of full-length Ci protein accumulate independently of Hh signalling in the wing cells of each mutation. Thus, each mutant identifies a gene required in some way for the normal processing of Ci, any one of which could therefore mediate the control of Ci by Hh. The product of the *slmb* gene (Jiang and Struhl, 1998) is an F box/WD40 repeat protein homologous to the *Saccharomyces cerevisiae* cdc 4 protein required for the ubiquitination of cell cycle regulators. By analogy, Slmb may target Ci for ubiquitination and hence for cleavage by the proteasome pathway (Jiang and Struhl, 1998), a possibility supported by the finding that proteasome inhibitors block the processing of Ci in tissue culture cells (D.Stark and P.W.Ingham, unpublished observations). Although the partial degradation of a protein via the proteasome is unusual, analogous proteasome-dependent processing of the NF- κ B and cystic fibrosis transmembrane regulator (CFTR) proteins has been reported previously (Palombella *et al.*, 1994; Jensen *et al.*, 1995). Since targeting of proteins to the proteasome is commonly regulated by their phosphorylation, a plausible scenario would be that Hh acts by modulating Ci phosphorylation. While the phosphorylation status of Ci has yet to be established, sequence analysis has revealed the presence of a number of consensus protein kinase A (PKA) phosphorylation sites that have been conserved in the vertebrate Gli proteins (Akimaru *et al.*, 1997; Chen *et al.*, 1998). The implication that PKA may thereby regulate Ci processing is supported by the fact that another of the wing duplication mutations inactivates the catalytic subunit of PKA (Jiang and Struhl, 1995; Lepage *et al.*, 1995; Li *et al.*, 1995). Moreover, mutating each of the putative phosphorylation sites in Ci results in increased stability of the full-length protein and a concomitant increase in its transcriptional activation activity (assayed in transfected tissue culture cells) (Chen *et al.*, 1998). Although this does not constitute definitive proof, the strong implication is that PKA phosphorylates Ci directly, raising the possibility that Hh might regulate Ci cleavage by regulating the activity of PKA (Chen *et al.*, 1998). While such a model would be consistent with Smo coupling to a G protein-regulated signalling pathway, the finding that a constitutively activated PKA catalytic subunit does not suppress Hh signalling suggests rather that Hh acts in parallel with PKA (Jiang and Struhl, 1995). In fact, it transpires that the processing of Ci is also regulated by its sequestration within the cytoplasm and this process depends upon the product of the *costal-2* (*cos-2*) gene, mutation of which, like *slmb* and PKA, causes wing duplication (Whittle, 1976; Grau and Simpson, 1987). Unlike Slmb and PKA, however, the *cos-2* product is a novel protein, though it does possess an N-terminal domain that shares significant sequence similarity with the motor domains of kinesin superfamily proteins (Sisson *et al.*, 1997). Through this domain, the Cos-2 protein binds to microtubules, taking with it both the novel serine-threonine kinase encoded by *fused* and Ci with which it forms a high molecular weight multimeric complex (Robbins *et*

al., 1997; Sisson *et al.*, 1997). Intriguingly, the association of this Cos-2–Fu–Ci complex with microtubules is itself regulated by Hh signalling, the entire complex dissociating intact from the microtubules when cells are exposed to Hh (Robbins *et al.*, 1997). Since the full-length form of the Ci protein accumulates in cells lacking Cos-2 activity (Sisson *et al.*, 1997) as well as in cells induced by Hh, it follows that the association of Ci with microtubules is essential for its processing (Figure 1). In this view, the critical step in the regulation of Ci activity by Hh is the modulation of its sequestration rather than its PKA-dependent phosphorylation. The latter process may be necessary to target Ci to the complex or, as suggested above, for its ubiquitination.

How Hh induces the dissociation of the Cos-2–Fu–Ci complex from microtubules remains a mystery for the present. One possibility is that Smo activates the Fu serine-threonine kinase which in turn could act by inhibiting microtubule binding by Cos-2, a possibility consistent with genetic epistasis analysis that places *cos-2* downstream of *fu* (Préat *et al.*, 1993). However, although Cos-2 does become hyperphosphorylated in response to Hh activity, this response is delayed relative to the release of the complex from the microtubules, and in any event it is independent of the activity of the Fu kinase domain (Robbins *et al.*, 1997). Moreover, the activity of Fu is entirely dispensable for normal Hh signalling when a fourth component of the complex, the so-called Suppressor of fused [Su(fu)] protein is also eliminated (Préat, 1992). In *in vitro* assays, Su(fu) can bind directly to Fu and to Ci; since there is no evidence for a direct interaction between the latter proteins or indeed between them and Cos-2, it may be that Su(fu) forms a bridge between them, stabilizing their association with one another and with Cos-2 (Monnier *et al.*, 1998) (Figure 1). Other as yet unidentified proteins almost certainly participate in complex formation, however, since Su(fu) is not essential for Hh signalling; until these are identified, a complete picture of how the complex is regulated will remain elusive.

Concluding comments

Genetic analysis in *Drosophila* has provided an outline of the Hh signalling pathway, the details of which are now beginning to be filled in at the biochemical level. Whilst a relatively clear picture is starting to emerge, many issues remain to be addressed. A major gap in our understanding lies in the events between activation of Smo and the dissociation of the Ci–Fu–Cos2–Su(fu) complex from microtubules, while the failure to detect significant levels of the full-length form of Ci in the nucleus—the high levels of this species that accumulate in response to Hh are located almost exclusively in the cytoplasm (Aza-Blanc *et al.*, 1997)—remains a long-standing puzzle. Another paradox concerns the role of PKA; although, as discussed above, inactivation of PKA results in activation of the pathway, recent studies in the *Drosophila* embryo have shown that hyperactivity of PKA can have the same effect (Ohlmeyer and Kalderon, 1997). Consistent with a role for PKA in promoting Ci cleavage, this PKA-induced activation occurs in the absence of an increase in the levels of Ci¹⁵⁵; one possibility is that PKA activates Ci¹⁵⁵ through phosphorylation of some other cryptic site or sites

or, alternatively, it may act on some other co-factor. Whatever the target, this effect of PKA appears to be dependent upon the activity of Smo (Ohlmeyer and Kalderon, 1997).

Finally, it is reasonable to ask just how far the lessons learned from *Drosophila* can be extrapolated to vertebrates. At first sight, the evidence that the pathway has been highly conserved seems quite compelling. The results of the expression and functional analyses of vertebrate Ptc homologues largely fulfil the predictions based on analogy with *Drosophila* studies (Goodrich *et al.*, 1996, 1997), while the biochemical characterization of the Hh–Ptc–Smo interactions has actually been performed using the vertebrate gene products (Marigo *et al.*, 1996a; Stone *et al.*, 1996). It is notable, however, that vertebrates have two homologues of Ptc (Concordet *et al.*, 1996; Takabatake *et al.*, 1997), and recent expression analyses of Ptc2 in mice reveal a pattern not predicted by analysis in *Drosophila* (Motoyama *et al.*, 1998). Similarly, where *Drosophila* has just one *gli* gene, in vertebrates there are at least three members of the Gli family, each of which exhibits its own distinctive pattern of expression (Hui *et al.*, 1994). One possibility is that in *Drosophila* the functions of all the Gli gene products have been subsumed by Ci; Gli3, for instance, appears to act as a repressor of Shh expression whereas Gli1 and 2 may act only as activators of Hh family target genes (Marigo *et al.*, 1996b). This begs the question as to whether the Gli proteins are subject to the same processing as Ci and, if not, is their regulation by Hh signalling exclusively at the level of transcription? Significantly, recent studies of the COUP TFII gene, a target of Shh signalling in the neural tube, have identified a Shh response element that does not bind Gli proteins, but does bind an unidentified nuclear factor that is susceptible to modulation by protein phosphatase IIA (Krishnan *et al.*, 1997). No parallel for such a factor has yet been described in *Drosophila*; however, evidence for Ci-independent regulation of a Hh target has been obtained recently with the identification of a *cis*-acting element upstream of *wg* that lacks Ci consensus binding sites yet drives transcription in a *hh*-dependent manner (Lessing and Nusse, 1998). Thus, it seems clear that transcription factors other than members of the Ci/Gli family are regulated directly by Hh signalling; the next challenge will be to identify these factors and the pathways that control them.

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References

- Akimaru, H., Chen, Y., Dai, P., Hou, D.-X., Nonaka, M., Smolik, S., Armstrong, S., Goodman, R. and Ishii, S. (1997) *Drosophila* CBP is a co-activator of cubitus interruptus in hedgehog signalling. *Nature*, **386**, 735–738.
- Alcedo, J., Ayzenzon, M., Von Ohlen, T., Noll, M. and Hooper, J.E. (1996) The *Drosophila* *smoothed* gene encodes a 7-pass membrane-protein, a putative receptor for the hedgehog signal. *Cell*, **86**, 221–232.
- Alexandre, C., Jacinto, A. and Ingham, P.W. (1996) Transcriptional activation of *hedgehog* target genes in *Drosophila* is mediated directly by the cubitus interruptus protein, a member of the Gli family of zinc finger DNA-binding proteins. *Genes Dev.*, **10**, 2003–2013.
- Aza-Blanc, P., Ramirezweber, F., Laget, M., Schwartz, C. and Kornberg, T. (1997) Proteolysis that is inhibited by hedgehog targets cubitus-interruptus protein to the nucleus and converts it to a repressor. *Cell*, **89**, 1043–1053.
- Basler, K. and Struhl, G. (1994) Compartment boundaries and the control of *Drosophila* limb pattern by hedgehog protein. *Nature*, **368**, 208–214.
- Bejsovec, A. and Wieschaus, E. (1993) Segment polarity gene interactions modulate epidermal patterning in *Drosophila* embryos. *Development*, **119**, 501–517.
- Bhanot, P., Brink, M., Samos, C.H., Hsieh, J.-C., Wang, Y., Macke, J.P., Andrew, D., Nathans, J. and Nusse, R. (1996) A new member of the *frizzled* family from *Drosophila* functions as a Wingless receptor. *Nature*, **382**, 225–230.
- Blair, S. and Ralston, A. (1997) *Smoothed*-mediated hedgehog signaling is required for the maintenance of the anterior–posterior lineage restriction in the developing wing of *Drosophila*. *Development*, **124**, 4053–4063.
- Borycki, A.-G., Mendham, L. and Emerson, C., Jr (1998) Control of somite patterning by Sonic hedgehog and its downstream signal response genes. *Development*, **125**, 777–790.
- Bumcrot, D.A., Takada, R. and McMahon, A.P. (1995) Proteolytic processing yields two secreted forms of Sonic hedgehog. *Mol. Cell. Biol.*, **15**, 2294–2303.
- Chen, Y. and Struhl, G. (1996) Dual roles for patched in sequestering and transducing Hedgehog. *Cell*, **87**, 553–563.
- Chen, Y., Gallaher, N., Goodman, R. and Smolik, S. (1998) Protein kinase A directly regulates the activity and proteolysis of cubitus interruptus. *Proc. Natl Acad. Sci. USA*, **95**, 2349–2354.
- Chidambaram, A., Goldstein, A., Gailani, M., Gerrard, B., Bal, S., Digiovanna, J., Bale, A. and Dean, M. (1996) Mutations in the human homolog of the *Drosophila* *patched* gene in Caucasian and African-American nevroid basal-cell carcinoma syndrome patients. *Cancer Res.*, **56**, 4599–4601.
- Concordet, J.-P., Lewis, K., Moore, J., Goodrich, L.V., Johnson, R.L., Scott, M.P. and Ingham, P.W. (1996) Spatial regulation of a Zebrafish *patched* homologue reflects the roles of sonic hedgehog and protein kinase A in neural tube and somite patterning. *Development*, **122**, 2835–2846.
- Diaz-Benjumea, F.J. and Cohen, S.M. (1994) Cell-interaction between compartments establishes the proximal–distal axis of *Drosophila* legs. *Nature*, **372**, 175–179.
- Dominguez, M., Brunner, M., Hafen, E. and Basler, K. (1996) Sending and receiving the hedgehog signal—control by the *Drosophila*-*gli* protein cubitus-interruptus. *Science*, **272**, 1621–1625.
- Forbes, A.J., Nakano, Y., Taylor, A.M. and Ingham, P.W. (1993) Genetic analysis of *hedgehog* signalling in the *Drosophila* embryo. *Development Suppl.*, 115–124.
- Forbes, A.J., Spradling, A.C., Ingham, P.W. and Lin, H. (1996) The role of segment polarity genes during early oogenesis in *Drosophila*. *Development*, **122**, 3283–3294.
- Goodrich, L., Milenkovic, L., Higgins, K. and Scott, M. (1997) Altered neural cell fates and medulloblastoma in mouse *patched* mutants. *Science*, **277**, 1109–1113.
- Goodrich, L.V., Johnson, R.L., Milenkovic, L., McMahon, J.A. and Scott, M.P. (1996) Conservation of the *hedgehog/patched* signaling pathway from flies to mice—induction of a mouse *patched* gene by *hedgehog*. *Genes Dev.*, **10**, 301–312.
- Grau, Y. and Simpson, P. (1987) The segment polarity gene *costal-2* in *Drosophila*. I. The organization of both primary and secondary embryonic fields may be affected. *Dev. Biol.*, **122**, 186–200.
- Hahn, H. *et al.* (1996) Mutations of the human homolog of *Drosophila* *patched* in the nevroid basal-cell carcinoma syndrome. *Cell*, **85**, 841–851.
- Hammerschmidt, M., Brook, A. and McMahon, A. (1997) The world according to hedgehog. *Trends Genet.*, **13**, 14–21.
- Hepker, J., Wang, Q., Motzny, C., Holmgren, R. and Orenic, T. (1997) *Drosophila* *cubitus interruptus* forms a negative feedback loop with patched and regulates expression of *hedgehog* target. *Development*, **124**, 549–558.
- Hidalgo, A. and Ingham, P. (1990) Cell patterning in the *Drosophila* segment: spatial regulation of the segment polarity gene *patched*. *Development*, **110**, 291–302.
- Hooper, J. (1994) Distinct pathways for autocrine and paracrine Wingless signalling in *Drosophila* embryos. *Nature*, **372**, 461–464.

- Hooper, J. and Scott, M.P. (1989) The *Drosophila patched* gene encodes a putative membrane protein required for segmental patterning. *Cell*, **59**, 751–765.
- Hui, C.C., Slusarski, D., Platt, K.A., Holmgren, R. and Joyner, A.L. (1994) Expression of three mouse homologs of the *Drosophila* segment polarity gene *cubitus interruptus*, Gli, Gli-2, and Gli-3, in ectoderm- and mesoderm-derived tissues suggests multiple roles during postimplantation development. *Dev. Biol.*, **162**, 402–413.
- Hynes, M., Stone, D., Dowd, M., Pitts-Meek, S., Goddard, A., Gurney, A. and Rosenthal, A. (1997) Control of cell pattern in the neural tube by the zinc finger transcription factor and oncogene *Gli-1*. *Neuron*, **19**, 15–26.
- Ingham, P.W. (1993) Localised *hedgehog* activity controls spatially restricted transcription of *wingless* in the *Drosophila* embryo. *Nature*, **366**, 560–562.
- Ingham, P.W. and Hidalgo, A. (1993) Regulation of *wingless* transcription in the *Drosophila* embryo. *Development*, **117**, 283–291.
- Ingham, P.W., Taylor, A.M. and Nakano, Y. (1991) Role of the *Drosophila patched* gene in positional signalling. *Nature*, **353**, 184–187.
- Jensen, T., Loo, M., Pind, S., Williams, D., Goldberg, A. and Riordan, J. (1995) Multiple proteolytic systems, including the proteasome, contribute to CFTR processing. *Cell*, **83**, 129–135.
- Jiang, J. and Struhl, G. (1995) Protein kinase A and hedgehog signaling in *Drosophila* limb development. *Cell*, **80**, 563–572.
- Jiang, J. and Struhl, G. (1998) Regulation of the *hedgehog* and *wingless* signaling pathways by the f-box/WD40-repeat protein *slimb*. *Nature*, **391**, 493–496.
- Johnson, R.L. et al. (1996) Human homolog of *patched*, a candidate gene for the basal-cell nevus syndrome. *Science*, **272**, 1668–1671.
- Krishnan, V., Pereira, F., Qiu, Y., Chen, C., Beachy, P., Tsai, S. and Tsai, M. (1997) Mediation of Sonic Hedgehog induced expression of COUP-TFII by a protein phosphatase. *Science*, **278**, 1947–1950.
- Lawrence, P., Sanson, B. and Vincent, J.-P. (1996) Compartments, wingless and engrailed—patterning the ventral epidermis of *Drosophila* embryos. *Development*, **122**, 4095–4103.
- Lee, J.J., Ekker, S.C., von Kessler, D.P., Porter, J.A., Sun, B.I. and Beachy, P.A. (1994) Autoproteolysis in *hedgehog* protein biogenesis. *Science*, **266**, 1528–1537.
- Lee, J., Platt, K., Censullo, P. and Altaba, A. (1997) Gli1 is a target of sonic hedgehog that induces ventral neural-tube development. *Development*, **124**, 2537–2552.
- Lepage, T., Cohen, S.M., Diaz-Benjumea, F.J. and Parkhurst, S.M. (1995) Signal transduction by cAMP-dependent protein kinase A in *Drosophila* limb patterning. *Nature*, **373**, 711–715.
- Lessing, D. and Nusse, R. (1998) Expression of *wingless* in the *Drosophila* embryo: a conserved cis-acting element lacking conserved Ci-binding sites is required for *patched*-mediated repression. *Development*, **125**, 1469–1476.
- Li, W., Ohlmeyer, J.T., Lane, M.E. and Kalderon, D. (1995) Function of protein kinase A in Hedgehog signal transduction and *Drosophila* imaginal disc development. *Cell*, **80**, 553–562.
- Ma, C., Zhou, Y., Beachy, P.A. and Moses, K. (1993) The segment polarity gene *hedgehog* is required for progression of the morphogenetic furrow in the developing *Drosophila* eye. *Cell*, **75**, 927–938.
- Marigo, V., Davey, R., Zuo, Y., Cunningham, J. and Tabin, C. (1996a) Biochemical evidence that *patched* is the hedgehog receptor. *Nature*, **384**, 176–179.
- Marigo, V., Johnson, R., Vortkamp, A. and Tabin, C. (1996b) Sonic hedgehog differentially regulates expression of *Gli* and *Gli3* during limb development. *Dev. Biol.*, **180**, 273–283.
- Marigo, V., Scott, M.P., Johnson, R.L., Goodrich, L.V. and Tabin, C.J. (1996c) Conservation in *hedgehog* signaling: induction of a chicken *patched* homolog by Sonic hedgehog in the developing limb. *Development*, **122**, 1225–1233.
- Martinez Arias, A., Baker, N.E. and Ingham, P.W. (1988) Role of segment polarity gene in the definition and maintenance of cell states in the *Drosophila* embryo. *Development*, **103**, 157–170.
- Mohler, J. (1988) Requirements for *hedgehog*, a segment polarity gene, in patterning larval and adult cuticle of *Drosophila*. *Genetics*, **120**, 1061–1072.
- Monnier, V., Dussillol, F., Alves, G., Lamour-Isnard, C. and Plessis, A. (1998) Molecular interactions between three members of the *Drosophila* Hedgehog signalling pathway: Suppressor of fused links the ser-thr kinase Fused and the transcription factor Cubitus interruptus. *Curr. Biol.*, **8**, 583–586.
- Motoyama, J., Takabatake, T., Takeshima, K. and Hui, C. (1998) *Ptch2*, a 2nd mouse *patched* gene is coexpressed with *Sonic hedgehog*. *Nature Genet.*, **18**, 104–106.
- Motzny, C.K. and Holmgren, R. (1995) The *Drosophila* cubitus interruptus protein and its role in the wingless and *hedgehog* signal transduction pathways. *Mech. Dev.*, **52**, 137–150.
- Nakano, Y., Guerrero, I., Hidalgo, A., Taylor, A.M., Whittle, J.R.S. and Ingham, P.W. (1989) The *Drosophila* segment polarity gene *patched* encodes a protein with multiple potential membrane spanning domains. *Nature*, **341**, 508–513.
- Nüsslein-Volhard, C. and Wieschaus, E. (1980) Mutations affecting segment number and polarity in *Drosophila*. *Nature*, **287**, 795–801.
- Ohlmeyer, J. and Kalderon, D. (1997) Dual pathways for induction of *wingless* expression by protein-kinase-A and *hedgehog* in *Drosophila* embryos. *Genes Dev.*, **11**, 2250–2258.
- Orenic, T.V., Slusarski, D.C., Kroll, K.L. and Holmgren, R.A. (1990) Cloning and characterization of the segment polarity gene *cubitus interruptus* Dominant of *Drosophila*. *Genes Dev.*, **4**, 1053–1067.
- Palombella, V., Rando, O., Goldberg, A. and Maniatis, T. (1994) The ubiquitin–proteasome pathway is required for processing the NFκB precursor protein and the activation of NFκB. *Cell*, **78**, 773–785.
- Porter, J.A., von Kessler, D.P., Ekker, S.C., Young, K.E., Lee, J.J., Moses, K. and Beachy, P.A. (1995) The product of hedgehog autoproteolytic cleavage active in local and long-range signalling. *Nature*, **374**, 363–366.
- Porter, J., Young, K. and Beachy, P. (1996a) Cholesterol modification of hedgehog signaling proteins in animal development. *Science*, **274**, 255–259.
- Porter, J.A. et al. (1996b) Hedgehog patterning activity—role of a lipophilic modification mediated by the carboxy-terminal autoprocessing domain. *Cell*, **86**, 21–34.
- Préat, T. (1992) Characterisation of *Suppressor of fused*, a complete suppressor of the *fused* segment polarity gene of *Drosophila melanogaster*. *Genetics*, **132**, 725–736.
- Préat, T., Théron, P., Limbourg-Bouchon, B., Pham, A., Tricoire, H., Busson, D. and Lamour-Isnard, D. (1993) Segmental polarity in *Drosophila melanogaster*: genetic dissection of *fused* in a *Suppressor of fused* background reveals interaction with *costal-2*. *Genetics*, **135**, 1047–1062.
- Quirk, J., van den Heuvel, M., Henrique, D., Marigo, V., Jones, T., Tabin, C. and Ingham, P. (1997) The *smoothed* gene and the anterior wing induction *Drosophila* and vertebrate development. *Cold Spring Harbor Symp. Quant. Biol.*, **62**, 217–226.
- Robbins, D.J., Nybakken, K.E., Kobayashi, R., Sisson, J.C., Bishop, J.M. and Théron, P.P. (1997) Hedgehog elicits signal transduction by means of a large complex containing the kinesin-related protein Costal2. *Cell*, **90**, 225–234.
- Rodriguez, I. and Basler, K. (1997) Control of compartmental affinity boundaries by hedgehog. *Nature*, **389**, 614–618.
- Sanchez-Herrero, E., Couso, J., Capdevila, J. and Guerrero, I. (1996) The *fu* gene discriminates between pathways to control *dpp* expression in *Drosophila* imaginal discs. *Mech. Dev.*, **55**, 159–170.
- Sisson, J., Ho, K., Suyama, K. and Scott, M. (1997) Costal2, a novel kinesin-related protein in the Hedgehog signaling pathway. *Cell*, **90**, 235–245.
- Slusarski, D., Corces, V. and Moon, R. (1997) Interaction of Wnt and a Frizzled homolog triggers G-protein-linked phosphatidylinositol signaling. *Nature*, **390**, 410–413.
- Slusarski, D.C., Motzny, C.K. and Holmgren, R. (1995) Mutations that alter the timing and pattern of *cubitus interruptus* gene expression in *Drosophila melanogaster*. *Genetics*, **139**, 229–240.
- Stone, D. et al. (1996) The tumor-suppressor gene *patched* encodes a candidate receptor for Sonic hedgehog in the developing limb. *Nature*, **384**, 129–134.
- Strutt, D. and Mlodzik, M. (1997) Hedgehog is an indirect regulator of morphogenetic furrow progression in the *Drosophila* eye disc. *Development*, **124**, 3233–3240.
- Takabatake, T., Ogawa, M., Takahashi, T., Mizuno, M., Okamoto, M. and Takeshima, K. (1997) *Hedgehog* and *patched* gene expression in adult ocular tissues. *FEBS Lett.*, **410**, 485–489.
- van den Heuvel, M. and Ingham, P.W. (1996) *smoothed* encodes a serpentine protein required for *hedgehog* signaling. *Nature*, **382**, 547–551.
- van den Heuvel, M., Klingensmith, J., Perrimon, N. and Nusse, R. (1993) Cell patterning in the *Drosophila* segment: *engrailed* and *wingless* antigen distributions in segment polarity mutant embryos. *Development Suppl.*, 105–114.

- Von Ohlen,T. and Hooper,J. (1997) Hedgehog signaling regulates transcription through gli/ci binding-sites in the wingless enhancer. *Mech. Dev.*, **68**, 149–156.
- Von Ohlen,T., Lessing,D., Nusse,R. and Hooper,J. (1997) Hedgehog signaling regulates transcription through *cubitus interruptus*, a sequence-specific DNA-binding protein. *Proc. Natl Acad. Sci. USA*, **94**, 2404–2409.
- Whittle,J.R.S. (1976) Clonal analysis of a genetically caused duplication of the anterior wing in *Drosophila melanogaster*. *Dev. Biol.*, **51**, 257–268.
- Wicking,C. *et al.* (1997) Most germ-line mutations in the nevoid basal-cell carcinoma syndrome lead to a premature termination of the patched protein, and no genotype–phenotype correlations are evident. *Am. J. Hum. Genet.*, **60**, 21–26.
- Xie,J. *et al.* (1998) Activating smoothened mutations in sporadic basal-cell carcinoma. *Nature*, **391**, 90–92.
- Zecca,M., Basler,K. and Struhl,G. (1995) Sequential organizing activities of *engrailed*, *hedgehog* and *decapentaplegic* in the *Drosophila* wing. *Development*, **121**, 2265–2278.

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