Distinct roles of Central missing and Dispatched in sending the Hedgehog signal

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SUMMARY

Secreted Hedgehog (Hh) proteins control many aspects of growth and patterning in animal development. The mechanism by which the Hh signal is sent and transduced is still not well understood. We describe a genetic screen aimed at identifying positive regulators in the hh pathway. We recovered multiple new alleles of hh and dispatched (disp). In addition, we identified a novel component in the hh pathway, which we name central missing (cmn). Loss-of-function mutations in cmn cause similar patterning defects to those caused by hh or dispatched (disp) mutations. Moreover, cmn affects the expression of hh responsive genes but not of hh itself. Like disp, cmn acts upstream of patched (ptc) and its activity is required only in the Hh secreting cells. However, unlike disp, which is required for the release of the cholesterol-modified form of Hh, cmn regulates the activity of Hh in a manner that is independent of cholesterol modification. Finally, we show that cmn mutations bear molecular lesions in CG11495, which encodes a putative membrane bound acyltransferase related to Porcupine, a protein implicated in regulating the secretion of Wingless (Wg) signal.

Key words: Hedgehog, Central missing, Dispatched, Acetyltransferase, Drosophila melanogaster

INTRODUCTION

The Hedgehog family of secreted proteins control cell growth and patterning in many key developmental processes in both vertebrates and invertebrates (Ingham, 1994). In Drosophila wing development, posterior (P) compartment cells express and secrete Hh proteins that act upon neighboring anterior (A) compartment cells to induce the expression of decapentaplegic (dpp), which encodes a member of the TGFβ/BMP family of secreted proteins (Basler and Struhl, 1994; Tabata and Kornberg, 1994). Dpp then diffuses bidirectionally into both compartments and functions as a long-range morphogen to control the growth and patterning of cells in the entire wing (Lecuit et al., 1996; Nellen et al., 1996). In addition, Hh activates other genes including patched (ptc), collier (col) and engrailed (en), which are required for proper patterning of the region near the AP compartment boundary (Chen and Struhl, 1996; Strigini and Cohen, 1997; Vervoort et al., 1999).

Hh is synthesized as a full-length protein that undergoes an auto-processing event to generate a cholesterol-modified N-terminal signaling ligand (Porter et al., 1996a; Porter et al., 1996b). The N-terminal fragment of Hh binds to Ptc, resulting in alleviation of Ptc inhibition of Smoothened (Smo) (Chen and Struhl, 1996; Stone et al., 1996). Smo then signals downstream to regulate the Zinc finger transcription factor, Cubitus interruptus (Ci), which transduces the Hh signal into the nuclei (Alexandre et al., 1996; Methot and Basler, 1999; Orenic et al., 1990).

Many components in the hh pathway have been identified through genetic screens for embryonic and adult phenotypes. Almost all of the identified components are required in cells that receive the Hh signal to control either the movement or transduction of the Hh signal. The only exception is dispatched (disp), which encodes a multi-span transmembrane protein with a cholesterol-sensing domain similar to Ptc (Burke et al., 1999). disp is required in the Hh secreting cells and it appears to control the release of cholesterol-modified Hh (Burke et al., 1999). We describe a genetic screen for positive regulators in the hh pathway and present genetic and molecular characterizations of a novel gene required in the Hh secreting cells for proper Hh activity.

MATERIALS AND METHODS

Genetic screen using the eyFLP system

In the primary screen, isogenic FRT-containing males (FRT80B or FRT82B) were treated with 25 mM EMS overnight. After 24 hours recovery, the males were mated with virgin females with corresponding eyFLP/FRT (y w eyFLP2 glass-lacZ; cl3L5 w+ FRT80B/TM6B, y+ for 3L screen; y w eyFLP2 glass-lacZ; FRT82B w+ cl3R3/TM6B, y+ for 3R screen). Mosaic flies with small-eye phenotypes similar to those caused by hh mutation were selected in the F1 generation and singly backcrossed with several eyFLP/FRT males or females. F2 mosaic males with the same phenotypes as the F1 mosaic flies were selected and mated with female balancers to establish stocks. About one third of fertile F1 flies
transmitted mutations to the F2 generation. In the secondary screen, males from individual mutant stocks isolated from the primary screen were mated with virgin females with corresponding hsFLP [y w hsFLP122; Dpp(1;3) x c216 y+ M(3) FRT80B/TM2 for 3L screen; y w hsFLP122; FRT82B hsCD2 y+ M(3)/TM2 for 3R screen]. First or second instar larva were heat shocked to induce FRT/FLP mediated mitotic clones. The F1 flies with mutant clones on the wings (marked by y) were examined for hh like phenotypes. From the 3R screen, we recovered 5 new alleles of hh (SH2, SH3, SH7, SH9, and SH17) and 2 new alleles of disp (SH21 and SH23) based on the eye and wing phenotypes. The identities of these mutants were assigned by complementation test with previously identified alleles.

Second instar larva were heat shocked to induce FRT/FLP mediated mitotic clones. The F1 flies with mutant clones on the wings (marked by y) were examined for hh like phenotypes. From the 3R screen, we recovered 5 new alleles of hh (SH2, SH3, SH7, SH9, and SH17) and 2 new alleles of disp (SH21 and SH23) based on the eye and wing phenotypes. The identities of these mutants were assigned by complementation test with previously identified alleles.

**RESULTS**

**Tissue-specific mosaic screen for positive components in the hh pathway**

We previously identified several novel components in the hh pathway including PKA (also known as DCO) and supernumary limbs (slimb) in a genetic mosaic screen for mutations that affect patterning of adult structures (Jiang and Struhl, 1995; Jiang and Struhl, 1998). In the previous screen, we utilized a heat inducible FLP/FRT system to randomly induce mutant clones and examined pattern abnormalities in adult structures such as wings and legs (Xu and Rubin, 1993). This screen efficiently identified inhibitory components in the hh pathway because clones of mutant cells ectopically activating the hh pathway induced pattern duplications if they were situated in the anterior compartment (Jiang and Struhl, 1995; Jiang and Struhl, 1998). In addition to PKA and slimb, we also identified new alleles of ptc and costal2 (cos2), two previously identified inhibitory components in the hh pathway (Grau and Simpson, 1987; Hooper and Scott, 1989; Sisson et al., 1997; Wang et al., 2000). However, we were unable to isolate positive components in the hh pathway such as disp or tout-velu (ttv), which are involved in either sending or moving the Hh signal (Bellaiche et al., 1998; Burke et al., 1999; The et al., 1999). A likely reason is that Hh signaling only occurs near the AP compartment boundary. Moreover, Hh as well as its major downstream effectors Dpp and Wg acts cell non-autonomously (Basler and Struhl, 1994; Lecuit et al., 1996; Nellen et al., 1996; Zecca et al., 1996). Thus, mutant clones that are defective in Hh signaling may cause significant phenotypes only when they are large enough and are situated near the AP compartment boundary, which is infrequent.

To identify additional positive components that regulate either sending or receiving of the Hh signal, several factors led us to explore the *Drosophila* compound eye. First, Hh signaling activity is required for the initiation and progression of the morphogenic furrow (Dominguez and Hafen, 1997; Greenwood and Struhl, 1999; Heberlein et al., 1993; Ma et al., 1993). Conditional loss of Hh signaling in eyes prevents furrow progression, resulting in a small-eye phenotype (Ma et al., 1993). Second, it is possible to generate mosaic flies that have mutant eyes but wild-type bodies, using the eyFLP system (Newsome et al., 2000). Third, eyes are dispensable for viability and fertility; thus, mutant flies with eye defects can be recovered in the F1 generation. To test the potential of the eyFLP system for identifying positive components in the hh pathway, we generated mosaic flies with eyes mutant for smoo or ttv using eyFLP. As shown in Fig. 2, smoo or ttv mutant eyes exhibit similar small-eye phenotypes to hh mutant eyes. We then conducted a two-step screen as illustrated in Fig. 1. In the primary screen, we used eyFLP to generate mosaic flies and screened in the F1 generation for eye phenotypes similar to those caused by the smoo or hh mutation. Once the mutants were bred true in the F2 generation, we conducted a secondary screen in which we generated mosaic flies carrying large mutant clones in the wing and screened for wing phenotypes similar to those caused by loss or reduction of Hh signaling activity (Basler and Struhl, 1994; Burke et al., 1999). We conducted an extensive screen of randomly introduced mutations on the third chromosome and recovered multiple new alleles of hh and disp (see Materials and Methods). In
Fig. 1. Tissue-specific mosaic screen for mutations in the hh pathway. (A) A two-step screen was designed to identify genes that positively regulate the hh pathway. In the primary screen, mosaic flies were generated using the eyFLP system and mutations that caused hh-like small-eye phenotypes were isolated in the F1 generation. (B) In the secondary screen, mosaic flies bearing large mutant clones in the wing were generated using hsFLP in conjunction with the Minute technique, and were screened for hh-like eye phenotypes. For details, see Results and Materials and Methods. The asterisk indicates a newly induced recessive mutation.

addition, we isolated multiple alleles of a novel gene, which we name central missing (cmn) based on the wing phenotype (see below). cmn mutant eyes, generated using eyFLP, exhibited similar small-eye phenotypes to those caused by mutations in other positive Hh signaling components (Fig. 2).

**cmn affects Hh signaling**

We identified 9 cmn alleles from the mosaic screen and one additional allele by complementation test with previously isolated lethal mutations mapped near the cmn locus (see Methods). We used the cmn<sup>M82</sup> allele for most of our analyses because cmn<sup>M82</sup> homozygotes exhibited similar phenotypes to cmn<sup>M82</sup> over deficiency (data not shown), suggesting that cmn<sup>M82</sup> is a genetically null allele. Wings carrying large clones of cmn mutant cells were often smaller and lacked patterning elements in the central region such as vein 2, 3, 4 and 5 (Fig. 3B). These phenotypes are similar to those caused by loss of hh in the P-compartment (Basler and Struhl, 1994). Despite the severe patterning defects along the AP axis, the wing margin appears normal, suggesting that cmn does not affect Wingless (Wg) signaling.

The similarity of the cmn and hh phenotypes in both eyes and wings suggests that cmn may affect the hh pathway. To further test this possibility, we examined whether cmn affects the expression of Hh-responsive genes including dpp and ptc. As cmn mutants are pupal lethal, we examined dpp and ptc expression in late third instar wing discs homozygous for cmn. cmn mutant discs show reduced levels of dpp expression, as indicated by the expression of both Dpp protein and dpp-lacZ (Fig. 3D, Fig. 4B). As expected, the upregulation of ptc expression at the AP compartment boundary is nearly abolished (Fig. 3F). cmn does not regulate hh expression because hh-lacZ expression is not affected in cmn discs (Fig. 3H). These observations suggest that cmn acts in the hh pathway rather than upstream of hh to control its expression.

**cmn acts upstream of ptc**

To place cmn in the hh pathway, we carried out a genetic epistasis analysis. To determine whether cmn acts upstream or downstream of ptc, we examined Hh responses in cmn ptc
double mutant cells. To do this, we generated ptc mutant clones in cmn homozygous mutant discs. As shown in Fig. 4, cmn singly mutant cells exhibit diminished levels of dpp-lacZ expression. In contrast, anteriorly situated cmn ptc double mutant cells ectopically activated dpp-lacZ at wild-type levels. Thus, ptc mutation can bypass the requirement for cmn in activating Hh signal transduction pathway, suggesting that cmn acts upstream of ptc.

cmn is required in the Hh sending cells
Cmn could act upstream of Ptc to regulate Hh movement or as a Hh coactivator in the Hh receiving cells. Alternatively, Cmn could regulate the production or secretion of Hh ligand in the Hh sending cells. To distinguish these two possibilities, we carried out a mosaic analysis in which we generated large clones of cmn mutant cells in either the A- or P- compartment and examined their effects on ptc-lacZ expression. As shown in Fig. 5, wing discs exhibited normal levels of ptc-lacZ expression even though anterior compartment cells near the AP compartment boundary are mutant for cmn (Fig. 5A). In contrast, wing discs lost ptc-lacZ expression if they contained large P-compartment cmn mutant clones that abut the AP compartment boundary (Fig. 5B). These results suggest that Cmn, like Disp, regulates the sending of Hh signal in the Hh producing cells.

Hh is not detected in A-compartment cells of cmn mutant discs
We then investigated whether cmn affects the secretion of Hh into the anterior compartment by examining Hh distribution in cmn mutant discs. To facilitate the detection of the Hh signal, we overexpressed Hh in P-compartment cells using the hh-gal4 driver line to activate a UAS-Hh transgene (Wang et al., 2000). As shown in Fig. 6, wild-type wing discs overexpressing Hh in P-compartment cells exhibited Hh staining in A-compartment cells near the AP compartment boundary. In these cells, Hh colocalized with Ptc in intracellular vesicles (Fig. 6A,A’). A similar pattern of Hh distribution has been described previously (Tabata and Kornberg, 1994). In contrast, cmn mutant discs that overexpressed Hh in P-compartment cells exhibit little if any Hh signal in neighboring A-compartment cells (Fig. 6B,B’). This observation suggests that secretion of Hh into the anterior compartment might be impeded in cmn mutant discs. cmn mutant discs exhibit lower levels of cell surface staining of Hh in the P-compartment.
expressing CiU expressed diminished levels of col (Fig. 7F).

We also examined Smo stabilization as a readout for Hh activity (Denef et al., 2000). As shown in Fig. 7, wild-type and disp mutant discs stabilized Smo in P-compartment cells at comparable levels (compare Fig. 7G with Fig. 7H). In contrast, cmn mutant discs stabilize Smo at levels much lower than wild-type or disp mutant discs (Fig. 7I). Taken together, these observations demonstrate that disp mutant discs produce normal levels of active Hh in P-compartment cells whereas cmn mutant P-compartment cells produce reduced levels of active Hh.

**cmn is required for both cholesterol modified and unmodified forms of Hh**

Hh is produced as a full-length precursor, which undergoes an auto-processing event to generate a cholesterol-modified N-terminal fragment that functions as a ligand (Porter et al., 1996a; Porter et al., 1996b). To determine if cmn affects Hh processing, we tested whether a pre-cleaved form of Hh (HhN) could rescue cmn mutant phenotypes (Porter et al., 1996a). We used the actin>C2G>Gal4 driver line to express UAS-HhN uniformly in wild-type, disp or cmn mutant discs and examined ptc upregulation as a readout for the Hh signaling activity. As shown in Fig. 8, indiscriminately expressing HhN in either wild-type or disp mutant discs caused ectopic ptc upregulation in the entire A-compartment (Fig. 8B,D). In contrast, uniformly expressing HhN in cmn mutant discs failed to induce upregulation of ptc (Fig. 8F). These results suggest that Cmn does not regulate the cleavage of the Hh precursor into the mature form of Hh. As HhN is no longer modified by cholesterol, this result also suggests that cmn is required for the activity of Hh, independent of cholesterol modification.

**P-compartment cells mutant for cmn produce reduced levels of active Hh**

It is possible that normal levels of active Hh are produced in P-compartment cells of cmn mutant discs but somehow Hh fails to be released into the anterior compartment. If this is true, one would expect that P-compartment cells should activate Hh responsive genes if provided with Ci (Methot and Basler, 1999; Wang et al., 1999). To test this possibility, we used an uncleavable form of Ci (CiU) that requires Hh for its activation (Methot and Basler, 1999). We used a wing-specific Gal4 driver (MS1096) to express UAS-CiU in wild-type, disp or cmn discs and examined these discs for the expression of a Hh-responsive gene col (Vervoort et al., 1999). In wild-type discs, Hh induces col expression in a stripe of cells in the A-compartment abutting the AP compartment boundary (Fig. 7A). This col expression is reduced or abolished in disp and cmn mutant discs (Fig. 7B,C). Consistent with the previous finding that the activity of CiU depends on Hh (Methot and Basler, 1999), expressing CiU in wild-type wing discs ectopically activated col only in P-compartment cells (Fig. 7D). Misexpressing CiU in disp mutant discs activated col in P-compartment cells at levels comparable to those in wild-type discs (Fig. 7E, compared with Fig. 7D). In contrast, P-compartment cells of cmn discs (compare Fig. 6B with 6A; n>50). Moreover, cmn mutant P-compartment cells appear to accumulate more punctate intracellular staining of Hh than wild-type P-compartment cells (Fig. 6A,B). These observations suggest that cmn may affect Hh trafficking.

![Fig. 5. cmn is required in the posterior compartment.](image)

(A,A') A wing disc carrying a large clone of cmn mutant cells was stained to show the expression of ptc-lacZ (red) and a GFP marker gene (green). cmn mutant cells are identifiable by the lack of green staining. Anterior compartment cells near the AP compartment boundary express ptc-lacZ (arrowhead) at wild-type levels even though they are mutant for cmn. (B,B') A wing disc carrying a large clone of cmn mutant cells (marked by the lack of green staining) in the posterior compartment has lost ptc-lacZ expression in adjacent anterior compartment cells (arrowhead).

![Fig. 6. Hh distribution in wild-type and cmn mutant discs.](image)

(A,A',A'') A wild-type wing disc expressing UAS-Hh under the control of hh-Gal4 was stained to visualize Hh (red) and Ptc (green) protein distribution. Hh staining can be detected in anterior compartment cells near the AP compartment boundary. In these cells Hh colocalizes with Ptc in intracellular vesicles (arrows). (B,B',B'') A cmn homozygous mutant wing disc expressing UAS-Hh under the control of hh-Gal4 was stained to visualize Hh (red) and Ptc (green) distribution. Hh staining can only be detected in P-compartment cells, with little, if any, staining in A-compartment cells.

![Image](image)
Molecular characterization of cmn

As a first step to determine the mechanism by which Cmn regulates the production of active Hh, we carried out molecular characterization of the corresponding gene. We mapped cmn to chromosomal region 63B6-63C1 by complementation test with deficiency stocks (see Materials and Methods). Complementation tests with available P elements failed to identify any P element insertion alleles of cmn. We decided to use a local hopping strategy to generate P element tagged alleles of cmn (Tower et al., 1993). We mobilized a P element inserted at 63B and screened derived hop lines by complementation test with cmnM82. We obtained 3 hop lines that failed to complement the lethality of cmnM82. We focused on one such line, P630, because its lethality over cmnM82 could be reversed by precise excision of the inserted P element. Using both plasmid rescue and inverse PCR, we found that P630 harbors a single new insertion between two annotated genes CG14964 and CG12734. However, sequence analysis of cmn mutant alleles as well as rescue experiments suggested that none of these two genes corresponds to cmn (data not shown). We hypothesized that P630 may affect the expression of cmn, which is located further away. We therefore applied RNAi to candidate genes located near the P630 insertion site. One annotated gene, CG11495, drew our special attention because it is related to a gene required for Wg secretion (see below). Injections of double-stranded RNA corresponding to CG11495 into wild-type embryos produced a weak segment polarity phenotype at low frequency (data not shown). Moreover, we found that 5 out 6 EMS induced cmn alleles we sequenced have a nonsense mutation in the coding region of CG11495 (Fig. 9B). These results strongly suggest that the phenotypes associated with cmn mutations are due to inactivation of CG11495. Of note, cmnM82 generates a stop codon at amino acid 163, resulting in a truncated protein that lacks the majority of coding sequence. Thus, cmnM82 appears to be a true null mutation.

CG11495 is predicted to encode a protein that is 500 aa long and contains 10 transmembrane domains (Fig. 9B). Blast search identified homologs in mouse and human, which share over 25% sequence identity to CG11495 and the conservation spans the whole proteins (Fig. 9D). Sequence analysis of the CG11495-encoded protein suggests that it belongs to a family of membrane bound acyltransferases (Fig. 9C) (Hofmann, 2000). Interestingly, this family also includes Porcupine, which is required for Wg secretion (Kadowaki et al., 1996; Tanaka et al., 2000).

Fig. 7. cmn mutant discs produce reduced levels of active Hh in the P-compartment. Late third instar wing discs were stained with anti-Col (green) or anti-Smo (red) antibody to monitor Hh signaling activity. (A-C). Wild-type wing discs activate col in A-compartment cells near the AP compartment boundary in the wing pouch region (A). disp mutant discs exhibit diminished levels of Col expression in a narrow stripe of A-compartment cells (arrowhead in B). Note that Col staining surrounding the wing pouch region is not controlled by Hh (indicated by asterisks in B). cmn mutant discs fail to express Col at detectable levels near the AP compartment boundary (C). (D-F) Expression of CiU in wild-type (D) or disp (E) discs activates Col expression at comparable levels in P-compartment cells, whereas expression of CiU in cmn mutant discs (F) induces ectopic Col expression in P-compartment cells at levels much lower than wild or disp mutant discs. (G-I). Wild-type (G) and disp (H) discs stabilized Smo at comparable levels in P-compartment cells whereas Smo was only slightly stabilized in P-compartment cells of cmn discs (I).

Fig. 8. cmn is required for the activity of cholesterol-free Hh. Late third instar wing discs of the following genotypes were stained with anti-Ptc to monitor the up-regulation of ptc in response to Hh. (A) Wild type, (B) act>CD2>Gal4/UAS-HhN, (C) disp 338037707, (D) act>CD2>Gal4/UAS-HhN; disp 338037707, (E) cmnM82, (F) act>CD2>Gal4/UAS-HhN; cmnM82. Misexpressing HhN in both wild-type and disp mutant discs induced ectopic ptc upregulation (B,D). In contrast, misexpressing HhN in cmn mutant disc failed to upregulate ptc expression (F).
**DISCUSSION**

In this study, we conducted a tissue-specific mosaic screen to identify additional components that act positively in the hh pathway. This screen allowed us to recover multiple alleles of hh and disp, demonstrating the validity of the strategy. Moreover, we identified multiple alleles of a novel positive regulator in the hh pathway, cmn. The number of alleles identified for each of these genes suggests that our screen has reached saturation. Previous saturation zygotic lethal screens for larval cuticle phenotypes failed to identify disp and cmn as segment polarity genes because both genes appear to be maternally expressed. Indeed, we observe hh-like segmental polarity defect in embryos devoid of both maternal and zygotic cmn product (data not shown).

Our experiments suggest that cmn acts upstream of ptc and its function is required in the Hh sending cells but not in cells that receive the Hh signal. Thus, cmn represents a second gene after disp that regulates sending of the Hh signal. The close
similarities between cmn and disp phenotypes prompted us to carry out comparative study of these two genes. We found that cmn and disp affect the sending of Hh in different ways. Whereas disp is specifically required for the cholesterol-modified form of Hh, cmn regulates Hh activity independent of cholesterol modification of Hh (Fig. 8).

A previous report proposed that disp is required for the release of Hh after it reaches the cell surface (Burke et al., 1999). This notion is primarily based on the observation that Hh appears to be trapped in disp mutant cells with no apparent change in subcellular localization of Hh (Burke et al., 1999). However, this study did not rule out the possibility that disp may affect intracellular trafficking of Hh in a subtle way because imaginal disc cells have very narrow cytoplasmic spaces and it is difficult to distinguish between cell surface staining and cytoplasmic staining. Here we provide evidence that Hh produced in P-compartment disp mutant cells can signal normally (Fig. 7), implying that Hh reaches the cell surface.

In cmn mutant discs, we did not detect Hh signal in anterior compartment cells near the AP compartment boundary (Fig. 6). However, unlike the case of disp, we did not observe accumulation of Hh staining in P-compartment cmn mutant cells (data not shown). In contrast, we observed that cmn mutant P-compartment cells consistently exhibited lower levels of cell surface staining of Hh with concomitant increase in the number and size of intracellular Hh aggregates as compared with wild-type cells (Fig. 6). This observation implies that cmn might affect cellular trafficking of Hh. Consistent with lower levels of cell surface Hh staining, we found that cmn mutant P-compartment cells produce lower levels of active Hh as compared with wild-type or disp mutant cells (Fig. 7).

In 5 out 6 cmn EMS alleles we sequenced, we identified a nonsense mutation in the coding region of CG11495, which encodes a putative membrane-bound acyltransferase (Fig. 9), suggesting that cmn phenotypes are caused by inactivation of CG11495. All biochemically characterized members of the membrane-bound acyltransferase family transfer organic acids, typically fatty acid, onto hydroxyl groups of membrane-bound targets (Hofmann, 2000). Although the target for Cmn is not known, a good candidate is Hh. Hh precursor is cleaved to targets (Hofmann, 2000). Although the target for Cmn is not known, a good candidate is Hh. Hh precursor is cleaved to targets (Hofmann, 2000).

It has been shown that mammalian Sonic hedgehog (Shh) acquires a palmitoyl modification on an N-terminal cysteine in cell culture (Pepinsky et al., 1998). N-terminal fatty acid modification of Shh appears to enhance its activity in certain developmental settings (Kohtz et al., 2001). In addition, mutation of a conserved cysteine residue in Drosophila Hh (C845-Hh) impairs its function in vivo, implying that Hh may also be modified by palmitoylation at its N-terminal region (Lee et al., 2001). Thus, one possible role for Cmn is to regulate Hh palmitoylation, which may control Hh activity or intracellular trafficking. Indeed, C845-Hh acts in a dominant negative fashion, implying that it is defective in signaling (Lee et al., 2001). Thus, the lack of detectable Hh signal in A-compartment cells of cmn mutant discs could be explained if palmitoyl-free Hh fails to bind and internalize Ptc efficiently.

It is interesting to note that Cmn is related to Porcupine, which is required for the secretion of Wg signal (Kadowaki et al., 1996). Like Cmn, Porcupine also belongs to the membrane bound acyltransferase family (Fig. 9C), suggesting that acylation of secreted proteins may be a more general mechanism than previously thought for regulating the activity or secretion of signaling molecules involved in animal development.

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