The C-terminal tail of the Hedgehog receptor Patched regulates both localization and turnover

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Patched (Ptc) is a membrane protein whose function in Hedgehog (Hh) signal transduction has been conserved among metazoans and whose malfunction has been implicated in human cancers. Genetic analysis has shown that Ptc negatively regulates Hh signal transduction, but its activity and structure are not known. We investigated the functional and structural properties of *Drosophila* Ptc and its C-terminal domain (CTD), 183 residues that are predicted to reside in the cytoplasm. Our results show that Ptc, as well as truncated Ptc deleted of its CTD, forms a stable trimer. This observation is consistent with the proposal that Ptc is structurally similar to trimeric transporters. The CTD itself trimerizes and is required for both Ptc internalization and turnover. Two mutant forms of the CTD, one that disrupts trimerization and the other that mutates the target sequence of the Nedd4 ubiquitin ligase, stabilize Ptc but do not prevent internalization and sequestration of Hh. Ptc deleted of its CTD is stable and localizes to the plasma membrane. These data show that degradation of Ptc is regulated at a step subsequent to endocytosis, although endocytosis is a likely prerequisite. We also show that the CTD of mouse Ptc regulates turnover.

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Hedgehog (Hh) signaling is essential to the development of many organs and tissues, and is implicated in many human diseases. Its role and mechanism are broadly conserved among metazoans. Target cells deploy two transmembrane proteins to receive and regulate Hh signals—Patched (Ptc) and Smoothened (Smo)—and genetic evidence suggests that the activities of these proteins are functionally linked. Smo is a seven-transmembrane domain protein that is essential to transduce the Hh signal (Alcedo et al. 1996; van den Heuvel and Ingham 1996), but Smo does not apparently function as a receptor. Instead, Ptc is proposed to be the Hh receptor (Ingham et al. 1991; Chen and Struhl 1996; Marigo et al. 1996; Stone et al. 1996; Fuse et al. 1999), and to inhibit Smo unless bound by Hh. Understanding the processes by which Ptc responds to Hh and gates signal transduction is the key to deciphering the mechanism of Hh signaling.

Ptc proteins have been identified in a number of species, and their roles in Hh signal transduction appear to be conserved. Ptc proteins are present at the highest levels in cells that are active in Hh signal transduction, where they are up-regulated in response to Hh (Capdevila et al. 1994a; Tabata and Kornberg 1994; Ingham and Fietz 1995; Goodrich et al. 1996; Marigo et al. 1996). Molecular evidence for Hh binding has been provided by studies showing that vertebrate Sonic Hh (Shh) binds to cells expressing vertebrate Ptc (Marigo et al. 1996; Stone et al. 1996; Fuse et al. 1999). Although equivalent data for *Drosophila* Hh and *Drosophila* Ptc has not been reported, genetic studies in *Drosophila* show that Ptc acts downstream from Hh to regulate signaling activity (Ingham 1993; Tabata and Kornberg 1994; Ramirez-Weber et al. 2000) and that Ptc and Hh colocalize in a punctate distribution in Hh-receiving cells (Bellaiche et al. 1998; Burke et al. 1999; Ramirez-Weber et al. 2000; Martin et al. 2001; Strutt et al. 2001). Genetic studies also indicate that up-regulating Ptc expression in Hh-receiving cells functions to sequester Hh, creating a barrier to further movement that limits the range of Hh action (Chen and Struhl 1996). Localization of Ptc to multivesicular bodies and endosomes (Capdevila et al. 1994b; Torroja et al. 2004) and removal of Ptc from the plasma membrane upon exposure to Hh (Denef et al. 2000; Zhu et al. 2003) support the proposition that Ptc scavenges Hh by ferrying it through the endocytic pathway.

It is unclear how Ptc carries out its other important roles: inhibiting Smo in the absence of Hh and activating signal transduction when Hh is present. The existence of

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