Hedgehog, Patched and Dispatched near the basement

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Cell signaling is arguably the most important characteristic of multicellular organisms. Without cell signaling, the different cells in the body of a plant or animal could not communicate with each other, and they could not coordinate their actions. Such coordination is essential: first, to build a complex body composed of thousands or millions of cells and second, for the correct performance of such a body in everyday life, whether acquiring nutrients, excreting toxins, or dealing with hostile or friendly interactions from other organisms. This coordination is corroborated by the many problems and diseases (such as developmental abnormalities and cancers) that arise from malfunction of the cellular machinery that deals with cell signaling and communication (1–3). Thus, the study of such cell signaling machinery is receiving a great deal of attention by biological and medical research. At the most basic level, any cell signaling process must involve a signal, synthesized or otherwise, generated by the sending cell and some kind of system to receive that signal and respond to it in the receiving cell. An article by Callejo et al. (4) in PNAS sheds light on the first aspect of the process by providing crucial information on the mechanism for the release and transport of the signaling protein Hedgehog (Hh). Their findings show that a crucial step in Hh signaling is trafficking and recycling within the sending cell itself. Their findings, however, resonate with and are relevant to research on other signaling proteins.

Cells in many tissues organize themselves in epithelia (that is, single cell-thick sheets of cells with distinctive apical, lateral and basal surfaces). Cells within epithelia are, thus, polarized, and this fact has clear functional consequences. For example, in the gut, the apical surface faces the lumen and secretes digestive enzymes and other products, whereas the basal surface attaches the cells to a basement membrane composed of extracellular matrix. Because of these precedents, it might be easier to think that the natural place for signaling molecules to be secreted is the apical surface. However, it is being increasingly recognized that this polarization is exploited in a much more complex way during cell signaling (5) and that the secretion and traffic of signaling molecules may happen apically, basally, or apparently, at both cell surfaces. For example, the secretion of the signaling protein Wingless (Wg; a member of the Wnt family) was deemed to occur

Fig. 1. Main steps in the trafficking of the signaling protein Hh. The Hh protein is expressed in the sending cell (on the left) and undergoes posttranslational modifications, particularly cleavage and the addition of palmitic acid and cholesterol (1). This lipid-coated form of Hh is secreted by the apical surface, but perhaps because of its high affinity for membranes, it is retained there (2). The Hh protein is subsequently internalized in endocytic vesicles, a process that requires the proteins Shi (Dynamin) and several members of the Rab family (3). In an internal vesicular compartment, most likely early endosomes, Hh binds the 12 transmembrane-pass protein Dispatched (Disp), which drives Hh to the basolateral plasma membrane (4). There, direct protein interactions between Disp, Ihog, and the glypican protein Dally-like (Dlp) lead to the release of Hh to the intercellular space, perhaps as part of a complex or a specialized type of extracellular vesicle (5). At the plasma membrane of the receiving cell (on the right), the Hh protein binds its receptor, the seven transmembrane protein Patched (Ptc) (6). This binding activates the Hh signaling pathway in the receiving cell, leading to the posttranslational activation of the Ci transcription factor (7). In turn, Ci activation leads to the expression of Hh target genes, such as *collier*, *dpp*, and *ptc* itself. Finally, binding by Ptc leads to internalization and degradation of the Hh protein in the receiving cell.

mostly apically (6) and subsequently, basally (7) all in the same tissue used in the work by Callejo et al. (4), the developing fly wing and likewise the signaling protein Decapentaplegic (Dpp; a member of the TGF- β family) (8). Because the properties of the extracellular medium change from the apical to the basal sides, the secretion of a signaling molecule at either side will affect its mode of transport, range, speed of diffusion, and even the type of receptors that it may have access to in the receiving cell; hence, knowledge of the place of secretion is a fundamental piece of the signaling jigsaw.

Secretion of Hh has been reported to occur apically (9), but the available data suggested a complex process. The Hh protein is first produced as a long precursor, which then needs to be cleaved, and the active or mature fragment needs to be modified by the addition of lipids (3).

The molecular purpose of these modifications has been hotly debated, because engineered versions of Hh that cannot have lipids added to them are able to travel even farther than WT Hh (11). In addition, the range of the Hh protein is limited by its receptor Patched (Ptc), because after binding, internalization, and signaling, the Hh protein is degraded in the receiving cell. In turn, the expression of Ptc is up-regulated by Hh signaling in a feedback control mechanism also shared by Wg and Dpp signaling (8, 10). Finally, the proteins Dally-like (Dlp), Dispatched (Disp), and Interference Hh (Ihog) have also been linked to Hh trafficking,

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particularly in helping Ptc to receive and internalize Hh (3, 11).

Callejo et al. (4) reexamine the roles of these proteins in Hh signaling and the apico-basal specificity of Hh secretion. In their quest, they are aided by a set of techniques. First, they use a set of tools for genetic manipulation developed by the *Drosophila* community over the years, which allows the selective manipulation of Hh, Disp, Dlp, and Ihog in either sending cells (the cells located in the posterior part of the developing fly wing) or receiving cells (located in the anterior part). Second, Callejo et al. (4) have developed in their laboratory a staining method to observe and distinguish between Hh protein located inside and outside the cells. This method relies on their specific fixation technique coupled with the use of a fusion Hh-GFP protein (11). Their report here shows that, in normal conditions, most of the secreted, extracellular Hh is found at the baso-lateral, not apical, side of the epithelium (4). However, Hh is initially secreted apically, and then, it is internalized by the sending cells and transferred to their baso-lateral membranes (Fig. 1). This recycling traffic (or transcytosis) in the sending cells involves the Shbire/dynamin protein and the Rab5, -4, and -8 proteins, which is most clearly shown by the apical accumulation of Hh in sending cells when these proteins are removed or blocked specifically in the sending cells. Each protein is required for a specific step of this traffic in agreement with their previously described functions (5). Shi affects the initial internalization of extracellular Hh in endocytic vesicles. Rab5 is required for the detachment of such vesicles from the plasma membrane to allow their traffic to early endosomes. Rab4 and -8 are required for the fusion of Hh-containing vesicles to early endosomes in preparation for the release of Hh protein to the baso-lateral cell membrane.

Such apical to basal traffic and recycling is also shown by other molecules in other epithelia, such as the GPI-linked proteins in the vertebrate gut (5). However, an even more interesting finding is that the proteins Disp, Dlp, and Ihog are also involved in this traffic in the sending cells apart from their previously described functions in the re-

ceiving cells. In particular, the 12-membrane pass protein Disp may function at the traffic from early endosomes to the baso-lateral membrane, and Callejo et al. (4) show how Disp colocalizes with (and directly binds to in co-immunoprecipitation experiments) Hh in vesicles that, after incubation with dextran, can be seen to move towards the basal side of the cell. At the baso-lateral plasma membrane, the protein Ihog and the glypican Dlp seem to be involved in the release of Hh, perhaps in some type of extracellular vesicle similar to the argosomes described previously (12).

What is the functional significance of this apical to basal traffic? A functional requirement for an apical traffic and recycling of Hh had already been noted (9), but it had not been linked to basal recycling. Callejo et al. (4) show that blocking the function of Rab5 by expressing a dominant-negative version of the protein leads to accumulation of Hh inside the sending cells. This sequestering correlates with a reduction in the expression of all targets of Hh signaling in the receiving cells (i.e., the expression of the receptor Ptc and the genes *Dpp*, *collier*, and *Ci*, which is the homolog of vertebrate Gli, is reduced). However, blocking the function of the dynamin Shbire leads to reduction of Ptc and *collier* but also to an expansion of *Ci* and *Dpp*. Because blocking Shi also leads to the accumulation of Hh apically, this observation may seem apparently contradictory with the proposal that most Hh diffusion and signaling occurs basally. Two observations help to solve this riddle. First, the observed accumulation of Hh in Shi-blocked cells is mostly extracellular, and thus, it is possible that a small amount of this abnormally accumulated Hh may be able to travel across the epithelium and activate some Hh targets. Second, the Hh targets expanded in Shi (*Dpp* and *Ci*) are deemed to require lower levels of Hh signal than those reduced (*Ptc* and *collier*). Thus, even if some amount of the apically accumulated Hh may be diffusing, its signaling ability is reduced in the absence of apical to basal traffic and recycling. This reduced signaling leads to reduction of Ptc levels and hence, reduced Hh degradation, which may, in turn, contribute to the expanded range of the limited, apically re-

leased Hh in this experiment. In conclusion, apical to basal recycling would seem essential for Hh to acquire its full signaling potency.

What happens to Hh during this recycling to increase its signaling ability? A similar requirement for secretion and recycling in the sending cells has already been shown for the transmembrane signaling protein Delta, which to signal to its receptor Notch, must be first internalized by the sending cell and glycosylated (13). A tempting scenario for Hh is postulated by Callejo et al. (4), which attempts to link the already known lipid modifications of Hh with the roles of Dlp and Rab proteins in the process. In synthesis, Callejo et al. (4) propose that the lipid modification of Hh may be essential to ensure its secretion; this secretion would happen apically, but the secreted lipid-linked protein would not be able to travel far [or at least, not as far as the lipid-free Hh, which was shown by an engineered lipid-free Hh (4)]. For long-range diffusion, the lipids have to be removed, and for this removal, Hh may take advantage of the apical to basal GPI-related trafficking and transcytotic machinery.

What are the next questions to be answered? The details of the scenario proposed by the work by Callejo et al. (4) have to be clarified, particularly the exact nature of the modifications (if any) undergone by Hh during the apical to basal traffic. Also, the difference between the roles of Dlp and Ihog in sending and receiving cells has to be ascertained, which might be best achieved by removing the function of these genes in receiving cells after apical to basal traffic has already been blocked in sending cells. Last but not least, this model of apical to basal traffic has to be extrapolated to other epithelia where proteins of the Hh family signal, including vertebrates where homologs of all of the proteins mentioned here exist, and are, in most cases, already linked functionally (3). However, the work of Callejo et al. (4) should also trigger a reexamination of the traffic of other signaling proteins, where apparently contradictory observations of apical and basal secretion have been reported, and a search for evidence of apico-basal traffic and recycling of more signaling molecules.

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