Hedgehog Signalling

Hedgehog is autocatalytically cleaved in the ER to produce an N-terminal and a C-terminal fragment where the N-terminal fragment is responsible for its biological activities.
During the cleavage reaction a cholesterol molecule is coupled to its C-terminal end and the N-terminal end is palmitoylated.
The lipophilic property of hedgehog proteins is one of the things that speak against a simple diffusion mechanism for long range signalling. Another factor that restricts hedgehog movement is that hedgehog stimulates transcription of its own receptor, Ptc.
Despite these hindrances the effects of hedgehog can be seen several cell-diameters away from where it is produced. During the last couple of years a number of reports have provided clues for how to solve this paradox.

Rietveld and others (Rietveld et al., 1999) have shown that HhNp accumulates in so called Lipid Raft Microdomains known to play an important role in polarized protein sorting and signal transduction.
These domains consist mainly of sphingolipids, sterols and GPI-linked proteins, and therefore have very distinct physical properties compared to the rest of the phospholipid membrane. The flat structure of the hedgehog-linked cholesterol is preferentially associated with sphingolipids rather than phospholipids, due to the higher degree of saturated fatty acids in sphingolipids, which allows for a more favourable packing.
Lipid rafts have been shown to participate in axonal trafficking of proteins in neurons and apical trafficking in epithelia. In analogy to this, hedgehog protein moves axonally in photoreceptor neurons and evidence suggests that hedgehog protein is actively transported through the plane of the epithelium.

Burke and others (Burke et al., 1999) have identified a segment polarity gene in *Drosophila*, called *disp*.
Disp acts exclusively in hedgehog producing cells to liberate the processed cholesterol tethered form of hedgehog from the membrane.
Hedgehog without the cholesterol is not affected by disp and can diffuse freely. Experiments have also been done with hedgehog associated to a GPI-anchor and these are not released from the cell surface.
Disp as well as ptc contain a sterol sensing domain (SSD) which underscore the importance that the cholesterol modification have in the intercellular trafficking of hedgehog.

Recent evidence has implicated Heparan Sulphate Proteoglycans as important factors in Wnt, Hh, FGF and TGF? signalling during embryonic development.
Perrimon and others (The et al., 1999) have reported the finding of tout velu (French for “all hairy”), a HS polymerase involved in HSPG biosynthesis.
Drosophila ttv mutants have a segmentation polarity phenotype reminiscent to hh or wg mutants.
What makes ttv mutants unique compared to other HSPG biosynthesis mutants (such as sgl or sfl) is that it seems to be specific for the hh protein, which accumulates within the producing cells.
For example Wg and FGF/Htl signalling (involved in *Drosophila* mesoderm migration) known to be defect in sgl and sfl mutants is not affected at all in ttv mutants.
This specificity can be interpreted in a quantitative or a qualitative model:
In the quantitative model the loss of hh signalling is due to that hh is more sensitive to a reduction of HSPG’s than FGF and Wg.
According to the qualitative model the specificity suggests the existence of hh-specific HSPG’s.

The fact that Wg and FGF signalling isn't affected at all in ttv mutants while hh signalling is similar to a total loss of hh activity favours the qualitative model. These results show that ttv is key enzyme in the specific transportation of hh between cells.
One theory of how this transportation can take place is that a GPI-anchored HSPG, such as a Glypican molecule, is required to localize hh in lipid rafts (where GPI-linked proteins are common). There have been observations of how GPI-anchored proteins can be transferred between cells, and this may in this case be a way to transport hh-molecules.
Another theory that involves the disp protein postulates ttv-modified HSPG's as co-factors required for the dissociation of hh from disp.
In this model disp on hh-secreting cells will sequester hh from lipid rafts and ttv modified HSPG's on adjacent receiving cells aids in the dissociation of hh from disp.
Once associated to the proteoglycan the hh molecule can either be transported on the membrane surface or interact with ptc.

Kornberg and associates (Ramirez-Weber and Kornberg, 1999) have shown that cells in the *Drosophila* wing imaginal discs have thin, actin-based extensions called cytonemes, that project towards the signalling centre at the A/P compartment border where they arborate at cell contact.
These cytonemes are induced and oriented by FGF, and it is hypothesized that hh and other morphogens can interact with the cytoneme and create an intracellular gradient within the cytoneme of the receiving cell.
This gradient would have the same effect as an extracellular gradient since the length of the cytonemes depends on the distance to the receiving cells from the signalling centre.
Cytonemes have also been found in mouse limb bud cells and chick embryo cells suggesting that this could be a common way of long-range cell-cell communication in multicellular eucaryotes.

References

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