



Study of Transcytosis and Cellular Trafficking on Hedgehog Signalling

Dr. Jhrna M*

Department of Cell Biology, University of the French West Indies and Guiana, French Guiana

Abstract

Hedgehog and Wingless morphogens specify cell fate in a concentration-dependent manner in the *Drosophila* wing imaginal disc. In this study, we demonstrate that the glycosyl-phosphatidyl-inositol anchor of the glypican Dally-like is required.

Keywords: Hedgehog; Wingless morphogens; Glycosyl-phosphatidyl-inositol; Glypican dally-like; Proteoglycans

Introduction

In vertebrates and invertebrates, tissue growth and patterning is controlled by molecules called morphogens. Hedgehog and Wingless/Wnt morphogens are secreted from a local source, forming a gradient of activity where target cells adopt specific cell fates depending on the distance from the source. Target cells interpret the secreted signal through a complex network of proteins leading to activation of a specific set of target genes. Powerful systems exist to study morphogens regulation, such as the limb bud in vertebrates or the wing imaginal disc in *Drosophila*. The wing pouch of the disc gives rise to the wing blade once metamorphosis is completed, and is patterned by several morphogens such as Wg and Hh. Hh is produced by the posterior (P) compartment and moves toward the anterior (A) compartment, whereas Wg is expressed into two rows of cells along the dorsoventral (D/V) axis and moves toward both the dorsal and ventral compartments [1].

Method

Morphogen movement and reception are tightly regulated by various proteins. HSPGs are composed of heparan sulfate glycosaminoglycan (HSGAG) chains attached to specific sites on a core protein.

Only genetic analyses of the lack of enzymes involved in the biosynthesis of HSGAG chains. The experiments carried out in *Drosophila* embryos and in cell culture suggest that Dlp may act at the level, or upstream, of the Hh receptor Patched. Indeed, in vertebrates, GPI-anchored proteins are mainly targeted to the apical surface of epithelia. GPI proteins can also be located within a plasma membrane subdomain enriched in cholesterol and sphingolipids, called lipid rafts. Such lipid raft localization allows "lateral" association of GPI proteins with other transmembrane proteins, predicting their future pathway of endocytosis in a dynamin- and clathrin-dependent manner. The GPI can be cleaved by phospholipase C enzymes. Based on the hh-like phenotype observed by Franch-Marro [2]. In dlp homozygote wings, we chose to examine in more detail the role of Dlp in Hh signaling. The former phenotype is reminiscent of a reduction of Hh activity, as the area between veins 3 and 4 is directly under Hh control. This prompted us to analyze the expression of Hh target genes in the wing imaginal discs of dlp homozygote L3 larvae. Only cells of the A compartment can interpret the Hh signal because P cells lack both the Hh receptor Patched and the transcription factor Cubitus interruptus which mediates Hh signaling. Close to the Hh source in the A compartment the patched and engrailed genes are upregulated, whereas far away the target gene decapentaplegic is activated. In dlp discs, En, Ptc, and dpp were reduced both in their intensity and width of expression and the stabilization of Ci was also narrower. These data strongly suggest an involvement of Dlp in Hh signaling [3].

To distinguish between these hypotheses, we randomly generated mutant clones for dlp either in the P or in the A compartment of the wing imaginal disc by mitotic recombination. dls is a protein null allele, as Dlp staining is absent from mutant clones. Large mutant clones in the P compartment did not affect En, Ptc, or dpp expressions in the A compartment or Hh stability at the apical or lateral side of the Hh-producing cells. Nevertheless, all three targets are decreased in A mutant clones although still expressed anterior to narrow clones. Also observed that small mutant clones as well as the first mutant cells inside larger clones still express the Hh target genes at the right levels. This observation suggests that WT neighboring cells can partially rescue the lack of Dlp in mutant cells, as already proposed by Callejo. Analyses of mutant clones for Tout velu/Ext1, an enzyme required for HSGAG biosynthesis [4].

Results

Analysis of the expression pattern of Dlp suggested that Dlp may be a target of the Hh pathway. Dlp is upregulated in the first few of cells in the A compartment, it is lower in the P compartment. It was proposed that the low level of Dlp along the D/V axis is a result of the shedding of Dlp by the activity of the secreted β -hydrolase Notum, a Wg target gene expressed in a few of cells surrounding the domain of Wg production. To test Dlp acts as an Hh coreceptor that is cointernalized with Hh and Ptc, we performed colocalizations in the wing imaginal disc [5].

To look at the presence of Ptc in the Hh/Dlp vesicles, we overexpressed GFP-Dlp in Hh-receiving cells. Detected a strong colocalization between Ptc, Hh, and GFP-Dlp in large subapical intracellular vesicles. To confirm that these large vesicles were of endocytic nature, we performed a live pulse-chase experiment with the endocytic marker. We found that GFP-Dlp never colocalized with Hh in large intracellular vesicles without the presence of Ptc, Hh colocalization with Ptc, but without Dlp, was observed, suggesting that Dlp was unable to internalize Hh by itself [6]. We generated transgenic flies carrying a construct in which the GPI anchor of GFP-Dlp is replaced by a CD2 transmembrane domain. It never colocalized with either Ptc or Hh in intracellular vesicles. The GPI anchor of Dlp is

*Corresponding author: Dr. Jhrna M, Department of Cell Biology, University of the French West Indies and Guiana, French Guiana, E-mail: m.jhrna8658@gmail.com

Received: 10-Apr-2023, Manuscript No: jbc-23-99735, **Editor assigned:** 12-Apr-2023, PreQC No: jbc-23-99735 (PQ), **Reviewed:** 26-Apr-2023, QC No: jbc-23-99735, **Revised:** 01-May-2023, Manuscript No: jbc-23-99735 (R), **Published:** 08-May-2023, DOI: 10.4172/jbc.1000183

Citation: Jhrna M (2023) Study of Transcytosis and Cellular Trafficking on Hedgehog Signalling. J Biochem Cell Biol, 6: 183.

Copyright: © 2023 Jhrna M. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

necessary for the cointernalization of Dlp together with Hh and Ptc in endocytic vesicles.

The extracellular labeling protocol was carried out at 4°C to diminish endocytosis, raising the possibility that under normal temperature conditions, Dlp rapidly cycles from the apical surface. The GFP-Dlp-tagged form using the dorsal driver ap-Gal4 and monitored Dlp endocytosis. The first internalized vesicles were observed 30 min after shifting to 22°C. Ninety minutes after endocytosis was restored, almost all the extracellular Dlp was relocated from the apical side to the basolateral compartment of the cells [7].

We blocked endocytosis by expressing a dominant-negative form of Shibire, the *Drosophila* homolog of dynamin, which is involved in clathrin- and caveolin-dependent mechanisms of endocytosis. As expected, expressing ShiDN promoted extracellular Dlp accumulation at the apical surface of cells but at the expense of basolateral staining. The demonstrate that once Dlp reached the apical surface of the epithelium, it is rapidly internalized through dynamin-dependent endocytosis and redirected to the basolateral compartment where it accumulates [8].

The expression of Ptc and dpp in ShiDN-expressing clones. Within these clones, we observed a stabilization of Ptc at both the apical and basal poles of the cells, which can be attributed to the inhibition of Ptc degradation when Ptc is blocked at the cell surface.

Discussion

Previous studies failed to observe Dlp at the apical surface of the wing disc epithelium despite the fact that it has been extensively shown in vertebrates that GPI-linked proteins are mainly targeted to the apical surface of epithelial cells. By performing extracellular labeling and kinetic experiments, we demonstrated that Dlp is targeted to the apical surface before being endocytosed and readdressed to the basolateral compartment. Blocking endocytosis allowed us to demonstrate that apical surface accumulation takes place at the expense of its basolateral location, showing that Dlp is first targeted to the apical domain of the epithelium before being sent to the basolateral compartment. We show that the GPI anchor of Dlp is essential for its internalization, as Dlp tethering by a transmembrane domain abolishes its capacity to be internalized [9].

Interestingly, we observed a rescue of the first row of **dlp** mutant cells by the wild-type surrounding cells. GPI-linked proteins are inserted in the outer leaflet of the plasma membrane and are exposed to the extracellular space Dlp from the outer leaflet of the plasma membrane of one cell to the next. low-density lipoproteins containing lipophorins, esterified cholesterol, and triglycerides surrounded by a phospholipid monolayer [10].

Whereas numerous data have clearly demonstrated the role of the HSPG in Hh signaling through regulation of Hh stabilization, movement, and reception, the function of Dlp in Hh signaling remained unclear. In this study, we clearly demonstrate that Dlp is exclusively necessary in Hh-receiving cells for full-strength Hh signaling. The role of Dlp is clearly different from other Hh coreceptors. Indeed, already identified Hh coreceptors such as Ihog and Brother of Ihog stabilize Hh

at the cell surface, Dlp overexpression does not increase Hh binding on receiving cells in vivo or in vitro.

Conclusion

We and others have demonstrated that Hh is apically secreted by wing disc epithelial cells. Nevertheless, the data suggest that endocytosis from the apical surface is necessary to sustain full-strength Hh. We observed a colocalization between Hh and extracellular Dlp at the apical side of Hh-receiving cells but not at the basolateral part of the cell. Blocking endocytosis impairs Hh signaling both in embryos and in wing discs. It has been previously published that inhibiting endocytosis in imaginal discs using a thermosensitive allele of **shi** does not impaired Hh signaling: **Ci** and **collier** are unaffected but Ptc is stabilized.

We demonstrate that Wg is mainly secreted via the apical pole of producing cells. Strikingly, in those cells, Wg is strongly localized in endocytic vesicles that are abundant apically but also in multivesicular. Therefore, we propose that Wg is secreted apically and is then endocytosed with the help of Dlp, Dlp targets Wg by transcytosis to the lateral compartment, where it is stabilized and can spread farther away to activate long-range target genes.

Acknowledgement

None

Conflict of Interest

None

References

- Nusslein-Volhard C, Wieschaus E (1980) Mutations affecting segment number and polarity in *Drosophila*. *Nature* 287: 795–801.
- Briscoe J, Therond PP (2013) The mechanisms of Hedgehog signalling and its roles in development and disease. *Nat Rev Mol Cell Biol* 14: 416–429.
- Chen MH, Li YJ, Kawakami T, Xu SM, Chuang PT (2004) Palmitoylation is required for the production of a soluble multimeric Hedgehog protein complex and long-range signaling in vertebrates. *Genes Dev* 18: 641–659.
- Panakova D, Sprong H, Marois E, Thiele C, Eaton S (2005) Lipoprotein particles are required for Hedgehog and Wingless signalling. *Nature* 435: 58–65.
- Matussek T, Wendler F, Poles S, Pizette S, D Angelo G, et al. (2014) The ESCRT machinery regulates the secretion and long-range activity of Hedgehog. *Nature* 516: 99–103.
- Gradilla AC, Gonzalez E, Seijo I, Andres G, Bischoff M, et al. (2014) Exosomes as Hedgehog carriers in cytoneme-mediated transport and secretion. *Nat Commun* 5: 5649.
- Bilioni A, Sanchez-Hernandez D, Callejo A, Gradilla AC, Ibanez C, et al. (2013) Balancing Hedgehog, a retention and release equilibrium given by Dally, Ihog, Boi and shifted/DmWif. *Dev. Bio* 376: 198–212.
- Bischoff M, Gradilla AC, Seijo I, Andres G, Rodriguez-Navas C, et al. (2013) Cytonemes are required for the establishment of a normal Hedgehog morphogen gradient in *Drosophila* epithelia. *Nat. Cell Biol* 15: 1269–1281.
- Rojas Rios P, Guerrero I, Gonzalez Reyes A (2012) Cytoneme-mediated delivery of Hedgehog regulates the expression of bone morphogenetic proteins to maintain germline stem cells in *Drosophila*. *PLoS Biol*
- Sanders TA, Llagostera E, Barna M (2013) Specialized filopodia direct long-range transport of SHH during vertebrate tissue patterning. *Nature* 497: 628–632.