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SPOTLIGHT

Glypicans and cytonemes unite to distribute Wnt ligands

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Hu et al. (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202009082) show that Glypican 4 participates in filopodia-mediated Wnt transport from endoderm to mesoderm in zebrafish embryos to facilitate intercellular communication between germ layers.

Signaling from one cell to another is fundamental for the development of multicellular organisms. Signaling is initiated by one cell releasing a ligand so that it can be recognized on the surface of another cell. To control cell signaling, then, it is imperative to control the ligand's extracellular distribution. Two prominent mechanisms have been identified that regulate the distribution of extracellular ligands: first, restricted diffusion mediated by glypicans, and second, direct communication between cells mediated by cytonemes or signaling filopodia, which are actin-rich conduits that transport ligands directly from the signal-generating cell to the signal-receiving cell. In the report by Hu et al., these mechanisms are united, as they show that Glypican 4 (Gpc4) promotes the function of signaling filopodia to signal from one tissue to another (Fig. 1; 1).

In a previous study, these authors found that Gpc4 was required for morphogenesis of the developing zebrafish embryo, specifically for convergence and extension (C&E) movements (2). In the current article, Hu et al. identify the mechanisms by which Gpc4 facilitates intercellular signaling, beginning with the interesting finding that supplying *gpc4* transgenically only in the endoderm of *gpc4* null animal rescues C&E not only in the endoderm but also in the other germ layers. This result indicates that Gpc4 acts both autonomously within the endoderm but also nonautonomously, somehow affecting the other germ layers of the transgenic animal even though it is not expressed in those layers (1).

How does Gpc4 function nonautonomously? Typically, glypicans localize on the cell surface where they are attached via a glycosylphosphatidylinositol (GPI) anchor, and one possibility was that Gpc4 leaves the cells that synthesize it by getting cleaved at its GPI anchor. To address this question, the authors injected one cell of a 16-cell blastula with messenger RNAs encoding both GFP-Gpc4 and nuclear mCherry, then looked later in embryogenesis to see if GFP-Gpc4 was found only at the plasma membranes of cells with mCherry-labeled nuclei. Interestingly, GFP-Gpc4 was able to spread to unlabeled cells distant from expressing cells. The authors tested whether this nonautonomous spreading could account for the ability of Gpc4 to rescue C&E in all germ layers by reengineering the Gpc4 protein so that it could no longer spread: They replaced the GPI anchor with a transmembrane domain and showed that this modification effectively anchored Gpc4 to the cells that express it. To the authors' surprise, however, when this transmembrane version of Gpc4 was expressed in the endoderm of transgenic embryos, it was still able to rescue C&E nonautonomously, in the mesoderm. Thus, the delivery of Gpc4 itself to other cells could not fully account for its nonautonomous function.

Glypicans function by promoting the diffusion of secreted ligands, such as Wnts, in the extracellular space, ensuring proper ligand availability to the recipient cells (3, 4). Given the previous findings that *Xenopus* Gpc4 interacts with Wnt5, Wnt8, and Wnt11 to regulate C&E (5), the authors investigated if the Gpc4-mediated rescue of a C&E defect in zebrafish was also Wnt dependent. After finding that loss of these Wnts mimicked a *gpc4* loss of function phenotype, they determined that Gpc4 physically interacted with functional Wnt5b and Wnt11f2 ligands in cultured cells, as they could be coimmunoprecipitated. Importantly, genetic interaction experiments showed that endodermal Gpc4 rescue of the C&E defect relied on endogenous Wnt5b and Wnt11f2 ligands. Based on these results, the authors tested a new model for the nonautonomous function of Gpc4—that the Wnts might spread to other cells.

Some signaling ligands can be transported to other cells by signaling filopodia, actin-rich cellular protrusions that deliver ligands directly to the recipient cell. In the most significant and surprising results of this study, the authors discovered that Gpc4 was required for the functioning of endodermal signaling filopodia, as loss of *gpc4* decreased their number and length. These filopodia were decorated with Wnt ligands Wnt5b and Wnt11f2, and live imaging showed labeled Wnt ligands being actively transported from cell to cell, released by and

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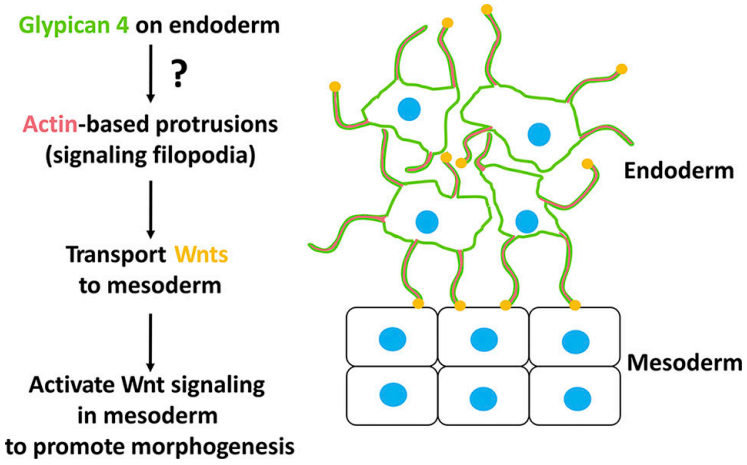

Figure 1. **Endodermal Gpc4 aids in filopodia-mediated transport of endodermal Wnts to mesoderm to facilitate morphogenesis.** Cell-surface Gpc4 is required for Wnt transport from endoderm to mesoderm in zebrafish embryos. Wnt transport occurs on actin-rich signaling filopodia, and Gpc4 is required for proper filopodial morphology to effectively transport Wnts.

taken up by endodermal cells in embryos and even transported to mesodermal cells. Although it might have been expected that without Gpc4, these filopodia could not transport Wnt ligands, the authors found that both Wnt ligands were still available in the signaling filopodia, and instead the problem is that without Gpc4, the filopodia are too short and too few to effectively transport Wnts from cell to cell. This conclusion is supported by experiments that interfere genetically or pharmacologically with the actin polymerization necessary for filopodia formation: When Gpc4 was supplied only in the endoderm, embryos were especially susceptible to interference with filopodia, as these treatments abolished the ability of endodermal Gpc4 to rescue C&E in other germ layers.

Thus, the conclusion of this work is that the nonautonomous effect of Gpc4 in rescuing C&E in all germ layers comes from the role of Gpc4 in promoting the function of signaling filopodia, likely delivering Wnt ligands from endodermal cells that express them to mesodermal and ectodermal cells that need them for morphogenesis to occur.

Signaling filopodia or cytonemes, as they were first discovered and called in flies, emerged as a novel mechanism of ligand transport in vivo about two decades ago (6). Much of cytoneme biology, other than the general actin-based cellular machinery required for their formation, is largely unknown, and the discovery that glypicans contribute to their formation and function is exciting. Two prior observations in flies supported the idea that glypicans could be involved in cytoneme function. First, cytonemes cannot extend over a patch of cells mutant for fly glypicans (7). Second, glypicans localize on cytonemes (8), suggesting that they might contribute to cytoneme function. The advance by Hu et al. alters how we think about cytoneme-like actin protrusions: at least in some contexts, their ligand-delivery function is impaired in the absence of a glypican. This work also has the potential to revise how we think of the standard role of glypicans, understood to be exchange factors that passively distribute ligands randomly through the extracellular space by reversible binding. Coupling glypicans with the machinery of the actin cytoskeleton provides an active mechanism for the directed delivery of signaling ligands.

There is still a lot to learn about how glypicans and actin protrusions work together. The big unanswered question is how glypicans can communicate with the actin cytoskeleton on a molecular level. Glypicans are GPI anchored and lack a transmembrane domain, so they cannot communicate directly with the inside of the cell. Therefore, there must exist a network of proteins that spans the membrane, connecting glypicans to the intracellular cytoskeletal machinery to facilitate changes in cell shape, which underlie cytoneme formation. The identity of these proteins, however, is not known. The lateral mobility afforded by GPI anchors could allow for focal accumulation of glypicans, initiating the underlying cytoskeletal machinery to form a protrusion. Another interesting possibility stems from the location of glypicans in the glycocalyx, a pericellular matrix made up of glycoproteins and glycolipids, which can alter cell membrane properties and shapes by altering physical forces experienced by the cell membrane (9). Perhaps glypicans and their unknown glycocalyx partners can promote actin protrusions at specific sites on the cell surface. Despite these black boxes, the discovery that glypicans can promote formation of filopodia is both significant and exciting because it opens avenues to investigate how specificities of signaling molecules and fine-tuning of development might be regulated.

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