Research Highlight in Developmental Biology

Morphogen Signaling—Filopodia Pave the Way

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Morphogen signaling is critical for pattern formation during embryogenesis. The concept of morphogens originated from the results of classic embryology experiments performed nearly a century ago by Morgan and Boveri (Lawrence, 2001). A century of work, and still ongoing, morphogens are understood to be signaling molecules diffusing out in a monotonic concentration gradient from the source cells, and influencing the developmental fate of recipient cells in a strictly concentration-dependent manner. Despite our challenges in precisely defining the core set of features that characterize morphogens, the concept of morphogen signaling remains a cornerstone in developmental biology that has led to major breakthroughs in our understanding of the cell biology of animal development.

During morphogen signaling, the basis for the specificity of response in the target cells is the establishment of a field of concentration gradient with the highest concentration seen locally at the morphogen source cells. A prevailing view in morphogen signaling holds that the morphogens are transmitted, across their field of action, by diffusion along the extracellular matrix (Crick, 1970; Gurdon and Bourillot, 2001). Meanwhile, it has also been shown that a class of filopodia imparts specificity to the transmission and uptake of morphogens within the field of concentration gradient (Ramirez-Weber and Kornberg, 1999;

Roy et al., 2011). This article highlights findings from two recent investigations (Bischoff et al., 2013; Danilchik et al., 2013) that provide additional evidence for morphogen signaling mediated by filopodia.

In the study by Danilchik et. al., published in Developmental Biology, the authors identified long filopodia, spanning the blastocoel of the cleavage-stage *Xenopus* embryo (Figure 1), connecting non-adjacent blastomeres. These filopodial connections spanned the expanding
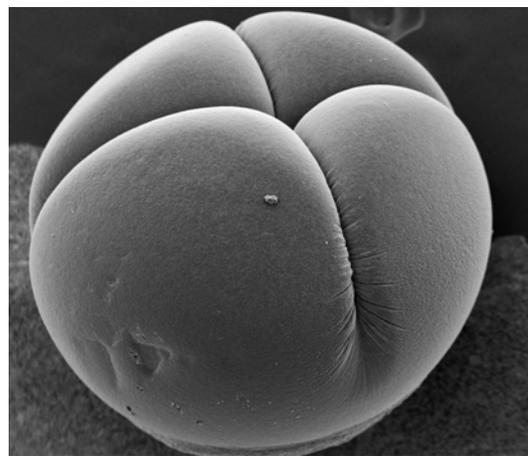

Figure 1: A cleavage stage *Xenopus* embryo consisting of four blastomeres. *Image courtesy of M. Danilchik*

blastocoel, and were maintained between blastomeres separated by as much as 250 μm in the 32-cell embryos. The filopodia were flask-shaped, and established contacts between the basolateral surfaces of blastomeres. Remarkably, the filopodia persisted through the passage of cleavage furrows until the development of 256-cell embryos. The filopodial arrays appeared to cluster at regions the authors identified as "hubs" of incoming and outgoing protrusions. The hubs and filopodial arrays were not any different, in the

frequency of occurrence, between the ventral and dorsal halves of the embryo.

Danilchik et. al. also noticed that the long filopodia bear vesicles of approximately 1.5 to 3 μm traversing—both anterograde and retrograde flow—along their length. Intriguingly, these vesicles were found to be membrane-bound distensions that share cytoplasmic continuity, and were shed at the sites of hubs suggestive of caveolin-mediated endocytosis. The results obtained by Danilchik et. al. suggest the possibility of a role for filopodia in the distribution of maternal morphogens beyond their initial range of localization in the early *Xenopus* blastula.

Meanwhile, the study by Bischoff et. al., published in Nature Cell Biology, provides evidence for the requirement of filopodial protrusions (cytonemes), in the establishment of Hedgehog (Hh) morphogen gradient in the *Drosophila* epithelium. The authors use the larval wing disc (an epithelial sheet that gives rise to the adult wing) and the abdominal epidermis to analyze the role of cytonemes in Hh morphogen signaling. Hh signaling is conserved from fly through mammals. The hallmark of Hh signaling is its ability to act over a long range in a concentration dependent manner, and hence is an exemplification of morphogen signaling (Jiang and Hui, 2008).

In the compartmentalized *Drosophila* wing disc (Figure 2) and abdominal epithelium, Hh is produced by the Posterior (P) compartment cells and acts upon the anterior (A) compartment cells. "A" compartment cells express the Hh receptor Patched (Ptc). The morphogen signaling is enabled by Interference hedgehog (Ihog) that is required for the membrane localization of Ptc (Zheng et al., 2010).

Bischoff et. al. showed that both wing disc and abdominal epithelial cells generate actin-based cytonemes studded with exovesicles that are marked by Hh pathway components. They also

presented data positively correlating cytoneme length/extent with the Hh signaling gradient length. Remarkably, their results also indicate a temporal correlation between the development of cytonemes and the development of the
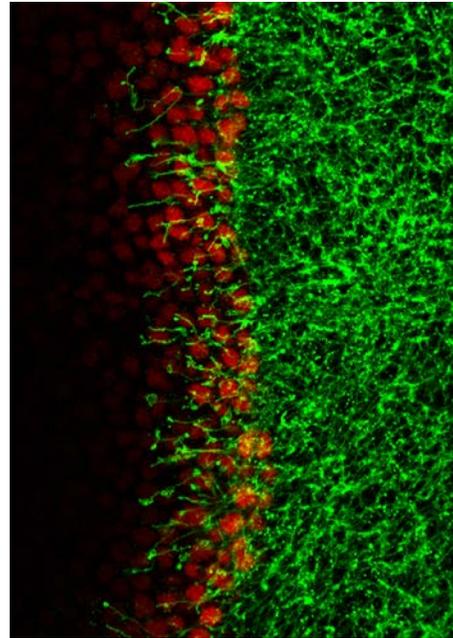

Figure 2: *Drosophila* Wing Imaginal Disc Epithelium. Posterior compartment cells (green) can be seen extending cytonemes into the Anterior compartment cells (red). The gradient of red fluorescence corresponds to the gradient in Hedgehog activity. *Image courtesy of M. Bischoff and I. Guerrero*

morphogen gradients. Compromising cytoneme length through RNAi of various cytoskeletal components interfered with the Hh gradient. Moreover, additional RNAi studies revealed that an intact Hh gradient relied also on intact exovesicle production/release machinery. Using clonal analysis, the authors also found that cytonemal transport of Hh for morphogen signaling relied on the stability of the extracellular matrix. The findings by Bischoff et. al. confirm the requirement of cytonemes in Hh morphogen signaling during fly development.

Together, these two studies indicate that a class of filopodia acts as dynamic bridges for morphogen transport connecting specific cell

groups spanning various stages of development. These studies also suggest that cell-cell contact and communication by cell extensions are not exclusive to neurons, but also occur in non-neuronal cells during morphogen signaling. These new results also redefine the role of filopodia to include morphogen signaling in addition to their roles in cell motility. With recent results of cytoneme functions in the developmental signaling of chick limb bud (Sanders et al., 2013) and the demonstration of cytonemes in isolated mouse cells (Koizumi et al., 2012), we might expect future studies to also investigate the molecular features that allow a filopodial structure to function as a cytoneme. More investigations are needed to shed light on the dynamics of signal transport and transfer mediated by filopodia engaged in morphogen signaling.

Acknowledgements

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