Intercellular calcium waves are controlled by morphogen signaling during organ development

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***Short Abstract* — Spontaneous and dramatic intercellular calcium waves are frequently observed during organ development, but are poorly understood. Calcium ions are ubiquitous second messengers that carry out a wide-range of functions, including the regulation of cell proliferation, metabolism and death. Regulation of calcium signaling encodes a significant portion of the cellular decision making state of cells through both amplitude and frequency-dependent regulation of transcription factors and key regulatory enzymes. Here we report that intercellular calcium waves exhibit spatiotemporal patterns at the organ-level using a quantitative image analysis pipeline. Intercellular calcium waves in the *Drosophila* wing disc require a specific phospholipase C, Plc21C. Further, we demonstrate that the morphogen signaling pathway, Hedgehog, controls frequencies of calcium oscillations uniformly in the tissue and is required for non-uniform spatial patterning of oscillation amplitudes. Thus, the dynamics of spontaneous intercellular calcium waves are regulated by morphogenetic signaling. Intercellular calcium waves propagate information at the organ-scale that reflects the differentiation state of the developing wing disc.**

***Keywords* — pattern formation, signal transduction, dynamic systems, reverse engineering, developmental biology, image analysis.**

I. Overview and Results

SPONTANEOUS intercellular calcium waves (ICWs) are observed in many developmental, wound healing and pathological contexts. However, it is not known whether spontaneous intercellular Ca^{2+} waves in epithelia are regulated, or by which mechanism. Recent results from both our group [1–3] and others [4,5] have reported gap-junction based intercellular Ca^{2+} transients in the wing imaginal disc. ICWs can be imaged in wing discs with the genetically encoded calcium indicator GCaMP6f sensor to visualize relative concentrations of cytoplasmic Ca^{2+}. Here we report the discovery that intercellular Ca^{2+} waves exhibit spatiotemporal patterns at the organ-level using a new image analysis algorithm to generate systematic spatiotemporal maps of intercellular Ca^{2+} wave signatures from a large data set of live-imaging experiments encompassing hundreds of hours of confocal microscopy. Time-averaged intercellular Ca^{2+} waves amplitudes in the wing disc are spatiotemporally patterned by the morphogen signaling pathway, Hedgehog, during organ development, suggesting that emergent intercellular calcium waves are developmentally regulated.

Surprisingly, Hedgehog also modulates ICW frequencies *uniformly and globally* in the organ through two distinct routes (both canonical and noncanonical). Further, we can selectively silence intercellular calcium waves by inhibiting the PLCβ1 homolog, *Plc21C*.

II. Conclusions

These discoveries position the wing disc as a powerful model system for studying regulation of organ-scale calcium signaling by morphogenetic pathways during development. These findings lead to significant implications on both organ development and homeostasis as they provide significant evidence of a spatial information storage pathway working in tandem with morphogen signaling. As one implication, these results could be an important step in uncovering new mechanisms that specify and maintain cell identities in tissues, which will be vital for unlocking the regenerative potential of organisms. An understanding of how Ca^{2+} signaling provides an integrated readout of multiple upstream signaling pathways also can inspire new assays for compound screening in multicellular systems.

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