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Cellular basis of limb morphogenesis: lessons from the crustacean Parhyale hawaiensis

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During development coordinated cell behaviors orchestrate tissue and organ morphogenesis to suit the lifestyle of the organism. We have used here the crustacean *Parhyale hawaiensis* to study the cellular basis of limb development. Transgenic *Parhyale* embryos with fluorescently labeled nuclei were imaged at high spatiotemporal resolution using multi-view light-sheet fluorescence microscopy over several days of embryogenesis spanning appendage morphogenesis from early specification up to late differentiation stages. To be able to analyze the terabyte-sized data sets we used a new tool called *Massive Multi-view Tracker* (*MaMuT*) for cell tracking that enabled us to reconstruct the complete cell lineage of an outgrowing thoracic limb with single-cell resolution. The quantitative analyses about cell behaviors show that the limb primordium in *Parhyale* becomes subdivided from an early stage into anterior-posterior and dorsal-ventral compartments whose boundaries intersect at the distal tip of the growing limb. Limb bud formation is associated with the spatial modulation of cell proliferation, while limb elongation is also driven by the preferential orientation of division of epidermal cells along the proximal-distal axis of growth. Our findings validate the boundary model originally proposed by Hans Meinhardt. This model postulates that a secondary developmental field, i.e. the proximodistal axis of a limb that is specified during embryogenesis de novo relative to the main anteroposterior and dorsoventral body axes, and is initiated around the intersection of the anteroposterior and dorsoventral compartment boundaries.

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