Aulehla group

Collective oscillatory large-scale calcium waves in the gastrulating Medaka embryo

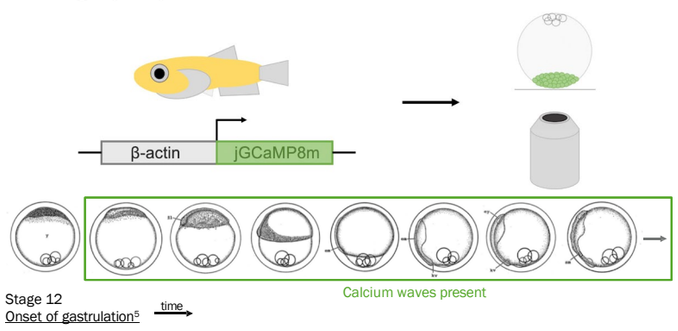
Simon Knoblich[1,2], Cielo Centola Buttigliero[3], Michael W. Dorrity[4], Juan R. Martinez-Morales[3], Alexander Aulehla[1]

Introduction

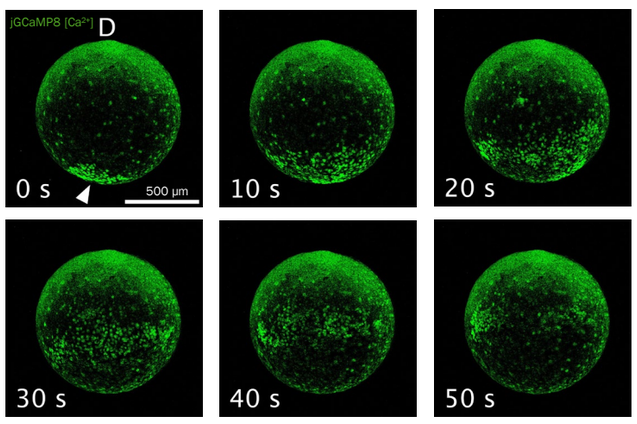
The development of organisms involves precise intercellular coordination in space and time. How cells communicate and transmit information in the presence of intrinsic and extrinsic noise has been a central topic of investigation for decades. Here, we address this question by studying spatiotemporal patterns of intra- and intercellular calcium dynamics during gastrulation in the teleost fish Medaka (*Oryzias latipes*). We combine a novel transgenic GCaMP reporter line with fast in-vivo imaging, allowing us to reveal widespread, oscillatory calcium dynamics in the gastrulating embryo undergoing epiboly. Over time, we find these oscillations to organize into striking large-scale wave patterns, which then correlate with rhythmical waves of contraction traversing the blastoderm. We demonstrate that these calcium contraction waves sweep through the Medaka non-neural ectoderm, previously termed the stellate cell layer, and that these cells synchronize their individual oscillatory calcium-contractility activity in order to collectively give rise to embryo-scale contractions. By inhibiting initial synchronisation during development, we propose a function of regulating early developmental progression in a checkpoint-like manner. Ultimately, we aim to further our understanding of the origin and function of collective calcium rhythms during vertebrate development.

Results

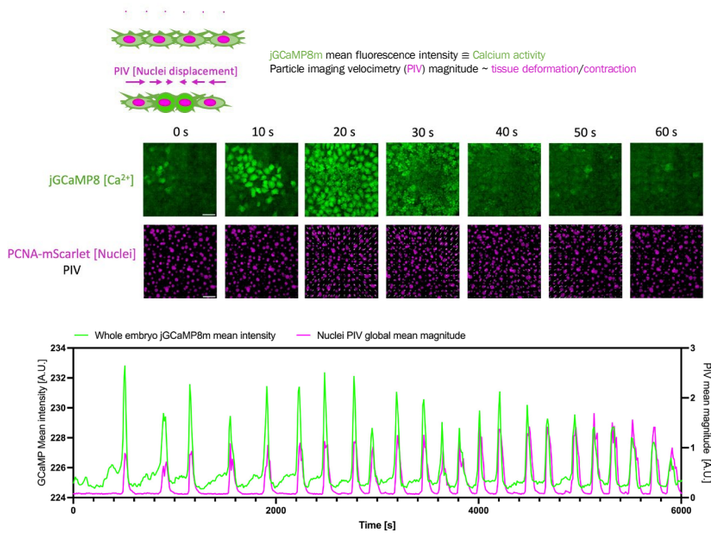
Large-scale intercellular calcium contraction waves traverse the gastrulating Medaka embryo in a highly rhythmic manner.



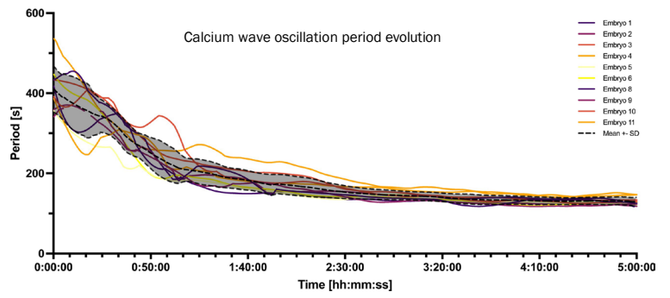
OlactB:jGCaMP8m transgenic Medaka line combined with high-resolution Spinning Disk Confocal microscopy allows for visualization of cellular calcium dynamics during early development



Representative timepoints of a single wave, Animal pole view. Scale bar = 500 µm



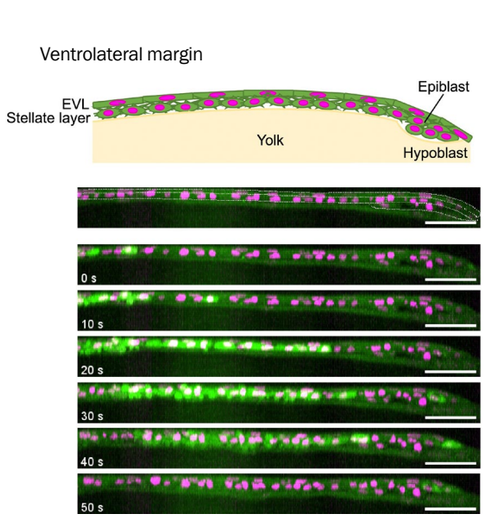
Whole-embryo mean GCaMP intensity and PIV mean magnitude over time, illustrating oscillatory calcium contraction waves



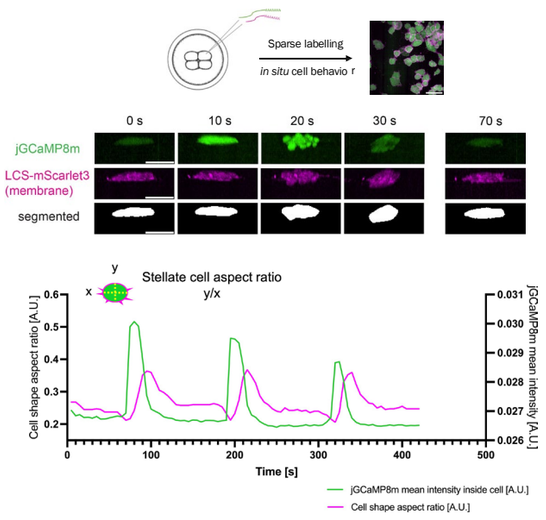
Stereotypic evolution of collective calcium wave oscillation period across 10 embryos

Oscillation period was quantified using pyBOAT Biological Oscillations Analysis Toolkit[6]. Parameters used for wavelet were a cut-off period of 600 for detrending, a lowest and highest period of 100 and 500 respectively, Number of periods 100. Period values plotted above were extracted using ridge detection, with a threshold of 1 and smoothing of 300. Embryos were aligned in time by the first visible wave.

Calcium contraction waves originate in the Medaka stellate cell layer[7].

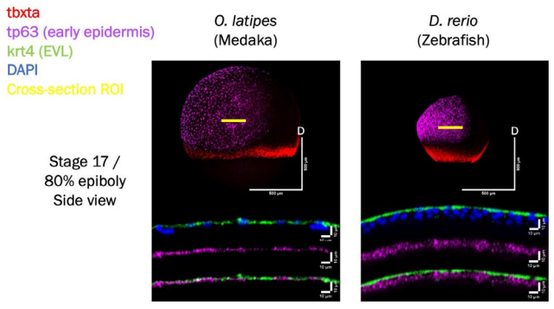


Schematic and representative timepoints of live-imaging cross-section inside calcium wave domain. Scale bar = 50 µm



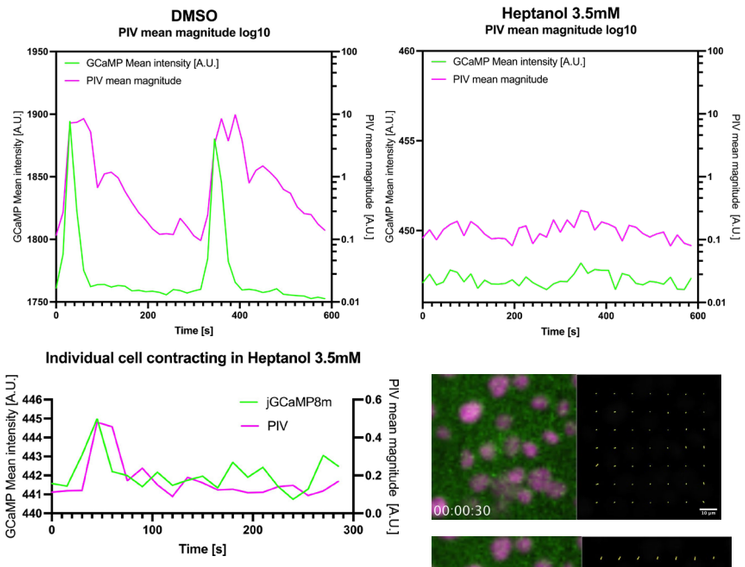
Sparse labelling by 4-cell-stage injection of fluorescent reporter mRNA allows for characterization of endogenous cell behavior. Stellate cells exhibit oscillatory cell shape changes, preceded by calcium transient. Scale bar = 20 µm

Stellate cells constitute the Medaka non-neural/epidermal ectoderm.



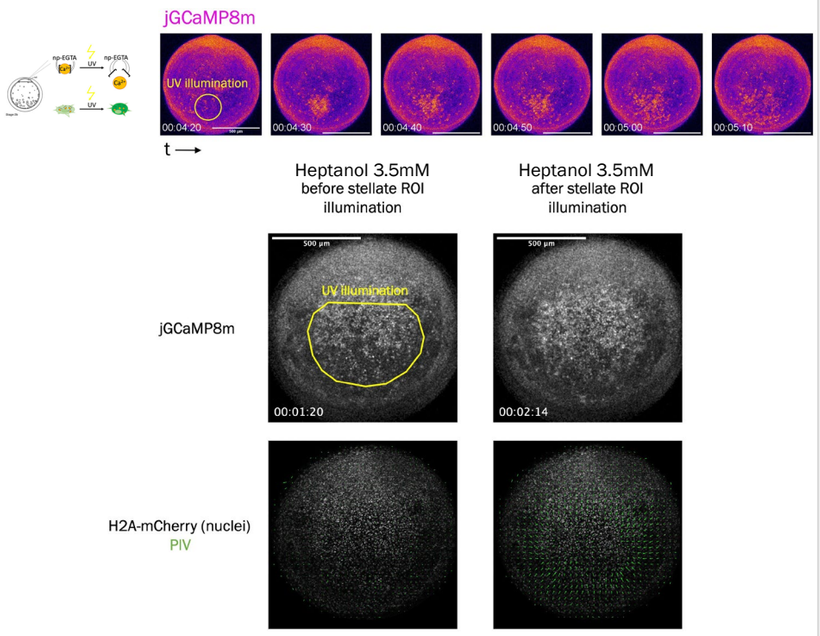
HCR-RNA-FISH staining of Medaka and Zebrafish embryos at epiboly stages revealed that the Medaka stellate layer constitutes the non-neural or early epidermal ectoderm, marked by expression of tp63 in magenta, despite the absence of comparable calcium wave activity in early Zebrafish developmental stages[8].

Inhibition of gap junction cell-cell coupling prevents synchronization into collective waves required for large-scale contractions.



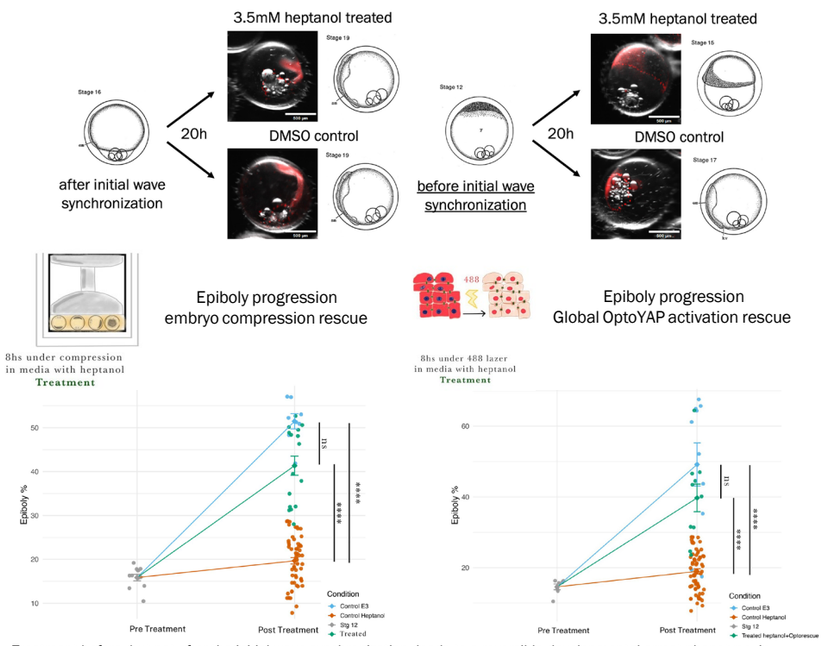
Calcium and contraction activity at the tissue level in DMSO control embryos follows oscillatory cycles of wave initiation and subsequent tissue contraction and relaxation orders of magnitude larger than in cells treated with heptanol 3.5mM, a pharmacological gap junction blocker[9], in which cells retain the individual potential to fire calcium transients and contract, but fail to synchronize.

Triggering of waves via optochemical intracellular calcium ion release reveals excitability characteristics and sufficiency of local calcium synchrony for tissue contractions.



np-EGTA-[Ca^{2+}] microinjected into the cytoplasm at the 1-cell-stage allows for spatiotemporal control of intracellular calcium release. A wave can be triggered by illuminating a small region within the stellate domain in an untreated embryo (top panel), revealing trigger wave characteristics. Illuminating a large patch of stellate cells forces them into calcium synchrony and is sufficient to cause a large tissue deformation/contraction (bottom panel).

Inhibition of initial wave synchronisation leads to a stage-specific, reversible developmental arrest phenotype rescuable both via embryo mechanical compression and optogenetic Yap activation.



Treatment before, but not after the initial wave synchronization, leads to a reversible developmental arrest phenotype, in which embryos are unable to proceed through epiboly stages (top panel). This arrest is rescuable both by rhythmical mechanical compression of embryos in a custom device, possibly mimicking endogenous contractions, and via optogenetic global YAP signaling activation, linking the calcium-contraction-mechanical signal axis to downstream signaling pathways.

Conclusion

Summary

- Intercellular oscillatory calcium waves traverse the gastrulating Medaka embryo in a rhythmic and spatiotemporally stereotypic manner.
- The large waves of calcium contraction activity reside in and are exclusive to the Medaka stellate layer, a monolayer of contractile cells constituting the epidermal ectoderm, closely adhered to the EVL above.
- Stellate cells undergo drastic cell shape changes in a calcium-dependent manner, giving rise to the previously observed waves of contraction traversing the Medaka blastoderm.
- Ectopic calcium waves can be triggered by optochemical intracellular calcium release, suggesting trigger waves within a system with excitable properties
- Preventing initial wave synchronisation leads to a stage-specific, reversible developmental arrest which can be rescued by rhythmical mechanical compression and optogenetic yap signalling, suggesting that the first large contractions might act as a signal, regulating early developmental progression in a checkpoint-like manner

Future Direction

- Synchronization in varying environments – What could be a physiological input for this early developmental checkpoint right before the sensitive gastrulation stage? Medaka embryos experience a wide range of temperature and salinity in their natural, fluctuating environment, making embryonic development both highly robust and, in several instances, plastic. We preliminarily find that in low temperatures – that is, temperatures below the range permissive for completion of the entirety of development, calcium activity fails to synchronize into oscillatory waves, concomitant with a previously reported nonlinear slowing-down, or partial arrest[10,11] of global development, reminiscent of the heptanol-treated condition. What rules govern the transition into synchrony in the stellate system, and what prevents this transition under unfavourable environmental conditions such as low temperature or early-on in development, when endogenous waves are not present yet but can already be triggered ectopically? Could coordination within the stellate system function as a sort of collective integrator of environment, acting as a critical embryo-wide signal for developmental progression?
- Fate – Where does the stellate lineage end up?

References and footnotes

[1] Developmental Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany
[2] Faculty of Medicine, Heidelberg University, Heidelberg, Germany
[3] Gene regulation and morphogenesis department, Centro Andaluz de Biología del Desarrollo (CABD), Seville, Spain
[4] Molecular Systems Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany
[5] Iwamatsu, T. *Stages of normal development in the medaka Oryzias latipes.* Mechanisms of Development (2004)
[6] Mönke, G., Sorgenfrei, F. et al. *Optimal time frequency analysis for biological data – pyBOAT.* Biorxiv (2020)
[7] Cope, J., Fluck, R. et al. *The stellate layer and rhythmic contractions of the Oryzias latipes embryo.* J Exp Zool. (1990)
[8] Chen, J., Xia, L. et al. *Imaging early embryonic calcium activity with GCaMP6s transgenic zebrafish.* Dev. Biol (2017)
[9] Rembold, M., Wittbrodt, J. *In vivo time-lapse imaging in medaka – n-heptanol blocks contractile rhythmical movements.* Mech Dev. (2004)
[10] Toulany, N., Morales-Navarrete, H. et al. *Uncovering developmental time and tempo using deep learning.* Nature Methods (2023)
[11] Sampetrean, O., Iida, S. et al. *Reversible whole-organism cell cycle arrest in a living vertebrate.* Cell Cycle (2009)g

simon.knoblich@embl.de

EMBL Heidelberg
Meyerhofstraße 1 · 69117 Heidelberg · Germany
T+49 6221 123456-78 · www.embl.org