P4

Quantitative imaging of the Wnt switch in *Clytia hemisphaerica* embryos at single cell resolution

GönomiX

State of the art

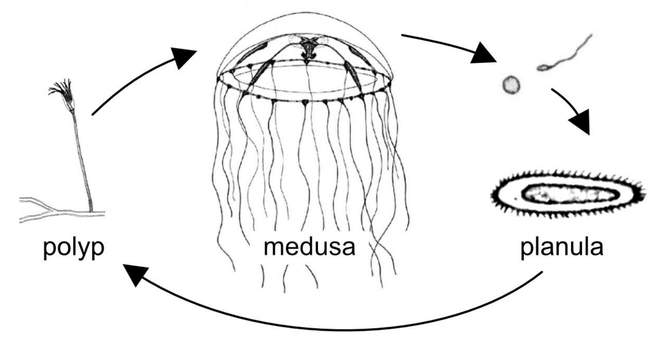
Cnidaria are among the earliest branching animal phyla in which functional analyses of Wnt in axis formation are available [1, 2]. The oral (head) region of the planula larva is determined by the conserved Wnt switch. Maternally deposited mRNA of the ligand Wnt3 is localized along the animal-vegetal axis of the egg. After fertilization, translation of this and other mRNAs (e.g. the inhibitory/activator receptors, Fz1 and Fz3) activate the canonical Wnt and the planar cell polarity (PCP) pathways [1, 2]. Wnt activation at the oral pole drives gastrulation by unipolar ingression.

Primary Question

- How does oral activation of Wnt signaling instruct gastrulation?

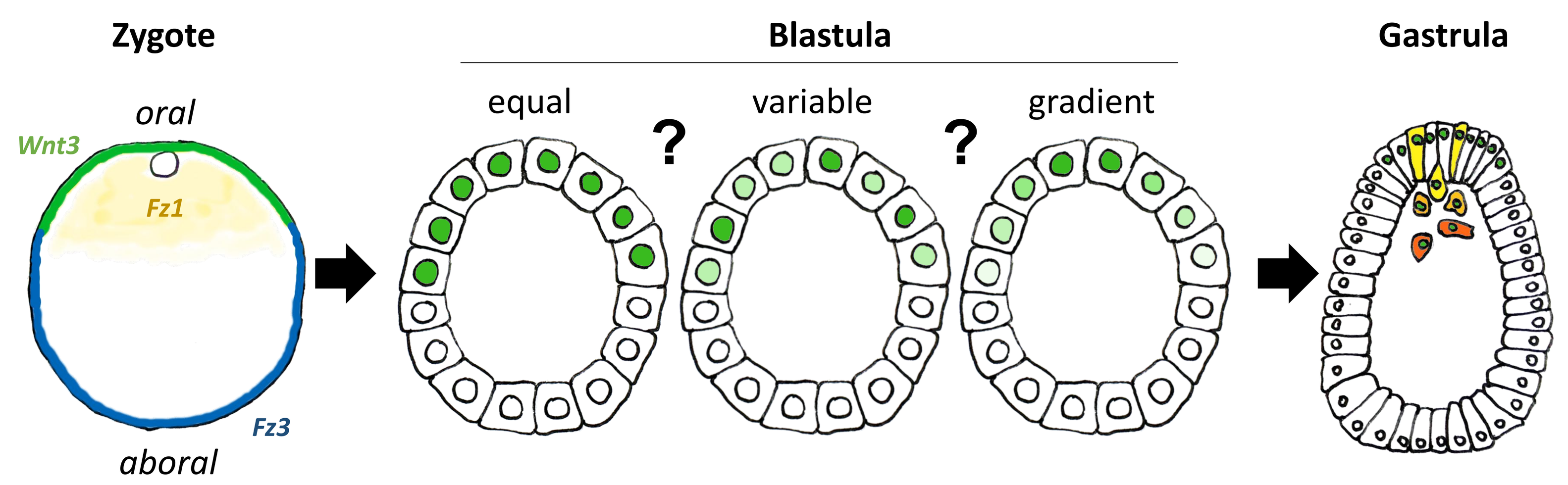
The model system

Clytia hemispaerica is a cnidarian model with sequenced genome, transcriptomic atlases, inbred strains, genomic tools, and knock-down techniques established [3]. *Clytia* jellyfish complete their sexual life cycle in 3-4 weeks, and vegetative, genetically identical polyp colonies can be cultured indefinitely [3].

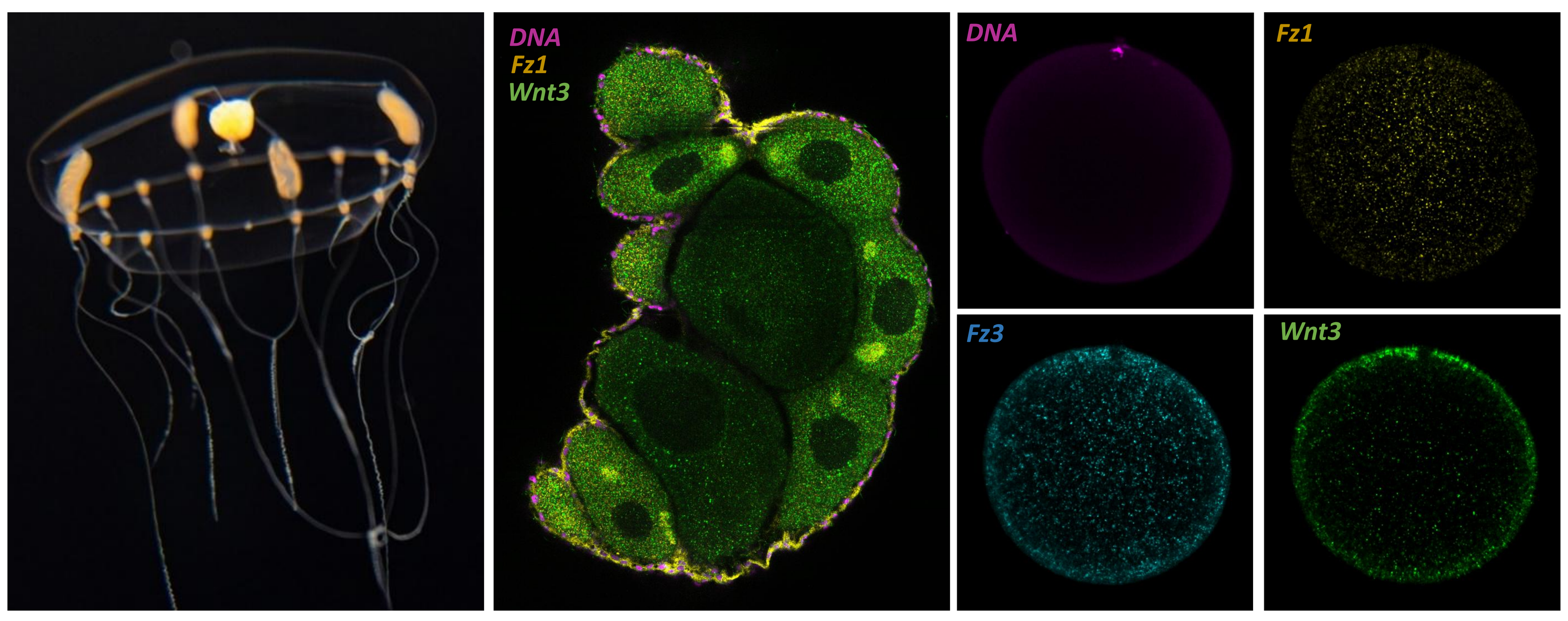


Objectives

- Quantitate Wnt activity in the blastula at single-cell resolution
- Localize known and identify new downstream Wnt targets
- Dissect mechanisms in functional, cellular assays



Preliminary data



Adult Clytia hemisphaerica jellyfish

Whole mount HCR RNA FISH of a Clytia ovary

Whole mount HCR RNA FISH of a mature Clytia oocyte

Work plan

A) Visualizing Wnt activity and downstream effectors at single-cell resolution: We will use β-catenin and other markers to quantitate Wnt activity *in vivo* and at single-cell resolution. We will visualize when and where transcripts of downstream effectors of the aGRN are present and translated using RNA FISH (WMISH) and RiboMAP, respectively. We will integrate data into a virtual embryo model to represent aGRN dynamics within the real 3D cellular geometry through early embryo development.

B) Identify novel downstream Wnt targets: To complement our cellular work, we will monitor aGRN dynamics by transcriptome sequencing of specific stages in untreated and perturbed embryos (e.g. Stbm-MO and pharmacological Wnt activators).

C) Dissecting the interplay between PCP and Wnt signaling: block of PCP signaling (Stbm-MO) impairs cell ingression at gastrulation [4]. We hypothesize that this effect is caused by perturbed apical-basal polarity leading to misorientation of cell division planes. We will test this by combining the above and other cellular assays with Stbm-MO and other perturbations.

Synergy and collaborations

- **Collaborative Project 1: Wnt signaling in anterior development**
  Providing data on cnidarian embryo development for comparative work (Wnt gradient dynamics, direct Wnt target genes); coordinated design of *in vivo* transgenic reporter and single molecule RNA FISH imaging with **GB, MA, JR**.
- **Collaborative Project 2: Reconstructing evolving GRNs**
  Provision of data on cnidarian embryo development. MO-mediated perturbation of novel genes identified by joint analyses with **GB, MA, JR, TB, ACH**.
- **Collaborative Project 3: Novel bioinformatics and genetic tools**
  Providing RNAseq data for bioinformatics tool development. Testing emerging hypotheses; expertise in transgenic and genome editing approaches (**TB, ACH, GB**).

Technical innovation

- *In vivo* imaging of Wnt dynamics at high spatial and temporal resolution
- Single molecule RNA FISH and RiboMAP
- Image-data integration and virtual embryo model

Specific qualification

- Imaging and image-data analysis
- Transcriptomics and data analysis

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References

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3. Lechable M, Jan A, Duchene A, Uveira J, Weissbourd B, Gissat L, Collet S, Gilletta L, Chevalier S, Leclère L, Peron S, Barreau C, Lasbleiz R, Houliston E, Momose T. An improved whole life cycle culture protocol for the hydrozoan genetic model Clytia hemisphaerica. Biol Open 2020:bio.051268. https://doi.org/10.1242/bio.051268.
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