P7

Characterization of a GRN that specifies a phylum-level synapomorphy - the molluscan shell

GönomiX

State of the art

The evolutionary success and diversification of the phylum Mollusca (the largest group of marine animals) was supported by the evolution of a highly plastic dorsal calcified shell. The amazing diversity of this phylum-level synapomorphy is unified by a set of highly conserved homologous morphogenetic events that initiate its construction during gastrulation (1, 2). Surprisingly, very little is known about the events that initially specify shell-forming cells and that coordinate their early movements and behaviours (invagination, secretion and evagination).

Fig. 1. A selection of gastropod and bivalve shells illustrates their diversity.

We have identified and validated ~100 shell matrix protein (**SMP**) genes that are uniquely expressed in both early and fully differentiated shell-forming cells of our chosen molluscan model ***Lymnaea stagnalis*** (Fig. 2). In addition, we have recently identified several Wnt components that are expressed in cells thought to specify the earliest shell-forming cells. Our library of downstream SMP genes and these Wnt components will act as landmarks for our single cell RNASeq approach that is aimed at identifying the complete shell-specifying GRN.

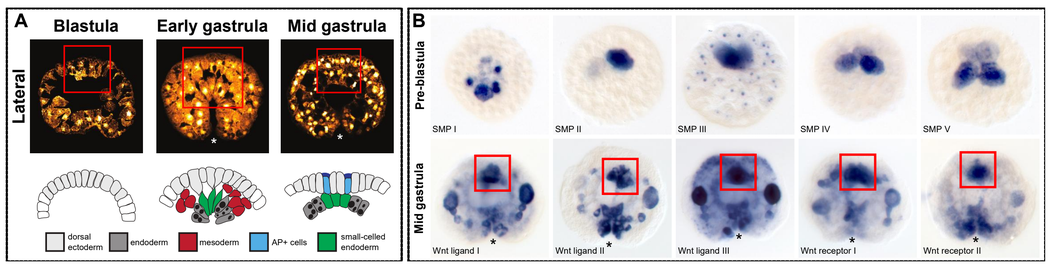

Fig. 2 (A): Optical sections illustrate contact between small-celled endoderm and the dorsal ectoderm (future shell-forming cells) during gastrulation. **(B).** Whole-mount *in situ* hybridisation demonstrates that several 'shell matrix proteins' (SMPs) are expressed in blastulae well before the shell gland has differentiated. We have also identified 3 Wnt ligands and 2 Wnt receptors that all appear to be co-expressed in cells located at the tip of the archenteron (red boxes correspond to those in **A**). Interestingly, we cannot detect the expression of other Wnt pathway ligands, receptors, canonical targets or other typical Wnt-interacting components (for example TCF/LEF, Dishevelled, Hippo) in the dorsal ectoderm where the shell gland will form. The blastopore in early- and mid-gastrulae is indicated by asterisks.

Primary Questions

- What is the nature of the molluscan shell GRN? What Wnt components, target genes and interactions comprise the GRN of molluscan shell formation?
- How conserved is the molluscan shell GRN?
- How did the molluscan shell-forming GRN arise evolutionarily?

Objectives

- Identify the full complement of signalling molecules and transcription factors co-expressed with known SMPs and Wnt components in shell-forming cells.
- Identify putative direct Wnt targets (additional SMPs) in shell-forming cells.
- Characterize the conservation of the shell GRN.
- Reconstruct the origins of the molluscan shell GRN.

Workplan

- **scRNASeq** to identify signalling pathway components and transcription factors that specify shell gland; cells in the scRNASeq data that express known Wnt components and SMPs (Fig. 2) will serve as markers for co-expressed genes of interest regarding shell-gland specification. Our recent publication of the *L. stagnalis* genome will be an invaluable resource for this exercise (3).
- Candidate regulatory genes with appropriate expression profiles identified in *L. stagnalis* will allow us to screen other conchiferans (shelled molluscs) in a **comparative approach** to ask whether orthologs of these genes display similar spatial and temporal expression patterns during gastrulation. Species such as *Crepidula fornicata* (collaboration with Deidre Lyons where CRISPR is established) and *Bithynia tentaculata* are distantly related to *L. stagnalis* (>400 million years) making them ideal candidate species.
- In the potential follow-up project, we will reconstruct the evolutionary origins of the molluscan shell with **phylum level genomic comparisons** of shell GRN genes. Collaborators (both within this RTG and international) will be invaluable here, as they provide the potential to gain functional insight to orthologous genes and novel gene structures using their respective model systems. Ongoing efforts to establish gene-specific functional assays in *L. stagnalis* (CRISPR, morpholinos) if successful would allow for specific scenarios of evolution to be tested.

Synergy and collaborations

- **Collaborative Project 1: Wnt signalling in anterior development**
  Contribute molluscan data with respect to Wnt signalling in anterior and posterior regions of mollusc embryos (**GB, PL, JR, MA**).
- **Collaborative Project 2: Reconstructing evolving GRNs**
  Contribute scRNASeq data on molluscan development with respect to Wnt signalling and respective target genes (**GB, PL, JR, NP, TB, MO, ACH**).
- **Collaborative Project 3: Novel bioinformatics and genetic tools**
  Provide scRNAseq data from an emerging model for bioinformatics tool development; potentially develop transgenic methods for *Lymnaea stagnalis* (**TB, ACH, GB**).

Technical innovations

- Contribute to an integration of scRNASeq, ATACseq and HiC data for understanding GRN dynamics and function.
- Develop scRNASeq and potentially transgenic methods for a molluscan model (*Lymnaea stagnalis*).

Specific qualifications

- Design and development of scRNASeq methods in a mollusc model.
- RNASeq, bioinformatics, phylogenetic analyses.
- Spatial gene expression analysis via whole mount ISH/HCR.

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References

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