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Spotlight

Many Ways to Build a Polyp

Detlev Arendt[1,2,*]

The freshwater cnidarian *Hydra* has been studied for centuries for its unique regenerative capacities. Whole-body single-cell transcriptomics now reveal cellular lineages and gene regulatory networks that build the *Hydra* polyp. For the first time, transcription factor signatures allow direct comparison of the polyp body plan between *Hydra* and sea anemone.

The *Hydra* polyp is a simple animal (Figure 1A). It is basically composed of an outer and an inner epithelial layer, called ectoderm and endoderm, which are separated by an extracellular matrix, the mesoglea (Figure 1B). In addition, *Hydra* has complex stinging cells, the nematocytes, and neurons – both of which reside in the interstitial spaces between the epithelial cells. Another frequent cell type, the gland cells, are integrated into the endodermal epithelium. The nervous system is composed of sensory neurons and of ganglion cells – multipolar neurons that send out axons to form a wide-meshed nerve net (Figure 1A).

All cells of the adult *Hydra* body are constantly replaced. Reflecting the simple anatomy, only three distinct cellular lineages – ectodermal, endodermal, and interstitial – build the polyp body. Ectodermal and endodermal cells remain mitotic along the body column and replace themselves. They terminally differentiate only towards the head, tentacles, or foot. This results in a constant flux of epithelial cells toward the extremities, with older cells being lost. In a separate lineage, nematocytes, neurons, and gland cells arise from the multipotent interstitial stem cells (ISCs) [1].

Benefitting from the clarity and simplicity of the *Hydra* anatomy and lineage, Siebert and colleagues have now used whole-body single-cell transcriptomics to molecularly characterize lineage trajectories and cell types of the entire *Hydra* body [2]. Collecting ~25 000 single-cell transcriptomes, their study has revealed the dynamics of gene expression that accompany cell specification and differentiation in the three *Hydra* lineages. Building on that, they have assembled the bifurcating trajectories of progenitor states that lead to the differentiating cell types; the Waddington landscape [3] of the adult *Hydra* polyp. For example, they have found that ISCs enter a single path that bifurcates towards neuronal or gland cell differentiation (Figure 1C). From this they have inferred that ISCs produce bipotential progenitors in the ectodermal layer that cross the extracellular matrix to supply the endodermal layer with neurons and gland cells.

Beyond that, the comparison of cellular transcriptomes across an entire body efficiently unravels gene sets that are uniquely coregulated within the genome. Juliano and colleagues have found ~60 of such gene sets. They then used ATACseq to identify regulatory regions for the genes of each set, detect transcription factor binding motifs that are enriched within the regions of coregulated genes, and finally match them to candidate transcription factors found in each set [2]. This yields first insights into the gene regulatory networks controlling cell type specification and differentiation in cell types and tissues. Figure 1D shows cell type-specific transcription factors selected from coregulated gene sets by the author.

Conserved Ectoderm – But How about Endoderm and Mesoderm?

The transcription factors that Siebert and colleagues have identified as specific for the ectodermal gene sets (*otx*, *six3*, *msx*, *hbn*, and *Dlx*; Figure 1D) have been shown to be active in ectodermal patterning in another cnidarian, the sea anemone *Nematostella* [4,5]. They also play well-established roles in ectodermal head patterning (otx, six3, and hbn) and mediolateral patterning (msx and Dlx) in the bilaterians [6]. This suggests overall conservation of ectodermal patterning in *Hydra* – with the exception of several factors (*Nkx6*, *PaxD*, and *rx*) that are conserved in sea anemone and bilaterians but missing from the *Hydra* genome [7].

The comparison of the endodermal gene sets sheds new light on endoderm evolution. On the one hand, specific transcription factors such as *Brachyury*, *Goosecoid*, *Pitx*, and *FoxA* (Figure 1C) make a strong signature matching the anterior endoderm of the vertebrate organizer [8]. On the other hand, *Pitx* and *FoxA* demarcate ectodermal pharynx in *Nematostella* [5], which has recently been homologized to

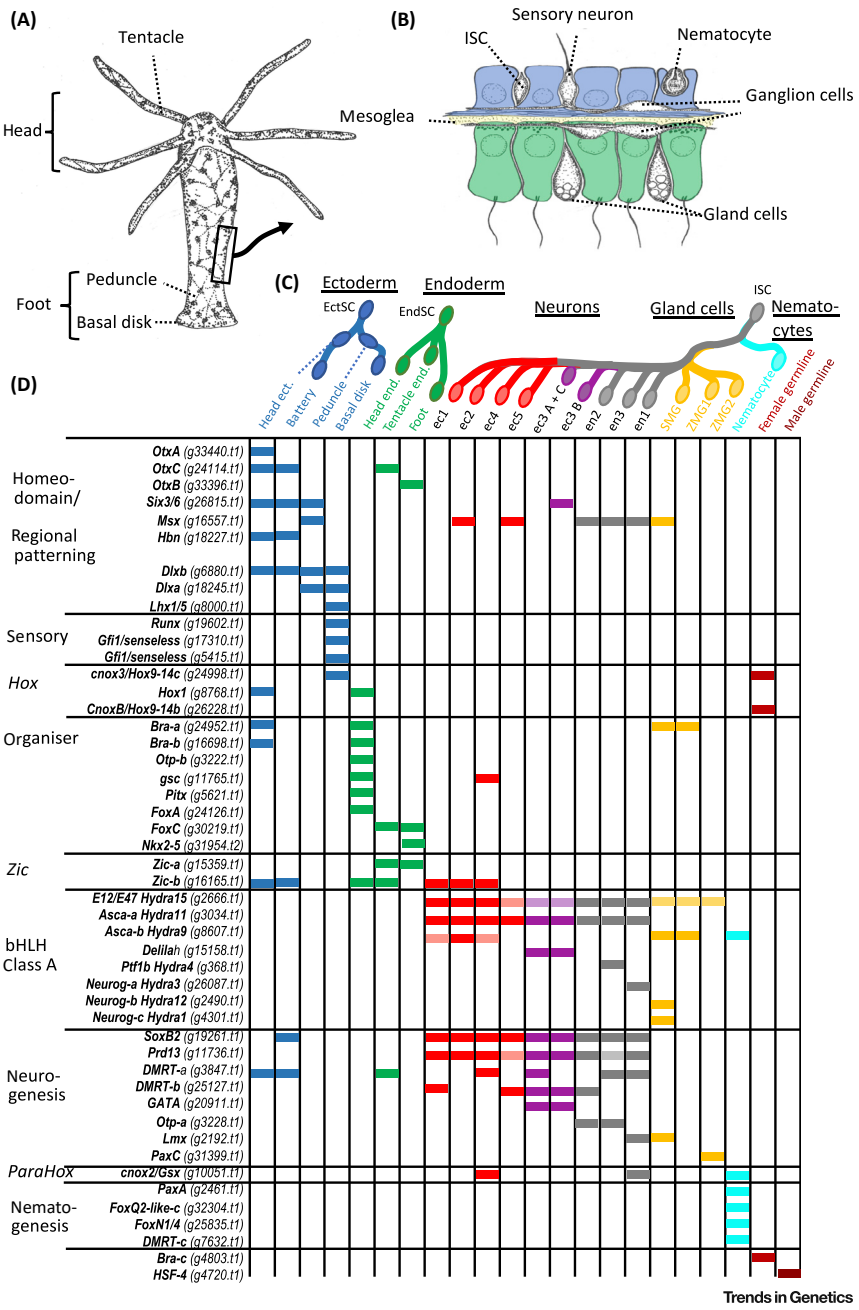

Figure 1. Cellular Lineages and Transcription Factor Signatures of Cell Type Families in *Hydra*. (A) Anatomy of the *Hydra* body. Box with arrow indicates position of longitudinal section in B. (B) *Hydra* ectoderm (blue) and endoderm (green). Labels identify cell types of the interstitial lineage. (C) The cellular lineage of *Hydra* as deduced from single-cell trajectories. (D) Transcription factor signatures of *Hydra* ectoderm, endoderm, and interstitial lineage, compiled from data by Siebert and colleagues made available under Dryad https://doi.org/10.5061/dryad.v5r6077 and in the Broad Single-Cell Portal (https://portals.broadinstitute.org/single_cell/study/SCP260/stemcell-differentiation-trajectories-in-hydra-resolved-at-single-cellresolution). Gene annotation follows [7]. Abbreviations: EctSC, ectodermal stem cell; EndSC, endodermal stem cell; ISC, interstitial stem cell.

bilaterian endoderm [9]. The new data by Siebert and colleagues clearly support this notion, as they suggest homology of large part of the *Hydra* endoderm to pharyngeal ectoderm in sea anemone (and to bilaterian endoderm).

In sea anemone, the endodermal layer expresses various mesodermal markers, suggesting that the mesoderm evolved from the endodermal layer [5,9]. In comparison, the endodermal gene sets in *Hydra* express only few markers for mesoderm (or gastroderm) [5], including *FoxC* and the heart specification factor *Nkx2-5* (Figure 1D). This is in line with the absence of mesodermal transcription factors (*twist*, *paraxis*, and *hand*) from the *Hydra* genome [7], and suggests that mesodermal characteristics are much reduced in *Hydra* in comparison to sea anemone.

Three Genetically Distinct Nerve Nets in the *Hydra* Polyp

The *Hydra* nerve net has recently been shown to comprise three nonoverlapping subnetworks that are associated with specific behaviors [10]. The contraction burst (CB) network and the rhythmic potential (RP)1 network are two opposed ectodermal networks that contract or relax longitudinal muscle fibers. The RP2 network is an endodermal network that integrates information about the gastric environment and activates circular endodermal fibers for radial contraction [10].

Siebert and colleagues used fluorescence-activated cell sorting to enrich for neurons and build a molecular map of the nervous system [2]. Via wholemount *in situ* hybridization of marker genes and transgenic reporter lines they have localized and characterized neuronal cell types. They have shown that the ectodermal clusters ec1 and ec5 represent ganglion cells

in the tentacles and peduncle, respectively, and by position likely represent the CB circuit (likely innervated by Ec2 and Ec4 sensory neurons in tentacles and hypostome). The ec3 ganglion cell clusters uniquely span the basal disk ectoderm and therefore likely represent the RP1 cluster active in the basal disk. Finally, they have proposed that the endodermal en1 ganglion cell neurons make up the RP2 circuit, likely innervated by en2 sensory cells [2].

Figure 1D shows the distinct transcription factor signatures of neuronal cell types. Some important lessons can be learned. First, all neurons (and related gland cells) specifically express transcription factors of the class A basic helix–loop–helix family (*E12/E47*, *AscA*, *Delilah*, *Ptf1b*, and *Neurogenin*), supporting the ancestral and conserved role of this family in neuronal specification. Second, neuron types representing the three subnetworks CB, RP1, and RP2, also differ by transcription factor signature, expressing *Zic* (ec1,2, and 4), *GATA* (ec3), *otp* (en2 and 3), or *Lmx* (en1), (red, violet, and gray in Figure 1D). Three of these factors have also been identified to specifically demarcate neuronal subgroups in sea anemone [5], opening up the exciting possibility that some subnetworks already existed in cnidarian ancestors. More comparative work in these and other cnidarian species will be needed to trace the evolution of nerve nets in molecular and cellular detail across the cnidarian phylum.

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Forum

A Genetic Instruction Code Based on DNA Conformation

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Flipons are sequences capable of forming either right- or left-handed DNA under physiological conditions, forming a class of dissipative structures that trade metabolic energy for information by cycling DNA between different chromatin states. Flipons enhance the diversity of transcriptomes, increasing entropy while enabling the evolution of features both new and unexpected.

The Unexpected Answer to a Question Nobody Was Asking

DNA comes in many different forms [1]. The Watson and Crick B-DNA conformer is a right-handed helix, while, in Z-DNA, the bases are flipped over, causing the phosphate backbone to zag left [2] (Figure 1A). Z-DNA was an accidental discovery found in the first-ever synthetic DNA crystal. Z-DNA forms dynamically under physiological conditions when certain enzymes untwist right-handed DNA while working to make RNA. The *in vivo* relevance of Z-DNA was an unresolved question until recently [3]. One early clue to its function was the nonrandom distribution, within the human genome, of sequences capable of adopting either right- or left-handed DNA conformations, referred to here as 'flipons'. Flipons are enriched in the promoters and 5′ untranslated regions of genes [4] and are associated with active histone marks [5]. By setting chromatin state, they provide instructions for compiling genetic information into RNA, a process dependent upon the consumption of free energy. As such, flipons are a class of dissipative structures, nonequilibrium states initially described by Prigogine [6] that, in the case of nucleic acids, convert the thermodynamic entropy of DNA structures (ΔE) into the information entropy of transcripts (ΔI) (Figure 1). Here, I describe how flipons work, how they are encoded, and how they accelerate evolution through the dissipative structures they form.

Experimental Approaches to the Z-DNA Question

One approach to understanding the biological role of Z-DNA has been to