



Opsin Expression in Disparate Cell types in *Hydra vulgaris*

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Introduction to *Hydra* and Opsins

Hydra do not have eyes yet can sense & respond to light.

The identity of the cells expressing these opsins in non-visual tissues is often unknown

- *Hydra vulgaris*: Small (10-30mm) **freshwater invertebrate** in phylum Cnidaria
- Opsins serve as the **light sensing protein** in the phototransduction cascade
- Using **single cell RNA sequencing** techniques we can study opsin **gene expression at the cellular level**

Bioinformatics Work Flow

Mine Data

- Find & download whole-organism single cell RNA seq dataset (1)
- Load tools onto computing cluster:
 - Packages: PIA, URD, Seurat

Find Opsins

- Phylogenetically Informed Annotation
 - PIA uses pre-calculated maximum likelihood trees to assign gene identities to sequences, generally in non-model organisms

Visualize Expression

- Use Seurat and URD to visualize opsin expression
 - Seurat: R program used to visualize gene expression in single cell datasets.
 - URD: reconstructs cell differentiation trajectories based on gene expression

Acknowledgements

Thank you to the Worster family for funding this summer research project

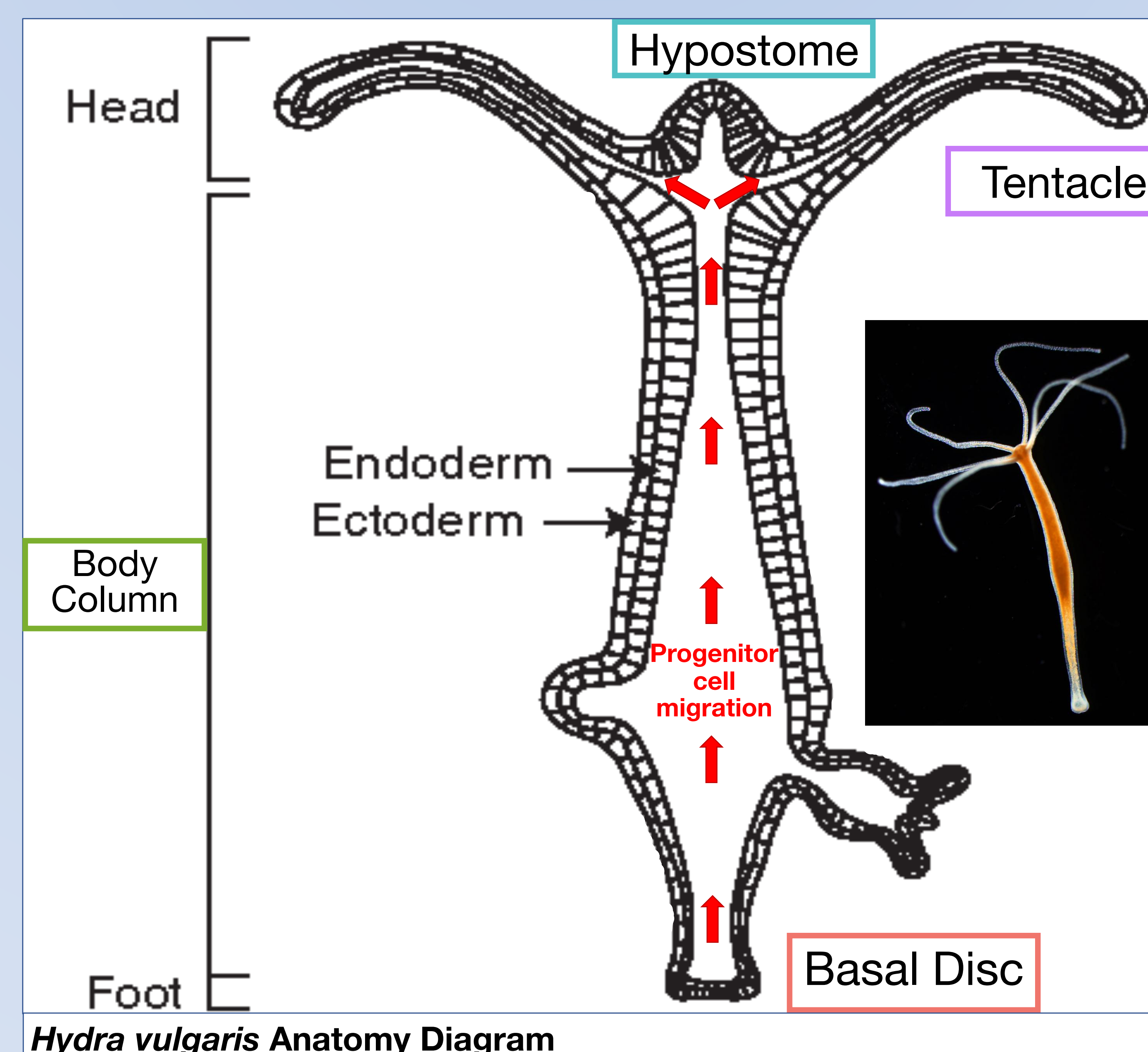
Hydra Dataset: Siebert, Stefan & Farrell, Jeffrey & Cazet, Jack & Abeykoon, Yashodara & Primack, Abby & Schnitzler, Christine & Juliano, Celina. (2019). Stem cell differentiation trajectories in Hydra resolved at single-cell resolution. *Science*. 365. eaav9314. 10.1126/science.aav9314.

Hydra Phototransduction genes: Macias-Muñoz, A., Murad, R. & Mortazavi, A. Molecular evolution and expression of opsin genes in *Hydra vulgaris*. *BMC Genomics* 20, 992 (2019). <https://doi.org/10.1186/s12864-019-6349-y>

At a Glance

What **types of cells** are expressing **light sensing protein genes**?

When in the **differentiation trajectories** are these genes being expressed?



What did we find?

28 opsins found in *Hydra* dataset

Some opsins were found expressed in **non-neuronal cell types** (Figure 1)

This could be :

- Non-neuronal cell types using opsins to sense and respond to light
- Opsins being used for purposes other than sensing light

Opsin expression increases in body column cells from ectodermal lineage before differentiation (Figure 2)

As *Hydra* progenitor cells differentiate, they migrate up the body column as seen in the *Hydra vulgaris* anatomy diagram

The increase in expression before differentiation may indicate progenitor cells using opsins in cell fate decision

Other phototransduction cascade genes were also expressed (Figure 3)

Opsins do not work alone; they need the other proteins in the phototransduction cascade in order to be used by cells to sense and respond to light

What next? Use Weighted Gene Co-Expression Network Analysis (WGCNA) to understand the degree and extent to which opsins are co-expressed with other genes in the phototransduction cascade

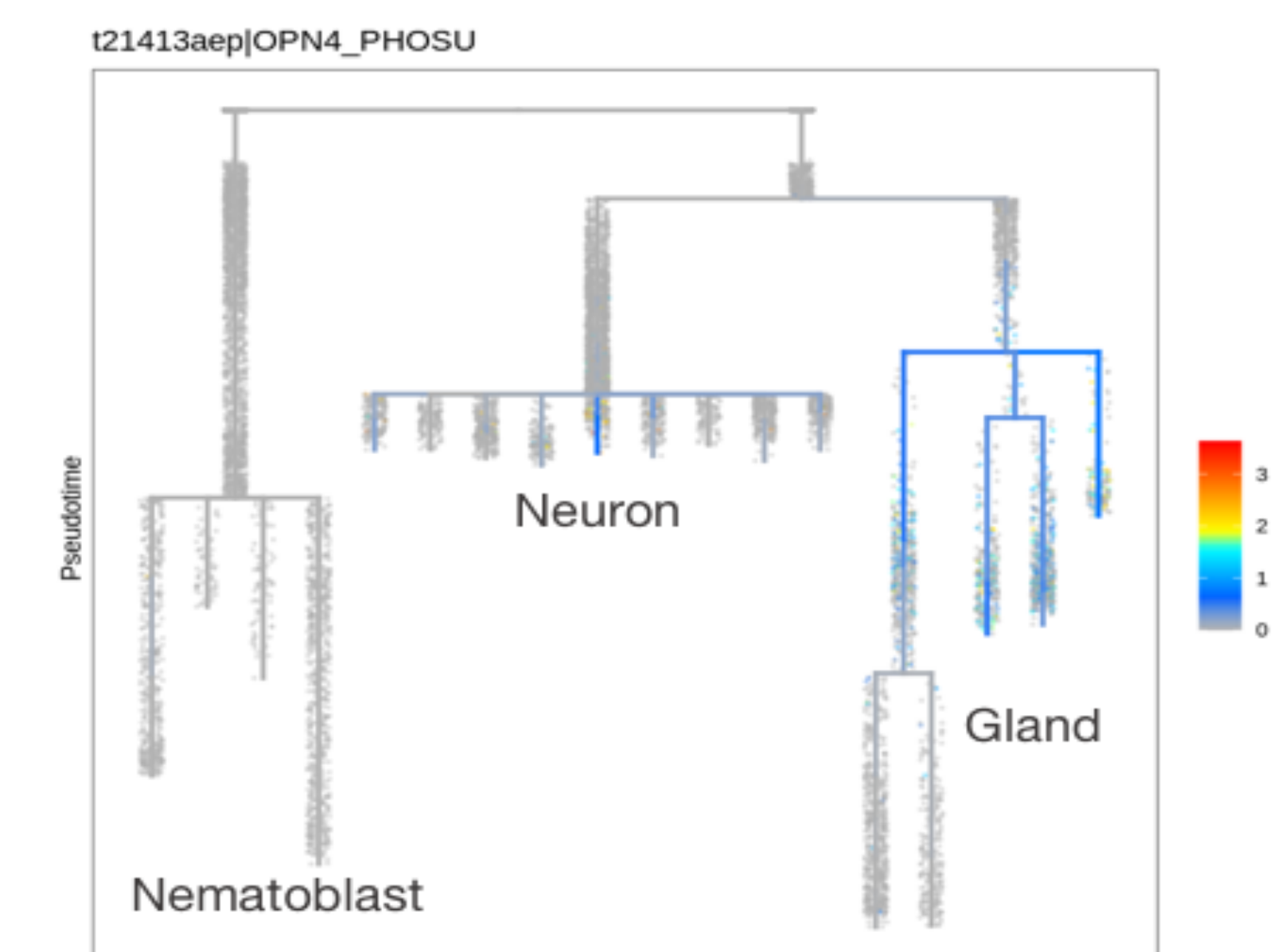


Figure 1: Dendrogram of a reconstructed differentiation trajectory plotting the expression of an opsin gene found in the *Hydra* dataset. Branch length in the plot denotes pseudotime and the color of the dots denoted expression level.

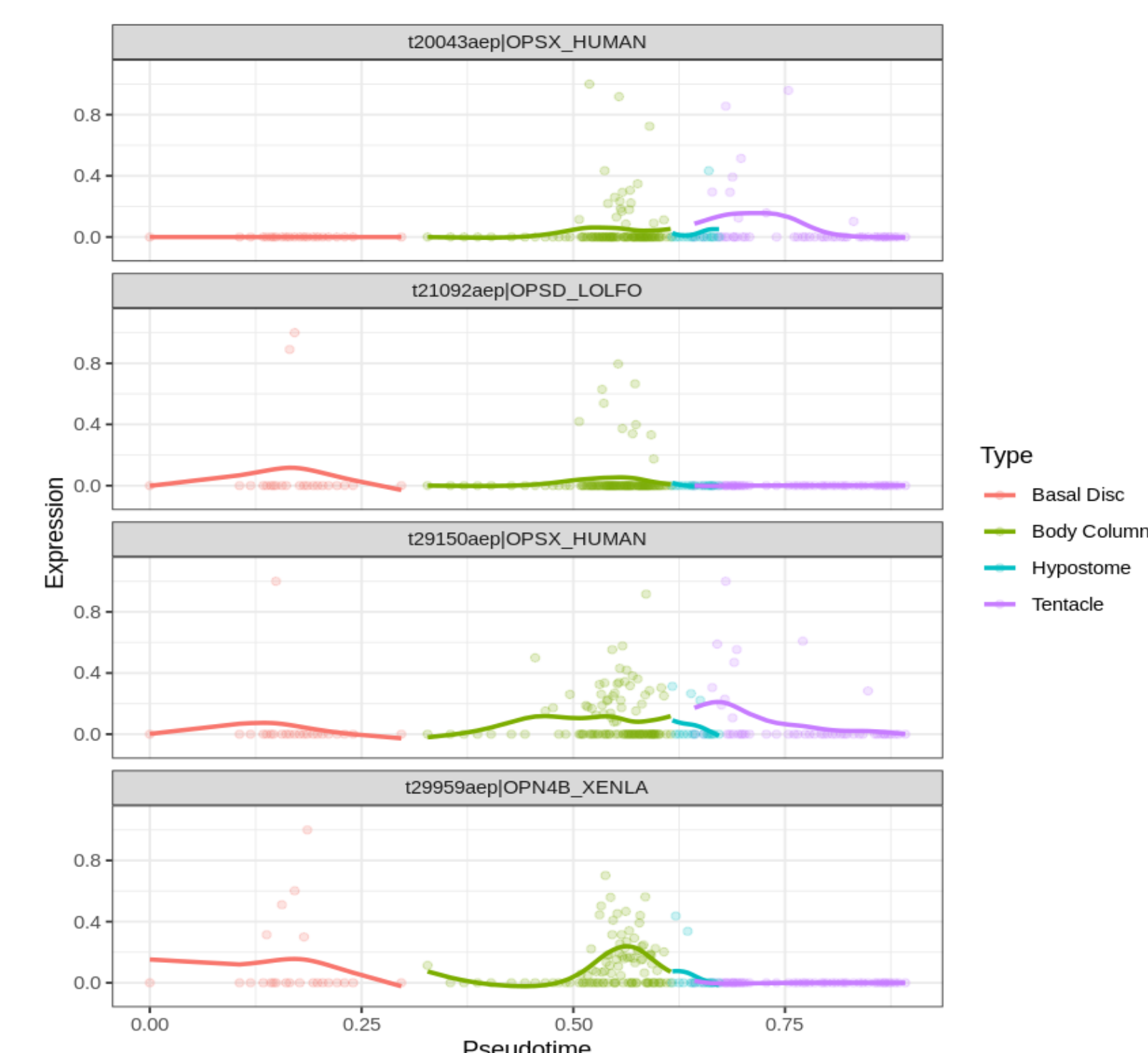


Figure 2: Multi-segment spline plot visualizes the expression of 4 opsin genes in cells from the ectodermal lineage over pseudotime, with spatially relevant cell type groups annotated as colored line segments.

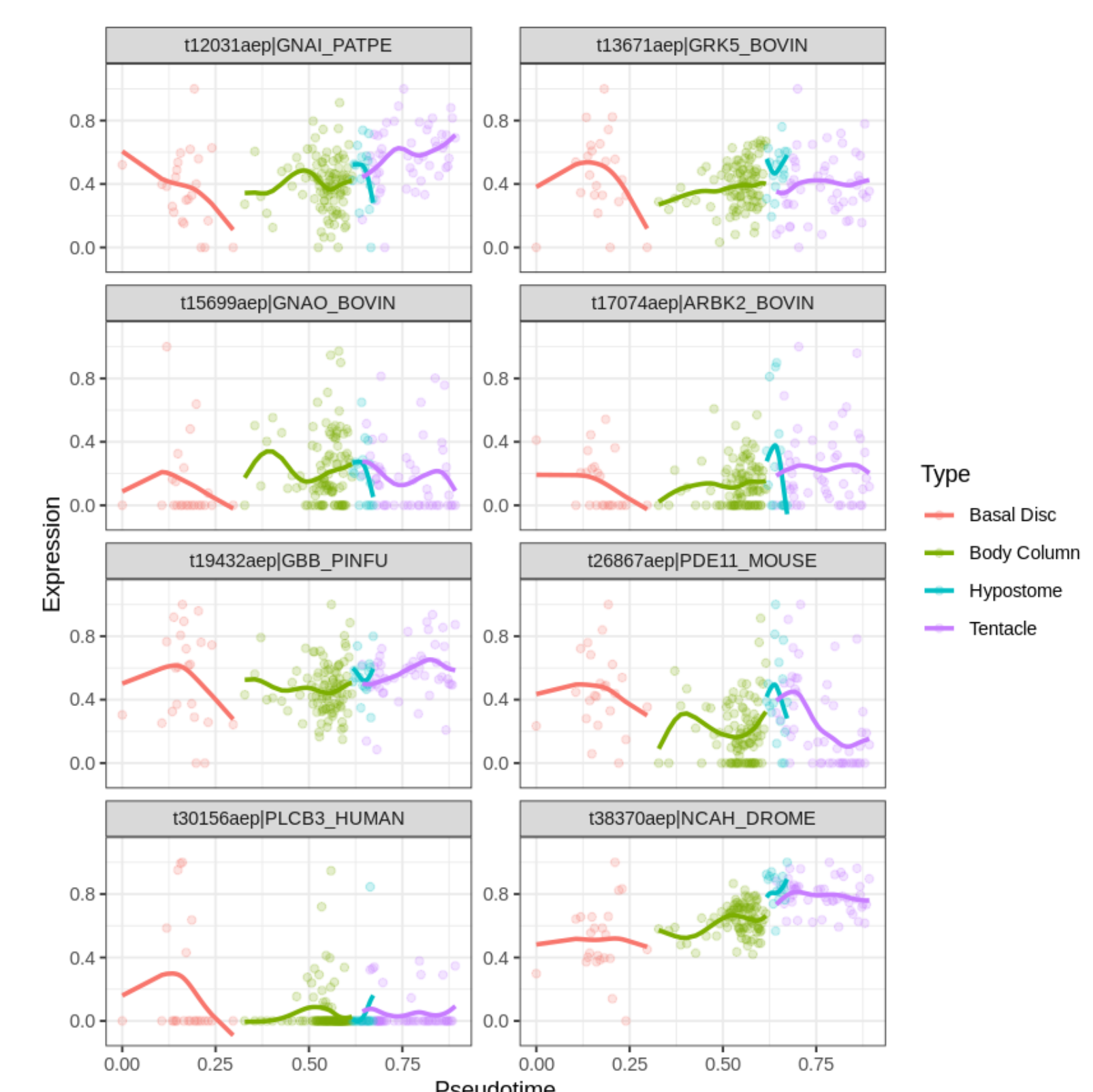


Figure 3: Multi-segment spline plot visualizing the expression of 8 known phototransduction genes in *Hydra* over pseudotime, with spatially relevant cell type groups annotated as colored line segments.