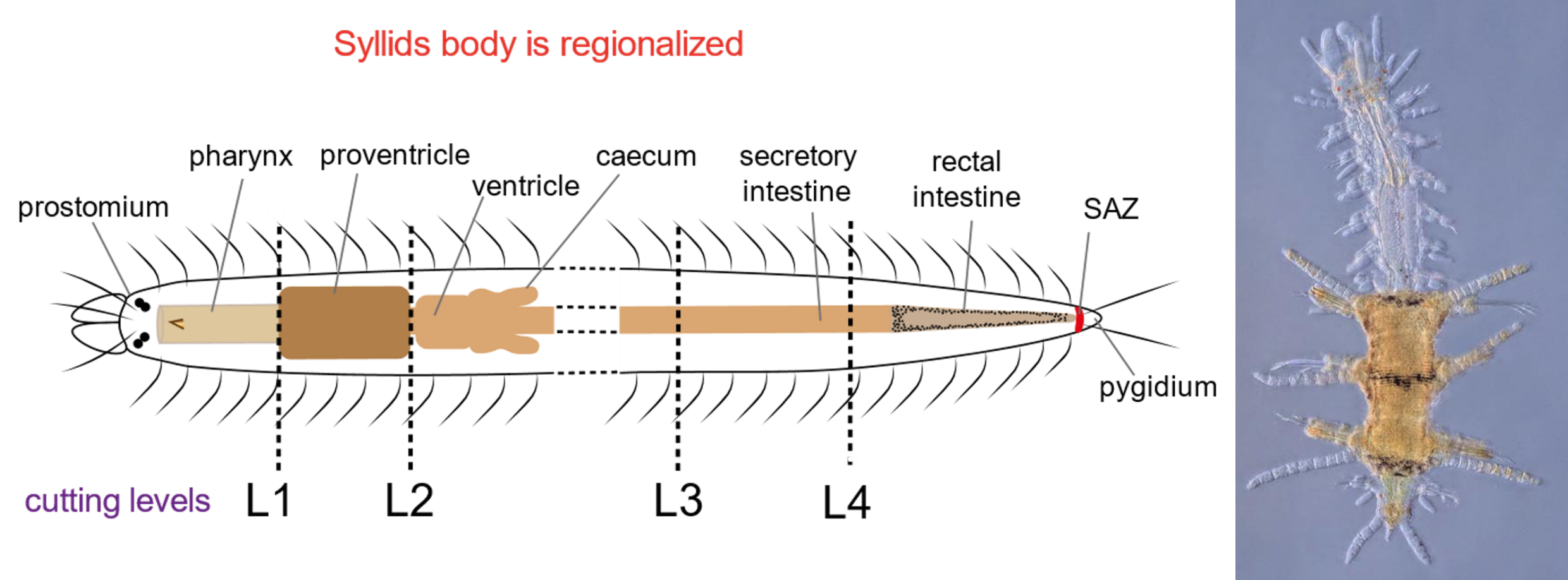


State of the art

Syllids are annelids with several reproductive modes and impressive regenerative capacities. Posterior regeneration (PR) is widespread; anterior regeneration (AR) is generally limited to a few species that reproduce by fission. Previous studies show Wnt pathway involved in annelids regeneration (1); however, it is still unknown, how far repression of Wnt signaling is required for head regeneration. Additionally, why some species can regenerate complete anterior ends, while their sister clades do not have this ability?



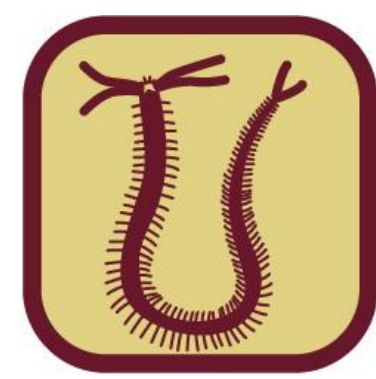
Primary Questions

- Are Wnt genes and β catenin regionally expressed along AP axis during homeostasis and regeneration? Is there a gradient?
- What Wnt activities are required at different regeneration stages?
- What genes are required for polarity?

Objectives

- Determine **regional** and **temporal** expression patterns of Wnt/ β catenin signalling pathway during homeostasis and regeneration.
- Identify effects of wnt **activation** or **inhibition** in neural differentiation of anterior **nervous system**.

Workplan



2 species:

- *Syllis malaquini* –Complete AR
- *Syllis prolifera* –Incomplete AR

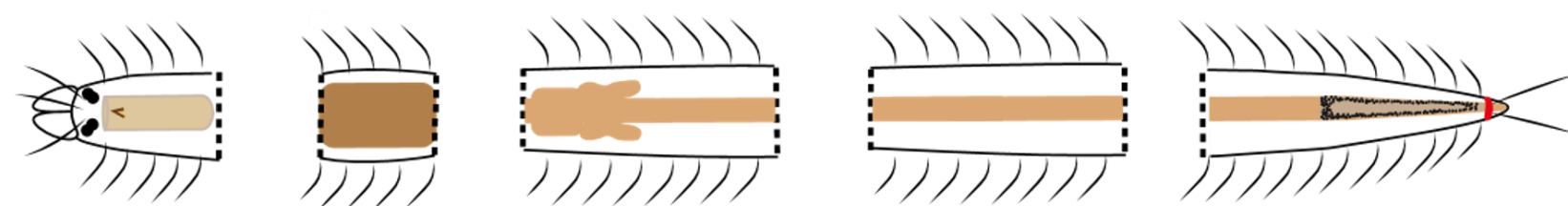
Comparing species:

- Species specific requirements?
- Conserved programs?
- Comparison of spatial patterns
- Phylogenetic context

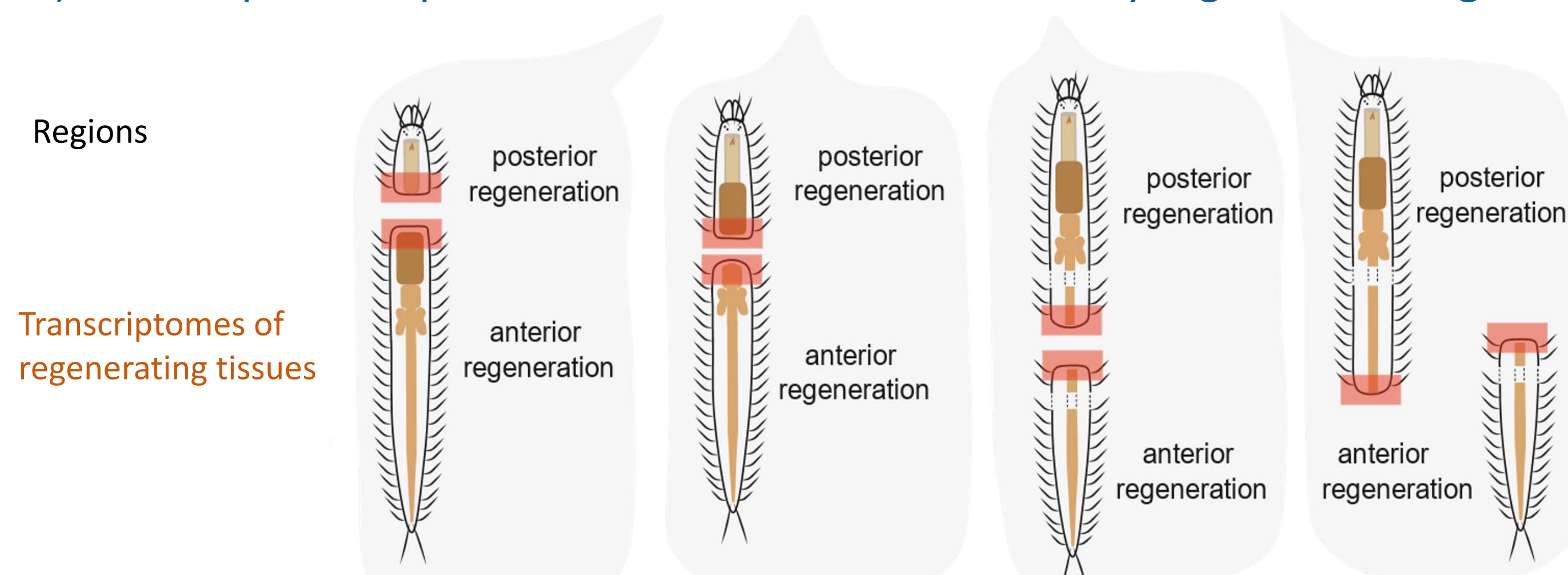
A) Cultures and experimental design.

B) Gene expression patterns along the A/P axis.

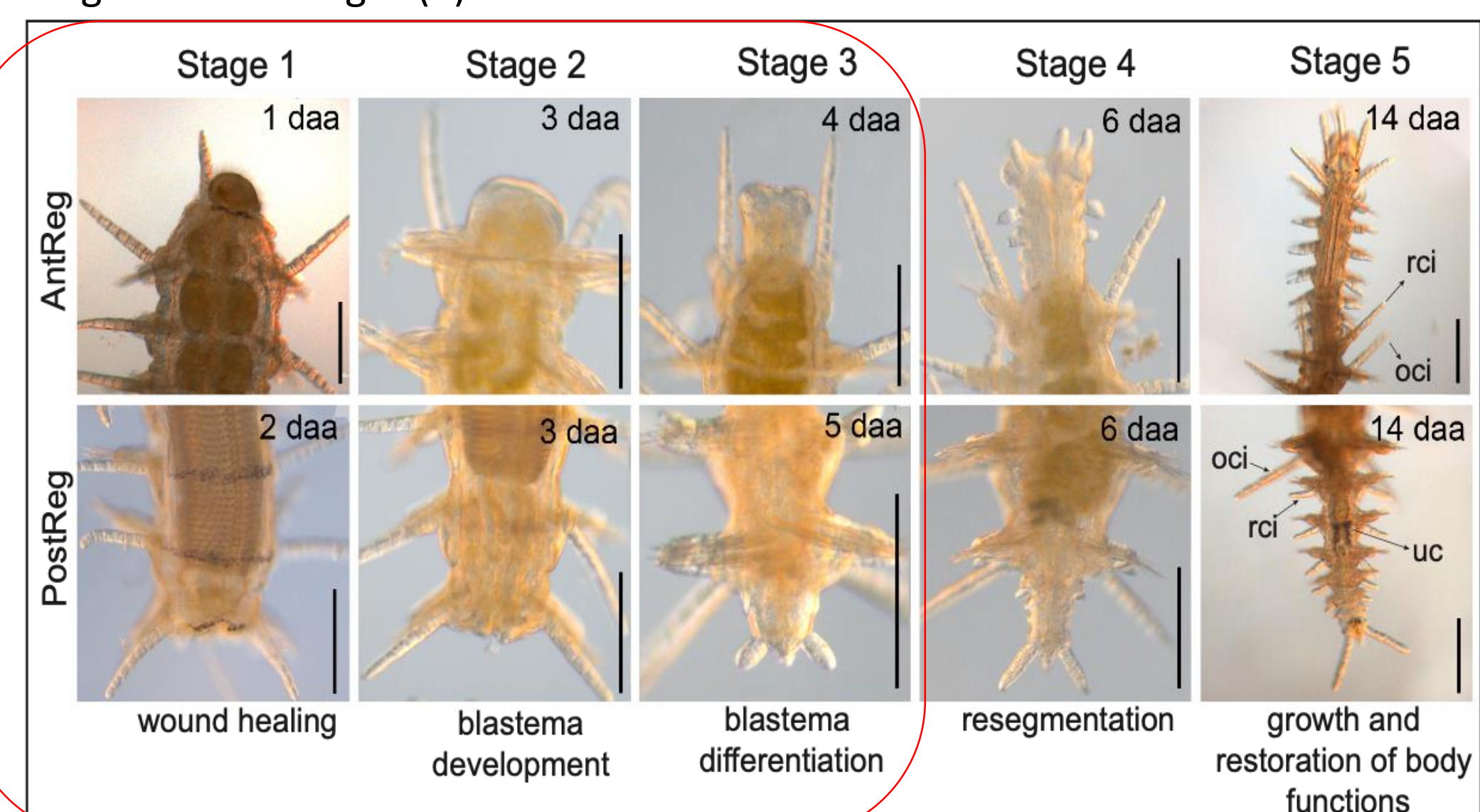
In homeostasis



C) Gene expression patterns in AR & PR in different body regions and stages.



Regeneration stages (2)



D) Detecting direct Wnt target genes. Genes differentially expressed between wildtype and Wnt up- or downregulation. Effects of Wnt/ β -catenin pathway modulation during regeneration and possible heteromorphic regeneration after treatments with GSK3 β modulators.

E) Visualization of Wnt pathway activity and target genes. WMISH protocols & single molecule imaging.

F) Comparing diverging GRNs: In species with and without complete AR.

G) Regenerating a brain: Role of the nervous system in successful anterior regeneration: immunohistochemistry and cLSM. With and without modulators.

Synergy and collaborations

- **Collaborative Project 1: Wnt signaling in anterior development**
Gene expression and chemical modulation data will allow us to study the dynamics of Wnt signaling during homeostasis and axis regeneration in syllids, identify putative direct Wnt targets and compare them with data from other clades (GB, PL, JR, DJ).
- **Collaborative Project 2: Reconstructing evolving GRNs**
The comparison between two species with and without complete anterior regeneration will allow us to discuss about possible evolutionary novelties in syllids and annelids. Comparison data with other groups and single molecule imaging techniques across clades will contribute to reconstructing evolving GRNs (GB, PL, JR, NP, TB, MO, ACH).

Technical innovation

- Establishing syllid model-systems to study related species with and without complete AR.
- Testing RNAi during regeneration.

Specific qualification

Syllid's biology and phylogenetic relationships, differential transcriptomics, use of pharmacological modulators, WMISH, live imaging, immunohistochemistry and cLSM. Good knowledge in bioinformatics GRN reconstruction.



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References

1. Chen C-Y, Yueh W-T, Chen J-H. Canonical Wnt signaling Is Involved in Anterior Regeneration of the Annelid *Aeolosoma viride*. *Developmental Biology*; 2020.
1. Ribeiro RP, Ponz-Segrelles G, Bleidorn C, Aguado MT. Comparative transcriptomics in Syllidae (Annelida) indicates that posterior regeneration and regular growth are comparable, while anterior regeneration is a distinct process. *BMC Genomics* 2019;20:855.