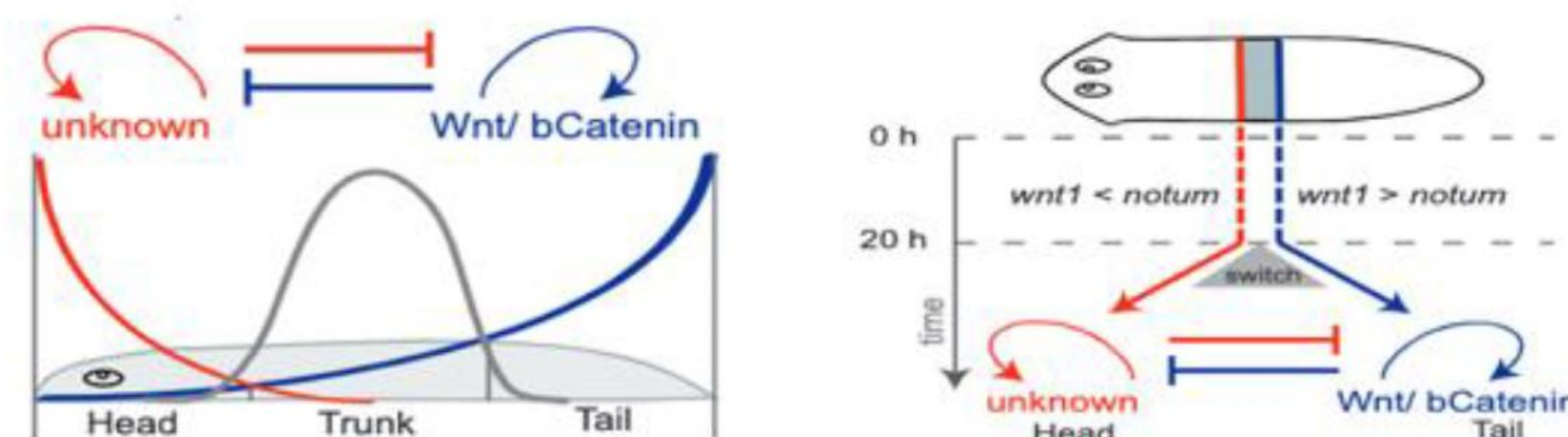




State of the art

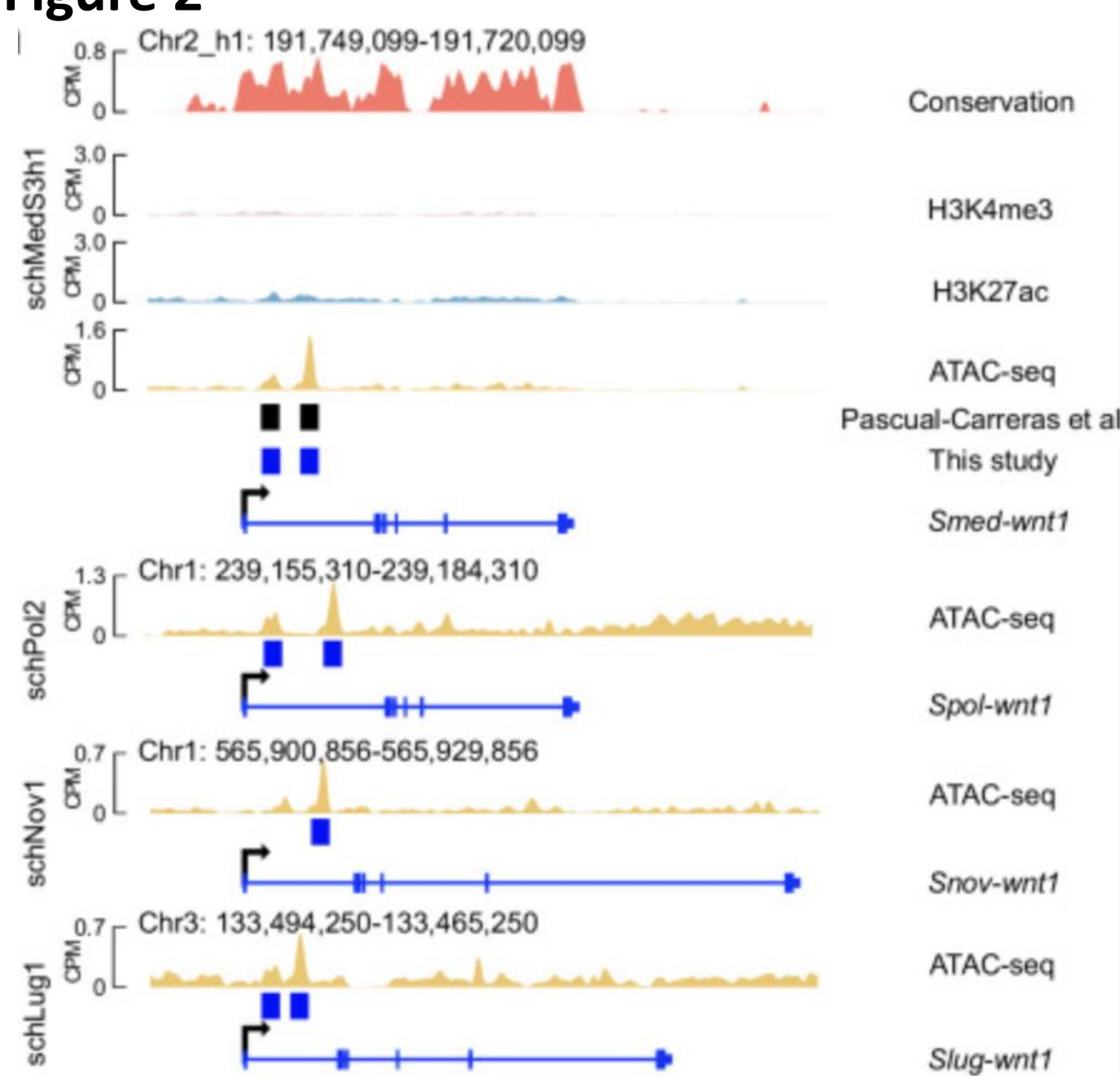
Planarian flatworms are ordinary animals in the sense that evolutionarily conserved signalling pathways pattern the major body axes of their triploblastic body plan (e.g. Wnt for A/P, Bmp for D/V). What makes planarians extraordinary is their ability to regenerate their entire bodies from random tissue fragments. This, in turn, involves the ability to deploy the conserved axial patterning signals not only from the well-defined and invariant conditions of the fertilised embryo, but also from arbitrary pieces of tissue resulting from catastrophic injuries.

Figure 1



We have previously shown that the maintenance and re-generation of the planarian AP axis involves a tissue-scale Wnt signalling gradient extending from the tail tip [1]. Our data further suggests that the tail Wnt gradient is a self-organizing pattern that exists in mutual antagonism with a similarly self-organizing signalling gradient emanating from the tip of the head, (Fig. 1, left and that the mutual antagonism of the two systems governs the mutually exclusive expression dynamics of head or tail genes during regeneration (Fig. 1, right). However, we still know very little regarding the underlying gene regulatory mechanisms.

Figure 2



Top: Annotation of the putative regulatory elements of Wnt1 in *Schmidtea mediterranea* by means of the indicated ChIPseq and ATACseq tracks. Bottom: Comparative ATACseq in the indicated *S. mediterranea* sister species.

For this reason, my group has been pioneering planarian functional genomics. Our recent publication of the chromosome-scale reference genome of the model species and chromosome-scale assemblies of its 3 closest sister species also provides regulatory element annotations by ATACseq, ChIPseq and evolutionary conservation [2].

Synergy and collaborations

- Collaborative Project 1: Wnt signalling in anterior development**
Contribute planarian data with respect to direct targets, general roles and dynamics of planarian Wnt signalling in head/tail specification.
- Collaborative Project 2: Reconstructing evolving GRNs**
Contribute RNAseq and scRNAseq data on the planarian head/tail GRN..
- Collaborative Project 3: Novel bioinformatics and genetic tools**
Provide scRNAseq time course data for regulatory inference; development of transgene expression tools in planarian flatworms.

Primary Questions

- What is the gene regulatory basis of the head and tail GRN in planarians?
- What makes head and tail specification mutually exclusive?
- Taxon-specific versus evolutionary conservation in planarian head/tail specification mechanisms?

Objectives

- Elucidation of the head/tail GRN in *S. mediterranea*.
- Understanding the mechanistic basis of the mutual inhibition between head and tail at the level of the GRNs.
- Development of transgenic Wnt reporters.
- Comparative analysis of planarian A/P-patterning

Workplan

A) To document the emergence and consolidation of the head/tail transcriptional states, we will carry out combined ATAC-seq/RNA-seq at multiple time points before and after the previously defined commitment point at 18 h post amputation (i.e., comparable stages defined in collaborative Project 2. Leveraging our preliminary regulatory region annotations (e.g., example in Fig. 2), we will carry out systematic motif searches within the top differentially expressed genes in activation sequence (e.g., early/late). Analysis objectives include i) systematic mapping of TCF binding sites for the further delineation of direct Wnt targets and Wnt-dependent Wnt expression during tail identity consolidation [1]; ii) cross-regulation between Wnt signalling and posterior Hox genes; iii) the identity of head-specifying transcription factor(s); iv) repressive head/tail network components as possible mediators of the mutual antagonism between the two expression states.

B) Functional validation of regulatory connections. We will use RNAi and the principle that the knock-down of regulatory element-binding transcription factors is often followed by the loss of the corresponding ATACseq peak and target gene expression. We will use this approach to confirm key edges in the emerging head/tail gene regulatory network and to confirm the expected hierarchical relationships between “pioneering” head/tail specifiers and their various downstream targets. In addition, we will use scRNAseq and scATACseq approaches at strategic time points to localize gene expression changes to specific cell types. And to ultimately understand the GRN-deployment in the tissue context.

C) We will address the dynamics of Wnt signaling and tail/head GRN deployment during regeneration by means of developing a transgenic Wnt reporter. Our previous demonstration of mRNA-based reporter expression [3] and unpublished proof of principle of Cas9-mediated reporter knock-in makes the construction of a planarian Wnt reporter feasible. In parallel efforts with → GB in *T. castaneum*, we will develop a Wnt reporter based on concatenation of several Wnt-responsive regulatory elements emerging from the ATACseq experiments in A) or artificial multimerized TCF binding sites. The Wnt-responsiveness of the reporter will be verified by RNAi and pharmacological tools. These efforts will benefit from our long-standing investments in developing live imaging approaches for planarians and the live imaging expertise of → PL.



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