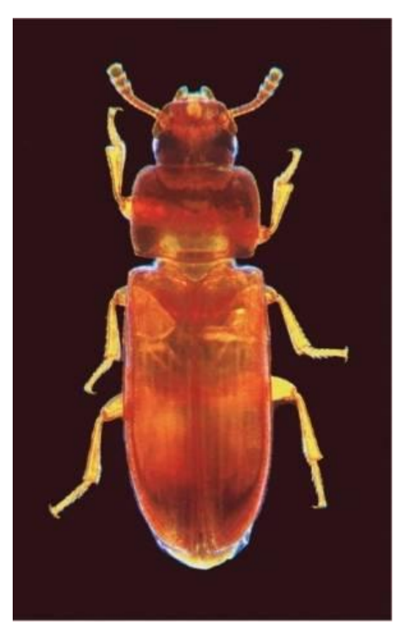
P1.1

Mechanism of Wnt signalling in insect axis formation

GönomiX

State of the art



Canonical Wnt-signalling is involved in axis formation in most animals but not in the main insect model, the fly *Drosophila melanogaster.* The red flour beetle *Tribolium castaneum* has a more insect-typical mode of development and we showed that Wnt-signalling is indeed essential for axis formation [1].

Hence, *Tribolium* is the key insect model for studying the diversification of Wnt signalling in axis formation. Surprisingly, maternal localization of *Tc-axin* mRNA and zygotic feedback-loops are required for axis formation [1,2] in contrast to the strictly maternal control in flies (Fig. 1).

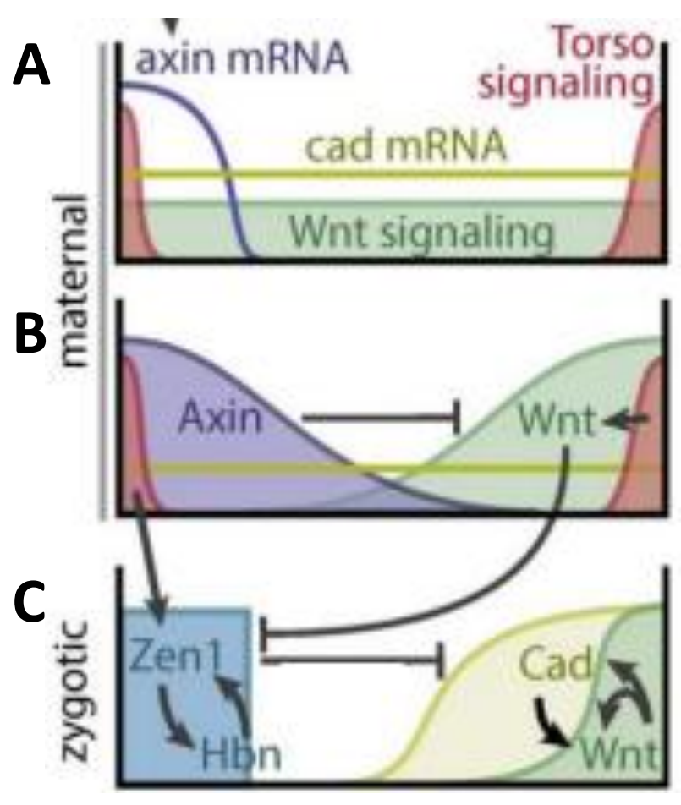


Figure 1 Current model of axis formation
Maternal Tc-axin at the anterior pole represses Wnt-signalling (A,B). A posterior Wnt-signalling gradient (B) activates a zygotic positive feedback loop to define the posterior (Tc-wg and Tc-cad; C). Another zygotic feedback loop (Tc-zen1 and Tc-hbn) define the anterior region.

Primary questions

Several key aspects of the model remain unclear:

- How does the Wnt-signalling gradient form and mature?
- What are the direct Wnt target genes and are they conserved?
- What gene sets determine the anterior and the posterior part of the embryo, respectively?
- How does insect axis formation compare to other protostomes?

Objectives

- Determine the dynamics of the early Wnt signaling gradient
- Distinguish head- versus tail-specific gene sets
- Identify the direct Wnt targets
- Compare our insect paradigm with other protostomes

Workplan

A) Determine the dynamics of the Wnt signaling gradient:

We will establish a transgenic Wnt-reporter both, by predicting an endogenous Wnt responsive enhancer (Fig. 2B) and by creating an artificial enhancer with *Tc-pangolin* binding sites (Fig. 2C). *in vivo* imaging will reveal the Wnt signaling dynamics.

As alternative, we will use single molecule imaging to detect the mRNA of a direct Wnt target gene throughout early stages.

This will allow us distinguishing, whether e.g. ubiquitous Wnt signaling retracts from the anterior or whether posterior Wnt signaling expands. Together with data from other projects (Collaborative Project 1) we determine conservation versus divergence of Wnt dynamics among protostomes.

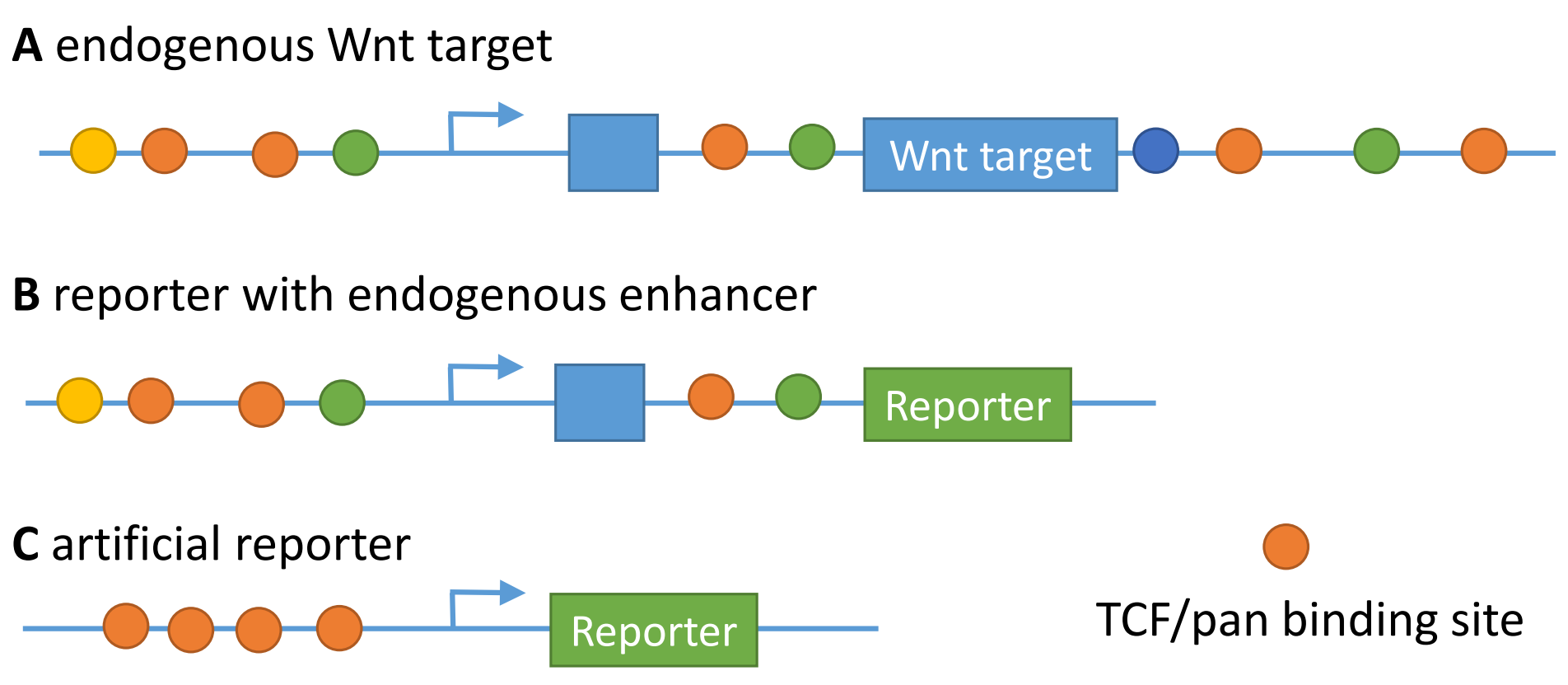


Figure 2 Wnt reporter
From a Wnt target gene (A), the endogenous regulatory region will be tried (B) as well as an artificial enhancer with multimerized TCF/pan binding sites (C).

B) Detecting head- versus tail-specific genes:

The gene sets specifying anterior vs. posterior are only partially known [2,3]. We want to identify them comprehensively by differential transcriptomics. We will compare wildtype with RNAi treated embryos that show either double abdomina (*Tc-gcl RNAi, Fig. 3A-D*) or double-heads (*Tc-cad/Tc-arrow* double RNAi; Fig. 3D,E) [2].

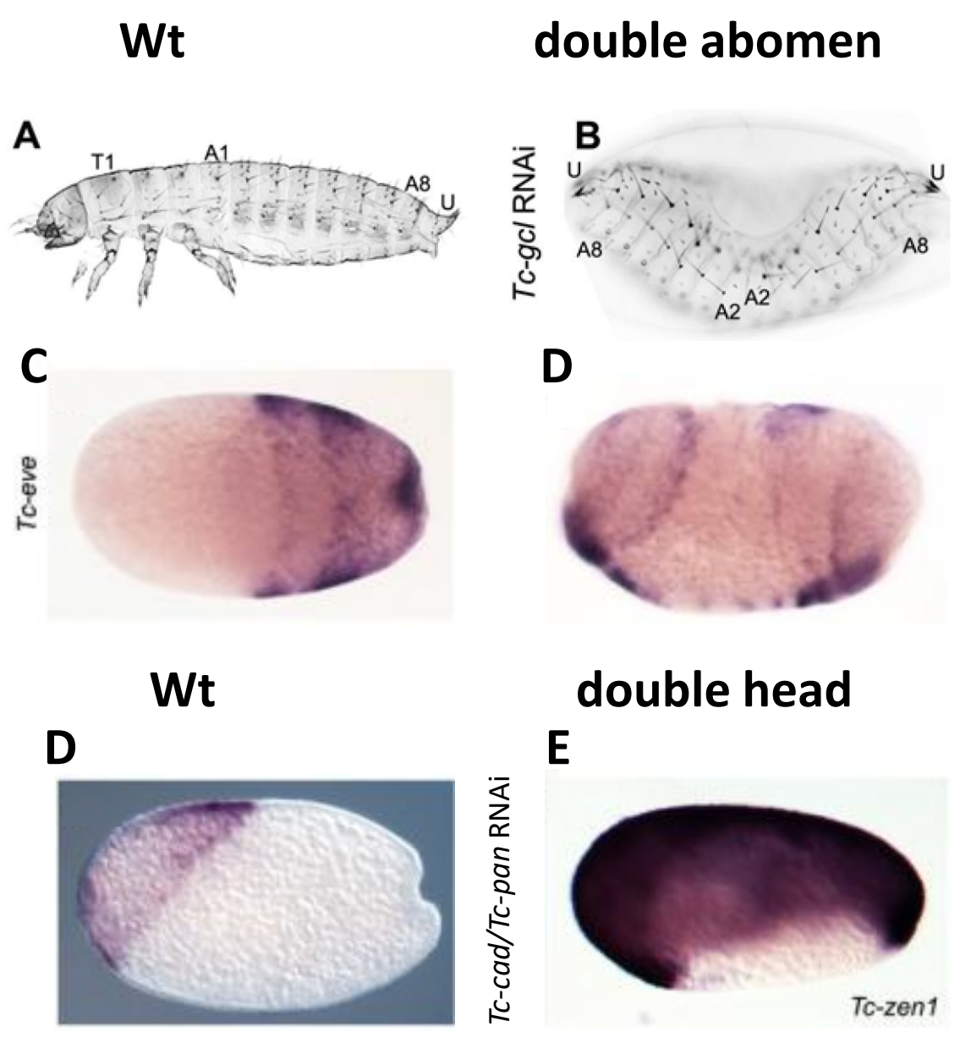


Figure 3 Axis duplication phenotypes
Tc-gcl RNAi leads to double abdomen phenotypes (compare B,D with A,C). Double RNAi targeting Tc-cad and Tc-pan leads to double-headed embryos (D, E).

C) Determining direct Wnt targets:

We will predict direct Wnt target genes based on their Wnt responsiveness (RNA-seq after RNAi; *Tc-axin* for increased and *Tc-dishevelled* for decreased Wnt signalling) and by enhancer analysis (ATAC-seq and binding site prediction). Our set of direct targets will be compared to other protostomes.

Synergy and collaborations

- **Collaborative Project 1: Wnt signaling in anterior development**
  Data on insect embryonic development for comparison with protostomes (Wnt gradient dynamics, direct Wnt target genes) (PL, JR, DJ, MA). Coordinated design of *in vivo* transgenic reporter, *in vivo* and single molecule imaging (PL, JR). Functionally testing new candidate genes emerging from joint analyses.
- **Collaborative Project 2: Reconstructing evolving GRNs**
  Provide RNAseq data from embryonic stages coordinated among groups for maximum comparability between model species (PL, MA, DJ, JR).
- **NP, TB:** ATAC analyses and detection of pan binding sites
- **NP:** Studying a novel axis formation gene from his previous work
- **MO:** Provision of embryos from the same stages for 3C analyses

Technical innovation

- Transgenic Wnt reporter in emerging model systems
- Single molecule imaging
- *in vivo* imaging of Wnt dynamics

Specific qualification

- Design and development of transgenic tools
- *in vivo* and single molecule imaging
- Bioinformatics integration of RNAseq, ATACseq
- Enhancer and binding site prediction
- RNAi mediated knock-down gene function

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References

1.Fu* J, Posnien* N, …, Kitzmann P, Brown* SJ, Bucher* G. (2012) Asymmetrically expressed axin required for anterior development in Tribolium. PNAS 109(20):7782-6

2.Ansari S, …, Bucher* G, Klingler* M. (2018) Double abdomen in a short-germ insect: Zygotic control of axis formation revealed in the beetle Tribolium. PNAS doi.org/10.1073/pnas

3.Oberhofer, Grossmann, Siemanowski, Beissbarth, and Bucher, G. (2014). Wnt/b-catenin signaling integrates patterning and metabolism of the insect growth zone. Development dev.112797

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