

ABSTRACT

The NF- κ B/Dorsal signaling cascade, which has a conserved role in innate immunity, governs the establishment of different cell fates along the dorso-ventral axis during *Drosophila* embryogenesis. The underlying gene regulatory network (GRN) is among the best understood, but it is not conserved outside of insects since dorso-ventral patterning is dominated by BMP signaling in most multicellular animals. The central question motivating this work is how the NF- κ B/Dorsal immune pathway evolved to replace the BMP signaling cascade in fly dorso-ventral patterning. Answering this question will yield insights into evolutionary plasticity and constraints underlying macroevolutionary change in a GRN.

The process of dorso-ventral patterning has diverged between the fly *Drosophila* and the beetle *Tribolium castaneum*, but still has many common features. In *Drosophila*, the Dorsal signaling cascade is regulated in a hierarchical fashion, while in *Tribolium* Dorsal signaling is more dynamically regulated due to feedback loops within the cascade. However, in both organisms the transcription factor Dorsal and one of its target genes, *twist*, play central roles in dorso-ventral patterning. Identifying genes regulated by *Tc-Dorsal* and *Tc-twist* will provide access to large parts of the *Tribolium* dorso-ventral GRN and is the objective of this work.

To that end two unbiased, genome wide, novel, next generation sequencing approaches were employed. For one approach, knockdown of *Tc-dorsal* (via *Tc-Toll*) and *Tc-twist* followed by comprehensive differential expression analysis (RNA-seq) was performed to find *Tc-dorsal* and *Tc-twist* target genes. For the other approach, this work is the first to establish and to prove the feasibility of chromatin immunoprecipitation followed by sequencing (ChIP-seq) in a non-classical model system outside mammals. *Tc-Dorsal* ChIP-seq comprehensively identified enhancers and respective genes regulated by *Tc-Dorsal*. Both approaches complemented each other and this study shows that combining these complementary approaches yields insights that exceed the value of either individual approach.

In total, several hundred enhancers and genes were identified. Extensive expression analysis by *in situ* hybridization and matching of the produced data sets led to the identification of 40 *bona fide Tribolium* dorso-ventral patterning genes, including basically all known ones. The dynamically regulated transcription factor *Tc-Dorsal* seems to establish expression patterns in three different domains within the ventral third of the *Tribolium* embryo. *Tc-Dorsal* appears to influence a smaller region and fewer, as well as different, target genes than *Drosophila* Dorsal. Initial contextualization of the data suggests a dynamic, regulative GRN downstream of *Tc-Dorsal*, the outputs of which are less dependent on *Tc-Dorsal* and more dependent on regulative interactions of *Tc-Dorsal* target genes, as compared to *Drosophila*.

The results and methods presented here constitute an essential foundation for future functional studies, including defined misexpression of genes, and for developing a thorough understanding of the enhancers comprising the core of the *Tribolium* dorso-ventral GRN.