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Exploring tissue-specific effects of 20-hydroxyecdysone through e23 hormone transporter expression in *Drosophila*

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Motivation and Aim: The main steroid hormone in *Drosophila* is ecdysone, with its active form 20-hydroxyecdysone (20H). Elevated level of 20H facilitates the progression of several crucial phases in fly development. Double peaks of 20H during metamorphosis control the transition of larva to prepupa and then to pupa, activating fundamentally different transcriptional programs in fly tissues [1–3]. While larval tissues are targeted for destruction through apoptosis or autophagy, adult tissues begin to proliferate from the imaginal discs, moving on to differentiation [4, 5]. Impressively, all these programs are triggered by 20H. However, the knowledge about the tissue-specific action of steroid hormone is insufficient.

The aim of our work was to describe the mechanism of 20H-dependent transcriptional activation by studying *Drosophila* tissues.

Methods and Algorithms: To describe the mechanism of 20H-dependent activation of transcription in tissues we used a natural element of the ecdysone cascade – the E23 transporter. Previously it was shown that E23 can export 20H from cells [6, 7]. We made a transgenic line of *Drosophila melanogaster* hspE23-F26 that can express the ecdysone transporter E23 exogenously after heat shock treatment. We expressed E23 at a stage preceding the increase of hormone concentration during metamorphosis to suppress the response to ecdysone. We used RNA-seq and ChIP-seq data of tissues from experimental and control (not expressing E23) drosophila larvae to determine 20H target genes and to reveal the changes in transcriptional factors binding profiles of control tissues comparing to E23 expressing tissues.

Results: We identified ecdysone targets in salivary glands, which are consistent with the results of previous studies [8]. We also found that brain tissue is more resistant to ecdysone exposure at the studied stage as the previously described 20H targets were not expressed in brains of wandering larvae.

Furthermore, we described the changes that occur at the promoters and enhancers of 20H target genes when hormone concentration was lowered during the expression of the E23 transporter. We established that a larger group of promoters is regulated by 20H through stimulation of productive elongation. The decrease in the transcription of these promoters upon E23 expression is accompanied by a reduction in the level of the Pol II elongation complex in gene bodies. For the remaining smaller group of promoters increasing the 20H titer opens chromatin at the TSS and promotes Pol II recruitment. We concluded that 20H promotes the transcription of its primary targets in the salivary glands by stimulating Pol II recruitment or productive elongation.

Moreover, we showed that ecdysone stimulates transcription of target genes by creating new regions of open chromatin – enhancers, which complements previously obtained data on the mechanism of activation of ecdysone-dependent genes [9].
In addition, we show that salivary gland enhancers, organized when the 20H titer increases, are active in a tissue-specific manner.
Conclusion: In our work, we present an experimental system for investigating the role of steroid hormone in physiological transitions of *Drosophila melanogaster*. We perform multiple genome-wide profiling experiments to examine the role of ecdysone in gene regulation. Thus, we have demonstrated the use of the E23 transporter as an effective tool for studying the genome's response to ecdysone and, using this tool, described new mechanisms of gene transcription activation by the steroid hormone in drosophila.
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