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Opponent assessment of thesis by Tereza Kozelková: **The importance of the insulin receptor signalling pathway in physiology of *Ixodes ricinus* ticks**

The master thesis by Tereza Kozelková explores key components of the insulin and insulin-like growth factor signaling (IIS) pathway in *Ixodes ricinus*. Experimentally it is reasonably rich work that includes gene identification, expression profiling by quantitative PCR, gene silencing employing RNA mediated interference (RNAi), injection of insulin receptor agonist, and artificial feeding with AKT and TOR inhibitors. The work also involved recombinant protein purification, antibody production and verification, western blotting, and SDS-PAGE.

Formal aspects: The thesis is written in very good English, presented in classical format with the introduction, material and methods, and joint chapter for results and discussion. Additional data are available in sufficiently detailed supplement. The whole product is logically organized and the orientation in the thesis is easy, too.

I also have some suggestions and comments to the written part. Introduction: When you mention bombyxins (page 6 of the intro), you may also indicate the remarkably high number of bombyxin genes (I believe it is 38). When you talk about duplication of InR in insects, the original papers by Xu (Xu et al., *Nature* 519:464–467; Xu HJ, Zhang CX. 2017. *Philos Trans R Soc Lond B Biol Sci* 372:20150489) might be mentioned. Although at the end of the introduction you do explain that InR has never been described before in any tick species, it is virtually one sentence “justifying” the topic for your entire thesis. I think there should be one small paragraph dedicated for that purpose. Obviously, these are more or less cosmetic issues, but given the reasonably quality of the thesis, I believe, that detailed feedback is worth it.

Material and methods. When protein domains are identified, I suggest using Interproscan instead of NCBI Blast output. NCBI is fine for a quick feedback which comes as a bonus of the BLAST search, but the identification of a particular domain is not reliable when it comes to publication purposes.

Cloning templates for dsRNA production. I am not familiar with plasmid P1L10. Does it have two inverted T7 promoters? Please clarify, particularly in the context of RNA synthesis done with MEGAscript T7.

Results, RNAi. I personally like the data presentation format with each value plotted as a dot, so one can see the real distribution of all phenotypes. It is great to see that RNAi silencing was efficient for all three targets, InR, Akt and Tor. However, it is a bit difficult to orient in the presented results. Phenotypic differences in weight were only revealed after Akt silencing. In Fig. 16 you talk about small and large group received upon InR knockdown. Please, clarify how animal size shown in Fig. 16 is (is not) connected to the weight data presented in Figure 13.

Western blots. InR is a dimer of two protein units. But each of these units consists of alpha and beta chains. Thus, four proteins are attached together to form functional InR. Given the number of bands detected by western in ovaries (Fig. 20), and that several of them seem to be silenced, can you correlate their size with predicted mass of alpha and beta chains, unprocessed monomer, and the complete dimer?

In addition to questions directly connected to ^{the} presented work, I have three additional requests stemming from the literature review:

1. You talk about eight Dilps (*Drosophila* ILPs) in the introduction. Can you comment on their reception, since not all of them are recognized by the canonical InR (with tyrosine kinase)?
2. Insulin receptor agonist – can you explain the mechanism of action?
3. Can you comment on Irs, *chico* in *Ixodes*?

Taken together, the thesis is a solid piece of work which apparently required regular and lengthy visits of the laboratory, the diversity of experimental work and tools is remarkable, as well. Quality of the text is also above the local standards, and it is written in solid English, which is a bonus. There are no doubts that the quality is above what we usually experience here. Therefore I definitely recommend the thesis for defense. Based on what I have seen so far, I suggest mark excellent.

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