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Posudek na magisterkou diplomovou práci
Bc. Terezy Dolejškové
The role of Gluconate shunt in the immunity of *Drosophila melanogaster*

General Thoughts:
This Thesis represents a substantial body of work on discovery and description of putative novel metabolic pathway – gluconate shunt – in the vinegar fly. The amount and quality of results presented in the Thesis greatly exceeds the average of the Faculty of Science, University of South Bohemia.

Strengths:
I especially appreciate the overall novelty and originality of the work. In addition:

1. Tereza does an excellent job of thoroughly presenting the complicated biochemical pathways of glycolysis, pentose phosphate shunt, and other related pathways in the Introduction.
2. Formal and graphic arrangements of the Thesis are above standards. I especially liked the nice charts of metabolic pathways.
3. The major strength, however, is the strength of presented research. I understand that the high quality of research is a result of team effort and good leadership by experienced supervisor. Nevertheless, Tereza's contribution to overall excellent result is apparent.

Weaknesses:
There isn't much I can say in this section. Just a few minor formal suggestions:

1. The first paragraph of Introduction could be little expanded to encompass the general relationships between parasitization, immune responses, metabolic stress, and reshuffling of metabolic pathways. The transition to concrete experiments with ^{13}C labelled metabolites is too abrupt. Moreover, the sentence: 'we found an interesting increase in ^{13}C-labeled gluconate upon infection with the parasitoid wasp' is not supported by data (see Fig. 7A).
2. The sections 1.1. to 1.5. of Introduction contain detailed descriptions of biochemical pathways, but the last section 1.6. is again too laconic and fails to present the goals and the results of the work in full.
3. The sentence on page 25: 'Regucalcin exists in two forms: secreted (encoded by the RD transcript with a signal peptide) and intracellular (encoded by the RA, RB, and RC transcripts) (Fig. 8.)' belongs to M&M section, should be added to the caption of Fig. 6, making the Fig. 8 redundant.
4. Similarly as above, several descriptions presented in early paragraphs of the sections 4.3.1., 4.3.2., and 4.3.3. do not belong to Results but rather repeat what was written in M&M.
5. The last paragraph of page 43 mentions the role of regucalcin in calcium homeostasis for the first (and last) time. It could have been included already in Introduction (section 1.5.), where the links of calcium signaling to parasitization and immune responses could be mentioned.
6. One thing was not clear to me: have you isolated the hemocytes from hemolymph and measured the concentrations of metabolites in the two tissues separately? One sentence in the

section 4.1. (page 24) would suggest so ('In the experiments with 13C6 glucose, after the 6 hours of feeding, about 22% of hemolymph glucose is 13C6 labelled ... In hemocytes the labelling is very similar ...'). In M&M section, however, I do not see any method for separation of hemocytes from hemolymph. Moreover, on page 23, I see a sentence suggesting the opposite: ('The samples were frozen in liquid nitrogen and reheated in 37°C tree times for the cells to burst'). The phoshorylated forms of metabolites should belong almost exclusively to the hemocyte pool. The non-phosphorylated forms, however, could belong to hemocyte and/or hemolymph pool, and moreover, could get to hemolymph from other tissues such as gut, fat body, or muscle.

Discussion Questions:
The observations collected from literature and the results of own experiments make an impressive and coherent chain of indirect evidence for the existence of a gluconate pathway in *Drosophila*. I appreciate that the authors remain modest and chose the conservative way of interpretation of their results.

1. The Figure 7A suggests that the total amounts of ^{13}C labelled gluconate are similar in the not infected and the infected larvae. What significantly changes after the infection, is the total pool of unlabelled gluconate. In fact, similar trends were observed for all other metabolites (Fig. 10A, Fig. 12A, Fig. 15). While the labelled metabolites must come from diet, what is your explanation for potential source of the flood of unlabelled glucose and other metabolites in infected larvae? Do they feed more or do they degrade the glycogen stores in fat body cells or muscles, or the trehalose stores in hemolymph?
2. I see that the question on origin of glucose and its katabolic products flooding the hemolymph after infection is perhaps not so important (for your specific goal). Important is the fact that all six ^{13}C labelled carbons travel from glucose to gluconate and ribose (Fig. 16) – a putative gluconate shunt, while only low levels of labelling occur in glucose 6-phosphate (Fig. 16) and phosphogluconate (Fig. 15D) – a classic pentose shunt. The evidence presented in Fig. 16 seems to be central to your hypothesis but it is not easy to follow for non-specialists. Could you, please, spent some time to explain it in common language?
3. The first enzyme of putative gluconate pathway is proposed to be the H6PD (hexose 6-phosphate dehydrogenase), which is known as luminal enzyme in endoplasmic reticulum. Is it possible that whole gluconate pathway can localize to ER? Would that make any physiological sense? Possibly linked to regulation of calcium (regucalcin?).

Conclusion:
I strongly recommend this Thesis as high quality basis for awarding the MSc degree to Tereza Dolejšková.

In České Budějovice, 26 April 2023

Vladimír Košťál