FYZIOLOGICKÝ ÚSTAV AV ČR

Mgr. Martin Sládek, PhD
Laboratoř Biologických Rytmů
Fyziologický ústav AV ČR, v.v.i.
Vídeňská 1083
Praha 4, 142 20

Oponentní posudek na magisterskou diplomovou práci: Bc. Václav Brabec

In silico and functional analysis of clock component timeless-m

The thesis of Václav Brabec focuses on an enigmatic clock gene, mammalian-type Timeless (*tim-m*). Unlike its paralog Drosophila-type Timeless, which is a crucial component of fruit fly circadian clock, *tim-m* plays a major role in development across the animal kingdom and its circadian function is unclear. The author used reverse genetics and locomotor activity monitoring to study its role in circadian clock. To avoid causing embryonically lethal mutations, author compared genetic and protein sequences of *tim-m* of Drosophila with many invertebrate and vertebrate species to identify a highly variable C-terminal domain, to which he introduced indels using CRISPR-Cas9 mutagenesis. The work resulted in 24 unique homozygous mutant lines that were tested by actigraphy in LD cycle and in constant darkness using three different environmental temperatures. Despite the mutations needed to be in a likely much less important part of the protein to avoid developmental failure, the author identified several homozygous lines with altered rhythms or with increased percentage of arrhythmic locomotor behavior, supporting the suspected role of TIM-m in the circadian clock mechanism.
For a diploma thesis, the work described here is interesting and valuable, presents a robust evidence and provides tools for future research. The author got familiar with many methods of molecular biology, genetics and chronobiology. The thesis is written in good English and with very few mistakes.
The introduction is the weakest part of the thesis. While generally well written and interesting, the theory part is only 6 pages long, with 3 additional pages describing the principles of CRISPR, which could have been easily moved to the Methods instead. For example, the chapter 1.4 introducing TIM-m should have been much more detailed and elaborate not only on the evidence described in the few available papers on its role in the clockwork mechanism in various organisms, but also summarize its other known roles in cell cycle and development. The thesis cites only about 60 papers, but the references are well chosen. Methods are adequate, though I would personally prefer less focus on the PCR or cloning and more focus on the fruit fly genetics. Results are concise with many great figures that nicely describe the findings. The discussion is again quite short (3 pages) and some of its parts work better as a summary, but this may be due to a lack of comparable research on the topic.
Overall, despite some criticism, I really enjoyed reading the thesis. I think it reflects a lot of work done during the studies. I would like to recommend the thesis for defense.

Minor:
Page 5, paragraph 2 – „as was mentioned in chapter 1.5" – reading this in ch. 1.4 is confusing

Questions:

Q1: Can you explain why were the F1 *tim-m*-mutant heterozygous flies crossed with chromosome 3 balancer strain for the second time (Fig. 7C) before trying to establish the homozygous line?

Q2: Can you better explain the mosaic fly screening so a person less familiar with fly genetics can understand it? I know that mosaicism can occur in mouse due to zygote being injected after DNA replication, resulting in more than 2 mutant alleles in the embryo. Where/when, does the mosaic arise in this case? During which step of the genetic crossing (Fig. 7) did you screen the gonads?

Q3: Is there any evidence of protein-protein interaction between TIM-m and any other clock protein in flies or other Protostomes?

Q4: Is genetic knockdown of *tim-m* embryonically lethal as well?

Q5: Speculative question, asking about your opinion – Do you think it is possible that TIM-m does not play any direct role in the fly TTFL and that the observed circadian phenotypes may be a product of some minor developmental defects?

Prague, 3 Jan 2023