Supervisor's evaluation of Ph.D. thesis by Mgr. Veronika Burešová – „Function of the α_2-macroglobulin protein family in the immune response of the tick *Ixodes ricinus*"

(Petr Kopáček, Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic)

Veronika Burešová joined our group as an undergraduate in 2002 and finished her Master diploma three years later in 2005. Thanks to her biological background she gained before in Dr. Berger's lab, I asked her to focus from the very beginning to the biological part of our tick research. Noteworthy, that time the lab was full of molecular biologists and biochemists doing their PCRs and sequencing but no one who was really ready to look inside the tick and help us to proceed from molecules back to the animal. Veronika at first looked at the problem of the *Chryseobacterium indologenes*, the goddamn bacterium which completely exterminated our huge laboratory colony of the soft ticks *Ornithodoros moubata*. I called this African species for its size a "tick Manduca" because it allowed us to purify proteins and subsequently clone the genes. This sad moment forced us to turn our attention to the Czech tick No.1 – namely *Ixodes ricinus* and exploit the reverse genetic approach. Veronika showed that *C. indologenes* are nicely phagocytosed by tick hemocytes and she developed a surprisingly reliable and reproducible phagocytic assay based on indirect immunofluorescence. Therefore we could afford to promise in the last sentence of her first paper that the yellow pigmented bacteria may become a valuable model for deeper research into tick-pathogen interaction.

Our molecular research of tick innate immunity has been mainly focused on plasmatic lectins named Dorin or Ixoderins and also on the role of the universal protease inhibitor of α_2-macrogloubulin (α_2M) class which we characterized before from the soft tick *O. moubata*. By a lucky coincidence about the same time, the group headed by Dr. Elena Levashina published their wonderful results showing that mosquito thioester containing protein (TEP1), which belongs to α_2M – protein family, functions in a complement-like manner in phagocytosis of bacteria. Moreover, they proved that TEP1 mediates killing of transmitted Plasmodium and it was (to my knowledge) the first molecule described to truly function as determinant of mosquito vectorial capacity. Other positive impulse was Elena's visit to our lab five years ago, which stimulates us to employ the freshly emerging method of RNA interference to perform functional studies in ticks. The realization team for introducing RNAi in ticks in our institute was made of two persons – Ondra Hajdušek, who designed and cooked the dsRNA according to Elena's protocol and Veronika Burešova who gradually become a master in tick microinjection and handling.

Having this in hand we could ask the question: Is tick α_2M somehow involved in cellular immune response against invading bacterium? Many publications anticipated that α_2M possibly guard organisms against pathogen proteases but the real proofs for this statement were in fact missing. Veronika designed and performed an RNAi-based experiment which demonstrated that phagocytosis of *C. indologenes* is mediated by *I. ricinus* α_2M (further referred to as IrAM) and that its phagocytosis is dependent on the metalloprotease secreted by the bacterium. Unfortunately, it was *C. indologenes* and not *Borrelia burgdorferi* which eventually limited our ambition to publish these results in a top-rank journal. I completely leave aside the tedious story of completing the puzzle of the IrAM full sequence, which finally allowed us to make a reasonable structural prediction. We are not going to sequence other members of tick α_2M family any more since they become available with the release *Ixodes scapularis* genome. Luckily, these proteins in both tick species are almost identical, so we plan now to perform a functional genomics study of other tick α_2M family proteins with *I. ricinus* and the results will be surely valid also for *I. scapularis*. I only regret that I could not persuade Veronika to incorporate into her thesis the results they have already achieved with Ondra on tick fibrinogen related lectins. It looks very likely that these molecules possibly act as pattern recognition molecules in a primitive complement-like system of ticks. At least I hope that we will be able to publish it soon.

Veronika Burešová had to learn all her skills alone from the very beginning and at the moment she is really an indispensable person in our tick immunity research. I can not afford to allow her to leave the lab before she train up a full-value successor. Veronika will probably need some more time to improve her scientific writing and formulating her own project proposals. On the other hand, if she already knows everything perfect, there will be no work left for her boss.

In conclusion, I would like to mention that I am very grateful to Veronika Burešová for her work and effort which substantially elevated the level of tick research in our laboratory. I am convinced that Veronika Burešová fulfilled by her published papers and yet unpublished data the criteria for awarding a Ph.D. title from Faculty of Sciences, University of South Bohemia in České Budějovice.

In České Budějovice, April 24, 2009

Petr Kopáček (supervisor)