

Supervisor's evaluation of Ph.D. thesis by Mgr. Lenka Grunclová – „Immune related molecules in the gut of the soft tick *Ornithodoros moubata*” –

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Lenka Grunclová has been working in our laboratory since the year 2000, when she has started her master diploma. The topics for her master diploma was focused on molecular cloning and phylogenetic analysis of lysozyme from the gut of the soft tick *Ornithodoros moubata* called as TGL. This enzyme has been previously purified and biochemically characterized by us in co-operation with Chris Weise and Ragnar Vogt from Free University Berlin.

The lysozyme paper was written and published already during the first year of Lenka's doctoral study and reflects the state of molecular biology in our laboratory at the very beginning of this millennium. Nevertheless, TGL from *O. moubata* gut was the first lysosyme sequence described till 2003 among *Chelicerata* and thus adding a phylogenetic analysis and post-blood meal kinetics reached the sufficient scientific merit to be published in the *Developmental and Comparative Immunology* (IF= 3.26). To the date, this article has been cited by other authors 10 times.

The presence of lysozyme and defensin (described by Japanese authors) in the gut of soft tick pointed to the existence of an active immune system within the gut lumen which likely guards tick against bacterial grow and decomposition of stored blood-meal. With this in mind, we decided to concern more about the lysosyme and defensin up-regulation upon challenge with infected blood-meal. Unfortunately, these plans have to be abandoned since our colony of soft ticks was devastated by the undesired infection with the Gram negative *Chryseobacterium indologenes* resistant not only to the endogenous tick immune molecules but also to the common antibiotics/antimycotics used as a supplement for the artificial feeding. Since almost all *O. moubata* died off due to the infection, we have to establish a very limited healthy colony fed naturally on rabbits or on the expensive sterile sheep blood. This unlucky event made us to change completely our classical way from proteins to genes and start to follow the reverse genetics approach.

As a first step, we have constructed an *O. moubata* gut-specific cDNA library (with a substantial contribution of Daniel Sojka) and begun a screening for genes coding for proteins having a potential function in the gut immune system or pathogen transmission. From the first set of genes identified by this pilot screening – five molecules were selected to be characterized in detail: Putative inhibitors of cystein proteases cystatin 1, 2 and thyropin, serine protease homolog and putative – mucin-like perithrophin.

The main task for Lenka Grunclová was the characterization of tick cystatins. The cystatin paper which is a core publication of her thesis was published in December 2006 in *Biological Chemistry* (formerly *Biological Chemistry Hoppe-Seyler*). This journal is quite well recognized among enzymologists, unfortunately the IF of this journal dropped from about 4-5 to 2.57 last year. Lenka's cystatin paper demonstrates well our current procedure we use to characterize molecules of tick origin. Isolation of full clones from cDNA library, sequencing, tissue expression profiling and post-blood meal kinetics by RT-PCR, preparation of recombinant protein in appropriate expression system, refolding to active molecule, biochemical characterization and immunolocalization in tick tissues using indirect immunofluorescent and/or immunogold electron microscopy. The similar approach has been applied also for research on thyropin and serine protease homolog. However, the stories about the latter two genes/proteins have to be completed before publication. To present the data obtained so far on these two molecules in Lenka's thesis, we decided to add two other chapters having roughly the form of short communication. The main problem of thyropin is

pure solubility of the recombinant fusion protein isolated from the inclusion bodies and low expression of non-tagged soluble recombinant protein. To overcome these problems and obtain a sufficient amount of active thyropin allowing to determine its inhibitory specificity is the main task for Lenka's current activity in the laboratory.


Serine protease homolog secreted into the gut lumen is also a very exciting molecule, however we still need to have any reliable information on its function. It is obviously non-active homolog, however there is still a chance that the molecule may serve as an antibacterial peptide due to the (HD)-rich domain and we have just not been able yet to prove it with our possibly incorrectly folded recombinant. Other possible function of this serine protease homolog which is also present in other tick species, that it may somehow interfere with active serine proteases of the host origin and preventing for instance undesired clotting of the ingested blood.

Due to above mentioned problems with the soft ticks, Lenka is also searching for orthologous molecules like cystatins and thyropins in the hard tick *Ixodes ricinus*. This species is much more relevant as a disease vectors and importantly, this species was already proved by us to be feasible for functional studies by RNA interference.

As a young scientist, Lenka is a type of a conscientious laboratory worker who patiently repeats and modifies established protocols until they lead to successful results. I also appreciate very much her sense for order in the lab and her endless effort to make us to keep the cleanlines. During her doctoral study, Lenka helped me a lot as a co-supervisor of two undergraduate students - Veronika Voš'íňáková (they worked together on the serine protease homolog) and currently Jan Perner, who is mainly focused on cystatins in *Ixodes ricinus*. Lenka also stayed for one month in Japan, in the laboratory of prof. Kozo Fujisaki, who in the mean-time become our major competitor in the research of tick gut-specific molecules.

I am convinced that Lenka Grunclová fulfilled by her published and yet unpublished work the criteria for awarding a Ph.D. title from Faculty of Sciences University of South Bohemia in České Budějovice.

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