

Supervisor's report on Ondrej Ballek's Diploma thesis:

**RACK1 as a candidate protein involved in the regulation of translocation of Lck to lipid rafts.**

The study describes an investigation of the translocation mechanism of Lck to lipid rafts in general, and the biochemical and genetic characterization of the first identified candidate molecules named RACK1 in this process, in particular. While the existence of Lck translocation machinery was proposed 5-6 years ago, the nature as well as its molecular components have not been revealed so far. This is the very first study providing evidence for an essential role of RACK1 in the regulation of Lck membrane redistribution by functioning as a linker between Lck and microtubular cytoskeletal network. This, in turn, provides an experimental framework for further studies in this emerging field of research in T cell signal transduction.

Ondrej Ballek joined my research group in summer 2007. He quickly adapted to laboratory life and routine. In several months he mastered many essential molecular and microscopic techniques. Thus, in a short period of time, he was able to conduct relatively complex experiments independently. Moreover, after Ondrej became familiar with details of his project, he often came up with suggestions and comments concerning the experimental design and approaches directed to solve particular methodological problems and efficiently address important scientific questions.

In his thesis, Ondrej presents his original data obtained by using two different cellular model systems: a fibroblast cell line NIH 3T3 and primary CD4<sup>+</sup> T cells. While the former system allowed the structure-function analysis of Lck-RACK1 interaction, the later system provided an important insight into the potential role of RACK1 in T cell proximal signalling. In aggregate, Ondrej's results provide a compelling evidence that RACK1 is, indeed, a critical component of Lck translocation machinery, suggesting that interaction of CD4-Lck complex with cytoskeletal system via RACK1 could be an indispensable event for the initiation of T cell activation. Data presented in the thesis warrant further investigation which will focused on mapping a precise physiological role of Lck-RACK1 interaction in T cell signalling using the transgenesis, knock-down and knock-out technology and modern microscopic techniques. On my view, the completion of such experiments will likely result in papers published in high impact journals.

The thesis is written in a standard format in English. Chapters are well balanced and transitions between them are smooth. The Introduction, Literature review and Materials and Methods sections highlight the rationale of the study, describe its design and the procedures and methods required for its analysis. The result section documents important observations in a clear way. Figures are labelled properly and easy to understand. The result section testifies to Ondrej's experimental skills: he was able to apply many complex, tricky and tedious techniques to obtain interesting results. The discussion section puts the new data into a context of well-known facts surrounding the initiation of T cell signalling and discusses an emerging model of Lck translocation mechanism to lipid rafts.

The obvious strength of the study is the discovery and characterization of RACK1 as candidate protein in the context of Lck membrane translocation machinery, opening a

whole new area of research in proximal T cell signalling. In this sense, the major objective of this investigation has been achieved.

**Conclusions and recommendation**

In my opinion, Ondrej Ballek is the prototype of a successful student: diligent, accurate, and well organized. Ondrej's work represents an important step towards an understanding of fundamental molecular events regulating T cell responses. Richness of experimental approaches, clear presentation of results and a thorough discussion fully attest that the author is well prepared for the scientific carrier he has chosen. Moreover, the fact that the thesis is written up in English further strengthens the potential of Ondrej Ballek as a successful PhD candidate.

Based on the above review, I recommend Ondrej Ballek's thesis to be accepted as the fulfilment of the requirements for the degree of Master of Science.

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