

## **Towards unraveling the role of carp cold acclimation protein 31 (Cap31) – a new important player in fish antimicrobial defense?**

**Objective.** The objective of this project is to obtain new knowledge about unknown to date carp protein Cap31 structure, characterization, localization, and kinetics during fish acclimation to cold and microbial infection. We believe that Cap31 is a new important player in fish antimicrobial defense. The research hypotheses assume that Cap31 has cytidine deaminase activity, an enzyme which can modify single stranded DNA and RNA of microbes and its expression is affected by microbial infection as well as ontogenetic and phylogenetic factors. It can be useful marker for evaluation of well-being of fish.

**Description of research.** Since the existence of protein Cap31 is only deduced from carp genome analysis and mass spectrometry identification of protein spot from two-dimensional electrophoresis, there is no information concerning its characteristics. Therefore, it is of utmost importance to isolate Cap31 from carp blood in order to understand its structure and functions. The planned research will start from isolation of Cap31 in order to obtain pure preparations for detailed characterization of this protein. In the next step we will develop analytical tools for measurements of Cap31 concentrations (enzyme-linked immunosorbent assay, ELISA) and its location in tissues (immunohistochemistry). These tools (together with measurements of Cap31 gene expression) will be used in several experiments aimed to obtain new knowledge about localization of Cap31 in fish tissues in order to learn where this protein is produced in fish body and how it is distributed. We will also test expression of Cap31 during fish development. This way we will learn when Cap31 appears during life stages. In next experiments we will focus on obtaining detailed information regarding appearance of Cap31 during acclimation to cold. We know that Cap31 is produced in response to cold, but we do not know the time of synthesis commencement and the dynamics of its production. In cooperation with Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine Hannover, we will perform several experiments focusing on Cap31 changes during bacterial, viral and fungi experimental infections at different temperatures. This way we would like to obtain the detailed information the possible role of Cap31 in microbial infection. Finally, using the gathered knowledge, we plan to evaluate usefulness of Cap31 for evaluation of well-being of fish in aquaculture conditions, such as stress and health status.

**Justification.** The primary justification of this project is our curiosity related to a lack of information concerning the “uncharacterized” protein discovered earlier in our study. Most importantly, results of our preliminary experiments and recent database analyses clearly and strongly suggest Cap31 can be a new important player in fish antimicrobial defense, that never been described before. We recently discovered that protein Cap31 has homology to secreted activation-induced deaminase AID/APOBEC (SNAD) which are enzymes able to convert cytidine to uridine in single stranded nucleic acids leading to mutation of DNA and/or RNA of both host and pathogen. As such, studies of Cap31 are highly justified in a view of possible importance of such defense mechanism in fish, which hasn't been described yet.

**Expected effects.** We believe that our project will provide a truly important contribution to knowledge regarding fish immunity by describing of unknown mechanisms of fish defense. As such our results should be important for better understanding of the mechanisms of fish infection with further possible role of Cap31 in coping with fish diseases. We also think that the use of Cap31 for better understanding of fish well – being may also contribute in future improvement of fish welfare, taking the advantage of using Cap31 as biomarker for monitoring fish health and defensive mechanisms.