Summary of Thesis

Functional studies of RNA interference as a tool for gene silencing in teleost fish

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This thesis addresses functional aspects of RNA interference in teleost fish. RNA interference (RNAi) is a conserved cellular mechanism which inhibits gene expression in specific manner. The active molecules to trigger the RNAi are the siRNA and miRNA which can be derived from double stranded RNA (dsRNA) endogenously or exogenously. RNAi has been widely used for gene function studies and has high potential to be used for therapeutic application. However, there are some fundamental problems related to therapeutic use of RNAi in humans and veterinary animals including farmed fishes. In fish, there is still limited knowledge of how the mechanism of RNAi-mediated gene silencing can be efficiently triggered *in vivo*. Also, delivery of therapeutic small exogenous dsRNA *in vivo* remains a challenge, especially when systemic delivery is requested.

This thesis aimed to investigate specific gene knock down by RNAi in cell cultures and fish and to assess the potency of different methods and administration routes for delivery of small silencing RNAs in fish. The gene specific knock down by RNAi in fish cell cultures, fish muscle tissue, and human cell cultures were examined using different types of small silencing RNAs targeting a reporter gene. Despite demonstration of efficient knock down in the human cells, no specific effect could be obtained in neither fish cells nor in live fish. This suggest that despite the conserved nature of RNAi, the individual small RNA molecules must be designed and optimized according to the host species conditions. Temperature is suggested to be one of the potentially important parameters which must be taken into account for RNAi in fish. Delivery of fluorochrome labeled small silencing RNAs into fish was assessed by the use of different physicochemical approaches including electroporation and chemical formulation with chitosan or poly-lactic-co-glycolic acid (PLGA) (nanoparticles). Some uptake could be demonstrated in fish tissues and/or cells following all delivery strategies, but further optimisation is needed, potentially by combining different formulation reagents. One alternative approach is to do further research into the natural mechanisms of transmission of small interfering RNAs

between cells and tissues in living organisms and in vertebrates in particular, in order to learn from nature how to design optimal delivery strategies. A review of the research in this field was performed as a part of the thesis.