Investigation of phosphorylation dynamics within the mTOR signalling pathway in zebrafish PAC2 cells by means of mass spectrometry-based targeted proteomics

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Currently, thousands of fish are being used annually to perform toxicity tests needed to inform environmental risk assessment of chemicals and products. To reduce the use of animals, alternative (non-animal) toxicity testing methods need to be developed. Recently, the first fish cell line-based assay for the determination of acute toxicity to fish was adopted as an OECD test guideline.[1] This assay provides a measure of chemical effects on cell viability, which can be linked to fish mortality *in vivo*. However, being relatively short-term, this assay does not measure effects on cell population growth and hence cannot be directly used to predict *in vivo* effects on fish growth, another important ecotoxicological endpoint. To obtain growth-relevant measures, one could extend the assay by several days and use the change in cell numbers as a proxy for fish growth.[2] Another approach could be to monitor chemically induced molecular changes known to affect cell growth and proliferation. One of the major cellular signalling pathways known to be involved in the regulation of cell growth and proliferation in eukaryotes is the mechanistic target of rapamycin (mTOR) pathway. There are also indications that the mTOR pathway could be involved in the mediation of chemical effects on growth, but these interactions have not yet been systematically explored, especially in aquatic organisms such as fish. In this project, we are using *in vitro* cultured zebrafish (*Danio rerio*) PAC2 cells as a model to investigate the architecture and functionality of the mTOR pathway, as well as its susceptibility to chemicals and the concomitant effects on growth and proliferation of fish cells. The initial steps included: i) selection of the zebrafish counterparts for the main proteins comprising the mTOR signalling pathway in mammals; ii) identification of putative phosphorylation sites on our targets through comparative analyses with help of PhosphoSitePlus;[3] (iii) development of mass spectrometry based assays to monitor the abundance and phosphorylation changes of selected proteins as described in.[4,5] Our approach will enable the monitoring of protein phosphorylation dynamics by using mass spectrometry instead of relying on antibody-based methods. This would provide a significant methodological advancement since the respective antibodies for non-mammalian proteins are not always available. The subsequent experiments will expand our knowledge about the mTOR-mediated signalling and its role in growth regulation in fish cells, as well as its potential disruption by chemicals. This knowledge in turn could provide an avenue to develop a new non-animal toxicity test for predicting chemical effects on fish growth without using the actual fish.

[1] OECD, **2021**, Test No. 249.
[2] J. Stadnicka-Michalak, K. Schirmer, R. Ashauer, *Sci. Advances* **2015**, e1500302.
[3] P.V. Hornbeck, B. Zhang, B. Murray, J.M. Kornhauser, V. Latham, E. Skrzypek, *Nucleic Acids Res*. **2015**, 43:D512-20.
[4] A. Tierbach, K.J. Groh, R. Schönenberger, K. Schirmer, M.J.F. Suter, *Toxicol. Sci.* **2018**, 162(2), 702-712.
[5] E.L. De Graaf, J. Kaplon, S. Mohammed et al., *J. Proteome Res.* **2015**, 14(7), 2906-2914.