Melanosome transfer, photoreception and toxicity assays in melanophores

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DISSERTATION ABSTRACT

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Many animals such as fish and frogs have developed the ability to change colour of their skin to adapt to the environment or to signal to other individuals. This ability is due to specialised skin cells called melanophores. Melanophores contain thousands of melanosomes, small membrane-enclosed organelles containing the black or brown pigment melanin. The melanosomes can aggregate to the cell centre rendering the cells pale or disperse throughout the cell to become dark. The intracellular transport of melanosomes is regulated by neuronal or hormonal external stimuli. Fast colour change is achieved by aggregation/dispersion of melanosomes but long-term colour change can also be achieved by melanosome transfer to surrounding skin cells.

An amphibian immortalized melanophore cell line was used from the African claw frog, *Xenopus laevis* to study transfer of melanosomes to co-cultured fibroblasts. Melanosome transfer was observed and up regulated by the hormone α-MSH . The transfer was quantified using light-, fluorescence and electron microscopy.

A new and powerful method for transfer experiments was developed. Fluorescent semiconductor nanocrystals, qdots, were used in combination with flow cytometry. The qdots were taken up by the cultured *Xenopus laevis* melanophores, localised to the melanosomes and transferred to co-cultured fibroblasts. The method is a step towards enabling large scale analysis of pigment transfer.

Xenopus laevis melanophores can be cultivated in 96-well culture plates which allow quantification of aggregation or dispersion in a fast and reproductive way. Glyphosate containing herbicides, i.e. Roundup, are commonly used in the world, but some toxic effects have been found on amphibians *in vivo* and human and mouse cells *in vitro*. To learn more about potential effects on intracellular transport and the cytoskeleton in animal Roundup, glyphosate, glyphosateisopropylamine and isopropylamine were tested on the transport of melanosomes to the cell centre by spectrophotmetry and by fluorescence microscopy on microtubules and actin filaments. All tested compounds inhibited the aggregation and affected the morphology of the cytoskeleton. The effect was found to be pH dependent.

Amphibian melanophores can be regulated directly by light via a melanopsin receptor. Photoreception was found in cultured early embryos of the zebrafish *Danio rerio.* Light was found to induce dispersion of the melanophores. In adults light causes aggregation of the melanosomes due to signals from the CNS. At least one subclass of melanopsin was detected in the zebrafish retinal pigment epithelial cells.

Key words: colour change, melanophore, melanosome, intracellular transport, pigment transfer, photoreception, toxicity assay, Roundup, glyphosate