Development of a novel biosensor to detect glucocorticoid signaling and stress response in vivo in zebrafish larvae

Stress response refers to complex adaptive physiological responses of an animal to environmental stimuli that threaten homeostasis. In vertebrates, an appropriate stress response involving primarily the hypothalamo-pituitary-adrenocortical (HPA) axis, is crucial for survival. Being the pleiotropic final effectors of the HPA-axis and having numerous targets that influence a wide range of processes, glucocorticoids are widely considered as an important readout of the HPA-axis activity and stress response. The available cortisol assays, like radioimmuno assay and enzyme linked immunosorbent assay (ELISA) are invasive and requires high sample numbers. Taking advantage of the transparency of zebrafish larvae, reporter based fluorescent biosensors will be useful to overcome these limitations. Therefore, this thesis aims at developing a non-invasive cortisol assay, using an in vivo biosensor to detect glucocorticoid signalling and stress response at the single larval level in zebrafish. Making use of the modularity of the glucocorticoid receptor (GR), a GAL4/UAS reporter system was developed, which upon binding cortisol drives green fluorescent protein (GFP) expression. A preliminary assessment of the available transgenic founder lines identified a sensitive transgenic biosensor line that produced significant increase in GFP expression upon exposure to different concentrations of the GR analogue Dexamethasone, whereas a decrease in GFP expression was observed with the GR antagonist Mifepristone. Though the biosensor had negligible cross-reactivity with estradiol, it was found that progesterone resulted in increase in GFP expression. Interestingly, the biosensor was responsive to osmotic stress, with dramatic increase in ubiquitous GFP expression when exposed to NaCl. In conclusion, these results suggest that the reporter system developed in this study is indeed a promising in vivo glucocorticoid biosensor that will be a valuable tool for glucocorticoid and stress research.