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Urocortins in the zebrafish brain and redox regulation of embryonic development through glutaredoxins

AKADEMISK AVHANDLING

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ABSTRACT

The cellular redox state is a central regulator of embryonic development. Redox homeostasis and thiol redox signaling are modulated through glutaredoxins (Grxs), glutathione dependent thiol-disulfide oxidoreductases. Grxs can reduce protein disulfides and protein-glutathione mixed disulfides (de-glutathionylation) via distinct reaction mechanisms. Although it has been shown that Grxs protect cells against oxidative stress induced apoptosis, known interaction partners are rare and physiological functions of this protein family are poorly understood.

This thesis represents the first investigation of dithiol Grxs during vertebrate embryonic development, and we demonstrate that vertebrate specific Grx2 is essential for the formation of a functional brain and cardiovascular system.

We characterized a redox circuit, in which Grx2 modulates the activity of a newly identified interaction partner, collapsin response mediator protein 2 (CRMP2), through the reduction of an intra-molecular disulfide. Thereby, Grx2 regulates axonal outgrowth and neuronal survival. Since CRMP2 and Grx2 have already been implicated in various neurological disorders, the redox circuit based on Grx2 might be a promising target for future therapeutic strategies.

Additionally, we unraveled that development of functional vessels, heart, and erythrocytes in the zebrafish embryo is dependent on the de-glutathionylation activity of Grx2. These results have high clinical relevance, as defects in the cardiovascular system are a major reason for human embryonic mortality.

Moreover, we sought to characterize zebrafish Grx2 (zfGrx2) biochemically and biophysically. Mössbauer spectroscopy as well as size-exclusion chromatography demonstrated the coordination of one $[2Fe2S]^{2+}$ cluster per zfGrx2 monomer. Further analysis indicated that two out of four additional cysteines, which are conserved across the infraclass of bony fish, are involved in cluster coordination. As this mode of cluster binding is different to all yet described [FeS] Grxs, zfGrx2 might represent a new class of iron-sulfur Grxs.

Embryonic development is also regulated by environmental factors, and the stress axis is responsible to mediate the body's response to those stimuli. Here we investigated the expression pattern of urocortin (UCN) genes, important regulators of the stress axis, in the embryonic zebrafish brain. The specific expression sites indicate that UCNs might modulate locomotor activity through noradrenergic and serotonergic systems.

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