CELLULAR MECHANISMS DRIVING MORPHOLOGICAL CHANGES DURING ANHYDROBIOSIS AND CRYOBIOSIS IN WATER BEARS (TARDIGRADA)

Water bears, or tardigrades, are microscopic invertebrates that belong to the phylum Tardigrada, currently comprising about 1400 species. Morphologically, tardigrades have a bilaterally symmetrical body with four pairs of legs, usually terminating in claws. Their average body length ranges from 40 μm up to 1,200 μm. Tardigrades inhabit almost all earth ecosystems, from polar regions to the tropics and from the highest mountains to ocean depths. They live in mosses, lichens, algae, soil, plants, leaf litter, and water sediments.

They have come to the fore mainly because of their ability to survive extreme environmental conditions such as high-intensity γ-radiation, space vacuum, both high and low temperatures, osmotic stress, high concentrations of various chemicals, and desiccation. Tardigrades survive these conditions through morphological, physiological, and molecular transformations and almost complete halt of metabolism. Their resulting state, popularly seen as between life and death, is called cryptobiosis. Tardigrades are among only a few animals phyla that can enter cryptobiosis at all stages of their life cycle. There are five types of cryptobiosis: anhydrobiosis (caused by dehydration), chemobiosis (caused by exposure to a toxicant), cryobiosis (caused by freezing), osmobiosis (caused by high osmotic pressure), and anoxybiosis (caused by a lack of oxygen). In osmobiosis, chemobiosis, and anhydrobiosis, the tardigrades actively contract to a specific smaller and compact morphology form called "the tun." In cryobiosis, they contract only partially or not at all. When external conditions return to the optimum, tardigrades resume activity. Remarkably, this can occur after decades in cryptobiosis. How tardigrades regulate the morphological and physiological transformations at the molecular level during cryptobiosis and then during revival, i.e., "waking up" from cryptobiosis, is still largely unknown.

The morphological changes of eukaryotic cells are mediated by the cytoskeleton, a dynamic network of protein filaments including microtubules (MTs), actin microfilaments, and intermediate filaments (IFs), with IFs being absent in invertebrates. The cytoskeleton drives cell division and movement, directs cell shape, protects the cell from mechanical forces, and influences intracellular transport and signalling. The organization and the role of the cytoskeleton during tardigrade cryptobiosis and revival are currently completely unknown.

Recently, more and more mechanisms crucial for successful cryptobiosis at the molecular level have been emerging. One of them is synthesis of protective molecules, e.g., proteins. Many terrestrial tardigrades synthetize proteins that withstand very high temperatures (without irreversibly denaturing like proteins in a fried egg), have very high affinity to water, i.e., they are hydrophilic, and are highly unstructured, which means that they can flexibly change their shape. Thus, this protein family is called Tardigrade intrinsically Disordered Proteins (TDP). TDPs can reversibly polymerize into filamentous networks and form gels, both in a test tube and in cells, under conditions that typically induce cryptobiosis. After return of the conditions back to normal, TDP filaments depolymerize and the gels dissolve. We would like to investigate how TDP filaments influence the classical cytoskeleton (actin filaments and microtubules) and *vice versa*, which system is the main driving force of changes in cellular morphology during entry into cryptobiosis and then revival and how they might work together or possibly antagonistically. However, first, we must learn how the cytoskeleton looks like during cryptobiosis and revival which is currently unknown.

Functional mitochondria, as the biggest cellular energy factories, defence units against dangerous reactive oxygen species, and major regulators of programmed cell death, are also indispensable for successful cryptobiosis. Interestingly, tubulin heterodimers, the basic building blocks of MTs found in the cytoplasm, regulate mitochondrial function. Namely, soluble tubulin can dock at and block voltage-dependent anion channel (VDAC). The VDAC is located in the mitochondrial outer membrane (MOM) and is central for regulating the transport of water-soluble compounds and ions across MOM, making it the master regulator of MOM permeability and thus mitochondrial functions. Moreover, MTs can assemble from similar yet different tubulin proteins, so called isoforms. It was shown that specific tubulin isoforms have different effects on VDAC function. We hypothesize that the composition (different isoforms) and the state of MT network (soluble/polymerized ratio) might contribute to successful cryptobiosis through VDAC regulation of mitochondrial functions.

Our teams bring interdisciplinary expertise ranging from invertebrate zoology to molecular and cell biology. We will employ a broad and smart combination of approaches to systematically study the tardigrade cytoskeleton and its role in cryptobiosis. Moreover, we will uniquely compare anhydrobiosis and cryobiosis, which exhibit different morphological transformations. By studying two tardigrade species that differ in their cryptobiotic abilities, we will be able to compare both intraspecies differences between anhydrobiotic and cryobiotic pathways and interspecies differences between strong and weak cryptobionts simultaneously.