Mitochondria are important cell organelles and their dysfunction is involved in many human diseases including cancer and neurodegenerative disorders. Mitochondria act as a cellular electricity producing energy in the form of azdenosine 5′-triphosphate (ATP), that is necessary for maintaining cellular functions. The process of ATP production, namely, oxidative phosphorylation, is regulated by calcium ions ($Ca^{2+}$) concentration. Moreover, mitochondria serve as a $Ca^{2+}$ sinks that uptake these ions and prevent cellular $Ca^{2+}$ overload. Importantly, $Ca^{2+}$ ions are essential messengers involved in the multiple cellular signaling pathways, but their excessive level is toxic for a cell. Therefore, mitochondrial $Ca^{2+}$ uptake is important for cellular signaling.

The only known protein that is responsible for transport of $Ca^{2+}$ to mitochondria to date is the mitochondrial $Ca^{2+}$ uniporter (MCU), but despite the important and obvious roles of mitochondrial $Ca^{2+}$ handling, Mcu deficiency in zebrafish and other species has no obvious phenotype. This suggests the existence of additional ways of $Ca^{2+}$ transport. Based on compelling preliminary data from our German collaborator Axel Methner, we now hypothesize that TMBIM5 protein, an ubiquitously found in the inner mitochondrial membrane, is an additional mitochondrial $Ca^{2+}$ channel important for the maintenance of mitochondrial function.

In this project we will use zebrafish to clarify the role of Tmbim5 in mitochondrial $Ca^{2+}$ homeostasis and physiology. Zebrafish larvae are the ideal tool for this as they are translucent and allow the visualization and quantification of mitochondrial $Ca^{2+}$ levels *in vivo* using fluorescent calcium probes. To study TMBIM5 function, we already generated TMBIM5-deficient zebrafish. We will characterize the effect of Tmbim*5* deficiency on fish development, morphology and behavior. Advanced microscopy tools and state-of-the-art molecular biology techniques, such as lightsheet microscopy and high-resolution respirometry will be used to study alterations in mitochondrial morphology, function and $Ca^{2+}$ handling. In the final stage of the project we will clarify whether the phenotype of Tmbim5 deficiency is affected by concurrent MCU deficiency.

This project will allow us to understand the function of TMBIM5, a novel candidate for a mitochondrial $Ca^{2+}$ transport. Based on the preliminary data we expect TMBIM5 to function as a $Ca^{2+}$ channel. The differences between wildtype and Tmbim5-deficient zebrafish mitochondrial $Ca^{2+}$ levels analyzed *in vitro* and *in vivo* will clarify this. Our experiments will also identify the conditions under which TMBIM5 is active. This project will also clarify whether it functions independently or cooperatively with MCU, the major mitochondrial $Ca^{2+}$ channel. We expect that changes in $Ca^{2+}$ homeostasis due to the lack of Tmbim5 will affect mitochondrial morphology, oxidative phosphorylation, cell death in the brain, structure of muscle fibers, and fish behavior. Thus, this data will help to understand neuronal and muscular pathologies induced by mitochondrial dysfunction.

This project is of utmost interest because it will answer fundamental questions on how mitochondrial functions are regulated by $Ca^{2+}$ ions. This will provide important insights into mitochondrial dysfunction which plays a major role in human disease.