Abstract

In a healthy cell, the ROS levels are stringently regulated by the action of various enzymatic or non-enzymatic antioxidant systems. Imbalance in the ROS homeostasis generates oxidative stress resulting in damage to cellular macromolecules. Besides, pro-oxidants, glyoxals which are normally generated as an intermediate compound in the glycolytic pathway and other metabolic activity are known to cause oxidative stress in the cell. Elevated oxidative stress is one of the prominent cellular aetiologies associated with premature aging, cardiovascular and retinal disorders, atherosclerosis, and several neurological disorders. Parkinson disease (PD) is one of well-known neurodegenerative diseases whose pathogenicity is correlated to mitochondrial dysfunction due to elevated oxidative stress in the neuronal cells. Several proteins which are associated with development of familial form of PD, DJ-1, a member of ThiJ/DJ-1/PfpI super family, is known to act as an oxidative sensor in humans. Interestingly, heat shock protein (Hsp)31 from *S. cerevisiae* which belongs to DJ-1 family was shown to provide a similar oxidative stress resistance in yeast. However, the mechanistic aspects how these family members functions as an oxidative stress sensor are not clearly defined. The main focus of my investigation is to understand the involvement of these DJ-1 proteins in regulation of redox homeostasis and mitochondrial health, which are major hallmarks in the pathogenesis of PD.

My major findings demonstrate the importance of Hsp31 family proteins in protecting cells against oxidative stress, which is induced by methylglyoxal (MG). The deletion of Hsp31 leads to a compromised growth phenotype in yeast upon MG induced stress. Moreover, Hsp31 exhibited robust GSH-independent glyoxalase activity both *in vivo* and *in vitro*. Besides, the glyoxalase activity is critical for glyoxal detoxification as well as suppression of ROS levels in cells. On the other hand, in agreement with the observed growth phenotypes, Hsp34 protein possesses a very mild glyoxalase activity as compared to Hsp31. Furthermore, active site mutational analysis reveals that methylglyoxalase activity of Hsp31 protein is critical for providing protection against oxidative stress in yeast. Importantly, endogenous expression of human DJ-1 could complement the growth of yeast under oxidative and glyoxal stress conditions signifying its functional conservation across species. Mechanistically, my findings highlight that Hsp31 regulates cellular GSH and NADPH homeostasis thereby protecting cells against oxidative stress. In addition, cellular localization experiment reveals that though Hsp31 is a cytosolic protein, it predominantly localizes into mitochondria under oxidative stress conditions and protects the organelle from severe oxidative damages.

Lastly, my findings uncover the role of Hsp31 paralogs in the maintenance of mitochondrial health integrity and other stress related pathways. To test their role in the mitochondrial health, I have analysed several parameters such as mass, dynamics and functionality. Interestingly, though the single deletions of these paralogs do not have significant effects over the mitochondrial phenotypes, the deletion of DJ-1 homologs in combination of *hsp31* and *hsp34* in yeast led to enhanced total as well as functional mitochondrial mass in cells. To address how mitochondrial mass enhancement occurs in the cells, the organelle turnover (mitophagy) was assessed. The microscopic and western analysis indicates, there was no alteration in mitophagy among the Δ*hsp31*Δ*hsp34* compared to WT. On the contrary, an enhancement in the basal levels of ROS stimulated increased biogenesis of mitochondria in Δ*hsp31*Δ*hsp34* cells was observed. Strikingly, Δ*hsp31*Δ*hsp34* cells also exhibit upregulation of mitochondrial fusion proteins resulting hyperfusion of mitochondria. Additionally, our results demonstrates that Δ*hsp31*Δ*hsp34* cells exhibited a long-term G2/M cell cycle arrest, which was rescued upon overexpression of mitochondrial fission protein, Dnm1. Lastly, absence of these paralogs in yeast, resulted in induction of apoptotic-like features in the cells and decreased lifespan in *Saccharomyces cerevisiae*. Altogether, my studies highlight the importance of DJ-1 class of proteins in maintaining the cellular redox status, mitochondrial integrity and cellular health in yeast.

In conclusion, overall my studies highlight that Hsp31 is a robust methylglyoxalase and regulates cellular NADPH and GSH pool thereby helps in the maintenance of redox homeostasis. Hsp31 predominantly translocate into mitochondria upon oxidative stress to protect the organelle from oxidative damages. Furthermore, my findings provide the first evidence over the involvement of DJ-1 family proteins in the regulation of mitochondrial health and dynamics, cell-cycle arres and reduced lifespan in yeast.