Abstract

Within the last decade, a vivid and diverse field of research on posttranslational control of mitochondrial function has developed. In particular, reversible lysine acetylation of mitochondrial proteins and its possible involvement in metabolic control has been studied in detail (Hofer and Wenz, 2014). Mitochondrial acetylation levels are regulated by SIRT3, a NAD^+-dependent protein deacetylase localized to the mitochondrial matrix (Lombard et al., 2007). SIRT3 plays an important role in integrating metabolism with the current state of nutrient supply, through controlling a global metabolic reprogramming process (Anderson and Hirschey, 2012; Choudhary et al., 2014; Hebert et al., 2013). Although, a vast number of target proteins, including several components of the respiratory chain, have been identified by large scale mass spectrometric approaches, functional studies demonstrating their physiological relevance are still lacking behind (Hebert et al., 2013; Hofer and Wenz, 2014; Rardin et al., 2013b; Weinert et al., 2015).

In this study, our main goal was to obtain a better understanding of how mitochondrial acetylation is involved in the metabolic adaptation of the OXPHOS (oxidative phosphorylation) system towards changes in nutrient supply. Therefore, we have analyzed the role of SIRT3 in metabolic stress adaptation of mitochondrial translation, combining *SIRT3* knockdown & overexpression with metabolic intervention strategies in HEK293T cells. We could clearly demonstrate that regulation of mitochondrial translation is independent of SIRT3 in these cell lines, even in response to metabolic stress conditions. Hence, this study provides profound evidence that SIRT3 dependent regulation of mitochondrial translation is not a general mechanism occurring in all cell types, tissues and species. Furthermore, we assessed the involvement of SIRT3 in starvation signaling as a potential retrograde signaling response, communicating the mitochondrial energy status towards the nucleus. Despite its role as nutrient sensor and metabolic switch, SIRT3 did not influence this signaling network consisting of the cell survival kinases Akt, AMPK and mTOR in our experimental setting. Furthermore, we have assessed the potential involvement of mitochondrial acetylation in OXPHOS remodeling upon changes in nutrient supply. Using an *in vitro* acetylation assay and blue native polyacrylamide gel electrophoresis (BN-PAGE) analysis, we could show that a sudden and uncontrolled increase in mitochondrial acetylation disrupts the structural and functional organization of the respiratory chain. However, this finding was not reflected *in vivo*, where mitochondrial acetylation levels were manipulated in a tissue specific way via genetic and metabolic intervention strategies. Further studies are needed to dissect, whether the expected OXPHOS remodeling process was prevented *in vivo* via additional secondary responses such as an altered composition of the inner mitochondrial membrane or due to the presence of a defined cristae structure.

In the last section of this work, we aimed at deciphering the role of GCN5L1 in mitochondrial acetylation *in vivo*. Therefore, novel mouse models of *GCN5L1* deficiency have been generated. Through a double knockout strategy of the *GCN5L1* and the *SIRT3* gene, encoding for the mitochondrial deacetylase, we could rule out opposing functions of these two proteins. Also in conditional knockout mice with restricted deficiency to either the skeletal muscle or forebrain neurons, we could clearly demonstrate that GCN5L1 is not an essential component of the mitochondrial acetyltransferase program as it was proposed by others. Instead, GCN5L1 has a robust effect on autophagy. Skeletal muscle specific knockout mice showed a normal life and health span, neuron specific loss of GCN5L1 in the forebrain however, caused sudden death only a few weeks after the *cre recombinase* was activated. This sudden death was likely caused by increased neuronal cell death. Hence, we could demonstrate for the first time that loss of GCN5L1 is more detrimental in the central nervous system than in skeletal muscle. Taken together, this study is not only the first report of a viable vertebrate model with *GCN5L1* deficiency but also points to a new tissue specific function of GCN5L1. Therefore, this work largely contributes to a better understanding of the *in vivo* role of GCN5L1 in vertebrates.