Mitochondrial diseases are very heterogeneous in their genetic cause and clinical presentation and the underlying pathomechanisms are poorly understood. The mitochondrial elongation factor G1 (EFG1), the gene product of *Gfm1,* and the mitochondrial translation optimization factor 1 (MTO1) are both crucial for mitochondrial translation and hence deficiencies are associated with severe mitochondrial dysfunction causing very diverse clinical manifestations with early onset and often fatal course.

The function of EFG1 was extensively studied in bacteria and yeast. However, the precise role has not yet been confirmed in mammals and pathological consequences of EFG1 deficiency are still to be elucidated. Therefore we used a novel primary patient fibroblast line and a neuron-specific *Gfm1* knockout mouse model to follow the spatial and temporal dynamics of pathological changes. The present study shows that the deficiency of EFG1 leads to a severe defect of mitochondrial *de novo* protein synthesis, resulting in a multiple OXPHOS deficiency *in vitro* and *in vivo*. Moreover, the neuron-specific *Gfm1* knockout seems to induce a neuroinflammatory response in the forebrain. However, secondary stress response pathways such as mitochondrial protein quality control and oxidative stress response are not activated in response to EFG1 deficiency.

MTO1 is an evolutionary conserved protein proposed to be required for the τm^5U modification at the wobble position of certain mitochondrial tRNAs, but the exact function *in vivo* remained elusive. Here we investigated the role of MTO1 in τm^5U modification in a mouse model with MTO1 deficiency that does not develop a severe disease phenotype, making it especially suited for mechanistic studies. The presented experiments demonstrate that the stability of mitochondrial tRNAs and their 2-thio modification are not affected by the loss of MTO1. Furthermore, RNA mass spectrometric analysis revealed that mammalian MTO1 is indispensable for τm^5U modification *in vitro* and *in vivo.* In conclusion, these results confirm the essential function of the τm^5U modification establishing the missing link between *MTO1* mutations and mitochondrial translation defect observed in patients.