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 $\text{Tm}^5\text{U}$ 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 $\text{Tm}^5\text{U}$ 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 $\text{Tm}^5\text{U}$ modification in vitro and in vivo. In conclusion, these results confirm the essential function of the $\text{Tm}^5\text{U}$ modification establishing the missing link between MTO1 mutations and mitochondrial translation defect observed in patients.