SUMMARY

The mitochondrion is an essential cytoplasmic organelle that provides most of the energy necessary for a eukaryotic cell. Mitochondrial structure and functions are maintained by proteins of both mitochondrial and nuclear origin. The entire mitochondrial population is in constant flux, driven by continual fusion and division of mitochondria. Defects in mitochondrial dynamics can cause deficits in mitochondrial respiration, morphology and motility leading to apoptosis under extreme conditions. Mutations in OPA1, a nuclear encoded mitochondrial protein, involved in the mitochondrial fusion mechanism cause autosomal dominant optic atrophy. OPA1 is expressed as eight mRNA splice variants in human cells generated by alternative splicing of exons 4, 4b and 5b (Delettre et al., 2001).

This study was undertaken to characterize the mouse OPA1 GTPase. Four mRNA splice variants 1, 5, 7 and 8, were found in mouse tissues generated by alternative splicing of exons 4b and 5b. In contrast to an earlier report, alternative splicing of exon 4 was not confirmed. While the overall level of OPA1 gene transcription seems to be constant throughout tissues, the individual expression level of the four splice variants in the mouse brain differs when compared to other tissues, indicating the existence of a post-transcriptional regulatory mechanism. Particularly, splice form 1 was predominantly expressed in the mouse brain. To study the expression of OPA1 protein isoforms, monoclonal antibodies were generated that identified six protein isoforms on western blots. Mass spectrometry and N-terminal microsequencing revealed the two longest forms to be OPA1 isoforms 1 and 7 which are cleaved by mitochondrial processing peptidase (MPP) during protein import into mitochondria. Furthermore, post-MPP processing by other unknown proteases in regions corresponding to exons 5, 5b and 6 generated three different short forms of OPA1. Only the long forms were tightly embedded into the mitochondrial inner membrane, whereas the short forms were extracted easily from the membranes presumably due to the loss of the transmembrane domain after post-MPP processing. The expression levels of OPA1 protein isoforms varied in a tissue dependent manner, though all the tissues contained identical set of isoforms. OPA1 isoform 1 was predominant in mouse nervous tissues. Gel filtration experiments showed that the longest of the three short forms distinctly formed a dimer of 184-kDa while all other OPA1 protein isoforms were part of a 285-kDa complex. The two coiled-coil domains present in the OPA1 protein isoforms showed a high affinity to self-associate (but not to hetero-associate) that likely mediated the complex formation. Yeast Two-Hybrid screens, co-immunoprecipitation and size exclusion chromatography experiments failed to identify proteins interacting with OPA1.