1 Abstract

The great majority of the mitochondrial proteome is nuclear encoded, thus proteins have to be imported from the cytosol into mitochondria. Most of the proteins of the inner mitochondrial membrane space are imported by the disulfide relay system. The oxidoreductase MIA40 has long been known to be crucial for import and folding of mitochondrial proteins via this import pathway. Recently, studies described AIFM1 as import factor of MIA40.

In the present study, an expanded functionality of AIFM1 could be shown, describing AIFM1 as the novel core component of the disulfide relay system. MIA40 forms a persistent functional complex with AIFM1. Functional separation of AIFM1 and MIA40 in human AIFM1*KO* cells permitted the functional analysis of the AIFM1-MIA40 complex. In this system, MIA40 becomes imported into mitochondria and assumes a redox-active state in absence of AIFM1. However, the levels of MIA40 substrates of the respiratory chain complex I are reduced and assembly of complex I is hampered. Complex I assembly stalls at the formation of early complex I intermediates. The integration of the MIA40 substrates NDUFA8, NDUFB7 and NDUFB10 into complex I intermediates is not affected, whereas conversely the integration of NDUFS5 is affected. NDUFS5 exhibits a slowed oxidation, reflecting an impaired mitochondrial import, while being subject to proteasomal degradation in the cytosol. This points to NDUFS5 as a key player in complex I assembly, whereby mitochondrial import and folding of NDUFS5 is critically controlled by the AIFM1-MIA40 complex. Although the assembly of complex I is hindered under these conditions, a minor fraction of fully assembled complex I exists. The impaired assembly of complex I results in reduced total respiratory activity of complex I. The activity of the further complexes of the respirasome is not affected. Supporting the findings of previous studies, it was also shown in this study that simple overexpression of MIA40 leads to rescued MIA40-substrate levels. Therefore, this study characterizes AIFM1 as the novel core component of the disulfide relay system. The AIFM1-MIA40 complex is necessary to increase the efficiency of MIA40 as oxidoreductase and facilitates proper integration of NDUFS5 into complex I.

Graphical abstract