1 Abstract

The disulfide relay is the major import pathway into the intermembrane space of mitochondria, with the key component being MIA40. Here, Adenylate Kinase 2 (AK2) could be identified as novel and unique substrate of this machinery. AK2 converts ATP and AMP in the intermembrane space of mitochondria to ADP, thus supplying substrate for oxidative phosphorylation.

AK2 could be shown to rely on MIA40-mediated oxidation for import, but it engages in a second round of internal disulfide isomerization to reach its mature redox state. This process is reminiscent of corrective mechanisms for non-native disulfides in other oxidative folding pathways, something which was previously not reported for the disulfide relay. Therefore, the import mechanism of AK2 emphasizes the increasingly diverse substrate spectrum of MIA40 and adds further complexity to the system by including first evidence of substrate isomerization in the mitochondrial disulfide relay. Additionally, it could be shown that AK2 is especially adapted to the hot environment of mitochondria.

Furthermore, the competition of mitochondrial import and cytosolic folding enables regulated dual targeting of AK2 to the cytosol. Cytosolic presence of AK2 is additionally tempered by proteolytic processing by the peptidase DPP9, adding an additional layer of control. In this way, the dual localization can be finetuned by the balance between these processes. This mechanism might be key to facilitate the signalling function of AK2 during proliferation and apoptosis. While loss of AK2 is devastating for immune cells, no negative impact of cytosolic AK2 was observed on immune cell viability and differentiation.

Finally, AK2 was shown to exhibit a very distinct enzymatic profile when compared with its cytosolic counterpart AK1. The high affinity to AMP as substrate and substantial substrate inhibition place AK2 as very specialized member of the AK family. It achieves catalysis with very low substrate concentrations, thus allowing steady supply of ADP for ATP synthesis with only low AMP concentrations. The abrupt substrate inhibition profile also allows AK2 to be active only in a narrow band of substrate concentrations, thus integrating an intrinisic shutoff mechanism into the enzyme which additionally might facilitate metabolic signaling in cells.

The regulatory mechanisms governing localization and activity of AK2 are novel findings that provide further insight into the physiological role of this essential enzyme.