The mitochondrial NME6, a member of the nucleoside diphosphate kinase family interacts with RCC1L (WBSCR16), a protein involved in coordination of the mitochondrial ribosome assembly.

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NME6 is a member of the nucleoside diphosphate kinase (NDPK/NME/Nm23) family, a group of proteins catalyzing the transfer of gamma phosphate from NTPs to NDPs. The ping-pong reaction is dependent on its hexamer assembly in eukaryotes, and involves the synthesis of high energy intermediate via the phosphorylation of a specific histidine residue within the catalytic site. The family is divided in two groups: Group I (NME1-NME4) members are highly homologous among themselves and exhibit NDPK activity; Group II (NME5-NME9) members display less homology and seem to lack NDPK activity. Extensive research has been conducted on Group I members after the discovery of NME1's role in metastasis suppression, while Group II remained largely unexplored. Although little is known about Group II members, these evolutionary old genes are presumed to participate in one or more basic cellular processes, therefore, our studies focused on the human NME6 protein. Building on our previous findings on expression of endogenous NME6 isoforms and its subcellular localization in mitochondria, we aimed to resolve the precise sub-mitochondrial localization, understand the enzymatic properties, determine quaternary structure and to reveal interacting partners. Fractionation of mitochondria by the swelling/shrinking procedure was analyzed by western blot and showed a NME6 distribution pattern highly similar to proteins facing the matrix. Western blot analysis revealed a lack of NME6 histidine phosphorylation, an indispensable prerequisite for enzymatic activity. Immunoprecipitation experiments showed that NME6 does not interact either with Group I NME members, or with itself which would, also, be mandatory for its enzymatic activity. Proximity ligation assay followed by immunoprecipitation showed that NME6 physically interacts with RCC1L, a protein involved in coordination of the mitochondrial ribosome assembly. Together, these results provide precious clues for understanding the NME6 function.