

Identification of Interacting Partners of Mrp20

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Mitochondria are present in all eukaryotic cells and responsible for the majority of energy production through the oxidative phosphorylation (OXPHOS) pathway. A number of key proteins of the OXPHOS enzymes are encoded by the mitochondrial DNA, are synthesized on mitochondrial ribosomes and become inserted into the inner membrane via the Oxa1 machinery. This insertion process is co-translational and involves the physical interaction between Oxa1 and the ribosome, in particular the large ribosomal subunit protein Mrp20. Mrp20 is a homolog of bacterial ribosomal protein L23, located near polypeptide exit tunnel and contains a C-terminal extension sequence termed a “mitospecific region”. This C-terminus contains a highly conserved α -helix sequence, proposed to be an important protein-protein binding motif. It is hypothesized that the mitospecific region interacts with an inner membrane protein to ensure correct assembly and positioning of the mitochondrial ribosome at the membrane. In this study the functional significance of the mitospecific region is explored through two studies: a genetic suppressor screen and a biochemical analysis. To examine how the mitospecific region of Mrp20 relates to function a truncation of the C-terminus was generated (Mrp20 Δ C), which is respiratory deficient. Through a suppressor screen a number of possible suppressors were identified which can overcome the growth phenotype of the Mrp20 Δ C mutant and are currently undergoing further characterization. In order to prepare for a biochemical study using an affinity purification approach, a histidine tag was attached to the N-terminal sequence of Mrp20 (HisMrp20) as well as the mitospecific region sequence (M.S.R. His Mrp20). These proteins were transformed into competent yeast cells and protein expression was analyzed through SDS-PAGE, Western blotting and parallel immunodecoration with Mrp20 and histidine specific antibodies. A preliminary characterization of the histidine-tagged mrp20 derivatives was performed.