IPD Project Details

Project ID: IPD1344

Project Title: QUATERNARY AND QUINARY ORGANIZATION OF RESPIRATORY COMPLEX SUBUNITS TO ADAPT PROTEOSTASIS-STRESS

Description: Phase separation and reversible aggregation of proteins is a well-recognized adaptive strategy to survive stress. Here, we show that RCC subunits are engaged into improved super-quaternary organizations inside mitochondria during proteostasis stress. Assembly and oligomeric organizations of Complex II and V are consolidated while Complex I, III and IV are increasingly incorporated into respiratory supercomplexes in multiple cell-lines with different proteostasis and metabolic demands. Further, our results suggest that improved supra-organization of respiratory complexes (iSRC) is an outcome of conformational optimization towards better enzyme activity and co-terminus to appearance of aggregates of RCC subunits in stressed cells. Simultaneous reversion of iSRC and disappearance of the aggregates during stress-withdrawal indicates complementarity between these quaternary and quinary proteome-reorganization mechanisms. iSRC appears to be the pre-emptive and deterministic ensemble over stochastic aggregation as it offers direct fitness-benefit.

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Sample Preparation: The total, soluble and insoluble mitochondrial fractions were separated on NuPAGE 4-12% Bis-Tris Protein Gels (Invitrogen). The gel was run, fixed and stained with Coomassie brilliant blue and cut into 6 slices each. The digitonin-solubilsed mitochondrial fractions were separated on NativePAGE 3-12% Bis-Tris Protein Gels (Invitrogen)and cut into 20 slices for complexome profiling experiments.

Peptide Separation: Preparation of gel slices, reduction, alkylation, and in-gel protein digestion was carried out as described by Shevchenko et al.. Finally, peptides were desalted and enriched according to Rappsilber et al.

Protein Characterization: For peptide identification, raw MS data files of individual slices were loaded and analysed as separate experiments for complexome profiling using MaxQuant (Ver. 1.3.0.5) and searched against Swissprot database of Mus

musculus (release 2018.10 with 25208 entries) or Homo sapiens (release 2019.03 with 42419 entries) and a database of known contaminants. MaxQuant used a decoy version of the specified database to adjust the false discovery rates for proteins and peptides below 1%. The search parameters included constant modification of cysteine by carbamidomethylation, enzyme specificity trypsin, multiplicity set to 3 with Lys4 and Arg6 as medium label and Lys8 and Arg10 as heavy label. Other parameters included minimum peptide for identification 1, minimum ratio count 1, re-quantify option selected and match between runs with 2 min time window. iBAQ option was selected to compute abundance of the proteins. Bioinformatics and statistical analysis was performed in Perseus environment (Ver. 1.5.2.4).

Experiment Type: Shotgun proteomics

Species: Homo sapiens - 9606, Mus musculus-10090

Tissue:

Cell Type: Permanent cell line cell

Disease: Unknown

Instrument Details: Q Exactive (MS:1001911)

Protein Modifications: No PTMs

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