IPD Project Details

Project ID: IPD8972

Project Title: Distinct Stress Response to Mitochondrial Inter-membrane Space and Matrix Proteotoxic Stress are Preferentially Modulated by TOM complex and Vms1, respectively

Description: Double-membrane-bound architecture of mitochondria is essential for its ATP synthesis function; simultaneously such structure sub-divides the organelle into inter-membrane space (IMS) and matrix. IMS and matrix are inherently different in protein folding milieu due to their contrasting oxido-reductive environments and distinctly different protein quality control (PQC) machineries. By inducing proteotoxic stress limited to IMS or matrix using varied stressor proteins, we decipher distinct cellular response to IMS and matrix stress. IMS stress leads to specific upregulation of IMS-resident chaperones and TOM complex components. In contrast, matrix stress leads to specific upregulation of matrix- chaperones and cytosolic PQC components. We report that cells respond to mitochondrial stress by an adaptive mechanism by adjourning mitochondrial respiration while upregulating glycolysis as a compensatory pathway. By systematic genetic interaction, we show that TOM complex components act as specific modulators of IMS-stress response while Vms1 preferentially modulates the matrix stress response

Principal Investigator: Dr Swasti Raychaudhuri

PI Affiliation: CSIR-Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad - 500007, India

Sample Preparation: Yeast strains (Wt, IMS-PMD and MM-PMD) were grown overnight in YPR. Next morning, secondary culture was inoculated at OD600 of 0.1 and grown till OD600 of 0.5. Each culture was divided in two culture tubes and in one half of each strain was induced with 1% galactose for 12 hours. The other half was grown in YPR as uninduced controls. After induction, equal number of cells were taken from uninduced and induced culture of each strain, cells were harvested by centrifugation and were re-suspended in resuspension buffer (50mM Tris-HCl, pH 7.5, 150 mM NaCl, 1mM EDTA, 5mM MgCl2, 1% NP-40, Protease inhibitor cocktail). The re-suspended cells were subjected to lysis by using glass beads in bead beater. Each cycle of bead beating of 3 minutes were followed by incubation in ice for 5 minutes and 3-4 cycles of bead beating were done. After cell lysis, the whole cell lysate was centrifuged at 18,000Xg for 15 minutes and the supernatant were collected in fresh tubes. All lysates were quantified by

BCA protein estimation kit (Thermo Scientific) and the concentrations were made equal for all samples (~10mg/ml). The whole cell lysates were boiled at 95°C for 10 minutes with 4X SDS loading buffer [0.2 M Tris–HCl (pH 6.8), 8% SDS, 0.05 M EDTA, 4% 2-mercaptoethanol, 40% glycerol, 0.8% bromophenol blue] and heating at 95? for 10 min and were centrifuged shortly to remove the debris. 150 µg of protein extract from each sample were ran on NuPAGE 4%–12% Bis–Tris Protein Gels (Invitrogen) using MES running buffer (100 mM MES, 100 mM Tris–HCl, 2 mM EDTA, 7 mM SDS) at 200 V for 40 min and fixed and stained with Coomassie brilliant blue.

Peptide Separation: Reduction, alkylation and In-gel trypsin digestion was done as described in Shevchenko et al 54. Trypsin digested peptides were eluted, desalted and vacuum dried as described in Rappsilber et al 55, and stored in -20? until Mass spectrometry analysis. Dried peptides were dissolved in 2% formic acid and sonicated in bath sonicator for 5 min. Each sample were loaded in reverse phase liquid chromatography followed by mass spectrometry analysis. Peptides were analysed on Q Exactive (Thermo Scientific) mass spectrometer interfaced with nano- flow LC system (Easy nLC II, Thermo Scientific). EasySpray Nano Column PepMapTM RSLC C18 (Thermo Fisher) (75 ?m× 15 cm; 3 ?m; 100Å) using 60 min gradient of mobile phase [5% Can containing 0.1% formic acid (buffer A) and 90% ACN (acetonitrile) containing 0.1% formic acid (buffer B)] at flow rate 300nL/min was used for separation of peptide. Full scan of MS spectra (from/z 400 to 1650) were acquired followed by MS/MS scans of top 10peptide with charge state 2 of higher.

Protein Characterization: Raw files obtained were analysed in MaxQuant Computational platform (Ver. 1.6.10.43) using UniProt database of Saccharomyces cerevisiae (released Nov, 2019) 56. LFQ option was selected for label free quantification with minimum 2 unique peptides for ratio count along with oxidation (M), acetylation (Protein N-term) as variable and carbomethylation as fixed modification. Additional parameters: 2 trypsin missed cleavages, 20 ppm peptide mass tolerance and 1% peptide false discovery rate (FDR) were allowed. Further data analysis and statistical tests were performed in Perseus (Ver. 1.6.0.2) 57. To compare the control and stressed samples, ratio of IMS-PMD by control and MM-PMD by control is calculated using average LFQ intensities of two biological repeat experiments. The ratio is converted into Log2 space and mean and standard deviation is calculated for both conditions. Z-score normalization was done for both conditions using the formula where X is single protein and “a” to “n” is dataset of proteins. Z-score cut-off ±1.96 indicates population lies outside the 95 % interval hence considered significant. The z-score cut-off (1? Z) and (-1? Z) of proteins is considered as enriched and depleted respectively. Heatmaps were prepared using Morphius (https://software.broadinstitute.org/morpheus).

Experiment Type: Top-down

Species: Saccharomyces cerevisiae-4932

Tissue: Unknown

Cell Type: Unknown

Disease: Unknown

Instrument Details: LTQ Orbitrap (MS:1000449)

Protein Modifications: No PTMs

PubMed ID: 35500842