IPD Project Details

Project ID: IPD6103

Project Title: Interactome identification of Histones H3 and H4 in the presence and absence of methyl methanesulfonate (MMS) in the pathogenic yeast Candida glabrata

Description: The main goal of this project is to identify proteins in Candida glabrata that interact with histones H3 (CgHht) and H4 (CgHhf) under normal and MMS-treatment conditions.

Principal Investigator: Dr. Rupinder Kaur

PI Affiliation: Laboratory of Fungal Pathogenesis, Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad, India

Sample Preparation: Cghht1?2?/CgHHT-SFB and Cghhf1?2?/CgHHF-SFB strains, used for the interactome analysis, were grown in CAA medium till logarithmic-phase, and further incubated for 3 h in the CAA medium lacking or containing 0.06% MMS. Cells were harvested and cell lysates were prepared using glass bead lysis method. Cell lysates were subjected to tandem affinity purification with streptavadin-agarose and S-protein-agarose beads. Cell lysates (5 mg protein) were incubated with streptavidin beads for 2 h, followed by elution with the biotin (2 mg/ml)-containing buffer. The resulting supernatant was incubated with S-protein agarose beads for 2 h at 4°C, followed by 3 washes with lysis buffer. Next, beads were boiled in 2X SDS-gel loading buffer, and protein samples were resolved on a 10% SDS-PAGE gel till bromophenol blue dye in the sample buffer entered about 3 mm into the gel. After staining with Coomassie Brilliant Blue, protein bands were excised and sent to the Taplin Biological Mass Spectrometry Facility, Harvard Medical School Spectrometry Facility, Harvard Medical School, Boston, USA (https://taplin.med.harvard.edu) for protein identification.

Peptide Separation: Samples were prepared and sent from two independent biological replicates. At the Taplin facility, gel pieces were subjected to in-gel trypsin digestion followed by microcapillary LC-MS/MS (Liquid chromatography-tandem mass spectrometry) using the LTQ Orbitrap Velos Pro ion-trap mass spectrometer. Sample with search name 69465 refers to Cghhf1?2?/SFB-vector_CAA grown, 69466 refers to Cghhf1?2?/CgHHF-SFB_CAA grown_Replicate-1, 69467 refers to Cghhf1?2?/CgHHF-SFB_MMS (0.06%) grown_Replicate-1, 69468 refers to Cghhf1?2?/CgHHF-SFB_CAA grown_Replicate-2, 69469 refers to Cghhf1?2?/CgHHF-SFB_MMS (0.06%)

grown_Replicate-2, 69470 refers to Cghht1?2?/SFB-vector_CAA grown, 69471 refers to Cghht1?2?/CgHHT-SFB_CAA grown_Replicate-1, 69472 refers to Cghht1?2?/CgHHT-SFB_MMS (0.06%) grown_Replicate-1, 69473 refers to Cghht1?2?/CgHHT-SFB_CAA grown_Replicate-2 and 69474 refers to Cghht1?2?/CgHHT-SFB_MMS (0.06%) grown_Replicate-2.

Protein Characterization: All generated fragmentation patterns were acquired and searched against the UniProt C. glabrata reference proteome database using the SEQUEST software to determine the peptide sequences. 1% false discovery rate was set as a cut-off to filter the identified peptides.

Experiment Type: Shotgun proteomics

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Data in cell_details No Data

Disease: Unknown No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: dehydrated residue

PubMed ID: 32134928