IPD Project Details

Project ID: IPD3061

Project Title: Phosphatidylinositol 3-phosphate (PI3P) interactome analysis under varied iron conditions in Candida glabrata.

Description: The project is aimed at identifying proteins that interact with PI3P in low-iron, regular-iron and high-iron grown Candida glabrata cells.

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Sample Preparation: C. glabrata wild-type cells were grown in iron-limited-CAA containing BPS (50 µM) medium at 30ºC for 12 h. For low-iron, high-iron, and regular-iron conditions, cells were again cultured in CAA-BPS (50 µM) medium, CAA medium containing sodium ascorbate (1 mM) and ferrous ammonium sulphate (500 µM; freshly prepared), and CAA medium, for 2h, respectively. Cells were harvested and proteins were extracted using the glass bead lysis method. For PI3P pull-down assay, 100 ?l of PBS-washed PI3P-agarose beads (Echelon #P-B003a; contain 10 nanomoles of bound PI3P per ml of beads) or control agarose beads were incubated with 10 mg cell lysates for 6 h at 4ºC. After washing beads with PBS, beads were boiled in 2X Laemmli sample buffer and the supernatant containing PI3P-bound proteins was run on the 10% SDS-PAGE for a short duration, till the dye front entered the resolving gel. The gel was stained with coomassie brilliant blue and protein bands were cut. Two biological replicate samples for each conditions were prepared, and sent to the Taplin Biological Mass Spectrometry Facility, Harvard Medical School, Boston, USA (https://taplin.med.harvard.edu) for protein identification using LC-MS/MS (liquid chromatography-mass spectrometry) analysis.

Peptide Separation: 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. Samples with the search name 69743 refers to C. glabrata wild-type sample grown in CAA medium and incubated with agarose beads; replicate 1 and 69727 refers to C. glabrata wild-type sample grown in CAA medium and incubated with agarose beads; replicate 2. Samples 69728-68733 were incubated with PI3P beads. 69728 refers to C. glabrata wild-type sample grown in CAA medium; replicate 1, 69729 refers to C. glabrata wild-type grown in CAA medium; replicate 2, 69730 refers to C. glabrata wild-type grown in CAA medium containing BPS; replicate 1, 69731 refers to C. glabrata wild-type grown in CAA medium containing BPS; replicate 2, 69732 refers to C. glabrata wild-type grown in CAA medium containing sodium ascorbate and ferrous ammonium sulphate; replicate 1, 69733 refers to C. glabrata wild-type grown in CAA medium containing sodium ascorbate and ferrous ammonium sulphate; 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.

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: LTQ Orbitrap Velos Pro (MS:1003096) Data in instrument_details

Protein Modifications: dehydrated residue

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