Project:
Investigation of putative HTT interacting proteins

Experiment:
Analysis of HTT BioID datasets

Date completed:
2019/04/09

Rationale:
BioID technology employs a promiscuous biotin ligase (BirA) fused to the terminus of the target protein, huntingtin, allowing proximal proteins to be biotinylated and then subsequently identified through mass spectrometry experiments. This technique has not been applied to assess huntingtin interactors to date in the published literature, so will provide a novel methodology to characterize the huntingtin interactome. As huntingtin is a large protein molecule and the precise location of the N and C-termini remain unresolved due to their flexible nature, both N and C terminally BirA-tagged constructs for full-length huntingtin will be generated for overexpression as well as a truncated construct spanning amino acids 80-3100, the region of the protein resolved in the recent cryo-electron microscopy structure which omits the flexible termini. Huntingtin fusion proteins will be overexpressed in cells subjected to different ROS stresses as well as control conditions. Resultant cell lysates will be analysed through collaboration with Prof. Anne-Claude Gingras (Lunenfeld Tanenbaum Research Institute, University of Toronto). From this work, we hope to obtain a list of putative huntingtin interactors which will be compared to previously published findings and assessed for stable complex formation with huntingtin.

Experimental approach:

1. Summary of work completed by Geoff Hesketh (Postdoctoral Fellow in the Gingras lab):

Construct Name	SGC Construct ID	Vector	Amino Acid Start	Amino Acid End	HTT protein
N-flag-BirA-HTT_1-3140	HTT:TOC011-C02	V9595	1	3140	Full-length
N-flag-BirA-HTT_93-3100	HTT:TOC011-C03	V9595	93	3100	Trimmed termini
N-flag-BirA-HTT_93-1715	HTT:TOC011-C04	V9595	93	1715	N-HEAT + exon1
N-flag-BirA-HTT_1-1715	HTT:TOC011-C05	V9595	1	1715	N-HEAT - exon1
N-flag-BirA-HTT_2094-3140	HTT:TOC011-C06	V9595	2094	3140	C-HEAT
N-flag-BirA-HTT_1731-2064	HTT:TOC011-C07	V9595	1731	2064	Bridge
HTT_1-3140-C-flag-BirA	HTT:TOC011-C08	V9596	1	3140	Full-length
HTT_93-3100-C-flag-BirA	HTT:TOC011-C09	V9596	93	3100	Trimmed termini
HTT_93-1715-C-flag-BirA	HTT:TOC011-C10	V9596	93	1715	N-HEAT + exon1
HTT_1-1715-C-flag-BirA	HTT:TOC011-C11	V9596	1	1715	N-HEAT - exon1
HTT_2094-3140-C-flag-BirA	HTT:TOC011-C12	V9596	2094	3140	C-HEAT
HTT_1731-2064-C-flag-BirA	HTT:TOC011-D01	V9596	1731	2064	Bridge

BioID HTT clones generated. NB: HTT amino acid numbering is according to a Q19 template sequence. Vector details can be found here: https://gingraslab.lunenfeld.ca/resources.php?cateName=Reagents listed as pDEST-pcDNA5-BirA-FLAG N-term (V9595) and pDEST-pcDNA5-BirA-FLAG C-term (V9596).

The HTT BioID clones (https://zenodo.org/record/1239045) have been used to generate stable Flp-In T-Rex HEK293 cell lines where HTT-BirA fusion protein expression may be selectively switched on by

supplementing the growth media with tetracycline. HTT fusion proteins overexpression was induced in all cell lines and the cells were harvested and pellets stored at -80 °C prior to further processing. Duplicates of each HTT-bait cell line were grown, induced for expression and harvested. 3 replicates of 4 different negative control cell lines were also grown. A total of 36 cell pellets were harvested.

Cell pellets were subjected to cell lysis and biotinylated proteins were purified by streptavidin–agarose affinity purification. Proteins were digested on-bead with sequencing-grade trypsin in 50 mM ammonium bicarbonate (pH 8.5). Peptides were then acidified by the addition of formic acid (2% (v/v) final concentration) and dried by vacuum centrifugation. Dried peptides were suspended in 5% (v/v) formic acid and analyzed on a TripleTOF 5600 mass spectrometer (SCIEX) in-line with a nanoflow electrospray ion source and nano-HPLC system. Raw data were searched and analyzed within ProHits LIMS and peptides matched to genes to determine prey spectral counts. High-confidence proximity interactions (BFDR ≤ 0.01) were determined through SAINT analysis implemented within ProHits. Bait samples (biological duplicates) were compared against 12 independent negative control samples (6 BirA-FLAG only and 6 triple-FLAG only expressing cell lines).

2. Analysis of the data:

The data were parsed by the following criteria – see associated spreadsheet for further details:

i) Data sorted by fold change and exclude anything less than 2
ii) Exclude hits which are only seen with 1 bait or not seen in the replicate experiment
iii) Exclude hits seen with the BRIDGE domain constructs which showed no expression
iv) Exclude hits with crapome score > 50/411
v) Highlight hits with 100+ fold change or log odds score 5+
vi) Exclude proteins as per Geoff's advice – common hits in BioID experiments which are likely non-specific to HTT or related to protein folding issues

Prey gene	Entry	Protein names	Gene names	Length
ARFIP1	P53367	Arfaptin-1 (ADP-ribosylation factor-interacting protein 1)	ARFIP1	373
APC	P25054	Adenomatous polyposis coli protein (Protein APC) (Deleted in polyposis 2.5)	APC DP2.5	2843
UBAP1	Q9NZ09	Ubiquitin-associated protein 1 (UBAP-1) (Nasopharyngeal carcinoma-associated gene 20 protein)	UBAP1 NAG20	502
LNPEP	Q9UIQ6	Leucyl-cystinyl aminopeptidase (Cystinyl aminopeptidase) (EC 3.4.11.3) (Insulin-regulated membrane aminopeptidase) (Insulin-responsive aminopeptidase) (IRAP) (Oxytocinase) (OTase) (Placental leucine aminopeptidase) (P-LAP) [Cleaved into: Leucyl-cystinyl aminopeptidase, pregnancy serum form]	LNPEP OTASE	1025
SH3GLB1	Q9Y371	Endophilin-B1 (Bax-interacting factor 1) (Bif-1) (SH3 domain-containing GRB2-like protein B1)	SH3GLB1 KIAA0491 CGI-61	365
NEBL	O76041	Nebulette (Actin-binding Z-disk protein)	NEBL LNEBL	1014
PASK	Q96RG2	PAS domain-containing serine/threonine-protein kinase (PAS-kinase) (PASKIN) (hPASK) (EC 2.7.11.1)	PASK KIAA0135	1323

Top BioID hits as determined by the data parsing protocol

Next steps:

I will compare these hits to my previously determined list of HTT interacting proteins and also search the global SGC database for available expression clones for these targets.