Joint Department of BIOMEDICAL ENGINEERING

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Engineering of proteins for analysis of protein-protein interaction in a cell system

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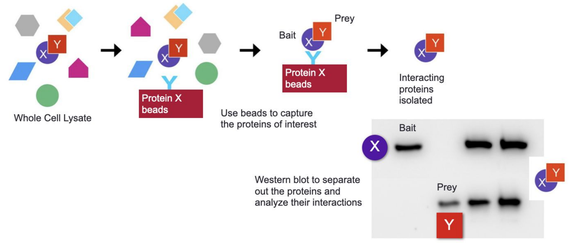
INTRODUCTION

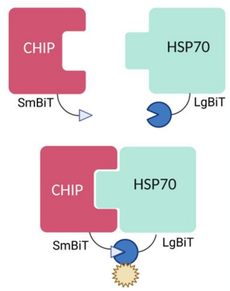
- Protein quality control (PQC) is important in homeostasis of a healthy cell especially in response to stress. One type of stress is metabolic stress, such as in an infected cancer cell (1).
- Important components of the PQC system are Heat shock protein 70 (HSP70), Carboxyl-terminus of HSP70 interacting protein (CHIP) and Hsp organizing protein (HOP).
- HSP70 can bind with many co-chaperones, the main ones being CHIP or HOP, to ensure PQC.
- The preference for CHIP versus HOP is influenced by the phosphorylation status of HSP70 (2). In an un-phosphorylated form, HSP70 preferentially binds to CHIP. In phosphorylated form, it preferentially binds to HOP.
- A mutation at glycine 132 in CHIP has been shown *in silico* to accommodate this phosphorylation of HSP70 and shift the preference away from HOP and back to CHIP.
- Using a new technology called NanoBiT in conjunction with co-IPs, I hypothesize that phosphorylated HSP70 will preferentially bind with G132N CHIP at an equivalent level to HOP while the non-phosphorylated form of HSP70 preferentially binds to wild-type (WT) CHIP.

METHODS

Co-Immunoprecipitation (Co-IP): Co-IP measures interaction of proteins via capturing the protein of interest out of cellular lysate with antibody tagged beads A western blot then separates out the proteins, which allows for the visualization and quantification of these proteins, such as CHIP, HOP and HSP70.



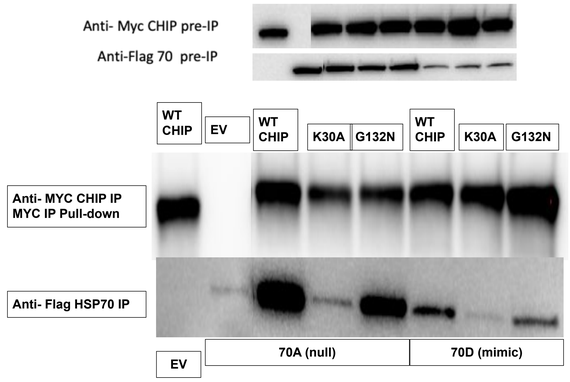
NanoBit Assay: A NanoBit assay involves attaching a larger truncated version of a protein (LgBit) to one of the proteins of interest, and a smaller 11 amino acid sequence (SmBit) to the other protein of interest (3).



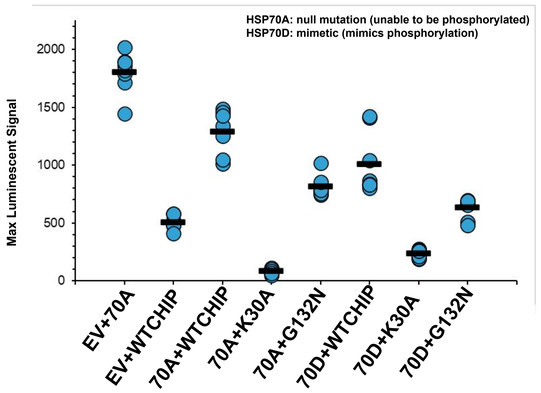
Engineering HA Tag: For engineering the HA tag to HOP, I used a mutagenesis system with primers directed to the N-terminus or C-terminus side of HOP. By using the primers and PCR, I annealed the HA tag to the end of HOP. To confirm proper reannealing, I ran a PCR gel and determine the product size. I then transform DH5-alpha bacteria, midi prepped to generate plasmid and confirmed via sequencing the HA tag was properly added.

RESULTS

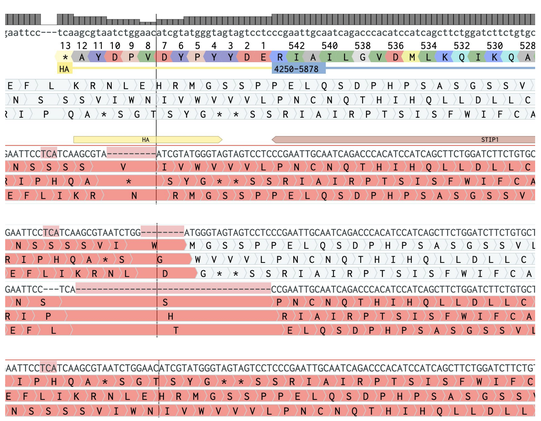
Co-IP confirmed the interaction between HSP70D and G132N CHIP



NanoBiT further confirmed the interaction between HSP70D and G132N CHIP



Addition of hemagglutinin (HA) tag to HOP was unsuccessful



CONCLUSIONS

-NanoBiT is an effective way of measuring protein interaction and is comparable to co-IP to understand protein interactions.
-G132N does not rescue the decrease in interaction caused by the phosphomimetic (HSP70D), as seen in both the co-IP and NanoBiT results.
-HSP70D has a lower expression, potentially influencing the results of the co-IP.

FUTURE DIRECTIONS

-Add HOP into NanoBiT system by inserting it into both Lg BiT and Sm BiT vectors, then asses the orientation by measuring the interaction with HSP70.
-Explore another cell system to measure interaction of proteins
-Repeat the HA tag addition using a gradient of melting temperatures for the primers to increasing insertion opportunity.

REFERENCES

[1] Jolly C., Morimoto R.I. Role of the heat shock response and molecular chaperones in oncogenesis and cell death. *J Natl Cancer Inst* 92(19), 1564-1572 (2000).

[2] Muller, P., *et al.* C-terminal phosphorylation of Hsp70 and Hsp90 regulates alternate binding to co-chaperones CHIP and HOP to determine cellular protein folding/degradation balances. *Oncogene* 32(25), 3101–3110 (2013).

[3] Dixon A.S., *et al*. NanoLuc Complementation Reporter Optimized for Accurate Measurement of Protein Interactions in Cells. *ACS Chem Biol*. 11(2), 400-408 (2016).