Joint Department of BIOMEDICAL ENGINEERING

NC STATE UNIVERSITY

INTRODUCTION

CHAPEL HILL

Î

- 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.

Engineering of proteins for analysis of protein-protein interaction in a cell system **Colleen McCann, Mariah Stewart, Jonathan Schisler**

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.

stern blot to separat



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.



NanoBiT further confirmed the interaction between HSP70D and G132N CHIP



Addition of hemagglutinin (HA) tag to HOP was unsuccessful

<pre>saattcctcaagcgtaatctggaacatcgtatgggtagtagtcctcccgaattgcaatcagacccacatccatc</pre>															gc													
	13 1	12 11	10	9	8	7	6	5	4	3	2	1		542		540		538		536		534		532		530	5	28
	*	AY	D	P	V	D	Y	P	Y	Y	D	E	R	I	A	Ι	L	G	V	D	M	L	K	Q	Ι	K	Q	A
	HA				Ì								42	50-5	878													
E>F>L	K	$\langle R \rangle$	N	$ \rangle$	E	$ H\rangle $	r >i	۱) ($\left \right\rangle$	s	s >	P	P	E	$L \rangle$	Q >	s >	D	P >	H	P〉	S >	A >	s >	G	s > s	\rangle V	\rangle
N S	\rangle s \rangle	s >v	\rangle I	\rangle W \rangle	N)I	$\langle \mathbf{v} \rangle$	$\langle W \rangle$	$\langle v \rangle$	V	\rangle V	\rangle L	$\rangle \mathbf{P}$	\rangle N	\rangle C	\rangle N	} Q ∣	$\rangle T$	H	ight angleI	\rangle H	Q	L	\rangle L	D	\rangle L \rangle	$L \rangle$	С
₹ \I \ P	Q	$\langle A \rangle$	× S	G	\rangle T	\rangle S	\rangle Y	G	*	>*	S	\rangle S	$\left \right\rangle$ F	s) I) A	$ \rangle$ I	R	:	\rangle T	\rangle S	\rangle I	\rangle S	\rangle F	\rangle W	\rangle I	\rangle F	C	\rangle A
					HA								\subset												ST	IP1		
GAATTCC TCA	TCAA	GCGT	۹			ATC	GTA ⁻	TGGO	GTA	GTA	GTC	СТС	0000	GAAT	TGC	AAT	CAG	ACC	CAC	ATC	CAT	CAG	СТТ	CTG	GAT	сттс	TGT	GC
$\rangle N \rangle S \rangle S$	\rangle S \rangle	s 〉	١	/		ÌI	$\langle \mathbf{v} \rangle$	$\langle W \rangle$	$\langle V \rangle$	V	\rangle V	\rangle L	$\rangle P$	\rangle N	C	\rangle N) Q ∣	angleT	H	ight angleI	angle H	Q	L	\rangle L	D	angleL $ angle$	$ L\rangle$	С
$\langle A I \rangle$	I)Q ∣	\rangle A \rangle		*		\rangle S) Y	G	> *	>*	\rangle S	\rangle S	$\left\langle \right\rangle$ F	${\tt I} ig > {\tt I}$) A	$ \rangle$ I) R	:	\rangle T	\rangle S	\rangle I	\rangle S) F	\rangle W	\rangle I	F	C	$ angle$ A \langle
E>F>L>	I K	$\rangle R \rangle$		Ν		\rangle	R)	M) ($\left \right\rangle$	s >	s >	P	P	E	$L \rangle$	Q >	s >	D	P >	${\tt H}$	P	S >	A	S >	G	s > s	\rangle V	\rangle
					!																							
							_																					
GAATTCCTC	ATCAA	GCGT	AATC	TGG			A	TGGC	STAC	GTA	GTC	СТС	CCG	GAAT	TGC	AAT	CAG	ACCO	CAC	ATCO	CAT	CAGC	TTC	CTGG	ATC	TTCT	GTG	IC1
$\langle N \rangle S \rangle S$	>S>	S V) I		W			M) (3 > 5	$S \rangle$	S >	P >	P	E	$L \rangle$	\mathbf{Q}	S ⟩I	D	> ⟩ ŀ	-l ⟩ F		S > A		S G	i ∕S	S	\rangle V	> L
R \ I \ P \ I	H)Q	$\langle A \rangle$	*) S	; >		G		W	V	V	V	}L_	} P	∕N	C	N)	Q)T)	H)	I)	H)	Q	L)	L	D	L	.) C	
<pre> >E >F >L > </pre>	⟩ı ⟩k	(\rangle N \rangle	L		D		G	*	> *	S ≤ 2	\rangle S	;	s > I) A	\rangle I	\rangle R		\rangle T	⟩ S	\rangle I	S) F	\rangle W	ight angleI	\rangle F \rangle	C	Α
GAATTCC	-TCA-												CCG	GAAT	TGC	AAT	CAG	ACCO	CAC	ATC	CAT	CAGC	TTC	CTGG	ATC	TTCT	GTC	C1
\rangle N \rangle S	\rangle					S							$\rangle P$	\rangle N	C	N)	Q)τ)	H)	I	H	Q >	L	$ L\rangle$	D	L>L	_) c	$: \rangle$
$R \setminus I \setminus P$	\rangle					Н							\rangle R	I (S) A	\rangle I	\rangle R) P	\rangle T	S	\rangle I	S	\rangle F	W	ight angleI	angleF $ angle$	C	Α
$\langle E \rangle F \rangle L \rangle$							Т						\rangle	E L Q S D P						H P S A S G S S						S	\rangle V	angleL
					1																							
	_																											
AATTCCTCA	TCAA	GCGT	AATC	CTGG	AAC	ATC	CGTA	TGG	GTA	AGT	AGT	CCI		CGAA	TTO	SCA/	ATC/	AGAC	CCC	ACA	тсс	ATC	AGC	TTC	TGG	ATCI	ТСТ	G
$ \rangle$ I \rangle P \rangle H	I ∕Q	\rangle A \rangle	*) 5	; ⟩G	i ⟩ T		5 > Y	/)0	k 🗸 i	* >	* >	s >	S	$ R\rangle$	$ \mathbf{I}\rangle$	$ A\rangle$	I	$\langle R \rangle$	P	T	S	angleI	S	\rangle F	W	angle I $ angle$	F	С
E $ angle$ F $ angle$ L $ angle$	I K	R	$\langle N \rangle$	L	E	$ H\rangle$	$ R\rangle$	M	G	S	S	P) P	E	\rangle L	Q	S	D) P	H	P	S) A	S	\rangle_{G}	S	\rangle S	\rangle
N S S	SC)	SV) T	W	N	T	V	W	V	V	V	\rightarrow			$1 \rangle c$	•) •		$\mathbf{n} > \mathbf{r}$		H)	тΣ	H)	$\left(\right)$				\rightarrow	N

CONCLUSIONS

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

-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

[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).