ERK2-Peptide Interaction on Yeast Cell Surface

Karl Barber, Jasdeep Mann and Sheldon J. Park

University at Buffalo Department of Chemical and Biological Engineering

ABSTRACT

Protein-peptide interactions are common in nature and play important roles in signaling. Therefore, modulating these interactions is a potentially powerful way of controlling cellular events. Here, we look specifically at the ERK2 protein-pepHePTP peptide interaction on the yeast cell surface. Displaying this complex on yeast cells eliminates the need to perform further purification techniques. However, the fast dissociation kinetics of the system make it difficult to detect the formation of the complex on the yeast surface. To address this shortcoming, we introduced cysteine mutations into ERK2 (T116C) and pepHePTP (V31C) to form an intermolecular disulfide bond. Both the protein and peptide were then labeled with fluorescent markers and analyzed by flow cytometry. We observe that both ERK2 and pepHePTP were successfully labeled, which indicates that the disulfide bond significantly helps with the detection efficiency. This technique can be used to screen therapeutic peptides and proteins to disrupt various signaling pathways.

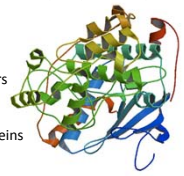
BACKGROUND

Yeast Surface Display and Disulfide Trapping

- Yeast cells are capable of expressing proteins that require post-translation modifications, such as **disulfide linkages**
- For protein-peptide pairings, it is often difficult to distinguish between an unstable interaction and no interaction
- Introducing an engineered disulfide crosslinking between subunits expressed in yeast cells can help identify weakly interacting molecules
- Display on yeast cell surfaces removes the need to purify the complex

ERK2 Protein

- Mitogen-activated protein kinase I
- Phosphorylates nuclear transcription factors
- Inactivation by dephosphorylation of pThr and/or pTyr
- Activity regulated by interactions with proteins and peptides at docking motifs



ERK2 Interactions with pepHePTP D-peptide

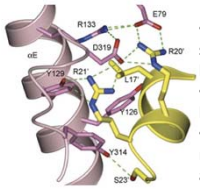


Image credit: Zhou et al.

- Docking interactions along CD site (network of hydrogen bonding and charted interactions)
- Van der Waals interactions along hydrophobic docking groove
- Disulfide bridge via site-directed mutagenesis between ERK2 T116C and pepHePTP V31C

Disulfide trapped ERK2/ D-peptide complex

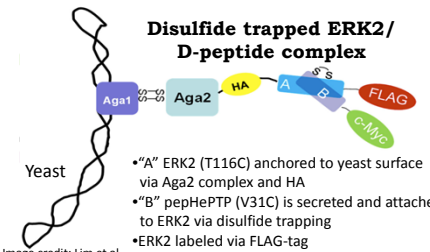
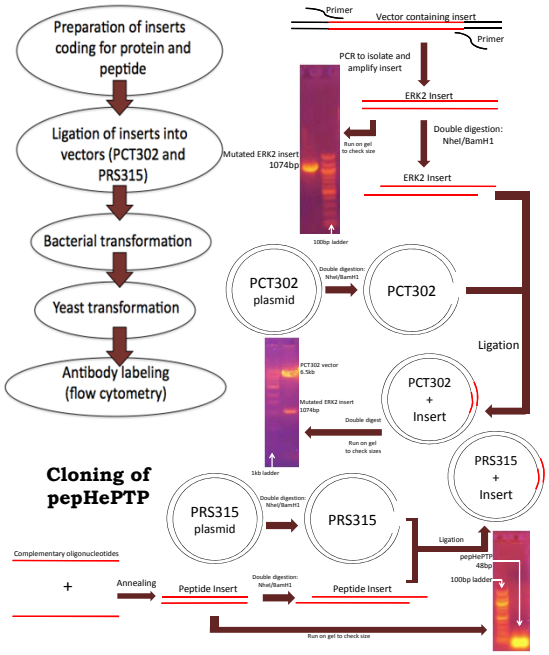


Image credit: Lim et al.

- "A" ERK2 (T116C) anchored to yeast surface via Aga2 complex and HA
- "B" pepHePTP (V31C) is secreted and attaches to ERK2 via disulfide trapping
- ERK2 labeled via FLAG-tag
- D-peptide labeled via c-Myc tag

OVERALL APPROACH



PCT302	PRS315
Proteins/peptides expressed on yeast cell surface via Aga2 and HA	Proteins/peptides secreted by yeast cells
Synthesizes Trp	Synthesizes Leu
FLAG-tag	c-Myc tag

Screening Conditions

Sample #	PCT-ERK2 (T116C)	PRS-pep (V31C)	PRS-pep (wt)	PCT-pep (wt)
1	✔	✔		
2	✔		✔	
3		✔		
4			✔	
5				✔

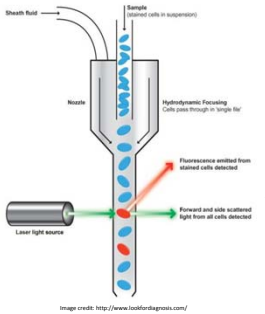
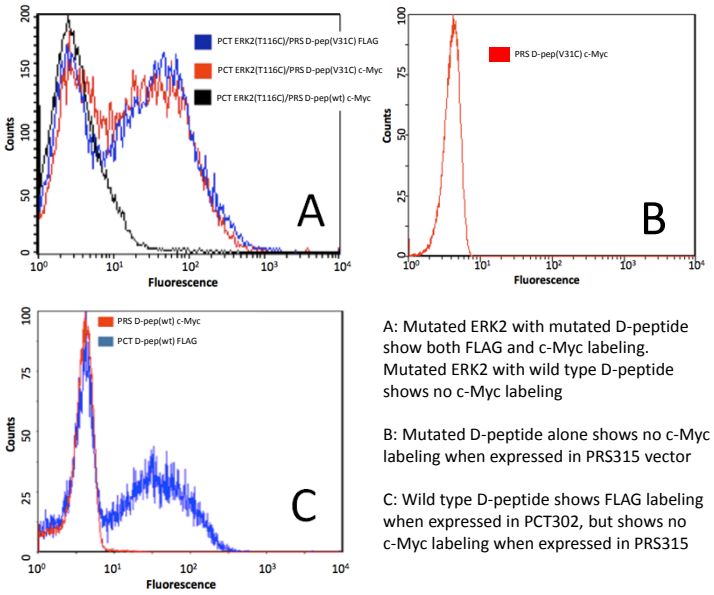


Image credit: http://www.lookfordiagnosis.com/

Flow Cytometry

- Yeast cells labeled with antibodies containing FITC (fluorescent marker)
- Cells passed through in single file
- Light at 494nm passed through pathway
- If cells are successfully labeled with FITC, they emit light at 521nm (fluorescence)
- Flow cytometer measures number of cells that emit light at this wavelength

RESULTS



A: Mutated ERK2 with mutated D-peptide show both FLAG and c-Myc labeling. Mutated ERK2 with wild type D-peptide shows no c-Myc labeling

B: Mutated D-peptide alone shows no c-Myc labeling when expressed in PRS315 vector

C: Wild type D-peptide shows FLAG labeling when expressed in PCT302, but shows no c-Myc labeling when expressed in PRS315

Yeast Surface Expression

Sample	ERK2 expression	D-peptide expression
PCT-ERK2(T116C)/PRS-D-pep(V31C)	+	+
PCT-ERK2(T116C)/PRS-D-pep(wt)	+	-
PRS-D-pep(V31C)	-	-
PRS-D-pep(wt)	-	-
PCT-D-pep(wt)	-	+

CONCLUSIONS

- Mutated ERK2 is expressed on the yeast cell surface
- **Mutated D-peptide binds to mutated ERK2 by engineered disulfide bond**
- Without engineered disulfide bond, the wild type D-peptide does not remain bound to the mutated ERK2 for labeling
- Mutated D-peptide does not show nonspecific binding to yeast cell surface
- Experiment should be repeated with wild type ERK2 with wild type D-peptide as a control

IMPLICATIONS AND FUTURE RESEARCH

- Specific drugs could target the ERK2 docking site and compete with other proteins and peptides *in vivo*
- Screening can be performed to find specific antibodies that are successful in this task

REFERENCES

- Lim, Kok Hong, Sri Ranganayaki Madabhushi, Jasdeep Mann, Sriram Neelamegham and Sheldon Park. "Disulfide Trapping of Protein Complexes on the Yeast Surface." *Biotechnology and Bioengineering (Wiley Periodicals, Inc.)* 106.1 (2010): 27-41. Print.

- Zhou, Tianjun, Liguang Sun, John Humphreys, and Elizabeth J. Goldsmith. "Docking Interactions Induce Exposure of Activation Loop in the MAP Kinase ERK2." *Structure* 14.6 (2006): 1011-019. Print.

ACKNOWLEDGEMENTS

- Dr. Jeffrey Errington
- The Center for Undergraduate Research and Creative Activities