ENGINEERING A LIGHT ACTIVATED CASPASE-3 FOR CELL BIOLOGY RESEARCH AND CANCER TREATMENT

Tricia Jensen
Mentor: Dr. Mark Gomelsky, Min-Hyung Ryu
EPSCoR 2011
Molecular Biology
University of Wyoming
EPSCoR Summer Fellowship Research Project

- **Goals**
  - Create an infrared light-activated caspase-3
  - Optimize it

- **Significance**
  - Study development and diseases in model organisms
  - Treatment of diseases through gene therapy
Optogenetics = use of genetically encoded photoactivated proteins to regulate biological processes *in vivo*

- Few, if any side effects
- Spatial precision (single cells)
- Temporal control
- Reversibility

light *versus* drugs
Photoreceptor modules for optogenetics

Bacteriophytochrome

cis→trans

BLUF
Opsin
Phytochrome
Crytochrome
LOV

Absorbance

0.7
0.45
0.2

P_r (dark)

P_{fr}

Wavelength (nm)

260
410
560
710

712 nm
Why bacteriophytochrome optogenetics?

(i) Near-IR light penetrates animal tissues much deeper than visible light
(ii) Near-IR light harmless
(iii) The chromophore is biliverdin, the first product of natural heme breakdown
(iv) Phytochromes can be instantly turned “off” (i.e. photoinactivated)
(v) Fluorescent phytochromes have been used for whole-body imaging in mice

Kimberly et al. 2005, Lasers in surgery and medicine 36:171-185

Skin
Loose connective tissue
Dense connective tissue
Muscle
Vertebral column and spinal cord
Why Caspase-3?

**Figure 1.** Apoptosis activation sequence. Signals cause a cascade of caspases. Caspase-3 is the final effector caspase that causes the cell to execute apoptosis. Figure by Faris Q Alenzi.
Caspase-3

Inactive WT

Active WT

Uncleavable
Constitutively Inactive

Constitutively Inactive

Mutant Procaspase-3
Activated by
Homodimerization
Creating a Light-Activated Caspase-3

**Figure 2.** Bph photoreceptor is fused to the caspase-3 mutant. Light causes a conformational change activating the caspase. Figure by Tricia Jensen
Construct

pET-23

BphG → C-3
Screening For Caspase-3 Activation

**Figure 3.** In the dark caspase-3 is inactive and the cells live. In the light caspase-3 is active and the cells die. Figure by Tricia Jensen
Caspase-3 (D3A, V266E) kills *E. coli*

- 0.01 mM IPTG
- 0.02 mM IPTG
- 0.05 mM IPTG
Screening System

- We inserted Caspase-3 DEVD recognition site into the Lac Z.
- Lac Z degrades X Gal medium resulting in a blue color.
- In theory the active caspase-3 should cleave the Lac Z protein resulting in white colony color and cells with inactive caspase-3 should be blue.
E. coli naturally contain the lac operon.

We constructed the Lac Z deleted mutant in BL21(DE3).

Therefore the Lac Z protein with the caspase-3 recognition site contained in our created plasmid will affect screening.
Caspase-3 test in B-lacZ- stain

0 mM IPTG

pET-CASP3
CS: C163S
D3A: D3A (Procaspase mutant)
D3A, V266E : active procaspase

0.01 mM IPTG
Infrared-light activated caspase-3 construction

<table>
<thead>
<tr>
<th>Variant</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>bphC3-1</td>
<td>AIAAEMAQRTSGISLDNSYKMDYPEMGLCIINNKNFHKSTGMSRTSRTSGTDVDAANLRETF</td>
</tr>
<tr>
<td>bphC3-2</td>
<td>AIAAEMAQRTNSYKMDYPEMGLCIINNKNFHKSTGMSRTSRTSGTDVDAANLRETF</td>
</tr>
<tr>
<td>bphC3-3</td>
<td>AIAAEMAQRTKMDYPEMGLCIINNKNFHKSTGMSRTSRTSGTDVDAANLRETF</td>
</tr>
<tr>
<td>bphC3-4</td>
<td>AIAAEMAQRTMDYPEMGLCIINNKNFHKSTGMSRTSRTSGTDVDAANLRETF</td>
</tr>
<tr>
<td>bphC3-5</td>
<td>AIAAEMAQRTNSYKMDYPEMGLCIINNKNFHKSTGMSRTSRTSGTDVDAANLRETF</td>
</tr>
<tr>
<td>bphC3-6</td>
<td>AIAAEMAQRTYPEMGLCIINNKNFHKSTGMSRTSRTSGTDVDAANLRETF</td>
</tr>
<tr>
<td>bphC3-7</td>
<td>AIAAEMAQRTPEMGLCIINNKNFHKSTGMSRTSRTSGTDVDAANLRETF</td>
</tr>
<tr>
<td>bphC3-8</td>
<td>AIAAEMAQRTEMGLCIINNKNFHKSTGMSRTSRTSGTDVDAANLRETF</td>
</tr>
</tbody>
</table>

Diagram showing the activation of caspase-3 under light and dark conditions.
Future Goals

- Create more fusions
- Random mutagenesis
- Once promising:
  - Sequenced
  -Contributors test in mice
Thank You

Special Thanks to:

EPSCoR
Dr. Gomelsky and
Min-Hyung Ryu