Abstract

Objectives:
- To engineer a novel yeast strain that can detect, attract and eliminate pests.
- To alleviate the environmental problems caused by chemical pesticides.
- To serve as a model system to screen for GPCRs that bind to certain ligands.

Project main parts:

- Odorant sensing
- Attractant production
- Toxin production

Experimental Design

Test of Chimeric Protein Function

To test the functional sensing and coupling to rat Gαolf or yeast endogenous Gpa1, we have made five constructs:

1. pESC-Fusion-FLAG, which is the chimeric protein with the FLAG tag at the C terminus in the expression vector pESC-HIS under the control of the GAL1 promoter.

2. pESC-R7-FLAG, which is the rat R7 receptor protein with the FLAG tag at the C terminus in the expression vector pESC-HIS under the control of the GAL1 promoter.

3. pESC-Gαolf-FLAG, which is rat Gαolf in the pESC-HIS under the regulation of the GAL1 promoter.

4. pRS426-FUS1P-GFP-FUS1T, which is the FUS1 promoter, GFP and FUS1 terminator in pRS426.

5. pESC-HIS empty plasmid, the negative control.

There are two parts of the functional coupling test:

A. Test whether our chimeric protein could be coupled to Gpa1.
B. Test whether our chimeric receptor could couple to Gαolf.

Test of the Reporter System

To functionally express the yeast ARO9 gene (2) and EGFP gene, we have designed a reporter and an attractant production construct in pRS426 yeast expression vector.

- pRS426-ARO9-FUS1T, pESC-His, respectively. After α-factor binding, we would expect to see the cells transformed with pESC-Fusion-GFP to have strong green chimeric protein with the GFP tag at the proteins, we have made two constructs in pESC-HIS.

Construction of Attractant production and Reporter pathway

To functionally express the yeast AR09 gene and EGFP gene, we have designed a reporter and an attractant production construct in pRS426 yeast expression vector.

- pRS426-EGFP, which is EGFP gene without the FUS1 promoter, cloned into the pRS426 yeast expression vector.

Construction of toxin Expression

We have designed several constructs to test the toxicity of BinA and BinB to insects as well as yeast:

- (1)BinA and BinB genes each fused with GST, cloned into pESC-Leu expression vector under the GAL10 promoter, respectively.
- (2)Only BinA gene is fused with GST, cloned into the same multi-cloning site of pESC-Leu expression vector under the GAL10 promoter.
- (3)Only BinB gene is fused with GST, cloned into the same multi-cloning site of pESC-Leu expression vector under the GAL10 promoter.

These constructs will be transformed into the yeast and induced by galactose to test the expression of toxins using western-blot and estimate whether they are resistant to the toxic from the yeast phenotype.

Odorant sensing

Functional expression of heterologous GPCR in yeast

Taking advantage of yeast pheromone sensing pathway, we can functionally express heterologous GPCRs by:

1. changing yeast GPCR to our ODR-10 or R7 receptor;
2. changing yeast Gα to a better coupled Gαolf;
3. knock out FAR1 to stop cell cycle arrest;
4. insert a reporter under the FUS1 promoter.

Test of Chimeric Protein Localization

To test the localization of chimeric G-protein coupled receptor responsive to a volatile odorant is coupled to the yeast mating pathway and yields 2-phenylethanol to lure the pests. Constitutively expressed binary toxin in the yeast would poison the attracted pests after their consumption.

Results

For odorant sensing:
- Chimeric receptor cassette has been successfully constructed in the yeast expression vector pESC-HIS.
- Chimeric receptor can localize onto the yeast membrane.

For attractant production:
- Chimeric receptor functions as a ligand sensing receptor for volatile molecule diacyl.

Future Work

- For odorant sensing:
  - To finish constructing pESC-Goa9 and pSrs426-FUS1P-GFP-FUS1T reporter.
  - To finish the chimeric receptor functional assay Part 2.
  - To optimize the coupling of chimeric receptor.

- For attractant production:
  - To finish constructing pRS426-FUS1P-AR09-FUS1T and pRS426-FUS1P-FUS1T reporter.
  - To finish the attractant production and reporter functional assay.
  - To further characterize the attractant production pathway.

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