Cling *E. coli*: Bacteria on target

A system for targeting bacteria to a specific substrate and effecting a cellular response

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Quorum Sensing

The motivation for this part of the project was to effect downstream activity after E. coli bind to a particular substrate, using the lux/lux bioluminescence system from Vibrio fischeri, which would turn on after the bacteria localize to the target.

Lux quorum-sensing works like a system of senders and receivers. In the sender, LuxCDABE codes for a protein that catalyzes the synthesis of 3-oxo-homoserine lactone (OHHL) which can diffuse freely out of the sender cell and into other cells. In the receiver, LuxR encodes for a non-permeating protein which, when bound to OHHL, upregulates the lux promoter. This occurs at a high enough OHHL concentrations, so a certain concentration of cells (quorum) is reached.

Initial characterizations of the lux/lux system and quorum sensing were done using GFP and RFP reporters, which were taken to quorum sensing. (1) A lux/lux production system in one cell acting as both sender and receiver would be simpler, but it’s possible that the cells might self-induce. (2) lux and luxR production in separate cells would ensure no cross-induction, but it requires monitoring two populations of cells.

Methods

We used a sender construct Lux and RFP under constitutive P promoters, and a receiver construct Lux and constitutive P promoters and GFP under lux promoters. We transformed these constructs into E. coli to create constitutive-RFP constitutiveSender and inducible-GFP constitutiveReceiver.

To characterize one-cell quorum activity, a non-RFP constitutive sender construct and an inducible-GFP constitutive receiver construct were assembled into a single plasmid and transformed into E. coli. An overnight culture was diluted and grown to OD 0.3, re-limited and grown to OD 0.3, etc. and fluorescence was detected after each dilution.

To characterize two cell quorum activity, constitutive-RFP senders were mixed with inducible-GFP receivers. Fluorescence and OD606 readings were taken every 15 minutes during incubation at 37s Celsius.

Results

In the one-cell system, we find that the overnight culture exhibited high GFP fluorescence, but with each successive dilution, the fluorescence decreased to a level comparable with no-GFP cells. When the cultures were allowed to grow past OD 0.3, the fluorescence increased again at OD 0.6.

In the two-cell system, we found that at a specific concentration of sender cells added to a mixed culture, the GFP fluorescence per OD (per cell) in receiver cells increased greatly.

Conclusion and Future Plans

We have conducted both one-cell and two-cell models of quorum-sensing activity. We determined that the one-cell model was not self-inducing and does exhibit a quorum response, making it a better candidate for future quorum-sensing applications. We determined that quorum-sensing activity can also be divided between two cells, one sender and one receiver. We will continue to characterize the one-cell system.

Bacterial Targeting

The motivation for this part of the project was to engineer bacteria to adhere to targets with a high degree of specificity. Initial targeting was done by displaying histidine and strep tags on the E. coli surface via fusion with the proteins LppOMP and AIDA-1, and screening were performed with binding to nickel and streptavidin targets, respectively. After characterization and high enrichment with these known substrates, random libraries were inserted into LppOMP and AIDA-1 constructs for screening peptides with affinity for novel targets. As we proceed with this experiment, we hope to characterize sequences that have specificity for calmodulin and EGFR.

Methods

Bacteria were engineered with histidine and strep tags displayed on the E. coli surface via fusion with LppOMP and AIDA-1 vehicles, LppOMP with C-terminus insertion, LppOMP with a loop 1 insertion, and AIDA-1 with a N-terminus insertion.

In order to test the tags and their ability to bind to specific antibodies and beads (nickel/streptavidin), two cell sorting assays were performed to ascertain the binding strength of the tagged cells against a background of untagged cells.

In Magnetic Activated Cell Sorting (MACS), cultures of white cells expressing histidine (nickel-targeting) or strep (streptavidin-targeting) tags on the surface were mixed with culture of RFP-expressing non-tagged cells. The mixture was incubated with nickel- or streptavidin-coated magnetic beads, then run through a magnetic column, so that non-tagged cells would flow through, and bead-bound tagged cells would stick to the column. After removal of the magnet, the bound fraction of cells was eluted and spread on agar plates. After overnight incubation, the numbers of white and red colonies were counted.

In Fluorescence Activated Cell Sorting (FACS), we added anti-histidin and anti-streptavidin fluorescent antibodies to the mixed cultures. The fluorescent fraction of cells was separated from the mixture with a flow cytometer and spread on agar plates. After overnight incubation, the numbers of white and red colonies were counted.

Results

We were able to construct LppOMP and AIDA-1 constructs with histidine and strep tags. We significantly enriched histidine and strep tagged cells through MACS, as there were many more white colonies (from tagged cells) than red (from non-tagged) on the plates spread with bound fractions. Similar results were found with FACs as well (data not shown).

Conclusions and Future Plans

LppOMP and AIDA-1 have proven to be effective vehicles for expressing tags on the surface of E. coli to bind specific targets, as shown by enrichment of tagged cells through MACS and FACS. We plan to try out new peptides for other targets, such as calmodulin (CaM, a calcium binding protein). We will also explore using a random library to select for novel targeting peptides, by which you introduce fixed-length random nucleotide sequences into the construct, express the random tag, and select for peptides with affinity to your target. This has important medical implications since we may be able to target "microbial factories" to harmful toxins or microbes, or to various areas of the body.

Fec Signal Transduction

The motivation for this project was to create a system of targeting and direct signal transduction/gene expression.

The Fec system was chosen because it is the only well-known characterized signaling system with an outer membrane lipid code for a biotin to extracellular tag system. The Fec system receptor is the outer membrane protein FecA, whose wild-type ligand is citrate in the presence of nickel. When binding occurs in iron-limiting conditions, FecA activates the inner membrane protein FecR, which activates cytoplasmic sigma factor FecJ; and FecJ induces gene expression in collaboration with the FecA promoter.

Structural papers detail the conformational changes that FecA undergoes after binding citrate and nickel. Two loops close over the ferric citrate. These large changes imply the importance of loops 7 and 8 for binding. We propose inserting a tag into loop 7 or FecA such that it would bind to a target and potentially transduce a signal. We also explored a computational approach with the Maranas lab (Penn State) to produce sequences for binding a target, and a random-library approach to select for binding sequences for a target.

Methods

The constructs we used came from Volkmar Braun at the University of Tuebingen, Germany. He provided A303 cells, strain of E. coli with the Fec system knocked out to isolate a re-engineered Fec system; a plasmid expressing FecA promoter; and the pCRA plasmid containing all the Fec system genes.

To test Pinduction, we transformed A303 cells with the P‐GFP and pCRA plasmids, and induced with sodium citrate. The Fec system is repressed by the P promoter repressor by free iron in LB. Sodium citrate was used instead of ferric citrate, so that the citrate could chelate free iron from the media without adding new iron. GFP fluorescence was detected over time with a plate reader.

Because pCRA is not well-characterized and the expression of the Fec system is controlled by its own P promoter, we thought it valuable to be able to control levels of FecA expression. We attempted to use a T7 regulatable system by cloning the FecA and S gene under the T7 promoter vector (repressing the A303 cells into A303Δcell) and transforming with the P‐GFP plasmid, so that Fec expression could be induced by IPTG/T7, and induction could be assayed by GFP fluorescence.

Results

We found significant increase in GFP fluorescence with P‐GFP / pCRA transformed A303 cells, after sodium citrate induction. Having tried different concentrations, we found that 10mM sodium citrate worked best.

Because pCRA/IpCRA system proved difficult to work with, Our cells had trouble surviving both leaky and induced system expression. Therefore, we decided to test this toxicity might be due to membrane disruption. So far, our assays have not yielded significant results.

Conclusions and Future Plans

We have confirmed that the P‐GFP / pCRA system works in A303 cells. Sodium citrate can effectively transduce a signal into the cell and upregulate P‐GFP expression. We are still working on inserting tags (histidine and strep) into loop 7 of FecA in an attempt to re-engineer FecA for targeting nickel and streptavidin, and potentially under the P‐GFP promoter. If this is successful, we will use the computer and/or random libraries to select for novel targeting/signaling sequences.

References

Quorum sensing


Fec Signal Transduction


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