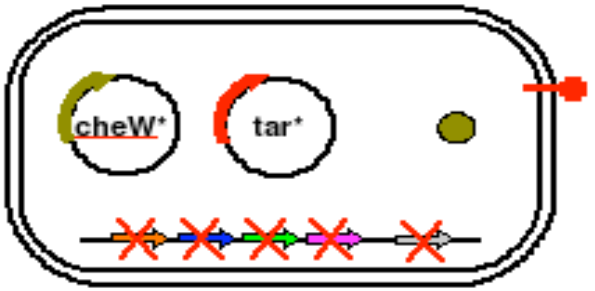
UCSF 2006 aims

- Steering *E. coli* through a maze.
- Switching the response of *E. coli* to external signals.
- Orthogonal pairs.
- Practically not achieve, prove of principle.
- Used:
  - Chassis lacking receptors (UU1250, parkinson), KO CheW.
  - CheW and CheW*
  - Tar* receptor (responsive to Asp)
  - Tar receptor (responsive to Phe)
  - FimE switch

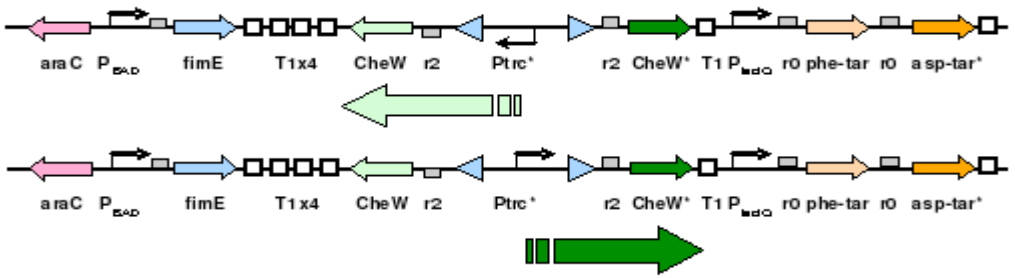


Receptor design

- Previously, orthogonal interaction discovered by Parkinson in Tsr.
- Computational design
  - UCSF mapped the alteration of the evolved Tsr to Tar resulting in Tar* (BBa_J56002 and BBa_J56006).
- Tar* (Asp) interacts with CheW* (BBa_J56001).
- Naturally mutated Tar responsive to Phe (BBa_56003).
  - Chemotactic response has been characterised to be comparable to the normal.
- Tar (Phe) binds to wt CheW
- Tar* binds to CheW*.

Genetic Switch

- Arabinose controls the genetic switch
- Recombinase FimE switches the expression of CheW to CheW* - switching *E. coli* response from Phe to Asp.
  - Irreversible
  - CheW is a small protein
    - Quicker degradation
    - Quicker rates of translation (lack requirement of membrane insertion)
- Unexpected result: after the log phase growth signal became stochastic.
  - Strain may already contain components e.g. FimE or FimB.
  - OR possibly due to unstable plasmid.



Lab work

- UU1250 then KO CheW

- BBa_J56017 composite seq. including CheW and genetic switch.

Problems

- Phe responsive Tar receptor not native.
- CheW/* may not have successfully cloned into the system.
  - But CheW is expressed at low levels in *E. coli* and therefore the problem could be resolved in future.
- The switch is irreversible
- Improving performance of the switch
  - Addition of degradation tags to CheW?
  - Building in a reversible mechanism (FimB?)
  - Increase sensitivity?

Modelling

- Computationally docked Tar and CheW together to characterise the interface identifying residues involved.

- Rate of degradation and translation of CheW?
- How quickly can switching between CheW and CheW* occur?

Similarities between projects

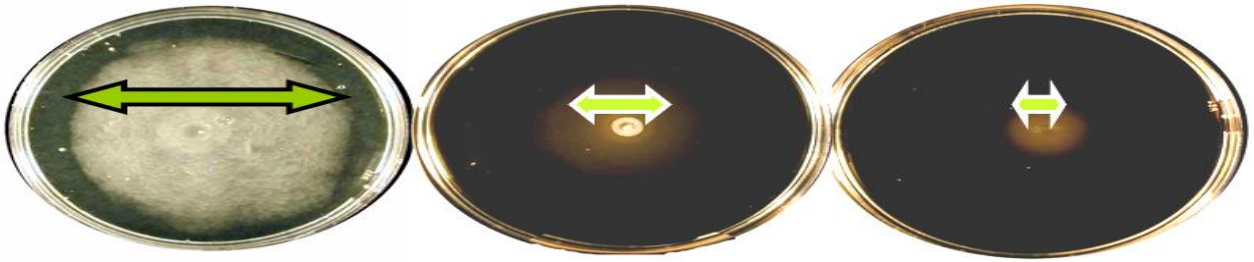
- Chemotaxis- Che pathway and receptors.
- Directed chemotaxis towards a “goal”, more sophisticated target- a maze.
- They did not achieve this.
- This project was not continued in 2007.

Cambridge 2006

- Artificial bi-directional signalling, AHL.
- Quorum sensing systems from other bacteria *Vibrio fischeri* and *Pseudomonas aeruginosa*.
- Unable to construct the whole system but did construct the cassettes LuxI/R and LasI/R
- Construction 3-Antibiotic assembly, described by Tom Knight et al.
- Purified standard plasmid backbone of vectors.
- Modelling of cellular behaviour.

Lab work

- *E. coli* strain MC1000, highly motile.
- *E. coli* strain MG-1655 suggested by Savery second most motile.
- *E. coli* optimum swimming agar medium. Optimum temperature pouring & incubating agar.
- They verified cells were producing lactones with assay *Chromobacterium violaceum* CVO26 plate assay described by McClean et al.
- 2 AHL cassettes:
  - Lux autoinducer BBa_J28032
  - Las autoinducer BBa_J28031



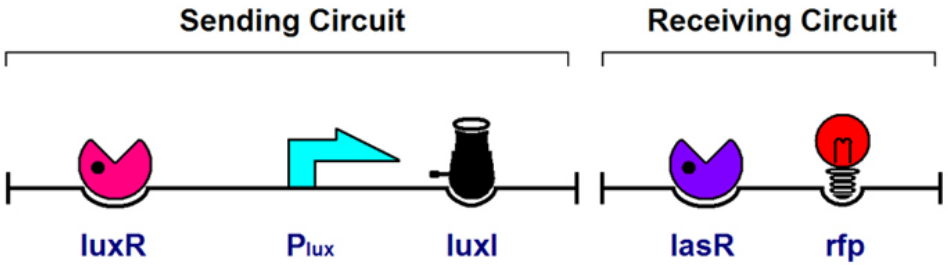
E. coli MC1000 0.5% Bactoagar

E. coli MG1655 0.3% Bactoagar

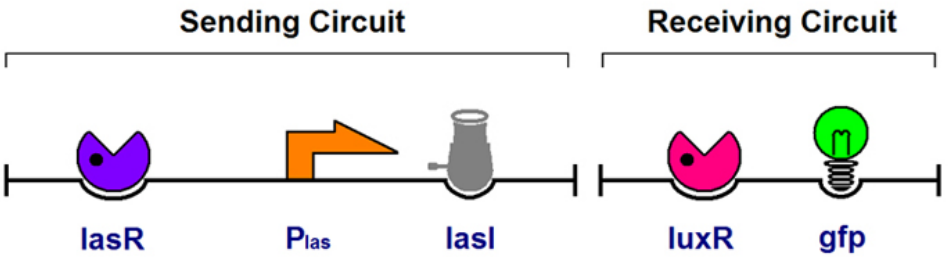
E. coli XL-1 Blue 0.3% Bactoagar

Genetic circuit

TYPE 1 CELL



TYPE 2 CELL



Modelling

- Adapted the experiments of Weiss et al., using cell motility, rather than a differential response to AHL concentrations

- Could modify the system by changing the simple agent resulting in a different range of parameter values, e.g.
  - time to shift chemoattractant
  - force of single bacteria
  - diffusion constants, etc

Modelling

- Single cell dynamics
  - Matlab model of the dynamic behaviour of Type 1 Cell.
- Multicell dynamics
- 2 experiments:
  - Swimming assay
  - AHL bioassay
- Autonomous pattern formation

Problems

- Always a clear zone at the interface of the bacterial population
  - Due to the depletion of nutrients?

Unable to construct the whole system but did construct the cassettes LuxI/R and LasI/R

Similarities

- Fates of cells visualised with fluorescent proteins
- cell motility in combination with position-dependent gene expression has the potential to generate complex patterns / directional movement
- Construction 3-Antibiotic assembly, described by Tom Knight et al.

Similarities

- Did use the chemotactic response
- Quorum sensing
- Two cell populations
- Lactone signalling is involved in biofilm formation *Pseudomonas aeruginosa.*
- Suggested future work equip *E. coli* populations with more complex genetic circuits allow subpopulations of bacteria to “battle” for dominance.
- Parts submitted that would allow construction of such a switch.