Protein Biosensor

We are creating a cell-based polypeptide biosensor that would be able to detect a specific protein present in a liquid or tissue specimen. This device could sense a protein found on the surface of a virus or bacterial cell, for example, and then change color when such a protein target is detected. In our case, an external protein on the malaria protozoan would be detected.

Our challenge:

- first, engineer a membrane receptor which is only activated by a specific target protein
- Second design a signal transduction apparatus to generate a cellular response when this protein is detected.

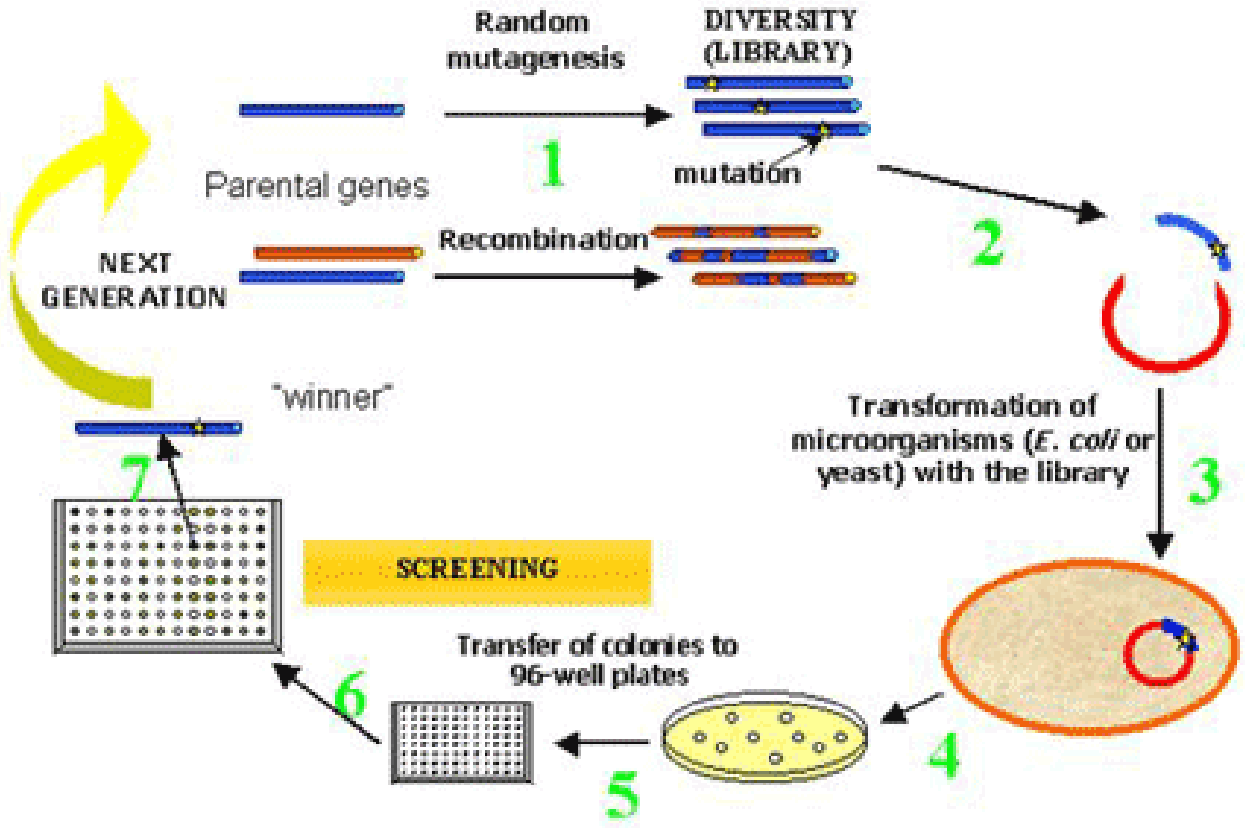
Motivation: Global Health

- In a developing world, there is widespread transmission of disease with inadequate (or no) treatment
- Our Strategy: Sense disease *before* it is spread
  - Detect pathogens for cheap
  - Stop transmission early
- Malaria: 1 million deaths per year
  - Water-bourne disease
  - 40% of malaria preventable by environmental monitoring
  - Very little or no water monitoring in much of the third world (<50%)
- United Nations Millennium Development Goal: Water Sanitation
  - Biggest need: cheap, low-tech biosensing

Directed Evolution

- Method to emulate natural evolutionary process in the laboratory
  - Enables the creation of enhanced or novel functions of proteins
- Input: natural gene that we wish to alter somehow
  - In our case: cell membrane protein for detection of extracellular ligands- heterologous GPCR from mammals
  - Contained on a plasmid or linear DNA segment
- Output: modified gene that has a desired functional profile
  - GPCR that responds specifically to a protein on the surface of malaria parasite (plasmodium species)
- Method: random mutation, then selection, then iteration
  - Mutation through error-prone pcr; recombination
  - Multiple commercial suppliers
  - Selection: either screen (ie FACS) or selection (ie His-)
    - Desired cells 'respond' only in presence of target protein
  - Iteration: re-mutate top performers iteratively
    - Eventually get at 'optimal' mutant

Directed Evolution



G-Protein Signal Transduction Pathway

GPCR
- "G-protein coupled receptor"
- Trans-membrane protein
- Activated by some protein or chemical called a ligand or agonist

G-protein
- Protein that has GTPase activity
- Is either "on" or "off" depending on whether it is GTP or GDP bound

Phosphorylation
- Adding a phosphate group to a protein
- Accomplished by kinases
- Changes the conformation of target protein to make it either active or inactive

Transcription factor (TF)
- Activates or represses transcription of particular genes

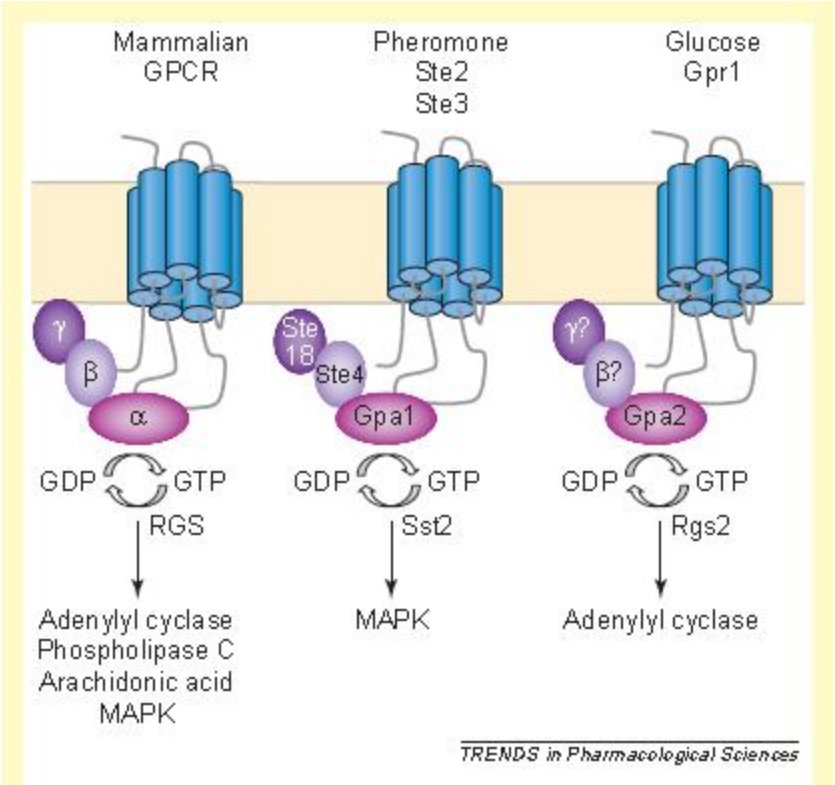
<http://bcs.whfreeman.com/lodish6e/pages/bcs-main.asp?v=chapter&s=15000&n=00010&i=15010.02&o=|00510|00520|00530|00540|00550|00560|00570|00580|00590|00600|00610|00010|00020|00030|00040|00050|00060|01000|02000|03000|04000|05000|06000|07000|08000|09000|10000|11000|12000|13000|14000|15000|16000|17000|18000|19000|20000|21000|22000|23000|24000|25000|99000|&ns=0>

GPCR pathways in yeast

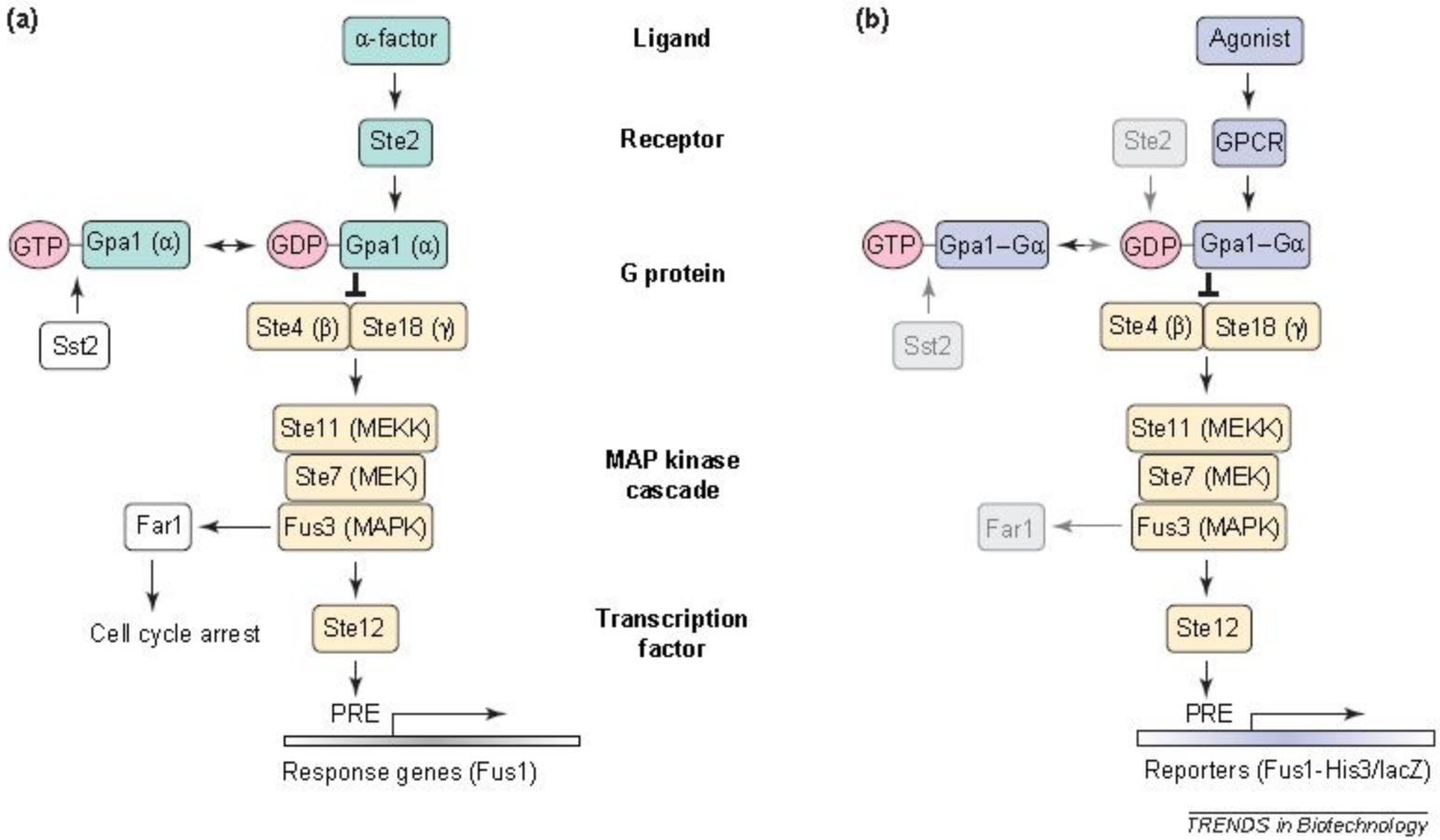
S. cerevisiae has two GPCR pathways

- the pheremone pathway
- the glucose pathway

Both of these pathways are easy to knock out so that foreign GPCRs can be studied



Engineering Yeast GPCR Pathways



Yeast GPCRs

† Contain GPCR signalling pathways similar to mammalian cells.
† Contain only two endogenous GPCR signalling systems that can be eliminated easily.
† Can functionally express heterologous signalling units of ligand-receptor-G protein that can be tested in isolation.
† Robust, fast growing and easy to manipulate robotically.
† Do not require elaborate sterile technique or expensive growth media.
† Highly amenable to manipulation by genetic and molecular biological procedures.
† Low cost, flexibility, rapid growth and ease of handling make yeast an ideal host for high-throughput screens.
† Availability of a variety of reporter systems, including absorbance, colour and growth.
† Allow multiplexing of assays by pooling cultures expressing different receptors within a given well

Further Research

- Protocol for growing yeast (S. cerevisiae)
  - Where to get yeast-talk to professors
  - Culture storage: media conditions
  - Yeast transfection methods
  - Equipment to borrow/purchase -Where?
    - cost
- Malaria
  - potential target proteins on the protozoan
  - potential sources of the proteins for experimental purposes
  - current methods of malaria detection(can be done later)
- Methods and Kits on Directed Evolution
  - Commercial vendors
  - Screen/selection methods
- Yeast GPCR Pathways
  - Endogenous/mating
  - Heterologous GPCRs in yeast

To-Do This Week

- Next meeting this Friday
- Til then: four groups
  - Yeast culture/genetic engineering
  - Malaria biology
  - Directed evolution
  - Yeast GPCR pathways
- Come up with actionable items:
  - Candidate proteins, kits, cell lines etc
- Post results on the wiki!
  - Start updating it regularly- part of the IGEM scoring
  - Post saved pdfs of papers on Netfiles
- Present at end of this week
- Next week:
  - Start ordering supplies
  - Planning first experimental protocols
  - Thinking of 'modularization' of the team