Abstract

Although there are many tools to manipulate DNA in vitro, synthetic biologists are often limited by the in vivo capabilities of the chassis organism. Our project intends to expand synthetic biology by developing the tools necessary for converting lower metazoans into new chassis organisms. Lower metazoans are advantageous because they are closer in relationship to humans, as compared to yeast and E. coli, allowing for more complex systems to be built. We engineered E. coli to deliver proteins and/or DNA payloads into phagocytic eukaryotes and tested our system in choanoflagellates, a robust but currently genetically intractable organism of potential interest as a chassis for synthetic biology. Once ingested by the choanocyte, our E. coli are programmed to self-lyse and release their payloads into the cytosol. This delivery mechanism has the potential to deliver payload to any phagocytic organism with a cholesterol-based membrane. As part of our parallel software effort to rework the Clotho plugin environment and API, we made automatic biosafety handling an intrinsic feature of the core. Together, these tools provide a foundation for metazoan synthetic biology and a framework for improving safety in our field.

Choanoflagellates

We used choanoflagellates, single-celled organisms, as proof-of-concept.

- Easy to culture and proliferate quickly
- Naturally leaf bacteria are an easy target for phagocytosis-based manipulation.
- Closest living relative to multicellular organisms
- No method to genetically modify them: transfection, electroporation, and retroviral infection all have failed
- A mechanism to genetically manipulate them would open an entirely new area of research for developmental biologists

Possible organisms to test in the future:

Amoeba Tetrahymena Vorticella Tricoplax

Our simple and nonspecific design makes it applicable to eukaryotes that:

- phagocytose E. coli into a vesicle that contains cholesterol
- exists in a media that is incompatible with our Self-Lysis device

We are now focusing our efforts on Tetrahymena, Blepharisma and Paramaecia

Delivery Scheme

We engineered E. coli to have two constructs

Payload: either proteins, nucleic acids, or a combination of both

Payload delivery device: the combination of a Self-Lysis part and a Vesicle-Buster part

The bacteria naturally enters a food vesicle through phagocytosis, once inside...

End of Digestion: This vesicle is transported to the food vacuole, where everything is destroyed. The payload must escape the food vesicle before digestion

Barriers to delivery: when the bacteria is in the phagocytic vesicle, there are two barriers, the bacteria’s membranes, and the vesicle membrane

To overcome these challenges, we built the Payload Delivery Device (PDD)

Self-Lysis: Once ingested, the bacteria lyses itself to release proteins into the vesicle

Vesicle-Buster: upon release, due to self-lysis, the Vesicle-Buster device punctures the food vesicle membrane to release the payload into the cytoplasm

Self-Lysis Device

Plbad promoter: fast, controlled induction by an exogenous molecule

Bacteriophage: releasing protein (BIP) degrades the outer membrane

Lysozyme: degrades the peptidoglycan layer

Holin/Anti-holin: creates pores in the inner membrane

A previous version of this lysis device was presented by Berkeley’s iGEM team in 2008. This version needed to be inducible, fast-acting, and functional in a media formulation friendly to the chassis organisms. We tested lysis in a variety of media, including Luria Broth (LB), Cereal Grass Media (CGM), and Artificial Sea Water (ASW), by inducing with arabinose and then monitoring the optical density of the culture (which decreases as the cells lysis).

Vesicle-Buster Device

Pcon promoter: constitutive promoter

Perfrinogen O (PFO): forms pores in cholesterol-containing membranes, does not affect bacteria because they lack cholesterol in their membranes

Phospholipase C (PLC): degrades phospholipids in eukaryotic membranes, does not affect bacteria because they target eukaryotic-specific phospholipids

Degradation tag (sDeg): Eukaryotic degradation tag, to protect health of eukaryote

Pre-pro: targets proteins to the periplasm of E. coli

Successful Delivery

Confirmed by confocal image: multiple Z-stacks show that GFP is diffused throughout the cytoplasm.

This phenotype was observed only when GFP payload was combined with a complete payload delivery device and induced with arabinose

An average of 7% (±2% standard deviation) of choanoflagellates had successful delivery of GFP (1 out of every 16), verified using a hemocytometer

Results

Vesicle-Buster Device

Derived from a construct built in the Anderson Lab originally intended for use in a mammalian system

Clotho Human Practices

Risk Group Description
1 Low-hazard
2 Moderate-hazard
3 Potentially lethal
4 Lethal aerosol transmission

The current biosafety rating system cannot identify potential threats from the combination of individually innocuous genetic parts:

- Self-Lysis Device (RG 1)
- Vesicle-Buster (RG 2)
- Transposome (RG 2)

This risk was realized before any parts were built and necessary precautions were taken. The lysis device is under inducible control and all organisms that would combine transposon devices and payload delivery are deficient in iron acquisition.

Our Clotho biosafety monitor is only the first step. As a community, we must develop techniques to predict dangerous combinations of non-virulent genetic parts. The enforcement of safety standards by the Clotho API is the type of automated checking system needed once such technical standards are developed.

Conclusions

- Characterized a new version of the self-lysis part in different media conditions to determine the optimal culture for both choanoflagellate health and self-lysis function
- Constructed a complete payload delivery device and demonstrated its functionality by successfully delivering GFP (7% success rate) to the cytoplasm of choanoflagellates
- Built 18 complete transposase and zinc finger payloads for the purpose of genetically engineering phagocytic eukaryotes
- Restructured Clotho’s infrastructure, workflow and data model, as well as implemented a scheme for automatic biosafety checks

Acknowledgements