Artificial Multi-cellular Self-organized System

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Abstract

It is an amazing process in nature that the evolution from Protozoa to Metazoa. Even in the development of each Metazoa, it is still unknown how the genome regulates stem cells to differentiate into different kinds of cells, which can compose different tissues or organs, according to where they are in the body. There should be a self-organized process. Here we are trying to build a self-organized multiple-cell system based on the quorum sensing system to understand the mechanism of this process. We employed small molecules in the AHL family as messengers to transmit the orders of differentiation, and we use Cre recombinase as the executor of differentiation. With the use of an artificially designed network, we are trying to construct a new kind of cells, through which a ring composed by GFP will be seen on the plate if the colony is big enough.

Our design

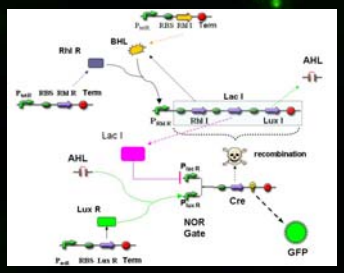


Fig. 1 Overview of the regulatory network

We design a time-dependent gene regulatory network to construct a self-organized system. The RhlI/RhlR system is employed to report the cell density. The "sender" cell will appear first when the density reaches a certain level. And it will command other cells around it to differentiate into "receiver" cells by sending the signal molecules into the environment. After receiving the signals, the Cre recombinase in "receiver" cells will be generated and "scissor" the capacity of sending signals. In this way, a spatial differentiation will be accomplished, and the result will be that the "sender" cells are surrounded by the "receiver" cells. The chemical gradient of th e signal molecules will be built up sometime after the differentiation process is finished. In this way, the response in a specific region will eventually result in a picture of a green ring in the black background.

Components

Differentiation Components

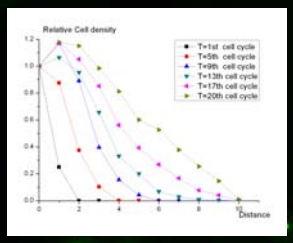


Fig 2. The changes of cell relative density related to the distance at different time points.

◆ **The cell density of the colony on the plate**

Cell Automata is employed to mimic the states of a colony growing on the plate. Fig 1 shows the changes of cell relative density related to distance at different time points.And the result shows that 1) the colony can be divided into two parts, the central region and the peripheral region, according to the density of cells; 2) the central region will expand during the growth of the colony; 3) the difference between the peripheral region and the central region is evident enough to be sensed by the concentration of signal molecules.

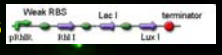
◆ **Quorum Sensing Systems**

We use RhlI/RhlR quorum sensing system as the messenger to inform a cell of the local environment where it lives. RhlI is the enzyme that catalyzes the synthesis of C4HSL and RhlR is the receptor of C4HSL. If the concentration of C4HSL reaches a certain level, the RhlR will increase the expression of pRhlR's downstream genes to a great extent, which marks the initiation of differentiation. There are three genes downstream: RhlI, LuxI and LacI. RhlI can upregulate the expression of downstream genes by forming a positive feedback cycle. The LuxI gene is transcribed to synthesize the signal molecule 3OC6HSL, sending the differentiation order to other cells. However, LacI acts against AHL inhibiting the expression of differentiation executor. In order to build up the system, we have designed and constructed several protein-coding units as follows, and more will be done in the next step.

1. K082035 

2. K082021 

3. K082020 

4. K082045 

Cross-talk of the two quorum sensing systems

In our project, we need to use two types of signal transduction systems, and we choose to utilize the RhlI/RhlR and LuxI/LuxR systems. Our selection is based on the proof that the two kinds of systems do not exchange the acyl-homoserine lactone signals.

NOR gate & Cre recombinase

NOR Gate

The executor part of differentiation is the Cre recombinase that is regulated by the NOR gate. The designed NOR gate is regulated by two proteins: LacI and LuxR. NOR gate will only be turned on with AHL present and LacI absent.

Here the logical gate is utilized to distinguish whether the signal molecule is endogenic or ectogenic. At the beginning, the expression will be inhibited by LuxR. Once LuxI is expressed, AHL will be synthesized and spread throughout the colony. If AHL is endogenic, LacI will be expressed simultaneously so that the executor is not activated. But if AHL is ectogenic, before the concentration of LacI in the cell reaches a sufficient level, the NOR gate is turned on and the executor will be expressed, leading to the shutting off of the AHL generator.

LuxR	LacI	Exp
0	0	1
1	0	0
0	1	0
1	1	0

Graph1: Truth table of NOR gate

• Recombination Unit

In order to utilize the recombination system repetitively, we design an adaptor where we can put in any part that needs recombining. See Fig. 3, 4.

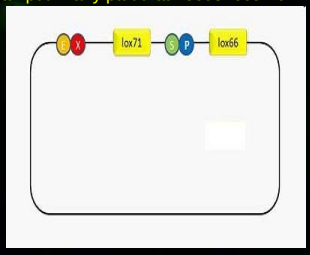


Fig 3.General recombination system

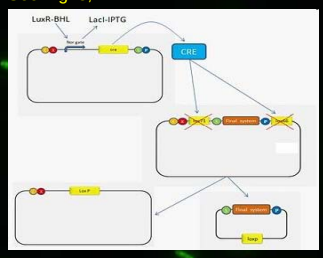


Fig 4. Process of recombination

Reporter Components

The response to distance requires that the reporter gene only responds to a narrow range of signal concentrations, which means the reporter gene will not be expressed when the concentration of signals is either too high or too low. There are some different ways to deal with the problem:

➢ **Multi-regulatory system**

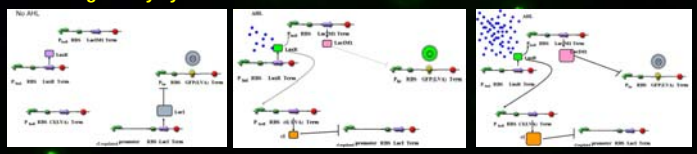


Fig 5. Distinct responses to different amounts of AHL.

➢ **XOR gate**

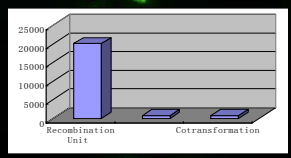
➢ **Computational protein design**

Results

We've already constructed at least 8 units that will be used in our system successfully and submitted 38 parts to the registry. Though time is not enough for us to finish the whole system , we get some intermediate result .

• Test of Recombination Unit

We inserted GFP expression gene into the Recombination Unit. While this unit is transformed into E.coli cells alone, the GFP expresses a lot , while it is cotransformed with Cre recombinase, there is nearly no GFP expresses. The recombination Unit is constructed successfully!



Recombination Unit

Cotransformation

Fig6. Test of recombination unit we designed

• Influence of LacI gene in E.coli genome

Because LacI is recruited here to regulate our system, IPTG should be absent. As it is already known that the E.coli strain TOP10 expresses LacI constitutively at a basal level, we performed some experiments to test whether the influence can be small enough to be ignored. Tests shows that though lacI gene exists in E.coli genome, GFP is still expressed without IPTG because of the high copy numbers of the plasmid.

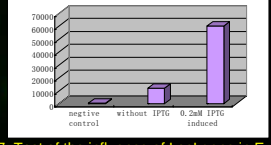


Fig7: Test of the influence of LacI gene in E.coli genome

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