

Teaching bacteria a new language

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In the nascent field of synthetic biology (1), the engineering of novel cell–cell communication capabilities will become critical. Synthetic biology involves the creation of artificial gene and metabolic networks to program new cell and organism behaviors. Recent accomplishments have demonstrated that cells can be engineered to carry out novel tasks (refs. 2–10; R.W. and S. Basu, www.hpcaconf.org/hpca8), and hint that someday we will be able to program cell behaviors with the same ease and capability that we now program computers. However, to achieve higher level functions, we need to program individual cells to coordinate their activities. Previous efforts achieved such coordination by taking existing quorum sensing (QS) components from a source host, *Vibrio fischeri*, and integrating them into a target host, *Escherichia coli* (5, 7, 8). The work of Bulter *et al.* (11) in this issue of PNAS describes a new form of engineering cell–cell communication based on manipulating metabolic pathways. Specifically, the authors engineered the nitrogen regulation system and the acetate pathway in *E. coli*. As the engineered cells grow, they broadcast their presence by producing and secreting acetate. This intercellular signal is then detected by neighboring cells that respond by elevating the expression of a GFP. Because the acetate level correlates with cell density, this process confers QS behavior on the cells without using traditional QS biochemistry.

Fig. 1 shows the circuit operation. First, during normal cell metabolism, amino acid biosynthesis results in acetate as a byproduct. Acetate, which functions as the communication signal, is readily converted between several forms in the cells. The protonated form of acetate, acetic acid, diffuses out of the cell to the growth media and then into neighboring cells. When in the cytoplasm, acetic acid is deprotonated to acetate, which in turn can be phosphorylated to acetyl phosphate by acetate kinase. Acetyl phosphate transfers its phosphate group to NR₁, a transcriptional regulator of *glnAp2* promoter (12). As a result, NR₁~P dimerizes, binds the *glnAp2* promoter at two enhancer regions, and activates transcription of GFP in the engineered system (11). Through the above processes, the cytoplasmic level of NR₁~P reflects the extracellular acetate concentration, which in turn correlates to cell density.

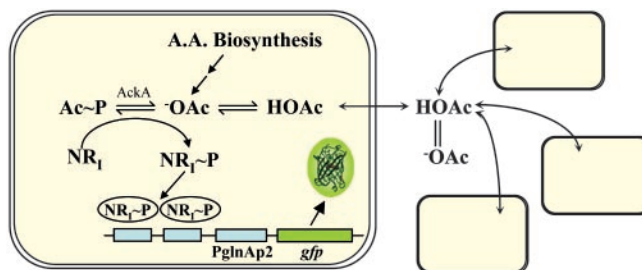


Fig. 1. Basic operation of the engineered acetate QS circuit. Ac~P, acetyl phosphate; OAc, acetate; HOAc, acetic acid; A.A., amino acid; NR₁, nitrogen regulator protein I; *gfp*, gene for green fluorescent protein; AckA, acetate kinase; *glnAp2*, *glnAp2* promoter.

Thus, GFP expression reports cell density by using acetate as a QS signal.

Although the nitrogen regulation system and acetate pathway exist naturally in *E. coli*, harnessing these mechanisms to QS requires several modifications. For example, the natural system is highly sensitive to oxygen; acetate production increases significantly under anaerobic conditions. By using a *pta*[−] strain (13), where *pta* is one of the main genes in the acetate pathway, the authors removed the influence of oxygen; the mutant showed similar acetate production under aerobic or anaerobic conditions (11). Another benefit to using this mutant is slower degradation of acetate (14) that increases the dynamic response range to acetate (15).

The authors also used a mathematical model to guide them in forward engineering and fine tuning of the system through additional genetic mutations and changes in environmental conditions. The model predicted the simultaneous effects of variations in ΔpH across the cell membrane and in NR₁~P binding affinity to the *glnAp2* promoter. According to the model, decreasing the medium pH will shift the acetate/acetic acid equilibrium toward the latter, resulting in higher intercellular acetate concentration. Of the two forms, only acetic acid permeates the cell membrane. Therefore, decreasing the pH should enhance the system sensitivity. The model also predicted that the detection sensitivity can be fine-tuned by changing the binding affinity of the NR₁~P dimer to the *glnAp2* enhancer regions. Stronger binding affinities increase the sensitivity to acetate, and weaker affinities have the opposite effect. These observations led to experiments under different pH conditions and the construction of three different

versions of the enhancer domains with varying binding affinities.

The experimental results with various pH and NR₁~P binding affinities confirmed the model predictions. First, to quantify the system's response under different pH, fluorescence was measured in a variety of extracellular acetate concentrations. As predicted, GFP expression in response to any given extracellular acetate concentration was significantly higher at lower pH. Accordingly, cells grown in lower pH were able to respond to lower cell densities. Experiments with a stronger enhancer region further improved the ability to detect lower cell densities. Conversely, a weaker enhancer resulted in higher detection thresholds. The experimentation along these two axes of control validated that the system could be fine tuned and optimized based on model predictions to achieve a particular task, a basic tenet of synthetic biology (ref. 6; R.W. and S. Basu).

To date, most synthetic biology efforts have focused on implementing novel, nonnative behavior at the single-cell level (refs. 2–4, 6, and 9–11; R.W. and S. Basu). These explorations have taught us lessons about the operating principles of biological systems and are enabling the creation of bioengineered systems with new functionalities. However, to fully exploit the potential of synthetic biology, we must also explore the realm of cell–cell communication. Such endeavors will allow us to realize sophisticated applications that simply

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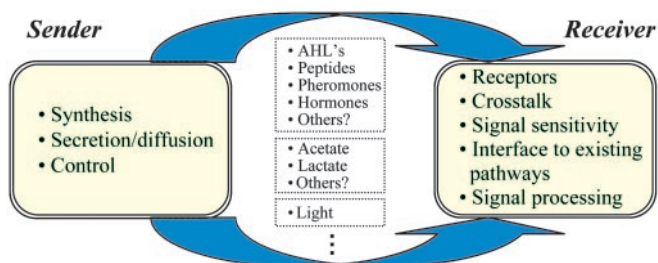


Fig. 2. Issues in architecting cell-cell communication.

cannot be accomplished by single cells acting alone.

Fig. 2 presents some of the issues central to the engineering of cell-cell communication. Any intercellular network consists of sender and receiver elements that communicate via the synthesis, transmission, and reception of signals. In many cases, the sender and receiver elements coexist in the same cell. For example, in the work of Bulter *et al.* (11), amino acid synthesis functions as the sender element because it produces the acetate signal. This signal is transmitted to other cells as well as the originating cell, and is finally received by the NR₁/glnAp2 pathway. This design demonstrates the general requirements that the signal must be readily synthesized and secreted, and, ideally, controllable (e.g., the effect of pH).

Having a diverse library of such signals affords the engineer flexibility and power. Different signals may be suitable for different environmental conditions (e.g., pH, temperature, light, and

solid-phase media density). Multiple signals also allow architecting complex interactions that involve simultaneous coexisting communication dialogs between cells. Appropriate signals may take different forms. These include traditional cell-cell signaling molecules such as acyl-homoserine lactones (AHL) used for QS in Gram-negative bacteria (16), peptides used in Gram-positive bacterial QS (17), or yeast pheromones (18). Another class of molecules includes metabolic byproducts such as acetate (11) or lactate (see below) that are typically not considered as cell-cell communication molecules but, as the present study demonstrates, can be used effectively for such purposes. Nonbiochemical signals are also conceivable, such as light or physical contact. One can envision the use of different classes of signals simultaneously, such as AHL and acetate. This approach will likely reduce crosstalk that often exists between related signals [e.g., different AHLs (19)].

On the receiver side, the signal has to be detected. The detection is accomplished by cell surface or cytoplasmic receptors. For example, epidermal growth factors in eukaryotes bind cell surface receptors, whereas AHLs diffuse freely into the cell and bind cytoplasmic receptors. A promising approach is taken by Hellinga's group (20) to design novel receptors by "rational mutagenesis" of existing *E. coli* periplasmic binding protein, allowing them to bind other substrates such as lactate, TNT, and serotonin. This approach can expand the choice of useful metabolites and can be integrated with engineered organisms that have been modified to secrete such metabolites, e.g., lactate (21). Finally, once the receptor detects a signal, it needs to be processed. This signal processing may include amplification, threshold detection, digitization, or combination with other signals. Biochemical networks can perform such signal processing by regulating transcription and translation, controlling phosphorylation, and coordinating metabolic activities.

The work of Bulter *et al.* (11) suggests a new paradigm for finding and exploiting preexisting nontraditional cell-cell signaling. As we improve our understanding of the crucial role cell-cell communication plays in biology and discover the rich complexities of communication protocols between cells, it becomes increasingly important to be able to engineer these protocols. Such engineering capabilities will benefit both the understanding of natural systems and the design of complex novel behaviors that require coordination between cells.

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