Point of VIEW

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A Whole More Than the Sum of Its Synthetic Parts

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ABSTRACT Synthetic biology is the realization of systems with desired behavior using biological materials. A recent addition to the field is a bipartite consortium of the bacterium *Escherichia coli* in which each species harbors complementary gene circuits that actuate only when both are present above a critical density. This bacterial "consensus" system, functional in liquid, solid, and biofilm niches, represents a novel strategy that raises the bar in terms of the specificity and complexity of tasks performed by engineered organisms.

Attempts to construct *de novo* gene circuits displaying various expression patterns in bacteria have provided insight into control of gene expression in engineered biological systems and in their more complex natural counterparts (*1, 2*). In an early effort to program cellular communication, Weiss and Knight (*3*) co-opted the quorum sensing (QS) module of a marine bacterium, *Vibrio fischeri*. QS systems, found in bacteria and other unicellular organisms, including yeast, allow cells within a population to ascertain their density *via* the concentration of diffusible messengers (*4*). One class of QS system employs molecules in the acyl-homoserine lactone (AHL) family. To date, AHL-based mechanisms have been employed to coordinate activities in single populations or to enable subpopulations to convey information to one another in a unidirectional fashion (*5–7*).

A new report by Brenner *et al*. (*8*) describes the generation of a pair of synthetic circuits that define two populations of bacteria able to activate expression of their respective target genes only in the other's presence. This division of labor, combined with a system for coordinating activities, demonstrates an approach to enhancing system flexibility and functional complexity and is akin to the specialization observed in multicellular organisms. In brief, Brenner *et al*. engineered a microbial consensus consortium (MCC) in *Escherichia coli* in which output gene expression is a function of the concentration of QS signals produced by two growing subpopulations. Their design consists of a pair of sender–receiver circuits, A and B, containing components derived from the LasI/LasR and RhlI/RhlR QS signaling system of *Pseudomonas aeruginosa* (Figure 1). These I- and R-proteins are involved in catalytic production of AHL (LasI catalyzes $3OC_{12}HSL$; RhlI catalyzes C_4HSL) and AHL-dependent transactivation ($3OC_{12}HSL$-bound LasR interacts with p(las), whereas C_4HSL-bound RhlR interacts with p(rhl)). By design, *E. coli* harboring circuit A (containing RhlR driving production of $3OC_{12}HSL$ *via lasI*) should respond to C_4HSL signals, whereas circuit B (containing LasR driving production of C_4HSL *via rhlI*) should respond to $3OC_{12}HSL$. However, because of their similarity, even these distinct QS systems experience unwanted interactions within individual cells. AHL-mediated activation of a noncognate R-protein or unactivated R-protein association with its cognate promoter could result in unsolicited "autoactivation".

A major design consideration was thus the specificity afforded to each circuit. However, instituting a high degree of substrate specificity invariably comes at a cost: reducing the sensitivity of a circuit to avoid autoactivation may reduce its sensitivity to its cognate signal and stifle consensus actuation. To facilitate circuit optimization, Brenner *et al*. investigated the consequences of altering circuit components (*e.g*., parameters such as strength of transcription and translation) by detailed mathematical mod-

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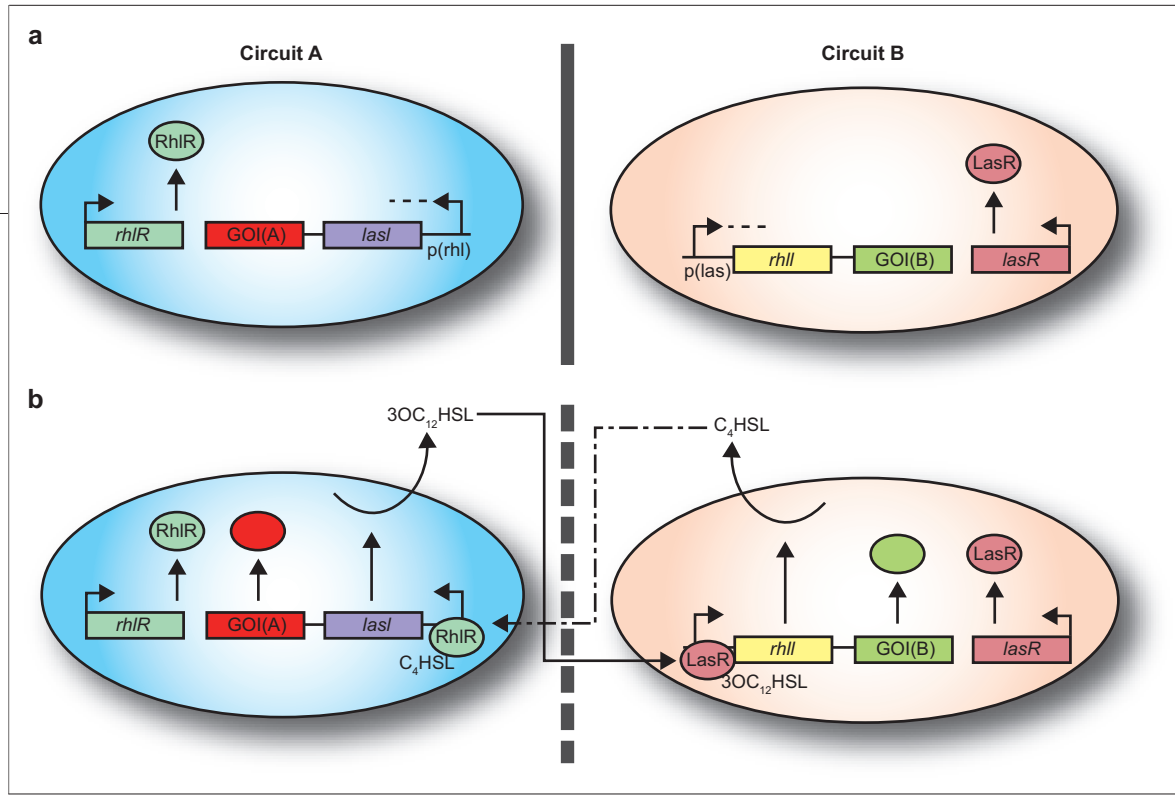

Figure 1. The microbial consensus consortium. Behavior of the MCC system (a) in isolation or (b) in conditions allowing acyl-homoserine lactone ($3OC_{12}HSL$, C_4HSL) signal exchange. Structurally identical sender–receiver gene circuits A and B contain I- (RhlI, LasI) and R (RhlR, LasR)-proteins from the *P. aeruginosa* QS system and effectively define two populations of *E. coli* (blue and pink). a) When transmission of $3OC_{12}HSL$ and C_4HSL is prevented, R-proteins are transcriptionally inactive. In theory, basal I-protein expression catalyzes leaky signal formation. In practice, appreciable autoactivation was observed in circuit A. b) $3OC_{12}HSL$ and C_4HSL produced from circuits A and B, respectively, are allowed to diffuse freely to their counterparts and bind with R-proteins. This results in transcriptional activation of I-proteins and their respective GOIs. Adapted from Brenner *et al.* (*8*).

eling. This analysis revealed that the undesirable effects of autoactivation could be mitigated by increasing I-protein degradation rates or instituting positive feedback on I-protein synthesis. In the final design, the authors decided to generate positive feedback by placing *lasI* and *rhlI* under the control of p(rhl) and p(las), respectively. Importantly, this example illustrates that modeling can greatly expedite the process of selecting between alternative system architectures.

An engineered gene expression system will likely enjoy wide usage if it is flexible enough to accommodate a wide range of inputs and outputs. An attractive feature of the MCC design is its modularity. In particular, induction of an R-protein is decoupled from its corresponding output, or gene of interest (GOI). In principle, each population is not restricted to the expression of a single target gene, nor is it necessary to couple an I-protein to GOI in transcription. The modularity permits these circuits to be rendered competent by independent conditions. For example, R-protein production could be tied to environmental cues such as pH and pO_2 levels that would be necessary for the function of a therapeutic prodrug–enzyme pair. This adds an additional layer of regulatory precision: without the aforementioned competency signals, the consensus mechanism would be inactive, irrespective of cell density. The decoupling of GOI expression from QS mechanisms makes it possible to swap in other QS components with, for example, more elaborate promoter specificity or to satisfy host compatibility. More than 50 such QS components have been identified in proteobacteria (*9*). Alternatively, it would be useful to employ synthetic QS signaling components to insulate signals exchanged between synthetic populations and increase the bandwidth available for information exchange (*10*).

In the parlance of digital electronics, a circuit that gives a high output only if all its inputs are high is said to behave as an AND-gate. The MCC system represents the biological equivalent of an AND-gate. Obligate cooperation can afford a process a great deal of specificity with regards to the conditions required for its initiation. In other words, the requirement for at least two distinct inputs to elicit a response provides a "sober second thought" that reduces inappropriate triggering. This would be an important safety measure for responses that have destructive consequences or involve a costly/lengthy postinitiation down-regulation. For example, this regulatory approach is employed by the mammalian immune system by the process of linked recognition in the thymus-dependent antibody response (*11*). Antibodies produced *via* B cells are crucial for eliminating potentially infectious diseases. However, before B cells can be fully induced to make an antibody to a pathogen in an infection, helper T cells must be activated by the pathogen. The activated helper T cells induce B cells to proliferate and differentiate into plasma cells that secrete a specific antibody. The AND-gate consisting of B cells and helper T cells helps to ensure self-tolerance, the absence of which has been known to lead to autoimmune diseases, including type

1 diabetes mellitus, multiple sclerosis, systemic lupus erythematosus, and some forms of hyperthyroidism. Here, synthetic biology has borrowed a regulatory mechanism from nature and repackaged it in a user-friendly form. The MCC represents a strategy that might be used in mission-critical applications.

Synthetic circuits in bacteria are often examined in liquid culture, with bacteria assuming a planktonic state or with bacteria immobilized on solid agar (*6*). It is remarkable that the MCC function was consistent in culture, solid agar, and when *E. coli* populations were forced to grow into a biofilm. First described more than 7 decades ago by Authur T. Henrici, biofilms are "matrix-enclosed bacterial populations adherent to each other and to surfaces or interfaces" (*12*). It has only been recently appreciated that biofilms exist as structured ecosystems, composed of a multitude of unicellular species that interact in a complex manner. The importance of biofilms in bacterial biology and pathogenicity cannot be overstated: >99% of all bacteria live in biofilms (*13*). Surprisingly, it has been estimated that 30–40% of proteins present in the bacterial cell walls differ between these sessile and planktonic bacteria (*14*), and they exhibit distinct patterns of gene expression (*15*). Therefore, the extrapolation of results observed in planktonic bacteria to biofilms is not a given. For example, biofilms cause a discrepancy between the mathematical description of a predator–prey relation and observations in a chemostat (*16*). Whereas biofilm bacteria have beneficial activities that include a crucial function in wastewater treatment, the role of biofilms in disease is significant. It has been suggested that 65% of human bacterial infections involve biofilms, including periodontitis, chronic sinusitis, chronic otitis, cystic fibrosis, and a variety of prosthetic device infections (*17*). Compounding this problem, bacteria in biofilms are inherently more resistant to a multitude of antibiotics and incredibly difficult to abolish (*18, 19*). The MCC system's comparable behavior in a biofilm speaks to its wide applicability. More importantly, the consensus gene expression was observed for >6 days in biofilms. In addition to the exquisite regulatory precision inherent in the MCC circuitry, such sustained behavior would be not only desirable but also necessary for applications requiring persistent gene expression, such as the elimination of infection or therapeutic drug delivery.

One benefit of using a consortium to engineer biological function, rather than a single engineered cell type, is the ability to sequester conflicting but complementary functions within different cells. Another benefit is the ability to control a function through space and time by the introduction or elimination of particular members of the consortium. One can imagine that engineered consortia could be applied to problems ranging from microfabrication to drug delivery. This system offers a great deal of promise, because this is the way that nature has solved these types of complex problems—community cooperation.

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