Invited Mini Review

Microbial linguistics: perspectives and applications of microbial cell-to-cell communication

Robert J. Mitchell[1,#], Sung Kuk Lee[1,#], Taesung Kim[2,#] & Cheol-Min Ghim[1,*]

[1]School of Nano-Bioscience and Chemical Engineering, [2]School of Mechanical and Advanced Materials Engineering, Ulsan National Institute of Science and Technology (UNIST), 50 UNIST-gil Ulju-gun, Ulsan 689-798, Republic of Korea

Inter-cellular communication via diffusible small molecules is a defining character not only of multicellular forms of life but also of single-celled organisms. A large number of bacterial genes are regulated by the change of chemical milieu mediated by the local population density of its own species or others. The cell density-dependent "autoinducer" molecules regulate the expression of those genes involved in genetic competence, biofilm formation and persistence, virulence, sporulation, bioluminescence, antibiotic production, and many others. Recent innovations in recombinant DNA technology and micro-/nano-fluidics systems render the genetic circuitry responsible for cell-to-cell communication feasible to and malleable via synthetic biological approaches. Here we review the current understanding of the molecular biology of bacterial intercellular communication and the novel experimental protocols and platforms used to investigate this phenomenon. A particular emphasis is given to the genetic regulatory circuits that provide the standard building blocks which constitute the syntax of the biochemical communication network. Thus, this review gives focus to the engineering principles necessary for rewiring bacterial chemo-communication for various applications, ranging from population-level gene expression control to the study of host-pathogen interactions. (BMB reports 2011; 44(1): 1-10)

INTRODUCTION

Cell-to-cell communication is essential for the coordinated action of cells in multicellular organisms (1). Failure in responding to the signals generated and transmitted by neighboring cells and environment signifies the onset of diseases, such as sclerosis, diabetes, and cancer. Considering that the inter-

cellular communication is a key step in the evolution toward the multicellularity, it is not so surprising that unicellular organisms have been found to communicate with their own species and others. As a matter of fact, cell density-dependent regulation of gene expression is quite prevalent amongst single-celled organisms.

A recurring pattern of regulation is the cascade of signal transduction mediated by the *autoinducer* molecules excreted by a local population of cells, which bind to the transcription factors to turn many bacterial operons on or off (Fig. 1). The so-called quorum sensing (QS) system regulates wide array of genes ranging from housekeeping genes to those related to pathogenesis, bioluminescence, exogenous DNA uptake, antibiotic synthesis, and many others (2). In the opportunistic human pathogen *Pseudomonas aeruginosa* as many as 616 genes have been reported to respond to the change of the local *autoinducer* concentration (3). As such, the autoinduction followed by signal transduction has been a popular target for controlling microbial communities for engineering purposes (4, 5) and for a novel non-antibiotic strategy (6) to cope with the adverse human health effects.

Yet another potential of QS comes from the inter-species communication mediated by another *autoinducer* termed AI-2. Being produced by both gram-positive and gram-negative bacteria, AI-2 is a widespread "trade language" across the microbial kingdom. Since the natural microbial communities are rarely clonal and the majority of bacteria have been found to have the well-conserved AI-2 synthase, LuxS, AI-2/LuxS seems to be a more ancient language than its intra-species counterpart (7). Hence, the *autoinducer* synthase combined with its receptor protein seems to be a universal paradigm for microbial communication within and across the species.

To make sense of quorum as the "minimal behavioral unit" (8), however, one needs to clarify what is really detected from the *autoinducer* concentration because it is not necessarily a perfect proxy for the population density of cells of the same or difference species. The *autoinducer* "signal" can be altered by environmental factors affecting the drift-diffusion and degradation, and by the crosstalk or interference that may be prevailing within highly diverse microbial communities. Another, but related, challenge that the QS-triggered gene expression regulation faces comes from the evolutionary implications of the

"cheaters", which take advantage of others' cooperation with no contribution to the community they belong to. Parts of these questions have been brought up under the new hypothesis of efficiency sensing (9), but many fundamental questions as well as the engineering applications still remain open. Here we review quantitative approaches to better understand the genetic circuits for QS and their evolutionary rationales that will serve as the basis for design and construction of novel engineering systems.
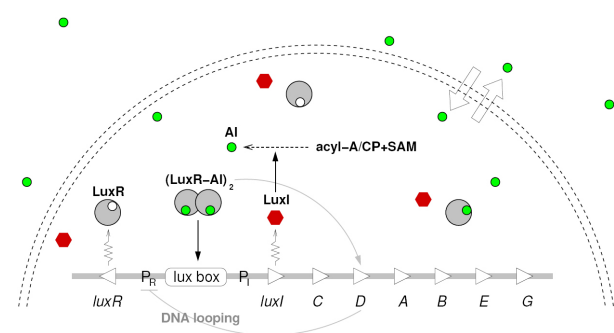
Autoinducers and the Genetic Circuit

Chemical communication between bacterial species was first observed within the luminescent symbiotic bacteria, *Vibrio fischeri*, which lives in a mutualistic relationship with the Hawaiian bobtail squid *Euprymna scolopes*. Within the lab, this strain is non-luminescent when present at low cell concentrations. When the culture surpasses a threshold number, which is approximately 10^{11} cells/ml for this bacterium, the phenotype of the whole culture shifts and it becomes highly bioluminescent. Initially thought to be the result of an inhibitor, studies into this phenomenon showed that a chemical signal was being produced by each of the members of the culture, that this production was auto-induced (10, 11) and that at the threshold cell number this signal reached a concentration which led to the transcription of otherwise unexpressed genes. Since this always occurred when the cell concentration was about the same, it was dubbed *quorum* sensing.

The first autoinducer molecule to be identified (*N*-(3-oxohexanoyl)-homoserine lactone) was from this bacterium (12). Since this time, numerous acyl-homoserine lactones (AHLs) and methods for their detection have been studied, including thin layer chromatography (13), colorimetric assays

(14, 15), gas chromatography (16), and bacterial reporter strains (17, 18), many of the latter by Paul Williams group at the University of Nottingham. AHLs, which are produced only by gram negative bacterial strains, have a similar structure but vary according to the fatty acid moiety and can carry a hydroxyl or oxo group on the third carbon position. Additional *autoinducer*s have been identified, including small polypeptides (19, 20), which are predominantly used by gram positive bacteria, and AI-2, which is produced by many different species of bacteria, both gram positive and negative. Both the AHL and AI-2 *autoinducer*s are produced using *S*-adenosylmethionine (SAM) as a precursor. For AHLs, a reaction between SAM and an acyl-acyl carrier protein (acyl-ACP) is catalyzed by a LuxI homolog. Since the acyl-ACP is involved in fatty acid biosynthesis, the LuxI homolog binds to the acyl-ACP of a specific length. This permits different bacteria to produce AHLs with varying acyl group lengths between C-4 and C-14 (17). In contrast, SAM is degraded to produce the AI-2 signaling molecule (21, 22). During this process S-adenosylhomocysteine (SAH) is generated, which can then be converted to S-ribosylhomocysteine (SRH) and then cleaved to form homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD). It is this latter compound which forms AI-2 as it spontaneously cyclizes (23, 24).

The formation of the polypeptide *autoinducer*s is a bit more complex involving the formation of a pre-protein that is processed post-translationally. This can involve either the production of a fully functional polypeptide or a precursor which needs to be modified prior to its activation, as in the case of the *Staphylococcus aureus* AgrD1 *autoinducer* which needs cyclized (20). Perhaps the most well-known polypeptide *autoinducer* is the pentapeptide competence-stimulating factor of *Bacillus subtilis*, which controls the genetic competence pathway within this bacterium and also has a role in sporulation (25, 26).

Autoinducer Degradation

It was found that between 8 and 20% of cultivatable soil bacteria produce AHLs (27, 28), suggesting that the production of AHL is widespread within soils. With so many bacteria producing autoinducers, a mechanism for their removal from nature should logically exist. This was found to be true by Wang and Leadbetter (29) and d'Angelo-Picard *et al.* (28). In their studies, they demonstrated that various bacterial strains are capable of rapidly degrading AHLs. AHLs can be inactivated by hydrolysis of either the lactone ring, which occurs chemically and is exacerbated by the pH (30) or via AHL lactonases. AHL lactonases have been identified in several bacterial species, including *Bacillus*, *Agrobacterium* and *Arthrobacter* (31-35). After lysis of the AHL, an acyl-homoserine is produced and can be utilized by the bacterial strain. Another method that bacteria use to inactivate the AHL is through the lysis of the acyl-amide linkage (36-38), producing a fatty acid and homoserine lactone, both of which can be used as an energy source

Fig. 1. QS network of gram-negative bacteria. Autoinducer (acyl-homoserine lactone, AI) is synthesized by the catalysis of LuxI to freely diffuse in and out of the cell membrane. AI binds to LuxR and LuxR-AI complex homodimerizes to upregulate the genetic activity of *luxR* and *luxI*, which closes a dual positive feedback circuit. Excessive LuxR-AI dimer may bind *luxD* to abolish the promoter region of *luxR* via DNA looping. SAM, S-adenosylmethionine acyl-A/CP, acyl carrier protein.

by the bacteria.

Likewise, the production of an AI-2-like molecule in *Salmonella typhimurium* was found to be expressed during the mid-exponential phase of growth and then degraded by this strain during the stationary phase (39, 40). This was also shown to be true for *E. coli* (41), where the presence of glucose was shown to extend the AI-2 lifetime through catabolite repression. This capacity of *E. coli* was utilized in (42) to demonstrate that the degradation of the AI-2 signal can interfere with the phenotype of other bacterial strains including *Vibrios*, in this case the production of bioluminescence by *V. harveyi* and activity of *V. cholerae*.

Quorum Sensing is Not Unanimous

The basic concept of QS, as described above, is that bacteria communicate with each other using the *autoinducer* in order to determine their cell numbers. When a certain threshold is achieved, a population-wide phenotypic change occurs. This idea has been used to explain certain natural phenomenon, such as diseases where a certain number of bacteria can elicit a response (virulence factors) that would normally be overwhelmed by the host defenses if produced by a single bacterium or a lower density culture. Due to the high population number, however, the triggered "attack" is more effective at overcoming the host defenses.

Attractive though the idea behind QS may be, it is not completely accepted by scientists, or even by bacteria. The main problem with the idea is that natural systems are rarely, if ever, clonal, meaning that within a given space many bacterial species will cohabitate and interact with each other. Furthermore, a flask is not representative of natural environments since it is, for the most part, a closed system. Due to these and other limitations with the "quorum sensing" concept, the idea of "diffusion sensing" was proposed (43).

In diffusion sensing, the bacteria uses the autoinducer as a measure of the diffusivity. Though diffusion sensing has basically the same concept as QS, it differs in at least one point: why the cells produce the signaling molecule. Diffusion sensing puts forward the idea that bacteria are not trying to determine their numbers but measuring the diffusivity of the surrounding environment. In an environment with a low rate of diffusion, the autoinducer molecule would be retained nearby and build up in concentration, eventually reaching the threshold concentration and leading to the phenotypic change. Conversely, when the diffusion is high, the autoinducer is quickly dispersed and cannot lead to phenotypic changes. Clearly both of these conditions would dramatically affect the QS results, which adhere to a definition based upon a closed system. Redfield (43), where the idea of diffusion sensing is first presented, further states that this point has to do with the idea of metabolic cost. Namely, the use of an autoinducer molecule costs little for the bacterial cell as compared with, for example, the production of extra-cellular proteases. In an envi-

ronment where the diffusion is low, the extra-cellular proteases would likewise be retained nearby the cell while, in the highly diffusive setting, the metabolically expensive proteases would be swept away, a waste energetically for the cell. This was further explored by (44) where they determined that the cost for the bacterium to produce AI-2, butyryl-homoserine lactone (the simplest and cheapest AHL produced) and the polypeptide ArgD from *S. aureus* to be 0~1 ATP, 8 ATP and 148 ATP, respectively, and concluded "based on the concentration of signal required for a response, it can generally be stated that the amounts (of each autoinducer) produced are inversely proportional to the production costs".

To address the issues between quorum and diffusion sensing, a recent article proposed "efficiency sensing" as a way of bridging these two ideas (9). In efficiency sensing, the bacteria would use the autoinducer as a proxy to determine the retention of costly effectors, such as proteases, once released into the surrounding media. The authors propose that there would be a high degree of correlation between the *autoinducer* and effector concentrations and demonstrate their proposed idea mathematically. Furthermore, their study focused on microcolonies, which are prevalent within nature and generally considered to be clonal though they may come in contact (45), and showed through their model that the bacterial cells located within or in close proximity of the microcolony would experience sufficiently high enough concentrations to elicit the phenotypic change while those further away would not.

Experimental Approaches to Cell-to-cell Communication

Miniaturized technologies

Microbial cell-to-cell communication assays can benefit from the miniaturization of technologies, particularly those based on microfabrication and microfluidics (46, 47). This is mainly because of recent advancements with these technologies enables a number of experiments in parallel, in high-throughput and with high resolution. Furthermore, using these systems, it is possible to investigate the activities of individual cells in a shorter time and with less effort than the typical bench-scale experimental instrumentations require (46).

Microfluidic technologies provide the cell-to-cell communication assays with *de novo* approaches which had not been possible until such technologies emerged. To further develop and understand cell signaling pathways, including cellular communication, it was also necessary for researchers to develop many novel investigative approaches with which microfabrication and microfluidics can contribute toward microbiology and synthetic biology. Among those recent technologies, our discussion mainly focuses on microfluidic devices (46, 48-50) and printing technology (51-54) both of which provide effective tools for regulating and monitoring cell-to-cell communication by adjoining bacterial cultures, both homogeneous and heterogeneous colonies of microbial cells.

Microfluidic devices for microbial cell-to-cell communication assays

Several microfluidic devices have been developed that provide a powerful means to investigate microbial cell-to-cell communication. As one of the representative devices, Kim *et al.* developed a "defined spatial structure" that separated three soil bacteria physically but allowed for a chemical interaction between them via diffusion of small biomolecules across the three colonies (55). They used a double-layered microfluidic structure separated by a nanoporous polycarbonate membrane (0.2 μm in pore size). Three species of bacteria were cultured separately in small wells located on the top layer so that each species cannot migrate to the other wells. However, the culture wells were connected through the bottom layer so that small molecules secreted by one species were able to diffuse into the other wells. They found that the spatially-separated wells provided an essential co-culture environment for multiple species and that the distance between the wells was an important factor for reciprocal syntrophy because it determined the diffusion rates of the secreted biomolecules.

Another microfluidic device was developed where different bacterial cells were selectively and locally added to chambers that were separated with hydrogel, which shows a similar range of diffusivity of small molecules as those in water (56). By culturing three synthetically engineered cells, which were referred to as a "sender", "receiver" and "inverse receiver", within the chambers in a physically separated, but chemically connected manner, they were able to demonstrate that these cells communicate with each other via diffusion of AHL. In this system, the sender cells express AHL, which diffuses and then activates the receiver cells to express GFP but causes the inverse receiver cells to stop expressing GFP. Hence, this device provides researchers with an easy method or platform to facilitate synthetic bacterial cell-to-cell communication assays.

Instead of using a series of microfabrication technologies, Yaguchi *et al.* reported on an aqueous two-phase system (ATPS) that utilizes both a mixture of polyethylene glycol (PEG) and dextran. Their process depends on the manual pipetting of the dextran-rich solution, which contains a high concentration of cell suspension, on a surface that is filled within a PEG-rich solution (57). The ATPS also physically segregates but chemically connects the pipetted bacterial micro-colonies (bacterial systems) and also permits each "micro-colony", *i.e.*, droplet, to contain various types of bacterial cells in suspension. In a similar manner as previous methods, this technique allows researchers to study the cellular responses of bacterial micro-colonies to various diffusible molecules, including autoinducers. Not only did they demonstrate that 6 different stains can be patterned within the dextran-rich phase but also that the pipetted droplets respond to the inductive molecules differently, via expression of GFP or bioluminescence, that are dissolved in and diffuse through out the PEG solutions. The authors also proved in their study that ATPS systems offer many benefits and can be used as a bacterial suspension-based biosensor array.

Microfabrication and microfluidic technologies have been applied further to studying the QS natures of single cells that were cultured in small and confined volumes (58). However, more attempts are being made to investigate cell-to-cell communication at the single cell level using another class of technology. Therefore, printing technology can make it possible to investigate cell-to-cell communication at the single cell level and with high resolution, printing.

Printing technologies for spatiotemporal cell patterning

Printing technologies make it possible to investigate cell-to-cell communication at a single cell level and with a high degree of resolution. Pattern formation using genetically engineered bacterial cells was demonstrated by manually seeding two types of cells (sender and receiver cells) into different areas of a cell culture plate and allowing them to communicate with each other (59). However, to generate multi-cellular patterns or conduct multi-cellular cell-to-cell communication studies at a high resolution (at a single cell level), printing technologies appear very promising when compared to the microfabrication and microfluidics technologies introduced above because it enables researchers to form various, multi-cellular microbial patterns. Moreover, printing technologies offer the unique capability of spatiotemporally printing patterns of cells, both homogeneous and heterogeneous, in a controlled manner (51-53). Due to these great advantages and characteristics over typical miniaturized technologies, printing technologies has been widely employed to print live bacterial cells.

In 2004, Xu, *et al.* reported that commercially available inkjet printers could be used to form bacterial arrays in complex patterns (54). They printed 100 bacterial "colonies" within a 1 cm^2 area and produced arrays with cell density gradients on an agar hydrogel surface. Inkjet printing technologies have also been used to create yeast patterns on a solid phase plate containing nutrients and shown that various microbial patterns can be produced with a resolution of tens of micrometers (51). Although it was demonstrated that the inkjet printing technology holds the potential to generate bacterial cell patterns and colony arrays, which could be useful in cell-based biosensors and high throughput pharmacological screening, the publication used only a single bacterial strain. However, in 2007, Merrin *et al.* formed multi-strain bacterial patterns with a piezoelectric inkjet printer (53). They not only quantified the number of cells per droplet but also characterized the viability of the printed *E. coli* cells. After securing a overall high viability, greater than 98.5%, they demonstrated that the printing technology can be further applied to many multi-cellular systems. This successful demonstration opened a new avenue to investigate inter-communication, cooperation and competition between multiple strains. In addition to printing multiple species on a surface, Cohen *et al.* modified a consumer inkjet printer for the spatiotemporal printing of inducer or inhibitor molecules on a cell-spread agar surface for control of gene ex-

pression (52). They employed a bi-responsive *E. coli* strain, which responds both to glucose (inhibitor) and lactose (inducer), and then perturbed the signaling pathways of the strain (the *lac* operon) by adding lactose using the printer. Since they demonstrated that the induction and inhibition of the signaling pathway could be controlled over time and space, printing technologies were proven to be a more promising approach for cellular communication studies.

Recently, Choi *et al.* utilized a commercially available materials printer to print multiple cell patterns on a surface and then reported corresponding cell-to-cell communication results (60). They adopted three synthetically programmed cells, the "sender", "receiver" and "inverse receiver" from (56), and characterized the effect of initial seeding number of cells and distances on their intercellular communication. The fluorescent intensities from the receiver and inverse receiver were quantified to characterize the communication and based upon these values they found that with a greater initial sender seeding number, more AHL signaling molecules were produced. They also found that the distances between the cells are another important factor governing the cell-to-cell communication because they determine the diffusion and response time. This work, seemingly the first application of printing technologies to study microbial cell-to-cell communication, demonstrates the feasibility of printing technologies to produce various spatiotemporal patterns of live cells. As such, these technologies provide another means of co-culturing different types of cells and studying interactions thereof. As an example, these cells were patterned as shown in Fig. 2, illustrating the multi-cel-
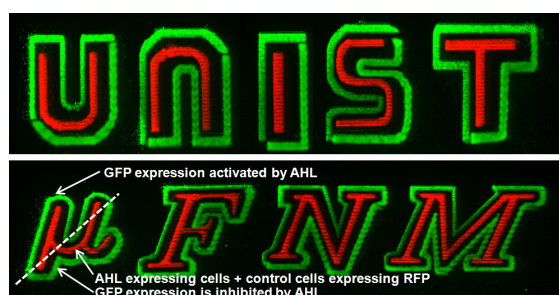
Fig. 2. Multi-cellular bacterial cell patterns produced by a materials printer (60). AHL-expressing cells that contain no fluorescence protein genes are printed together with control cells that constitutively express RFP to visualize their printed location. The AHL-expressing cells activate the cells printed in the upper left part of each letter to express GFP, resulting in brighter GFP signals. On the other hand, the cells printed in the lower right part of the diagonal line initially express GFP but their fluorescence intensities decrease with time because AHL molecules produced by and diffused from the AHL-expressing cells inhibit the expression of GFP. These cells were sequentially printed and then grown on an agar surface containing nutrients to demonstrate synthetic bacterial cell-to-cell communication. 'UNIST' stands for Ulsan National Institute of Science and Technology and 'μ*FNM*" for Microfluidics and Nanomechanics Laboratory at UNIST. The images are approximately 5.0 mm by 1.2 mm in size.

lular patterns that can be produced by a materials printer. Hence, printing technologies are a very powerful means to facilitate investigations into synthetic multi-cellular cell-to-cell communication (59, 61, 62).

In addition to non-contact printing technologies, micro-contact printing/stamping technologies have been developed to produce cell patterns using soft materials, like poly-dimethylsiloxane (PDMS) (63-65). Briefly, these technologies ink cells on the junctions and then write them onto another surface so that they enable the patterning of cells at the single cell level and with a high resolution (64). Among these methods the stencil method provides a means to co-culture two types of cells, allowing researchers to investigate cell-to-cell interactions and communication. However, to date, this method has not been widely applied to microbial cell-to-cell communication although similar methods have been used to form microbial biofilm arrays (66).

Cell-to-Cell Communication and Synthetic Biology

Most of the physiological functions of an organism at the cell level are mediated by multiple, interlinking signal transduction cascades followed by the complex circuits of genetic and metabolic regulation. So far, quantitative and reductionist approaches adopted from physical science have shown a limited success in these inherently complex systems. To understand the emergent properties of a biological system based on the characteristics of its "parts", we need to understand the working of the parts, which is a founding theme of synthetic biology.

Synthetic biology is an emerging field of research that helps to program cellular behavior through rational design and the construction of biological components/parts. It provides valuable tools to uncover the principles of natural biological systems and at the same time to generate novel functions in a systematic manner. The ability to engineer living cells that perform a desired task using the synthetic biological systems holds a great promise for a wide range of applications, from therapeutics to green chemistry. From an engineering perspective, "eavesdropping" on the cell-to-cell communication has a tremendous potential in the rapidly growing field of synthetic biology since bacterial QS systems can serve as a versatile regulatory module that enables spatiotemporal control of gene expression coupled with the cell population.

Furthermore, synthetic cell-to-cell communication modules can enhance our understanding of the population driven cellular behavior in natural systems. They allow us to design and implement artificial cellular interactions that will tune the robustness of microbial populations. Natural bacterial QS mechanism can be used in a synthetic system to control gene expression and growth in a population of cells by tuning the signal between populations of cells (67, 68). The LuxR/I-type QS system has been well-characterized and is widely used in synthetic circuit designs (8, 69, 70). Fig. 3 lists a couple of syn-
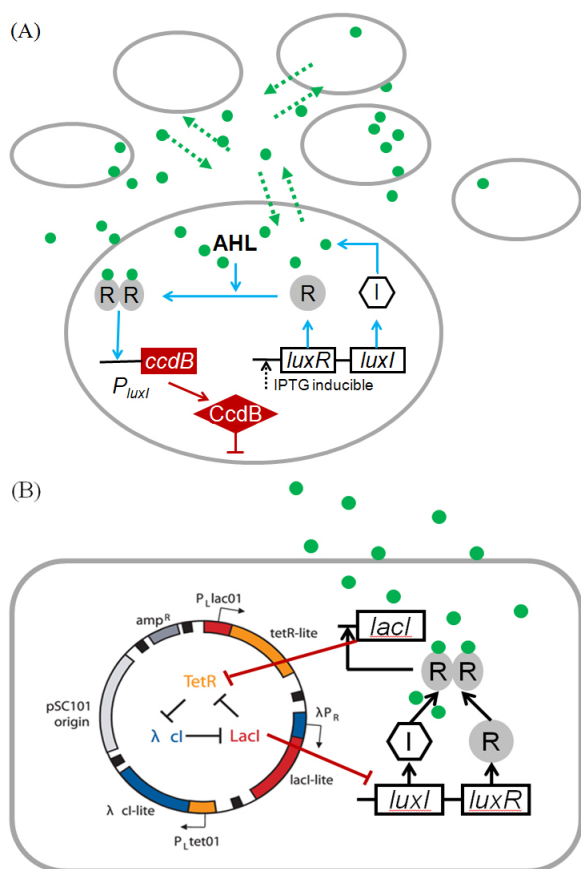
Fig. 3. (A) A population control circuit used by You *et al.* (4) programs population dynamics by broadcasting, sensing and regulating the cell density using QS network combined with the negative feedback control. *ccdB* is activated by LacR-AI dimer to kill susceptible cells by poisoning the DNA gyrase complex. More or less constant population density. *luxI* and *luxR* are induced by IPTG. Green filled circles represents autoinducer (AHL) molecules. (B) Repressilator circuit (left) coupled by QS system (right) can be used as a multicellular clock (90).

thetic biological applications that have been realized so far.

Apart from the LuxR/I system there are diverse natural communication modules yet to be discovered. Some recently discovered systems, like the oligopeptide-based QS system (71), need to be explored in depth for any practical applications. Bacterial QS regulatory modules can serve as building blocks for combinatorial synthesis of genetic circuits. For instance, recent advances in the engineering of light-inducible biological parts and systems make it possible to characterize optical-based circuits, the spatio-temporal control of gene expression, and increases the number and type of tunable knobs that are available to synthetic biologist (72-74). Levskaya *et al.* have devised a light-inducible genetic circuit in *E. coli* by fusing a light sensing domain of cyanobacterial photoreceptor with the intracellular histidine kinase domain of osmoregulatory *envZ*

gene of *E. coli* (75). This chimeric receptor is able to regulate transcription from the OmpR-dependent *ompC* promoter in the presence/absence of light.

Microbial consortia are symbiotic in nature, particularly in environments where more complex functions are difficult or even impossible for individual strains or species. Although not strictly "communicating", these systems do share biomolecules between the members of the population. As such, a synthetic consortium mimicking the microbial consortium is an attractive platform for synthetic biologists. For example, Eiteman *et al.* constructed two substrate-selective strains of *E. coli*, one which is unable to consume glucose and one which is unable to consume xylose, and showed that xylose and glucose are consumed more quickly by combining these two strains in a single process than that obtained with a single population of cells (76, 77). The efficient and simultaneous metabolism of pentoses and hexoses is one of key economic bottlenecks in the generation of fermentation products from biomass hydrolysates. Mixed culture fermentation with substrate specific strains provides important clues to solving this problem. Another major advantage of using a microbial consortium is their ability to simplify the performance of a multi-step process. Multi-step tasks can be accomplished by using dedicated cell-types at each step. For example, some plant enzymes for alkaloid synthesis are not necessarily expressed in bacteria in an active form but are active when they are compartmentalized in the cytosol and endoplasmic reticulum within the yeast cell. Minami *et al.* used a two-step synthesis of alkaloids with *E. coli* producing reticuline from dopamine and Saccharomyces cerevisiae producing various types of benzylisoquinoline alkaloids from reticuline (78). Such applications require greater overall understanding of both the dynamics and stability of the mixed populations. Aided by the synthetic consortia, the behavior of individual microbial population can be reprogrammed to perform the specific function. For the production of drugs and fuels, engineering synthetic consortia is an important new frontier in order to optimize their ability to persist and withstand environmental fluctuations.

Synthetic biology and quorum quenching
The term quorum quenching (QQ) was coined to describe numerous enzymatic (32, 38) and non-enzymatic (79, 80) signal interference mechanisms, which lead to the disruption of QS, thereby preventing or limiting colonization (81). Some animals and plants have evolved multiple defense mechanisms against bacterial pathogens which are mostly to interfere with the QS system (82). QQ has recently been proposed to be used as a drug target. One of the most attractive features of the signal interference approach is to allow the host to gain valuable time to activate defense reactions before the elimination of the pathogenic invaders. Lesic *et al.* demonstrated that analogues of QS molecules of *P. aeruginosa*, 2-alkyl-4(1H)-quinolone (AQ), such as methyl-anthranilate or halogenated derivatives of anthranilate, have been shown to inhibit AQ synthesis due to
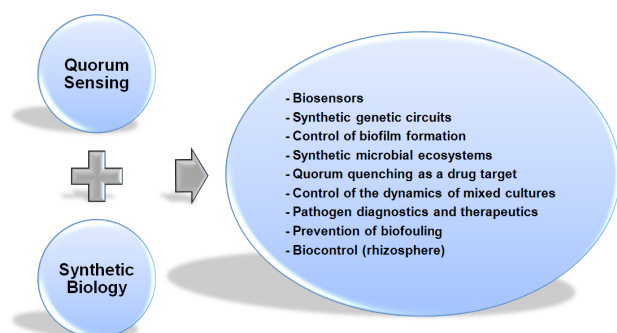
Fig. 4. Microbial QS system built on the synthetic biological parts or modules lends itself for a wide array of genetically engineered systems.

their interference with the signaling system by acting as competitive inhibitors of PqsA (the first enzyme of the 4-quinolone synthesis pathway) and result in a limited systemic proliferation of *P. aeruginosa* infections in mice (83). Additionally, natural and synthetic QS inhibitors have been used to prevent the formation of harmful bacterial biofilms (84) that might lead to contamination, colonization, and corrosion of machine parts exposed to water. QQ molecules have also been used in engineering plants that are resistant to infection by common pathogens, like *Erwinia carotovora* (85). Interestingly, environmental factors can influence the QS responses as well since AHLs are sensitive to elevated temperature (86) or alkaline pH (30). More recently, Byers *et al.* found that the half-life of N-(3-oxo) hexanoyl-HSL (3O, C6-HSL) was 30 min at pH 8.5, while it was seven hours at pH 7.8 (87).

Microbial ecosystems have increasingly attracted our attention for their important roles in the ongoing formation and maintenance of the Earth's biosphere. It also has great application in the industrial production of value-added products. The communication network within and between organisms isn't completely explored due to the complexity and diversity of the natural microbial ecosystems. The recent progresses in synthetic biology show that the construction of an artificial ecosystem where relationship between each species is comparatively clear could help us further understand complex interactions between the environment and the microbial ecosystem. Hu *et al.* (88) designed, simulated and constructed a synthetic ecosystem where various population dynamics are formed by changing the environmental factors such as antibiotics levels and initial cell densities. They showed that changes in any two factors can result in correlated population dynamics such as extinction, obligatory mutualism, facultative mutualism and commensalism. Furthermore, Bulter *et al.* (89) constructed a novel cell-to-cell communication system using artificial gene and metabolic networks where acetate acts as a communication signal.

As covered above, these mechanisms are currently being dissected and utilized through the discipline of synthetic

biology. Fig. 4 summarizes the ongoing research objectives emerging from the convergence of QS and synthetic biology. Clearly many areas of research can benefit from the marriage of these two fields. As such, *de novo* synthesis and the tuning of the parts responsible for signal processing (generation, detection, and amplification) require a quantitative knowledge of the dynamics of the genetic circuits in molecular detail (91, 92), based upon which scalable functional modules can be built and utilized in various population control systems.

Summary and Outlook

As discussed in this review, microbial cell-to-cell communication plays a central role in the coordinated multicellular behavior of microbial communities. Despite its high relevance to wide range of applications as well as to fundamental biological processes (Fig. 4) and the number of articles published, understanding of the QS systems and other chemo-communication mechanisms within bacterial cultures remains limited. Though it is agreed upon by all groups that microbes generate chemical signals to modulate their gene expression in the population level, even the basic precepts of QS are under debate, with the ideas of diffusion and efficiency sensing being proposed as a replacement concept.

However, thanks to the recent progress made in the fields of microfluidics, inkjet printing, and synthetic biology, the construction and characterization of different QS modules has become more feasible. As reviewed in this article, the use of miniaturized and printing technologies, combined with micro-chemostat technology, provides unprecedented experimental methods for manipulating cell-to-cell communication and sheds light on single cell- and population-level information processing. They also minimize evolutionary instability in such systems by shrinking the population size of the cells needed for each study. Another promising avenue is to study these mechanisms and apply them through synthetic biology. Under the umbrella of this discipline, researchers are trying to develop and archive standard biological parts that can be assembled with other functional gene circuits, such as bi-responsive switches, oscillators, or homeostatic devices, to form integrated biological circuits based upon chemo-communicative pathways. The expansion of cell-to-cell communication in this direction offers many exciting downstream applications, including a variety of genetic control circuits combined with diverse reporter genes that can be used for controlling and monitoring the expression levels (93).

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