Trends in Microbiology

CellPress REVIEWS

Review

Synthetic Biology for Manipulating Quorum Sensing in Microbial Consortia

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Bacteria exist as communities in diverse multispecies environments. Quorum sensing, a process for cell–cell communication, allows individual bacteria to glean information about their surroundings and coordinate activities with their neighbors. Recent studies indicate the importance of quorum sensing in microbiomes, but many questions remain regarding how quorum sensing may influence the composition and function of these communities. Synthetic biology, a field where scientists seek to design biological systems with predictable behavior, may provide tools to probe and manipulate quorum sensing behavior in natural consortia. In parallel, quorum sensing processes can be used as a tool in synthetic biology to construct synthetic cocultures with desired behavior. Here, we review recent synthetic biology strategies for manipulating quorum sensing processes in microbial consortia.

Highlights

In nature, microbes exist in diverse communities where interactions amongst and between species contribute to the makeup and function of the community. A cell–cell communication process called quorum sensing is often important in these complex communities.

Synthetic biology can be used to manipulate quorum sensing processes in natural microbiomes for positive health or environmental outcomes.

Knowledge of quorum sensing processes can be applied to design synthetic consortia. These synthetic ecosystems can be used for manufacture of molecular products or to study social interactions in well-defined systems.

Synergy between Quorum Sensing (QS) Research and Synthetic Biology

Although bacteria are unicellular organisms, it is now well understood that they are social and display population-based behavior. Cell–cell communication can occur through QS. In this process, bacteria secrete signaling molecules called autoinducers that accumulate in the extracellular milieu as the local cell density increases. Once a threshold autoinducer level is reached, the autoinducers alter gene expression within the cells collectively across the population. Early investigations of QS primarily consisted of growing a single strain in standard (well-mixed) laboratory conditions [1,2]. Many recent studies, however, focus on the impact of QS between species and within consortia. The rapid expansion of microbiome research is likely to further reveal the impact of QS in natural environments.

Research on QS, in addition to revealing important fundamental science, was also instrumental in the early years of synthetic biology [3]. Synthetic biologists seek to design biological systems with programmable or predictable behavior. A hallmark of the field is the incorporation of engineering design principles. Many early studies in synthetic biology made use of QS circuits for programming cell behavior [4,5] and QS processes have continued to be a staple for synthetic biologists when creating synthetic circuits. This interest in QS by synthetic biologists lead to many QS processes and the parts (i.e., promoters, genes, and proteins) that make up those processes being well characterized with a focus on understanding ways to precisely and predictably manipulate responses to QS molecules. That is, QS and synthetic biology research have been highly complementary, with QS research expanding the synthetic biology toolkit and synthetic biology providing new tools for investigating QS. In recent years, both of these fields have been shifting focus away from research using single strains towards consortia and microbiome research.

In this review we discuss ways in which synthetic biology can be used to manipulate QS processes in microbial consortia. We first cover ways in which synthetic biology tools have been used to manipulate signal transduction and cell response to autoinducers. Then, we discuss

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how synthetic biology can be used to manipulate and interrogate QS processes in microbiomes and to create synthetic consortia.

Synthetic Biology Provides Tools to Manipulate QS Signal Transduction and QS-Mediated Cell Phenotypes

Several types of QS systems have been discovered. Here, we cover the AI-1 or acyl-homoserine lactone (AHL) and AI-2 QS systems. We give brief backgrounds on the genetic and molecular pathways that make up each QS process and then focus on examples of how these processes have been manipulated in order to engineer cellular responses.

AHL

The AHL systems are perhaps the most well-known QS systems. The process was originally discovered in the marine bacterium *Vibrio fischeri* and found to control luminescence production [6]. In *V. fischeri*, LuxI synthesizes the AHL molecule, which is able to freely diffuse across the cell membrane (Figure 1). AHL level increases as cell density increases, and once a threshold of AHL is reached, the AHL molecule binds LuxR and the bound complex activates the bidirectional *lux* promoter. Promoter activity results in transcription of the luciferase genes *luxCDABE* and

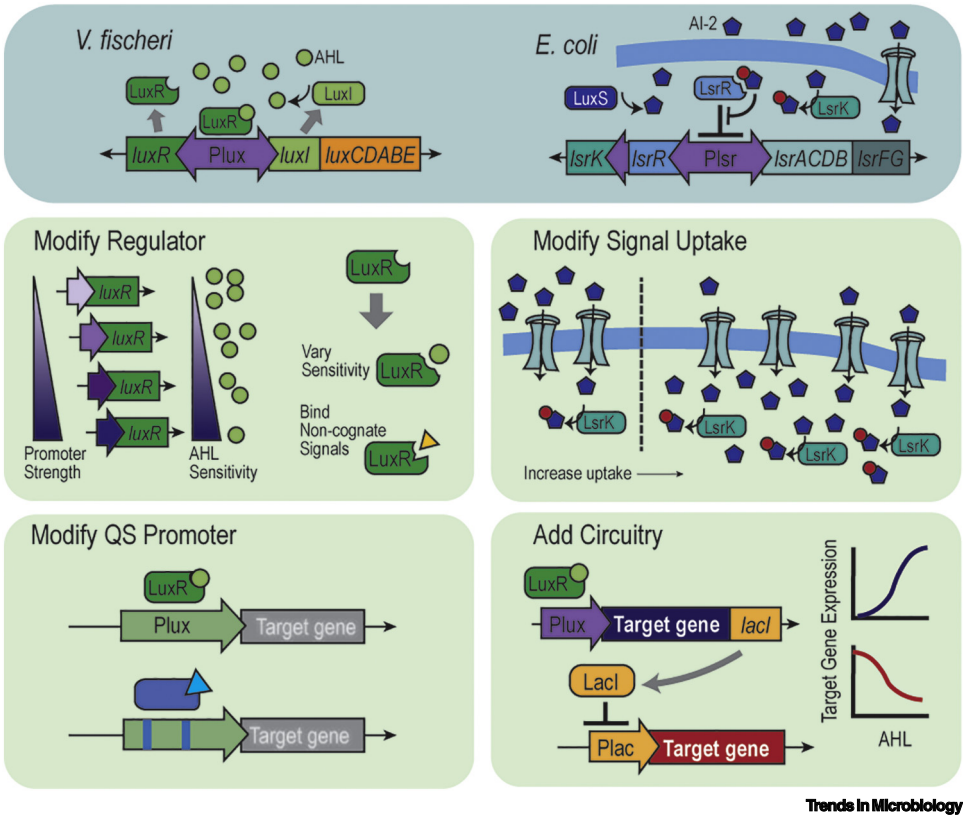

Figure 1. Synthetic Biology Allows Manipulation of Cell Response to Quorum Sensing (QS) Signals. The native acyl-homoserine lactone (AHL) and AI-2 QS pathways are shown in *Vibrio fischeri* and *Escherichia coli* respectively (top panel). Several strategies for manipulating cell response are illustrated (bottom panels). The regulator (LuxR) can be manipulated to control sensitivity to AHL signals, either by regulating transcription to control the number of copies of LuxR or by modifying the AHL binding site. Signal uptake can be altered by, for instance, increasing the number of copies of proteins involved in AI-2 transport and processing. The QS promoter can be mutated to alter response to the relevant autoinducer. Additional circuitry can be incorporated into the cell response to create varied or programmed responses to the autoinducer.

additional transcription of *luxI* and *luxR*. This positive feedback loop is a hallmark of many QS systems. Several homologous AHL systems have been discovered in other species, including *Pseudomonas aeruginosa* and *Vibrio harveyi* [7]. Each organism produces a different AHL molecule, usually differing in the length of the fatty acid chain. Generally, organisms only recognize the AHL molecule that they produce and so each AHL system is considered species-specific. In addition, several species use multiple QS signals and bacteria can use these to integrate multiple pieces of information, which is likely important in consortia or different niches (see reviews [1,8]).

Synthetic biologists frequently use the AHL QS systems. These systems require relatively few components, namely LuxR (or the homologous regulator), the AHL synthase, and the relevant promoter. AHL molecules cross the cell membrane without requiring specific transporters. These qualities allow the different components to be easily assembled in a range of hosts. The International Genetically Engineered Machines (iGEM) organization maintains a library of genetic 'BioBricks' that can be used to build synthetic circuits and the components comprising the AHL systems are amongst the most commonly used parts in the database (http://parts.igem.org/Frequently_Used_Parts).

Many efforts have been made to characterize responses to AHL and to engineer cells that respond to specific concentrations of AHL. This is frequently done by manipulating the regulator protein LuxR. For instance, Wang *et al*. expressed LuxR under a series of constitutive promoters with varying activity. The varied expression levels resulted in populations that detected different ranges of AHL, with high constitutive expression of LuxR resulting in cells with the most sensitivity to AHL [9]. Alternately, directed evolution of the regulator can change the sensitivity to its cognate AHL [10] or increase its sensitivity to noncognate AHL molecules [11]. Shong and Collins mutated an AHL responsive promoter by adding an additional binding site for the regulator in different locations [10]. This resulted in varied promoter activities and in one case reversed the effect of adding the AHL molecule. A mathematical approach can be used to rationally engineer QS cell response. For instance, Zeng *et al*. developed an approach that combines network enumeration with parameter optimization, incorporating known information on biological parts to design an ultrasensitive QS switch [12].

The AHL systems are frequently used by synthetic biologists to engineer cells where the phenotype is dependent on cell density. For instance, You *et al*. engineered AHL-producing cells where AHL activated a toxin within the cell, resulting in programmable stationary phase cell density [4]. Liu *et al*. linked chemotaxis with cell density for patterned behavior [13], and Swofford *et al*. engineered *Salmonella* that turn on gene expression in tumors where they accumulate at higher density than in other organs [14]. QS has also been used by metabolic engineers to autonomously redirect cell metabolism at a certain cell density [15,16]. Gupta *et al*. engineered cells that produced the AHL signal at different rates, with higher rates of AHL production causing the metabolic switch to occur at lower cell densities [16]. They then selected the strain with the highest titers for the desired product. Additional genetic circuitry is also frequently added to develop more complex phenotypes [17–19]. Basu *et al*. engineered a system where the cells fluorescence only at medium concentrations of AHL and not low or high concentrations [5]. Danino *et al.* used QS to synchronize a genetic clock amongst the cells in a culture [20]. Andrews *et al*. engineered a system for sequential or check-point controlled activation of target genes, using AHL QS components along with other small molecule induction systems [21].

AI-2

Unlike the AHL QS systems, the AI-2 QS system is used by multiple species. LuxS synthesizes AI-2 as a byproduct of the activated methyl cycle. In *E. coli* (Figure 1), AI-2 is imported into the cell by

the transporter LsrACDB [22]. It is then phosphorylated by the LsrK kinase. Phosphorylated AI-2 binds the repressor LsrR and relieves repression of the bidirectional *lsr* (luxS regulated) promoter. This causes transcription of the *lsr* operon and overexpression of LsrACDB, LsrK, LsrR, and LsrFG, leading to rapid uptake of AI-2 and depletion of AI-2 from the extracellular media. LsrFG eventually metabolize the AI-2.

Due to the relatively complex signal transduction process and the fact that AI-2 does not diffuse across cell membranes [23], several more components are required for reconstructing the AI-2 system compared with the AHL systems. However, the additional complexity allows for multiple control points to regulate cell response. Overexpressing or deleting specific genes in the cascade can result in interesting dynamics. For instance, in a clonal population of *E. coli*, only a subset of the culture responds to AI-2. Deleting *lsrFG* from the genome, however, causes cells to be more sensitive to AI-2 and also changes the fraction of the population that responds to AI-2 [24]. It was also found that overexpression of LsrACDB or LsrK leads to rapid uptake of AI-2 from the extracellular environment and decreased variability in cell response across the population [25]. Combining these two strategies lead to a suite of cells with varied responses to AI-2 [26].

The AI-2 receptor may also be an avenue for manipulating cell responses to AI-2. AI-2 is derived from 4,5-dihydroxy-2,3-pentanedione (DPD), and can spontaneously cycle among a collection of molecules all known as AI-2. In *Vibrio* species, LuxP is responsible for initiating cell responses to AI-2 via a phosphorylation cascade, while in *E. coli* and *Salmonella*, LrsB binds AI-2 and initiates transport via an ABC-like transporter. These two receptors, importantly, bind different forms of AI-2. Recently, it was found that *Clostridium saccharobutylicum* has an LsrB-like receptor in which the binding site for AI-2 contains amino acid variations from what was previously determined to be critical for AI-2 binding, which may lead to discovery of additional species with ability to bind AI-2 [27]. This same study revealed that *C. saccharobutylicum* begin to uptake AI-2 at a lower AI-2 threshold than *E. coli*. This difference in AI-2 uptake, along with the ability of different organisms to respond to different forms of AI-2 is interesting when considering these species may exist together in medically important niches. It may also provide avenues for synthetic biologists to manipulate the AI-2 signal in a given environment in ways that affect certain species more than others [28].

The *lsr* promoter in *E. coli* is also sensitive to carbon catabolite repression and is not active when glucose is present in the media [29]. It's been understood for many years that the promoter contains cAMP–cAMP receptor protein binding sites. However, it was also more recently shown that a cytosolic phosphotransferase system protein involved in sugar transport, HPr, can post-translationally regulate AI-2 QS [30]. HPr can bind LsrK and lower LsrK activity. The phosphorylation state of HPr, which is indicative of glucose transport, determines how effectively HPr binds LsrK. Others have similarly demonstrated a strong link between metabolism and AI-2 QS [31]. These results present challenges when engineering cells using the AI-2 system. That is, the cells may behave drastically differently depending on whether the media contains glucose. Zargar *et al*. showed that by decoupling expression of the AI-2 uptake and processing genes from the *lsr* promoter, cells could be engineered to uptake AI-2 even in media containing glucose [32]. HPr mutants are also able to uptake AI-2 in the presence of glucose [30], as are catabolite repression insensitive strains [31].

Another challenge of using the *lsr* system is that the promoter is relatively weak. This has been overcome by coupling the *lsr* promoter with the strong T7 expression system [33]. T7 RNA polymerase is placed under the *lsr* promoter and activates expression of the gene of interest under the T7 promoter. Alternately, directed evolution of the promoter showed that mutations in the region thought to be regulated by cAMP lead to higher promoter activity [34].

The aforementioned works illustrate how synthetic biology techniques can be used to understand the function and relative importance of specific components of the AI-2 QS pathway and to manipulate AI-2 levels or cell response to AI-2. Several mathematical models have also been developed from these results to predict and understand AI-2 QS [35–38].

Manipulating QS in Microbiomes Using Synthetic Biology

It is likely that QS plays an important role in niches where microbial communities exist, such as the gastrointestinal (GI) tract or plant microbiome. While experiments with single strains in shake flasks have been useful in identifying the molecular pathways that make up QS processes, it is likely that QS processes play as yet undiscovered roles in these complex niches. Synthetic biology may provide tools for understanding both the role of QS in microbiomes and for manipulating QS processes for positive health outcomes (Figure 2). Here we discuss recent studies indicating the importance of QS in natural consortia and preliminary studies that use synthetic biology to manipulate QS processes in these environments.

GI Tract

The microbiome of the GI tract is an exciting area of research due to its importance for human health. The human GI microbiome is important for nutrient and drug adsorption and digestion [39,40], is the site of many pathogenic diseases, and has even been shown to affect depression and mood through its connection to the central nervous system [41]. Although it is understood that commensal bacteria are important for a healthy microbiome, there are many open questions about how the microbiome community forms and how or why the microbiome community becomes dysregulated. It is currently unclear the extent to which QS plays a role in these processes. Thompson *et al*. showed that AI-2 may play a role in the composition of the gut microbiome [42]. They engineered *E. coli* to either overproduce AI-2 (by eliminating the ability for uptake) or not produce AI-2 (through elimination of the AI-2 synthase) and showed that treatment with these two strains in the antibiotic-treated mouse changed the resulting composition of the species in the mouse gut. AI-2 shifted the relative levels between Bacteroidetes and Firmicutes phyla. Another study suggested that probiotic *Bacillus* can interfere with QS systems and prevent pathogen infection [43]. These early investigations show potential for manipulating the microbiome using engineered bacteria that can manipulate QS processes within the gut. Many questions remain, however. There is currently not a clear idea of autoinducer levels in the gut and whether these levels vary drastically spatially, over time or naturally, from person to person. Potentially, synthetic biology could be used to probe for these signals in order to begin to answer these basic questions.

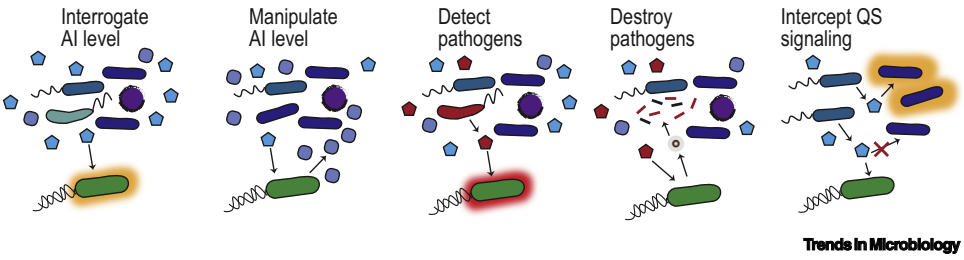

Figure 2. Synthetic Biology for Manipulating Quorum Sensing in Microbiomes. Cells can be engineered to operate in native environments as diagnostic or therapeutic vehicles. As depicted, these strains can be designed to read varied autoinducer (AI) levels and respond with a measurable output (such as fluorescence). They can manipulate autoinducer levels by uptaking or secreting specific signals. Engineered cells can detect autoinducers from pathogens and respond by releasing molecules to destroy the pathogen. They can also intercept cell–cell communication that may be occurring between different strains by uptaking the relevant signal.

Synthetic biology can also be used to probe for pathogens or halt infections from pathogens that rely on QS to initiate virulence. *Vibrio cholerae*, for instance, uses multiple QS systems to control production of virulence factors and biofilm formation and dispersal [44,45]. At low cell density, *V. cholerae* attaches to the intestinal wall and produces virulence factors. At high cell density, *V. cholerae* disperses. Duan *et al*. engineered a commensal *E. coli* strain, Nissle, to overexpress CAI-1, the *V. cholerae*-specific autoinducer [46]. They showed that prophylactic treatment with this Nissle strain reduced *V. cholerae* virulence and cell number in the infant mice model. More recently, Mao *et al*. used a probiotic strain that naturally interrupts the *V. cholerae* QS process to prevent infection [47]. They also engineered their strain to report on *V. cholerae* autoinducer CAI-1 as an early detection of *V. cholerae*. Another strategy is to design sense-and-kill bacteria that detect QS molecules produced by pathogens and then produce molecules (often released by cell lysis) that kill the pathogen [48–50]. Hwang *et al*. showed their engineered probiotic strain could treat *P. aeruginosa* infections in the mouse gut [48]. A different strategy is to use phage. Silpe *et al* recently showed that a *V. cholerae* phage encodes a homologous receptor for a *V. cholerae* autoinducer [51]. Interestingly, the phage-encoded autoinducer receptor can activate the relevant promoter in both the phage and *V. cholerae* genomes. However, the *V. cholerae* receptor cannot activate the promoter in the phage genome. The authors then used this information to design a species-specific kill switch for *V. cholerae*.

Synthetic biologists have also begun to investigate QS for engineering cell–cell signaling in the gut. Kim *et al*. engineered bacteria that can secrete and respond to AHL molecules within the mouse gut [52]. Sedlmayer *et al*. engineered mammalian cells that are able to detect autoinducers and interfere with QS controlled processes in microbes [53,54]. While these studies were completed *in vitro*, they suggest opportunities to use QS for interkingdom communication.

Oral and Skin Microbiomes

Studies of the human microbiome thus far have predominately focused on the GI tract. However, research shows that the oral and skin microbiomes play a role in human health as well. QS is important in these communities. *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* (*A. act*) are both associated with dental cavities. *A. act* was found to induce the QS regulon of *S. mutans* through an unknown mechanism in dual species biofilms grown on saliva [55]. Further, the authors found this only occurs when the species are cocultured and does not occur if *A. act* are cultured separately and cell free conditioned medium added *to S. mutans*. Muras *et al*. studied the effect of exogenously supplied AHL molecules to *in vitro* biofilms resembling the oral microbiome and found that some of the signals changed cell metabolism and shifted the consortia composition [56].

The makeup of the skin microbiome is also thought to be a contributing factor to various diseases, including the common skin disease atopic dermatitis, although direct causes are still generally unknown. *Staphylococcus aureus* is often associated with atopic dermatitis. Williams *et al*. showed that *S. aureus* QS-controlled protease production, which contributes to skin inflammation, could be inhibited by a peptide secreted by *Staphylococcus epidermidis* [57].

Plant Microbiome

Plant microbiomes affect plant health and crop yield. As environmental concerns about pesticide and land use related to crops increase, forward engineering microbiomes that promote plant health is a new and potentially promising approach to reducing the environmental impacts of agriculture [58,59]. QS plays a role in plant microbiomes and there is potential to positively impact plant health by monitoring or manipulating QS processes in these communities. In crop microbiomes, some pathogens rely on QS for virulence factor production and community interactions can inhibit or aggravate virulence. *Lysinibacillus*, a soil bacterium, can attenuate virulence

from the pathogenic species *Pectobacterium carotovorum* by degrading AHL signals and interrupting QS processes of the pathogen [60]. By engineering overexpression of the AHL degrading enzyme, the authors were able to inhibit virulence of the pathogen. In another example, Valente *et al*. showed that crosstalk between species could actually cause virulence of plant pathogens [61]. That is, they found autoinducers from *P. carotovorum* could induce virulence in the pathogen *Pectobacterium wasabiae*. Engineers have also begun to use QS to engineer commensal bacteria with desired, density-dependent behavior. Zuniga *et al*. engineered the rhizobacterium *Cupriavidus pinatubonensis* with autoinducer-regulated production of indoleacetic acid [62]. In this way, the bacteria autonomously produced indoleacetic acid at the appropriate time (i.e., a specific bacterial cell density) to promote plant growth.

QS may also be important for facilitating interactions between species in other ecologically important microbiomes. For instance, in the coral microbiome, the microbiome composition is different in communities affected by blank band disease and this may be in part due to differences in QS between communities with and without the disease [63]. Cyanobacteria prevalent in the disease state can produce the metabolite, lyngbic acid, which is able to inhibit QS in *V. harveyi* and may contribute to the disease.

Creating Synthetic Consortia Using QS Processes

QS provides an opportunity to design sophisticated or robust synthetic microbial communities. The synthetic consortia can be used to explore social behavior in complex ecosystems in order to make hypotheses or even draw conclusions about natural consortia [64,65]. Metabolic engineers are also interested in using cocultures and multipopulation systems for production of molecular products [66,67]. Often products synthesized from complex pathways cannot be produced in high titers from pure cultures of single strains. This can be due to a high metabolic burden, or sometimes one part of the pathway inhibits a different part of the pathway. Use of cocultures can alleviate many of these issues, but introduces the additional challenge of regulating the behavior of individual populations and their composition within the consortia. Here, we discuss how engineers can use QS to construct synthetic consortia. Rewired QS circuits can be used to coordinate gene expression between subpopulations, control consortia composition, or enable communication between distant cell populations (Figure 3).

Controlling and Coordinating Gene Expression

QS can be used to autonomously control or to coordinate gene expression within cocultures or consortia. For instance, QS can be used to engineer cocultures where the two populations express target genes only when cultured together [68,69]. Others have engineered artificial cells that can send and receive signals to and from bacteria [70–72] and others have created Gram-negative *E. coli* that are able to communicate with Gram-positive *Bacillus megaterium* [73]. Terrell *et al*. engineered a coculture made up of two strains that respond to different levels of AI-2 [64]. Each strain produced a different fluorescent protein in response to AI-2 and constitutively expressed a magnetic nanoparticle that allowed all of the cells to be collected after surveying a complex environment. Comparison of expression profiles of the two strains after collection resulted in a color 'pattern' and information about the environment surveyed by the cells. The cell network was able to detect AI-2 that had been secreted by *Listeria*. This work also demonstrated the close relationship between fundamental research on QS mechanisms and the potential for synthetic biology to be useful for studying and manipulating QS phenomena. The engineered design used in the manuscript relies on foundational knowledge of the AI-2 QS process and the final design allowed for sophisticated interrogation of AI-2 levels in different environments.

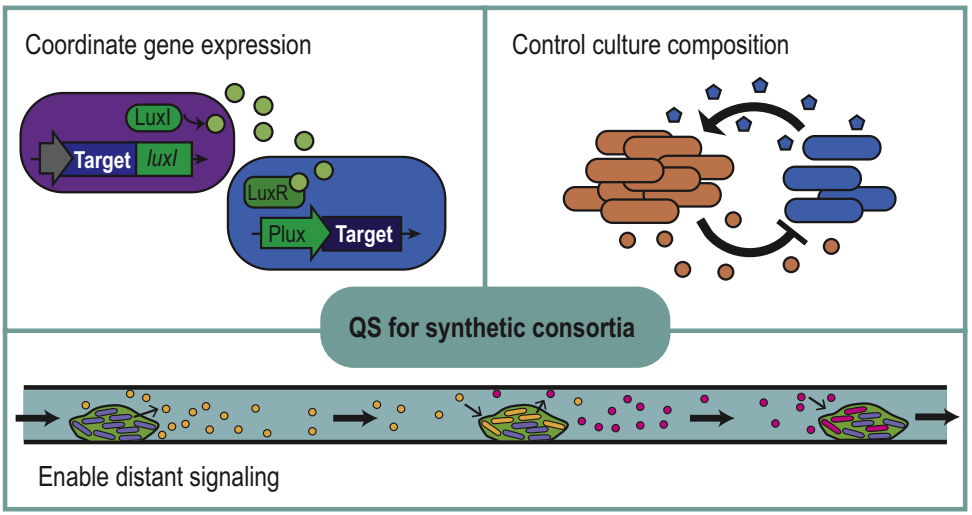

Figure 3. Quorum Sensing (QS) for Designing Synthetic Microbial Consortia. QS is used by synthetic biologists to design synthetic microbial consortia in order to coordinate cell behavior and allow for cell–cell signaling. QS can be used to coordinate gene expression (top left) between subpopulations. It is also used to control consortia composition (top right). This can be accomplished by using autoinducer production as a measure of cell density in one population in combination with autoinducer-regulated synthesis of a molecule that either enhances or inhibits growth in a second population. QS is also used to enable cell–cell communication over a distance (bottom), for instance between two populations located in different places in a microfluidic device.

A challenge in incorporating greater numbers of members or subpopulations within synthetic consortia is that many of the QS systems are not completely orthogonal. Studies that mix and match the different QS regulators, autoinducers, and promoters in *E. coli* have been conducted to characterize cell responses for a range of constructs [74]. Others have developed computational models to aid in designing systems using multiple QS signals in order to optimize signal-to-noise ratio and minimize crosstalk [75,76]. Interestingly, recently Wellington and Greenberg studied QS receptor sensitivity and promiscuity to different AHL signals in the native species and compared them with results in *E. coli*. They concluded that expressing AHL QS systems in non-native *E. coli* hosts, where the QS receptor is often overexpressed, leads to increased signal promiscuity compared to what occurs natively [77].

Controlling Consortia Composition

Consortia composition is a critical parameter in many synthetic coculture systems and methods for controlling composition may be beneficial. Stephens *et al*. designed a coculture where the composition of the coculture varies depending on the level of AI-2 in the environment [78]. The authors accomplished this through use of rewired QS pathways and through autoinducer-modulated cell growth rate. Growth rate of an individual strain in the coculture was modulated by controlling transcription of a sugar transport protein, HPr, using a species-specific AHL. Others have controlled cell density of individual populations or strains using autoinducer-controlled cell lysis [4,79,80] or production of toxins [81]. These strategies have been used to stabilize a coculture, preventing one population from outgrowing the other [79], and to create oscillating behavior [81]. Kong *et al*. created cocultures displaying a range of social behavior using small molecules that accumulate with cell density (as in QS processes) and activate genes that help or hurt growth of specific populations [82]. Wu *et al*. designed a coculture using QS that relied on mutualism to survive [83]. They used a model to describe the resulting conditions for survival or population collapse. Many studies have also been conducted using QS-component mutant strains that are able to 'cheat' on their

wild type counterparts by benefiting from, but not producing, QS-controlled public goods (see review [84]). Recently, Ozkaya *et al*. studied a *ΔluxR* cheater population of *P. aeruginosa* that causes population collapse when cultured with wild type cells [85]. Interestingly, adding a third *ΔpvdS* population, that was able to cheat on the *ΔluxR* cheaters, resulted in stable cultures.

Thus far, studies on controlling population density in engineered cocultures has primarily resulted in platforms to control culture composition or systems that mimic social behaviors (mutualism, competition, etc.). Questions remain about whether these strategies could be broadly applied by metabolic engineers. Honjo *et al*. used a QS-based technique to control a coculture for production of isopropanol [86]. The first population produced enzymes required to break down sugars in the extracellular media and then secreted a QS signaling molecule. After accumulation of the autoinducer (at a specific cell density), the autoinducer caused lysis of the first population, releasing the sugar-degrading enzymes and decreasing the composition of the first population in the coculture. A second population then was able to use the digested sugars to produce the target chemical.

Enabling Cell–Cell Communication between Localized Populations

QS systems are also used to enable cell–cell communication between distant populations. Luo *et al*. showed, in a microfluidic device, populations upstream could signal to populations downstream, even as modified by intermediate populations, all by modulating the QS signaling molecule as a function of distance [87]. Alternately, QS can allow for recruitment of one population to a specific location. For instance, Wu *et al*. engineered AI-2 synthases that dock to a specific locale (cancer cell receptor) and recruit a bacterial population to that locale [88]. The synthesized AI-2, which bacteria naturally chemotax towards, served both as a molecular beacon recruiting cells at one concentration and as a QS autoinducer altering gene transcription at another. Others have used QS to control microbial biofilms. Wood *et al*. engineered bacteria that prevent biofilm formation by other bacteria [89] and Hong *et al*. engineered bacteria that are able to cause dispersal of specific populations in a biofilm [90].

Concluding Remarks

Currently there is a high level of interest in understanding microbiomes and the interactions and contributions of individual members, along with an interest in forward engineering multipopulation systems and consortia. QS likely plays a key role in these consortia, both allowing members within the consortia to act in a population-based manner and by enabling interspecies and even interkingdom communication. However, many questions remain regarding how microbiomes form, how their compositions may shift over time, and how QS contributes to the composition and function of consortia (see Outstanding Questions). Synthetic biology provides tools to study and manipulate these processes, including in their native environments. Synbio constructs, if designed using native strains and with minimal alteration, can be used to eavesdrop on native environments and report on their findings. Such efforts represent a new strategy for influencing human health, agriculture, and the environment. At the same time, QS processes are being used by synthetic biologists to design and assemble synthetic consortia composed of discrete subpopulations that communicate amongst each other and work together to achieve a designed objective function. This strategy will surely extend the capabilities of systems currently using single strains. In summary, research on the role of QS within microbiomes and the use of QS to build synthetic consortia are still in the early stages of development, with many exciting avenues for exploration and high potential to influence human health and the environment.

Outstanding Questions

How do QS processes vary in diverse environments?

Can QS or interferences with QS processes alter the composition and subsequently the function of natural microbiomes? Preliminary studies indicate that this may be the case. However, questions remain. How and why does this happen?

Can synthetic biology provide tools for manipulating QS in natural microbiomes? Cell-based systems can be designed to manipulate QS signaling in predicable manners in the lab. It remains to be seen whether these systems will be robust enough to perform similarly in natural microbiomes where the environment (nutrients, etc.) may be unknown and variable. If they can be engineered to perform robustly, will manipulating QS processes change health outcomes for the host?

Can synthetic biologists use quorum sensing to design synthetic cocultures or consortia that behave predictably over long periods of time? Can these systems be applied by metabolic engineers for manufacture of valuable products?

Acknowledgments

Partial support of this work was provided by the Defense Threat Reduction Agency (DTRA) (HDTRA1-19-0021), National Science Foundation (NSF) (DMREF #1435957, ECCS#1807604, CBET#1805274), and the National Institutes of Health (R21EB024102).

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