Review

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Control of bacterial metabolism by quorum sensing

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Bacterial quorum sensing (QS)-dependent gene expression is a dynamic response to cell density. Bacteria produce costly public goods for the benefit of the population as a whole. As an example, QS rewires cellular metabolism to produce oxalate (a public good) to enable survival during the stationary phase in *Burkholderia glumae, Burkholderia thailandensis,* and *Burkholderia pseudomallei*. Recent reports showed that QS serves as a metabolic brake to maintain homeostatic primary metabolism in *B. glumae* and readjusts the central metabolism of *Pseudomonas aeruginosa*. In this review, we emphasize the dynamics and complexity of the control of gene expression by QS and discuss the metabolic costs and possible metabolic options to sustain cooperativity. We then focus on how QS influences bacterial central metabolism.

Dynamics and complexity of coordinated gene regulation in a cooperative population

QS is a system controlling the expression of groups of genes in a cell-density-dependent manner. Thus, the bacterial lifestyle is no longer individualistic, but rather social [1,2]. Many species of Proteobacteria feature a QS system controlled by *N*-acyl-homoserine lactone (AHL) and a LuxR-type regulator; this system is the QS paradigm [3–5]. Many research articles and reviews have discussed how QS bacteria control gene expression, rendering such bacteria social in nature [6,7]. Many authors have explored QS-dependent differential gene expression and the accompanying phenotypic changes [8–12]. Mechanistic and phenotypic studies on QS have concluded that QS controls bacterial cooperativity (see Glossary); again, the organisms are social in nature [13–16].

The coordination of cell-density-dependent gene expression may not be as straightforward as originally thought. The long-standing question is: how do bacterial cells coordinate gene regulation at different cell densities? It is conceivable that temporal control of QS-dependent genes plays a role. Systematic explorations of the dynamic nature of gene expression in a cooperative population have been conducted in some studies [17–23]. Interestingly, many QS-dependent genes are either activated or repressed after the mid- or late-exponential phase of growth rather than at earlier times [17–23]. This is important because cell densities of 1×10^8–1×10^9 cells/ml, which may be attained in the early exponential stage, are high [22]. The control of target genes by QS seems to depend on both the concentrations of QS signaling agents and the growth stage as observed in *P. aeruginosa*, *Pectobacterium atrosepticum*, *B. glumae*, and *B. thailandensis* [17–23], which reflects the dynamics of QS-mediated gene regulation. The take-home message of these studies is that the physiological status of bacterial cells at certain growth stages can be integrated into the gene regulation circuits operated by QS.

Other factors, including small RNAs (sRNAs) of *Vibrio* species and *P. aeruginosa*, nutritional conditions, and multiple QS systems, may also influence the dynamics of QS-dependent gene regulation in bacteria in which QS is mediated by AHL [6,24,25]. The modulation of QS activity is mediated by sRNAs and the chaperone Hfq in *Vibrio harveyi*, *Vibrio cholerae*, and *Sinorhizobium meliloti* [26,27]. Hfq mediates interactions between sRNAs and their specific mRNA targets in *V. harveyi* and *V. cholerae* [26]. It has been demonstrated that Qrr sRNAs modulate regulatory circuits to optimize the dynamics of QS in *V. harveyi* [24]. The modulation of QS in *S. meliloti* is achieved by interactions between Hfq and transcripts of *expR* encoding a receptor for a long-chain AHL [27]. In legume-nodulating rhizobia, QS-dependent regulation is highly diverse between species and strains, probably due to differences in rhizobial ecology and physiology in the rhizosphere [28]. Other examples of QS modulation include nutritional limitations and changes in the membrane properties of *P. aeruginosa* [29] and the antiactivation of QS in *Agrobacterium tumefaciens* and *Mesorhizobium loti* [30,31]. In *P. aeruginosa*, nutrient starvation preferentially induces the *rhl* system, with the *las* system appearing to respond predominantly to AHL signal accumulation, whereas the *rhl* system integrates nutritional cues. This may explain why one organism needs to possess multiple QS systems with split functions [6].

It is therefore becoming clear that QS is much more dynamic than previously thought, and that traditional

Glossary

Cooperativity: a social behavior that gives a benefit to individuals in a population by producing common good.
Cheaters: individuals who do not cooperate but who obtain benefits from the cooperators.
Defectors: individuals who do not produce costly public goods as a cooperative activity.

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bacterial physiology should be re-evaluated from the perspective of population biology to allow us to better understand metabolic fluctuations as functions of cell density, growth phase, and nutritional conditions. QS may control not only transcription but also post-transcriptional steps. Multifaceted mechanisms, including positive or negative regulation by transcriptional regulators and noncoding RNA, may need to be invoked to explain the dynamic nature of QS-dependent metabolic fluctuations. We propose that additional systematic analyses should be performed to determine the dynamic nature of gene regulation, taking into consideration cell density, growth stage, nutritional conditions, other genetic regulators, and ecological niches in cooperative populations.

The metabolic costs of cooperativity

Cooperation among cells in a monogenic population is required if individuals are to be successful and the population is social in nature [13–16]. In bacteria, it is accepted that QS controls cooperativity [6,7,14]. Bacteria engage in the QS-dependent production of a variety of public goods that are shared by all members of the group [7,13,14]. Density-dependent gene expression and the cooperativity required for the production of public goods might burden individual QS bacteria with metabolic expenses; these are the costs of a social existence in a cooperative population. However, it is not clear how bacterial cells manage metabolic costs to maintain cooperativity and metabolic homeostasis. The costs and benefits of exoenzyme production in terms of growth rate have been evaluated in *Escherichia coli* expressing a synthetic QS system. However, the direct costs of QS-controlled cooperativity have not been assessed [32].

Some basic questions regarding the metabolic expenses of QS arise. First, how do individuals within the group determine the optimum levels of public goods under various culture conditions? This issue is directly linked to the invasion of cheaters; selection pressure and nutritional limitations may encourage certain individuals to not contribute to the expense of producing public goods. Second, it is necessary to explore whether the metabolic costs to individual bacterial cells are sufficiently high to affect primary metabolism and growth in either a nutrient-rich medium, such as Luria–Bertani broth [33], or in minimal medium. Third, if the metabolic cost of social activities seriously affects bacterial growth or fitness, it is necessary to study how individual QS bacteria manage nutrient utilization and energy metabolism to maintain both homeostasis and cooperativity under crowded conditions. Finally, cell density may not be the only factor involved in the expression of genes that act for the public good; other growth and/or physiological features may play key roles in the production of public goods. This issue has been explored in *P. aeruginosa* growing in either batch or continuous culture [6]. In this organism, regulation of the production of public goods is dependent on the nutrient supply; it is metabolically prudent to minimize the cost of producing public goods [34]. In *P. atrosepticum*, the ppGpp starvation signal is integrated into QS signaling circuits to ensure that costly virulence factors, including plant-cell-degrading enzymes, are produced at times of appropriate cell densities and under nutrient-limited conditions [23]. In addition, if the costs of cooperative metabolism affect primary metabolism, it will be necessary to explore whether cooperative bacterial cells have metabolic alternatives permitting efficient energy metabolism.

Metabolic options to maintain cooperativity

In crowded environments, and thus at high cell densities, nutrients are limited and the environment is unfavorable for growth. Thus, bacterial cells would be expected to experience serious physiological stress. The means by which bacterial cells survive stationary phase stress is not the principal topic of this review; several relevant research and review articles have been published to date [34–37]. Rather, we are interested in how bacterial cells coordinate nutrient utilization and manage energy metabolism in a cooperative population. Do such cells have molecular mechanisms similar to the calorie restriction evident in mice? The answer is yes: wild type *B. glumae* self-restricts glucose uptake in a QS-dependent manner [38]. The *deft* mutant (i.e., a *tofR* mutant that cannot recognize *N*-octanoyl homoserine lactone) of *B. glumae* utilizes more glucose than is necessary and outcompetes the wild type strain in co-culture [38]. It might be assumed that QS mutants thus have advantages in terms of both nutrient utilization and growth compared to the wild type strain. However, this is not true for QS mutants of *B. glumae*, *B. thailandensis*, or *B. pseudomallei*; the mutation of QS genes triggers uncontrolled nutrient consumption accompanied by the accumulation of toxic compounds, including ammonia from amino acid catabolism, in Luria–Bertani medium [22]. QS mutants of the above three species of *Burkholderia* thus suffer catastrophic population crashes in the stationary phase, triggered by alkaline toxicity [22]. To counteract ammonia-mediated high-pH toxicity, wild type strains exercise a QS-dependent metabolic option. They produce oxalate (a public good), via the branched TCA cycle, in a QS-dependent manner. Specifically, oxalate production is mediated by the QS-dependent transcriptional regulator QsmR, and oxalate neutralizes the toxic alkaline environment [22] (Table 1, Figure 1). We term the branched TCA cycle a 'putative oxalate cycle' because oxalate and acetoacetate are produced from acetyl-CoA and oxaloacetate [22,39,40]. It is suggested that acetoacetate is converted to acetoacetyl-CoA, and then to acetyl-CoA, in *B. glumae* [40]. The gene responsible for this conversion seems to be positively controlled by QS [20,22] (Table 1). It is thus clear that *Burkholderia* species use an alternative metabolic pathway to render the growth environment favorable; this is an example of cooperativity. A particular metabolic option is chosen to further social existence. Such findings suggest that bacterial cells exhibit a metabolic preference for a social existence under crowded environments, analogous to calorie restriction by mice [41]. It will be interesting to explore whether the QS-mediated self-restriction of nutrient utilization is widespread in QS bacteria.

Do bacterial cells have any metabolic options whereby they maintain cooperativity and police cheaters in stressful environments? This can be explored in monogenic or isogenic cultures of a single species or in complex culture