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

Table 1. *N*-acyl homoserine lactone-dependent quorum sensing-regulated genes involved in primary metabolism

Description	*Burkholderia glumae*[a]			*Burkholderia thailandensis*[b]			*Yersinia pestis*[c]			*Pseudomonas aeruginosa*[d]		
	Locus tag	Gene	Fold change[e]	Locus tag	Gene	Fold change	Locus tag	Gene	Fold change	Locus tag	Gene	Fold change
(i) Glucose metabolism												
Phosphoenolpyruvate-protein phosphotransferase	bglu_1g31820	*ptsI*	Down				YPO2994	*ptsI*	Down			
Phosphocarrier HPr protein	bglu_1g31830	*ptsH*	Down				YPO2993	*ptsH*	Down			
Fructose-1,6-bisphosphate aldolase	bglu_1g29760		Down									
Triosephosphate isomerase	bglu_1g25960		Down									
Glyceraldehyde-3-phosphate dehydrogenase	bglu_1g05360		Down				YPO2157	*gapA*	Down			
Phosphoglycerate kinase	bglu_1g29780		Down									
Phosphoglycerate mutase	bglu_1g31790		Down				YPO1133	*gpm*	Down			
Enolase	bglu_1g24510		Down									
Pyruvate kinase	bglu_1g29770		Down				YPO2393	*pykF*	Down			
Glucose-6-phosphate 1 dehydrogenase				BTH_I1552	*zwf*	Down				PA3183	*zwf*	Up
Phosphogluconate dehydratase	bglu_1g05520		Down							PA3194	*edd*	Up
2-Keto-3-deoxyphosphogluconate aldolase	bglu_1g05530		Down							PA3181		Up
(ii) TCA cycle												
Type II citrate synthase	bglu_2g08280	*citA*	Up				YPO1108	*gltA*	Up			
Succinyl-CoA synthetase							YPO1115, YPO1116	*sucCD*	Up			
Succinate dehydrogenase							YPO1109-1112	*sdhCDAB*	Up			
(iii) Putative oxalate cycle												
Oxalate biosynthesis enzyme	bglu_2g18790, bglu_2g18780	*obcAB*	Up	BTH_II1071	*obc1*	Up						
3-Oxoadipate CoA-succinyl transferase subunit	bglu_1g21560		Up	BTH_I2607	*scoB*	Up						
3-Oxoacid CoA-transferase subunit A	bglu_1g21570		Up	BTH_I2608	*scoA*	Up						
(iv) Glyoxylate cycle												
Isocitrate lyase	bglu_1g12420	*aceA*	Up				YPO3725	*aceA*	Up			
Malate synthase	bglu_2g07500	*glcB*	Up				YPO3726	*aceB*	Up			
(v) Electron transport and oxidative phosphorylation												
NADH dehydrogenase subunits	bglu_1g25810-25940		Down				YPO2545, YPO2547-2549, YPO2541, YPO2542	*nuoEFHIJL*	Up			
F_0F_1-type ATP synthase subunits	bglu_1g00700-00780		Down				YPO4122, YPO4124-4128	*atpGHFEBI*	Down			
Cytochrome bd ubiquinol oxidase subunit I	bglu_1g31470		Down	BTH_I0454	*cydA*	Down						
Cytochrome d ubiquinol oxidase subunit II	bglu_1g31480		Down									
Nitrate reductase subunits	bglu_2g17390-17420		Down							PA3872-3875	*narIJHG*	Down
(vi) Acetate metabolism												
Acetate-CoA ligase	bglu_1g25360		Down				YPO0253	*acs*	Up	PA0887	*acsA*	Down
Acetate kinase							YPO2566	*ackA*	Down			

Table 1 (*Continued*)

Description	*Burkholderia glumae*[a]			*Burkholderia thailandensis*[b]			*Yersinia pestis*[c]			*Pseudomonas aeruginosa*[d]		
	Locus tag	Gene	Fold change[e]	Locus tag	Gene	Fold change	Locus tag	Gene	Fold change	Locus tag	Gene	Fold change
(vii) Activated methyl cycle												
S-Adenosyl-L-homocysteine hydrolase	bglu_1g01990	*ahcY*	Up	BTH_I3165	*ahcY*	Up						
S-Ribosylhomocysteinase							YPO3300	*luxS*	Up			
Hypothetical protein	bglu_1g02000		Up	BTH_I3164		Up						
5,10-Methylenetetrahydrofolate reductase	bglu_1g02010	*metF*	Up	BTH_I3163	*metF*	Up	YPO0287		Up			
5-Methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase	bglu_1g08430	*metE1*	Up							PA1927	*metE*	Up
S-Adenosylmethionine synthetase				BTH_I0174	*metK*	Up	YPO0931	*metK*	Down			
(viii) Nucleotide metabolism												
Adenylosuccinate lyase	bglu_1g05560	*purB*	Down				YPO1636	*purB*	Down			
Phosphoribosylaminoimidazole-succinocarboxamide synthase	bglu_1g29750	*purC*	Down				YPO3059	*purC*	Down			
Adenylate kinase	bglu_1g29120	*adk*	Down				YPO3118	*adk*	Down			
GMP synthase	bglu_1g13000	*guaA*	Down				YPO2870	*guaA*	Down			
Guanylate kinase							YPO0040	*gmk*	Down			
Orotate phosphoribosyltransferase	bglu_1g02280	*pyrE*	Down				YPO0045	*pyrE*	Down			
Uridylate kinase	bglu_1g12740	*pyrH*	Down				YPO1046	*pyrH*	Down			
Cytidylate kinase	bglu_1g08790	*cmk*	Down	BTH_I1637	*cmk*	Down						
Ribonucleotide reductase subunit	bglu_2g05290		Down	BTH_I1153	*nrdA*	Down	YPO2648	*nrdF*	Down			
(ix) Amino acid metabolism												
- Glutamine biosynthesis												
Glutamine synthetase, type I	bglu_1g25000	*glnA*	Down				YPO0024	*glnA*	Down			
- Tyrosine degradation												
Maleylacetoacetate isomerase	bglu_2g18600		Down							PA2007	*maiA*	Down
Fumarylacetoacetase	bglu_2g18610		Down	BTH_I1398	*fahA*	Up				PA2008	*fahA*	Down
Homogentisate 1,2-dioxygenase	bglu_2g18620		Down	BTH_I1397	*hmgA*	Up				PA2009	*hmgA*	Down
- Tryptophan degradation												
Anthranilate dioxygenase reductase	bglu_2g21210		Down							PA2514	*antC*	Up
Anthranilate 1,2-dioxygenase subunit beta	bglu_2g21220		Down							PA2513	*antB*	Up
Anthranilate 1,2-dioxygenase subunit alpha	bglu_2g21230		Down							PA2512	*antA*	Up
(x) Rhamnolipid biosynthesis												
Rhamnosyltransferase 1 subunit A	bglu_2g05650	*rhlA*	Up	BTH_II1075	*rhlA-1*	Up				PA3479	*rhlA*	Up
Rhamnosyltransferase 1 subunit B	bglu_2g05660	*rhlB*	Up	BTH_II1076	*rhlB-1*	Up				PA3478	*rhlB*	Up
Rhamnosyltransferase 2	bglu_2g05680	*rhlC*	Up							PA1130	*rhlC*	Up
(xi) Glycogen biosynthesis												
Glycogen synthase	bglu_1g16580		Up				YPO3939	*glgA*	Up	PA2165		Up

[a]Data from [22].

[b]Data from [20].

[c]Data from [53].

[d]Data from [17,18,54].

[e]The mean up and down fold changes indicate that gene expression was activated or repressed by QS, respectively [20,22,53].

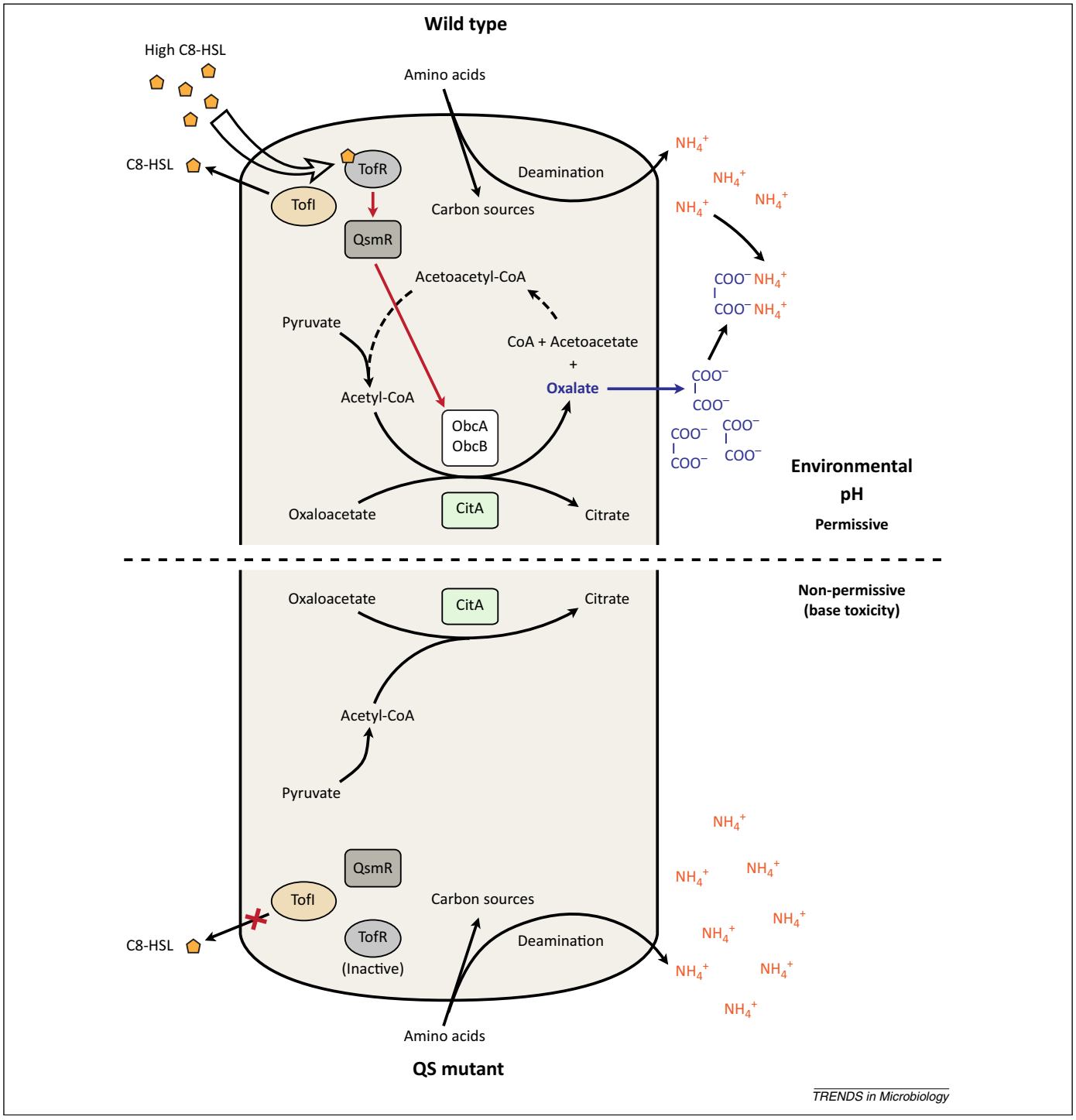

Figure 1. A working model of QS-dependent production of a public good, oxalate, by the branched TCA cycle of *Burkholderia* species. There is one LuxI-LuxR type QS system in *Burkholderia glumae*. TofI is responsible for the synthesis of *N*-octanoyl homoserine lactone (C8-HSL) whose receptor is TofR [9]. A wild type strain of *B. glumae* produces C8-HSL, which can freely diffuse into the culture medium. The concentration of C8-HSL increases as cell density increases, whereas QS mutants fail to produce C8-HSL. When the concentration of C8-HSL reaches a threshold in the culture medium, C8-HSL diffuses back into the cell and is recognized by TofR. The complex of TofR and C8-HSL activates a transcriptional regulator gene, *qsmR*. QsmR subsequently activates the oxalate biosynthetic genes *obcA* and *obcB*. Acetyl-CoA and oxaloacetate are used as substrates for ObcA and ObcB and citrate synthase (CitA). In the wild type strain, oxalate is produced in a QsmR-dependent manner whereas QS mutants produce significantly less oxalate than the wild type. The secreted oxalate presumably forms ammonium oxalate, which neutralizes the ammonium ion-mediated high-pH environment around wild type *B. glumae*, *Burkholderia thailandensis*, and *Burkholderia pseudomallei*. The inability of QS mutants to produce oxalate causes population crashes that are attributable to alkaline toxicity [22].

systems featuring other species or organisms. Multispecies competition has been reported to stabilize cooperativity [42–44]. Bacteria cooperating with *Pseudomonas fluorescens* CHA0 produce specific toxins suppressing phylogenetically related QS defectors [43]. Such phylogenetic constraints support the notion that *P. fluorescens* cooperativity is maintained by kin-dependent inhibition [43]. *P. aeruginosa* strain PAO1-UW has been reported to metabolically control the rate of cheater development, sustaining cooperative life under certain conditions [45]. The

policing mechanism of *P. aeruginosa* PAO1-UW features cyanide production by cooperators to kill cyanide-sensitive cheaters [46]. Such cheaters may be inevitable in very dense populations [47–50]. However, the number of cheaters seems to be very low in other QS bacteria, suggesting that QS-mediated cooperativity is stringently controlled. If so, common (and currently unknown) policing mechanisms may exist. Alternatively, policing mechanisms may be species-specific. It remains unclear how individuals or populations control, or determine the threshold of, cheater levels. It will be interesting to explore whether a choice between metabolic options plays a role in this context. The interaction between cooperativity and tolerance (or not) of cheaters is of general interest in bacterial population biology.

If metabolic constraints control the rate of cheater appearance, is it possible to detect the so-called 'tragedy of the commons' – are cheaters very prevalent in bacterial cultures grown in rich medium (e.g., Luria–Bertani broth)? Today, this tragedy may be difficult to discern as evolution will have selected against organisms that tolerate cheaters. Populations that ultimately perish because cheaters are not policed can be artificially created; reasonable and testable hypotheses can then be explored. What physiological conditions or selection pressures destabilize QS-controlled cooperativity in wild type QS bacteria? The answer may suggest a means of controlling QS bacteria at the population level.

Influence of persistent cooperativity on primary metabolism

At the population level, the maintenance of bacterial metabolic homeostasis is important in terms of energy conservation and resource utilization. Under crowded and nutritionally limited conditions, bacteria must use resources more efficiently and adapt to unfavorable growth environments. Little was known until recently about whether individual bacterial cells change their primary metabolism under crowded but cooperative conditions. However, An *et al.* [38] showed that, in *B. glumae*, QS controls nutrient acquisition and helps to maintain the homeostatic primary metabolism of individuals within a cooperative population [38]. Nuclear magnetic resonance spectroscopy-based analysis of ^{13}C-glucose uptake by *B. glumae* showed that QS restricted such uptake [38]. Turning to the multicomponent phosphoenolpyruvate-dependent sugar phosphotransferase system (PTS), the *ptsI* and *ptsH* genes (encoding phosphoenolpyruvate-protein phosphotransferase and phosphocarrier HPr protein, respectively [51,52]) were downregulated by AHL-mediated QS in *B. glumae* [38] (Table 1). The glucose uptake rate of wild type *B. glumae* was lower than that of QS mutants [38], strongly supporting the idea that QS slows glucose uptake under crowded conditions. This phenomenon does not seem to be confined to *B. glumae*. In *Yersinia pestis*, RNAseq analysis indicated that *ptsI* and *ptsH* were downregulated by AHL-mediated QS [53] (Table 1). Other genes involved in glucose metabolism, including those encoding fructose-1,6-bisphosphate aldolase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, and pyruvate kinase, appear to be downregulated by QS in both *B. glumae* and *Y. pestis* (Table 1). Some metabolic genes of *P. aeruginosa* are regulated via QS [17,18,54] (Table 1). The *zwf* gene (encoding glucose-6-phosphate 1 dehydrogenase) is downregulated by QS in *B. thailandensis*, whereas a *P. aeruginosa* homolog of that gene appeared to be upregulated by QS in the microarray study of Schuster *et al.* [17,20]. This discrepancy may be explained by either differences in regulation between the two organisms or the use of different tools in the analyses. More detailed real-time RT-PCR data are required. Nonetheless, it is clear that certain genes of glucose metabolism are controlled by QS. In addition to QS-dependent transcriptome analyses of *B. glumae*, *B. thailandensis*, *Y. pestis*, and *P. aeruginosa*, systematic analyses of primary metabolite levels (using capillary electrophoresis time-of-flight mass spectrometry) have been conducted to determine whether QS affects the primary metabolism of *B. glumae* [38]. The results from both wild type and QS mutants of *B. glumae* were well correlated with QS-dependent transcriptome data in terms of glucose metabolism [22]. Specifically, the levels of glucose-6-phosphate, fructose-6-phosphate, 3-phosphoglycerate, 2-phosphoglucerate, and phosphoenolpyruvate were significantly higher in the QS mutants than in wild type, and the genes encoding the enzymes synthesizing these compounds were downregulated by QS in *B. glumae* [38].

In contrast to the downregulation of genes of glucose metabolism, *citA* and *gltA* (encoding citrate synthase in *B. glumae* and *Y. pestis*, respectively) were upregulated by QS (Table 1). Other genes of the TCA cycle, *sucCD* and *sdhCDAB* (encoding succinyl-CoA synthetase and succinate dehydrogenase, respectively), were upregulated by QS in *Y. pestis* (Table 1). Genes of the glyoxylate cycle, including *aceA* and *glcB* (encoding isocitrate lyase and malate synthase G, respectively) were upregulated by QS in *B. glumae* (Table 1). In *Y. pestis*, homologs of *aceA* and *aceB* (encoding malate synthase A) were also upregulated by QS [53] (Table 1). In terms of genes of TCA branch pathways, *obcAB* and the gene encoding 3-oxoadipate CoA-succinyl transferase (mediating oxalate biosynthesis and the conversion of acetoacetate to acetoacetyl-CoA, respectively) were upregulated by QS in both *B. glumae* and *B. thailandensis* [20,22] (Table 1). These data indicate that the TCA, putative oxalate, and glyoxylate cycles are positively and directly controlled by QS (Figure 2). However, the biological significance of positive cycle control by QS remains unclear, with the exception of the putative oxalate cycle.

In addition to the elevated glucose uptake of, and glucose utilization in, QS mutants, the levels of pentose phosphate pathway metabolites were increased in QS mutants compared to wild type [38] (Figure 3). As the pentose phosphate pathway is the main source of bacterial NADPH, higher concentrations of $NADP^+$ would be expected in QS mutants, in agreement with the fact that the expression levels of pathway genes were higher in QS mutants than in wild type [22,38]. It is important to note that bacteria maintain a homeostatic level of ribose-5-phosphate to control nucleotide concentrations; intracellular ribose-5-phosphate is the primary regulator of *de novo* purine and pyrimidine biosynthesis [55–58]. Therefore, the higher levels of ribose-5-phosphate in QS mutants may

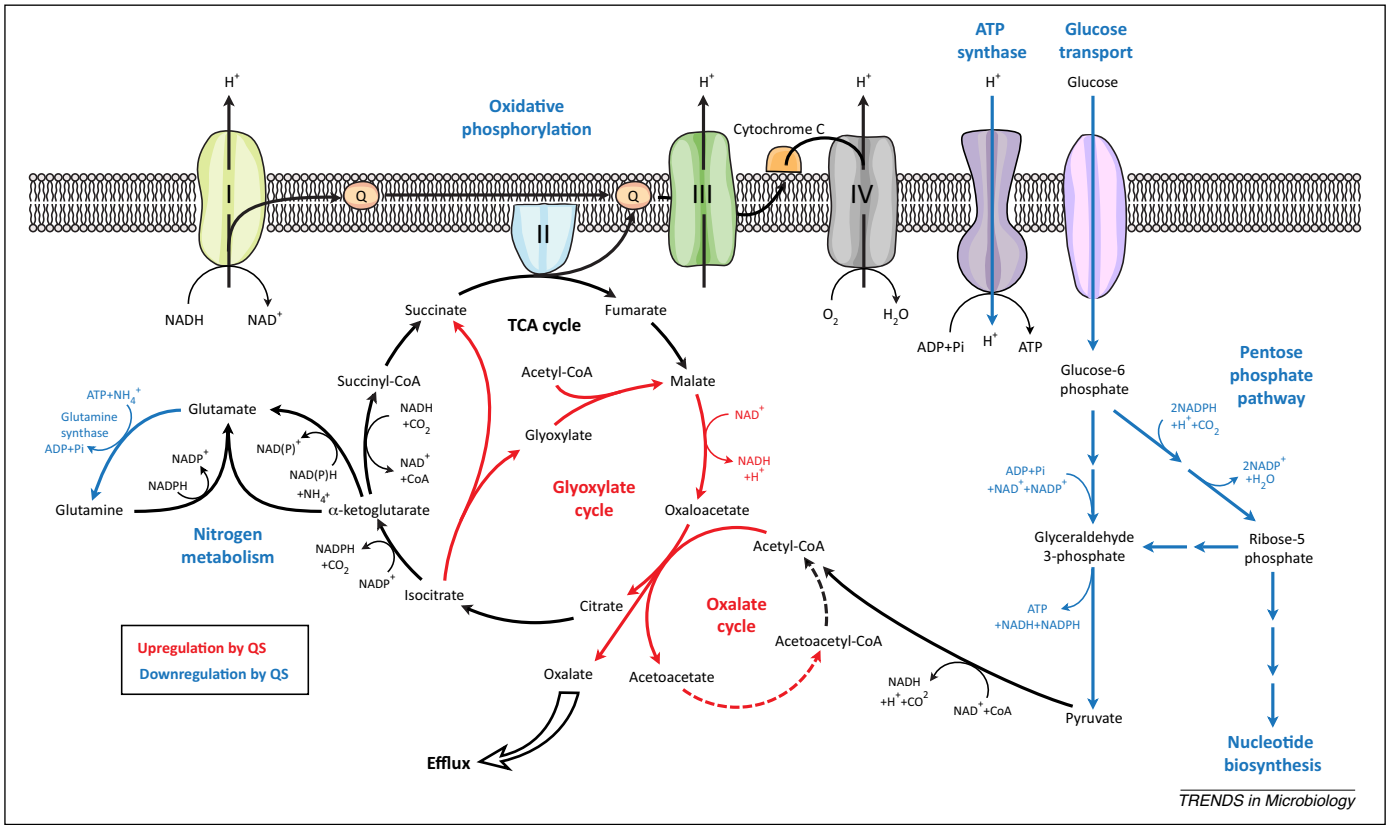

Figure 2. Schematic diagram of the *N*-acyl-homoserine lactone-mediated QS control of metabolism in *Burkholderia glumae*. Glucose uptake and metabolism, oxidative phosphorylation, and nucleotide biosynthesis are downregulated, whereas the glyoxylate and putative oxalate cycles are upregulated by QsmR [38]. The red and blue colors denote activation and repression via QS, respectively.

explain imbalances in the nucleotide concentrations of such mutants [38].

In addition to the QS-mediated suppression of glucose metabolism and the pentose phosphate pathway, the biosynthesis of ribonucleoside 5′-triphosphates (NTPs) and 5′-diphosphates (NDPs) is also influenced by QsmR of *B. glumae* [38] (Figure 3). The concentrations of all NTPs were significantly higher in QS mutants than in wild type cells [38] (Figure 3). The ATP:ADP ratio remained constant in wild type, but not in the QS mutants [38]. The imbalance in the ATP:ADP ratios of QS mutants was associated with the altered expression of two key genes. Thus, the levels of both adenylate kinase (encoded by *adk*) and nucleoside-diphosphate kinase (encoded by *ndk*), which are involved in *de novo* purine biosynthesis, were higher in QS mutants than in wild type cells [38]. The ATP:ADP imbalance also extended to the other NTPs, associated with changes in the expression levels of two genes – those encoding phosphoribosylglycinamide synthetase D (*purD*) and carbamoyl phosphate synthase A (*carA*); these genes are involved in *de novo* purine and pyrimidine biosynthesis, respectively [38,59]. The expression levels of both genes were higher in QS mutants than in wild type cells [38]. Furthermore, the levels of deoxyribonucleoside 5′-triphosphates (dNTPs) were not in balance in QS mutants, possibly because of the higher levels of ribonucleotide reductase B (encoded by *nrdB*), which catalyzes deoxyribonucleotide formation from ribonucleotides, in QS mutants compared to wild type cells [38]. The imbalance in dNTPs suggests that a QS deficiency triggers high-frequency mutation, as observed in *E. coli* [60,61], culminating in reduced genetic stability. These data suggest that bacterial QS plays critical roles in genomic evolution.

Apart from the observed imbalances in core primary metabolite levels in QS mutants of *B. glumae*, the QS-dependent fluctuations in the levels of such metabolites suggest that QS mutants experience more oxidative stress than do wild type cells. Thus, the levels of important reduction-related agents ($NADP^+$ and glutathione disulfide) that protect cells from toxic reactive oxygen species were significantly higher in QS mutants than in wild type cells [38] (Figure 3), consistent with data from a previous study indicating that the key catalase gene *katG* is positively controlled by QS [62]. In *P. aeruginosa*, the concentrations of polyamines, multifunctional molecules associated with responses to oxidative stress, are elevated in the *lasI*/*rhlI* mutant (which is unable to make AHL-QS signaling molecules) because QS activates the expression of *katA* and superoxide dismutase, which are involved in the detoxification of reactive oxygen species [63,64].

Recently published results from GC- and LC-MS-based analyses of intracellular metabolites in *P. aeruginosa* showed that a deficiency in QS perturbed the cellular concentrations of TCA cycle intermediates, amino acids, and fatty acids [63]. TCA cycle intermediates, including citrate, malate, and succinate, were more abundant in QS mutant cells compared to wild type cells, as reported previously in *B. glumae* [38,63]. The uptake of carbon sources, especially alanine, was faster in the *lasI*/*rhlI* mutant than in wild type, suggesting that

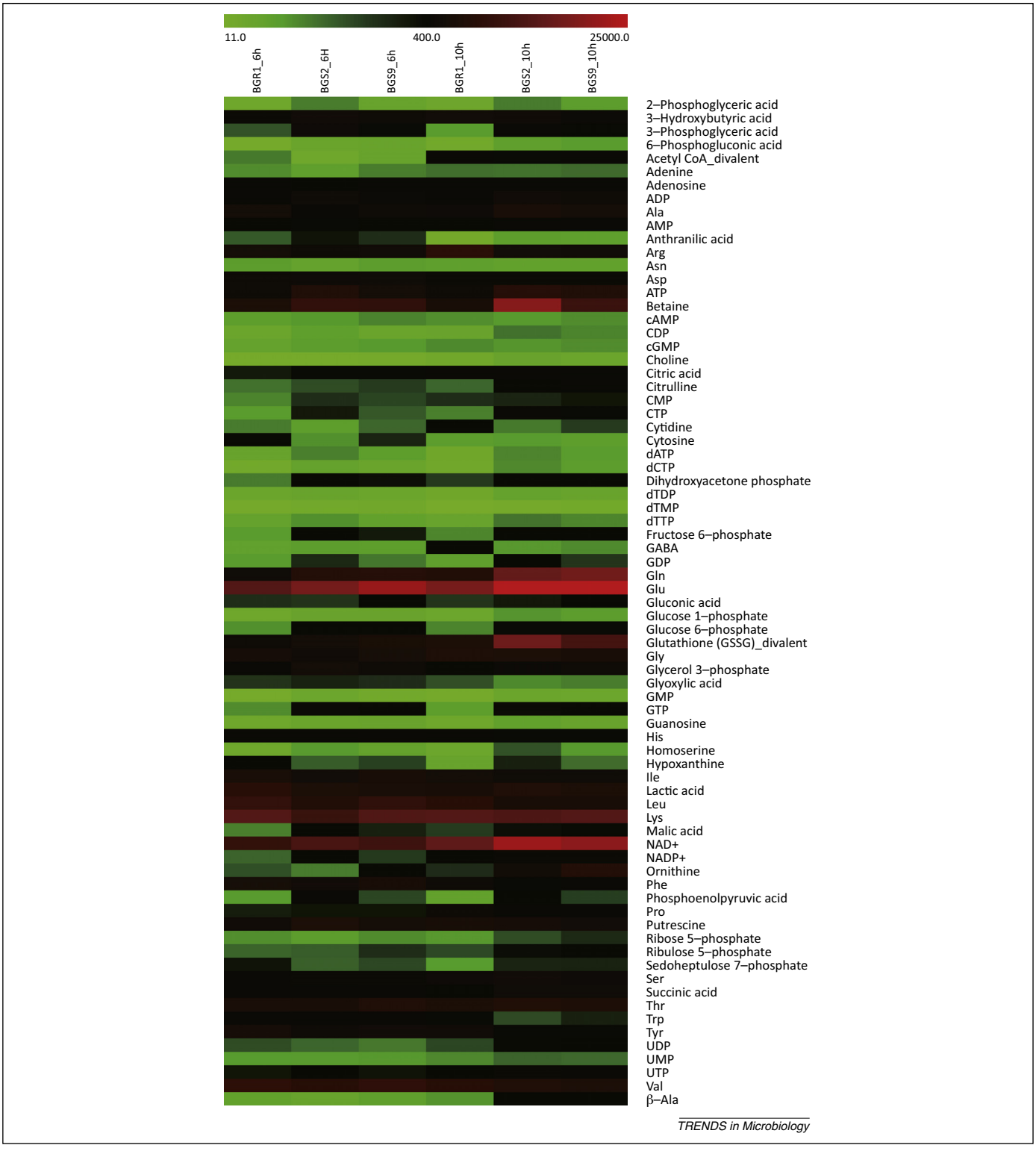

Figure 3. A heatmap of QS-dependent metabolomes reflecting the outcomes of QS-mediated gene regulation in *Burkholderia glumae*. Core metabolome levels measured via capillary electrophoresis time-of-flight mass spectrometry 6 and 10 h after subculture are shown [38]. The BGR1, BGS2, and BGS9 strains are the wild type, a signal synthase mutant, and a *qsmR* mutant, respectively.

carbon assimilation is negatively controlled by QS [63]. Differences in the fatty acid profiles of wild type and QS mutant cells were more apparent after the onset of QS during stationary phase growth [63]. Furthermore, the extent of fatty acid saturation and chain length were increased in wild type cells, and the degree of fatty acid cyclopropanation in the *lasI*/*rhlI* mutant was less than that in wild type [63]. These results suggest that multiple regulatory systems impact the cellular physiology of *P. aeruginosa* at different levels. The observation that QS mediates changes in the central metabolism of *P. aeruginosa* is consistent with data showing that QS mediates metabolic slowing in *B. glumae* [38,63]. Taken together, systematic analyses of QS-dependent metabolomes suggest that a loss

of QS causes serious physiological problems, especially with regard to nutrient acquisition, central metabolism, and cellular oxidative stress, in *B. glumae* and *P. aeruginosa*. It would be interesting to know whether the role of QS as a metabolic brake is a common phenomenon in other QS bacteria.

Concluding remarks

Intensive studies of bacterial cell–cell communication, as exemplified by AHL-mediated QS, have revealed new facets of bacterial behavior. Traditionally, bacterial phenotypes have been averaged over populations; individual contributions have been ignored. The phenotypes are the outcomes of the combined or average activities of all cells in a population. However, it is conceivable that bacteria are social organisms that cooperate within either monogenic or multigenic populations, both in the laboratory and in their natural habitats. The observed cooperative activities of individuals within a group have changed the long-lasting notion that bacteria act selfishly, rather than together. The realization that bacteria are social organisms has yielded new insights into bacterial physiology and gene regulation, from the viewpoints of both population and evolutionary biology. Bacterial QS is now a subfield of population biology; social population behaviors are easily studied by manipulating growth conditions and monitoring the experimentally induced evolution of later generations. The concepts of systems and population biology will further our understanding of the dynamic nature of AHL-mediated QS and reveal how individuals behave as social organisms in various sophisticated environments.

In addition to cell-density-dependent gene regulation, growth stage and nutritional conditions should be incorporated into QS regulatory circuits. Factors involved in cell-density-dependent control may communicate with those regulating growth stage specificities and starvation. In addition to the sRNAs of *Vibrio* species and *P. aeruginosa*, other factors may be involved in the control of gene expression dynamics by AHL-mediated QS.

QS bacteria may restrict calorie usage via a mechanism similar to that in mice. The QS-dependent self-control of nutrient acquisition mimics dietary restriction and is a feature of bacterial cooperation. Such restraint may improve survival in the stationary phase and prolong bacterial longevity under crowded conditions. In addition to restricting nutrient acquisition, QS serves as a metabolic brake in the primary metabolism of *B. glumae* and *P. aeruginosa*. Thus, QS functions to optimize energy and resource utilization by individuals living in crowded environments. Future research should focus on the significance of QS-mediated homeostatic primary metabolism and evolutionary aspects of QS (Box 1). Future studies of the QS-mediated control of primary metabolism and survival under various nutritional conditions may indicate how pathogens can be successfully controlled.

Box 1. Outstanding questions

- How can physiological conditions experienced by bacterial cells at different growth stages, or under varying growth conditions, be integrated into QS-mediated gene regulation circuits?
- How do individual bacterial cells in a cooperative population determine the optimum level of production of public goods under various culture conditions?
- How do bacterial cells coordinate efficient nutrient utilization and the management of homeostatic energy metabolism within a cooperative population?
- Do the calorie restriction-mimicking molecular mechanisms of bacterial cells further long-term survival under crowded conditions?
- Do bacterial cells have other metabolic options to sustain cooperativity?

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