Conversation game: talking bacteria

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Abstract The story of autonomous unicellular organisms, bacteria with unimaginable computational and evolutionary capabilities along with collective behavior has been running since the first six decades of the twentieth Century. However, do not consider them to be small and simple, because they possess the generic term quorum sensing adopted to describe the cell communication process which co-ordinate gene expression, when the population has reached a high cell density. Bacteria release diffusible signal molecules known as autoinducers or quorum sensing molecules. In recent research, the direction for activating or deactivating nature of a wave of gene expression is predicted experimentally which control bacterial populations subject to a diffusing autoinducer signal. On the other hand, it has been observed that the accumulation of the quorum sensing molecules leads to a negative diffusion coefficient in the solution of governing differential equation.

Keywords Autoinducers · High cell density · Quorum sensing · Wave solution

Introduction

We think that a multiple of bacteria are stronger than a few and thus by union are able to overcome obstacles too great for the few. However, there are many situations when the bacterial

population behave co-operatively and recognize self and non-self which can be highly advantageous, particularly in the contexts of sex, symbiosis, niche adaptation, production of secondary metabolites combined with the defense mechanisms of higher organisms and for facilitating population migration where the prevailing conditions in a specific environmental niche have become unfavorable. Besides cell to cell interaction, the production of small biochemical molecules probably offers and enhances the most obvious strategic communication between bacterial cells (Shapiro 2007; Shapiro 1998). These signal molecules secreted outside the producer organism facilitate communication between individual organisms. Bacteria release a wide variety of molecules like metabolic end products, siderophores and cell–cell signaling molecules which function as autoinducers. As the bacterial colonies get dense, signal molecules are released into extracellular locations and accumulated. When a threshold concentration of the molecules is achieved, a coordinated change in bacterial behavior is initiated. The term “quorum sensing” was introduced in 1994 to describe this biochemical complex phenomenon. It has been observed that quorum sensing, as determinant of cell population density, is only one of many different environmental signals which bacterial cell must integrate in order to determine their optimal survival strategy (Williams et al. 2007).

Autoinduction languages

Quorum sensing is considered in the context of cell–cell signaling talk sense and non-sense with intra and interspecies coordinated regulation of behavior. In case of intra communication it can be perceived but not vice versa because it has been characterized as unidirectional. The communication process is coordinated by quorum sensing molecules

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(autoinducers or pheromones). QSM possess some characteristic features to trigger the networking system. First, the production of the quorum sensing signal takes place during specific stages of growth, under certain physiological conditions, or in accordance to environmental changes. Secondly, the quorum sensing signal diffuses in the extracellular space and is recognized by specific bacterial receptors. Thirdly, the accumulation of a critical threshold concentration of the quorum sensing signal initiates a firm response and fourthly, the cellular response further proffer beyond physiological changes that metabolize or detoxify the molecule. When all the four benchmarks are satisfied, a molecule can be classified as QSM. There are two different types of mediated quorum sensing observed so far. One is acyl homoserine lactone (AHL) mediated and quinolone mediated quorum sensing which is very common in gram negative bacteria; another is modified

oligopeptides (pheromones) mediated quorum sensing in gram positive bacteria. This peptide, known as autoinducing peptide (AIP), ranges from 5 to 34 amino acids in length and typically contain unusual chemical architectures (Williams et al. 2007; Shapiro 1998).

The figure schematically represents AHL-mediated QS-dependent regulation of multiple target gene expression, where luxR and luxI are orthologues and code for the AHL receptor and AHL synthase respectively. In many but not all systems, the AHL is an autoinducer since it drives its own production via the amplification loop leading to the AHL synthase gene. The LuxR–LuxI system is the more dominant and best-studied circuit regulator of bioluminescence (Miyashiro and Ruby 2012). In the LuxR–LuxI system (Fig. 1), the luxR gene encodes the transcription factor LuxR. The luxICDABEG operon encodes the LuxI enzyme as well as

Fig. 1 Quorum sensing circuit with operon regulatory mechanism

components necessary for synthesis of the luciferase, the light-producing enzyme, and production of its substrates. LuxI catalyzes the synthesis of the acyl-homoserine lactone (AHL) 3OC6-HSL, an autoinducer that interacts with LuxR to form the LuxR/3OC6-HSL complex. The LuxR/3OC6-HSL complex activates the expression of the *luxICDABEG* operon, creating a positive feedback loop (Fuqua et al. 1996). This acts as a switch which turns over genetic expression from an inactivated state to an activated state.

Since AHLs are freely diffusible through the cell membrane, their concentrations are locally approximately equal extracellularly and intracellularly. Hence, we expect that if one bacterium is experiencing an AHL level high enough to be activated, then so are its nearest neighbours. As more bacteria become activated, we will see a switch on the colony level from the inactivated state to the activated state. In a spatially extended system, the activated region grows in the spatial dimension as the AHL signal diffuses from its source. Similarly, since LuxR binds to 3OC6-HSL reversibly (Dilanj et al. 2012) if we remove 3OC6-HSL from the system at a fast enough rate, we expect to see a colony-wide switch from the activated state to the inactivated state. AHL biosensors based on a given LuxR protein and the promoter of a target gene fused to a reporter such as *lux* (Bainton et al. 1992a; Winson et al. 1998). LuxR-type proteins consist of two functional domains, an N-terminal AHL-binding and a C-terminal DNA-binding domain. They reversibly bind their cognate AHLs and activate or repress gene expression by binding to specific DNA motifs containing dyad symmetry at their target promoters. It was experimentally exhibited that spatially extended patterns of QS activation were initiated by a diffusive signal. This raises the question of whether or not a spatially extended QS system with a diffusing AHL signal could exhibit a traveling wave of activation, where such a wave takes a specific mathematical form (Danino et al. 2010). To answer this question, many mathematical models were developed that described the LuxR–LuxI system which incorporated into biologically relevant components. It was proved in a few cases studied that there exists a travelling wave solution.

What's out there: quorum sensing

As animals and plants, the small creature bacteria also monitor the biotic and abiotic features of their surrounding environment using a wide range of sensory systems. They perceive their behavior to stay alive and reproduce. Nevertheless, the ecological niche of some bacteria is fairly stable. Sometimes in extreme conditions many inhabit highly mutable environments and conduct highly complex challenges, some of which could be anticipated and others not. Like all organisms, bacteria do not gather information in vacuum, but are able to assess the significant signals within their milieu, because it

is worth nothing that the power spectrum of signals remains unknown for any bacterium (Pamela 2015).

Bacterial coordinated phenotypes are driven via quorum sensing systems, beneficial only at certain cell densities. Meanwhile, quorum sensing systems are also threatened by imitators that may exploit regulated cooperation, which ask the question of how quorum sensing systems are maintained in nature. We first recapitulate the two remarkable observations on quorum sensing evolution: density-dependent benefits favour quorum sensing but competition and cheating will destabilize it. Recently, Schluter et al., studied the model of quorum sensing in a dense community like a biofilm, which revealed a novel benefit to quorum sensing that is intrinsically stable. They developed a realistic model and showed that quorum sensing can function as a way to outcompete neighbours in patches occupied by many different genotypes. In these communities, competing microbial genotypes gradually segregated over time leading to positive correlation between density and genetic similarity between neighbouring cells (relatedness). This enabled quorum sensing to track the way by genetic relatedness surrounded by similar mates. Bacteria secrete signal molecules into their environment and use these to regulate many of their key phenotypes. Growing aggressively made quorum sensing genotypes are a match for competitors. This strategy allowed recognizing clone mates. During growth, cells secrete autoinducers, that accumulate in the environment and high autoinducer concentration around cells induces expression as well as promotes metabolic traits (Schluter et al. 2016). Cells can tune the expression of density-dependent phenotypes, like virulence factors or secreted enzymes, so that they are only expressed when there are enough cells. The benefits of quorum sensing can also be affected by diffusion conditions, which may turn down to multiple quorum sensing signals (Cornforth et al. 2014).

Among numerous mediating QS signalling pathways, the Lsr (LuxS regulated) QS system is elucidated in scores of bacteria, and its signal molecule, autoinducer-2 (AI-2), is synthesized as a product of 1-carbon metabolism. Using mathematical simulations, it was examined how desynchronized Lsr QS activation, came up from cell-to-cell population heterogeneity resulting in bimodal Lsr signalling and fractional activation. This has been previously observed experimentally. These processes are governed by asynchronous AI-2 uptake, where positive intracellular feedback in Lsr expression is combined with negative feedback between cells. To show the difference, Quan et al., simulated both QS systems, where cell populations densifies and signal each other via traditional growth and diffusion equations. The results demonstrated that the LuxIR QS system produced an 'outward wave' of autoinduction, and the Lsr QS system yields dispersed autoinduction from spatially-localized secretion and uptake profiles. On the other hand, these models inform QS observations along with synthetic biology designs (Quan et al. 2016).

Modeling and quantum aspects

In the last decades, several researches have been carried out on mathematical models of the quorum sensing system. Variant new mathematical models are proposed based on dynamical systems (system of ordinary differential equations) and partial differential equations. Furthermore, analyzed models found bifurcation of the systems, stability of the models and asymptotic behavior of this complex biological phenomenon. On the other hand, analysis of noise in quorum sensing circuit plays a pivotal role in gene functionality. Past researches demonstrated that a tight coupling between exact stochastic simulation and functional density analysis provided insights into the structure and function relationships in the QS circuit (Tanouchi et al. 2008). But it still remains unclear how crosstalk between C8HSL and 3OC6HSL affects the information that the bacterium obtains through quorum sensing. Apart from that, origin of noise in QS system is still yet to be discovered.

In the year 2014, research revealed traveling waves in response to a diffusing quorum sensing signal in spatially-extended bacterial colonies (Langebrake et al. 2014). The results predict the direction and activating or deactivating nature of a wave of gene expression in experimentally controlled bacterial populations subject to a diffusing autoinducer signal. Although QS has been extensively studied in well-mixed systems, the ability of diffusing QS signals to synchronize gene expression in spatially extended colonies is not well understood. In order to investigate this synchronization, they developed an analytically tractable model of spatial QS in the LuxR–LuxI circuit. They proved the existence of a traveling wave solution to this model, which implies that the LuxR–LuxI circuit enables highly efficient QS synchronization, likely limited by cell growth. They showed that when omitting cell growth there is a maximum QS signal decay rate above which a traveling wave is no longer sustainable. For QS signal decay rates less than this maximum, they additionally found a critical rate that determines whether a QS system becomes completely upregulated or completely down-regulated. As expected, they find that the speed of the traveling waves of QS activation increases with the diffusion rate of the QS signal and decreases with the decay rate of the QS signal. The findings give insight to how quickly a colony will undergo a QS-mediated shift in gene expression and, when limited by cell growth, how much of a colony will become activated (or deactivated) before entering quiescent phase. From the biomolecular point of view, we can study the quorum sensing system with quantum effect, which is the most important in the twenty-first century. It is only now, however, with modern experimental techniques, that it is possible to observe quantum mechanical effects in bio-molecular complexes directly. Indeed, recent experiments have provided evidence that quantum effects such as wave like motion of excitonic energy flow,

delocalization and entanglement can be seen even in complex and noisy biological environments. Theoretical work has highlighted the importance of interplay between environmental noise and quantum coherence in such systems. All of this has led to a surge of interest in the exploration of quantum effects in biological systems in order to understand the possible relevance of non-trivial quantum features and to establish a potential link between quantum coherence and biological function. The full understanding of the dynamics at biomolecular length and timescales in noisy biological systems can uncover novel phenomena and concepts and hence present a fertile ground for truly multidisciplinary research. Quantum effect must play a crucial role to find the origin of noise and hidden signature in the wide range of quorum sensing molecules.

The recent work on the quorum sensing mechanism finds an important observation, we get a negative diffusion coefficient in the proposed mathematical model which shows that anti diffusion plays an important role in the quorum sensing system. Due to the negative diffusion coefficient, the bacterial population density continuously rises to a certain level. The diffusible quorum sensing molecules are released, then they accumulate and increase the population density until the threshold concentration is reached. If all the molecules are diffused, then quorum never occurs. So anti diffusion is an important cause of quorum sensing. The chemical diffusion occurs in a presence of the concentration (or the chemical potential) gradient and it results in net transport of mass. This is the process described by the diffusion equation. This diffusion is always a non-equilibrium process, increases the system entropy, and brings the system closer to equilibrium. The thermodynamic factor is related to the Gibbs free energy. A negative diffusion coefficient means that the flux of concentration diffuses up against the concentration gradient (though still along the chemical potential or free energy gradient). This is known as uphill diffusion, which is important for a special phase transformation, called Spinodal Decomposition (Majumdar 2016).

References

- Bainton, N. J., Bycroft, B. W., Chhabra, S. R., Stead, P., Gledhill, L., Hill, P. J., Rees, C. E. D., Winson, M. K., Salmond, G. P. C. & other authors (1992a). A general role for the lux autoinducer in bacterial cell signalling: control of antibiotic synthesis in *Erwinia*. *Gene* 116, 87–91.
- Cornforth DM, Popat R, McNally L, Gurney J, Scott-Phillips TC, Ivens A, Diggle SP, Brown SP (2014) Combinatorial quorum sensing allows bacteria to resolve their social and physical environment. *Proc Natl Acad Sci U S A* 111:4280–4284
- Danino T, Mondragón-Palomino O, Tsimring L, Hasty J (2010) A synchronized quorum of genetic clocks. *Nature* 463(7279):326–330

- Dilanji GE, Langebrake JB, De Leenheer P, Hagen SJ (2012) Quorum activation at a distance: spatiotemporal patterns of gene regulation from diffusion of an autoinducer signal. *J Am Chem Soc* 134(12): 5618–5626
- Fuqua C, Winans SC, Greenberg EP (1996) Census and consensus in bacteria/ecosystems: the LuxR–LuxI family of quorum-sensing transcriptional regulators. *Annu Rev Microbiol* 50:727–751
- Langebrake JB, Dilanji GE, Hagen SJ, De Leenheer P (2014) Traveling waves in response to a diffusing quorum sensing signal in spatially-extended bacterial colonies. *J Theor Biol* 363:53–61
- Majumdar S, Classical and numerical foundation theory: parabolic evolution problems with application, Thesis (2016).
- Miyashiro T, Ruby EG (2012) Shedding light on bioluminescence regulation in *Vibrio fischeri*. *Mol Microbiol* 84(5):795–806
- Pamela L (2015) The cognitive cell: bacterial behaviour reconsidered. *Front Microbiol* 6(264):1–18
- Quan DN, Tsao C-Y, Wu H-C, Bentley WE (2016) Quorum sensing desynchronization leads to bimodality and patterned behaviors. *PLoS Comput Biol* 12(4):e1004781
- Schluter J, Schoech AP, Foster KR, Mitri S (2016) The evolution of quorum sensing mechanism to infer kinship. *PLoS Comput Biol* 12(4):e1004848
- Shapiro JA (1998) Thinking about bacterial populations as multicellular organisms. *Annu Rev Microbiol* 52:81–104
- Shapiro JA (2007) Bacteria are small but not stupid: cognition, natural genetic engineering and socio-bacteriology." *Studies in History and Philosophy of Science Part C. Stud Hist Phil Biol Biomed Sci* 38(4): 807–819
- Tanouchi Y, Tu D, Kim J, You L (2008) Noise reduction by diffusional dissipation in a minimal quorum sensing motif. *PLoS Comput Biol* 4(8):e1000167
- Williams P, Winzer K, Chan WC, Camara M (2007) Look who's talking: communication and quorum sensing in the bacterial world. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 362(1483):1119–1134
- Winston MK, Swift S, Fish L, Throup JP, Jorgensen F, Chhabra SR, Bycroft BW, Williams P, Stewart GSAB (1998) Construction and analysis of luxCDABE-based plasmid sensors for investigating N-acyl homoserine lactone-mediated quorum sensing. *FEMS Microbiol Lett* 163:185–192