An Environment Aware P-System Model of Quorum Sensing

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Abstract. "Quorum Sensing" has been identified as one of the most consequential microbiology discoveries of the last 10 years. Using Quorum Sensing bacterial colonies synchronize gene expression and phenotype change allowing them, among other things, to protect their niche, coordinate host invasion and bio-film formation. In this contribution we briefly describe the elementary microbiology background and present a P-systems based model for Quorum Sensing which includes environmental rules and a topological representation.

1 Introduction

Recent advances in analytical biotechnology, computational biology, bioinformatics and computational modeling promise ever deeper understanding of the complexity of biological systems, particularly the computations they perform in order to survive in dynamic and hostile environments. These insights will ultimately enable researchers to harness the living cell as a computational device with its own sensors, internal states, transition functions, actuators, etc, and to program them as "nano-bots" for particular tasks such as targeted drug delivery, chemical factories, nano-structures repairs, bio-film scaffolding and self-assembling, to name but a few.

In this paper we will focus on one of the most important mechanisms for bacterial cell-to-cell communication and behavior coordination under changing environments: "quorum sensing (QS)". QS have been described as "the most consequential molecular microbiology story of the last decade" [21, 3]. It relies on the activation of a sensor kinase or response regulator protein by a diffusible, low molecular weight, signal molecule (a "pheromone" or "autoinducer") [12]. In QS, the concentration of the signal molecule reflects the number of bacterial cells in a particular niche and perception of a threshold concentration of that

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signal molecule indicates that the population is "quorated", i.e. ready to make a behavioral decision [20].

Natural QS is a powerful computational mechanism[5] that endows *Pseudomonas aeruginosa* with the capabilities to coordinate a population-wide attack necessary to breach host's immunological defences. Other bacteria (both Gram-negative and Gram-positive), like *V. fischeri*, *A. tumefaciens*, *E. carotovora*, *V. harveyi*, *B. subtilis*, *S. aureus*, *S. pneumoniae*, etc., also use QS for different purposes and it is usually mediated by a variety of sensors/receptors, regulons, etc.

In this paper we will present an overview of Quorum Sensing in *P. aeruginosa* and we will also show a more "computationally flavored" approach for Quorum Sensing which is based on a modified version of P-systems that takes into consideration topological aspects of the environment where cells live.

2 Cell-to-Cell Communication by Means of Quorum Sensing

The QS mechanism is a communication strategy based on diffusible signals, S, which kick-in under high cellular density. Bacteria use this mechanism to obtain a population-wide coordination of infection, invasion, and evasion of a host's defenses.

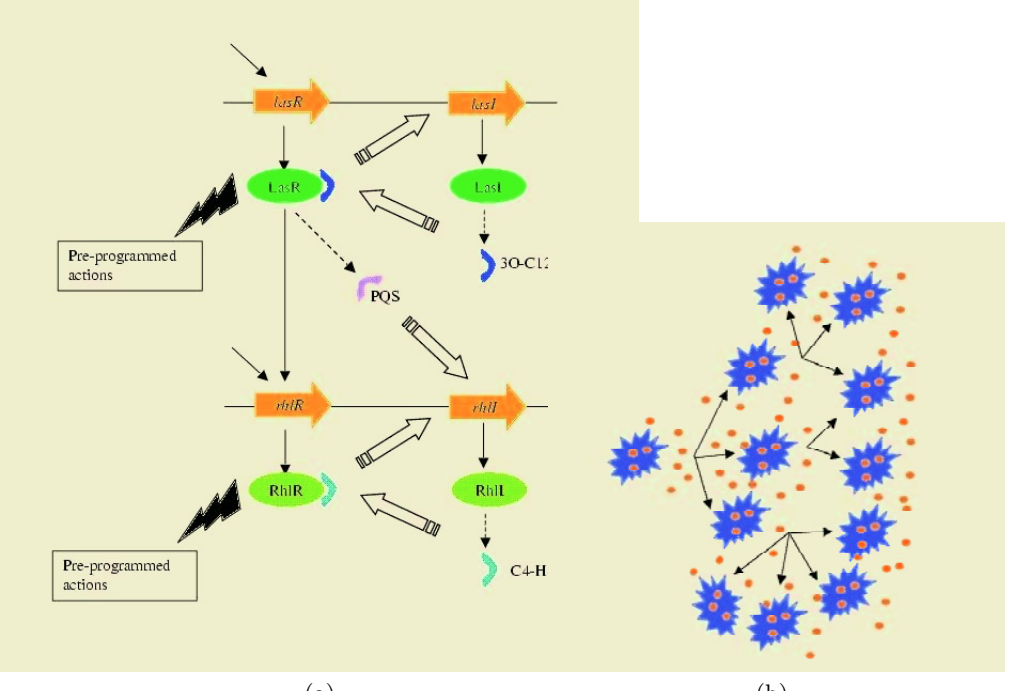
Once the mechanism is activated it usually triggers a cascade of transcriptional activity which results in phenotypic changes that are frequently related to the activation of virulence encoded regulons. As we mentioned before both Gram-negative and Gram-positive bacteria employ similar coordination mechanism albeit with different messenger molecules. The messenger molecules are often called (auto)inducers or pheromones(to be denoted by S). Under low bacterial densities molecule S is synthesized and accumulate. According to the specific geometry of the inducer molecule, more precisely its length, the synthesized S are either pumped out of the cell or simply diffuse into the surrounding environment[1].

Once in the environment, the inducer molecules that are usually much smaller than small proteins (and certainly tiny compared to the bacterium itself), disperse quickly and sometimes get in contact with other individual bacteria who occasionally ends up absorbing the inducer molecule. In addition to the inducer molecule, bacteria also produce a receptor molecule R. At high inducer's concentrations (within the cellular membranes) and once a specific threshold concentration is reached, the receptor molecules R binds to the inducers S forming a molecular complex. In turn the pheromone bound version of R, $R \circ S$, binds to a specific chromosome region thus activating or repressing the transcription of certain genes. Moreover, as the gene encoding the syntethase I for the inducer S (denoted with *i*, i.e. it is represented with the same letter as the synthetase, I, but in italics) is positively regulated by the complex $R \circ S$, a rapid signal ampli-

[1] In Gram-positive bacteria S is always actively transported out of the cellular membrane.

fication, i.e. positive feedback and hence the name autoinducer, takes place. The transcription of i into I results in the synthesis of excess molecules S that diffuse out of the bacteria and into the local environment. It is important to note that although it is possible to speak of "diffusion" in the case of Gram-negative, in Gram-positives the signal molecules don't diffuse out of the cell, instead they are secreted using active transport systems which in some cases activate the appropriate signals as they are getting out (e.g.*Staphylococcus aureus*). Also in Gram-positives the active molecule does not get into the cell; instead it activates cellular surface receptors which in turns relays the activation to other proteins resulting in the transcriptional response.

The amplification loop is shown in Figure 1(a). Under high cellular density, and once the QS is activated, the positive feedback effectively triggers a chain reaction that bridges the gap between various physical scales. That is, Quorum Sensing is a mechanism which processes and integrates information that ranges from the nano-level of the cell interior to the macro-level of a bacterial colony (sometimes visible with the naked eye) in a short period of time. Figure 1(b) gives a graphical representation for this phenomenom.



(a) (b)

Fig. 1. (a) Overlapped Quorum Sensing systems in *P. aeruginosa*s. (b) Multi-scale effects mediated by Quorum Sensing. A cell (from among a group of cells) senses an increase in inducer molecules (*small dots*) in the surrounding environment. The internal feedback loop is activated and, in turn, deposits more inducers into the external medium. The increase in inducers concentration in turn triggers other cells to react leading to a chain reaction

The chain reaction, mediated by the high mobility of S in the environment, ensures that more and more individual bacteria are activated within a short period of time producing a population wide behavioral shift. This behavioral shift is possible because QS activates the coordinated transcription of multiple genes. Consequently, in QS, the concentration of the signal molecule reflects the number of bacterial cells in a particular niche and perception of a threshold concentration of that signal molecule indicates that the population is "quorated", i.e. ready to make a behavioral decision. Wagner et al. [17] report that in *P. aeruginosa* up to 394 genes are activated by QS while 222 are repressed. In a recent review [7] it is estimated that between 6% to 10% of the whole genome is affected by this mechanism. Some of the phenotypic changes actuated by QS are increase in virulence, changes in the production of secondary metabolites, conjugation, growth inhibition, motility, swarming and bio-film formation. The reader must note that the autoinducers mentioned before are mainly used for bacterial intraspecies communication. A newly reported autoinducer called AI-2 has been proposed as a potential universal signals that mediate interspecies communication.

Important in governing the size of the "quorum" is 'compartment sensing' [21]. As noted above, the concentration of a given QS signal molecule may be a reflection of bacterial cell number, or at least the minimal number of cells (quorum) in a particular physiological state. To achieve the accumulation of a QS signal there is a need for a diffusion barrier, which ensures that more molecules are produced than lost from a given microhabitat. This 'compartment sensing' enables the QS signal molecule to be both a measure of the degree of compartmentalization and the means to distribute this information through the entire population. Likewise, the diffusion of QS signal molecules between detached sub-populations may convey information about their numbers, physiological state and the specific environmental conditions encountered. QS is thus a natural efficient, robust and simple mechanism for cell-to-cell communication.

3 An Environment-Aware P-System for Quorum Sensing

In this section we present an environment-aware P-system to simulate the process which occur in bacterial colonies which are capable of quorum sensing communications.

An environment-aware P-system Ω is defined as a collection of "environments", which contain both cells and metabolites, and communication channels between the environments. Both the environments and the channels are limited in their capacity of metabolite storage and transmission respectively. Formally: $\Omega = (\Pi_1, \ldots, \Pi_n, \tau_1, \ldots, \tau_n, \Gamma_1, \ldots, \Gamma_n, \Theta_1, \ldots, \Theta_n)$ where:

1. Π_i is an environment defined as $\Pi_i = (V, w_{E_i}, R_{E_i}, C_{E_{i_1}}, \ldots, C_{E_{i_n}})$
2. τ_i is the maximum amount of metabolites that Π_i can contain. The limit could arise for example from diffusion rate constraints. The metabolites are represented by objects in w_{E_i}.

3. $\Gamma_i = (\Pi_o, \Pi_t) 1 \leq o, t \leq n$ is a transmission channel between 2 environments.
4. Θ_i is the "bandwidth" of channel Γ_i.

An environment Π_i has:

1. V is a finite alphabet of symbols which represent secreted "metabolites" (e.g. signaling molecules or mRNA molecules).
2. $w_{E_i} \in V^*$ is a finite multiset of metabolites initially assigned to it.
3. R_i is a finite set of transformation rules associated with the environment. These rules can be of the form:
   - synthesis rules, $a \rightarrow y$, for $a \in V$, and $y \in V^*$
   - carriers construction rules (see [13]), $v_i m_1, \ldots, m_p \rightarrow [v_i m_1, \ldots, m_p]$ for $v_i \in V$, and $m_i \in V$.
   - carriers deconstruction rules, $[v_i m_1, \ldots, m_p] \rightarrow v_i m_1, \ldots, m_p$ for $v_i \in V$, and $m_i \in V$.
4. $C_i = (w_i, S_i, R_i, >)$, for each $1 \leq i \leq m_i$, a cell with:
   (a) $w_i \in V^*$ is a finite multiset of metabolites internal to cell C_i;
   (b) S_i is a finite set of communication rules; each rule has the form $(x; y, enter)$, where $x, y \in V^*$. These rules are used by the cell C_i to receive objects y from the environment when x is present in the cell.
   (c) R_i is a finite set of transformation-communication rules of the form $b_1 \ldots b_r \rightarrow (a_1)_{t_1} \ldots (a_q)_{t_q}$, for $b_i \in V$, and $1 \leq i \leq r$, $a_i \in V$, and $t_i \in \{here, out\}$, $1 \leq i \leq q$;
   (d) $>$ is a partial order on R_i. These rules are used by a cell to consume a multiset $b_1 \ldots b_r$ in order to produce a new multiset $a_1 \ldots a_q$ of which those with $t_j = here$ remain inside of the cell C_i and those with $t_j = out$ go out in the environment. A rule r_1 from R_i is used in a step if there is no rule r_2 in R_i which can be applied at the same step and $r_2 > r_1$.

In turn, each environment Π_i has a set of neighboring (i.e. overlapping regions) environments. This neighborhood set is involved in the "channel rules":

$N(\Pi_i) = \{\Pi_j | \exists \Gamma = (\Pi_i, \Pi_j)\ or\ \Gamma = (\Pi_j, \Pi_i)\}$. The channel rule is composed of three steps:

- $\Pi_i \circ [v_i m_1, \ldots, m_p] \overrightarrow{\Gamma_i} N(\Pi_i)$, for $v_i \in V$, and $m_i \in V$
- $\Pi_j = (V, w_{E_j} + m_1 + \ldots + m_p, R_j, C_{E_{j_1}}, \ldots, C_{E_{j_n}})$.
- $\Pi_i = (V, w_{E_i} - m_1 - \ldots - m_p, R_i, C_{E_{i_1}}, \ldots, C_{E_{i_n}})$

These rules state that a channel $\Gamma_i = (\Pi_i, \Pi_j)$ will be able to transfer already formed carriers (e.g. $[v_i m_1, \ldots, m_p]$) from, let say, Π_i to Π_j if the capacity of the Γ_i channel , Θ_i, is large enough to contain the p metabolites in the carriers and if the available storage in the target environment is enough to contain the additional metabolites once the carrier is unbuild. The target environment Π_j is non-deterministically chosen from $N(\Pi_i)$. After the movement of the carrier from one of the environments to the other, the appropriate number of metabolites

are substracted and added in each one[2]. Please note that this is just one rule composed of various steps not three independent rules, as such it should be considered atomic.

In general, biological Quorum Sensing once switched on it is never turned off but rather fine tuned and regulated by other concurrent processes within the cells. As such, we will not consider here halting computations but rather non-halting processes, we thus refrain from specifying an output membrane. This simple P-system model can mimic the basic behavior of a "quorated" system.

4 Conclusions and Future Research

A deeper understanding of biological Quorum Sensing could have an important impact not only in the biological sciences but also in computer science applications. Quorum sensing is a mechanism that, although complex in its biological details, is simple in its fundamental principles. Its appeal resides in the fact that, by exploiting a simple feedback loop and the limited capacity of both the cell and the environment to diffuse and carry a signal molecule, it is possible to bridge the "scale gap" between the individual bacterium and the colony. Such a mechanism should be useful in many applications beyond biological ones where multiple agents needs to robustly and efficiently coordinate their collective behavior based only on very limited information of the local environment. We are actively following several lines of research on both biological Quorum Sensing per se, modeling techniques based on P-systems, and we are also considering a range of computational applications. In particular we will investigate in the future the computational power of environment-aware P-systems and we will extend them to allow cell migration through the channels and channel/environment creation and removal. All of these will be reported elsewhere.

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[2] The '+' operator indicates the addition of an object to the multiset w_{E_j} and similarly with '-'.

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