Molecular Communication through Stochastic Synchronization Induced by Extracellular Fluctuations

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We model a synthetic gene regulatory network in a microbial cell, and investigate the effect of noises on cell-cell communication in a well-mixed multicellular system. A biologically plausible model is developed for cellular communication in an indirectly coupled multicellular system. Without extracellular noises, all cells, in spite of interaction among them, behave irregularly due to independent intracellular noises. On the other hand, extracellular noises that are common to all cells can induce collective dynamics and stochastically synchronize the multicellular system by actively enhancing the integrated interchange of signaling molecules.

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Intercellular communication and intracellular signal processing are essential for coordinated cell behaviors in multicellular systems. Many collective phenomena, such as complex pattern structures in multicellular organisms, various social behaviors in bacteria, and macromolecular transport between neighboring cells within the plasmodesmata [1], result from cell-cell communication. In particular, molecular communication among bacteria is widespread and involves complicated gene regulatory networks that serve to fine-tuned expression of a diverse group of genes. Several fundamental experiments [2,3] have indicated that cellular communication is generally accomplished by first transmitting individual cell information via signal molecules to neighboring cells, then exchanging information among these signal molecules and further generating a global cellular response at the level of tissues and organs.

Several theoretical models have been successfully established by studying natural or synthetic gene regulatory networks (SGN) [2–4] to examine the basic mechanism of cellular communication. It has been shown that a natural bacterial quorum-sensing mechanism can be used in a synthetic system to communicate between two populations of cells [3]. Recently, the fast threshold modulation [4] has been proposed as a possible scheme for cellular communication in coupled systems of a simple SGN based on the quorum-sensing apparatus of the marine bacterium *Vibrio fischeri*. However, such a communication mechanism neglects the effects of noises and diffusion that may play key roles in cooperative behaviors of biological systems. In fact, all cellular components exhibit intracellular noises due to the random birth and death of individual molecules [5,6] and extracellular noises due to environment perturbations. Such stochastic noises not only have been shown to affect the biological activity of an individual cell, but also may be exploited by living organisms to positively facilitate certain functions [7] like communication.

In this Letter we first report our design and construction of a SGN in a microbial cell by using an operon with two genes in *Vibrio fischeri*. A theoretical model is then proposed for cellular communication in an indirectly coupled multicellular system as an extension of our former two models: a globally interconnected model [7] and a mean field approximation model [8]. We especially consider the effects of both stochastic fluctuations and signal diffusion processes on cellular communication. We show that, in contrast to the noncooperative effect of intracellular noises, extracellular noises, if common to all cells, can induce a collective behavior, typically stochastic synchronized oscillation (SSO). Such a behavior is actually a phase-locking phenomenon from the viewpoint of probability distribution, which is similar to the effect of interacting coherence resonance oscillators [9].

We begin by describing the SGN in a cell [8], as shown in Fig. 1. Genes *luxI* and *luxR* that were initially discovered in *Vibrio fischeri*, are constructed as an operon, and both are under control of promoter P_{lac}Lux0. Protein LuxI is a synthase of protein AI (autoinducer), and produces AI. Proteins LuxR and AI are first dimerized and then form a

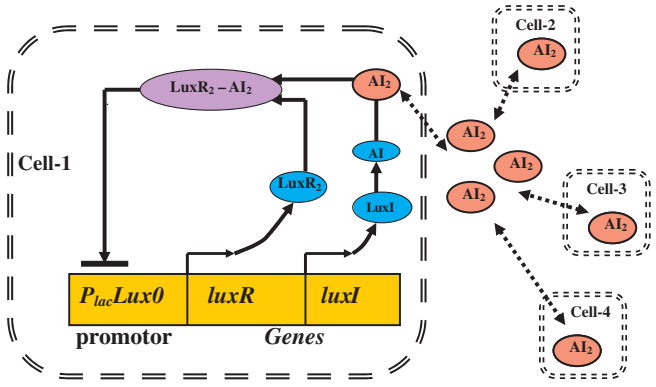

FIG. 1 (color). A schematic diagram of a gene network.

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TABLE I. Biochemical reactions in the SGN

Fast reactions	Slow reactions
$\mathrm{AI} + \mathrm{AI} \rightleftharpoons^{k_1}_{k_{-1}} \mathrm{AI}_2$	$\mathrm{DNA} \rightharpoonup^{k_m} mRNA_{\mathrm{LuxI}} + mRNA_{\mathrm{LuxR}} + \mathrm{DNA}$
$\mathrm{LuxR} + \mathrm{LuxR} \rightleftharpoons^{k_2}_{k_{-2}} \mathrm{LuxR}_2$	$\mathrm{ALD} \rightharpoonup^{\alpha k_m} mRNA_{\mathrm{LuxI}} + mRNA_{\mathrm{LuxR}} + \mathrm{ALD}$
$\mathrm{AI}_2 + \mathrm{LuxR}_2 \rightleftharpoons^{k_3}_{k_{-3}} \mathrm{AL}$	$mRNA_{\mathrm{LuxI}} \rightharpoonup^{k_{pi}} \mathrm{LuxI} + mRNA_{\mathrm{LuxI}}$
$\mathrm{AL} + \mathrm{DNA} \rightleftharpoons^{k_4}_{k_{-4}} \mathrm{ALD}$	$mRNA_{\mathrm{LuxR}} \rightharpoonup^{k_{pr}} \mathrm{LuxR} + mRNA_{\mathrm{LuxR}}$
	$\mathrm{LuxI} \rightharpoonup^{k_a} \mathrm{AI};\ \mathrm{AI} \rightharpoonup^{e_a} \emptyset$
	$\mathrm{LuxI} \rightharpoonup^{e_i} \emptyset;\ \mathrm{LuxR} \rightharpoonup^{e_r} \emptyset$
	$mRNA_{\mathrm{LuxI}} \rightharpoonup^{e_{mi}} \emptyset;\ mRNA_{\mathrm{LuxR}} \rightharpoonup^{e_{mr}} \emptyset$

complex of a hetero-tetramer that inhibits the activity of promoter P_{lac}Lux0. As a signaling molecule, AI_2 that is a dimer of AI, freely diffuses into the extracellular environment to exchange information with other cells, and then enters each cell to regulate expression of target genes. We assume that this circuit is engineered on plasmids [3,8,10] and grows further in *E. coli*.

Define LuxR_2 as the dimer of LuxR. Let AL and ALD represent AI_2-LuxR_2 and AI_2-LuxR_2-DNA complexes, respectively. All of the biochemical reactions are listed in Table I, and are divided into two groups: "fast" and "slow" reactions [7]. The former group includes multimerization reactions of proteins and binding reactions on the regulatory region of DNA, whereas the latter group is composed of transcription and translation, autoinducer synthesis, and degradation reactions [7]. On the right-hand side of the table, the last five reactions represent degradation. Constant $\alpha(0 < \alpha < 1)$ is a repression coefficient, and n_D is the copy number of plasmids with operon *luxI* and *luxR*. We set the parameter values below: $k_a = 3.0\ \mathrm{min}^{-1}$, $e_i = e_r = 1/6 \times 10^{-1}\ \mathrm{min}^{-1}$, $e_a = 1/6 \times 10^{-2}\ \mathrm{min}^{-1}$, $e_{mi} = e_{mr} = 1.0\ \mathrm{min}^{-1}$, $k_1 = k_2 = k_4 = 0.8/(\mathrm{nM} \cdot \mathrm{min})$, $k_3 = 6.0 \times 10^{-5}/(\mathrm{nM} \cdot \mathrm{min})$, $k_{-1} = k_{-2} = k_{-4} = 8.6\ \mathrm{min}^{-1}$, $k_{-3} = 0.75\ \mathrm{min}^{-1}$, $k_m = 5.4\ \mathrm{min}^{-1}$, $k_{pi} = k_{pr} = 6.5\ \mathrm{min}^{-1}$, $\alpha = 0.42$, $n_D = 10$, the individual *E. coli* cell volume $\upsilon = 1 \times 10^{-15} l$, and the total culture volume $V = 2 \times 10^{-3} l$, from [11,12] with slight modifications.

The origin of intracellular noises can be traced to random transitions among discrete chemical states due to low copy numbers of species in a living cell [5]. Theoretically, the master equation can be adopted to represent the random and discrete nature of biochemical reactions [13] [see Appendix A of supporting material [14]]. If the numbers for LuxI, LuxR, AL, ALD, AI_2, LuxR_2, $mRNA_{\mathrm{LuxI}}$, $mRNA_{\mathrm{LuxR}}$, and AI are denoted by R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, and R_9, respectively, then by appropriate approximations [also see Appendix A [14]], we can derive the Langevin equations for a single cell in terms of concentrations:

$$\frac{dx_i(t)}{dt} = f_i(x(t)) + \eta_i(t) \quad \text{for} \quad i = 1, \ldots, 9, \qquad (1)$$

where x_i represents the concentration of R_i, i.e., $x_i = R_i/\upsilon$, and η_i, called as intracellular noises, are Gaussian white noises with $\langle \eta_i(t) \rangle = 0$, $\langle \eta_i(t)\eta_j(t') \rangle = K_{ij}(x(t))\delta(t - t')$.

TABLE II. Expressions of f and K in the Langevin equations.

Function expressions $f_i(x)$

$f_1(x) = -k_a x_1 - e_i x_1 + k_{pi} x_7$

$f_2(x) = -2k_2 x_2 (x_2 - \frac{1}{\upsilon}) + 2k_{-2} x_6 + k_{pr} x_8 - e_r x_2$

$f_3(x) = k_3 x_5 x_6 - x_3 (k_{-3} + k_4 (\frac{n_D}{\upsilon} - x_4)) + k_{-4} x_4$

$f_4(x) = k_4 x_3 (\frac{n_D}{\upsilon} - x_4) - k_{-4} x_4$

$f_5(x) = k_1 x_9 (x_9 - \frac{1}{\upsilon}) - k_{-1} x_5 - k_3 x_5 x_6 + k_{-3} x_3$

$f_6(x) = k_2 x_2 (x_2 - \frac{1}{\upsilon}) - k_{-2} x_6 - k_3 x_5 x_6 + k_{-3} x_3$

$f_7(x) = k_m (\frac{n_D}{\upsilon} - x_4) + \alpha k_m x_4 - e_{mi} x_7$

$f_8(x) = k_m (\frac{n_D}{\upsilon} - x_4) + \alpha k_m x_4 - e_{mr} x_8$

$f_9(x) = -2k_1 x_9 (x_9 - \frac{1}{\upsilon}) + 2k_{-1} x_5 + k_a x_1 - e_a x_9$

Covariances $K_{ij}(x)$

$K_{11} = k_a x_1 + e_i x_1 + k_{pi} x_7$

$K_{22} = 4k_2 x_2 (x_2 - \frac{1}{\upsilon}) + 4k_{-2} x_6 + k_{pr} x_8 + e_r x_2$

$K_{33} = k_3 x_5 x_6 + x_3 (k_{-3} + k_4 (\frac{n_D}{\upsilon} - x_4)) + k_{-4} x_4$

$K_{44} = k_4 x_3 (\frac{n_D}{\upsilon} - x_4) + k_{-4} x_4$

$K_{55} = k_1 x_9 (x_9 - \frac{1}{\upsilon}) + k_{-1} x_5 + k_3 x_5 x_6 + k_{-3} x_3$

$K_{66} = k_2 x_2 (x_2 - \frac{1}{\upsilon}) + k_{-2} x_6 + k_3 x_5 x_6 + k_{-3} x_3$

$K_{77} = k_m (\frac{n_D}{\upsilon} - x_4) + \alpha k_m x_4 + e_{mi} x_7$

$K_{88} = k_m (\frac{n_D}{\upsilon} - x_4) + \alpha k_m x_4 + e_{mr} x_8$

$K_{99} = 4k_1 x_9 (x_9 - \frac{1}{\upsilon}) + 4k_{-1} x_5) + k_a x_1 + e_a x_9$

$K_{19} = -k_a x_1$

$K_{26} = -2k_2 x_2 (x_2 - \frac{1}{\upsilon}) - 2k_{-2} x_6$

$K_{34} = -k_4 x_3 (\frac{n_D}{\upsilon} - x_4) - k_{-4} x_4$

$K_{35} = -k_3 x_5 x_6 - k_{-3} x_3$

$K_{36} = -k_3 x_5 x_6 - k_{-3} x_3$

$K_{56} = k_3 x_5 x_6 + k_{-3} x_3$

$K_{59} = -2k_1 x_9 (x_9 - \frac{1}{\upsilon}) - 2k_{-1} x_5$

Expressions of f_i and K_{ij} are listed in Table II according to the Fokker-Plank equations (A3) and Table III of supporting material [14]. Note that all $K_{ij} = 0$ except those in Table II.

For simplicity, we consider a well-mixed homogenous culture with the identical cells. While x_5 represents the concentration of intracellular AI_2, y is assumed to be the concentration of extracellular AI_2 in the environment. Then, the coupled multicellular system corresponding to Fig. 1 can be mathematically expressed as:

$$\begin{aligned} \frac{dx_i^j(t)}{dt} &= f_i(x^j(t)) + \eta_i^j(t) + d_i[y(t-\tau) - x_i^j(t)] + \xi_i(t), \\ \frac{dy(t)}{dt} &= -k_y y(t) + \beta \sum_{j=1}^{n} d_5[x_5^j(t-\tau) - y(t)] + \xi_{m+1}(t), \end{aligned} \tag{2}$$

where $\beta = v/V$ and $1 \le i \le m(= 9)$. Here the superscript j represents the jth cell. Coupling coefficients are assumed as follows: $d_i \neq 0$ if $i = 5$, and 0 otherwise for all j. The extracellular noises, $\xi_k (1 \le k \le m + 1)$, are assumed as independently and identically distributed Gaussian noises with $\langle \xi_k(t) \rangle = 0$ and $\langle \xi_k(t), \xi_{k'}(t') \rangle = \sigma^2 \delta_{kk'} \delta(t - t')$. τ is a time delay, which is due to the diffusion and transport process of AI_2 between the environment and a cell, and k_y is the degradation rate of y. In simulation, we set $\tau =$ 15.6 min and $k_y = 0.26$. The time evolution of y represents a process of diffusion and transport of AI_2, and the coupling term in the first equation of Eq. (2) describes the interplay between a cell and the common environment through the signal molecule. The assumption that each $\xi_i(t)$ is uncorrelated with all $\eta_k^j(t)$ is reasonable since the intracellular noises in a cell are generally independent of the extracellular noises and vice versa.

Now, let us investigate effects of extracellular noises on cell-cell communication. Note that the extracellular noise intensity σ plays the role of a control parameter that governs the onset and the peak frequency of oscillations. For $d_5 = 4.35$, we set $\sigma = 5.43$, and plot the time evolution of signal molecules (AI_2) in Fig. 2(a). This figure shows the typical SSO which is actually the locking phenomenon of the peak frequencies in the power spectra [refer Figs. 3(a) and 3(b)]. It was observed that the frequency-locked region of σ tends to become broader with the increase of the coupling strength d_5. In the extreme case of $\sigma = 0$, i.e., without the extracellular noises, the system behaves irregularly in a noncooperative manner due to the intracellular noises [Fig. 2(b)].

In Refs. [15,16], it has been shown how the instantaneous phases of stochastic oscillations can be locked. Han *et al.* [9] have successfully used instantaneous phase difference to describe synchronization of two coupled stochastic oscillators. The similar treatment can be extended to our case. Thus, to describe the SSO we calculate instantaneous phase difference between stochastic oscillators, where each cell can be viewed as one stochastic oscillator [9]. Once an instantaneous phase is defined for a stochastic oscillator [17], it can be applied to the synchronization of many interacting stochastic oscillators. Because of the nonuniformity of the phase defined geometrically, we use the instantaneous phase $\phi(t) = 2\pi(t - \tau_k)/(\tau_{k+1} - \tau_k) + 2\pi k$, where τ_k is the time of the kth firing [for details, see [9,17]]. Furthermore, without loss of generality, we define the instantaneous phase difference between two stochastic oscillators as $\Delta\phi = \phi_i - \phi_j$. As the coupling strength d_5 is increased for a fixed σ, we observed a transition from a regime where the phases rotate with different velocities ($\Delta\phi \sim \Delta\Omega t$), to synchronous state where the phase difference oscillates around some mean value [refer Fig. 3]. For strong coupling (e.g., $d_5 = 4.35$), the phase locking in Eq. (2) is observed for a broad range of σ, as demonstrated in Fig. 3(a) in which the ratio of the peak frequencies or the winding number is stabilized near 1.0. Figure 3(b) shows how the peak frequencies of three stochastic oscillators approach each other and become coincident at $d_5 \approx 4.35$. Moreover, for the time evolution of synchronization, the phase difference, e.g., at $d_5 = 4.35$, of two stochastic oscillators rapidly reduces into a narrow range but fluctuates slightly around the mean value [see the inset of Fig. 3(a)].

According to the simulation, it was found that both the variances and covariances of the variables are small, compared to the amplitude of the oscillation. Hence, to examine the SSO qualitatively, we use the Gaussian approximation for all the stochastic variables [18]. Generally, when the noise intensity is strong, this approximation is quantitatively not justified, but may still reproduce the main features of the dynamics [18]. Following the approxi-

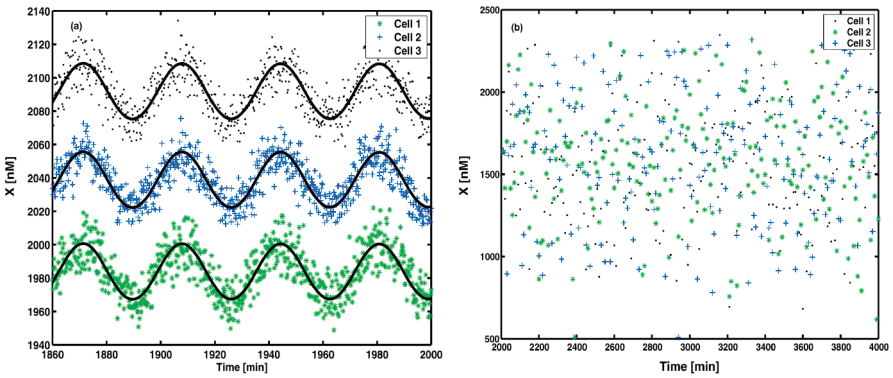

FIG. 2 (color). Time evolution of signal molecules (AI_2) for three cells according to the stochastic Eq. (2) at $d_5 = 4.35$. (a) Synchronous stochastic oscillation in which the signal molecules oscillate around their means for $\sigma =$ 5.43; (b) irregular dynamics due to intracellular noises for $\sigma = 0$.

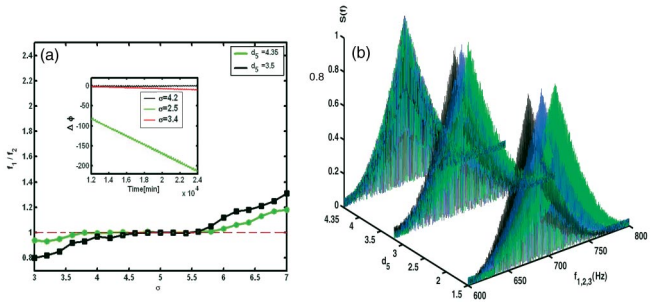

FIG. 3 (color). The frequency locking observed in Eq. (2). (a) The ratio of the peak frequencies or the winding number, which is stabilized near 1.0 for some ranges of the extracellular noise σ at different coupling strengths $d_5 = 4.35$ (green) and $d_5 = 3.5$ (black). The inset displays the time evolution of the phase difference for nonsynchronous ($\sigma = 2.5$), nearly synchronous ($\sigma = 3.4$), and synchronous ($\sigma = 4.2$) states at $d_5 = 4.35$ after the transient relaxation has finished, where the synchronous difference is bounded but fluctuates around the mean value. (b) The normalized power spectra S for different coupling strengths at $\sigma = 4.2$, where f_i is frequency of the ith cell.

mation, we first derive deterministic equations corresponding to Eq. (2) for cumulants [see Appendix B [14]] and then investigate their synchronization behaviors, and give a synchronous mechanism based on an extended version of Global Hopf Bifurcation Theorem [19] [see Appendix C [14], and the details will be published elsewhere]. In addition, the sufficient conditions of the synchronous solution are also derived in [14].

In conclusion, we have investigated both numerically and analytically the effect of extracellular noises on cell-cell communication by considering a well-mixed homogeneous system of microbial cells. We have shown that such noises act as a compensating signal source to enhance an integrated exchange of information and force all the cells to be stochastically synchronized. As a result, cellular communication is fulfilled in a synchronous manner. Our work can be viewed as a first step towards understanding natural communication processes in living organisms. The main features of this Letter are summarized as follows.

The intracellular noises η_i in Eq. (1), which are additive and white, are derived directly from the master equation by taking the second order approximation. Theoretically, when the jump or change of the individual R_i is small, such an approximation can approach an accurate result [13]. Otherwise, the Ω expansion technique or other approximation methods should be adopted to approximate the master equation. In the numerical example, all of the jumps are 1 or 2, which are small in contrast to R_i but still have introduced errors in the simulation.

The intracellular and extracellular noises can play different roles in molecular communication. The former has a tendency to disturb cooperative behaviors among cells whereas the latter, if common to all cells, has the effect to synchronize the cells by exerting the same fluctuations on each cell through signal molecules.

We have proposed and analyzed a general interaction model of Eq. (2) in a multicellular system, where any two cells are not directly but indirectly coupled through the environment y, in contrast to conventional star-type model or interconnected model.

Finally, although the extracellular noises here are assumed to be common to all cells under the well-mixed condition, they may be heterogeneous under a more realistic condition. It is an important future problem to analyze spatio-temporal dynamics of cells in such a heterogeneous circumstance.

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