Effects of bidirectional phenotype switching on signal noise in a bacterial community

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Cells can sense and process various signals. Noise is inevitable in the cell signaling system. In a bacterial community, the mutual conversion between normal cells and persistent cells forms a bidirectional phenotype switching cascade, in which either one can be used as an upstream signal and the other as a downstream signal. In order to quantitatively describe the relationship between noise and signal amplification of each phenotype, the gain-fluctuation relationship is obtained by using the linear noise approximation of the master equation. Through the simulation of these theoretical formulas, it is found that the bidirectional phenotype switching can directly generate interconversion noise which is usually very small and almost negligible. In particular, the bidirectional phenotype switching can provide a global fluctuating environment, which will not only affect the values of noise and covariance, but also generate additional intrinsic noise. The additional intrinsic noise in each phenotype is the main part of the total noise and can be transmitted to the other phenotype. The transmitted noise is also a powerful supplement to the total noise. Therefore, the indirect impact of bidirectional phenotype switching is far greater than its direct impact, which may be one of the reasons why chronic infections caused by persistent cells are refractory to treat.

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I. INTRODUCTION

Antibiotics have saved countless lives since they were introduced into modern medicine because they provide good treatments for many diseases, including serious infections caused by bacteria. However, due to widespread use and abuse, antibiotic resistance has gradually increased, while the discovery of new antibiotics has decreased, bringing about a global health crisis [1–3].

Persistence is believed to be the underlying cause of antibiotic resistance [4]. It is a reversible phenotype switching associated with heterogeneous bacterial populations [1,5], which can lead to nongenetically encoded and reversible loss of antibiotic susceptibility [6]. Persistence has become a new method of controlling antibiotic resistance, and it has attracted more and more attention.

The terms "persistence" and "persistent cells (PCs)" were proposed by Bigger [7] in 1944. By exposing genetically homogeneous strains of *Staphylococcus aureus* to a bactericidal concentration of penicillin for a long time, a small part of bacteria can escape the killing of antibiotics and survive, but their offspring are still sensitive to antibiotics [8–10]. This is the persistence of the cell. The small part of bacteria is called persistent cells. PCs are only temporarily resistant to antibiotics. When antibiotic pressure drops, they return to normal cells (NCs) [11]. PCs are not genetic variation, but phenotype switching [12–16]. In response to environmental triggers such as resource pressure or the presence of antibiotics, NCs and PCs can be transformed into each other [17–19] to form a bidirectional phenotype switching cascade.

Random fluctuations in the level of biomolecules are called "noise." In this bidirectional phenotype switching cascade, NCs and PCs can self-proliferate and undergo procedural death. Each biochemical reaction occurs randomly, which creates inherent noise. Changes in the microenvironment will generate external noise. So, noise is inevitable.

Cells can sense and process various signals that control their basic and specific cellular processes [20,21]. Cell signal transduction transmits intracellular and extracellular signals to different cellular compartments to regulate various cell reactions, thereby responding to environmental and intracellular changes [22]. It plays a vital role in almost all cell functions.

In the bidirectional phenotype switching cascade of the bacterial community, each phenotype can be used as an upstream signal, and the other as a downstream signal. Now interesting questions are pointed out: What is the relationship between cellular noise and signal amplification? What are the effects of phenotype switching on the signal noise? To address these issues, the gain factor [21,23] is introduced so that the gain fluctuation equation is obtained. Through simulation of these theoretical formulas, the characteristics of signal noise propagation can be studied.

The relevant experimental results in published papers so far are so few that theoretical results cannot be compared with actual data. In order to test whether the theoretical results are correct, Gillespie's algorithm [24,25] is used, which is a classic approach for stochastic simulation of chemical systems [26]. Gillespie's algorithm uses a strictly derived Monte Carlo program to numerically simulate the time evolution of a given chemical system and can correctly explain the inherent fluctuations and correlations in the deterministic formula [24].

The paper is arranged as follows. We start by constructing a bidirectional phenotype switching cascade in a bacterial

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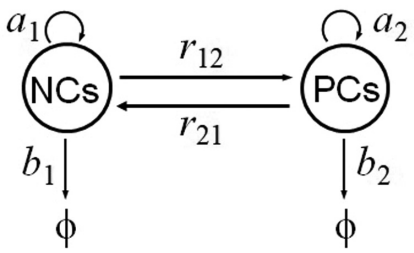

FIG. 1. The bidirectional phenotype switching cascade in a bacterial community [29–31]. Normal cells (NCs) and persistent cells (PCs) can undergo self-proliferation, death, and switching with the rates of a_i, b_i, and r_{ij} $(i, j = 1, 2; i \neq j)$, respectively. Normally, the death rate b_i, and the switching rate r_{ij} are taken as constants. Due to limited nutrition and exploitative competition, the self-proliferate rate of phenotype i is usually taken as $a_i = k_i[1 - (N_1 + N_2)/N_0]$, where k_i is the maximum self-proliferation rate, N_0 is the environment carrying capacity, and N_i $(i = 1, 2)$ are the numbers of two phenotypes.

community in Sec. II. In Sec. III, the gain-fluctuation relation for theoretically analyzing is derived by using the linear noise approximation of the master equation [27,28]. Using these theoretical formulas, the effects of gain factors on signal noise propagation is studied in Sec. IV. We end with conclusions and discussion in Sec. V.

II. BIDIRECTIONAL PHENOTYPE SWITCHING CASCADE IN A BACTERIAL COMMUNITY

The bidirectional phenotype switching cascade with exploitative competition in the bacterial community [29–31] is shown in Fig. 1.

PCs and NCs can self-proliferate, and their maximum self-proliferation rates are k_1 and k_2, respectively. Due to the limited nutrition, the self-proliferation rate will be affected by the environment carrying capacity, that is, the maximum total number of cells N_0. In addition, in order to obtain more resources, there is an exploitative competition between different cell phenotypes, that is, one cell phenotype maximizes the use of resources and reduces resource sharing, thus exhausting the resource availability of other cell phenotypes [29–31]. Based on the classic competitive Lotka-Volterra equation, the actual self-proliferation rates of two phenotypes are taken as $a_1 = k_1(1-\frac{N_1+N_2}{N_0})$ and $a_2 = k_2(1-\frac{N_1+N_2}{N_0})$, respectively, where N_i $(i = 1, 2)$ are the numbers of two phenotypes.

PCs and NCs can be switched mutually. The switching rates are described as r_{12} and r_{21}, respectively. Since PCs are only a small part of the bacterial community, the switching rate is relatively small. Therefore, r_{12} and r_{21} are usually taken as constants.

PCs and NCs can undergo death with the probabilities of b_1 and b_2 per unit time, respectively. Normally, the death rate b_i is taken as a constant.

In the deterministic description, the time evolution is

$$\frac{dN_1}{dt} = a_1N_1 - b_1N_1 - r_{12}N_1 + r_{21}N_2, \tag{1}$$

$$\frac{dN_2}{dt} = a_2N_2 - b_2N_2 - r_{21}N_2 + r_{12}N_1. \tag{2}$$

Taking $dN_i/dt = 0$ $(i = 1, 2)$, the steady states N_i^s are obtained as follows:

$$N_1^s = \frac{\rho_1 s_2 + r_{12}}{k_2}\frac{N_0}{\rho_1(1+\rho_1)},\ N_2^s = \frac{\rho_1 s_2 + r_{12}}{k_2}\frac{N_0}{1+\rho_1}, \tag{3}$$

with

$$\rho_1 = \frac{k_1 s_2 - k_2 s_1 + \sqrt{(k_1 s_2 - k_2 s_1)^2 + 4k_1 k_2 r_{12} r_{21}}}{2k_2 r_{21}}. \tag{4}$$

Here, $s_1 = k_1 - b_1 - r_{12}$, $s_2 = k_2 - b_2 - r_{21}$, denoting the inherent net (per-capita) growth rates.

If there is no mutual switching, that is, $r_{12} = r_{21} = 0$, the steady-state value is 0 when $a_i \neq b_i$, and an arbitrary value when $a_i = b_i$. Both of these situations are impossible in biology. Therefore, the case of $r_{12} = r_{21} = 0$ is not included in the following discussion.

III. NOISY SIGNAL PROPAGATION FORMULAS

A. The reaction flux elasticity

To measure how the balance between production and elimination of N_i is affected by N_k, the reaction flux elasticity [32–34] is defined by

$$H_{ki} = \left\langle \frac{\partial \ln(J_i^-/J_i^+)}{\partial \ln N_k} \right\rangle, \tag{5}$$

where $J_i^+ = a_i + \sum_{j\neq i} r_{ji}N_j$ is the pure production rate of phenotype i, $J_i^- = b_i + \sum_{j\neq i} r_{ij}N_i$ is the pure elimination rate of phenotype i. Angle bracket indicates the average value, which can be replaced by the number of stationary population in the mean-field theory, that is, $\langle N_i\rangle = N_i^s$.

Equation (5) can be rewritten as

$$H_{ki} = -\left\langle \frac{N_k}{J_i^+}\frac{\partial}{\partial N_k}(J_i^+ - J_i^-) \right\rangle. \tag{6}$$

Here, $J_i^+ - J_i^-$ is the net production rate of phenotype i. Taking into account Eqs. (1) and (2), we have

$$H_{11} = \frac{k_1\langle N_1\rangle/N_0 + \rho_1 r_{21}}{a_1 + \rho_1 r_{21}}, \tag{7}$$

$$H_{21} = \rho_1\frac{k_1\langle N_1\rangle/N_0 - r_{21}}{a_1 + \rho_1 r_{21}}, \tag{8}$$

$$H_{12} = \frac{k_2\langle N_2\rangle/N_0 - r_{12}}{\rho_1 a_2 + r_{12}}, \tag{9}$$

$$H_{22} = \frac{r_{12} + \rho_1 k_2\langle N_2\rangle/N_0}{\rho_1 a_2 + r_{12}}. \tag{10}$$

In the bacterial community, the switching rate is relatively small, so the sum of the two switching rates is always less than the difference between the maximum growth rate and the death rate of each phenotype, that is, $r_{21} + r_{12} < k_1 - b_1$, and $r_{12} + r_{21} < k_2 - b_2$. Then, the value of each reaction flux elasticity will be larger than zero, that is, $H_{ij} > 0$.

B. The gain factor

In signal transduction systems, the gain factor is used to quantify the noise amplification. It is defined as the ratio of the

relative change of the output signal to the relative change of the input signal [21,23]. In the phenotype switching cascade, the upstream phenotype is regarded as the input signal and the downstream phenotype is regarded as the output signal. Obviously, input and output signals are affected by the reaction process, which is different from the gene-based signal transduction systems. Thus, when the populations change very little, we redefine the gain factor based on the reaction flux as

$$g_{ik} = \left\langle \frac{\Delta N_k/N_k}{\Delta N_i/N_i} \right\rangle = \left\langle \frac{\partial \ln N_k}{\partial \ln N_i} \right\rangle = \left\langle \frac{\partial \ln(J_k^-/J_k^+)}{\partial \ln N_i} \right\rangle \left\langle \frac{\partial \ln N_k}{\partial \ln(J_k^-/J_k^+)} \right\rangle = \frac{H_{ik}}{H_{kk}}. \tag{11}$$

In the bacterial community including NCs and PCs, the switching between the two phenotypes is bidirectional. Each phenotype can be used as an input signal, and the other phenotype is an output signal. Therefore, there are two gain factors:

$$g_{12} = \frac{H_{12}}{H_{22}} \equiv g_1, \qquad g_{21} = \frac{H_{21}}{H_{11}} \equiv g_2. \tag{12}$$

Substituting Eqs. (7)–(10) into Eq. (12) and simplifying, we can get

$$g_1 = \frac{k_2\langle N_2\rangle - r_{12}N_0}{\rho_1 k_2\langle N_2\rangle + r_{12}N_0}, \qquad g_2 = \rho_1 \frac{k_1\langle N_1\rangle - r_{21}N_0}{k_1\langle N_1\rangle + \rho_1 r_{21}N_0}. \tag{13}$$

C. The average lifetime

In the quantitative biology, the average lifetime τ_i is defined as the population size divided by its total elimination rate. Under the steady state, $\langle J_i^+\rangle = \langle J_i^-\rangle = \langle J_i\rangle$, so

$$\tau_i = \left\langle \frac{N_i}{J_i^-} \right\rangle = \left\langle \frac{N_i}{J_i^+} \right\rangle = \left\langle \frac{N_i}{J_i} \right\rangle. \tag{14}$$

For NCs and PCs in the bacterial community, their average lifetimes are as follows:

$$\tau_1^{-1} = b_1 + r_{12}, \quad \tau_2^{-1} = b_2 + r_{21}. \tag{15}$$

D. Gain-fluctuation relation

In stochastic dynamics theory, the master equation gives the joint probability distribution $P(N_1, N_2, t)$ of population dynamics [27]:

$$\frac{\partial P(N_1,N_2,t)}{\partial t} = \left\{ \sum_{i=1}^{2} \left[\left(\Gamma_i^{-1} - 1\right) a_i N_i + \left(\Gamma_i^{1} - 1\right) b_i N_i \right] + \left(\Gamma_1^1\Gamma_2^{-1} - 1\right) r_{12}N_1 + \left(\Gamma_2^1\Gamma_1^{-1} - 1\right) r_{21}N_2 \right\} \times P(N_1,N_2,t). \tag{16}$$

Here, $\Gamma_{i\,(j)}^{\pm m}$ is the step operator that increases $N_{i(j)}$ by $\pm m$, i.e., $\Gamma_i^{\pm m} f(N_i, N_j) = f(N_i \pm m, N_j)$, or $\Gamma_j^{\pm m} f(N_i, N_j) = f(N_i, N_j \pm m)$.

The master equation is almost of no direct use to us because it cannot be solved. However, it can be expanded using van Kampen's Ω-expansion method. For large system size Ω, let $N_i(t) = \Omega x_i(t) + \Omega^{1/2}\xi_i(t)$, and $P(N_1, N_2, t) = \Omega^{-1}\Pi(\xi_1, \xi_2, t)$. Collecting the terms of Ω^0 in the expansion of Eq. (16), the Fokker-Planck equation [28] can be obtained:

$$\frac{\partial}{\partial t}\Pi(\xi_1,\xi_2,t) = -\sum_{i,k}^{2} A_{ik}\frac{\partial}{\partial \xi_i}[\xi_k \Pi(\xi_1,\xi_2,t)] + \frac{1}{2}\sum_{i,k}^{2} B_{ik}\frac{\partial^2\Pi(\xi_1,\xi_2,t)}{\partial\xi_i\partial\xi_k}. \tag{17}$$

$\boldsymbol{A}$ is the drift matrix and $\boldsymbol{B}$ is the diffusion matrix. Their matrix elements are

$$A_{ik} = \frac{\partial}{\partial N_k}(J_i^+ - J_i^-) = \frac{\partial}{\partial N_k}\left(a_i N_i - b_i N_i - \sum_{j\neq i} r_{ij}N_i + \sum_{j\neq i} r_{ji}N_i\right), \tag{18}$$

$$B_{ii} = 2\left(a_i N_i + \sum_{j\neq i} r_{ji}N_j\right), \qquad B_{ik} = -(r_{ik}N_i + r_{ki}N_k) \quad (k \neq i). \tag{19}$$

The coefficients of the Fokker-Planck equation [Eq. (17)] at the steady state satisfy the fluctuation-dissipation relationship:

$$\mathbf{AC} + (\mathbf{AC})^{\mathrm{T}} + \Omega\mathbf{B} = \mathbf{0}, \tag{20}$$

where $\boldsymbol{C}$ is the covariance matrix. In order to quantify the noise propagation, the fluctuation-dissipation relationship [Eq. (20)] is usually normalized [35–37] as

$$\mathbf{MV} + (\mathbf{MV})^{\mathrm{T}} + \mathbf{D} = \mathbf{0}, \tag{21}$$

with

$$V_{ik} = V_{ki} = \frac{C_{ik}}{\langle N_i\rangle\langle N_k\rangle}, \tag{22}$$

$$M_{ik} = A_{ik}\frac{\langle N_k\rangle}{\langle N_i\rangle}, \tag{23}$$

$$D_{ik} = \frac{\Omega B_{ik}}{\langle N_i\rangle\langle N_k\rangle}. \tag{24}$$

Angle brackets indicate average values. In the mean-field theory, the stationary population number can be replaced by its mean value, namely $N_i^s = \langle N_i\rangle$. Equation (21) is named the normalized fluctuation-dissipation formula. Here, $\boldsymbol{V}$ includes normalized variance V_{ii} and normalized covariance $V_{ik}\,(k \neq i)$. V_{ii} describes the fluctuation (or noise) in the ith phenotype, and V_{ik} describes the correlation between the fluctuations in the ith phenotype and in the kth phenotype.

$\mathbf{M}$ is the normalized drift matrix. Substituting Eq. (18) into Eq. (23), and considering Eq. (11), we can get its elements:

$$M_{ik} = -g_{ki}\Xi_i, \tag{25}$$

where $\Xi_i = H_{ii}/\tau_i = \langle \partial(J_i^- - J_i^+)/\partial N_i \rangle$, which is the net average death probability of the ith phenotype per unit time, indicates the change rate of net death rate with its own population.

D is the normalized diffusion matrix, and its element can be obtained based on Eqs. (19) and (24):

$$D_{ii} = \frac{2\left[a_i\langle N_i\rangle + \sum_{j\neq i} r_{ji}\langle N_j\rangle\right]}{\langle N_i\rangle^2}, \tag{26}$$

$$D_{ik} = -\left(\frac{r_{ik}}{\langle N_k\rangle} + \frac{r_{ki}}{\langle N_i\rangle}\right) \quad (k \neq i). \tag{27}$$

Substituting Eqs. (25)–(27) into Eq. (21), we get

$$\sum_{j=1}^{2} V_{ji} g_{ji} = \frac{1}{\langle N_i\rangle \tau_i \Xi_i}, \tag{28}$$

$$\sum_{j=1}^{2} (V_{jk}\Xi_i g_{ji} + V_{ij}\Xi_k g_{jk}) = -\left[\frac{r_{ik}}{\langle N_k\rangle} + \frac{r_{ki}}{\langle N_i\rangle}\right] \quad (k \neq i)\ . \tag{29}$$

By adopting the noise decomposition method used in gene networks [32–34] and expanding Eqs. (28) and (29) with $V_{ij} = V_{ji} (i \neq j)$, $g_{12} \equiv g_1$, and $g_{21} \equiv g_2$, we can get

$$V_{ii} = \overbrace{\frac{1}{\langle N_i\rangle H_{ii}}}^{\text{Pure}} + \overbrace{\frac{\Xi_j \Lambda}{\langle N_i\rangle H_{ii}} g_i g_j}^{\text{Additional}} + \underbrace{\frac{\Xi_i \Lambda}{\langle N_j\rangle H_{jj}} g_j^2}_{\substack{\text{Transmitted noise}\\ \text{from the other phenotype}}} + \underbrace{\left(\frac{r_{ji}}{\langle N_i\rangle} + \frac{r_{ij}}{\langle N_j\rangle}\right)\Lambda g_j}_{\substack{\text{Interconversion noise}\\ \text{between two phenotypes}}} \quad (i, j = 1, 2; i \neq j), \tag{30}$$

$$V_{12} = V_{21} = \underbrace{\left(-\frac{\Lambda \Xi_2 g_1}{\langle N_1\rangle H_{11}}\right)}_{\substack{\text{From additional intrinsic}\\ \text{noise in one phenotype}}} + \underbrace{\left(-\frac{\Lambda \Xi_1 g_2}{\langle N_2\rangle H_{22}}\right)}_{\substack{\text{From additional intrinsic}\\ \text{noise in the other phenotype}}} + \underbrace{\left[-\Lambda\left(\frac{r_{21}}{\langle N_1\rangle} + \frac{r_{12}}{\langle N_2\rangle}\right)\right]}_{\substack{\text{From interconversion noise}\\ \text{between two phenotypes}}}, \tag{31}$$

with $\Lambda^{-1} = (1 - g_1 g_2)(\Xi_1 + \Xi_2)$, which is determined by two net average death probabilities Ξ_i and two gain factors g_i. Except for the first term in Eq. (30), the other six terms all contain factor Λ, indicating that the mutual switching between NCs and PCs provides a fluctuating environment with a global impact on the two phenotypes.

Equation (30) is the gain-fluctuation relationship [23,38] of the bidirectional phenotype switching cascade. It is shown that the total noise in one phenotype includes intrinsic noise, transmitted noise from the other phenotype, and interconversion noise between the two phenotypes. In addition to pure intrinsic noise, intrinsic noise also includes additional intrinsic noise. The pure intrinsic noise is equal to the reciprocal of the product of the average number $\langle N_i\rangle$ and the reaction flux elasticity H_{ii}. Obviously, the smaller the average number, the more significant the pure intrinsic noise. The additional intrinsic noise in the ith phenotype is $(\Xi_j \Lambda g_i g_j)$ times its pure intrinsic noise, reflecting the indirect influence of the mutual switching between NCs and PCs. The transmitted noise comes from additional intrinsic noise in the other phenotype, indicating that the noise can be transmitted in this bidirectional phenotype switching cascade. The interconversion noise is related to the two switching rates and reflects the direct influence of the mutual switching between NCs and PCs.

Equation (31) describes the correlation between the signal fluctuations in NCs and in PCs. It can be found the correlation is related to the additional intrinsic noise in each phenotype and the interconversion noise between the two phenotypes.

IV. SIMULATIONS AND RESULTS

A. The character of gain factors

Stationary numbers of two phenotypes N_i^s are given in Eqs. (3) and (4). We can see that the stationary number of each phenotype is determined by all rate parameters. Generally, the death rate b_i and the switching rate r_{ij} are considered constants. Moreover, the maximum self-proliferation rate of PCs k_2 does not change much. Therefore, the parameter k_1 is selected as the control variable. Here we take the dimensionless parameters $k_2 = 0.121$, $b_1 = b_2 = 0.02$, $r_{12} = r_{21} = 0.001$, and $N_0 = 1000$ [29–31]. Considering the biological significance of the parameters, let k_1 vary within the range of [0.1, 0.24].

The gain factors $g_i (i = 1, 2)$ as a function of k_1 are given in Fig. 2. In Fig. 2(a), lines are theoretical predictions of Eq. (13), where $\langle N_i\rangle$ is replaced by N_i^s. Hollow markers are from simulation results of Eq. (13), where $\langle N_i\rangle$ is replaced by the output of the Gillespie method [24,25]. It can be seen that when Ω (about 1000) is selected appropriately and the number of iterations is large enough (about 10^9 times), the above two simulation results are in good agreement. In addition to the two change curves of g_i, the change curve of $1/g_2$ is also shown in Fig. 2(b), which is represented by a dotted line. We can find that $g_1 \neq 1/g_2$, which is different from the definition in signal transduction system, because the gain factor we define is related to the reaction process, rather than a simple data ratio.

It can be seen that as k_1 increases, g_1 increases and gradually reaches a constant value, while g_2 decreases. It can be seen from the partial enlargement that although $g_2 \neq 0$, it will eventually become very small within the permitted parameter range. When $k_1 = 0.12$, the two gain factors are equal, that is, $g_1 = g_2 = 1$, indicating that the relative change of the output signal at this point is equal to the change of the input signal. In addition, it is a turning point. Before and after $k_1 = 0.12$, the sign of the difference between the relative change of the output signal and that of the input signal will be opposite.

B. Effects of gain factors on stationary numbers

It can be seen from Eqs. (3) and (4) that although the expressions of stationary numbers do not obviously include the gain factors g_i, according to the definition of g_i, the

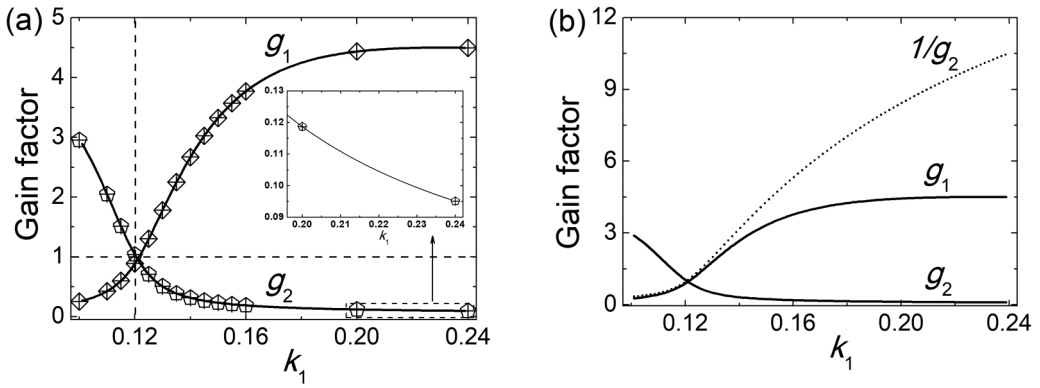

FIG. 2. Gain factors g_i $(i = 1, 2)$ as a function of k_1. All the parameters are dimensionless. The values of other parameters are $k_2 = 0.121$, $b_1 = b_2 = 0.02$, $r_{12} = r_{21} = 0.001$, and $N_0 = 1000$ [29–31], respectively. (a) Lines are theoretical predictions according to Eq. (13). Hollow markers are from simulations using the Gillespie method [24,25]. (b) The dotted line shows the change curve of $1/g_2$.

relationship between the two can be given by numerical simulation, as shown in Fig. 3. Lines are theoretical predictions according to Eqs. (3) and (4). Hollow markers are from simulations using the Gillespie method [24,25]. Two simulation results are in good agreement.

We can find that as g_1 increases, the stationary number of PCs decreases, while that of NCs increases. On the contrary, as g_2 increases, the former increases, while the latter decreases. This fact is attributed to the exploitative competition between the two phenotypes, that is, one phenotype maximizes the use of resources to allow itself to survive, thereby depleting the resources of the other phenotype and making it annihilate [29].

Moreover, whether it is viewed from g_1 or g_2, when the stationary number of NCs is large, that of PCs is not zero, indicating that PCs cannot be completely killed by NCs. This is consistent with the fact that NCs can coexist with PCs [17–19].

C. Effects of gain factors on the covariance

From Eq. (31), we can find that V_{12} seems to have a linear relationship with g_1 or g_2. However, both g_1 and g_2 are closely related to the average numbers of NCs and PCs. Therefore, the true relationship between them should be judged through numerical simulations.

Effects of gain factors $g_i (i = 1, 2)$ on normalized covariance V_{12} are given in Fig. 4. Lines are theoretical predictions according to Eq. (31). Hollow markers are from simulations using the Gillespie method [24,25]. Two simulation results are in good agreement, too. It is found that $V_{12} < 0$. So, there is a negative correlation between the fluctuations in NCs and in PCs. The responses of V_{12} to changes in g_1 or g_2 are similar. Each curve is approximately a parabola with upward opening. When $g_1 = 1.0$ or $g_2 = 1.0$, the absolute value of V_{12} is the largest. Therefore, within the permitted range of g_i, the dependence between the two phenotypes is the strongest, and their interaction is the least likely to be destroyed.

D. Effects of gain factors on fluctuations

Effects of gain factors $g_i (i = 1, 2)$ on the normalized variance V_{ii} are given in Fig. 5. Lines are theoretical predictions according to Eq. (30). Hollow markers are from simulations using the Gillespie method [24,25]. Two simulation results are also in good agreement.

It can be seen that with the increase of g_1, the normalized variance of NCs V_{11} gradually decreases, while the

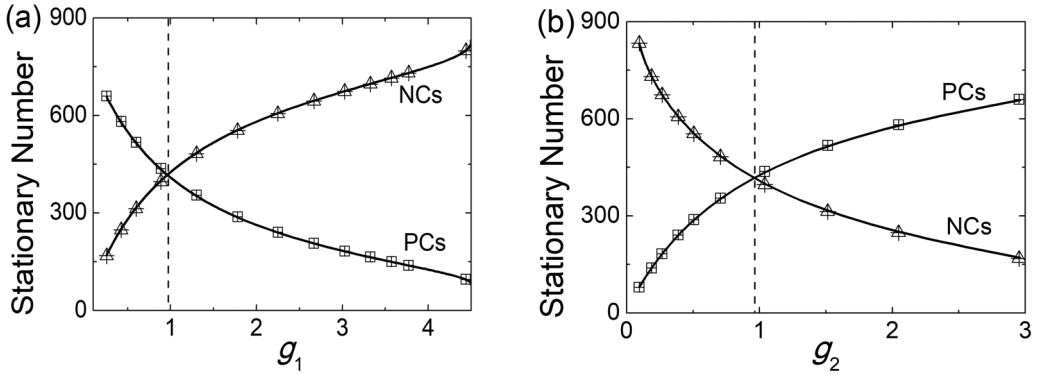

FIG. 3. Effects of gain factors g_i $(i = 1, 2)$ on stationary numbers N_i^s. Lines are theoretical predictions according to Eqs. (3) and (4). Hollow markers are from simulations using the Gillespie method [24,25]. All the parameters are dimensionless. The values of other parameters are $k_2 = 0.121$, $b_1 = b_2 = 0.02$, $r_{12} = r_{21} = 0.001$, and $N_0 = 1000$ [29–31], respectively.

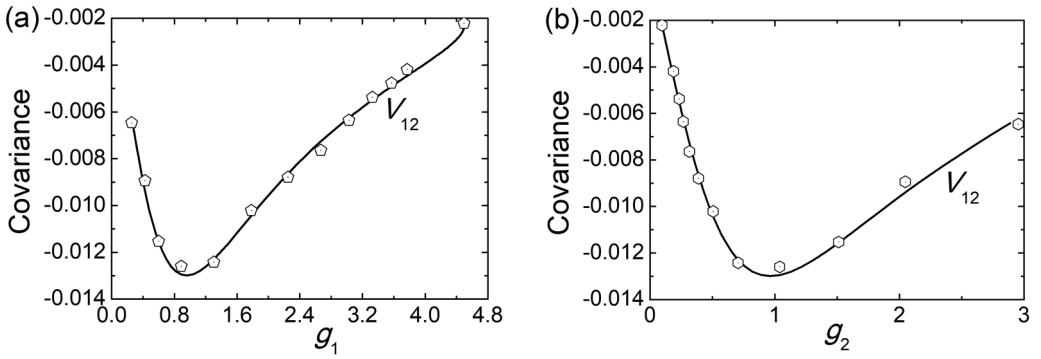

FIG. 4. Effects of gain factors g_i $(i = 1, 2)$ on normalized covariance V_{12}. Lines are theoretical predictions according to Eq. (31). Hollow markers are from simulations using the Gillespie method [24,25]. All the parameters are dimensionless. The values of other parameters are $k_2 = 0.121$, $b_1 = b_2 = 0.02$, $r_{12} = r_{21} = 0.001$, and $N_0 = 1000$ [29–31], respectively.

normalized variance of PCs V_{22} gradually increases and reaches a constant. With the increase of g_2, V_{11} gradually increases and reaches a constant, while V_{22} first increases slightly, reaches the maximum value, then gradually decreases, and reaches a constant.

In the theory of statistical physics, the relative fluctuation of the number of particles (called noise in quantitative biology) is inversely proportional to the number of particles. When the number of particles is large enough, the noise will be zero, and as the number of particles decreases, the noise will gradually increase. However, it can be found from Fig. 5(a) that as g_1 increases [while the number of PCs gradually decreases; see Fig. 3(a)], V_{22} will reach a constant instead of gradually increasing. In addition, it can be seen from Fig. 5(b) that as g_2 increases, V_{22} has an extreme value; when g_2 is large enough [while the number of PCs is large enough; see Fig. 3(b)], V_{22} is indeed small but not equal to 0. Why are these data results inconsistent with the theory of statistical physics? For understanding, noise decomposition is used; see the next section.

E. Effects of gain factors on noise propagation

The numerical simulation of Eq. (30) is carried out, and the results are given in Fig. 6. The influence of gain factors g_i $(i = 1, 2)$ on the noise propagation in the bidirectional phenotype switching cascade of the bacterial community are discussed.

It can be seen from Fig. 6(a) that for NCs, with the increase of g_1, both the pure intrinsic noise and the additional intrinsic noise decrease and eventually reach zero. And, at the same value of g_1, the additional intrinsic noise is greater than that of the pure intrinsic noise. The transmitted noise from PCs increases first, reaches the maximum value, and then decreases. Although relatively small, it will not be reduced to zero at the end of the permitted range of g_1 (see the line with circles in the partial enlarged view). The interconversion noise between the two phenotypes is almost zero and can be omitted. The change trend of the total noise is the same as that of the addition intrinsic noise. Due to the transmitted noise from the PCs, the total noise in NCs is greater than its addition intrinsic noise, and it is not zero even if g_1 is large.

From Fig. 6(b), it is found that for PCs, as g_1 increases, the pure intrinsic noise increases monotonically. The additional intrinsic noise increases first, reaches the maximum value when $g_1 = 3.0$, and then decreases. When $g_1 < 4.4$, the additional intrinsic noise is greater than the pure intrinsic noise. The transmitted noise from NCs increases first, reaches a maximum quickly, and then decreases. The interconversion noise between the two phenotypes is also almost zero and can be omitted. Therefore, in the region of $g_1 > 3.0$, due to the increase of pure intrinsic noise, even if the additional intrinsic

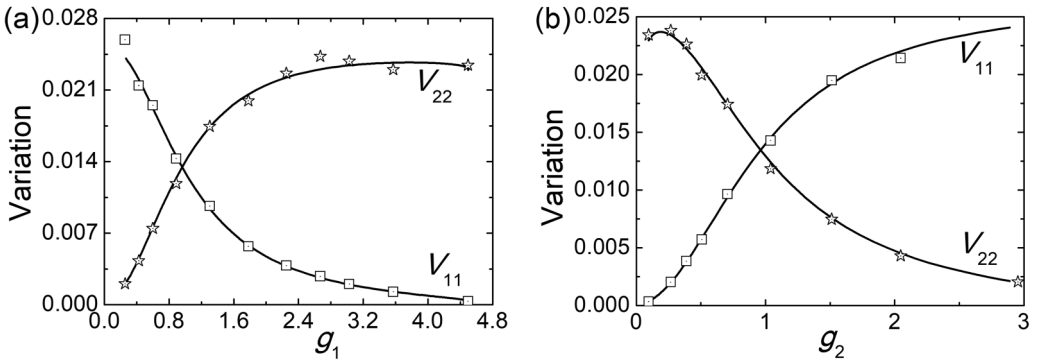

FIG. 5. Effects of gain factors g_i $(i = 1, 2)$ on normalized variances V_{ii}. Lines are theoretical predictions according to Eq. (30). Hollow markers are from simulations using the Gillespie method [24,25]. All the parameters are dimensionless. The values of other parameters are $k_2 = 0.121$, $b_1 = b_2 = 0.02$, $r_{12} = r_{21} = 0.001$, and $N_0 = 1000$ [29–31], respectively.

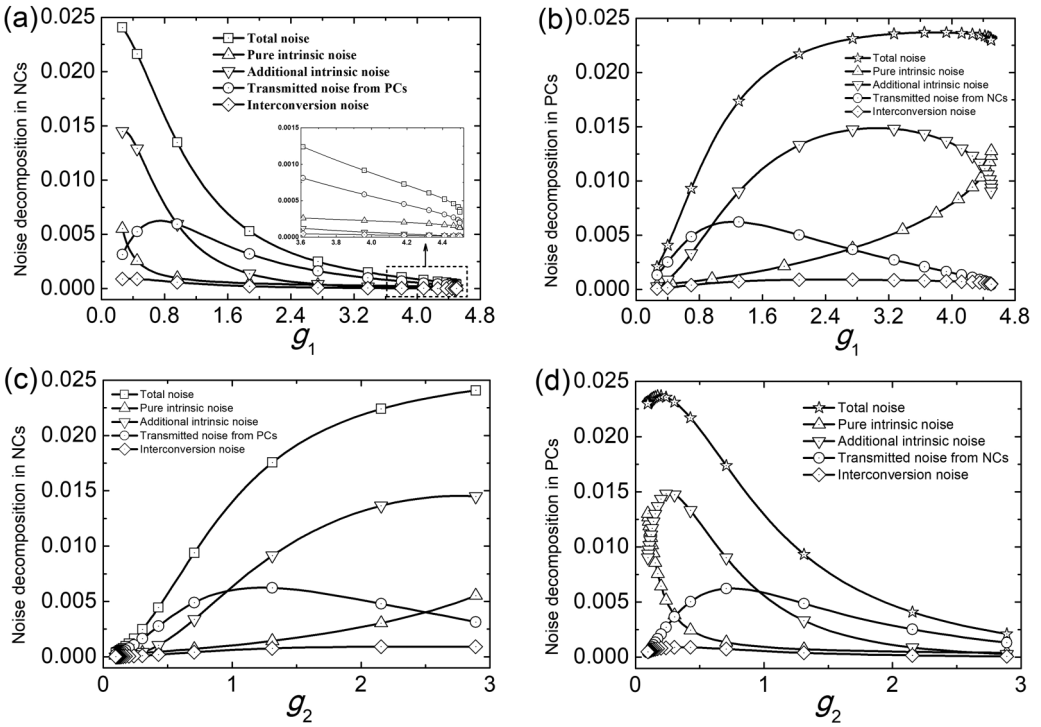

FIG. 6. Effects of gain factors g_i $(i = 1, 2)$ on the noise propagation. Lines are theoretical predictions according to Eq. (30). All the parameters are dimensionless. The values of other parameters are $k_2 = 0.121$, $b_1 = b_2 = 0.02$, $r_{12} = r_{21} = 0.001$, and $N_0 = 1000$ [29–31], respectively.

noise decreases, the total noise in PCs will not decrease, but will reach a constant value. In addition, due to the transmitted noise from NCs, even if g_1 is small and the number of PCs is large [see Fig. 3(a)], its total noise is small but not zero.

From Fig. 6(c), we can find for NCs, with the increase of g_2, both the pure intrinsic noise and the additional intrinsic noise increase. At the same value of g_2, the additional intrinsic noise is greater than the pure intrinsic noise. The transmitted noise from PCs increases first, reaches the maximum value, and then decreases. The interconversion noise is almost zero. The change trend of the total noise is the same as that of the addition intrinsic noise. Due to the transmitted noise from PCs, the total noise in the NCs is greater than its addition intrinsic noise.

From Fig. 6(d), it is found that for PCs, as g_2 increases, the pure intrinsic noise quickly drops to zero. The additional intrinsic noise and the transmitted noise from NCs both increase first, reach the maximum values, and then decrease. However, the additional intrinsic noise reaches its maximum value before the transmitted noise, and its maximum value is greater than that of the transmitted noise. The interconversion noise between the two phenotypes is also almost zero. Since the extreme value of the additional intrinsic noise cannot be offset by other noises, the total noise in PCs has an extreme value. Due to the transmitted noise from NCs, even if g_2 is large and the number of PCs is large [see Fig. 3(b)], the total noise in PCs is small but not zero.

All in all, whether it is for NCs or for PCs, and whether it is viewed from g_1 or g_2, the additional intrinsic noise is the main part of the total noise, and the transmitted noise is a powerful supplement to the total noise, while the interconversion noise between the two phenotypes is almost zero and can be omitted.

In the bacterial community, NCs and PCs can convert into each other. The direct effect of this mutual switching is the generation of interconversion noise, but the interconversion noise is generally very small and almost negligible. The indirect effect of this interconversion is to provide a global fluctuating environment, which affects the values of transmitted noise and interconversion noise. Especially, the fluctuating environment can produce additional intrinsic noise, which is the main part of the total noise. It can be seen that the indirect impact of interconversion is far greater than its direct impact.

V. CONCLUSIONS AND DISCUSSION

Currently, PCs have been found in human pathogens (such as *S. aureus*, *Mycobacterium tuberculosis*, and *Pseudomonas aeruginosa*), eukaryotic microorganisms (such as *Candida albicans* and *Saccharomyces cerevisiae*), and even tumor cell populations [39]. Because PCs can restart cell division after cessation of antibiotics [40], they greatly contribute to the refractory of chronic infections [11]. There is now convincing experimental evidence to prove their clinical relevance [41]. Therefore, insights into PCs will help us to cope with the ongoing antibiotic crisis [7]. And, strategies to eliminate PCs may improve the outcome of infection treatment [42].

In the bacterial community, NCs and PCs can switch into each other, resulting in a bidirectional phenotype switching cascade, in which either one can be used as an input signal, and the other phenotype as an output signal. The bidirectional phenotype switching provides a fluctuating environment that

has a global impact on the two phenotypes, so the results are very interesting. The global fluctuating environment will not only affect the values of transmitted noise and interconversion noise, but also generate additional intrinsic noise, which is the main part of the total noise. Moreover, the additional intrinsic noise in each phenotype can be transmitted to the other phenotype, and the transmitted noise is a powerful supplement to the total noise. However, the interconversion noise directly caused by the mutual switching is generally very small and almost negligible. Therefore, the indirect impact of interconversion is far greater than its direct impact, which may be one of the reasons why chronic infections caused by persistent cells are refractory to treatment.

In Ref. [32], one species can affect the growth rate of the other species, but the latter cannot affect the growth rate of the former (such as messenger RNAs and proteins). The author derived the noise formulas of the two species in detail. In Ref. [33], several models of stochastic gene expression were reviewed and the corresponding noise formulas were deduced. In Ref. [34], a synthetic network composed of four genes was designed, in which upstream genes can regulate their neighboring downstream genes, while downstream genes cannot regulate their neighboring upstream genes. Since the interactions between adjacent genes can be externally controlled and quantified at the single-cell level, authors verified the noise formulas in gene expression through experiments. In our recent paper on the propagation of noisy signals in colon cells [35], upstream cells can differentiate into downstream cells, but downstream cells cannot dedifferentiate into upstream cells. In above cases, the interaction between genes or cells is unidirectional. The unidirectional conversion can only generate conversion noise, but cannot provide a global fluctuating environment. Therefore, there is no additional intrinsic noise in the total noise of the cell. There is also no factor similar to "Λ" in the formulas of transmitted noise, interconversion noise, and covariance.

In addition, the bidirectional phenotype switching can cause the interconversion noise, which is not present in gene expression because although upstream genes can affect the expression of downstream genes, there is no direct phenotype switching between cells. Of cause, in this paper, the interconversion noise is relatively small and can be ignored.

The bidirectional phenotype switching cascade in the bacterial community includes only two phenotypes. In fact, there are three or more phenotypes in other cascades. For example, there are luminal cells, basal cells, and stemlike cells in breast cancer cell lines [43]. The mutual switching between any two phenotypes will provide a more complex fluctuating environment, and the characteristics of noisy signal propagation will be more interesting. It will be one of our future works.

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