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Relevance of phenotypic noise to adaptation and evolution

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Abstract: Biological processes are inherently noisy, as highlighted in recent measurements of stochasticity in gene expression. Here, the authors show that such phenotypic noise is essential to the adaptation of organisms to a variety of environments and also to the evolution of robustness against mutations. First, the authors show that for any growing cell showing stochastic gene expression, the adaptive cellular state is inevitably selected by noise, without the use of a specific signal transduction network. In general, changes in any protein concentration in a cell are products of its synthesis minus dilution and degradation, both of which are proportional to the rate of cell growth. In an adaptive state, both the synthesis and dilution terms of proteins are large, and so the adaptive state is less affected by stochasticity in gene expression, whereas for a non-adaptive state, both terms are smaller, and so cells are easily knocked out of their original state by noise. This leads to a novel, generic mechanism for the selection of adaptive states. The authors have confirmed this selection by model simulations. Secondly, the authors consider the evolution of gene networks to acquire robustness of the phenotype against noise and mutation. Through simulations using a simple stochastic gene expression network that undergoes mutation and selection, the authors show that a threshold level of noise in gene expression is required for the network to acquire both types of robustness. The results reveal how the noise that cells encounter during growth and development shapes any network's robustness, not only to noise but also to mutations. The authors also establish a relationship between developmental and mutational robustness.

1 Introduction

Phenotypes of isogenic individual organisms are not identical, as they all differ to some degree from each other. Cell motility in *Paramecium* exhibits differences between cells as evidence of individuality, as studied in pioneering papers by Spudich and Koshland, and Oosawa [1, 2]. Differentiation in isogenic bacterial cells has been measured through enzyme activities [3], suggesting that it is controlled by a dynamic mechanism [4]. With the recent advent of fluorescent proteins, measuring fluctuations in protein numbers among cells has become much easier. In fact, measuring stochasticity in protein abundance between cells sharing the same genotype has become a hot topic [5–16]. The sources of fluctuations can be distinguished and attributed to either extrinsic or intrinsic factors [5–17], and can be analysed both theoretically and experimentally. Besides the degree of variance in protein abundance, it is important to recognise that the distribution is not Gaussian but log-normal [15, 16], which suggests a multiplicative nature of stochastic processes in a cell.

Indeed, the existence of noise in a cell is inevitable, as biological processes consist of a huge number of reactions in which the number of molecules is not necessarily large. In cellular reproduction, molecules have to be synthesised, which involves positive feedback processes. The noise can be amplified via this positive feedback, which may introduce a log-normal distribution [15, 18].

In view of this, does stochasticity in gene expression have any relevance in biology? Often, such phenotypic noise is thought to be an obstacle in tuning a system to achieve and maintain a state with higher functions. Indeed, the question most often asked is how some biological functions can remain robust to phenotypic noise: examples include robustness of cell signalling [10, 19], chemotaxis in a noisy environment [20] and cell differentiation under molecular noise [11, 21]. Noise reduction is generally possible by temporal or spatial averaging, whereas optimal information processing in a signal network under noise is studied by using Shannon's information theory [12, 13].

Given that fluctuation by noise is inevitable, and considering that a relatively large amount of phenotypic noise has been preserved through evolution, it is important to investigate any positive role of phenotypic noise for biological functions. In nonlinear dynamics, the use of stochasticity has been discussed for decades. This includes a noise-induced transition to produce an ordered state [22–24], stochastic resonance [25, 26], attractor selection by noise [27] and so forth. Even though such mechanisms may work in a biological system (in particular in neural systems), whether they are relevant to a cell system is rather questionable. In studying the relevance of noise to cell function, we need to remember that cells grow and reproduce, in contrast to physical systems. It is crucial to discuss the relevance of noise to a cell in relationship with its capacity to grow. In other words, we need to consider the relevance of noise to adaptation, development and evolution.

As for development, the role of noise in robust cell differentiation processes has been described in isologous diversification theory. This theory addresses the question of robustness in cell types and the number and distribution of each type under noise. It has been shown that differentiation to a few discrete cell types progresses as a result of an interplay between intracellular dynamics and cell–cell interactions. In particular, differentiation of cell states triggered by noise leads to noise-tolerant developmental processes [18, 21]. With regard to adaptation to a fluctuating environment, the relevance of stochastic switching in bacterial phenotypes has been studied by Kussell and Leibler [14].

In the present paper, we briefly review the relevance of noise to adaptation and evolution, as they are basic phenomena enabling cells to survive under different environments. We consider adaptation to be the change in cellular states that does not involve genetic change, whereas evolution is a process over much longer time scales involving genetic changes. For both cases, it has been shown that noise is relevant in shaping cellular states to achieve higher growth speeds with robustness to a system's change. Here, we mainly focus on recent progress, particularly studies by ourselves and our collaborators.

2 Spontaneous adaptation by noise

2.1 Necessity of a general adaptation mechanism

In this section, we review a novel mechanism for cellular adaptation, which was proposed recently [28, 29]. According to this adaptation mechanism, noise in intracellular reaction dynamics drives the selection of actively growing states among all possible cellular states. This enables cells to adapt to a variety of environmental conditions without any fine-tuned signal transduction mechanism.

As is well known, cells adapt to a variety of environmental conditions by changing their pattern of gene expression or distribution of metabolic flux. Such adaptive responses are generally explained by signal transduction mechanisms, for example, the regulation of lactose metabolism by the Lac operon of *Escherichia coli* cells [30]. This is a programmed response depending on the input concentration. However, such 'if-then' type descriptions as used in computer programs may not always be sufficient to explain cellular adaptation, because the number of possible environmental conditions to which a cell must adapt is very large compared with the limited repertoire of gene regulatory mechanisms. In fact, experiments using phenotype microarrays [31] revealed that *E. coli* cells grow in hundreds of different environmental conditions, including different carbon and nitrogen sources and stress environments, in which they have altered their gene expression patterns in order to achieve growing states [32]. Considering that there are only a few hundred genes categorised as 'signal transduction mechanisms' in the *E. coli* genome [33], it is rather unlikely that gene regulatory programs can adapt to such a wide variety of environmental conditions. Of course, such regulatory programs could have a huge number of possible combinatorial states. However, since cellular processes have to work coordinately to achieve an adaptive state, the regulatory mechanisms for such processes should be tightly coupled. In that case, the selection of an adaptive state among such a huge number of possible states could cause a difficult computational problem. In this sense, the search for an alternative mechanism other than combinatorial signal transduction, if any, will be relevant to understanding the cellular adaptation process.

Furthermore, two recent studies revealed the possibility that cells can respond to environmental changes adaptively without preprogrammed signal transduction mechanisms. Using yeast cells, Braun and colleagues demonstrated that even when the promoter of the essential gene (*HIS3*) is detached from the original regulatory system, the expression of the gene is regulated adaptively in response to environmental demands [34, 35]. Kashiwagi *et al.* demonstrated that *E. coli* cells select an appropriate intracellular state according to environmental conditions

without the help of signal transduction mechanisms [28]. In their study, an artificial gene network composed of two mutually inhibitory operons was introduced into *E. coli* cells, such that the states of gene expression were bistable. Corresponding to each of the bistable states, two specific proteins were produced to carry out functions that were necessary for survival under the two given environmental conditions. Even without a corresponding signal transduction network, the cells were found to shift to an adaptive state in which the protein necessary for survival was expressed.

These experimental observations strongly suggest that there is an alternative adaptation mechanism in addition to the signal transduction machinery. Indeed, such a mechanism – if it exists – should be important in adapting to novel environments that a species has not experienced in the course of evolution, because organisms must survive by adapting to new environments even before specific signal transduction networks have been developed. As for environmental adaptation without sophisticated if-then type signal transduction mechanisms, there is a preceding study by Narang [36] in which a simple Lotka–Volterra type model was used to represent metabolic dynamics. In this study, it was shown that the selection of an adaptive state is possible by the change of stability of each state caused by environmental changes, thus explaining microbial growth behaviour on mixtures of substrates. Recently, Yomo and colleagues demonstrated that the selection of an adaptive attractor between bistable states by noise is possible by introducing phenomenological activity that governs the synthesis and degradation of proteins [28]. These studies showed that when there are multiple cellular states having a variety of growth rates, the selection of adaptive states can naturally emerge. Beyond the simple two-state model, it has recently been demonstrated that cells select states most favourable for their survival among a large number of other possible states as an inevitable outcome of the very fact that cells grow and that gene expression is inherently stochastic [29]. In the next section, we survey the adaptive mechanism of our model, which enables the selection of growing states by noise without any finely tuned signal transduction.

2.2 General scheme

Suppose that the internal state of a cell is represented by a set of concentrations of n proteins $(x_1, x_2, \ldots, x_n)$, which are regulated by each other. The change in concentrations of proteins over time is determined by: (i) the synthesis of proteins, (ii) the dilution of proteins by the growth in cell volume and (iii) fluctuations in protein expression arising from stochasticity in chemical reactions. (Besides dilution, there can also be degradation of proteins. However, the inclusion of protein degradation does not change the result of adaptation qualitatively, as long as growth-dependent dilution dominates the decrease of protein concentrations.) The dilution of proteins is proportional to the growth rate in cell volume v_g, which is determined by expression profiles of proteins and the environmental conditions. Also, it is natural to assume that the rates of protein synthesis are proportional to the growth rate v_g, since a decrease in protein concentration by dilution because of cell growth has to be compensated by synthesis to maintain a steady state. In fact, some experimental studies have shown that the total protein concentration is relatively unchanged with the growth rate [37, 38], which suggests that the change of the protein dilution rate is compensated by changing the protein synthesis rate. We assumed the proportionality of protein synthesis and dilution rates to the growth rate of the cell volume, but even if rigorous proportionality is replaced by a mere positive correlation between the synthesis and growth rates, the adaptation mechanism presented below still works. Following this argument, the dynamics of concentration of the ith protein is chosen as follows

$$\frac{\mathrm{d}x_i(t)}{\mathrm{d}t} = f_i(x_1, x_2, \ldots, x_n)v_g - x_i v_g + \sigma\eta_i(t) \qquad (1)$$

The first and second terms in the right-hand side (r.h.s.) represent synthesis and dilution of the proteins, respectively, where $f_i(\cdots)$ represents the regulation of protein expression by other proteins. The third term represents the noise in protein concentration with a certain amplitude σ satisfying $\langle \eta_i(t)\eta_j(t')\rangle = \delta(t-t')\delta_{ij}$, where i and j represent different proteins. For simplification, we assume that the amplitude of the noise is independent of the growth rate v_g, whereas the inclusion of v_g dependence does not alter our results qualitatively. (Even if the noise amplitude depends on the growth rate v_g, the noise-driven adaptation will work as long as the noise amplitude does not vanish with the growth rate, in other words, as long as a certain amplitude of the noise is maintained in the non-adaptive state).

Let us consider the case that the expression dynamics represented by (1) has multiple possible states (i.e. attractors) and the growth rate v_g is determined by the expression dynamics. In this case, the influence of noise depends on the growth rate v_g for each attractor. When the cellular state falls into an attractor that has a small v_g, the deterministic part of protein expression dynamics (i.e. the first and second terms of the r.h.s. of (1)) is small, and so the stochastic part is relatively dominant in the protein expression dynamics. In this case, the probability to escape the attractor by noise is large. In contrast, when the growth rate v_g is large in the attractor, the magnitude of the deterministic part of the expression dynamics is much larger than that of the stochastic part. Thus, the probability to escape that state becomes small. As the result of this negative correlation between the cellular growth rate v_g and the probability to escape a state, the cells can select a state with a relatively higher growth rate, under the presence of an appropriate noise level of gene expression.

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2.3 Simulation results

In this section, we present a simulation of a noise-driven selection process. In this simulation, we adopt a cell model with two networks. One is a regulatory network that controls the expression levels of proteins acting through each other. The other is a metabolic network in which the metabolic reactions are regulated by the concentrations of the proteins. The cell takes some metabolites as nutrients, and these are transformed to other metabolites by the metabolic reactions. The growth rate v_g is determined by the profile of metabolic fluxes and the environmental conditions. We assume that some metabolites are required for cellular growth and that a metabolite having a minimal concentration among these metabolites limits the growth rate. Thus, we use the simple rule that the growth rate v_g is determined to be proportional to the minimum concentration of these metabolites.

Also, we assume that the synthesis of proteins is given by the sigmoidal regulation function with regulatory inputs (activation or inhibition) from other proteins. We used the sigmoidal function $f_i(z) = 1/(1 + exp(-\mu z_i))$, where $z_i = (\sum_j J_{ij} x_j(t) - \theta)$ is the total regulatory input with the regulatory matrix J_{ij}, the threshold θ for activation of synthesis and μ indicates the gain parameter of the sigmoid function. (The form of the single sigmoidal regulation function with regulatory inputs was adopted here for simplification. However, the behaviour of the model was unchanged when other forms of regulation are adopted, as long as there are multiple attractors in the gene regulatory dynamics.) As the result of these regulations, the gene expression dynamics has multiple attractors. For a specific form of the noise term, we add a Gaussian white noise term with an amplitude of σ to the expression dynamics. We perform thousands of simulations starting from randomly determined initial conditions (i.e. gene expressions and metabolic concentrations) and investigate the general behaviour of cellular adaptation (for details of the model, see [29]).

In Fig. 1, an example of the selection process of rapidly growing states is shown by taking an adequate noise amplitude in the expression dynamics. Time series using arbitrarily chosen protein concentrations and cellular growth rates v_g are plotted in Figs. 1*a* and Fig. 1*b*, respectively. In the example, cells are set initially at a state with a low-growth rate. In such a state, stochasticity dominates the evolution of protein concentrations with time. After itinerating among various expression patterns, the cellular dynamics arrives at a state with a higher growth rate. Such a transition repeats until the growth rate becomes sufficiently high. Once a gene expression pattern supporting optimal growth is reached, the system maintains it over time.

This selection of higher growth states was observed for all of the one thousand networks that we simulated. It also

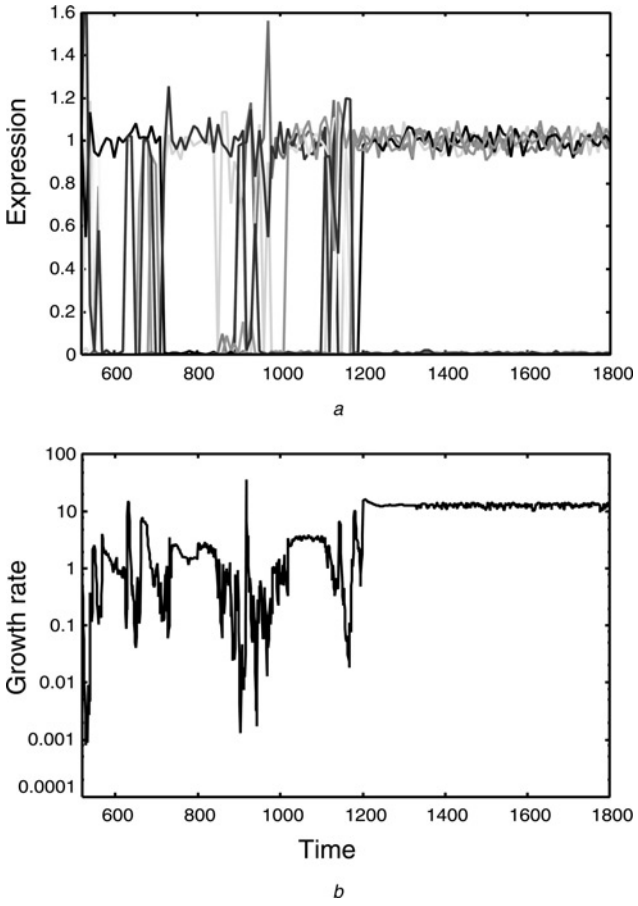

Figure 1 *Time series of protein expression levels and cellular growth rate v_g*

a Time series of protein expression levels $x_i(t)$; 10 of 96 protein species are displayed. Vertical axis represents the expression levels of proteins and the horizontal axis represents time

b Change in growth rate v_g observed during the time interval shown in Fig. 1*a*. Initially, the growth rate of the cell fluctuates owing to the highly stochastic time course in protein expression. After a few short-lived nearly optimal states (4800–5600 time steps), the cell finds a state of protein expression that realises a high rate of growth

worked independently of initial conditions. As the final state depends on the initial condition, we have computed the distribution of the final growth rate reached from randomly chosen initial conditions. The histograms of the final growth rate, thus obtained, are plotted in Fig. 2. In the case $\sigma = 0$, the cellular state converges rapidly and deterministically into an attractor. In such a case, the final growth rates exhibit a broad distribution as shown in Fig. 2, representing a wide variety of the final cellular states. In contrast, in the presence of noise ($\sigma = 0.02$ and $\sigma = 0.2$), the final growth rates exhibit a relatively sharp distribution because of the selection of faster growth states, as we have seen in Fig. 1.

The relationship between the noise amplitude σ and the final growth rate v_g is plotted in Fig. 3. For a small noise amplitude ($\sigma < 10^{-2}$), the final growth rates are distributed

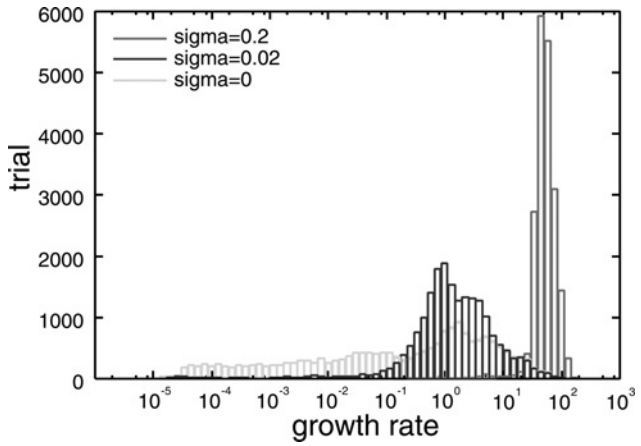

Figure 2 *Histogram of growth rate with different noise strength* σ

Starting from 2×10^4 randomly chosen initial conditions (i.e. protein expression levels), growth rates after 10^5 time steps have been computed with different noise strengths $\sigma = 0$, 0.02, and 0.2

broadly, as the cells cannot escape from the first attracting state they encounter. On the other hand, when the noise amplitude is larger ($\sigma > 1$), the final growth rates again exhibit a broad range distribution, because the cellular state continues to change without settling into any attractor. In the intermediate range of the noise strength $10^{-2} < \sigma < 1$, cellular states are selected that are associated with growth rates significantly higher than those found in the other noise ranges. This shift of the final growth rate is caused by the selection of cellular states by fluctuations, as shown in Fig. 1. In fact, we measured the 'depth' of an attractor, defined as the smallest noise amplitude that can eventually kick the state out of the attractor in a certain time period,

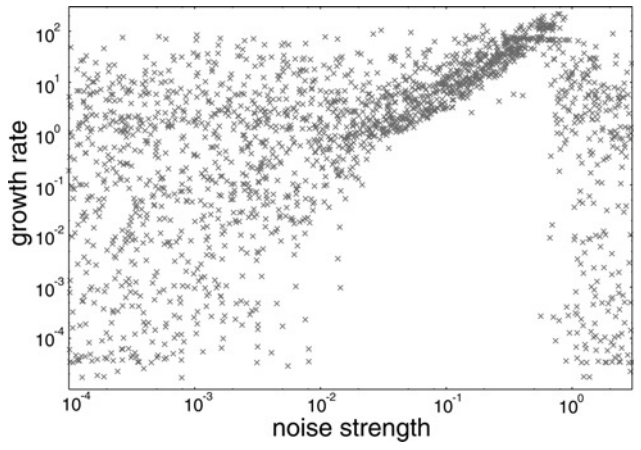

Figure 3 *Relationship between the noise amplitude* σ *and the growth rate* v_g

Starting from randomly chosen initial conditions against noise amplitudes σ values ranging over $10^{-4} < \sigma < 3$, the growth rates v_g after 10^5 time steps have been plotted. In the intermediate range of noise strength, $10^{-2} < \sigma < 1$, cellular states with high growth rates are selected among many possible cellular states, as depicted in Fig. 1

by changing the noise amplitude. As a result, we found that there is a positive correlation between the depth of the attractor and the growth rate (data not shown).

The time required to reach an adaptive attractor is not necessarily short, compared with that needed for the signal transduction mechanism. If the number of attractors is not very large, the selection is completed within a generation, whereas for successive selections among a thousand attractors as in Fig. 1, several generations are required. In this case, the expression pattern of proteins $(x_1, x_2, \ldots, x_n)$ has to be inherited epigenetically after cell division, at least to some degree.

2.4 Significance

We have carried out numerical experiments with our model using several sets of parameter values, which allow for multiple attractors in expression dynamics, and have evaluated thousands of different randomly generated reaction networks. The adaptation process triggered by noise is observed generally independently of the details of the model. In fact, it emerges as long as the following four requirements are satisfied: (i) the coexistence of multiple attractors, (ii) the dependence of the growth rate on attractors, (iii) an increase of cellular reaction processes with the speed of growth and (iv) the presence of stochasticity in reaction dynamics. We have confirmed the robustness of our results against changes in model parameters and rules. For example, the results did not change when model parameters such as coefficients of reactions were changed, provided the above requirements were satisfied. The robustness of the results against changes in the properties of reaction networks, such as the path density or distribution of the numbers of paths has been confirmed [30]. Also, the specific form of dependence of the growth rate on the expression dynamics is not important for the result; instead, the same results are obtained as long as the growth rate is somehow determined by the expression dynamics.

This study provides a possible explanation for the establishment of the optimal growth rate in metabolic reaction networks, proposed by Palsson and colleagues [39–41]. These studies suggested that a metabolic network is organised such that the growth rate is optimised under given conditions. For example, it was shown that *E. coli* strains with the deletion of a single metabolic gene can adapt to several environmental conditions, and that the value of the final growth rate is consistent with that calculated as an optimal growth rate in such perturbed metabolic networks and environmental conditions [41]. Their results suggest that these bacteria can adjust their intracellular state to optimise their growth rate, even against an environment they have never experienced. Indeed, by the adaptation mechanism surveyed here, a cellular state with an optimal growth rate is selected from those among a variety of environmental conditions. Provided the cellular states are perturbed sufficiently by stochasticity in gene

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expressions, there will be a negative correlation between the growth rate and the probability of escape from the corresponding cellular state. Thus, the adaptive attractor selection may be at work behind the observed regulations of metabolic fluxes leading to optimal growth rates.

Even though such adaptive attractor selection by noise is relatively slow, it works generally without the requirement of a finely tuned signal transduction network. Hence, for the environmental conditions that an organism encounters frequently, cells have likely developed a sophisticated sensory and signal transduction network, whereas the present mechanism enables the adaptation of cells even to environments that they have never faced.

The adaptation mechanism reviewed here has not been confirmed experimentally so far. Standard experimental studies have focused only on adaptation processes based on signal transduction networks, and therefore we need novel experimental setups to justify the proposed adaptation mechanism. There are two possibilities. One is the use of artificial gene networks, as demonstrated in [28]. In this approach, one can introduce a gene network disconnected from the existing signal transduction networks, and investigate whether the artificial gene network shows adaptive behaviour. Other possibility is the study of cellular response against environmental changes that cells have never faced, or the response of cells in which known regulatory mechanisms are destroyed. In both cases, by investigating the response of the cells, one can examine if they show adaptive behaviour to environmental changes without the sophisticated regulatory mechanisms, but by utilising the fluctuation-based selection of a higher growth state as presented in this paper.

3 Role of phenotypic noise in genetic evolution

3.1 Motivation and general framework

Now, we discuss the relevance of phenotypic noise to evolution. There have been long-lasting discussions on the possible relationships among phenotypic plasticity, robustness and evolution.

In general, plasticity is the changeability of a phenotype in response to environmental change. This change itself does not involve genetic changes because of mutation or recombination, but rather can appear within one generation. In this sense, the relationship between plasticity and evolution is not a logical consequence, and one has to examine if evolution decreases or increases plasticity, and if plasticity increases evolution [42–45]. In spite of long-term efforts, most of the studies to address this question remains rather qualitative.

If we borrow a concept from physics, one may expect that susceptibility to environmental changes is proportional to, or positively correlated with, phenotypic fluctuation [46, 47]. Indeed, in statistical physics near an equilibrium state, the ratio of response to an external force is proportional to the degree of fluctuation at the equilibrium state. The generalisation of such a fluctuation–response relationship has also been proposed [18, 48, 49]. Therefore phenotypic plasticity, which is a degree of phenotype change to different environments, is expected to be positively correlated (or proportional to) the isogenic phenotypic fluctuation that is now being quantitatively measured in the laboratory.

In evolution under a fixed environment, the phenotype changes to adapt to that environment. The evolutionary process is, thus, regarded as a response to the environment. Accordingly, it is of relevance to study possible relationships between the phenotypic fluctuation of isogenic individuals and the speed of genetic evolution. Borrowing a formulation from statistical physics, Sato *et al.* [50] previously proposed an evolutionary fluctuation–response relationship in which the speed of evolution increases in proportion to the variance of the isogenic phenotypic fluctuation. This proposition is supported by the results of an experiment on bacterial evolution in the laboratory, and is also confirmed by simulations of a reaction network model of a growing cell population [51].

The fluctuation of phenotype, on the other hand, is related with the robustness of a system. Robustness is generally defined as the ability to function in spite of changes in various parameters of a system [52–56]. Since a system's sensitivity to change increases with its fluctuation, the robustness increases as the system's fluctuation decreases. Historically, increases in robustness through evolution were proposed by Schmalhausen to stabilise evolution [57] and by Waddington to lead to canalisation [58, 59]. Since then, whether robustness increases through natural selection has long been debated in the context of developmental dynamics and evolutionary theory [42, 52, 54, 60–62]. Recalling that measurements of fluctuation are now available, it is possible to explore the relationship between fluctuation and evolution, and to study the evolution of robustness quantitatively. There are two questions to be addressed. Does fluctuation (robustness) decrease (increase) through evolution? And is fluctuation relevant to the evolution of robustness?

For the former question, there is direct evidence from experiments and simulations [50, 51]. As for the latter question, if and why stochasticity in gene expression is needed to establish the evolution of robustness has not been elucidated. To examine this, one must first recall the general structure of biological evolution. Evolutionary fitness is determined by dynamic processes that provide phenotype. In multicellular organisms, the phenotype is a result of complex developmental processes. Even in unicellular organisms, it is a result of stochastic gene expression dynamics that determines the rate of growth of the cell.

Such dynamics is governed by the genotype, whereas the dynamics of determining phenotype is stochastic in nature.

Now, one needs to consider the following structure of evolutionary processes. (i) There is a population of organisms with a distribution of genotypes. (ii) Phenotype is determined by genotype, through 'developmental' dynamics. (iii) The fitness for selection is given by the phenotype. (iv) The distribution of genotypes for the next generation is a result of reproduction, mutation and selection. Mutations change the genotype, whereas the offspring number will be based on the fitness value.

During evolution *in silico*, procedures (i), (iii) and (iv) are adopted as genetic algorithms. However, it is important to note that the phenotype is determined only after complex 'developmental' dynamics of (ii), which are stochastic because of the noise therein. By adopting this framework, a few models have been studied to examine the relevance of noise to evolution, such as a reproducing cell model with a catalytic reaction network [51], and a gene network model [63]. Here, we review the results from the latter model.

3.2 Gene network model

Gene expression dynamics is governed by regulatory networks. Each expression profile changes in time, and eventually reaches a stationary pattern that determines evolutionary fitness. Selection occurs after the introduction of mutations at each generation in the gene network. Among the mutated networks, we select those networks with higher fitness values. By including a noise term in the gene expression dynamics, we discuss how this noise influences the evolution of the network to increase fitness.

To be specific, typical switch-like dynamics with sigmoid input–output behaviour [64–66] was adopted, although several simulations in the form of biological networks would give essentially the same result. In this simplified model, the dynamics of a given gene expression level x_i is described by

$$\frac{\mathrm{d}x_i}{\mathrm{d}t} = \tanh\left[\frac{\beta}{\sqrt{M-k}}\sum_{j>k}^{M} \boldsymbol{J}_{ij}x_j\right] - x_i + \sigma\eta_i(t) \qquad (2)$$

where $\boldsymbol{J}_{ij} = -1, 1, 0$ and $\eta_i(t)$ is Gaussian white noise given by $\langle \eta_i(t)\eta_j(t')\rangle = \delta_{i,j}\delta(t-t')$. M is the total number of genes, and k is the number of output genes that are responsible for fitness to be determined. These output genes are fixed to $i = 1, 2, \ldots, k$. The value of σ represents the noise strength that determines stochasticity in gene expression. By following a sigmoid function tanh, x_i has a tendency to approach either 1 or -1, which is regarded as being 'on' or 'off' for gene expression. The initial condition is set at $(-1, -1, \ldots, -1)$; that is, all genes are off.

The fitness is determined by setting a target gene expression pattern. As a simple example, we adopt the target such that gene expression levels (x_i) for the output genes $i = 1, 2, \ldots, k < M$ reach 'on' states, that is, $x_i > 0$. The fitness F is at its maximum if all k genes are on after a transient time span T_{ini}, and at its minimum if all are off. F is set at 0, if all the target genes are on, and is decreased by 1 if one of the k genes is off. Note that fitness is calculated only after time T_{ini}, which is chosen at a sufficiently large value so that the gene expression dynamics reaches a stationary state. This initial time can be considered as the time required for developmental dynamics (see [63] for details).

Selection is applied after the introduction of mutations at each generation in the transcriptional regulation network. Among the mutated networks, we select those networks with higher fitness values. Because the network is governed by $\boldsymbol{J}_{ij}$ that determines the 'rule' of the dynamics, it is natural to treat $\boldsymbol{J}_{ij}$ as a measure of genotype. Individuals with a different genotype have a different set of $\boldsymbol{J}_{ij}$.

At each generation, there are $N(=200)$ individuals. We compute the average fitness $\overline{F}$ for each network by carrying out $L(=200)$ runs for each. Individuals with a different set of $\boldsymbol{J}_{ij}$ have different fitness values according to the dynamics given by (2), and those with higher fitness values are selected for the next generation. To be specific, $N_{\mathrm{s}} = N/4$ networks with higher values of $\overline{F}$ are selected for the next generation, from which $\boldsymbol{J}_{ij}$ is 'mutated', that is, a single path (a single pair of i, j) is changed. Here, we make N/N_{s} mutants from each of the top N_{s} networks, such that there are N networks again for the next generation. Following mutation, the N individuals at each generation have slightly different network elements, $\boldsymbol{J}_{ij}$, so that the values of $\overline{F}$ differ. From this population of networks, we repeat the processes of developmental dynamics, their mutation and selection of networks with higher fitness values.

As the model contains a noise term, fitness can fluctuate at each run, which leads to a distribution in F, even among individuals sharing the same network. This leads to the isogenic phenotype variance denoted by $V_{\mathrm{ip}}(I)$ for a given genotype (network) I that is

$$V_{\mathrm{ip}}(I) = \int p(F; I)(F - \overline{F}_I)^2 \mathrm{d}F \qquad (3)$$

where $p(F; I)$ is the fitness distribution over isogenic species I sharing the same network $\boldsymbol{J}_{ij}$, and $\overline{F}_I = \int Fp(F; I)\mathrm{d}F$ is the average fitness of the species I. $V_{\mathrm{ip}}(I)$ generally depends on the individual (genotype) I. Later, we use the average of $V_{\mathrm{ip}}(I)$ over all genotypes I existing at each generation, as V_{ip}.

At each generation, there exists a population of individuals with different genotypes I. The distribution $P(\overline{F})$ is obtained over all existing individuals (networks) including those that are not fitted. By using this distribution, fitness variance

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because of genetic variation is defined as

$$V_g = \int P(\overline{F})(\overline{F} - \langle \overline{F} \rangle)^2 \mathrm{d}\overline{F} \quad (4)$$

where $\langle \overline{F} \rangle = \int P(\overline{F})\overline{F}\mathrm{d}\overline{F}$ is the average of average fitness over all networks.

3.3 Result of numerical evolution

Let us first see how the evolutionary process changes as a function of the noise strength σ. Within a hundred generations, the top fitness among the network population reaches 0, the maximum possible value in our model. On the other hand, the temporal evolution of the distribution function $P(\overline{F})$ depends essentially on the noise strength σ. When σ is small, the distribution is broad. There remain individuals with very low-fitness values $\overline{F}$, even after many generations of evolution (Fig. 4). On the other hand, for large σ, even those individuals with the lowest fitness approach $\overline{F} = 0$. There is a threshold noise σ_c, below which the distribution $P(\overline{F})$ is broadened, as shown in Fig. 4. As a result, the average fitness over all individuals, $\langle \overline{F} \rangle$, is low. $\langle \overline{F} \rangle$ and the lowest fitness over individuals $\overline{F}_{\min}$, after a sufficiently large number of generations, is plotted against σ in Fig. 5. The sharp decrease in fitness suggests threshold noise σ_c, below which low-fitness mutants always remain in the distribution.

Why does the system not maintain the highest fitness state under small phenotypic noise, $\sigma < \sigma_c$? Indeed, the dynamics of the top-fitness networks that evolved under low noise has dynamic behaviours distinct from those that evolved under high noise. First, the highest-fitness network that evolved at low σ often fails to reach the target if simulated under a higher noise level. The expression often has a few oscillations

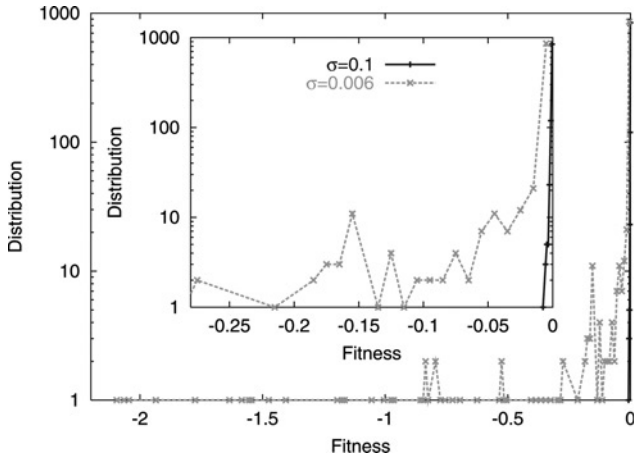

Figure 4 *Distribution $P(\bar{F})$ after 200 generations, for a population of 1000 individuals*

Inset is the magnification for $-0.27 < \langle \bar{F} \rangle < 0$. For a high σ value (solid line, with $\sigma = 0.1$), the distribution is concentrated at $\bar{F} = 0$, whereas for a low σ value (dotted line, with $\sigma = 0.006$), the distribution is extended to large negative values, even after many generations

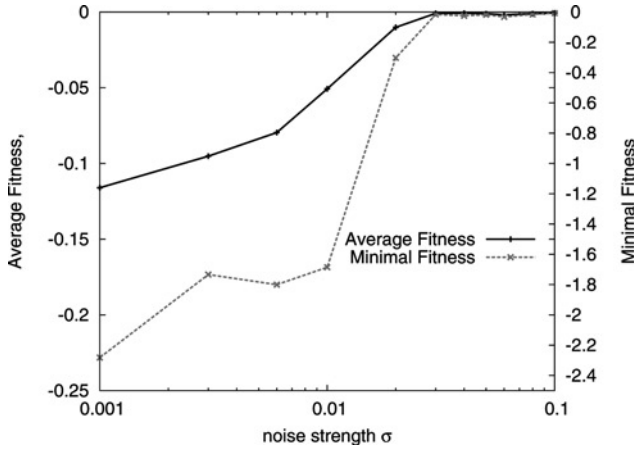

Figure 5 *Mean of average fitness $\langle \bar{F} \rangle$ and minimal fitness plotted against the noise strength σ*

$\langle \bar{F} \rangle$, the average of the average fitness $\bar{F}$ over all individuals was computed for 100–200 generations
The minimal fitness was computed from the time average of the least-fit network present at each generation

before reaching the target state and noise might cause the expression of the output genes to switch to off states. In contrast, the temporal course of gene expression evolved for $\sigma > \sigma_c$ is much smoother, and is not affected by noise. This distinction is confirmed by simulating gene expression dynamics by cutting off the noise term over a variety of initial gene expression conditions, and checking if the orbit is attracted to the original target. It was found that, for networks evolved under $\sigma > \sigma_c$, a large portion of the initial conditions is attracted to the target pattern, whereas for those evolved under $\sigma < \sigma_c$, only a tiny fraction (i.e. the vicinity of all off states) is attracted to the target.

When the time course of a given gene expression pattern to reach its final pattern is represented as a motion falling along a potential valley, our results suggest that the potential landscape becomes smoother and simpler through evolution and loses ruggedness after a few hundred generations. This 'developmental' landscape is displayed schematically in Fig. 6. For networks evolved under $\sigma > \sigma_c$ there is a large, smooth attraction to the target, whereas for the dynamics evolved under $\sigma < \sigma_c$, the initial states are split into small parts (basins), from each of which the gene expression patterns reach different steady states. Now, consider a mutation to a network. The change in the network leads to slight alterations in gene expression dynamics. In smooth dynamics, as in Fig. 6 (upper), this perturbation influences the attraction to the target only slightly. By contrast, under the dynamics as shown in Fig. 6 (lower), a slight change easily destroys the attraction to the target attractor. For this latter case, the fitness of mutant networks is distributed down to lower values, which explains the behaviour observed in Figs. 4 and 5.

In other words, evolution to eliminate ruggedness in developmental potential is possible only for sufficient noise

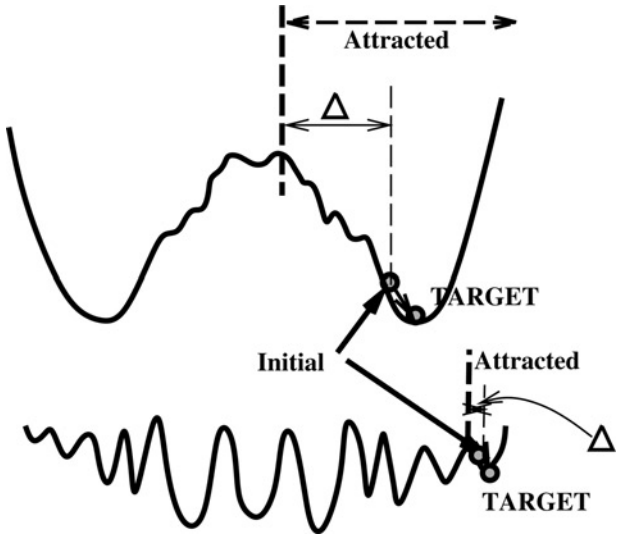

Figure 6 *Schematic representation of the basin structure, represented as a process of descending a potential landscape*

Δ is the magnitude of perturbation needed to jump over the barrier to a different attractor from the target. A smooth landscape evolves under a high level of noise (above), and a rugged landscape evolves under a low level of noise (below)

amplitudes, whereas ruggedness remains for small noise values and the developmental dynamics often fails to reach the target, either by noise in gene expression dynamics or by mutations to the networks. It is interesting to note that a greater set of initial conditions is attracted to a target pattern for networks evolved under large noise. The existence of such global attraction in an actual gene network has recently been reported for the yeast cell cycle [67] and others [68]. In fact, the existence of such global attraction was proposed in protein folding dynamics by Abe and Go [69], and is now termed as a funnel landscape [70]. Such a 'developmental landscape' is expected to be a result of evolution, as most random interaction yields rugged landscapes as in the lower diagram of Fig. 6. The existence of the landscape of folding dynamics as in the upper diagram suggests that it is shaped through the evolution under thermal noise.

3.4 Evolution of robustness

For a network evolved under large noise, the target pattern is reached even if the developmental dynamics is perturbed by noise or by a mutation to the network. In other words, the fitness is rather insensitive to such perturbations. This is nothing but robustness of the system.

As discussed already, robustness is the insensitivity of phenotype to a system's change. In a biological system, these changes have two distinct origins: genetic and epigenetic. The former concerns genetic robustness, that is, the rigidity of phenotype against mutation. The latter concerns robustness against stochasticity in gene expression or the external environment.

Corresponding to genetic and epigenetic robustness, there are two types of variances, as introduced in Section 3.2. The variance corresponding to genetic change is V_g, the phenotype variance caused by the distribution of genes. As the variance decreases, the system increases its robustness to genetic change (mutation). On the other hand, epigenetic robustness is measured by the phenotypic variance of isogenic organisms, V_{ip}.

Under large noise, the selection process favours a developmental process that is robust against it. This robustness to noise is then embedded into robustness to mutation. Indeed, both V_g and V_{ip} decrease through the course of evolution (Fig. 7) while proportionality is maintained between the two. Such proportionality between the two has been discussed from an analysis of evolutionary stability under a condition of low mutation rates [51]. This proportionality is consistent with observations from an experiment in bacterial evolution [18, 50].

Such proportionality suggests that 'developmental robustness' and 'genetic robustness' evolve in coordination. In fact, the correlation between phenotypic plasticity in development to genetic change in evolution was proposed as genetic assimilation by Waddington [58, 59], where phenotypic change in response to the environment is later fixed in genes through Baldwin's effect [71, 72]. Since then, the possible role of genetic assimilation to evolution has been extensively investigated [73, 74]. The study reviewed here quantitatively demonstrated a correlation between developmental robustness to noise and genetic robustness to mutation, in terms of phenotypic variance, when the system is evolved under sufficient noise. The

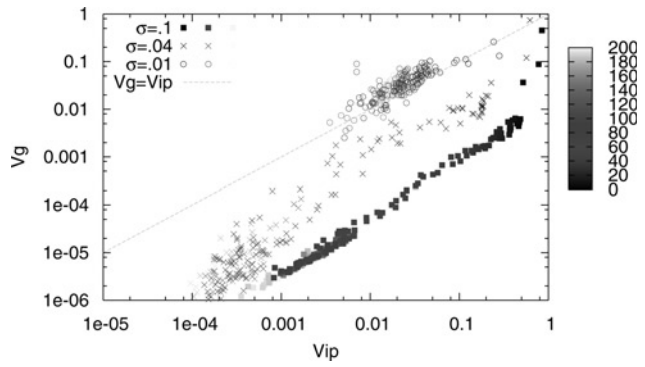

Figure 7 *Relationship between V_g and V_{ip}*

V_g is computed from $P(\bar{F})$ at each generation, and V_{ip} by averaging the variance of $p(F; gene)$ over all existing individuals. We also checked using the variance for a gene network that gives the peak fitness value in $P(\bar{F})$, but the overall relationship is not altered. Points are plotted over 200 generations. The colour is changed gradually with the number of generations, as displayed. $\sigma = .01$ (circle), .04 (cross), and .01 (square). For $\sigma > \sigma_c \simeq .02$, both decrease with successive generations

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relationship between phenotypic fluctuations and evolution implies a relationship between phenotypic plasticity and evolution akin to genetic assimilation.

4 Summary

Despite recent quantitative studies on stochastic gene expression patterns, their positive roles have not yet been fully understood. Here, we have reviewed recent studies on the relevance of noise to adaptation and evolution. First, it has been shown that growing cells have a general ability for adaptation by taking advantage of stochasticity in gene expression. On the one hand, this attractor selection mechanism by noise explains recent experiments showing adaptation without signal transduction. On the other hand, it also explains how cells can adapt to a variety of environments that they may never have encountered before.

Second, the evolution of robustness to developmental noise and mutation has been shown to be possible under the presence of noise. Indeed, developmental robustness to noise leads to mutational robustness. Although we have demonstrated this evolution of robustness using gene expression network models, we expect this behaviour to be observable generally if fitness is determined through developmental dynamics that is sufficiently complex so that a given developmental process, when deviated by noise, may fail to reach the fittest target pattern. The present work opens the possibility of quantitative studies on robustness, in terms of phenotypic variance, while the relevance of noise in shaping smooth developmental landscapes is elucidated.

In the studies surveyed here, evolution is concerned with a single fitness condition under a fixed environment. Then, the fluctuation or biological plasticity decreases over generations. Of course in present organisms, a relatively large degree of phenotypic fluctuations is preserved. Phenotypic plasticity or evolvability (the ability for evolution) is sustained. It is important to consider the influence of environmental fluctuations, interactions with other individuals and interference among different processes in developmental dynamics, which may restore or sustain phenotypic plasticity, evolvability and fluctuations.

Both adaptation and evolution are responses of organisms to their environment, which is crucial for their survivability. In the adaptation discussed here, a gene expression pattern is selected such that cell growth is optimal under given environmental conditions. In evolution, a genotype is selected such that the population growth is optimal under a given environment.

In both cases, consistency between two different levels is achieved, that is, gene expression and total cellular activity (growth speed) for adaptation, and genotype and phenotype for evolution. In both cases, the relation between the levels is mutual. Gene expression (protein concentration) controls cell growth, whereas cell growth influences protein concentration through dilution. Genotype determines the phenotype, but which genotype is selected depends on the phenotype concerning fitness. In both cases, the gene expression dynamics is stochastic. To sum up, consistency between different levels is achieved through a mutual relationship between levels and stochasticity. When the environmental condition is altered, the consistency between levels may be broken. In the new environment, gene expression dynamics may no longer lead to optimal growth, nor can the genotype produce a high fitness phenotype. Then, by taking advantage of stochastic dynamics, the phenotype is changed to fit the new environment, so that consistency between levels is later recovered. Stochastic dynamics, thus, provides flexible adaptation or evolution to the environment in biology [75].

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