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The stochastic nature of biochemical networks

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Cell behaviour and the cellular environment are stochastic. Phenotypes vary across isogenic populations and in individual cells over time. Here we will argue that to understand the abilities of cells we need to understand their stochastic nature. New experimental techniques allow gene expression to be followed in single cells over time and reveal stochastic bursts of both mRNA and protein synthesis in many different types of organisms. Stochasticity has been shown to be exploited by bacteria and viruses to decide between different behaviours. In fluctuating environments, cells that respond stochastically can out-compete those that sense environmental changes, and stochasticity may even have contributed to chromosomal gene order. We will focus on advances in modelling stochasticity, in understanding its effects on evolution and cellular design, and on means by which it may be exploited in biotechnology and medicine.

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Introduction

Stochasticity pervades cellular behaviour. Even in an isogenic population, every cell is unique, whether in their responses or in the shape of their organelles or in the expression of their genes. We define stochasticity as randomness: any phenotype measured from a population of cells or from a single cell at different times will not have a unique value, but a collection of values. Such collections will often, but not always, be distributed around a single most probable value, and systems with high stochasticity will have broader distributions than those with low stochasticity.

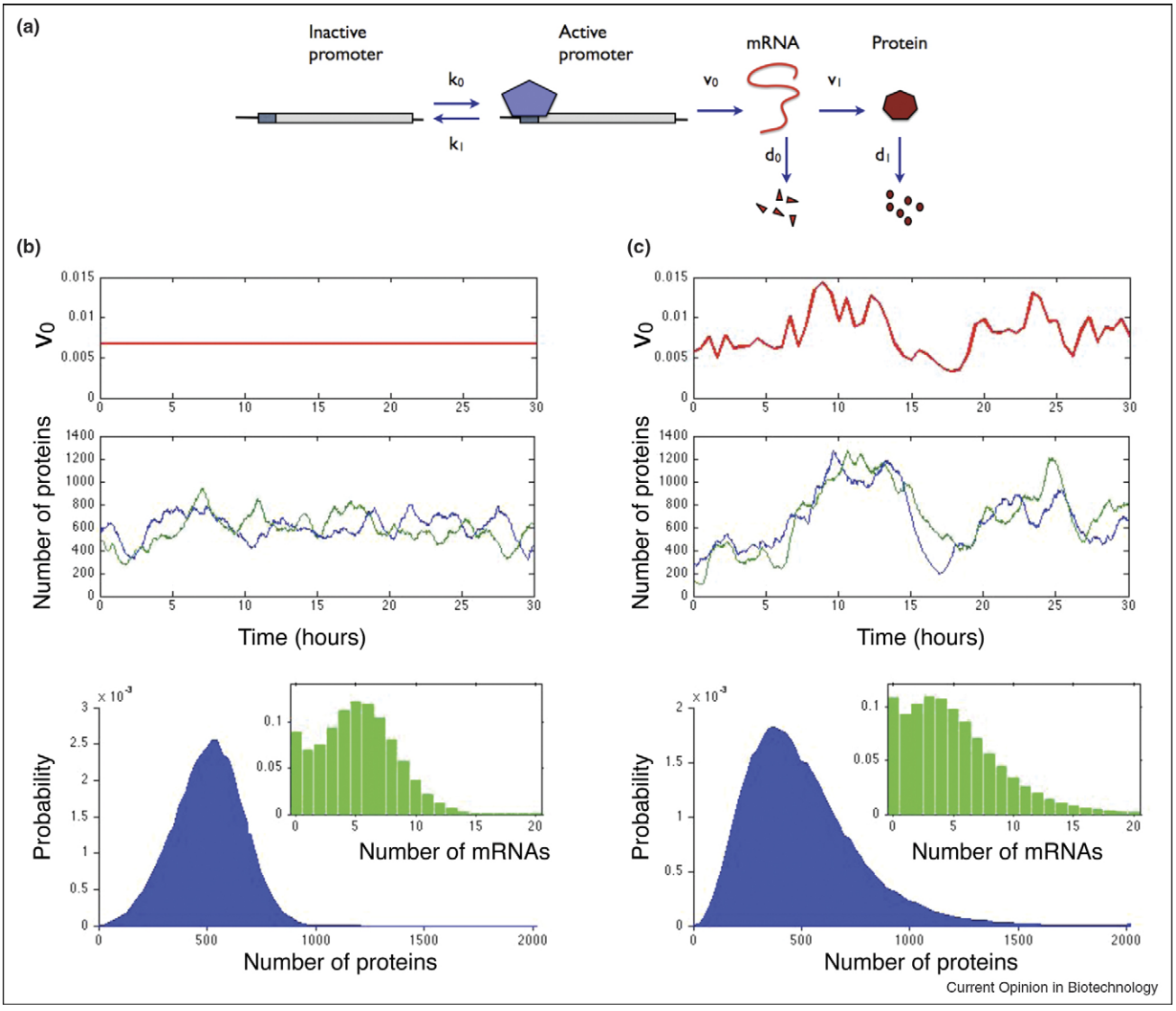
Ultimately, stochasticity arises because random intermolecular collisions make any biochemical reaction stochastic. Stochasticity can be negligible in some biochemical networks and substantial in others. For any system, it has two sources [1,2]. Intrinsic stochasticity is generated by the dynamics of the system from the random timing of individual reactions. It is enhanced by low numbers of molecules because low numbers make individual reaction events, which change molecular numbers by one or two, more significant. Extrinsic stochasticity is generated by the system interacting with other stochastic systems in the cell or its environment.

Both forms of stochasticity can be measured by creating a copy of the system of interest in the same cellular environment as the original system [2]. Stochasticity in gene expression has been most studied. Defining 'noise' to be an empirical measure of stochasticity, the total noise can be quantified by inserting a fluorescent protein downstream of the promoter of interest and then measuring the coefficient of variation of fluorescence (the standard deviation divided by the mean) across a population of cells. Using two copies of the promoter each upstream of a different allele of green fluorescent protein (GFP) allows the extrinsic noise to be measured by the correlation between the fluorescence from the two alleles across the cell population [2]. The intrinsic noise is a measure of the difference between the fluorescence from the two alleles (Figure 1), and the square of the intrinsic noise and the square of the extrinsic noise sum to give the square of the total noise [1].

Using synthetic promoters, total noise and both types of stochasticity have been quantified for gene expression in prokaryotes and eukaryotes [2–5]. These studies showed that intrinsic noise increased as numbers of molecules decreased and that extrinsic noise was usually greater than intrinsic noise. By demonstrating that stochastic effects are not negligible *in vivo*, they set the stage for more in-depth experimental and modelling studies.

It has now been demonstrated, conclusively in our opinion, that stochasticity is significant in endogenous biochemical networks. High-throughput studies have been carried out in yeast [6,7]; three-colour experiments have been used to quantify different contributions to extrinsic fluctuations [8]; stochasticity has been measured in mammalian cells, both in gene expression [9•,10•] and in the p53 network [11,12], in slime moulds [13], in HIV transactivation [14,15••], in bacterial chemotaxis [16], and in the timing of mitosis [17,18], meiosis [19], and lysis by phage lambda [20]. Typically, protein fluorescent reporters are used to detect fluctuations in protein concentrations, but mRNA levels can also be followed in live cells using fluorescently tagged mRNA-binding proteins [13,21].

Figure 1



Intrinsic and extrinsic fluctuations in stochastic gene expression. (**a**) A model of gene expression that includes active and inactive states of the promoter, transcription and translation. As an example, we show the TATA-box-binding protein driving activation of the promoter with probability $k_0 = 2.0 \times 10^{-4}\,s^{-1}$. The probability of inactivation is $k_1 = 6.0 \times 10^{-4}\,s^{-1}$. The probability of transcription is $v_0 = 0.0067\,s^{-1}$ and of translation is $v_1 = 0.02\,s^{-1}$. Both mRNA and protein degrade: mRNA with a probability $d_0 = 10^{-3}\,s^{-1}$ and protein with a probability $d_1 = 2 \times 10^{-4}\,s^{-1}$. We model all reactions as first-order processes. (**b**) Simulation results with only intrinsic fluctuations. The probability of transcription per unit time, v_0, is a function of the concentration of RNA polymerase and so will fluctuate because this concentration fluctuates. If we ignore such extrinsic fluctuations, v_0 is constant over time (top panel). We show fluctuations for two identical proteins, following a 'two-colour' experiment [2] (middle panel). Each protein is regulated in the same way, and genes for both proteins are in the same intracellular environment. Protein fluctuations are intrinsic and independent: $\eta_{tot} = \eta_{int} = 0.30$. Both the distributions of the numbers of mRNA and protein for any one of the genes are asymmetric (lower panel). The mRNA distribution has two peaks reflecting the active and inactive states of the promoter. These peaks are averaged away by the longer living protein. (**c**) Simulation results with both intrinsic and extrinsic fluctuations [37•]. The probability of transcription, v_0, is now stochastic with a log-normal distribution with a coefficient of variation of 0.5 and fluctuations of lifetime 10^4s (upper panel). The mean value of v_0 is unchanged. Both reporter genes experience the same extrinsic fluctuations in v_0 and their fluctuations become correlated (middle panel); $\eta_{int} = 0.32$; $\eta_{ext} = 0.40$; $\eta_{tot} = 0.51$. The mRNA and protein distribution broaden with the additional extrinsic fluctuations and become more asymmetric. The mode of the protein distribution decreases from 520 to 375 molecules.

We believe that the important research questions are first, what should be included to quantitatively model stochasticity; second, how has stochasticity affected evolution and cellular design and third, can we exploit stochasticity for medicine and biotechnology. We shall consider work on each in turn.

Modelling stochasticity

Models should include both intrinsic and extrinsic fluctuations. If diffusion can be ignored, intrinsic fluctuations are well understood, at least at steady-state. We believe that a future focus will be extrinsic fluctuations and including diffusion.

Perhaps the largest change in models of intrinsic fluctuations in gene expression is the now general acceptance that gene expression often occurs in bursts. Innovative experiments have quantified bursting both in mRNA [10•,13,21] and in protein synthesis [22•,23•]. Bursting in protein synthesis was first predicted 10 years ago [24] and is implicit in models which include transcription and translation as first-order processes (Shahrezaei *et al.*, unpublished data). Although such effects may in reality be more complicated [25], comparisons with experiments show that bursts in mRNA synthesis can be effectively modelled by the promoter transitioning between active and inactive states (Figure 1a) [4,5,21,10•], in agreement with earlier work [26,27]. Bursts of mRNA synthesis could be from changes in chromatin structure or from binding and unbinding of proteins involved in transcription [4,5,21] or from pausing by RNA polymerase [28]. For mammalian cells, stochasticity seems dominated by these transitions [10•]. In addition, we now know the mean and standard deviation of protein levels for arbitrary complex promoters [29] and the probability distributions for both protein and mRNA numbers for constitutive expression [10•,25,30•]. All of these results are at steady-state though, and only a few time-dependent predictions exist [1,31,32].

Extrinsic fluctuations have lifetimes comparable to the cell cycle [33]. They are non-specific, affecting many components in a network [34], and dominate stochasticity, at least in single cell studies [2,5]. Extrinsic fluctuations cause fluctuations in the parameters of models because these parameters are often functions of protein concentrations [35,36]. Consequently, extrinsic fluctuations add non-linearities to a biochemical system because parameters usually multiply a fluctuating intrinsic variable. These non-linearities can cause the protein mean, mode, and the intrinsic noise to vary with both the magnitude of the extrinsic fluctuations and their lifetime (Figure 1) [37•,38] — so-called deviant effects [39]. We have proposed a modification of the Gillespie algorithm for simulating intrinsic fluctuations [40] that includes extrinsic fluctuations with arbitrary properties [37•].

Diffusion is important in cell signalling [41] and contributes to extrinsic fluctuations, though its effects have been little studied. An increase in the stochasticity of ligands binding to receptors caused by the diffusion of ligands to the receptors has been calculated using the fluctuation–dissipation theorem [42] and the diffusion of repressors to their operator sites is predicted to increase fluctuations in gene expression [43]. Biological phenomena should be robust to local fluctuations, and testing the robustness of a model's predictions in a stochastic reaction–diffusion simulation can be an effective means to distinguish between competing models [44].

Consequences of stochasticity

Considering the effects of stochasticity on evolution and the design and function of biochemical networks is perhaps most interesting. Although this area has attracted modellers, there has been relatively little experimental work. To test if stochasticity influences the function of a network, experimenters need to control its magnitude. Fluctuations can be reduced by including multiple copies of the gene of interest [10•,36] or modified by varying levels of a relevant transcription factor or other regulatory input. With such an approach, however, the concentration of the protein product also changes, and changes in both noise and concentration can affect the function of biochemical networks.

New innovations allow stochasticity to be altered while concentrations remain unchanged. Suel *et al.* used a mutant of *B. subtilis* that undergoes gene replication and cell growth, but not cell division. They were thus able to show that stochasticity determines the fraction of cells in a population that become competent to uptake extracellular DNA [45••]. Studying the same system, Maamar *et al.* drew similar conclusions by decreasing intrinsic fluctuations through a mutant with a high rate of transcription, but with an initiation codon of poor translational efficiency [46••]. They thus increased mRNA levels and so decreased intrinsic fluctuations [1,35,47], while maintaining protein concentrations. Another approach, similar to that of Suel *et al.*, is to introduce multiple copies of the whole genome which in yeast increases proportionally both protein copy numbers and cell volume and so again keeps concentrations fixed while decreasing stochasticity [18].

Stochasticity is controlled and exploited by cells. Most work has focused on network designs that may have evolved to reduce fluctuations, such as negative feedback, both transcriptional [31,37•,48–50] and translational [47], dimerisation [51], co-expression [47,52,53], and feed-forward loops [37•,54,55]. Fluctuations also corrupt the information received by cells, and biochemical networks may have evolved to infer the most probable state of the cellular environment from the chemical signals they receive using a biochemical implementation of Bayes's rule [56] or of a Kalman filter [57].

Maintaining stochasticity is predicted to have an advantage in fluctuating extracellular environments [58–60]. If the environment changes suitably quickly, bacteria that switch stochastically between states, where each state of the bacterium is optimum for a particular

environment, were predicted to have an evolutionary advantage over bacteria that pay the metabolic costs of sensing the environment, even though these bacteria can always match their state to the environmental state [60]. These predictions were confirmed experimentally in yeast using a mutant that randomly transitions between two phenotypes with different abilities to metabolise galactose [61]. Stochasticity also allows an isogenic cell population to transiently explore different phenotypes. Such variation has been shown to help yeast survive a sudden environmental stress, providing that stress is severe [62••]. High stochasticity both creates larger numbers of cells able to respond to high stress and larger numbers of cells unable to respond to weak stress.

Stochasticity has been thought to enhance the robustness of the behaviour of a network in response to changes in the network parameters. From the fluctuation–dissipation theorem, we might expect that a network whose behaviour is robust to stochastic fluctuations also should be robust to parameter changes. This expectation has been confirmed by simulation where both types of robustness co-evolved [63,64].

Exploiting stochasticity

We are aware of only a few studies that indicate how we can exploit biochemical stochasticity. The magnitude of fluctuations is determined by numbers of molecules independently of how these molecules are measured. By following and fitting fluctuations in the partitioning of a fluorescent protein between daughter cells at cell division, Rosenfeld *et al.* were able to infer *in vivo* numbers of fluorescent proteins [65]. Such techniques provide measurements in absolute units. They therefore facilitate comparison between different experiments and the assimilation of different results into larger models.

Stochasticity also contributes to disease, and inhibiting stochasticity could provide possible therapies. Stochastic switching between different cellular states allows bacteria to survive antibiotics [66]. Stochasticity also controls the probability of the HIV virus entering either the replicative or the latent state [15••]. In agreement with more general predictions [49,67], positive feedback in the genetic network of the virus generates fluctuations in the levels of a transcription factor with lifetimes long enough for the virus to enter the replicative state. Weakening the positive feedback increases the number of latent viruses.

Conclusion

Biological evolution has always been the evolution of stochastic systems in stochastic environments. Stochasticity is a fundamental property of every biochemical network and of the signals and nutrients cells detect. Stochasticity can explain chromosomal gene order because essential genes may cluster in regions of open chromatin to avoid bursts of mRNA synthesis [68•]. It is heritable and as such may generate genetic predisposition to mutations [69]. It may even play a role in aging [70]. With such pervasive effects, we believe that accurate prediction and control of the behaviours of cells will only be possible through understanding their stochastic nature.

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