STOCHASTICITY IN GENE EXPRESSION: FROM THEORIES TO PHENOTYPES

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Abstract | Genetically identical cells exposed to the same environmental conditions can show significant variation in molecular content and marked differences in phenotypic characteristics. This variability is linked to stochasticity in gene expression, which is generally viewed as having detrimental effects on cellular function with potential implications for disease. However, stochasticity in gene expression can also be advantageous. It can provide the flexibility needed by cells to adapt to fluctuating environments or respond to sudden stresses, and a mechanism by which population heterogeneity can be established during cellular differentiation and development.

ISOGENIC
Genetically identical. Individual cells within an isogenic population are typically the progeny of a single ancestor.

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Stochasticity in gene expression arises from fluctuations in transcription and translation, despite constant environmental conditions. This phenomenon has attracted interest for many years because of its implications for cellular regulation and non-genetic individuality[1–7]. Recent advances in techniques for single-cell analysis have provided an impetus for novel experimental and theoretical investigations that, in turn, have led to fundamental new insights in this field. As a result, a coherent picture of stochasticity in prokaryotic and eukaryotic gene expression is beginning to emerge.

Here, we discuss the theoretical mechanisms that are thought to cause fluctuations in the expression levels of single genes and the experiments that have been used to validate these ideas. We also describe experimental studies of stochastic effects in gene-regulatory networks. Special emphasis is given to stochastic mechanisms that can lead to the emergence of phenotypically distinct subgroups within ISOGENIC cell populations. We conclude by discussing the possibility that stochasticity in gene expression is an evolvable trait, and the growing evidence for a role of stochasticity in development and disease.

Origins and consequences of stochasticity

Modelling the expression of a single gene. FIGURE 1 illustrates some of the main steps in gene expression. The control of transcription is mediated by factors that bind at upstream promoter elements or influence the binding of other molecules to *cis*-regulatory elements within or near the promoter. Because such binding events are the result of random encounters between molecules, some of which are present in small numbers, the biochemical processes that regulate transcription initiation are inherently stochastic. In addition, the multi-step processes that lead to the synthesis and degradation of mRNA and protein molecules are subject to similar molecular-level noise. The model in FIG. 1 is simple in comparison with the true complexity of gene expression[8]. However, it has provided a good theoretical framework for understanding the effects of stochasticity on prokaryotic and eukaryotic gene expression[9–49], and underlies the theoretical investigations used to design and interpret many of the experiments discussed in this review.

The origins and consequences of molecular-level noise on the expression of a single gene can be demonstrated by comparing the intracellular protein concentrations obtained from stochastic and deterministic simulations of the model in FIG. 1. Deterministic simulations typically use rate equations (BOX 1) — which do not take stochastic processes into account — to describe changes in mRNA and protein abundances. By contrast, stochastic simulations typically consider

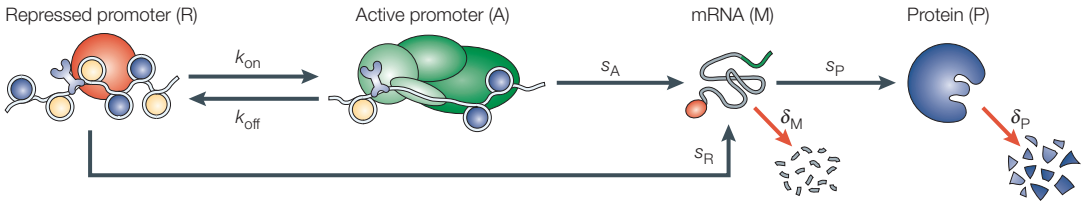


Figure 1 | **A model of the expression of a single gene.** Each step represents several biochemical reactions, which are associated with mRNA and protein production, transitions between promoter states and the decay of mRNA and protein. k_{on}, k_{off}, s_A, s_R, s_P, δ_M and δ_P are the rate constants associated with these steps, as indicated. These reactions involve binding and dissociation events that occur at random at the molecular level. This is ignored in deterministic models of gene expression, which typically describe the different steps in terms of reaction rates. Stochastic models generally describe each step as a single random event, with a reaction time that shows an exponential distribution. All steps are assumed to obey first-order kinetics. The ratios s_P/δ_M (the average number of proteins produced per mRNA) and s_A/k_{off} (the average number of mRNA produced between successive promoter activation and inactivation events) are referred to as the translational and transcriptional efficiency, respectively.

the random formation and decay of single molecules and multi-component complexes explicitly. As a result, the deterministic approach cannot capture the potentially significant effects of factors that cause stochasticity in gene expression.

In certain circumstances, deterministic simulations of the model in FIG. 1 predict intracellular protein concentrations that are similar to those predicted by stochastic simulations. The conditions that need to be satisfied for the predictions of the two approaches to be similar are large system size (high numbers of expressed mRNA and proteins, and large cell volumes) and fast promoter kinetics (BOX 1; see below). These conditions are met in the example illustrated in FIG. 2a, in which the protein concentration (the overall measure of gene expression) predicted by a stochastic simulation fluctuates with very low amplitude around the average level predicted by a deterministic simulation. Correspondingly, the relative deviation from the average, measured by the ratio η of the standard deviation σ to the mean N, is quite small. This ratio η (or, alternatively, η^2) is typically referred to as the coefficient of variation, or the noise.

When the conditions required for good agreement between deterministic and stochastic simulations are not fulfilled, the effects of molecular-level noise can

Box 1 | **Deterministic rate equations and stochastic models of gene expression**

Rate equations

One mathematical framework for describing gene expression uses deterministic rate equations to calculate the concentrations of mRNA [M] and proteins [P]. For the model in FIG. 1, with a single gene copy, these equations are:

$$\frac{d[\mathrm{M}]}{dt} = \frac{k_{on}}{k_{on} + k_{off}}\frac{s_A}{V} + \frac{k_{off}}{k_{on} + k_{off}}\frac{s_R}{V} - \delta_M[\mathrm{M}] \quad (1)$$

$$\frac{d[\mathrm{P}]}{dt} = s_P[\mathrm{M}] - \delta_P[\mathrm{P}] \quad (2)$$

where V is the cell volume; the terms $\delta_M[\mathrm{M}]$ and $\delta_p[\mathrm{P}]$ are the degradation rates for mRNA and proteins, respectively; and the term $s_p[\mathrm{M}]$ is the rate of protein synthesis. The rate constants k_{on} and k_{off} govern transitions between the active and repressed states of the promoter. Therefore, the ratios $k_{on}/(k_{off} + k_{on})$ and $k_{off}/(k_{on} + k_{off})$ in equation 1 are the fraction of time that the gene spends in the active and repressed states, respectively (that is, the promoter is assumed to be in chemical equilibrium). Consequently, mRNA production occurs at a constant rate, which is given by the weighted average of the activated synthesis (s_A) and repressed synthesis (s_R) mRNA synthesis rates.

The macroscopic limit and promoter kinetics

The above equations represent a valid approximation of the stochastic description when two limits are satisfied (FIG. 2a). The first is the macroscopic limit in which s_R, s_A and V become large, with the ratios s_R/V and s_A/V remaining constant. The second is the limit of fast chemical kinetics in which k_{on} and k_{off} become large, with their ratio remaining constant. Note that these limits do not alter equations 1 and 2. In FIG. 2b, the limit of fast chemical kinetics is satisfied, whereas fluctuations that are due to small system size are large (see main text). The reverse is true in FIGS 3a,b, where the number of expressed molecules is high, but the transitions between promoter states occur less frequently. Typically, concentration fluctuations scale in the form $1/\sqrt{V}$ for small system size effects (corresponding to $1/\sqrt{N}$ scaling, as $[N] = N/V$), and in the form $1/\sqrt{k_{off} + k_{on}}$ for slow chemical kinetic effects[19].

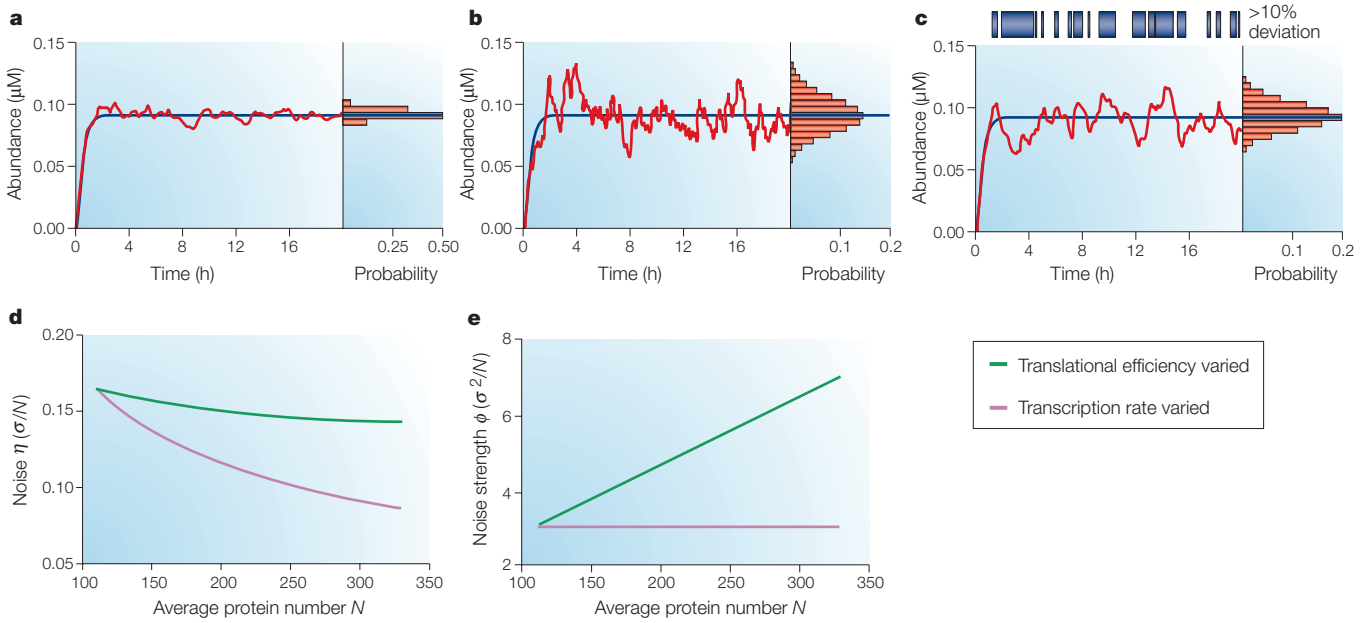


Figure 2 | **Finite-number effects and translational bursting. a**–**c** | Time series of protein concentrations generated from deterministic and stochastic simulations (blue and red curves, respectively). Histograms that show the probability that a cell will have a given intracellular protein concentration are also shown (right-hand panels). The parameters used yield protein concentrations in the μM range. The rate of promoter transitions is high ($k_{off} = k_{on} = 10$ per min). **a** | Low-amplitude fluctuations with high numbers of expressed mRNA and protein molecules (~3,000 and ~10,000, respectively) and a large cell volume (200 μm³). The other parameter values are $s_A = 50$, $s_R = 5$, $s_P = 0.2$, $\delta_M = 0.1$ and $\delta_P = 0.05$ in units per min. **b** | Increased fluctuations in protein concentration are due to a decrease in the number of expressed mRNA and protein molecules (to ~30 and ~100, respectively). The transcription rates and cell volume were decreased 100-fold compared with **a** ($s_A = 0.5$ units per min, $s_R = 0.05$ units per min, $V = 2$ μm³). **c** | Large fluctuations in protein abundance are due to low mRNA abundance (~30 molecules). Transcription rates were decreased 100-fold compared with **a** ($s_A = 0.5$ units per min, $s_R = 0.05$ units per min) and the translation rate was increased correspondingly ($s_P = 20$ units per min) to keep the protein abundance at ~10,000 molecules. The difference between **b** and **c** is a 100-fold increase in the average number of expressed proteins. Because low mRNA abundance is the dominant source of noise in gene expression (as described in the main text), the fluctuation amplitude and population distribution are almost the same in the two simulations. The 'bar-code' above the graph is obtained by drawing a vertical line at each time point where the protein concentration deviates by more than 10% from the average. **d**,**e** | The predicted dependencies of the coefficient of variation η, defined as the standard deviation σ over the mean N (the noise) (**d**), and the noise strength ϕ, defined as the variance σ^2 over the mean N (**e**), on the average protein abundance when the transcription rate and translational efficiency are increased. The initial parameters are as in part **b**.

be large. The simulations discussed below show how molecular-level noise can have pronounced effects on gene expression, and provide insights into factors that contribute to fluctuations in the abundance of expressed protein.

Finite-number effects and translational bursting. System size is an important factor contributing to stochasticity in gene expression. The effects of decreasing the cell volume are illustrated by the time series shown in FIG. 2b. These were obtained from stochastic and deterministic simulations for which the volume of the cell and the average rate of transcription were decreased 100-fold compared with the simulations in FIG. 2a. This proportional change in parameters does not affect the rate equations, and the average mRNA and protein concentrations remain the same. Comparison of the stochastic simulation results in FIGS 2a,b therefore demonstrates the effects of a smaller system size at fixed concentrations. In FIG. 2b, the protein concentration fluctuates with increased amplitude, causing significant deviations from that predicted by the deterministic model. This also causes a broader distribution of protein abundance and, therefore, increased population heterogeneity.

These results highlight a fundamental relationship between system size and noise[50]: namely that noise tends to increase when the size of the system is decreased. To understand this relationship, consider a protein that can move freely between the nucleus and cytoplasm. At equilibrium, the nuclear and cytoplasmic concentrations are, on average, equal. However, because the volume of the nucleus is less than that of the cytoplasm, translocation of a protein molecule across the nuclear membrane has a more significant effect on the nuclear concentration than on the concentration in the cytoplasm. If 10 molecules are present in the nucleus and 1,000 in the cytoplasm, the translocation of 1 molecule causes a 10% change in nuclear concentration, but only a 0.1% change in cytoplasmic concentration. This differential effect arises from the different number of molecules in the two compartments and is referred to as the 'finite-number effect'. In general, when N denotes average

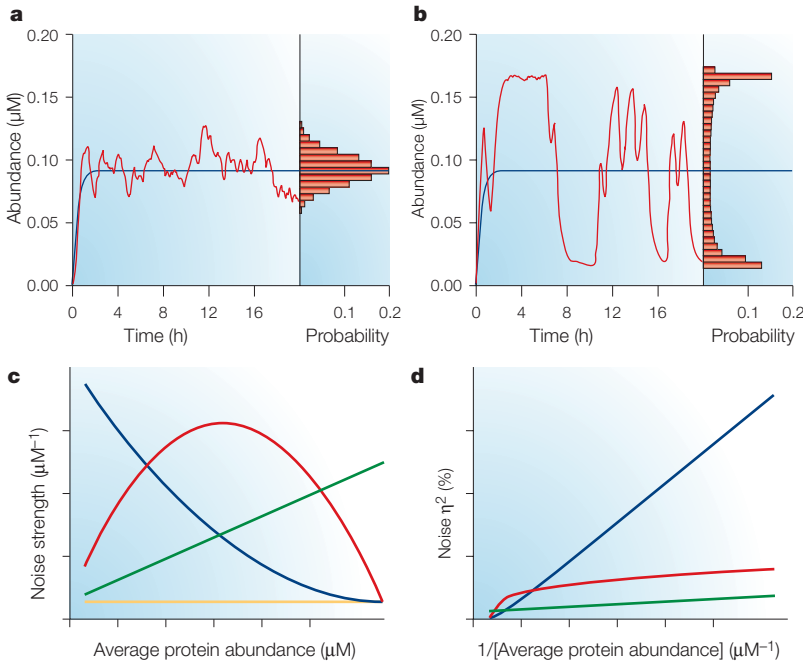


Figure 3 | **Slow promoter transitions and transcriptional bursting. a**,**b** | Time series and histograms were obtained as in FIG. 2. The parameters used were as follows: $s_A = 50$, $s_R = 5$, $s_P = 0.2$, $\delta_M = 0.1$ and $\delta_P = 0.05$. Parameters are given as units per min. High numbers of expressed mRNA and protein molecules (~3,000 and ~10,000, respectively) and a large cell volume (200 μm^3) were used. **a** | A decrease in the half-life of the two promoter states from ~4 seconds (FIG. 2) to ~1 minute ($k_{on} = k_{off} = 0.7$ per min) yields an increase in fluctuation amplitude that is comparable with those obtained following a 100-fold decrease in the number of expressed molecules. **b** | Promoter-transition rates that correspond to stable promoter states (half-life ~1 hour) yield random transitions between low and high expression states and a bimodal distribution in protein concentration. **c** | The curves show the predicted dependencies of the noise strength on the average protein abundance with different modes of transcriptional induction. The rate of transcription varies owing to changes in: (mode I) the promoter-deactivation rate (k_{off}) (red curve), (mode II) the promoter-activation rate (k_{on}) (blue curve), and (mode III) the transcription rate from the active promoter state (s_A) (green curve). No change is observed when the transitions are fast (yellow curve), regardless of how the average transcription rate is varied. **d** | The qualitative differences seen in **c** are less apparent in more traditional plots[34] of the noise (measured as η^2) against the average protein abundance (plotted as 1/*N*). Note that the average protein abundance decreases from left to right in this figure. Data in **d** and **c** were generated by the equation derived by Raser and O'Shea[36] for the model in FIG. 1, with $s_R = 0$. A general form of this equation is given by Paulsson[34]. Identical dependencies were observed in the model by Blake *et al.*[29].

molecular abundance, a decrease in abundance results in a characteristic $1/\sqrt{N}$ scaling of the noise ($\eta \sim 1/\sqrt{N}$ and $\eta^2 \sim 1/N$). The finite-number effect is perhaps the most commonly recognized manifestation of molecular-level noise in cellular regulation.

Comparing FIGS 2a,c highlights how finite-number effects at the level of mRNA comprise a second important factor contributing to gene-expression noise. In FIG. 2c, the rate of transcription is decreased 100-fold (compared with FIG. 2a) without changing the cell volume (in contrast to FIG. 2b). Moreover, the rate of translation is increased 100-fold, such that the average protein concentration (and the number of expressed protein molecules) remains the same as in FIG. 2a. This proportional change in transcription and translation rates captures a 'translational bursting' mechanism[12,20,21], in which the amplitude of fluctuations in protein abundance depends on the number of proteins produced per mRNA, referred to as the burst parameter[21] or translational efficiency[23,29,36]. According to this mechanism[21], for two genes expressed at the same average abundance, the one with the higher translational efficiency and lower mRNA abundance is predicted to display greater fluctuations in protein concentration and a broader population distribution than the gene with the lower translational efficiency and higher mRNA abundance. Because the average number of protein molecules and the cell volume are kept fixed, this increased gene-expression noise is attributable to increased fluctuations in mRNA abundance, causing increased fluctuations in the rate of protein synthesis.

Although finite-number effects at the protein level do contribute to stochasticity in gene expression, it is generally expected that variability is more strongly linked to changes in the number of mRNAs than of protein molecules. This can be seen by comparing the three simulations in FIG. 2a–c. Although a 100-fold difference in the average number of mRNAs at a fixed protein abundance causes a large change in the fluctuation amplitude and protein distribution (compare FIGS 2a,c), a 100-fold difference in the average number of protein molecules at a fixed mRNA abundance causes only a minor effect (compare FIGS 2b,c). Correspondingly, as illustrated in FIG. 2d, varying the rate of transcription causes a greater change in gene-expression noise than varying the translational efficiency.

Translational bursting and noise strength. The relative deviation from the average, measured as the standard deviation divided by the mean ($\eta = \sigma/N$), is the most direct and unambiguous measure of gene-expression noise (FIG. 2d). However, it is sometimes advantageous to use a different measure, the noise strength, which is defined by the variance divided by the mean ($\varphi = \sigma^2/N$). This measure is used primarily to reveal trends that would otherwise be obscured by the characteristic $1/\sqrt{N}$ scaling of noise arising from finite-number effects[21]. For example, in FIG. 2d, varying the rate of transcription and the translational efficiency yields qualitatively identical dependencies of the noise on the average protein abundance. On the other hand, measurements of noise strength, as described below, might yield dependencies that differ qualitatively, depending on how the abundance of expressed protein is varied. For example, the translational bursting mechanism predicts that the noise strength should increase linearly with the average protein abundance when translational efficiency is increased, but remain constant when the rate of transcription is increased[21] (FIG. 2e). Because of its ability to discriminate between these situations, the noise-strength measure has proved useful as a tool to interpret experimental data.

It is important, however, to note that increased noise strength does not imply that the relative variability (the coefficient of variation η) is also increased. This is clear when comparing FIGS 2d,e, which were obtained from the same data set using the two different noise measures. In fact, for two genes characterized by low and high noise-strength measures, it can only be concluded that the

gene with the highest noise strength has increased variability — that is, a broader protein distribution — when the two genes are expressed at similar abundances.

Slow promoter kinetics and transcriptional bursting. As mentioned previously, in addition to a large system size, a second requirement for a small effect of molecular-level noise on gene expression is to have fast transitions between promoter states. This is demonstrated in FIG. 3a, which shows simulation results obtained using the same parameters as in FIG. 2a (that is, high molecular abundances), but with reduced promoter transition rates. Here, the promoter is in the active state for longer, allowing an increased number of mRNAs to be synthesized in rapid succession. The size of these 'bursts' in transcription depends on the average number of transcripts produced between promoter activation and deactivation (the ratio s_A/k_{off}; see FIG. 1), referred to as the transcriptional efficiency[29,36].

The simulation results shown in FIG. 3b demonstrate that the effect of transcriptional bursting can be pronounced[35,41]. In this case, the transition rates are so slow that the protein abundance (and that of mRNA) tracks the state of the promoter. Consequently, protein (and mRNA) is produced at either very high or very low rates, resulting in random transitions between high and low expression states and a bimodal distribution of intracellular protein concentration. Because of this bimodality, a snapshot of a large cell population would show a mixture of cells that express the protein at low and high levels[9,10,35,41].

Similar stochastic simulations of the effects of promoter transition rates have led to predictions about the relative importance of sources of noise in prokaryotic and eukaryotic gene expression. The previously mentioned predictions of the translational bursting mechanism are valid when the transitions between promoter states are rapid. This is consistent with the general view for prokaryotes that the biochemical processes regulating transcription initiation occur frequently in comparison with synthesis and degradation events. Indeed, most models of prokaryotic gene expression assume that the transition rates are so fast that the promoter states are always in steady state and the rate of transcription is constant (BOX 1). The model in FIG. 1 therefore suggests that translational bursting is probably a dominant source of stochasticity in the process of prokaryotic gene expression.

By contrast, slow transitions between promoter states are expected to be particularly important in eukaryotic gene expression, for which the presence of NUCLEOSOMES and the packing of DNA-nucleosome complexes into chromatin generally make promoters inaccessible to the transcriptional machinery. Transition between open and closed chromatin structures, corresponding to active and repressed promoter states, can be quite slow[51]. In the context of the model in FIG. 1, the result is increased heterogeneity within a cell population (FIG. 3a), or even stochastic all-or-nothing responses in single cells (FIG. 3b).

Mixed populations and bimodal population distributions — which, as described above, might arise from slow promoter transition rates — are often observed in eukaryotes[52–59]. This has led to the proposal of two distinct modes of transcription regulation in eukaryotes[60] — a graded mode, in which all cells respond in proportion to the inducing signal, and a binary mode, in which the inducing signal changes the probability of a stochastic all-or-nothing response in an individual cell. In the context of the model in FIG. 1, a graded response becomes binary when the rate of promoter transitions is decreased. This indicates that the experimentally observed graded and binary modes of transcription might arise from differences in transition rates between promoter states[9,10,35,41].

Modes of transcriptional induction. Analyses of theoretical models[29,36] predict that transcriptional bursting arising from slow promoter transitions affects the dependence of the noise strength on the average protein abundance. Based on the model in FIG. 1, there are three possible modes of transcriptional induction, each yielding a characteristic dependency of noise strength on abundance (FIG. 3c). In mode I, the average transcription rate is increased by decreasing the rate of promoter inactivation. In this case, the noise strength has a maximal value at intermediate expression levels. In mode II, the average transcription rate is increased by increasing the rate of promoter activation. Here, noise strength decreases as a function of the average protein abundance. In mode III, the average transcription rate is increased by increasing the rate of transcription from the active promoter. In this case, the noise strength increases with the average protein abundance. The effect of transcriptional bursting on fluctuations in protein abundance and on population heterogeneity is therefore predicted to depend sensitively on the molecular details of the induction and on the strength of the inducing signal. These theoretical results have been used in experimental studies to interpret how both induction and mutations affect the kinetics of eukaryotic promoters.

In the following sections, we describe experimental approaches in prokaryotes and eukaryotes that have been used to test the theoretical basis of stochasticity in gene expression that is described above. We also discuss experiments showing that noise in gene expression is influenced, sometimes to a large degree, by factors that might be considered to be external to the process of gene expression.

Stochasticity in prokaryotic gene expression

Translational bursting in prokaryotes. The predictions described above concerning the effects of translational bursting on noise in gene-expression levels indicate that noise strength is more sensitive to variation in translational efficiency than to the rate of transcription. This was validated by Ozbudak *et al.* in a study of gene-expression noise in *Bacillus subtilis*[23]. Population heterogeneity was measured by expressing a gene encoding *GFP* from an inducible promoter, which provides a simple method of varying the rate of

NUCLEOSOME
The fundamental unit into which DNA and histones are packaged in eukaryotic cells. It is the basic structural subunit of chromatin and consists of 200 bp of DNA and an octamer of histone proteins.

Box 2 | **Intrinsic and extrinsic sources of gene-expression noise**

Intrinsic versus extrinsic sources

In the analysis of stochastic processes, it is often beneficial to separate contributions arising from fluctuations that are inherent to the system of interest (intrinsic noise) from those arising from variability in factors that are considered to be external (extrinsic noise). In the phenomenological model of gene expression in FIG. 1, intrinsic noise is defined by the fluctuations generated by stochastic promoter activation, promoter deactivation, and mRNA and protein production and decay. Extrinsic-noise sources are defined as fluctuations and population variability in the rate constants associated with these events.

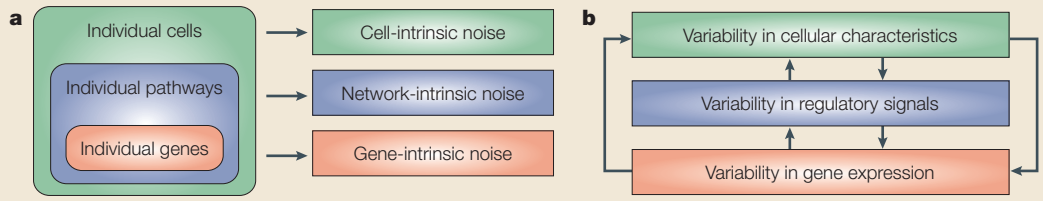
Context-dependent definitions of intrinsic noise

The definition of intrinsic noise is problem-dependent, and varies from one context to another, as illustrated in part **a** in the figure. Gene-intrinsic noise refers to the variability generated by molecular-level noise in the reaction steps that are intrinsic to the process of gene expression. Network-intrinsic noise is generated by fluctuations and variability in signal transduction and includes gene-intrinsic noise in the expression of regulatory genes. Cell-intrinsic noise arises from gene-intrinsic noise and network-intrinsic noise, as well as fluctuations and variability in cell-specific factors, such as the activity of ribosomes and polymerases, metabolite concentrations, cell size, cell age and stage of the cell cycle. Part **b** in the figure shows some of the paths that can propagate and potentially amplify fluctuations and population variability, demonstrating the interdependence of variability at different levels of organization.

Measuring gene-intrinsic noise

Noise intrinsic to gene expression has been defined operationally as the difference in the expression of two identical genes from identical promoters in single cells averaged over a large cell population[28,62]. This definition relies on the assumptions that the two genes are affected identically by fluctuations in cell-specific factors and that their expression is perfectly correlated if these fluctuations are the only source of population heterogeneity. With these assumptions, the contribution of gene-intrinsic noise can be investigated in two-reporter assays (FIG. 4). These assays evaluate, in single cells, the difference in the abundances of two equivalent reporters, such as cyan and yellow fluorescent protein, expressed from identical promoters, located at equivalent chromosomal positions[28].

The two-reporter assay represents a significant scientific advance as it allows measurements of the absolute magnitude of fluctuations generated by the biochemical reaction steps that are intrinsic to the process of gene expression, and allows the characterization of promoter kinetics on the basis of changes in intrinsic noise that are caused, for example, by mutations or gene deletions[36]. It does, however, have certain limitations. For example, contributions from extrinsic factors, such as imperfect timing in replication[28] and intracellular heterogeneity[122,123], might be measured as gene-intrinsic noise. Moreover, because increased variability in regulatory signals might cause cells to adapt distinct expression states, the measured population-average gene-intrinsic noise and the extrinsic regulatory noise might not always be independent. As an alternative, experiments have successfully used indirect methods that are based on single-gene reporter assays[23,29,58,63,65–67,81] to measure differences in population variability, which, with appropriate controls, can be attributed to changes in specific processes or parameters.



transcription. Point mutations that affect the affinity of ribosome binding to the *GFP*-encoding mRNA were introduced to vary translational efficiency. In agreement with the theoretical predictions described above, the noise strength showed a strong, positive linear dependence on the average protein abundance when the translational efficiency was varied. By contrast, changing the rate of transcription yielded a considerably weaker linear dependence.

These results confirm the translational bursting hypothesis that, for genes expressed at similar levels, a combination of low transcription rate and high translational efficiency causes increased gene-expression noise compared with a combination of high transcription rate and low translational efficiency. This led Ozbudak and colleagues to speculate that the inefficient translation of several regulatory genes in another prokaryote, *Escherichia coli*, although it is energetically unfavourable, might have been selected during evolution to guard against the potentially detrimental effects of fluctuations in protein concentrations.

Distinguishing between sources of noise. Stochasticity that is inherent to the biochemical process of gene expression, known as gene-intrinsic noise (BOX 2), is not the only source of variability in the abundance of expressed molecules. Factors that influence transcription rates — such as gene-regulatory signals and the abundances of polymerases and ribosomes — also cause variability in gene expression. Moreover, because of differences in gene copy number at different points in the cell cycle, transcription rates inevitably change as

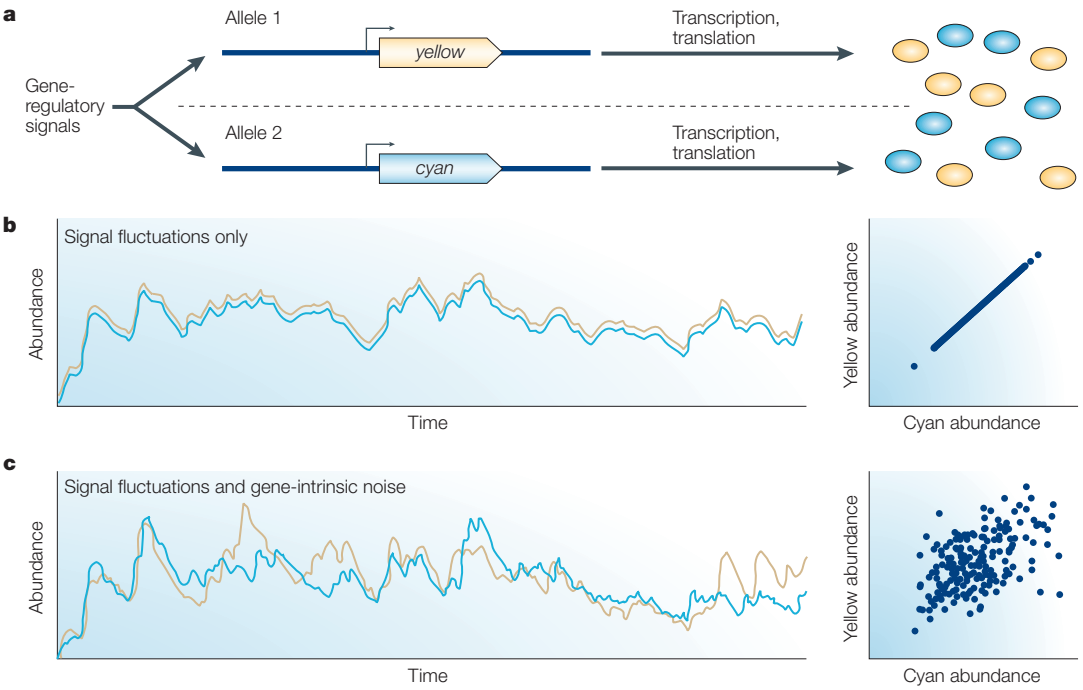


Figure 4 | **Measuring gene-intrinsic noise. a** | Two almost identical genes, which encode yellow and cyan fluorescent proteins, are expressed from identical promoters, and are influenced identically by cell-specific factors, such as gene-regulatory signals. **b** | The abundances of the two expressed proteins are perfectly correlated when stochasticity in the biochemical steps that is intrinsic to the process of gene expression (gene-intrinsic noise) is absent and the effects of intracellular heterogeneity are negligible (left panel). A scatter plot of protein abundance that was obtained from a 'snapshot' of a cell population contains points that are only on the diagonal (right panel). **c** | Asynchronous protein abundances in the presence of gene-intrinsic noise are shown (left panel). Because the biochemical steps in the expression of the two genes are independent, gene-intrinsic noise causes the number of expressed proteins to differ, giving rise to a scatter plot that contains off-diagonal points (right panel). Evaluating the differences in expressed protein abundance within individual cells, and averaging these differences across a sufficiently large cell population can therefore provide a measure of the absolute magnitude of gene-intrinsic noise[28,62].

cells grow and divide[61]. Variability arising from sources that are external to the biochemical process of gene expression is referred to as gene-extrinsic noise.

To measure the impact of both gene-intrinsic and gene-extrinsic noise on population heterogeneity directly, Elowitz *et al.*[62] and Swain *et al.*[28] developed a two-reporter assay that can discriminate between the two. In this assay, two almost identical fluorescent proteins are simultaneously expressed from identical promoters in the same cell (FIG. 4a). In the absence of gene-intrinsic noise, the expression of the two reporter proteins should be strongly correlated (FIG. 4b). Because biochemical steps that are intrinsic to the expression of the two reporters are independent, stochasticity in these steps should be manifested as differences in expression levels (FIG. 4c), and the absolute magnitudes of gene-intrinsic and gene-extrinsic noise can therefore be measured.

In the study by Elowitz *et al.*[62], *E. coli* strains were engineered to express cyan and yellow fluorescent proteins from identical inducible promoters. Measurement of the contributions of intrinsic and extrinsic noise in strains with different genetic backgrounds and varying levels of transcriptional induction showed that both sources of noise contribute to variation within cell populations. Moreover, when the rate of transcription was decreased, the observed dependence of the gene-intrinsic noise on the average protein abundance was found to be in agreement with the $1/\sqrt{N}$ scaling of the noise that is expected to arise from finite-number effects (FIG. 2d). As pointed out by the authors[62], this demonstrates how low molecular abundances can fundamentally limit the precision of gene expression. These studies in *B. subtilis*[23] and *E. coli*[62] populations have therefore confirmed the long-standing theoretical prediction[1–3,5–7] that the finite-number effect increases noise in prokaryotic gene expression.

Stochasticity in eukaryotic gene expression

Translational bursting in eukaryotes. The study described above by Ozbudak *et al.*[23] validated the translational bursting hypothesis experimentally, therefore demonstrating that the finite-number effect at the level of mRNA contributes significantly to gene-expression noise in prokaryotes. To investigate whether this conclusion extends to eukaryotes, Blake *et al.*[29] examined how altering the transcription rate and translational efficiency affects total population variability in the yeast *Saccharomyces cerevisiae*. In this study, the expression of *GFP* variants was placed under the control of a modified galactokinase 1 (*GAL1*) promoter, which could be naturally induced with galactose or artificially induced with anhydrotetracycline. The *GFP* variants had different translational efficiencies, owing to differences in SYNONYMOUS CODON usage. The

SYNONYMOUS CODONS
Codons that have different nucleotide triplets, but which encode the signal for incorporation of the same amino-acid residue during translation. Differences in synonymous codon usage can result in differences in translation rates because codon-specific tRNAs have different abundances.

Box 3 | **Robustness and the architecture of genetic networks**

Engineered gene networks have provided insights into how the architecture of the network influences its ability to function in noisy environments. For example, the two stable expression states of a bistable genetic toggle switch, designed from two mutually repressing genes, were observed to be stable against gene-expression noise[124–127]. This robustness to stochastic effects resulted in cells with epigenetic memory of a given expression state that could be maintained through successive cell generations. By contrast, the positive-feedback system implemented by Isaacs *et al.*[67], and discussed in the main text, yielded mixed populations for bistable conditions, indicating that gene-expression noise in this case was sufficient to cause random transitions between the two expression states. This difference in noise robustness is probably due to a higher transition threshold in the toggle-switch network.

Two oscillatory circuits studied in *Escherichia coli* provide another example of the importance of network architecture for the functioning of gene-regulatory networks. Whereas cells that carry an oscillatory network consisting of three transcriptional repressors showed rapid desynchronization[128], another oscillator design consisting of an activator and a repressor enabled dampened but synchronized oscillations across the cell population[129]. In circuits with the latter design, fluctuations in gene expression can, in theory, cause the emergence of oscillations that would not appear otherwise[27,130]. Therefore, stochasticity in gene expression might sometimes have a constructive effect, making a desired property, such as oscillatory gene expression, easier to obtain.

Although it can be solved on a case-by-case basis[124,127,129,131], one of the most difficult challenges in genetic network engineering is the design of circuits that can operate reliably despite noisy environments and stochasticity in gene expression. From the perspective of the genetic systems engineer, the robustness of natural cell-regulatory networks is as striking as it is intriguing. The design strategies used by natural gene networks to achieve robust function are being uncovered through investigations of model systems — such as bacterial chemotaxis[132–136] — and systems-level approaches[137–141], but are generally poorly understood. Past and future studies of noise in engineered gene circuits have and will aid the design of more reliable engineered networks. Perhaps more importantly, such studies will provide a more detailed understanding of how complex biological systems reduce the effects of, or possibly even exploit, the stochasticity that is inherent to gene expression.

experiments and data analysis were conducted to allow direct comparison with the *B. subtilis* study[23].

In agreement with the results from *B. subtilis*, the noise strength measured in *S. cerevisiae* increased linearly with the average protein abundance when translational efficiency was altered. In fact, the data obtained in the *S. cerevisiae* study[29], with different combinations of transcription rates and translational efficiencies that yield similar expression levels, directly confirm the validity of the translational bursting hypothesis in eukaryotes.

Transcriptional bursting in eukaryotes. In *B. subtilis*[23], a weak linear dependence of noise strength on transcription rate was observed. By contrast, in *S. cerevisiae*[29], the noise strength was found to be strongly and non-linearly dependent on transcription rate, with noise strength reaching a maximum at intermediate protein abundances. This observed dependency is similar to that shown by the red curve in FIG. 3c. It was suggested that the most likely explanation for this effect is a combination of variability in upstream signalling (network-intrinsic noise; BOX 2) and slow transitions between promoter states[29] (transcriptional bursting). This was supported by stochastic simulations, which demonstrated that both factors might result in increased noise strength at intermediate protein abundances.

To investigate stochastic effects in eukaryotic gene expression further, Raser and O'Shea[36] used a two-reporter assay in *S. cerevisiae* to determine how transcriptional induction and *cis*- or *trans*-acting mutations affect gene-intrinsic noise in expression from the *PHO5* promoter. Transcriptional induction had significant effects on the intrinsic-noise strength, which steadily decreased at increasing population-averaged expression levels. This dependency is consistent with that which is predicted to arise from transcriptional bursting when transcriptional induction increases the rate of promoter activation as described earlier (mode II; FIG. 3c).

To explore this further, Raser and O'Shea measured variability in strains that carry mutations in the TATA BOX of the *PHO5* promoter or in its UPSTREAM ACTIVATING SEQUENCE (UAS). TATA box mutations are expected to decrease the rate of transcription from the active promoter, whereas UAS mutations are expected to decrease the rate of promoter activation by slowing down the recruitment of chromatin-remodelling factors. Consistent with the corresponding dependencies of noise strength on protein abundance that are predicted for these different modes of transcriptional induction (as described earlier; see FIG. 3c), TATA-box and UAS mutations resulted in decreased and increased intrinsic-noise strength, respectively. The observation that induction and UAS mutations had similar effects — increased intrinsic-noise strength at lower population-averaged expression rates — indicates that slow promoter activation owing to chromatin remodelling has an important role in generating stochasticity in eukaryotic gene expression. This was further supported by observations in strains that lack different components of chromatin-remodelling factors, which had increased intrinsic noise at decreased expression levels[36].

Experimental studies of stochasticity in prokaryotes and eukaryotes[23,29,36,62] have therefore confirmed the theoretical mechanisms that are predicted by the model in FIG. 1. These mechanisms operate at the level of single genes and are intrinsic to the biochemical processes of gene expression. The direct measurements of

TATA BOX
A consensus sequence within promoters that is enriched in thymine and adenine residues, and is generally important for the recruitment of the transcriptional machinery.

UPSTREAM ACTIVATING SEQUENCE
A sequence that is located upstream of a promoter at which transcriptional activators bind and subsequently facilitate the expression of downstream genes.

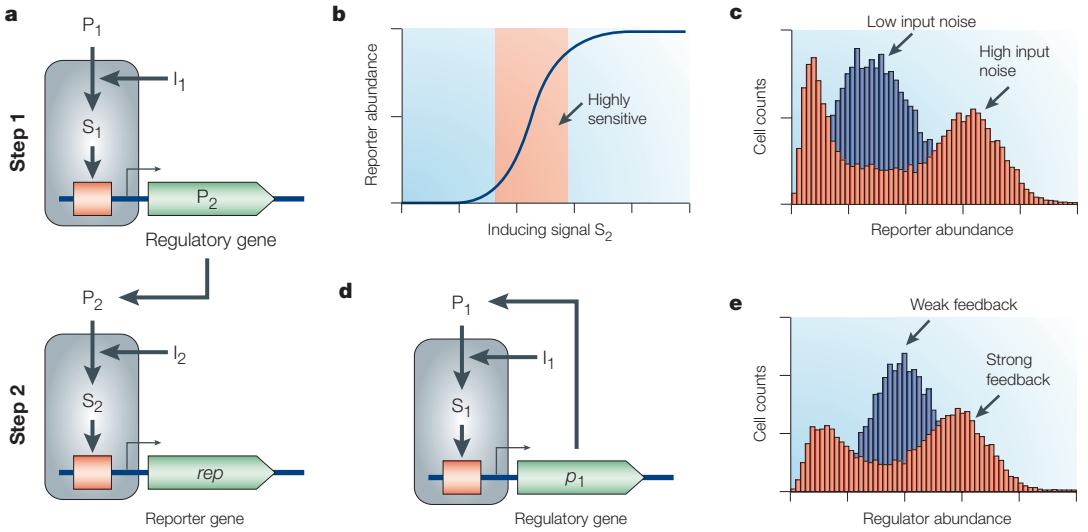


Figure 5 | **Noise in gene networks. a** | This shows a transcriptional cascade that has two regulatory steps. P_1 and P_2 represent regulatory proteins, I_1 and I_2 are their inducers, and S_1 and S_2 represent the effective activities of the regulatory proteins. **b** | The population-averaged dose response to transcriptional induction by S_2 is shown. The highest signal sensitivity occurs at intermediate values of the input signal where the dose-response curve has the highest slope. **c** | Increased variability in the S_2 signal in the region of high signal sensitivity causes a transition from a unimodal to a bimodal population distribution. The population histograms were obtained by simulations of the model in FIG. 1, with the noisy signal S_2 having a non-linear effect on the rate of promoter deactivation. Similar effects are observed experimentally[29]. **d** | An auto-regulatory single-gene network is shown. **e** | The effects of varying the strength of the positive-feedback loop in **d** are shown. A unimodal population distribution that is obtained in the presence of weak feedback becomes bimodal when the strength of the feedback is increased. The histograms were obtained from simulations of the model in FIG. 1, with the gene product P_1 acting as a noisy signal that affects the rate of promoter activation. Similar effects have been observed experimentally[66,67].

gene-intrinsic and extrinsic noise[36,62] have further demonstrated that there are factors extrinsic to the biochemical processes of gene expression that contribute significantly to fluctuations and population heterogeneity in protein abundance. One such factor is variability in gene-regulatory signals. It is therefore also important to investigate gene-expression noise in the context of gene-regulatory networks, as we discuss below.

Stochasticity in gene networks

Genes and proteins are organized into extensive networks that allow cells to respond and adapt to their environment. The complexity of these systems can hinder attempts to study how the architecture of regulatory networks enables cells to deal with or take advantage of unreliable, fluctuating signals. Here, we focus on engineered genetic cascades[29,63,64] and synthetic gene networks that involve negative and positive auto-regulation[65–67]. Studies of these networks have allowed more direct investigations of how stochastic effects at different parts of a network affect expression outcomes. Further insights can be gained from studies of synthetic gene networks that are based on different design strategies; these designs are not discussed in detail here, but an overview is given in BOX 3.

Stochastic effects in gene-regulatory cascades. To study noise propagation, Blake *et al.*[29] engineered a transcriptional cascade with two regulatory steps in *S. cerevisiae* (FIG. 5a). In this system, the expression of a target reporter gene depends on the activity of an upstream transcriptional regulator. The level of noise in the expression of the transcriptional regulator and its average activity can be controlled independently to study the effects of noise in a gene-regulatory cascade. As the level of transcriptional induction was increased, the population-averaged expression level was observed to follow a non-linear dose-response curve, with the greatest change occurring at intermediate levels of induction (FIG. 5b). Increased noise in the transcriptional regulatory signal increased the population heterogeneity in this range, but had little or no effect at high and low induction levels. This is perhaps intuitive, as the observed population-averaged dose-response curve (FIG. 5b) predicts that the rate of transcription should be most sensitive to variations in the regulatory signal at intermediate induction levels.

Interestingly, in the same study, a high level of regulatory noise gave rise to population distributions with distinctly separated high and low expression states (a binary response)[29]. By contrast, a low level of noise in the regulatory input yielded unimodal distributions, regardless of the induction level. This indicates that the binary response is directly linked to variability in the level of transcriptional regulatory signal received by the downstream promoter. Indeed, as illustrated in FIG. 5c, a steep dose-response curve for the regulatory input, coupled with large differences among cells in the regulatory signal, is sufficient to generate bimodal population distributions and all-or-nothing responses in single cells[68].

Box 4 | **Noise minimization as an evolvable trait**

The translational bursting hypothesis discussed in the main text proposes that organisms could evolve to minimize stochasticity in gene expression by using a high transcription rate and low translational efficiency if there was a selective pressure for noise reduction. Motivated by results in *Saccharomyces cerevisiae*[29], which showed decreased noise in the protein levels produced from genes with a high transcription rate and low translational efficiency, Fraser *et al.*[82] tested the hypothesis that noise in gene expression could be an evolvable trait. They used data from high-throughput experiments to estimate the rates of transcription and translation for groups of proteins with similar abundances in *S. cerevisiae*. It was proposed that essential genes, for which deletions are lethal to the organism, should be particularly sensitive to random perturbations in protein abundance. This is because survival could be compromised by large fluctuations that bring protein concentrations to dangerously low levels, thereby transiently mimicking the effect of genetic deletion. Fraser *et al.* also proposed that genes encoding components of multi-protein complexes should show a similar sensitivity to fluctuations, because fluctuations in any one of the subunits could compromise the integrity of the complex, wasting the energy invested in the synthesis of its components.

Indeed, in 14 of the 15 rates of protein production that were tested, essential genes showed a statistically significant bias towards high transcription and low translation. Similar results were found for genes that encode components of multi-protein complexes[82]. Taken together, these findings provide strong support for the hypothesis that noise in gene expression is an important biological variable that might be subject to natural selection. Moreover, there are mechanisms that operate at the level of gene and cell-regulatory control systems that might have evolved specifically to minimize the impact of intra- and extracellular fluctuations and noisy signal transduction. Such mechanisms, which include feedback regulation and redundancy of regulatory pathways, have recently been reviewed by Stelling *et al.*[136] and Kitano[140] in the broader context of biological robustness as a property of complex, evolvable systems.

Recent studies have provided further insights into the propagation of gene-expression noise in transcriptional cascades. Hoosangi *et al.*[63] measured variability in *E. coli* populations that carry cascades engineered with one, two or three regulatory steps. High population heterogeneity was observed in all three cascades at intermediate levels of transcriptional induction, with each additional regulatory step roughly doubling the expression noise. Little or no change was observed at high and low induction levels, regardless of the cascade length, consistent with observations in *S. cerevisiae*[29]. This was also found by Pedraza *et al.*[64] in a study of a two-step transcriptional cascade that involved an important difference from other studies of this type of system[29,63] — variability at each regulatory step was measured simultaneously in single cells, allowing the separation of the gene-intrinsic and regulatory noise that was transmitted through the cascade. It was found that the latter, which is defined as being extrinsic to the biochemical processes of gene expression (BOX 2), was the dominant source of noise in the expression of the target gene.

Hoosangi *et al.*[63] also showed that the three-step cascade yields a more non-linear dose-response curve than the one-step cascade, allowing a more precise on–off switching behaviour. However, this improved steady-state response characteristic comes at the price of a more asynchronous initial population response. This was attributed to each additional step in the cascade adding variability to the response time of individual cells[63]. This intriguing finding shows experimentally that there is a trade-off between cascade length and synchrony. Together with the observed increase in noise for longer cascades, this indicates that signals generated by short cascades generally are less noisy than those generated by long cascades.

As well as cascade length, it is also important to consider the influence of negative- and positive-feedback loops on the effects of gene-expression noise. Endogenous gene networks frequently rely on such feedback regulation. The effects of noise in the presence of feedback loops have been studied experimentally using synthetic networks as described below.

Gene networks with negative feedback. It is a commonly held idea that negative feedback provides a noise-reduction mechanism[21,32,34,40,43,65,69,70]. To test this in the context of gene regulation, Becskei and Serrano[65] engineered a single-gene negative-feedback system in *E. coli*. They compared the variability generated by this regulatory network with that generated in the absence of feedback control. This comparison revealed a decrease in gene-expression variability in the feedback network, therefore confirming that negative auto-regulation provides a noise-reduction mechanism.

Negative feedback might also minimize the effects of fluctuations on downstream processes. In general, when fluctuations in a regulatory signal occur at a high frequency, a slower downstream process can only 'feel' a time-average signal, effectively functioning as a low-pass filter. It has been shown theoretically[32] that negative feedback shifts the fluctuation frequency to higher values, thereby potentially minimizing its effects on slower processes. This prediction awaits further experimentation.

Despite the stabilizing effects described above, negative feedback can also have a destabilizing effect and so result in dampened or sustained oscillations if it involves a time delay[71]. Examples of delay-driven genetic oscillations include the DNA-damage response that is mediated by the P53–MDM2 FEEDBACK LOOP[72,73] and the SEGMENTAL-CLOCK oscillator that functions in early vertebrate development[74–77]. Stochastic simulations indicate that oscillations in the latter are sensitive to molecular-level noise, and that noise reduction by cell–cell communication is required to achieve the precision that is necessary for normal development[76].

P53–MDM2 FEEDBACK LOOP
One of the best-studied negative-feedback regulatory networks in human cells. The tumour-suppressor p53 activates the synthesis of Mdm2, which in turn targets p53 for degradation.

SEGMENTAL CLOCK
The gene-regulatory network that allows the periodic and population-synchronous expression of genes in the primitive streak and posterior presomitic mesoderm of developing vertebrates. This allows the formation of a periodic pattern of gene expression in the anterior presomitic mesoderm.

Gene networks with positive feedback. Autocatalytic reactions that result in positive feedback generally amplify fluctuations and population heterogeneity[78,79]. In a recent investigation, Isaacs *et al.*[67] reconstructed a single-gene autocatalytic network from the LYSOGENIC/LYTIC DECISION PATHWAY of bacteriophage-λ to study the effects of varying the strength of feedback activation on population heterogeneity. A hallmark of positive-feedback regulation is bistability, which gives rise to distinct cellular states with high and low expression levels. Stochasticity in gene expression can cause random transitions between the two states, yielding bimodal population distributions[16,66,67,80,81]. Indeed, Isaacs *et al.*[67] observed bimodal population distributions in protein abundance, and showed experimentally that the population distribution can change from being bimodal to unimodal when the strength of the feedback is varied. Similar results were obtained by Besckei *et al.*[66] in a study of an artificial single-gene autocatalytic system in *S. cerevisiae*, and these findings are captured by stochastic simulations of a simple positive-feedback network (FIGS 5d,e).

Positive-feedback regulation therefore provides yet another mechanism for generating phenotypically distinct subpopulations from cells that have identical genotypes[66,67]. Such mechanisms can have pronounced phenotypic consequences that might be involved both in disease and in normal cellular processes such as differentiation.

Biological significance of stochasticity

Stochasticity in gene expression is generally believed to be detrimental to cell function, because fluctuations in protein levels can corrupt the quality of intracellular signals, negatively affecting cellular regulation. One possible benefit of randomness, however, is that it can provide a mechanism for phenotypic and cell-type diversification. It is therefore interesting to consider the cases in which increased or decreased noise can be advantageous, and whether an evolutionary advantage is provided that would subject these processes to natural selection. A recent study by Fraser *et al.*[82] (discussed in BOX 4) provides strong support for the hypothesis that gene-intrinsic noise is subject to natural selection. Here, we focus on potentially beneficial roles of stochasticity in gene expression, and possible implications for development and disease.

Benefits of stochasticity in microorganisms. There are several examples in which stochasticity in gene expression has been proposed as a useful mechanism for generating phenotypic heterogeneity[12,23,25,70,83–90]. This is expected to be particularly beneficial to microbial cells that need to adapt efficiently to sudden changes in environmental conditions[85,86]. Fluctuations in gene expression provide a mechanism for 'sampling' distinct physiological states, and could therefore increase the probability of survival during times of stress, without the need for genetic mutation. These intuitive ideas were examined in a recent theoretical investigation that considered the response of a cell population that stochastically transitions between distinct phenotypic states in a randomly changing environment[86]. This study concluded that in a fluctuating environment, a heterogeneous bacterial population of isogenic cells (for example, created as a result of stochastic gene expression) might achieve faster growth rates than a homogeneous population, provided that the time taken to respond to sudden changes in environmental conditions is sufficiently slow.

Switching between phenotypic states with different growth rates might be an important factor in the phenomenon of persistent bacterial infections after treatment with antibiotics[84,88]. Although most of the population is rapidly killed by the treatment, a small genetically identical subset of dormant 'persistor' cells can survive an extended period of exposure. When the drug treatment is removed, the surviving persistors randomly transition out of the dormant state, causing the infection to reemerge. Although it remains to be demonstrated that stochasticity in gene expression is involved in switching to and from the physiological states associated with the persistor phenotype[90], random variation in intracellular factors has been proposed as a likely source of physiological diversification in these populations[84,87].

Stochastic transitions between distinct phenotypic states have also been observed in *S. cerevisiae*. A recent paper by Acar *et al.*[81] presented a comprehensive analysis of the role of three feedback-control loops, two positive and one negative, in the *S. cerevisiae* galactose-utilization network. The primary purpose of the network is to increase the uptake and metabolism of galactose. Because the negative-feedback loop downregulates these processes, its presence seems counterproductive. A possible functional role of the negative feedback, and therefore an explanation for the design of the regulatory network, is revealed when cellular regulation is considered in a stochastic framework. In agreement with the findings obtained using engineered networks, Acar *et al.*[81] found that the positive-feedback loops are important for the establishment and separation of two distinct expression states. In the absence of negative feedback, these states were found to be highly stable and to endow cells (and their progeny) with long-term EPIGENETIC MEMORY of past galactose-consumption states. Negative feedback reduces this memory by increasing the rate at which cells randomly switch between different phenotypic states that are associated with different expression of the galactose-utilization genes. As a result, the biological function of negative feedback might be to prevent cells from being trapped in sub-optimal phenotypic states.

Stochasticity in development and disease. There has also been speculation that stochasticity has a constructive role in development and cellular differentiation in higher organisms[51,57,91–101]. For example, during *Drosophila melanogaster* development, stochastic fluctuations in the turnover of two proteins, Notch and Delta, might underlie the random emergence of neural

LYSOGENIC/LYTIC DECISION PATHWAY
The gene-regulatory network that allows bacteriophage-λ to switch between a dormant (lysogenic) state, in which phage DNA is integrated into the chromosome of the host cell, and an active (lytic) state, in which the cellular machinery of the host is used to rapidly produce phage progeny.

EPIGENETIC MEMORY
The ability to transfer information through successive generations without modification of the DNA sequence. Common mechanisms of epigenetic inheritance are covalent modifications of DNA and altered chromatin structure that affects gene expression.

HAPLOINSUFFICIENCY
The inactivation of one of two alleles in diploid cells to produce a heterozygote that is insufficient to assure normal function.

DENDRITES
Short, tree-like extensions that are features of many neurons and allow the transmittance of nerve impulses between cells.

OPTICAL WELL ARRAY
An emerging technology for temporal single-cell fluorescence measurements. Each cell is contained within a well etched into the tip of a single optical fibre, with thousands of such fibres arranged in an array. This allows simultaneous measurements across large cell populations.

precursor cells from an initial homogenous cell population[54]. Differentiation in mammalian haematopoietic stem cells[57,93,98] and during *Caenorhabditis elegans*[92] and *Xenopus laevis*[99] development has also been linked to similar mechanisms. In these examples, stochasticity again establishes an initial population heterogeneity that allows the selection and propagation of cell-type-specific gene expression and, eventually, differentiation from an initially homogenous cell population.

Investigations of other epigenetic factors[102,103] are uncovering other phenomena that might be linked to stochastic gene expression. Mechanisms such as DNA methylation[104,105] and chromatin-structure alterations[106,107] can modify transcriptional activity in a stochastic manner, both in single cells and across populations. Transcriptional control in eukaryotes can involve silencing mechanisms, such as the formation of repressed chromatin states (heterochromatin) and DNA methylation, which enable the stable transmittance of functional states in cell lineages and prevent low-level basal expression of genes[108–110]. Because heterochromatin might be better viewed as a dynamic rather than a static structure[51,111–113], slow fluctuations in chromatin states might lead to variability in gene expression[114,115].

Stochasticity in gene expression might also be involved in disease, particularly in the context of diseases that arise from transcription-factor HAPLOINSUFFICIENCY[116], in which one allele is non-functional or inactivated[117–120]. Elaborating on previous studies[9,10], Cook *et al.*[117] used stochastic simulations of a model of gene expression with slow promoter transitions (transcriptional bursting) to show that diploid cells have a higher probability than haploid cells of maintaining the abundance of an expressed gene product above a low threshold value. This led the authors to propose that haploinsufficiency might increase the lifetime susceptibility for disease by increasing the probability, at a given time, that expression of an essential factor drops below a crucial threshold, causing the onset of disease. Although such events would occur only rarely, the probability would gradually accumulate, making the onset of disease more likely later in the life of the organism.

Following this study[117], it was confirmed experimentally[119] that cells that are haploinsufficient for the tumour-suppressor gene neurofibromatosis type 1 (*NF1*) are associated with increased population heterogeneity. This was measured by increased population variation in the number of DENDRITES. However, a direct link between increased noise and haploinsufficiency syndromes remains to be determined. Further investigations of epigenetic mechanisms in development and disease are likely to uncover other cases in which stochasticity in gene expression is relevant.

Future directions

The past few years have seen tremendous progress in our understanding of stochasticity in gene expression, and it is likely that the rapid pace within this field will continue. To facilitate further advancements, however, several technological limitations must be addressed. For example, our ability to measure the random synthesis and decay of single molecules in single cells across a sufficiently large cell population is limited. Recent technological advances towards this goal include the simultaneous, real-time measurement of single-cell fluorescence and gene-expression noise using OPTICAL WELL ARRAYS[121] and time-lapse microscopy[61]. The latter was recently employed by Rosenfeld *et al.*[61] to measure variability in single-cell transcriptional input–output relationships across cell populations and fluctuations in single-cell expression rates. Further developments in fluorescence techniques and high-throughput microscopy might also open up the possibility of studying the spatial effects of stochastic fluctuations in gene expression.

As noted above, most of the experimental studies on gene networks have so far focused on relatively simple artificial networks. There is a need to develop experimental and data-analysis techniques to allow the study of stochasticity in more complex regulatory systems, particularly endogenous gene networks. As demonstrated in the study by Acar *et al.*[81], a comprehensive understanding of design strategies used by endogenous transcriptional regulatory programmes might require a stochastic perspective. We have barely scratched the surface of this intriguing topic, and there is a clear need to address in greater detail how gene expression responds to fluctuations in signal transduction, how gene-expression noise is transmitted through regulatory circuits and control loops, and how the architecture of regulatory networks allows cells to deal with or take advantage of unreliable, fluctuating signals.

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Competing interests statement

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