Stochastic modeling of biological reactions

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Abstract | Several biological systems comprise of reaction networks where the low number of molecules makes it inappropriate to completely characterize system behavior using a continuous approach. Biological systems are also characterized by discrete states (e.g. infected, dead) that are not amenable to the use of a continuous descriptor. This review discusses the need for adopting a discrete stochastic modeling approach for analyzing biological reactions networks. Various stochastic simulation procedures and theoretical studies are presented. The challenges in the theoretical and computational analysis of discrete stochastic biological reaction networks are discussed.

Introduction

Biological processes involve a complex set of events that have to be coordinated in time and space in order to yield the correct functionality. The development of an embryo into an adult organism involves the correct spatio-temporal positioning and differentiation of nearly 10^{14} cells in humans. Members of a species interact with themselves, their predators and prey species to generate patterns of disease, population growth and even extinction. It is increasingly recognized that theoretical and computational approaches are essential for the expedited understanding of complex biological networks[1–3]. Various modeling formalisms are adopted based on the degree of accuracy that is desired, the modeling objective, computational and time constraints, and the availability of experimental data against which the model is calibrated and validated.

In this review, I present an overview of modeling and simulation methods used for analyzing biological systems and reactions that view these processes as being inherently probabilistic in nature. The review is not intended to be an exhaustive compilation of all stochastic modeling approaches and all implementations of the approaches. The goal is to serve as a useful starting point for stochastic modeling in biology, with particular reference to cellular and molecular biology problems. Stochastic modeling of single molecules, e.g. nucleic acid or protein conformation predictions, is not included in this review. Modeling approaches are presented here from the perspective of the forward-problem, i.e. modeling systems where the component interactions are known. Modeling approaches for the inference of biological networks from experimental data have been recently reviewed[4] and are not included in this review.

The article is organized as follows: first, examples illustrating the complexity and inherent heterogeneity of biological systems are presented. Next, a summary of various deterministic and stochastic modeling formalisms used to analyze biological systems is presented to provide a context for discrete stochastic modeling methods. Different methods for stochastic modeling are presented and Chemical Master Equation based approaches for simulating biochemical reaction dynamics are discussed in detail. A discussion of some of the challenges and issues in this field concludes the survey.

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Biological processes are stochastic

In this section, I present a few examples to illustrate the complexity and stochastic nature observed in most biological systems. These examples span length scales from a single cell to an ecosystem.

The small size of single cells leads to small volumes where intracellular reactions occur. Because of these small sizes, typical protein concentrations in the nanomolar range imply that there are only .02 to 20 protein molecules per prokaryotic cell. Proteins at picomolar concentrations will be present as a few molecules per eukaryotic cell. A recently discovered archea species has a volume in the atto-liter (10^{-18} liter) range[5], and signaling proteins in this species are likely to be present in amounts in the 100 molecules/cell range or lower. In all species, DNA exists as one or two copies and there are a finite number of promoter and repressor sites on the DNA where regulatory proteins bind and influence the transcription process. Several experimental studies involving tracking of concentrations at a single-cell level, whether of a particular mRNA or protein species[6], or a large number of species at the genome/proteome scale[7] have shown that the transcription process is stochastic. Experimental and theoretical investigations of stochastic gene expression have been recently reviewed[8,9].

An example of a complex biological process involving a large number of interacting cells is the development of a mature organism from a single zygote. In the worm *Caenorhabditis elegans*, which is a model organism used for studying development, the lineage of each of the 959 cells from the zygote has been identified. Detailed connection maps of each of the 302 neurons in the nervous system[10] have been meticulously constructed. However, understanding of the functioning of the nervous system is still unclear. Ageing of these cells is found to be stochastic[11]. In the developing retinal of the fly *Drosophila*, it has been shown that cell fate decisions are stochastic processes that are biased by signal transduction events[12,13]. It has been proposed that the concentration gradients of morphogens serve to control the inherent stochastic behavior of RNA regulatory networks[14]. During the development of the mouse olfactory system, each sensory neuron expresses only one of the approximately 1000 odorant receptor proteins. It has been proposed that a stochastic expression process is responsible for generating this one neuron-one odorant receptor rule[15].

Just as stochasticity in protein production is believed to generate diversity during development, it has also been shown to be the mechanism responsible for imparting different phenotypes to genetically identical individuals in a population. Fluctuations in DNA binding have been shown to be a potential mechanism that accounts for the induction of competence in a certain fraction of genetically identical bacterial cells[16,17]. This diversity is thought to be the beneficial in a varying environment[18]. Stochastic formalisms have also been used to model interactions between two populations[19,20]. Interactions between bacteria have been studied in biofilms, and it has been shown that there is heterogeneity in the phenotypes of neighboring cells[21,22]. Stochastic sources of variation in biofilms have been recently reviewed[23].

In addition to exploring the structure and function of existing regulatory mechanisms, scientists and engineers have developed artificial 'circuits' that can be introduced into a suitable host. Several reviews on the ethical and practical aspects of the field have been published[24,25]. These synthetic biology circuits range from simple three or four gene constructs[26–28] to an entire synthetic genome[29]. For small constructs, functioning *in vivo* has been reported as being consistent with stochastic models corresponding to the designed construct[26].

Modeling approaches

Different modeling approaches for biological processes have been reviewed in several textbooks[30–35]. Here, I briefly discuss the various approaches in order to place discrete stochastic models in the context of other modeling methods.

A class of modeling approaches that is implicitly adopted for simple systems and can be formalized for larger systems is a non-quantitative graphical[36] or database approach. Such models, and various software implementations, are reviewed elsewhere[4,37,38]. These models are an essential starting point in analyzing biological systems. Modeling approaches for analyzing biological processes may be divided into four categories[4] as shown in Table 1 depending on whether they model discrete/continuous states and whether the model output is a defined deterministic value or a probability distribution. In this survey, one element of the second row of Table 1, viz. discrete stochastic modeling approaches, are reviewed, with particular emphasis on the Chemical Master Equation (CME) based models. The review by de Jong[39] discusses in detail most of the formalisms that are briefly mentioned here. Other reviews have a narrower focus on modeling of metabolic pathways[40], cell biology[41,42] developmental biology[43], or gene networks[44–46].

For most modeling applications in biology, the most appropriate formalism is a continuous deterministic description of the system using mass-action kinetics[47]. For each system component,

Table 1: Classification of mathematical modeling approaches

	Continuous	Discrete
Deterministic	ODE models PDE models	Boolean models Petri nets
Stochastic	Master equation approximations (Fokker-Planck, SDE) Dynamic Bayesian models	Master Equation approaches Probabilistic Boolean models Dynamic (discrete) Bayesian

a mass balance between the rates of increase and decrease in concentration due to production, degradation and other reactions gives the rate of change of component concentration. This set of ordinary or partial differential equations (ODE or PDE) for all system components can be solved to predict system behavior. The principal shortcoming of the continuous deterministic modeling approach is the requirement for rate parameters. However the rich body of dynamical systems work in mathematics allows analysis of system behavior, such as existence of two steady states[48], independent of knowledge of exact parameters. Several reviews[42,49] present examples where such mathematical models have been successfully used to further the understanding of specific biological systems. In addition to the dynamics, the steady state behavior of biological systems can be explored through these models. The largest class of such steady state models are metabolic flux models, recently reviewed by Llaneras and Pico[50]. A steady-state analysis can also be used to estimate the range of behaviors exhibited by the biological system[51,52]. Steady state analysis methods have been used to study the behavior of gene regulatory networks[53].

Ordinary and partial differential equations lead to predictions of system behavior that is deterministic. Continuous stochastic models have been developed for predicting the observed variance in a system. These are almost always in the form of an additive (weighted) noise term to the deterministic ODE or PDE formulation. The form of the additive noise term ranges from variance-weighted white noise[54], to rigorously derived weighted noise[55] based on an approximation of the CME. In all the cases, the individual trajectories are (numerically) evaluated and several such trajectories are used to calculate the moments of the distribution. For nonlinear reaction kinetics, continuous stochastic models do not guarantee that the individual trajectories are exact representations of the actual trajectories expected from the CME solution. The advantage of such methods is the large reduction in computational requirements. The steady state distributions can be estimated using continuous stochastic modeling methods. In a linear chain of reactants with deterministic transitions but with a stochastically varying input, it can be shown that the variance of the flux decreases with chain length at steady state[56]. Bhat and Venkatesh[57] have used a deterministic steady state model to explore the effect of having a distribution of one of more pathway components, and used this analysis to explain certain experimental observations of the *gal* operon behavior.

Although a continuous description is adequate when the number of molecules is large, systems with discrete phenotypes or small number of molecules are accurately represented by discrete-state models. The largest class of discrete deterministic models is Boolean models, where each variable is assumed to be capable of taking values from a finite number of states. The transitions between these states are governed by a set of rules. Circuits are constructed that link each network constituent to its input constituents through these rules. To completely specify the model, the initial state and the updating strategy have to be specified. Typically, a range of initial states is used for simulation and the trajectory and final state is simulated. In synchronous updating, the states of each variable are updated simultaneously based on the current values of the (input) states. However, actual interactions occur at different timescales, e.g., binding events probably occur at much faster rates than transcription events[58], and so asynchronous updating should be used. It has been shown that not only the dynamics, but core network properties such as the number of attractors changes when the updating scheme is changed[59,60]. As such, the utility of a Boolean dynamics approach for prediction of system behavior is unclear. As quantitative rate information is not needed, these models are suitable for modeling data-poor situations, or for exploring the robustness of biological systems to changes in the kinetic rate behavior[61–63]. Boolean models have been extended to allow multiple values for each variable to represent multiple gene activity levels[64] or receptor protein levels[65].

Another modeling formalism that is capable of handling multi-valued variables is the Petri-net approach. This is a directed graph method consisting of place nodes (reactants) and transit nodes (reactions) connected with weighted arcs (rate constant). Each place node has a certain number of tokens (molecules). In the simplest situation, a reaction always occurs if sufficient tokens are present in the input place nodes, making it a discrete deterministic system. If the weights are based on the reaction rate constant and transitions are probabilistic, the approach is exactly equivalent to the Chemical Master Equation approach.

Modeling chemical reactions as discrete stochastic processes

In order to fully capture heterogeneity of states as well as their discrete nature, biological processes are modeled as discrete stochastic systems. The Chemical Master Equation (CME) is the equation that defines the probability that the system is in a particular state at a particular time, and describes the rate of change of this probability with time as a function of the various reaction rates and system probabilities. As such it is a fundamental description of the system, and the stochastic behavior of the system can be completely understood if the CME can be accurately solved. The rest of this article will cover in detail CME based modeling of chemical reaction systems. This modeling approach is the predominant method used for discrete stochastic analysis. A few researchers use other methods to model discrete stochastic systems. A Bayesian approach considers the whole system (model equations, initial conditions, and parameters) in a probabilisitic sense. One example of the use of this approach with discrete states to model vulva development in the worm C elegans is presented by Sun and Hong[66]. Another modeling method is the stochastic Paun systems approach[67] which is a modification of P-systems modeling[68] that considers nested sets (compartments) containing objects (species, or other compartments). The change in set composition is specified using sets of rules representing chemical changes or transport. Akin to the Boolean rule problem, there are several ways of defining the sequence in which the rules are applied. If correctly defined based on the reaction rates, the simulation can be shown to be similar to the Gillespie first-reaction method discussed later. These and other discrete stochastic modeling methods such as stochastic Petri nets[69], which are not widely used and whose theoretical underpinnings have not been as exhaustively analyzed, will not be discussed further. Several reviews[70,71] address one or multiple aspects of the CME-based modeling approaches discussed here, and the reader is referred to those for more details on specific aspects.

The Chemical Master Equation

The most exact molecule-level model of chemical kinetics is the microscopic dynamics model that tracks the behavior of individual molecules based on the forces acting upon them, their collisions with other particles and solvent, and the chemical transformations resulting from some such collisions. However, for even a small number of reacting molecules, this approach is computationally intractable, and it is convenient to think about probabilities of transitions, assuming that the system is well-mixed and the number of reactive collisions is low.

Given a certain number of chemical species capable of undergoing a certain set of transformations, it is possible to define a probability that the system is at a particular state (i.e. has a particular number of molecules of each species) at a certain time. After an infinitesimal amount of time has passed, so small that at most one reaction can occur, the probability that the system is at a particular state is given by (i) the probability that the system was in one of the precursor states that lead to this state via a particular reaction, and the specified reaction does occur; and (ii) the probability that the system was in the same state and no reaction occurred. This 'probability balance equation' can be re-written in differential form as the chemical master equation (CME). The relationship between the continuous deterministic form and the CME has been reviewed earlier[70–73]. The CME describing the change in the probability $P(n;t)$ that the system is in a state n at time t is given by

$$\frac{\mathrm{d}P(n;t)}{\mathrm{d}t} = \sum_{m_1 \in S(n)} \Re(m_1 \rightarrow n) P(m_1;t) - \sum_{m_2 \in \Omega(n)} \Re(n \rightarrow m_2) P(n;t)$$

where $S(n)$ and $\Omega(n)$ are the sets of progenitor and successor states of the state n, i.e., their members are the states that are one reaction away from the state characterized by $n \equiv (n_1, n_2, \ldots n_{N_S})$ molecules of species $M_1, M_2, \ldots, M_{N_S}$ respectively. The first term represents the probability of situation (i), i.e. system being in a precursor state and one reaction occurring while the second term represents the probability of situation (ii), i.e. no reaction. This CME completely defines the distribution of spatially homogeneous systems. For spatially distributed systems, the CME can be extended to include changes in state due to physical transitions or transport processes[73].

Solving for the infinite number of possible states for 'open' systems with species introduced into the system, or species that are 'born', or the large but finite number of states for 'closed' systems where the total mass of the system does not change, leads to the detailed probability distributions for all species at all times. However, solving the CME is not possible even for relatively small systems as the number of possible states, and hence the number of coupled differential equations, is very large. For example, a reaction system of 3 reactants each having 0–9 molecules will result in a system of 1000 coupled differential equations. As such, for most systems being studied, it is impractical to evaluate the probability distribution through a direct integration of the CME. Hence analytical and numerical or computational techniques are used to estimate the distribution of all the chemical species as a function of time.

Analytical methods for calculating the probability distribution

There is a long history of theoretical approaches for analyzing the CME by calculating the dynamics of the moments of the distribution of each species in the chemical reaction reaction network. In 1940, Delbruck[74] studied a one-component autocatalytic system and derived an expression for the distribution of the number of molecules for the single species. In 1948, Kendall[75] formulated a CME for one species undergoing birth (autocatalytic production) and death (first-order degradation). In 1949, Siegert[76] derived a probability distribution for the momentum of a gas as a function of time, and derived a general model which can be used to represent a system of first-order conversion reactions without formation or annihilation, i.e. where the total mass of the system is conserved. This result was re-derived in the context of a chemical reaction network more than a decade later by Krieger and Gans[77]. This was the start of a prolific decade of work on analytical studies of the CME for specific reaction systems. Bartholomay[78,79] showed how to relate transition rate constants to observed first-order rate constants. It was shown that a multinomial distribution characterizes the steady-state[80] as well as the dynamics[77] of a first-order closed system undergoing a transition from one equilibrium situation to another. Other work on specific chemical reaction systems including for instance those with reversible kinetics[81,82], bacteriophage kinetics[83], cyclic ternary conversions[84] analyzed the moments of the distribution of the reacting species using the generating-function approach for analyzing the CME.

In the generating-function approach[85,86], through an algebraic manipulation, the CME is recast as a system of equations for the moments (or mean, variance, kurtosis, skew, etc) of the distribution. This has been a very profitable line of analysis and is still used for investigating the time-dependent behavior of the moments of the distribution. For systems where the reactions or transitions are first-order, the resulting set of equations is tractable in the sense that the equation for each moment only includes terms for lower moments, and so the equations can be sequentially solved. Analytical expressions for the dynamics of the first two moments have been derived for a closed system of conversion reactions[77] and specific systems at steady state[87] as well as general[72] systems of transient first-order reactions that may be a combination of conversion and catalytic reactions that include production and degradation processes. Recently expressions for the complete probability distribution for first-order conversion reactions have been derived[88].

Analysis of the dynamics of the probability distribution of species undergoing second-order reactions is not as tractable. Even for simple second-order reactions, the equations for the moments are coupled, so that for instance the equation for the mean includes terms for the variance, the equation for the variance includes terms for the third moment, and so on. Thus an approximation has to be adopted in order to avoid a situation requiring the simultaneous evaluation of an infinite set of equations. This approximation is called the "moment closure" approximation, and is used for analyzing the stochastic behavior of systems undergoing higher-order reactions. For instance, Kepler and Elston[89] use an approximation for the third moment to calculate the mean and variance for a bimolecular reaction.

For general second order systems, Michaelis-Menten kinetics has been used as a 'model system' for analyzing the stochastic dynamics. Because of the moment closure problem discussed earlier, exact solutions without any assumptions are available only for systems that can be reduced to one variable, or in general closed systems with a small number of reactions and species. Several such solutions are available, from initial work considering one substrate or enzyme molecule[90], or the initial reaction rate where product formation is not considered[91], or restricting the analysis to steady state[92]; to more recent results on generic reversible second order[93] and catalytic[94] reactions. However, analytical solutions for systems of second order reactions, or second-order reactions in open systems (with production and degradation of one or more species) are yet not available.

Computational estimation of the probability distribution

It is clear that there are significant constraints in obtaining analytical expressions for the probability distributions of species in a chemical reaction network for most systems with second-order or more complex reactions. Several methods have been devised for using the Master Equation to numerically estimate the evolution of the distributions of the number of molecules of each species. Most methods assume that the initial state of the system is completely defined, and estimate the distribution during evolution of the system to a steady state. These methods may be classified into two groups: those that are exact representations of the CME, i.e. individual trajectories calculated using these methods are consistent with the predicted behavior based on the CME; and approximate

methods that sacrifice this individual trajectory accuracy for achieving reasonable predictions about the moments at a lower computational cost.

The first exact simulation method was proposed by Dan Gillespie[95] in 1977—long before the computational power to carry out simulations for systems of modest size was available. In his 'direct reaction' method, two random numbers are used to pick the time of the next reaction and the identity of that reaction respectively. These values are chosen from distributions of times and reaction identities that are functions of the reaction rate constants, current number of molecules of each species, and the system volume. A comprehensive physical justification of the assumptions involved regarding the nature of the distributions has also been presented[96]. Most current reports on the numerical evaluation of the probability distribution use this method (this paper has been cited over 950 times since 1996) for calculating sample trajectories and hence the moments. Another method called the first-reaction method[97] is also an exact simulation method, but it involves drawing as many random numbers at each time as the number of reactions, and so is computationally inferior to the direct method when the number of reactions is large.

The main disadvantage of the method is that time is not discretized, and hence there is no a priori bound on the number of steps taken by a particular realization. In particular, in reaction systems where there are fast reactions possibly at pseudo-equilibrium, and slower reactions that influence system response at larger time scales, the bulk of the computing time is spent in calculating the fast transitions, leading to the use of very small time increments. As such, computing the10,000 or more realizations that are needed to calculate accurate moments is not feasible with computational resources that are typically available. Therefore several algorithms have been developed to reduce the computing time. These methods are typically more complicated to implement, but lead to significant decreases in the computing time requirement, especially for large systems or stiff systems. Among such exact methods are the next reaction method by Gibson and Bruck[98], the optimized direct method[99], the logarithmic direct method[100], sorting direct method[101], and the fast kinetic Monte Carlo method[102]. Of these, the next reaction method seems to be the most popular. A comparison of the various methods has been given in the recent review by Gillespie[70]. It should be noted that all these methods represent improvements in the software or implementation, and not a conceptual advance in the form of a new algorithm.

Irrespective of the speed-up procedure adopted, for the simulation scheme to be exact, it has to account for every reaction event individually. This places a natural bound on the decrease in the computation time that is achievable by exact simulation methods. Approximate simulation methods achieve this decrease through an approximation that results in individual trajectories no longer being exactly according to the CME. In the tau-leaping method[103], a finite increment in time (tau) is used. It is assumed that in this time none of the propensity functions changes its value significantly, and yet many reactions occur in this period. Several extensions to this method have been presented that seek to avoid some of the problems associated while implementing this method, including negative species concentrations[104,105]. An analysis of the basic method and its recent improvements has been given by Li *et al* [106].

Several approximate methods[107–110] achieve a decrease in computational time by switching between continuous deterministic calculations and discrete stochastic computations. Such 'hybrid' methods use rules to determine the switching between the two regimes, and have been shown to achieve impressive computational efficiency for specific systems. There are also methods[111] that switch between the tau-leap method and an exact method such as the next reaction method. However it is important to note that these algorithms cannot be guaranteed to provide accurate solutions for a general reaction network, and so should be used with caution and at least some of the results cross-checked using an exact method.

Software implementations of most of these exact and approximate methods are available. Some of the more popular packages that are available include StochKit[106], Kinetikit[110], BioNetS[112], STOCKS[113], and Dizzy[114]. Several other packages are also available, though these five should be adequate for most non-specialized purposes.

Future directions

The convergence of increased computing power and quantitative high-throughput biological assays make this a unique period in terms of feasibility of computational analysis of biological systems. The field of stochastic analysis, despite the rich mathematical heritage, is as yet a young and evolving one in the context of stochastic analysis of biological systems. In this section, some of the areas that are not fully explored are presented as an example of possibly fruitful and relevant lines of research in this exciting field.

Given the many possible frameworks for modeling biological systems (Table 1), it is

important to define the differences in predictions of the different methods for the same system with equivalent parameter values. There has been a great deal of literature on the differences in the predictions of continuous deterministic and discrete stochastic approaches. It is generally expected that the stochastic approach will be consistent in the mean with the deterministic approach, i.e. the mean concentrations predicted by the stochastic model will exactly match the deterministic model predictions. However, it has been theoretically[115–117] and computationally[20,118] shown that this consistency is not observed for certain systems. In fact, there is a whole class of systems (with reaction kinetics such that there is an absorbing or death state) where the predictions of the mean given by the stochastic model can be proved to be different from the prediction of the corresponding deterministic model[119]. For these systems, the only stable steady state for the stochastic model is the absorbing state, which may be inaccessible to the deterministic model due to its unstable nature. The calculation of extinction times as a function of system parameters and initial state conditions is an open problem that will serve to quantify the expected differences in the mean for particular systems.

It is important to include all sources of heterogeneity (fluctuations in environmental conditions, fluctuations due to unequal partitioning, and inherent fluctuations due to stochastic reaction kinetics) while analyzing biological systems. There have been a few efforts at identifying the contribution to heterogeneity due to inherent stochastic kinetics and fluctuations in the environment[120–122]. Population balance modeling[123] is a well-established method that models the cell heterogeneity arising from unequal partitioning of cellular contents during cell division. Recently a framework for incorporating stochastic kinetics in the population balance modeling framework has been developed[124,125], which will account for the contributions of inherent stochastic kinetics and unequal partitioning to the overall heterogeneity. A modeling framework that includes all three sources of heterogeneity has not yet been developed.

Several problems in developmental biology involve patterning resulting from transport of morphogens and their reactions at various locations. This is deterministically modeled using a system of partial differential equations. However, variations in the concentration gradient require the use of a stochastic modeling approach to investigate the effect of noise on the observed pattern. The intuitive approach[126] is to divide the space into compartments and replicate the reactions in each compartment. Transport 'reactions' account for movement of species between compartments, with the transport reaction rate constant being a function of the species diffusivity. This results in coupling of the reactions in individual compartments, and can be analyzed as one large system of stochastic reactions using any of the methods mentioned earlier. This approach has also been implemented in a software package called SmartCell[127]. If all the reactions are first-order, a theoretical analysis is also possible[72]. There have been several approaches that extend the Stundzia/Lumsden algorithm[128] or use Smoluchowski models for reaction and diffusion[129]. However, there are unresolved issues including definition of an appropriate compartment size, and computational cost that will result from the compartment size being defined on the basis of the mean free path of the fastest-diffusing species. Efficient and accurate simulation of stochastic reaction-diffusion processes remains an open problem.

The stochastic behaviors of several specific systems have been analyzed through numerical simulations. However it is seen that a generic understanding of the effect of model components (number of components in a chain, control motifs) on stochasticity is not yet fully understood, though there have been some reports on the positive effects of negative feedback loops[28] and the effects of positive and negative feedback loops[130] on the noisiness of the controlled species. At a simpler level, the sensitivity of simulation results to parameter values is not routinely reported, though there have been some attempts to provide a framework for such an analysis[131]. The problem in using the results of these simulations is that they seem to be very specific to the system that is simulated. One of the more popular systems is the gene transcription control network[58]. There are several models that have been used to understand the contributions of constituent steps to the noisiness of the regulated gene transcript, or protein numbers. The Kierzak model[132] and another from the Collins group[133] conclude that the transcription step, where mRNA is formed from the DNA template, is the source of most of the noise observed in protein numbers. This conclusion is different from that arising from studies conducted by the van Oudenaarden group[87,134], which states that the translation step, where mRNA is converted to protein, is the dominant source of the noise, with only a weak positive correlation between the transcription efficiency and the noise. Both sets of studies are supported by experimental evidence supporting the conclusions. It should be noted that different measures of the noisiness

(ratio of standard deviation to mean, and ratio of variance to mean, respectively) are adopted by the two groups, and this may influence the qualitative nature of the results. The point is that despite the fact that gene transcription is the best-studied system in terms of stochastic dynamics, there is no definitive model, or a comprehensive model that is able to explain the differing results in terms of the model structure, noise measure and the parameter values. A comparative analysis of different modeling approaches is also lacking.

The question of the "right" modeling approach, at least for biological systems, is defined by the advantages of a particular approach in generating additional insights about the system. Ultimately, as has been recognized since the Gillespie method was developed, the choice of the method is governed by the following question[73]: "Given a particular system of chemical reactions, what level of microscopic detail is actually required to explain the macroscopic properties of interest, and how may the desired information be obtained from a theoretical formulation of the chemical kinetics at the required level?"

There is a general consensus in several biology subareas, e.g. developmental biology[1] and cell biology[49], that quantitative models coupled with experiments are essential for explaining system behavior. The focus so far has been on deterministic models. As experiments get more precise, there will be a need for models that can describe the variation and not just the average behavior. Besides, in several systems, stochasticity will also affect the mean behavior. Therefore it is necessary for experimentalists to be familiar with stochastic modeling techniques, and for modelers to adopt these tools routinely in order to simulate biological systems. Both these goals are challenging, but essential for a better understanding of complex biological systems.

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