Determining the Limitations and Benefits of Noise in Gene Regulation and Signal Transduction through Single Cell, Microscopy-Based Analysis

Marie D. Harton and Eric Batchelor

Systems Biology Unit, *Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA*

Correspondence to Eric Batchelor: *Laboratory of Pathology, CCR, NCI, NIH, 10 Center Drive, MSC 1500, Bethesda, MD 20892, USA. batchelore@mail.nih.gov.*
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Abstract

Stochastic fluctuations, termed "noise," in the level of biological molecules can greatly impact cellular functions. While biological noise can sometimes be detrimental, recent studies have provided an increasing number of examples in which biological noise can be functionally beneficial. Rather than provide an exhaustive review of the growing literature in this field, in this review, we focus on single-cell studies based on quantitative microscopy that have generated a deeper understanding of the sources, characteristics, limitations, and benefits of biological noise. Specifically, we highlight studies showing how noise can help coordinate the expression of multiple downstream target genes, impact the channel capacity of signaling networks, and interact synergistically with oscillatory dynamics to enhance the sensitivity of signal processing. We conclude with a discussion of current challenges and future opportunities.

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Introduction

At any given moment, cells perform a multitude of complex functions based on diverse biochemical reactions that can be highly susceptible to stochastic fluctuations in molecular reactant levels [1–3]. Such fluctuations in either a single or many molecular reactants across multiple reactions are referred to as molecular noise [4]. Biological noise can arise from multiple sources, including molecular reactants being present in small quantities [1,2], variability in internal states between cells due to differences in cell cycle progression [5], differences in levels of cellular components due to uneven segregation during cell division [6], stochasticity in gene expression [7,8], or differences in local microenvironment [4]. In some cases, variations from many sources of noise accumulate and produce cell-to-cell variability in observable phenotypes, while in other cases, even a single varying source can produce distinct cellular outcomes for populations that are genetically identical [7,9,10].

In many fields, such as electrical engineering, noise is often viewed as a detriment, causing a reduction in the transmission of information. However, from even the early days of molecular biology, biological noise was observed to confer benefits for some biological systems. For example, stochasticity has long been appreciated as serving a key role in regulating the lysis/lysogeny state switch of bacteriophage lambda in which the decision between infection and dormancy states is determined by biochemical fluctuations [11–14]; however, even in this relatively well-characterized system, the exact mechanisms and extent to which stochastic biochemical reactions impact the state switch are still being investigated (for a discussion, see Ref. [15]). As another classic example, early studies of microbial communities showed that cell-to-cell variability caused by biological noise can result in cellular bet-hedging, a process where a fraction of cells randomly enter a state of reduced fitness to increase the likelihood of a subpopulation surviving environmental stresses. Bet-hedging in microbial populations was first observed during studies in the 1940s, in which populations of genetically identical bacterial cells were not completely killed when treated with antibiotics [16]. A subpopulation of cells was able to survive, or "persist,"

in the presence of antibiotic; however, the persistence phenotype was nonheritable. Bacterial cultures derived from the persistent cells spontaneously reverted to the normal, non-persistent state, regaining sensitivity to antibiotics [17]. Variations in genetically identical cell populations were also observed in bacterial competence studies, in which only 10–20% of cells entered into a specialized competent state at the expense of cell growth to uptake and incorporate environmental DNA into their chromosome [18,19]. Although these population-level studies demonstrated the favorable role of noise in cellular bet-hedging strategies, the advent of technologies to study individual cells have illuminated the complex, and often conflicting role of noise in multiple regulatory networks, particularly those involved in signal transduction.

In recent years, the investigation of non-genetic cell-to-cell variability has been propelled by the development of sophisticated tools to analyze individual living cells [20,21]. Single cell methods deliver invaluable insights into the mechanisms of regulation and function that are concealed in bulk, population-level studies measuring ensemble-averaged cellular responses [22–25]. Single cell studies have not only furthered our understanding of the process of bet-hedging in single-celled organisms [26] but also revealed new mechanisms in which signaling networks have evolved to either circumvent or exploit noise to process inputs. Technologies based on fluorescent protein reporters have been particularly useful in challenging the notion that noisy expression is detrimental to signal processing.

The number of groundbreaking studies over the recent years in the area of biological noise precludes an exhaustive analysis of this important topic. Therefore, in this review, we choose to focus on recent work in which microscopy-based single-cell approaches have generated new insights into the regulation and function of biological noise in well-studied regulatory networks. We first review select studies that enabled better characterization of general features of biological noise, focusing on both live-cell fluorescence microscopy and fixed cell single molecule RNA fluorescence in situ hybridization (smRNA FISH) studies. We then describe select experimental and computational studies showing how variability in transcription factor dynamics may provide benefits of coordination in target gene expression. We next describe recent work analyzing the effects of noise in biological signal transduction systems, revealing how noise generates specific limitations for the transmission of information and how some cells circumvent such limitations. In addition, we discuss specific examples of how cells use biological noise to generate functional benefits for information processing tasks in well-studied signaling networks. Finally, we conclude with a discussion of future research challenges and opportunities.

Characterization of Noise in Gene Expression

In a broad sense, biological noise can be categorized as being composed of intrinsic and extrinsic components. Intrinsic noise refers to fluctuations caused by stochastic events in biochemical reactions within any given cell, for example, the random binding of a transcription factor to a promoter or the number of transcripts produced in a given unit of time. Extrinsic noise refers to variations among different cells in a shared environment, for example, variation due to cells having different numbers of ribosomes. Extrinsic noise can be further specified as either global extrinsic noise (i.e., fluctuations in molecular components that are the basis for essential biochemical reactions that widely affect gene expression) or pathway-specific extrinsic noise (i.e., fluctuations in the level of a key transcription factor involved in a specific signal transduction pathway) [8,27].

Fluorescence microscopy-based analysis of single cells provided a method to determine the extent to which varied gene expression in a cell population is generated by extrinsic or intrinsic noise sources. Elowitz and colleagues developed a method that employs two distinguishable fluorescent reporter genes from the same origin, that is, the cyan and yellow fluorescent protein variants derived from the green fluorescent protein (GFP) [9]. To differentiate between intrinsic and extrinsic noise contributions, the reporter genes are expressed from identical promoters but at distinct integration sites in the genome, such as distinct loci in the *Escherichia coli* chromosome, as in the work by Elowitz and colleagues [9], or two different alleles of the same gene for a diploid organism, as applied in follow-up studies [8,28]. Intrinsic noise sources produce differential expression between the reporter genes in a single cell. Extrinsic noise leads to equivalent reporter gene expression within a single cell, but not between two cells (Fig. 1). The dual reporter method enabled the determination of the contributions of intrinsic and extrinsic noise sources to non-genetic variation in cell populations and identified general mechanisms by which the sources of noise can influence global gene expression and regulation [8,9,28,29].

To identify more specific molecular mechanisms contributing to extrinsic and intrinsic noise sources in individual cells, several groups have made use of a variety of single-cell microscopy-based approaches. While technically challenging due to the often low copy numbers of biomolecular species being analyzed, such studies have enabled the precise quantification of mRNA and proteins with single molecule sensitivity. One particularly noteworthy phenomenon identified and characterized through such approaches is transcriptional bursting, a source of noise in gene expression for a variety of organisms [7,30,31].