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Identifying noise sources governing cell-to-cell variability

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Identifying noise sources governing cell-to-cell variability

Simon Mitchell and Alexander Hoffmann

Abstract

Phenotypic differences often occur even in clonal cell populations. Many potential sources of such variation have been identified, from biophysical rate variance intrinsic to all chemical processes to asymmetric division of molecular components extrinsic to any particular signaling pathway. Identifying the sources of phenotypic variation and quantifying their contributions to cell fate variation is not possible without accurate single cell data. By combining such data with mathematical models of potential noise sources it is possible to characterize the impact of varying levels of each noise source and identify which sources of variation best explain the experimental observations. The mathematical framework of information theory provides metrics of the impact of noise on the reliability of a cell to sense its environment. While the presence of noise in a single cellular system reduces the reliability of signal transduction its impact on a population of varied single cells remains unclear.

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Single-cell, Noise, Variability, Computational modeling, Signal transduction.

Introduction

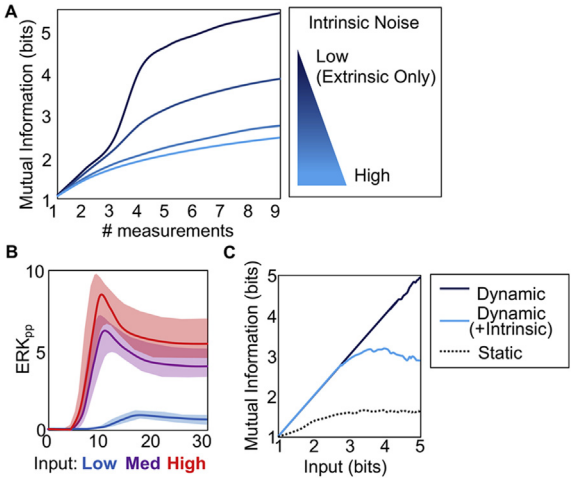
Cells must reliably sense their environment in order to behave and respond appropriately. Sources of information in the environment are highly varied and can include nutrient availability, cytokine and chemokine levels, pathogens and combinations thereof. Sensing the cellular environment typically involves receptor activation and transduction of receptor state information through a signaling network resulting in an appropriate response. Such responses can take the form of gene expression [1], or cell fate decisions such as differentiation [2], division and death [3•]. Mechanistic computational models of signaling networks are valuable tools to explain how a cell can respond to environmental cues with stimulus specific gene expression [4], differentiation [5,6], division [7] and death [3,8].

However, single cell studies have revealed a high degree of variability in the responses of genetically identical cells grown and treated in identical conditions. Numerous theoretical and experimental studies have addressed the sources and physiological consequence of this variability [9–11]. A useful distinction is whether the source of the variability is intrinsic or extrinsic to the regulatory system and timescale being considered. That distinction has indeed important implications to both the biology and our study of it. As intrinsic molecular variability reflects thermodynamic noise of molecular interactions within the regulatory network, modeling, it requires stochastic mathematical representation (e.g. Gillespie formalisms), and may limit the predictability of biological phenotypes, or reliability of signal transduction (Figure 1A). In contrast, extrinsic noise reflects distinct starting conditions (initial concentrations and/or kinetic rate constants) of the molecular networks of individual cells within the population, or distinct time-dependent inputs (changes in the environment) to the system [12,13]. In principle, biological outcomes that are only subject to extrinsic noise can be predicted and modeled with deterministic mathematical formalisms (e.g. ordinary differential equations) so long as the starting conditions and inputs to the system are known. Further, information loss through extrinsic noise can be mitigated through a Dynamical Signaling Code [14,15]. By leveraging information contained in time trajectories, the reliability of signaling is not diminished by extrinsic noise (Figure 1B and C) [16,17].

In a seminal study, an engineered bacterial system was developed to quantify the contribution of extrinsic and intrinsic noise sources to the expression of identical duplicate reporter genes [18]. However, such elegant engineering of signaling systems is not possible in many biological contexts such as the immune system [19,20]. For natural biological systems computational models can provide a tool to elucidate how different sources of variability can result in distinct phenotypes.

Here we will describe how recent advances in single-cell imaging, combined with computational models enable

Figure 1



Dynamic information can increase channel capacity and overcome extrinsic variability [16]. A) Mutual information (the information carried by a channel) calculated as a function of the number of input measurements integrated and the level of intrinsic noise. By integrating multiple measurements from a dynamic input mutual information can be increased to overcome pre-existing cell-to-cell variability. Increasing intrinsic noise reduces the mutual information and cannot be overcome by integrating more measurements of the same biochemical species. B) Trajectories from a computational model of ERK activity show highly variable responses [17]. C) Line graph of mutual information showing that a static single-timepoint measurement is incapable of encoding high mutual information. Dynamic information encoded by integrating measurements from multiple time points can overcome pre-existing variability to achieve high mutual information, this is limited by intrinsic noise.

disentangling the sources and impact of variability on a cell responding to its environment.

Non-genetic origins of cell-to-cell variability in TRAIL-induced apoptosis

Cell death decision pathways are well-suited for investigations into the molecular basis of cell fate heterogeneity due to the distinct and unambiguous phenotype. TNF-related apoptosis-inducing ligand (TRAIL) induces apoptosis through the TRAIL-receptor which activates initiator caspases (caspase-8 and caspase-10) followed by activation of effector caspase (caspase-3) either directly or via mitochondrial outer membrane permeabilization (MOMP) [8]. In response to a fixed dose of TRAIL, a wide distribution of death times is observed within clonal cell populations. By recording and tracking division events that occurred prior to administration of TRAIL a strong correlation between the death times of recently divided sibling cells was found. This indicated the predominance of extrinsic variability present prior to the treatment as substantial intrinsic noise would manifest as a poor correlation between siblings (Figure 2A). Further, by measuring the decay of concordance of death times among siblings as time after division increased, the investigators could quantify the effect of intrinsic noise (Figure 2B). The prevalence of pre-existing extrinsic variability over intrinsic noise motivated the search for determinants of death time in individual cells.

A computational model trained on live-cell imaging and flow cytometry data could recapitulate observed cell-death responses to TRAIL [21]. By incorporating experimentally determined distributed abundance of molecular regulators into the computational model, SL Spencer et al. [8] were able to show that cell-to-cell variable steady-state pre-stimulus protein concentrations were sufficient to explain the variable death times. Through the use of fluorescent reporters the investigators found that variability in the signaling network upstream of MOMP accounts for the distribution of death times. While such results show that a cell's pre-stimulus steady state determines its death time in response to TRAIL, no single protein abundance had predictive power over death time in either the model or through fluorescent reporter assays.

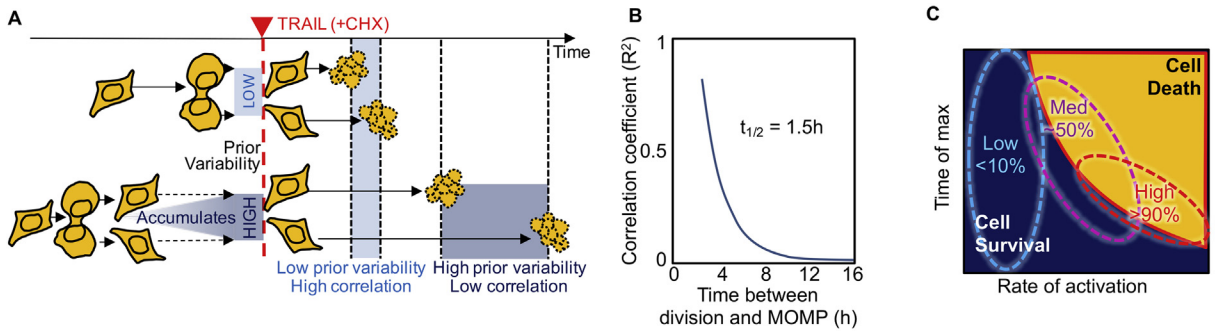
A combination of mechanistic modeling and single-cell Fluorescence Resonance Energy Transfer (FRET) by Roux et al. [22••] revealed initiator caspase trajectory variation within a population controls cell death fates. Insight from a simplified computational model of the apoptosis signaling pathway was used to reduce the complex variety of caspase 8 trajectories to a two variable space represented by the initial rate of caspase 8 activation and time of maximal caspase 8 activity. By mapping populations of single cells to this space a boundary could be found that separates cells within a population that survive from those that die. At different doses of TRAIL the fraction of cells on each side of this boundary explain the fraction of cells dying. An important consequence of the predominance of existing cell-to-cell variability within a population is the sensitivity of fractional killing (the proportion of cells undergoing cell death) to ligand dose (Figure 2C). Cells that survive the fractional killing were also found to maintain a pro-survival phenotype to subsequent stimuli [23].

The potential role of pre-existing cell-to-cell heterogeneity in controlling fractional phenotypes within a population is an enticing one. While it is tempting to extrapolate this finding to other biological scenarios it may not be true in other contexts. For example, stochastic decisions-making in lymphocyte proliferation has been long regarded as the process by which distinct fates are achieved.

Single cell lineage tracking enables quantification of intrinsic and extrinsic noise sources in affecting lymphocyte expansion

Snap shot assays at single cell resolution (such as flow-cytometry) have revealed a high degree of cell-to-cell heterogeneity in B lymphocyte proliferation [24].

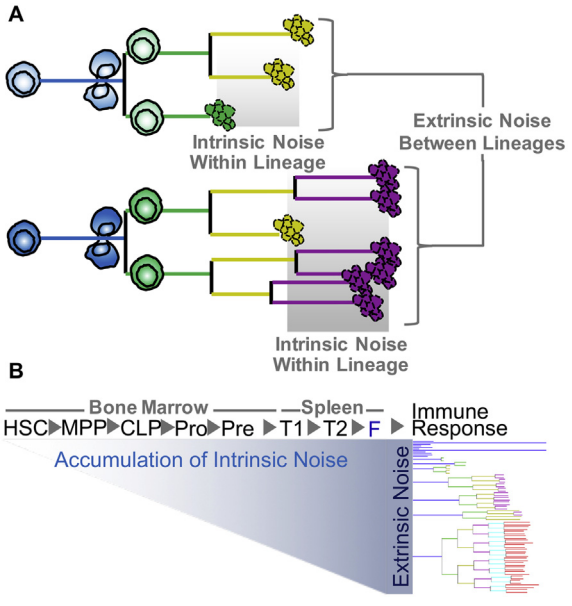
Figure 2



Sibling analysis reveals the predominance of pre-existing variability over intrinsic noise which enables dose-dependent fractional killing [8,22]. A) Schematic of the sibling analysis performed by SL Spencer et al. [8] in which treatment with TRAIL leads to highly variable cell death with no correlation between the time since division and the death time. However sister cell death times were found to be highly correlated indicating the predominance of pre-existing variability over intrinsic noise. B) Representative decay of correlation coefficient (R^2) between sister cells as a function of time since division. The decreasing correlation enables quantification of intrinsic noise. The addition of cycloheximide slows the loss of correlation indicating that intrinsic noise requires protein synthesis. C) Schematic representing the space created by time of each cells maximal FRET activity along with the rate of activation. By plotting each cell within a population within this space the role of pre-existing variability on increasing fractional killing with increasing TRAIL dose can be explained.

While genetic variability in the variable regions of the B-cell receptor (BCR) may contribute to variable proliferative capacity [25], highly varied proliferative fates are also observed in clonal cell populations, or in response to mitogenic stimuli that do not target the BCR. This has led to the hypothesis that B cell fate decision-making is highly stochastic and dominated by intrinsic noise. Probabilistic models based on characterizing variability of cell fate decisions timings may recapitulate population dynamics with great accuracy [24,26–29].

Figure 3



Lineage analysis reveals intrinsic and pre-existing variability that is difficult to distinguish at the population scale [3,31]. A) Example lineage trees created by single-lymphocyte tracking through live-cell microscopy. Intrinsic noise is visible as diverse generations are reached within a single lineage while pre-existing variability is displayed as differences in terminal generation between founder-cell lineages. B) Schematic of lymphocyte development indicating that while intrinsic noise is minimal there are many generations among which it can accumulate during hematopoiesis. Accumulation of intrinsic noise is a potential generator of the pre-existing variability seen in a population during an immune response.

However, initial short-term microscopy tracking of live cells indicated concordance of the fate decisions of siblings [30]. As an alternative, a mechanistic modeling approach treated the division and death decision as a predictable process determined by underlying regulatory networks, which are inherited throughout a lineage, with cell-to-cell variability introduced as pre-existing differences in the kinetic rates between founder cells [3]. Both probabilistic and mechanistic modeling approaches can provide accurate fits to the population scale experimental data [31•]. However, these two models predict very different single-cell lineages. The assumption that intrinsic noise is the main source of cell-to-cell variability results in highly irregular lineage trees with varied terminal generations within a single lineage (Figure 3A upper). A mechanistic modeling approach in which extrinsic, pre-existing variability is the main source of variability results in highly regular lineage trees in which the terminal generation within each lineage is constrained by the founder cell (Figure 3A lower). The lack of long-term single cell tracking prohibits distinction between these two hypotheses. A recently developed experimental and image analysis workflow reveals complete, long-term lineage information which is consistent with a predominance of extrinsic noise. By fitting the width of the distribution from which kinetic rates of founder cells are sampled an estimate of pre-existing extrinsic noise can be

generating using the mechanistic model. Similarly, by fitting the width of the distribution from which resampling occurs during proliferation an estimate for intrinsic noise can be obtained. Comparing the two a 3-fold higher coefficient of variance can be estimated for pre-existing variability than intrinsic noise [31]. These findings suggests the exciting opportunity to predict and perturb lymphocyte proliferative outcomes with high accuracy given sufficient characterization of the state of the founder cells. Modeling can predict specific signaling molecules that are indicative of proliferative capacity. By these signaling molecules in the naïve cells, it should be possible to predict the cells that will proliferate to late generation, and perturbing these targets experimentally may increase the number of cells proliferating to late generation.

It remains unclear how the preexisting cell—cell variability within a population of naïve B lymphocytes comes about. While only small amounts of intrinsic noise affect cellular growth and division, a cell must undergo many divisions during hematopoietic development from a stem cell to a naïve B cell. Recent studies have combined genetic barcoding and mathematical modeling to quantify hematopoietic developmental rates [32]. Incorporation of intrinsic variability into these models may reveal whether an accumulation of small amounts of intrinsic noise during development is sufficient to generate the pre-existing variability seen in naïve B lymphocytes (Figure 3B).

Both terminal generation and cell death are highly discrete phenotypes that show varied single cell responses to environmental perturbations due to preexisting variability in molecular networks. While transcription factor responses to stimuli are less discrete the predominance of preexisting variability may also explain the highly varied dynamics seen through recent single-cell analyses.

Identifying the sources of cell-to-cell variable NFκB signaling dynamics in response to LPS

The response of immune cells to pathogen-associated molecular patterns (PAMPs) is not only graded compared to the all-or-none response to apoptosis inducing ligands, but it is also dynamic [33,34]. Indeed, signaling dynamics have been hypothesized to constitute a code that carries information about environmental perturbations to coordinate cellular core machineries for ligand-appropriate responses. Macrophages sense PAMPs through a repertoire of toll-like receptors (TLRs) which recognize specific pathogenic stimuli [35]. Just two adaptor proteins are critical: myeloid differentiation marker 88 (MyD88) and TIR domain-containing adaptor protein-inducing interferon-β (TRIF). Whether the two branches have overlapping or distinct functions in LPS-to-NFκB signaling, and their relative roles in producing cell-to-cell variable NFκB dynamics, was the question addressed by Z Cheng et al. [36].

While average cellular dynamics can be observed in population assays [4], characterizing cellular variability requires accurate single-cell assays. Fluorescent reporters and time-lapse imaging enables quantification of the translocation of transcription factor NFκB in response to TLR ligands and intuitive visualization of the variability in response. Z Cheng et al. [36] found that there was little variability in the MyD88 branch, as the pathway showed a high degree of ultrasensitivity (due to the presence of a signalosome) and at typical doses of ligand the intra-cellular signal generated was substantially above the inflection point. At these ligand concentrations it thus showed reliable "digital" activation (Figure 4). In contrast, variability in the TRIF pathway (which showed a more linear dose response) affected the duration of signaling (determined by endosomal maturation time) and thus had a substantial impact on the phenotypic variability in NFκB activity dynamics.

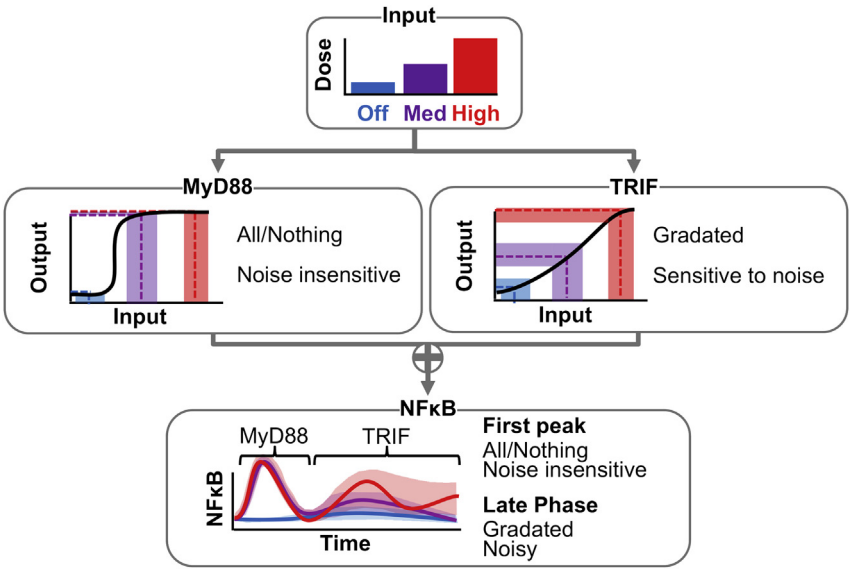
When the variability in a pathway is well characterized, it is possible to quantify the reliability of information transfer possible through that pathway using an information theory formalism [16•,37]. The MyD88 pathway was found to have higher reliability to distinguish between low and medium doses than the TRIF pathway, and therefore higher channel capacity (the maximum possible information that can be transmitted through a pathway). In contrast the TRIF pathways was found to be susceptible to noise but maintained scalable response (increasing response to increasing doses) beyond the saturation point of the MyD88 pathway. NFκB dynamics result from combining the different noise characteristics and temporal control of the MyD88 and TRIF pathways to reliably activate early but retain scalable activity with dose at later time points (Figure 4).

Signaling systems appear to have evolved to exploit extrinsic noise, enabling robust graduated responses or fractional killing. Combining this insight with the information theoretic analyses requires careful consideration of how such theoretic frameworks should be viewed in the context of populations of individual cells.

Intrinsic noise sources limit channel capacity of single-cell signal transduction however the effect on the population remains unclear

Whether it be a cell's imperfect ability to measure the environmental TRAIL concentration or the effectiveness of anti-cancer drugs, the effect of noise is widely regarded as deleterious [38]. This impact of noise is often quantified by the reduced channel capacity in the presence of noise [16,39]. Initial information theoretic studies focused on the ability of extracellular TNF to be

Figure 4



The characteristics of the MyD88 and TRIF pathways combine to ensure the early phase of NFκB activity is noise insensitive while the late phase exploits noise to enable a gradated dose-response [36]. Schematic shows how the switch-like MyD88 pathway shows all-or-nothing response which is highly insensitive to noise. The TRIF pathways is sensitive over a wider-range and thus susceptible to cellular variability. The two pathways combine and impact distinct phases of NFκB activity.

sensed by NFκB transcription factor and identified channel capacities of almost 1 bit, meaning a cell can only distinguish between the presence or absence of TNF but cannot accurately detect dose [39,40]. More recent work discovered that by integrating dynamic information from multiple time points within a response a cell can encode more information about the dose detected. It was discovered that a channel capacity of between 1 and 2 bits could theoretically be achieved by a single cell in by decoding such dynamic information, however a biological mechanism by which a cell could integrate such information and the effects of noise on this process remains poorly understood [16].

While much of the literature has focused on the deleterious effect of noise and how biological systems may mitigate noise, the ubiquitous nature of noise in biological systems along with the observation of a link between cell-to-cell variability and fractions of cell populations displaying a phenotype hints at an advantageous role for biological noise [20]. Single-cell analysis of TRAIL-mediated apoptosis, a process with established susceptibility to noise, by Suderman et al. [41••] results in limited channel capacity of ~0.56 bits. However, by analyzing channel capacity on a population scale, with ligand dose encoding the fraction of cells undergoing apoptosis rather than a single cell's ability to determine TRAIL dose, a much larger channel capacity is obtained (3.4–4 bits). Through analysis of the computational model described in the first section of this review, a fundamental tradeoff was identified: increasing noise on the single cell level decreases the channel capacity of the single cell while increasing the channel capacity of the population as a whole. The presence of existing cell-to-cell variability allows a population of cells, which individually have highly switch-like responses that are susceptible to noise and limit channel capacity, to demonstrate a more graded fractional killing dose-response on the population scale that is robust to noise. In the biological context where input signals such as TRAIL are cytokines expressed by other cells and therefore expressed with some variability it would be advantageous for a population of cells to respond over a wider range of cytokine concentrations to mitigate noise while reliably responding to a changing environment.

Conclusions

Recent advances in single cell imagine have revealed different phenotypic effects from intrinsic and extrinsic noise sources. This enables existing and new biochemical models to be fit with accurate levels of pre-existing variability and intrinsic noise incorporated into the correct processes. The resulting models enable the characterization of the effect of different noise sources along with quantification of the impact on the ability of a cell to detect accurately its environment.

While the approach of noise quantification using biochemical models is widely applicable, care should be taken when carrying quantitative results across

biological systems, as the regulation of noise may differ widely between biological contexts.

In many biological systems, the link between cell-to-cell variability and functional outcome may be amplified, such as individual lymphocytes undergoing clonal expansion after affinity selection, and in such scenarios the effect of pre-existing variability may be vastly different to contexts in which the phenotype results from an average of the entire population.

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