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Infection by bacteriophage lambda: an evolving paradigm for cellular individuality

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Since the earliest days of molecular biology, bacteriophage lambda has served to illuminate cellular function. Among its many roles, lambda infection is a paradigm for phenotypic heterogeneity among genetically identical cells. Early studies attributed this cellular individuality to random biochemical fluctuations, or 'noise'. More recently, however, attention has turned to the role played by deterministic hidden variables in driving single-cell behavior. Here, I briefly describe how studies in lambda are driving the shift in our understanding of cellular heterogeneity, allowing us to better appreciate the precision at which cells function.

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> '*I am no friend of the probability theory; I have hated it from the first moment [. . .]. For it could be seen how easy and simple it made everything, in principle — everything ironed out and the true problems concealed*' (Erwin Schrödinger, writing to Albert Einstein in 1946; quoted in [1]).

Introduction

Among the many contributions of bacteriophage lambda to molecular biology [2•], not least has been to serve as a paradigm for phenotypic cellular heterogeneity within a clonal population [3•,4•]. Dating back to Ellis and Delbrück's finding, that the number of viruses released following cell lysis varied dramatically between individually infected *Escherichia coli* cells [5], the life cycle of bacteriophage, and especially lambda, has been at the forefront of elucidating cellular individuality. Here, I will briefly describe how lambda is leading the shift from a view in which single-cell behavior is considered random and indeterminate, to one characterized more by precision and predictability.

Single-cell biology: between 'noise' and determinism

Twenty years ago, the field of cellular individuality was revitalized, again owing to phage. Arkin *et al.* [6] used numerical simulations to demonstrate that stochastic (random) fluctuations in biochemical processes can lead to diverging cell fates — lysis or lysogeny — among individual *E. coli* cells infected by lambda phage. Motivated by Arkin's work, and empowered by the new ability to measure the amount of a fluorescent protein in a single cell under the microscope, researchers proceeded to use promoter fusions to examine cell-to-cell variability in gene expression across different biological systems (recently reviewed in [4•]).

These experimental endeavors were paralleled by the development of a theoretical framework for interpreting the single-cell data. The theory centered on the engineering-inspired idea of 'noise', referring to the unavoidable random fluctuations that cause any physical system to deviate from its average behavior. The view put forward was that all cellular processes, and consequently cell behavior, are mostly random. Accordingly, the phenotype of a single cell can only be described in probabilistic, rather than deterministic, terms. From phage and bacteria, the idea of 'noisy cells' soon propagated to the other end of organismal complexity, and was applied to embryonic development and human virus infection [4•].

However, as foretold by Schrödinger above, the success of the 'noise' concept in describing cellular heterogeneity also points to its weakness: It is all too easy to describe single-cell properties as 'stochastic', and map them into statistical distributions. But invoking stochasticity does not imply that we understand the underlying cellular process. On the contrary, by creating a façade of understanding, a stochastic description may impede our effort to uncover the *deterministic* factors (so-called 'hidden variables' [7]) that drive single-cell behavior. In other words, the notion of 'noisy', indeterminate cellular function is circular, and leaves the alternative hypothesis, that cells can function precisely and predictably, unexplored.

Recent years have seen a growing awareness of this caveat, and, accordingly, an increase in efforts to identify

the deterministic drivers of cellular individuality [4•,8,9]. This shift in focus was enabled by further improvements in single-cell methods, in particular, the ability to detect and count individual molecules within the cell [4•]. Most notably, single-molecule fluorescence *in situ* hybridization (smFISH) is now routinely used for measuring RNA numbers in individual cells from bacteria [10•] to mammals [11], and can be performed at high throughput [12] or spectrally multiplexed [13]. New theoretical approaches were applied to interpret the data using deterministic, rather than probabilistic, ansatzes [12,14,15]. The result of these efforts has been the continuous uncovering of hidden variables that drive cellular individuality across the spectrum of organism complexity [4•], with lambda again at the forefront [3•].

In a true reflection of the field of single-cell biology as a whole, lambda may now be considered an 'ambiguous paradigm', with different elements of its infection cycle providing seemingly conflicting evidence regarding the degree of predictability (conversely, randomness) of cellular processes. Below I highlight a few examples. Additional ones are surveyed in Figure 1.

Elements of the lambda life cycle

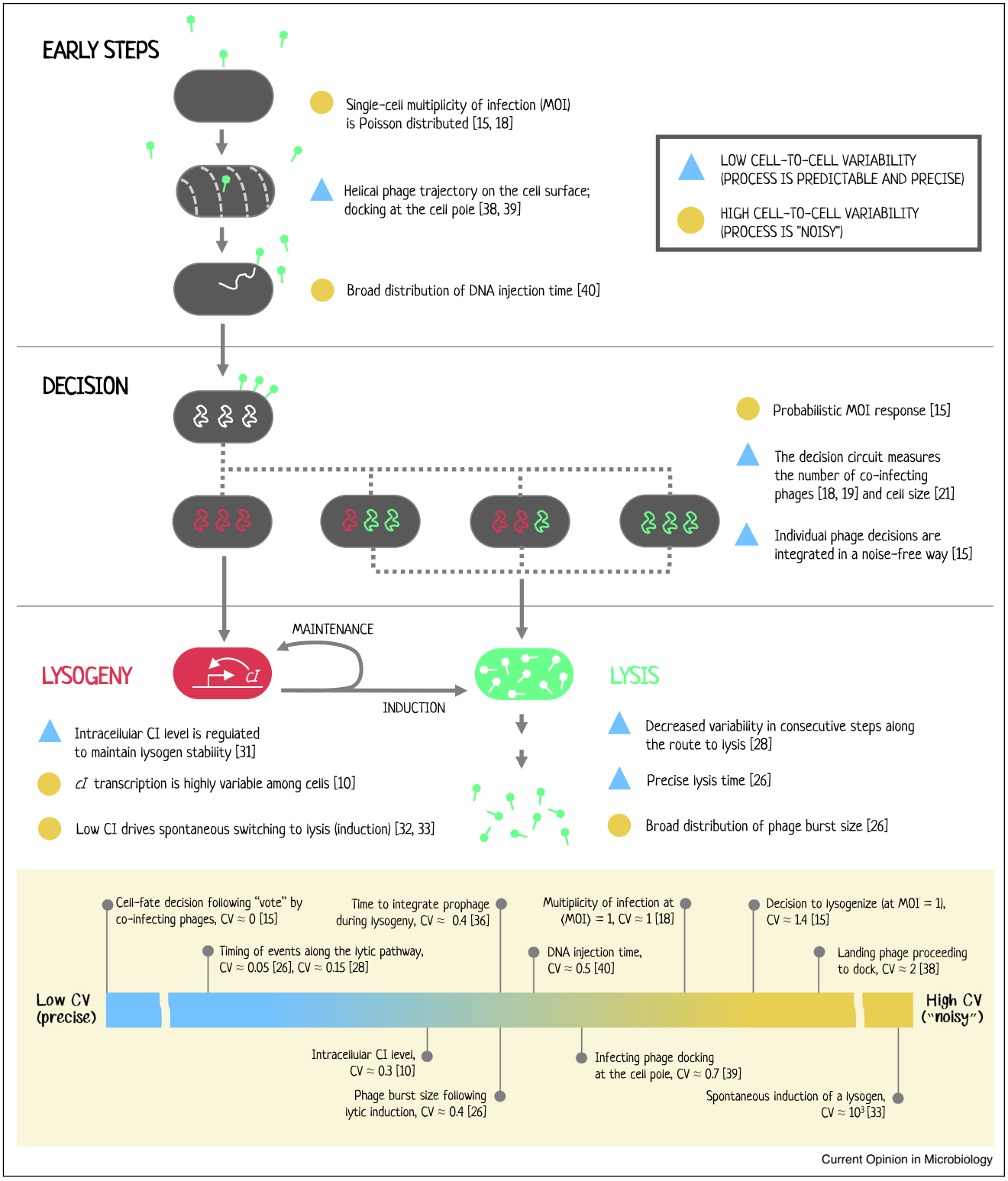
The postinfection decision. Perhaps the most famous aspect of lambda is the postinfection decision, whereby a phage-encoded genetic circuit, receiving inputs from the *E. coli* host, chooses between two possible outcomes: rapid viral reproduction resulting in cell death (lysis), or viral integration into the bacterial genome and long-term dormancy (lysogeny) [2•,16]. Following Arkin's assertion, it became widely accepted that stochastic fluctuations render the postinfection decision unpredictable at the single-cell level. However, the claim of indeterminacy was not based on any experimental evidence, but rather on computer simulations of the lambda decision circuit. These simulations used many *ad hoc* assumptions, reflecting our ignorance of the relevant cellular dynamics [17]. And in fact, the data available at the time was equally explainable by a fully deterministic model [18–20]. Thus, that the lambda decision is driven by noise was a hypothesis, not a proven fact.

Not surprisingly, as scientists began to examine the infection process at the single-cell level, a higher (albeit incomplete, for now) level of determinism was revealed, reflecting the effect of previously hidden variables driving the decision outcome [3•,21]. By using phages whose capsid was fluorescently labeled, Zeng *et al.* [15] identified two stages in the postinfection decision. In the first step, each phage chooses between lysis and lysogeny. This single-phage decision depends on the total concentration of viral genomes inside the infected cell, as predicted by theoretical models of the decision circuit [19]. In the second stage, the fate of the infected cell is decided via a 'vote' by all co-infecting phages, such that only if all phages choose lysogeny, that path is pursued; otherwise, lysis occurs (Figure 1). Importantly, while the single-phage decision still contains a probabilistic element, the outcome of the multi-phage vote appears to be deterministic, that is 'noise free'. Later studies using fluorescent indicators for phage capsids, DNA, and gene expression [22•,23], as well as studies applying bulk methods [24], provided further evidence for the interactions between multiple phages present in the same cell. However, we still lack a mechanistic picture for how phage genomes maintain their individual identity within a shared bacterial cytoplasm.

The lytic pathway. As noted above, the dramatic cell-to-cell variability in phage burst size was one of the earliest quantitative demonstrations of cellular individuality [5,25,26] (Figure 1). This observation also motivated early theoretical thinking on viral stochasticity [27]. In contrast, the duration of the lytic cycle, that is the time until cell lysis, was found to be highly uniform among different cells ([26], and see also [25]) (Figure 1). Prior to lysis, the durations of consecutive steps in the lytic pathway (measured using reporters for the relevant phage promoters) display gradually decreasing levels of cell-to-cell variability [28]. Thus, the two key observables of the lytic cycle, namely its duration and viral product, diverge in terms of cell heterogeneity. The mechanistic basis of this disparity, as well as its possible ecological and evolutionary consequences, remain unresolved [25,26,29].

Lysogeny. A similar ambivalence between noise and precision presents itself when one examines the lysogenic state, in which a lambda prophage lies dormant inside the *E. coli* chromosome. Lysogeny has long served as a paradigm for the epigenetic maintenance of cellular state [16,17,30]. In the canonical picture, the cellular concentration of the fate-determining transcription factor, CI, is finely tuned via autoregulation to provide stability against perturbations, while also allowing efficient switching into lysis (induction) in response to the proper environmental signals [17,31]. However, when CI regulation was recently measured at the single-cell level using a combination of immunofluorescence and smFISH [10•], this hypothesized precision was hard to detect: Both *cI* transcription and the resulting protein levels showed large cell-to-cell variation [10•] (Figure 1). While, *prima facie*, the fluctuations in CI expression may point to the dominance of stochasticity, some of their statistical properties suggest that these fluctuations also reflect deterministic cellular variables, yet to be elucidated [8,10•]. Notwithstanding its origins, the heterogeneity in CI levels is likely to be phenotypically relevant, since cells at the low-CI end of the distribution are the ones most likely to switch into lysis [32,33].

Figure 1



A schematic of lambda infection, distinguishing processes exhibiting large cell-to-cell differences ('noise') from those showing high uniformity between individual cells. The panel below depicts the coefficient of variation (CV), defined as the ratio of the standard deviation to the population mean, for different steps during the infection. Figure by Thu Vu Phuc Nguyen.

A continuing search for hidden variables

What the examples above show is that, at our current state of knowledge, some aspects of the lambda life cycle — for example the viral 'vote' during the choice of cell fate, and the regulation of lysis time — display a high level of uniformity among cells, while other elements exhibit significant cell-to-cell variability. In the latter case, cellular heterogeneity is typically assumed to reflect the stochasticity of the underlying cellular process. However, it just as likely signifies that the proper hidden variables have not yet been identified, which would reveal the deterministic underpinnings of the single-cell phenotype.

In seeking those hidden variables, we must look carefully to the state of the infected cell. The host's metabolic state (as indicated, for example, by its growth rate) has long been known to affect the infection outcome [18,21], but this relation has not been examined at the single-cell level. Similarly, multiple studies point to a coupling between the infecting phage and the bacterial cell cycle [34–36], but this issue too remains largely unexplored. Now, with microfluidic devices allowing precise control and long-term tracking of bacterial growth [37], one can begin to quantify precisely how parameters of the infected *E. coli* cell impact the lambda decision.

It is also evident that uncovering the drivers of lambda behavior depends on elucidating the spatial dynamics of infection at sub-cellular resolution. Repeatedly over the last decade, it has been found that these dynamics deviate significantly from the simple picture of homogenous, diffusion-driven events [3•] (Figure 1). By fluorescently labeling the phage capsid and genome, researchers were able to show that the infecting phage, landing on the *E. coli* cell, moves in a helix-like pattern, rather than diffusing uniformly on the bacterial cell surface [38]. The phage motion then leads it to the cell pole, where it injects its DNA [38–40]. Later, during the process of prophage integration, the incoming lambda genome again does not randomly diffuse in the cell in search of the designated integration site in the *E. coli* chromosome. Instead, it remains localized near the site of injection, until the bacterial locus arrives in its vicinity as part of the chromosome replicative cycle, and integration then proceeds [36]. These newly uncovered spatial dynamics have been shown to impact the infection outcome [15,36]. As for the lytic cycle, a recent study using fluorescence microscopy and cryo-electron tomography [41•], as well as earlier work [42], suggests that (non-lambda) phage replication, too, takes place in a defined spatial geometry. However, until our spatial picture of the lambda life history advances beyond its current rudimentary state, we are likely to attribute cell–cell differences to randomness even when we should not [3•].

Conclusion

As is invariably the case in biology, intellectual progress will come about by advancing our experimental capability. Specifically, future studies of lambda must detect and quantify multiple cellular and viral observables — genomes, transcripts, proteins, etc. — at the resolution of individual molecules within the single cell. Fluorescence microscopy, thus far the primary interrogation tool [3•], will have to be applied at 'super resolution' [43] and supplanted by complementary approaches, for example cryo-electron tomography [41•] and sequencing-based methods [44]. The experimental progress must be matched by improved theoretical tools: Mechanistic ('bottom-up') [10•] and phenomenological ('top-down') [15,19] theoretical models, as well as machine-learning methods [45], should be used to analyze and conceptualize the increasingly multi-dimensional data sets. Ultimately, by peeling off successive layers of hidden variables, we should strive to reveal the cellular precision at which lambda operates, leading the way to similar discoveries in higher systems.

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