Decision Making by Temperate Phages

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Glossary

Induction A process by which a temperate phage switches from the lysogenic state to the lytic pathway, typically in response to a perturbation such as damage to the host cell's DNA.
Lysogenic pathway One of the two possible pathways following temperate phage infection, characterized by suppression of viral replication and lytic functions. The bacterial cell carrying the dormant phage (e.g., in the form of a prophage) is termed a lysogen.
Lytic pathway The other possible pathway following temperate phage infection, in which phages replicate rapidly and eventually kill (lyse) the host cell, releasing progeny viruses.
Multiplicity of infection (MOI) The number of viruses co-infecting the same cell.
Prophage A phage in the lysogenic state, which has integrated into the host genome. Replication of the viral DNA occurs passively during replication of the bacterial chromosome.
Temperate phage A bacteriophage that is capable of the dormant (lysogenic) pathway, in addition to the active (lytic) one.

Introduction

The defining lifestyle of most bacteriophages, analogous to the behavior of higher viruses, is one in which infection is followed by rampant viral replication and the release of numerous mature progeny, often accompanied by death of the host cell (lysis). However, a subset of phages, denoted as *temperate*, are capable of an alternative lifestyle called lysogeny, where the violent cataclysm is replaced by viral dormancy: Following infection, the phage genome is maintained inside the bacterial host – either integrated (as a *prophage*) into the bacterial chromosome, or replicating extra-chromosomally – with all virulent functions shut off. The dormant phage, now an integral part of the bacterial cell, is inherited from generation to generation. However, while dormant, the phage typically maintains the potential for lytic induction: a switch back to the virulent pathway in response to specific signals indicating stress to the host cells.

Multiple aspects of the lysogenic lifestyle are the subject of current studies. These include the physiological costs and benefits, to both phage and host, of viral dormancy, as well as the ecological, medical, and evolutionary consequences of lysogeny. Here we focus on a single element: The decision between lysis and lysogeny, made by temperate phages upon infection of the host. In particular, we will devote most of our attention to the decision by bacteriophage lambda, which infects *Escherichia coli* (Fig. 1). Through more than half a century of genetic, biochemical, and biophysical studies, lambda has become arguably the best-characterized biological system, albeit one that still presents many open questions. As we review the lambda lysis/lysogeny decision, we will also provide examples for some of the ways that other temperate phages make this choice. In light of the overwhelming diversity of phage lifestyles, it is certain that many more ways in which phages pursue their lysis/lysogeny decisions remain to be discovered.

Owing to the relative compactness and tractability of bacteriophages, many of their functions have been studied not only for their intrinsic value, but also as simplified models for the behavior of higher biological systems. This is also the case for the lysis/lysogeny decision, which serves as a paradigm for the way genetic circuits, receiving external inputs, make binary cell-fate choices. In that role, for example, the decision by phage lambda has provided insights about the transition in and out of dormancy by HIV, and, beyond viruses, regarding the process of cellular differentiation during metazoan development. At the heart of lambda's ability to inform us on higher organisms are common features of binary decision circuits across biological systems, notably, the utilization of auto-regulating, fate-determining genes to provide high cell-state stability while simultaneously allowing efficient fate switching in response to external signals.

As part of its paradigmatic role, lambda has also served as a testbed for the idea of creating a quantitative description, formulated in mathematical terms, for the function of the genetic circuit, with the goal of predicting the decision outcome for given initial conditions. While efforts in this direction have yielded significant progress, they have also been limited by the phenomenon of cellular individuality, whereby genetically identical cells, in a uniform environment, nevertheless end up pursuing different paths. To explain the indeterminacy of single-cell choice, researchers invoked the effect of stochastic biochemical fluctuations ("noise") on the decision circuit. With this concept, too, lambda has paved the way for the elucidation of cellular stochasticity and its consequences across the fields of microbiology, development, ecology and medicine.

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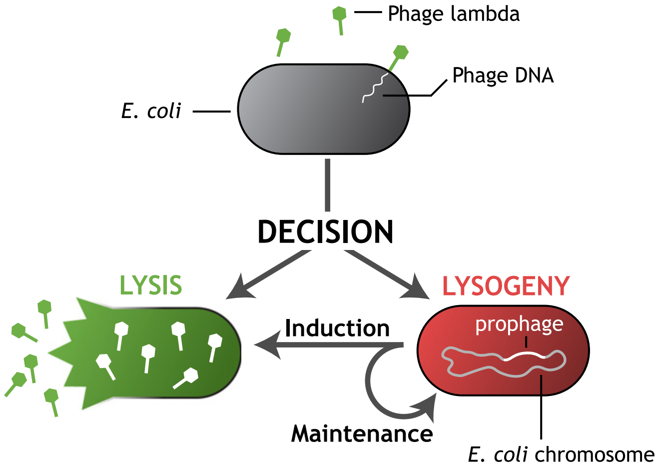

Fig. 1 The post-infection decision of bacteriophage lambda. Following infection of an *E. coli* cell, a binary choice is made between lysis, defined by rampant viral replication and cell death, and lysogeny, in which the viral DNA is integrated into the host's genome to become a dormant prophage. The lysogenic state is stably maintained, but a switch back to lysis can be induced by cellular damage. Adapted with permission from Golding, I., 2016. Single-cell studies of phage λ: Hidden treasures under Occam's rug. Annual Review of Virology 3 (1), 453–472, permission conveyed through Copyright Clearance Center, Inc.

The Post-Infection Decision of Phage Lambda

A decision between lysis and lysogeny first takes place following lambda infection, upon entry of the viral genome into the *E. coli* cell (**Fig. 1**). A genetic circuit encoded by the phage, integrating inputs from the host (and, indirectly, from the extracellular environment), converges on one of the two developmental pathways. If lysis is chosen, the decision is irreversible and, hence, the role of the decision circuit is complete. If, however, the choice is lysogeny, that fate needs to be actively maintained over the long term, using a small subset of the decision gene network. In addition to repressing all virulent functions, this maintenance circuit (known as a genetic "switch") perpetuates a continuous version of the lysis/lysogeny decision: at any given time, whether to maintain stable dormancy or, if cellular conditions change, undergo lytic induction, switching back to the lytic pathway by relieving the repression of virulent functions.

The choice between lysis and lysogeny can be seen as one of timescale, namely, whether to reproduce (and kill the host) immediately, or at a later, yet to be determined, time. An optimal choice, one which maximizes – in the long term – the expected number of progeny, requires knowledge of the state of the infected cell, e.g., how likely it is to support successful viral reproduction, as well as its environment, e.g., what the chances are that newly-released phages will find additional targets to infect. Below we discuss how lambda and a few other phages measure these critical parameters. From a theoretical point of view, the optimization problem that underlies decision making by temperate phages continues to be an area of considerable interest.

The genetic circuit governing the lambda decision is depicted in **Fig. 2**. The decision involves a dense network of regulatory interactions between phage genes, as well as multiple inputs from various host functions. The regulatory interactions involve diverse molecular mechanisms, modulating all stages of gene expression: transcription initiation (through transcription factors, alternative promoters, and interference between neighboring promoters) and elongation (anti-terminators), post-transcription (antisense RNA), translation, and post-translation (protein lifetime). The role of some of these interactions in the decision process has been elucidated through genetics, biochemistry, and mathematical modeling of the circuit. The utility of other interactions, however, is less clear. It is possible that those come into play under infection conditions that are not emulated by standard laboratory conditions, serving to increase the robustness of the decision to environmental and biochemical fluctuations.

To gain insight into the decision process, it is helpful to follow the cascade of transcriptional events taking place on the lambda genome following infection, and how these events diverge en route to each of the two possible outcomes (**Fig. 3**). Upon entry of the viral genome into the *E. coli* cell, transcription begins from the left (P_L) and right (P_R) early promoters. Transcription is initially attenuated at the t_{L1} and t_{R1} terminators, such that only a single protein is expressed from each promoter: N from P_L and Cro from P_R. One of these proteins, N, is an anti-terminator that allows readthrough at t_{L1} and t_{R1}, as well at another terminator, t_{R2}, further downstream of P_R. This leads to the expression of additional lambda genes. These "delayed early" genes include those that allow progression of the lytic pathway: *O* and *P*, required for phage genome replication, and *Q*, which controls the expression of multiple lytic genes. However, *cII* and *cIII*, whose products drive the establishment of lysogeny, are also produced from those same left and right transcripts.

If the Q protein accumulates to a sufficient level, it abrogates termination at the $t_{R'}$ site, to enable expression from the late lytic promoter $P_{R'}$ of genes responsible for the production of new viral particles and lysis of the host cell, and thus completion of the lytic cycle. For the alternative route of lysogeny to be taken, Q production needs to be reduced. This is achieved by CII, which activates the P_{aQ} promoter to produce an antisense transcript to Q, resulting in inhibition of Q expression and thus preventing the

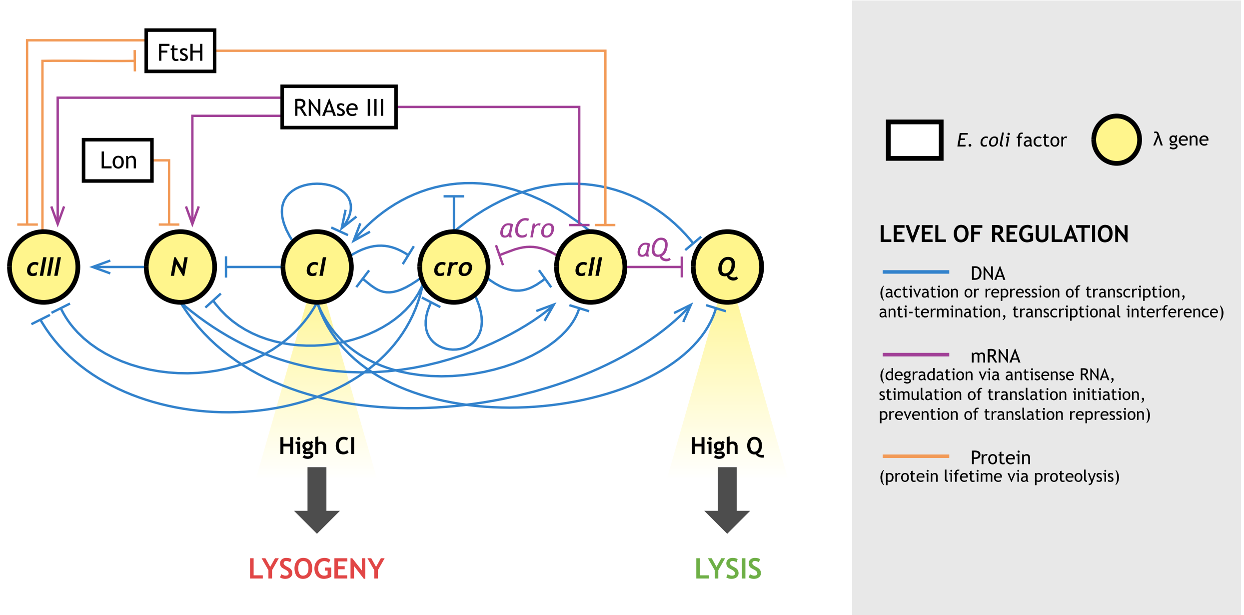

Fig. 2 Key lambda genes and host factors involved in the lysis/lysogeny decision. Regulatory interactions between the various nodes rely on diverse molecular mechanisms, at the level of phage DNA, mRNA and proteins.

expression of the late lytic genes. In addition, CII promotes the lysogenic pathway by activating the P_I promoter, whose product Int catalyzes integration of the phage genome into the bacterial chromosome. Finally, CII activates the repression establishment promoter P_{RE}, to produce CI. CI shuts off transcription from P_L and P_R and regulates its own transcription, now from the repression maintenance promoter, P_{RM}. In the lysogenic state, P_{RM} expression of CI is the only transcriptional activity from the decision network of the dormant prophage.

What makes the post-infection decision elusive, even after decades of meticulous interrogation, is that, qualitatively, the expression patterns of early genes are similar in lytic and lysogenic cells, and therefore appear unpredictive of the eventual outcome. Consider *cII*, the key driver of lysogenic choice. Its expression from P_R takes place in a limited time window of ~15 min, regardless of the eventual choice. Whether this transient expression will result in lysogeny depends on the exact timing and on the maximal CII level obtained. CII kinetics are regulated at multiple levels – at the promoter (P_R) and terminator (t_{R1}), as well as the mRNA and protein lifetimes. This multi-layer regulation provides the means by which the lysis/lysogeny decision is modulated by host and phage parameters, as discussed later.

Whereas the decision by lambda is by far the one best characterized, progress has also been made towards understanding the decision circuits of other phages. In multiple cases, the decision network is quite reminiscent of lambda's, with the *cI-cII-cIII* module conserved, and an orchestrated gene expression cascade taking place. Even in phages that are otherwise very different from lambda, a dual repressor motif similar to the *cI*/*cro* pair (discussed in more detail below) is often present, and an auto-regulatory viral "repressor", which both establishes and maintains the lysogenic state, appears to be near universal. Among temperate phages whose decision process has been studied in some detail, a few offer slight variations on lambda, for example, P22, a "lambdoid" phage (i.e., one with a similar genome architecture to lambda), where the Cro-analog (*ant*) directly inactivates the CI-analog (*c2*) by binding to it and preventing it from effectively binding to DNA. Others exhibit dramatically different behavior. P4, for example, is a "satellite phage", a genetic element that requires other viruses for its own propagation. Rather than having a global repressor, which establishes and maintains lysogeny by regulation of transcription initiation, the outcome and maintenance of the decision in P4 are driven by control of transcription termination, mediated by RNA-RNA interactions. The default pathway in P4 appears to be lysogeny, with the alternative state, in the absence of a co-infection by a helper phage, being that of a multi-copy plasmid. In that state, infection by a helper phage induces the transition into lysis.

Counting by Infecting Phages

The perceived role of the decision circuit, as stated above, is to choose between lysis and lysogeny based on the conditions of infection. A key question is, thus, how these conditions are sensed by the infecting phage and processed by the decision circuit to yield an optimal outcome. This question is far from settled. To begin with, which aspects of the infection event are pertinent to the outcome? The parameter best characterized in terms of its effect on the lambda lysis/lysogeny choice is the multiplicity of infection (MOI), namely, the number of phages co-infecting the cell. It was found long ago that, the higher the MOI, the higher the probability of lysogeny. Fig. 4 depicts the results of an experiment measuring the relation between the two observables. A known

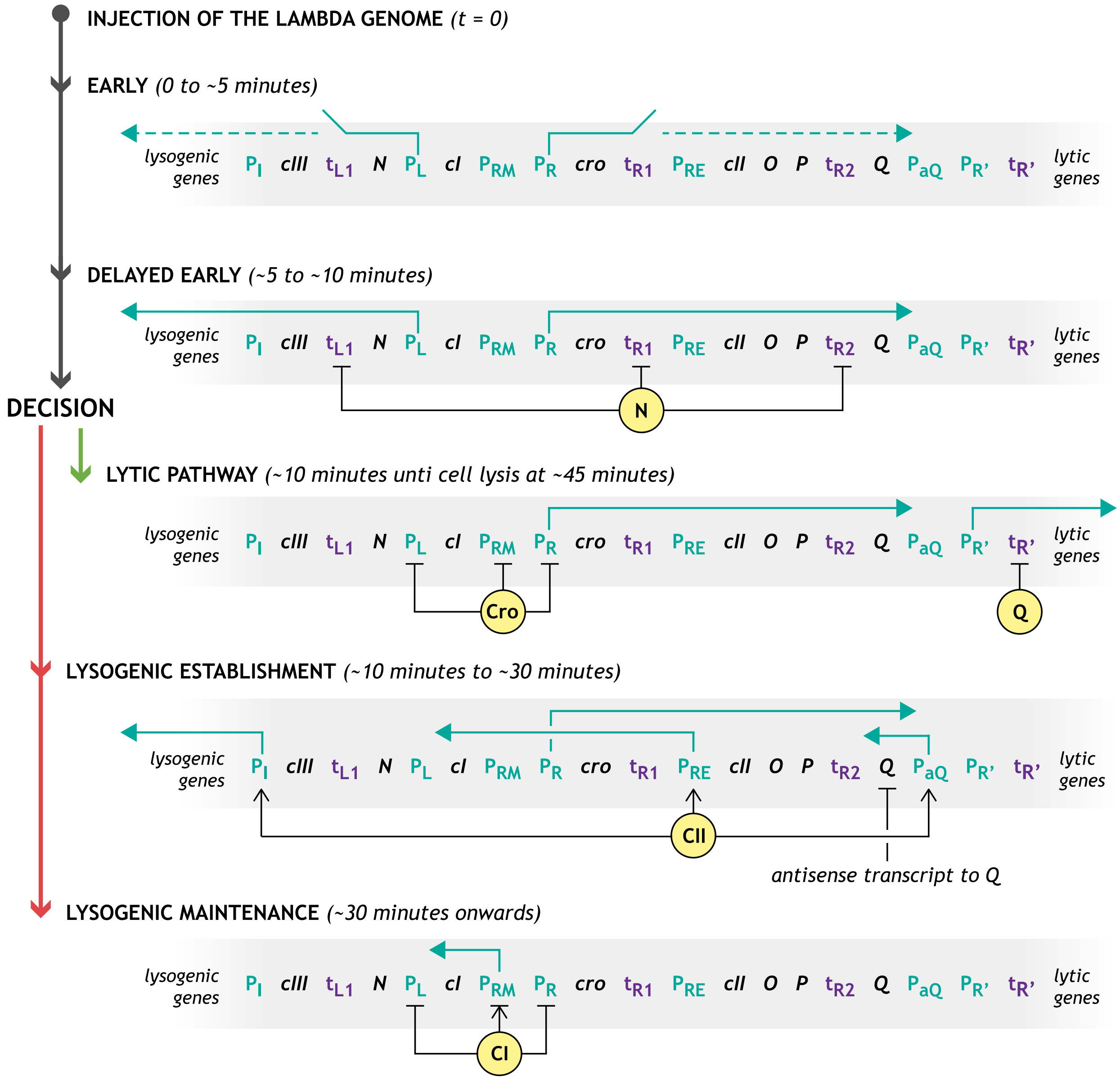

Fig. 3 The cascade of transcriptional events during the lambda lysis/lysogeny decision. Regulatory elements and their interactions are depicted on the relevant region of the lambda genome. The expression pattern of early genes is qualitatively similar irrespective of the eventual fate. After a decision has been reached, different sets of genes are expressed to execute the lysis/lysogeny choice.

number of bacteria is mixed with varying concentrations of phages, and, once infection is allowed to proceed, the number of resulting lysogens is measured using selection for an antibiotic marker that was engineered into the viral genome. To interpret the experimental results, the measured values are compared to a simple mathematical model, in which random phage-bacteria encounters (following mass-action probability and Poisson statistics) result in lysogeny if the number of phages co-infecting a single cell reaches some number m^*. As seen in Fig. 4, the measured data is consistent with a value of $m^* = 2$, i.e., a scenario where infection by a single phage leads to lysis, whereas simultaneous infection by two or more phages results in lysogeny.

The notion that infecting phages are able to count their numbers in the cell, and then decide on the mode of action based on that number, is intriguing both mechanistically – how do viruses count? – and in terms of its utility – why do they do so? In terms of mechanism, it is commonly held that counting is mediated through the level of CII reached during infection. The MOI affects both CII production (through increased gene dosage) and degradation (through production of CIII, which protects CII from degradation by FtsH, see below). As described earlier, high CII level triggers lysogeny by driving the expression of the integrase and CI, and inhibiting late-lytic gene expression from $P_{R'}$. However, an alternative hypothesis, motivated by recent single-cell experiments and mathematical modeling, posits that phage counting simply reflects the number of *cI* gene copies, whereas CII levels respond only weakly to MOI, possibly due to the auto-repression of P_R by Cro. If enough CI accumulates, it will then activate

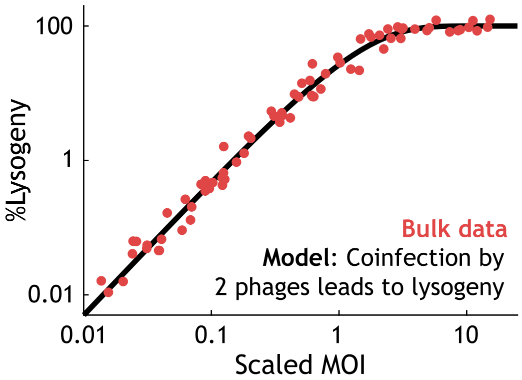

Fig. 4 The dependence of lysogenization on the multiplicity of infection: bulk data. A known number of *E. coli* bacteria is infected with varying concentrations of lambda phage, and the number of resulting lysogens is measured using selection for an antibiotic marker that was engineered into the viral genome. The experimental trend is reproduced by a simple mathematical model, where infection by a single phage leads to lysis, whereas simultaneous infection by two or more phages results in lysogeny.

its own transcription from P_{RM} and shut down expression of the lytic genes. Elucidating phage counting is complicated considerably by the fact that the lambda genome begins replicating shortly after infection, i.e., during, not after, the lysis/lysogeny decision. That early replication plays a role in the decision is evidenced by the fact that replication-deficient lambda mutants require a higher number of initial viruses to achieve lysogeny, compared to wild type phages.

As for the biological utility of choosing lysogeny at higher MOI, the common interpretation is that the number of co-infecting phages is used by lambda as a proxy for the ratio of phage-to-bacteria abundance in the surrounding environment. That ratio, in turn, serves to assess the chances of successful infection by the next generation of phages, should the lytic pathway be chosen. Specifically, high MOI indicates that phages outnumber bacteria and that, therefore, releasing more phages into the environment is futile, since they are unlikely to find new bacterial targets. Hence, high MOI advocates lysogeny. Conversely, low MOI indicates the availability in the environment of yet-uninfected bacteria, thus promoting the release of more viral particles through lysis.

Consistent with the idea that lambda measures the multiplicity of infection in order to assess the abundance of uninfected bacteria in the environment, recent studies found additional ways by which phages can infer this kind of information. During initial rounds of infection of *Bacillus subtilis*, phage phi3T expresses the genes *aimR* and *aimP*. AimR binds to the phage DNA and activates the transcription of *aimX*, which blocks the lysogenic pathway in a mechanism not yet elucidated. The lytic pathway is thus favored in the initial infections. Meanwhile, AimP, a short peptide, is secreted into the extracellular medium, where it accumulates and is taken up by uninfected cells. When some of these cells are later infected, the intracellular AimP, now at high concentration, binds to and inactivates AimR, resulting in repression of *aimX* expression. Consequently, lysogeny is favored in later rounds of infection. Using this intercellular communication system (termed "arbitrium"), infecting phi3T phages are able to record past infections of other cells and tune their lysis/lysogeny decision based on this knowledge.

The ability to hijack the host's quorum sensing system is also found in VP882, a temperate phage that infects *Vibrio cholerae* and other *Vibrio* species. The outcome of infection by VP882 depends on a repressor (Gp59) that inhibits the expression of the lytic regulator (Gp62), thus promoting the lysogenic pathway. During infection of *Vibrio cholerae*, the phage also expresses $VqmA_{Phage}$, a homolog of the host's endogenous quorum sensing receptor, VqmA. When bound by *Vibrio*'s autoinducer, DPO, $VqmA_{Phage}$ promotes the expression of an antirepressor (Qtip), which sequestrates the repressor Gp59, allowing genes involved in the lytic pathways to be expressed. Consequently, when the bacterial density is high, the increased DPO level in the medium promotes the lytic pathway of infecting VP882. Note that, in contrast to phi3T above, which relies on a phage-specific secreted molecule, here, VP882 assesses the bacterial density via an autoinducer encoded by the host.

The cases described above, in lambda and other phages, as well as the ecological argument mentioned, all support the idea that increased phage-to-bacteria ratio promotes lysogeny. However, whether this rule applies universally is still unclear. The propensity for lysogenization by bacteriophage P1, for example, is reported to be insensitive to the number of viruses infecting the host cell. In infections by phage Mu, the probability of lysogeny appears to decrease with the multiplicity of infection, although this trend may reflect the virus' toxicity to the host at high MOI.

There is also an ongoing debate whether, outside the artificial lab environment, bacterial density is correlated – positively or negatively – with the occurrence of lysogeny, and how to interpret the observed trends. Ecological studies of lytic infections support a "kill the winner" model, in which viral infection increases host diversity by preventing overabundance. However, the dynamics of temperate viruses are much harder to interpret, and the relationship between host density and outcome frequencies is unclear. Previous studies of prophage induction found that lysogeny is more prevalent at low host density, consistent with the picture above of increased lysogenization at high MOI. A more recent work, using metagenomics analysis, reported an inverse trend, but the interpretation of these newer findings is a subject of some controversy.

Whereas the multiplicity of infection is the best-characterized driver of the lambda lysis/lysogeny decision, it is definitely not the only one. Recall that multiple host factors interact with the decision circuit (**Fig. 2** above). One such factor is FtsH, a

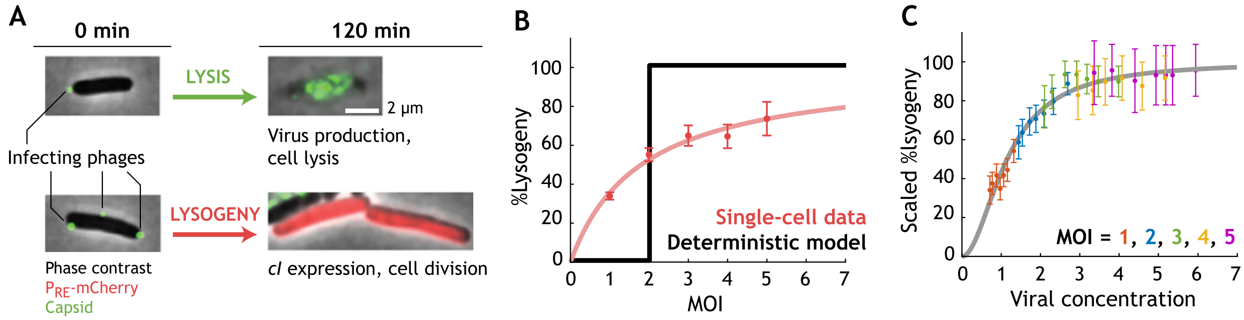

Fig. 5 The lysis/lysogeny decision at the single-cell level. (A) Images from a live-cell movie following the fate of two *E. coli* cells, infected by fluorescently-labeled lambda phages. The upper cell, infected by a single phage, proceeds to produce new viral particles and undergo lysis. The lower cell, co-infected by three phages, enters lysogeny, as indicated by a fluorescent reporter for P_{RE} activity, and proceeds to divide normally. (B) The fraction of cells undergoing lysogeny as a function of the multiplicity of infection, as measured from >1000 infection events. In contrast to the original modeling of the bulk data (**Fig. 4** above), the single-cell curve rises gradually, suggesting that the MOI dependence is probabilistic rather than deterministic. (C) Incorporating the effect of intracellular viral concentration (MOI divided by cell volume) captures the experimental data and yields a decision curve that is markedly more step-like. Adapted from Zeng, L., Skinner, S.O., Zong, C., *et al.*, 2010. Decision making at a subcellular level determines the outcome of bacteriophage infection. Cell 141 (4), 682–691, Copyright 2010, with permission from Elsevier.

membrane-bound ATP-dependent protease. During lambda infection, FtsH degrades CII and thus impacts the choice between lysis and lysogeny. Part of FtsH's influence comes about through the MOI, specifically, the dosage-dependent production of CIII, which is believed to protect CII by serving itself as a target for FtsH. However, beyond the response to MOI, the level and activity of FtsH are regulated by the physiological state of the cell, thus providing a means for the condition of the host cell to inform the lysis/lysogeny decision. For example, the increase in lysogenization at low temperature can be attributed to a decrease in FtsH levels, as well as an increase in the thermodynamic stability of CII, to which FtsH is highly sensitive. Temperature also impacts the decision circuit through its effect on another bacterial protease, Lon, which is expressed in a temperature-dependent manner and targets the N anti-terminator. Two other cellular sensors, cyclic adenosine monophosphate (cAMP) and guanosine tetraphosphate (ppGpp), have also been reported to affect the lysis/lysogeny decision, possibly by inhibiting FtsH. Cellular state also influences the lambda decision through RNase III, whose levels are modulated by the *E. coli* growth rate. RNase III promotes degradation of *cII* transcripts, stimulates ribosome binding and translation initiation of *cIII*, and blocks auto-repression of *N* translation during early infection, thus affecting multiple nodes of the decision network. The ways in which the decision circuit assesses the state of the cell via these and other physiological sensors remain a promising direction for future interrogation.

The View From the Single Cell

Most of what we know about lambda's post-infection decision comes from studies that used traditional genetic and biochemical assays, performed in bulk cultures, and thus involving the averaging of all measured observables over millions of cells. But these individual cells may, in fact, exhibit very different phenotypes. Over the last decade, traditional bulk assays have begun to be supplemented by microscopy-based studies, in which the infection process is followed in real time, at the level of individual cells and phages. **Fig. 5(A)** shows an example of such an experiment. Here, the lambda capsid was labeled using multiple copies of a fluorescent protein, such that each phage particle appears under the microscope as a diffraction-limited spot. The infected cells were simultaneously imaged using phase contrast microscopy. Time-lapse images show the infection and its outcome for two individual cells. The first cell, infected by a single phage, proceeds to produce more viral proteins (green) and lyse within two hours. The second cell, co-infected by three phages, survives to grow and divide. That cell has chosen the lysogenic pathway, as indicated by the production of a red fluorescent protein, here expressed from the lysogeny establishment promoter, P_{RE}.

Following many infection events in this manner allows one to determine how the decision outcome – lysis or lysogeny – depends on the infection parameters, such as the MOI. **Fig. 5(B)** shows that the fraction of cells choosing lysogeny increases with MOI, a trend consistent with the observations in bulk. However, in contrast to our original interpretation of the bulk data (**Fig. 4** above), the single-cell data suggests that the MOI dependence is probabilistic rather than deterministic: At MOI of 2, for example, an infected cell has about a 50% chance of going either lytic or lysogenic. In other words, when we observe a cell co-infected by two lambda phages, we have no way of telling what route will be chosen!

We are thus confronted with the indeterminacy of single-cell behavior: genetically identical cells, all subject to the same environment, exhibiting different phenotypes from each other. This phenomenon is observed throughout biology, and its origins are a subject of intensive interrogation. According to the prevailing picture, cellular individuality reflects the inherent randomness of biochemical reactions in the cell. In this view, the unavoidable fluctuations in molecular copy number and in the timing of events render the lambda decision "noisy" and unpredictable, rather than precise and deterministic. The plausibility of this argument was first demonstrated using a computational simulation of the lambda decision circuit, showing that fluctuations in

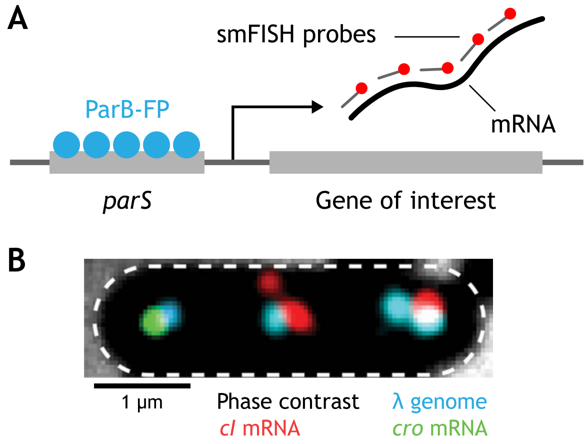

Fig. 6 Detecting the transcriptional activity of individual lambda phages. (A) Each phage is detected through the binding of fluorescently-tagged ParB proteins to the *parS* sequence, engineered into the lambda genome. mRNA molecules transcribed by the phage are simultaneously detected using single-molecule fluorescence *in situ* hybridization (smFISH). (B) Four lambda genomes (cyan) inside a single infected cell, at 10 min after infection. Individual phages vary in their transcriptional activity, with one transcribing *cro* (green) and two others producing *cI* (red).

biochemical reactions can result in diverging cell fates among infected cells. The concept of noise-driven decisions then evolved and was used to explain cell-fate indeterminacy in higher systems, including the transition in and out of HIV latency, as well as the differentiation and reprogramming of metazoan cells.

But the fact that we can describe cell fate decisions probabilistically does not necessarily mean that we should settle for such a narrative and give up seeking a deterministic description of the decision process. While biochemical stochasticity is undisputedly present, automatically attributing all cellular indeterminacy to unknowable "noise" may be taking the easy path. One must consider the alternative hypothesis, which is, that our inability to predict the decision outcome reflects a failure to account for additional cellular variables that have a deterministic effect on the decision. So long as these "hidden variables" remain unknown to us, the decision will appear more random that it truly is, and our understanding of it remain limited.

And, in fact, a number of lambda studies suggest that incorporating additional variables can reveal a more precise decision at the single-cell level. It was first found that, for a given MOI, smaller cells are more likely to be lysogenized than larger ones. This should not surprise us, since decreasing the size of the infected cell is, to a first approximation, the same as infecting with a larger number of phages: both result in an increased concentration of viral gene products in the cell. Detailed analysis revealed that a unique arithmetic combination of the MOI and cell size yields a more step-like (and therefore, more deterministic) probability of lysogenization (**Fig. 5(C)**). The way in which the infection parameters combine to yield a sharp decision curve points to a nonlinear interaction between the co-infecting phages as they converge on the cell's fate. Elucidating the nature of this interaction will require characterizing the spatiotemporal dynamics and genetic activity of individual phages within the infected cell. Fluorescent reporters for phage capsid, genome, RNA and protein products, needed for such an investigation, are now becoming available (**Fig. 6**).

The Decision to Remain Dormant

If, following infection, the lysogenic route is chosen, control of cell fate is then handed over from the post-infection decision circuit to a smaller circuit, whose role is to maintain dormancy by repressing all virulent functions. The maintenance circuit must also be able to trigger a switch back to lysis (induction) when cellular conditions change. Thus, the dormant virus continuously re-evaluates its lysis/lysogeny decision. Some elements of lysogenic maintenance in lambda and other phages are covered by a separate article in this encyclopedia (Shearwin). Here we focus on the decision-making aspect of the maintenance circuit.

In lambda, lysogenic maintenance is handled by a subset of the post-infection decision network discussed above. The smaller maintenance circuit, known as the lambda "switch", consists of two phage genes, *cI* and *cro*, transcribed respectively from two diverging promoters, P_{RM} and P_R (**Fig. 7**). The two gene products, CI and Cro, compete for binding to six operator sites (O_{R1-3} and O_{L1-3}) that regulate P_{RM} and P_R transcription, resulting in mutual repression by the two proteins. In the prophage state, high cellular level of CI represses transcription from P_R and P_L, thus maintaining viral dormancy. The lysogenic state is further stabilized by the formation of a DNA loop between O_{R1-3} and O_{L1-3}, secured by oligomerization of CI dimers bound at the two loci. Perturbations that reduce the level of CI (such as activation of the bacterial SOS response, discussed below), can lead to lytic induction. In this process, the inhibition of P_R and P_L is relieved, leading to transcription of early lytic genes, including *cro*. Cro then represses P_{RM}, leading to further reduction of CI level and allowing the lytic cascade to proceed. Despite decades of meticulous studies, recent experiments continue to reveal new features of the lambda maintenance circuit, such as the role of mechanical coupling between transcription, DNA supercoiling, and looping, and how this coupling may affect the stability of the lysogenic state.

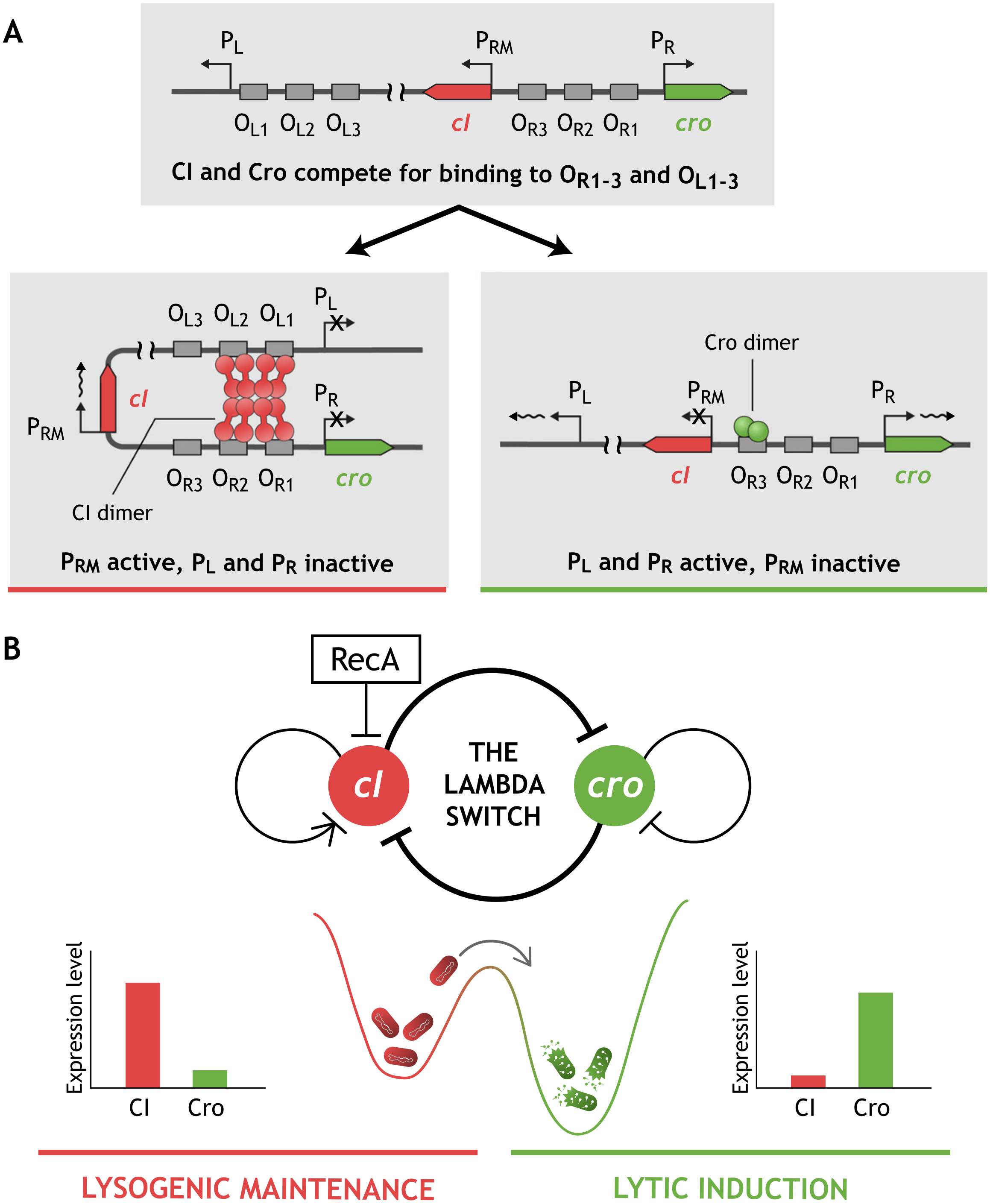

Fig. 7 The maintenance of lambda lysogeny. (A) The lysogenic state is maintained by a regulatory circuit consisting of CI and Cro, expressed from the P_{RM} and P_R promoters, respectively. CI and Cro compete for binding at six operator sites ($O_{R1–3}$ and $O_{L1–3}$), to determine which promoters (P_{RM}, or P_R and P_L) are active, and thus decide whether lysogeny is maintained or, instead, lytic genes induction takes place. Two examples of binding configurations are shown. On the left, CI dimers bind to four operator sites, resulting in DNA looping that ensures repression of P_R and P_L during lysogeny. On the right, binding of Cro to O_{R3} represses transcription of CI from P_{RM} and allows lytic genes to be expressed. (B) The regulatory interactions between CI and Cro form a bistable switch. The system can alter its state in response to a large perturbation, such as depletion of CI by RecA, leading to lytic induction, but is immune to small perturbations. Adapted with permission from Golding, I., 2016. Single-cell studies of phage λ: Hidden treasures under Occam's rug. Annual Review of Virology 3 (1), 453–472, permission conveyed through Copyright Clearance Center, Inc.

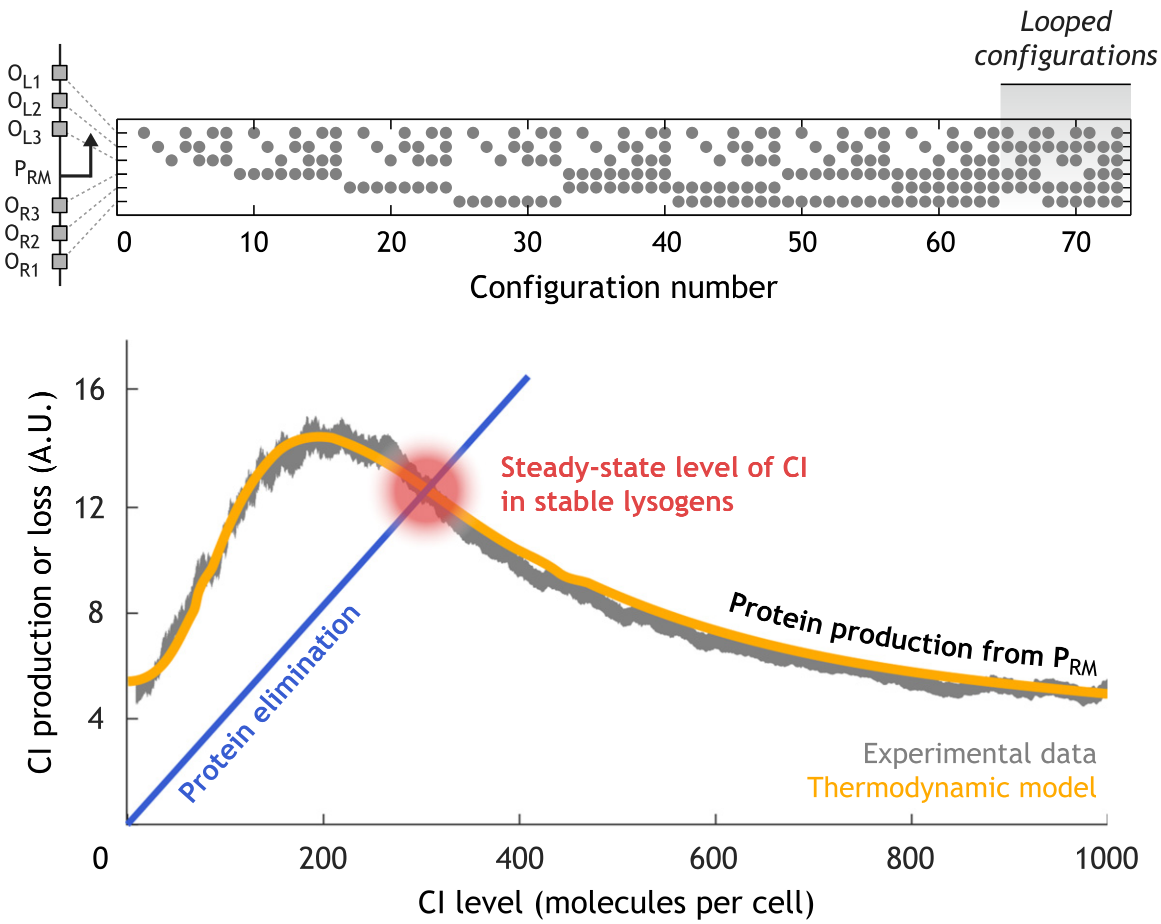

Fig. 8 A theoretical biophysical model captures P_{RM} regulation by CI. The regulatory curve relating CI concentration in the cell to P_{RM} activity can be predicted from a thermodynamic description of the possible binding configurations of CI at the O_{R1-3} and O_{L1-3} operator sites. The theoretically predicted curve agrees with the experimental measurement of the regulatory relation. The average amount of CI protein present in each lysogenic cell can be estimated by requiring that CI production is exactly balanced by CI elimination (via dilution, due to cell growth and division). Adapted from Sepúlveda, L.A., Xu, H., Zhang, J., Wang, M., Golding, I., 2016. Measurement of gene regulation in individual cells reveals rapid switching between promoter states. Science 351, 1218–1222, reprinted with permission from AAAS; and from Golding, I., 2011. Decision making in living cells: Lessons from a simple system. Annual Review of Biophysics 40 (1), 63–80 with permission conveyed through Copyright Clearance Center, Inc.

As in the case of infection, the phage (now, prophage)-encoded circuit requires input from the bacterial host in order to sense the state of the infected (now, lysogenized) cell and use that information to choose optimally between lysis and lysogeny. Specifically, during lysogenic maintenance, the role of host input is to alert the prophage when the bacterial cell is in danger, indicating that it is time to escape the host through the lytic pathway. Lambda receives this information via *E. coli*'s SOS system, which, in response to cellular DNA damage, halts progression of the cell cycle and triggers DNA repair and mutagenesis. Under normal growth, expression of the SOS response genes is repressed by LexA. However, in the presence of DNA damage (due to, e.g., UV radiation), regions of single-stranded DNA accumulate, leading to recruitment and activation of RecA. Activated RecA facilitates LexA self-cleavage, de-repressing SOS genes, including RecA itself. Phage lambda is coupled to the SOS response through the self-cleavage of CI by activated RecA. The consequent drop in cellular CI concentration leads to the relief of *cro* repression, activation of the lytic pathway, and prophage induction. Many other temperate bacteriophages are also induced following treatment with UV radiation or DNA-damaging agents. However, some, like P2, appear to be non-inducible, and immune to the bacterial SOS system.

The *cI*/*cro* pair serves as a canonical example for a so-called "toggle switch", a genetic module exhibiting two stable states, here corresponding to lysogeny and to the onset of lysis. Despite consisting of only two genes, the lysogeny maintenance circuit of lambda captures key features of cell-fate choice, as observed across the spectrum of biological complexity. Through the use of feedback (P_{RM} autoregulation by CI), the system achieves extremely high stability in the absence of external perturbations, with fewer than one spontaneous switching event per 10^6 cell doublings. At the same time, almost 100% of lysogenic cells switch to lysis in response to an inducing signal. These properties have made the lambda maintenance circuit an attractive starting point for understanding cellular differentiation and reprogramming in metazoans. In particular, lambda has served as a fertile test ground for the attempt to formulate a detailed biophysical description of cellular behavior, in the form of a mathematical model that uses the known molecular interactions to predict the resultant cellular phenotype. This effort has been, at least partly, successful. For example, a thermodynamic model can be written, describing the different binding configurations of CI at the operator sites that control transcription from P_{RM}, and this model used to predict the regulatory curve relating CI concentration in the cell to P_{RM} activity. The theoretically predicted curve shows good agreement with experimental measurements of this regulatory relation (**Fig. 8**). The theoretical calculations can next be utilized to estimate the amount of CI protein present in a lysogenic cell, by requiring that CI production is exactly balanced by CI elimination (via dilution, due to cell growth and division) (**Fig. 8**).

The eventual test for a theory of the lambda switch is to successfully predict the key phenotype, namely, whether a given cell will remain in the lysogenic state or, instead, switch to lysis. In attempting to answer this question, we are again confronted with the challenge of cellular individuality: In the absence of an external signal, only one out of a million lysogens in a growing culture will spontaneously switch. Can we predict which cell this will be? It is widely believed that the answer is negative, and that we may only aspire to predict the probability of induction, not the actual fate of an individual cell. This is because spontaneous induction is considered a stochastic process, driven by random fluctuations of CI levels in the cell. Small drops in CI number will be corrected by the negative feedback in the P_{RM}-CI circuit, raising CI level and thus reverting to the mean. However, rare, larger drops will overcome the feedback and lead to de-repression of P_R, Cro production and onset of the lytic pathway. In this picture, spontaneous lytic induction is analogous to the way random thermal motion drives a physical system to transition from one stable state to another (**Fig. 7** above). The challenge of theoretically predicting the behavior of the *cI*/*cro* switch dwarfs in comparison to the larger goal of predicting cell fate following infection, when the full decision network (**Fig. 2** above) comes into play.

Conclusion

The prevailing narrative for the lambda lysis/lysogeny decision, both following infection and during lysogenic maintenance, offers only a probabilistic prediction, rather a deterministic one, as to which path an individual cell will choose. This probabilistic point of view is similarly applied, further afield, to the choice of latency by mammalian viruses and to cellular differentiation and reprogramming. These decisions are all held to be indeterminate, noise-driven processes. Bacteriophage lambda, where this picture originally emerged, also bears the potential to challenge the probabilistic view by revealing previously-hidden variables that bias the decision outcome, or even determine it in full. Future studies, describing the lysis/lysogeny decision in individual phages and cells, in real time, will be key to delineating true randomness from the hidden precision of cellular decision-making.

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Further Reading

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