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From Bench to Keyboard and Back Again: A Brief History of Lambda Phage Modeling

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Abstract

Cellular decision making is the process whereby cells choose one developmental pathway from multiple possible ones, either spontaneously or due to environmental stimuli. Examples in various cell types suggest an almost inexhaustible plethora of underlying molecular mechanisms. In general, cellular decisions rely on the gene regulatory network, which integrates external signals to drive cell fate choice. The search for general principles of such a process benefits from appropriate biological model systems that reveal how and why certain gene regulatory mechanisms drive specific cellular decisions according to ecological context and evolutionary outcomes. In this article, we review the historical and ongoing development of the phage lambda lysis–lysogeny decision as a model system to investigate all aspects of cellular decision making. The unique generality, simplicity, and richness of phage lambda decision making render it a constant source of

mathematical modeling–aided inspiration across all of biology. We discuss the origins and progress of quantitative phage lambda modeling from the 1950s until today, as well as its possible future directions. We provide examples of how modeling enabled methods and theory development, leading to new biological insights by revealing gaps in the theory and pinpointing areas requiring further experimental investigation. Overall, we highlight the utility of theoretical approaches both as predictive tools, to forecast the outcome of novel experiments, and as explanatory tools, to elucidate the natural processes underlying experimental data.

Contents

1. INTRODUCTION: CELLULAR DECISION MAKING

Cellular decision making is ubiquitous across all forms of life. It occurs when cells with identical genomes exposed to identical environments develop different, stable phenotypes (8, 49, 61, 62, 83, 94). As the anthropomorphism "decision" suggests, comparing cellular and human decisions can be insightful. Some human or cellular decisions are more predictable than others, depending on evolutionary history or past and current internal and external circumstances. Just as the human brain (a vast network of neurons) can rationally weigh the benefits and costs based on past experience when deciding between two competing options, the gene regulatory network (GRN, a vast network of genes) can also process inputs to drive cellular decisions according to evolutionary history encoded in the genome (2, 6, 7, 41). Despite the fundamental differences between a human brain and a cell's GRN, they have certain similarities in structure and function (59, 92). Microscopic cells can use their GRNs to sense the environment, then adapt by changing their molecular composition and phenotype accordingly. GRNs can store information nongenetically, maintaining the levels of certain molecules despite internal or external changes (62), or genetically, keeping

a record of the cell's environmental exposure and mutation history in the genetic sequence that encodes GRN structure. Since the principles of decision making for cells, mammals (86), or other animals (23) are not fully understood, it seems useful to seek common questions, such as (*a*) what the mechanisms or algorithms that trigger the selection of one decision over another are, (*b*) how such mechanisms integrate information depending on internal and external parameters, and (*c*) how the quality of one decision-making scheme can be compared to that of another. Evolutionary fitness, a sum of individual- or isogenic population-level costs and benefits encoded by genes (20), may offer useful clues to the answers to these questions, but multiple optimizations may exist, and conflicts can arise. What is best for a multicellular organism or cooperating unit may not be best for individuals and vice versa (46, 58). Since decisions contribute to fitness, understanding the mechanisms and outcomes of decision making in cells could inform evolutionary understanding of decision making in various living or artificial systems, including humans (53) and machines (66, 77).

To unravel the principles of cellular decision making, a collaborative approach iterating between modeling and experiment is critical (60). The typical scenario for such iterative-collaborative approaches starts with experiments producing data on the biological system in diverse scenarios. From the experiments, general principles emerge that can be formulated mathematically to collectively comprise an initial theory of system behavior. Mathematical analysis can make modeling assumptions more rigorous. It can also help compare quantitative predictions with experimental data. At this point, two possibilities arise. First, major discrepancies between the model's outcomes and existing experimental data indicate faults in the theory, requiring a search for specific assumptions or conditions that cause the discrepancy. Directed experiments designed to further test these assumptions then lead to a revised theory. Second, if the model's results agree with existing measurements, then the theory is consistent with the data, and it can be used to make novel, experimentally testable predictions. In either case, the model directs future experiments, which further refine the model or validate its predictions. This process (**Figure 1*a***) continues iteratively until a robust model based on a robust theory emerges.

The bacteriophage (phage) lambda lysis–lysogeny fate choice exemplifies how the iterative interdisciplinary process can reveal principles of cellular decision making. Upon infecting a host cell, lambda phages choose among three outcomes (lysis, lysogeny and lyso-lysis; **Figure 1*b***), relying on environmental information-sensing, -processing and -storing phage and host GRNs. From an evolutionary perspective, phage lambda decision making is comparable to decisions of microbes to grow or sporulate, of mammalian viruses to be active or latent, etc. Below, we review the historical progression of phage lambda research since the 1950s, summarizing important experiments relevant to modeling, and important models that explained or predicted experimental outcomes, along the way. We illustrate the historical use of models to validate the consistency of decision-making theories with experimental data and to predict novel experimentally testable aspects of the decision-making process. The history of phage lambda research (**Figure 2*a***) demonstrates that increasing experimental resolution and capabilities do not diminish the need for quantitative modeling. On the contrary, the demand for new, different modeling approaches increases as the experiments progress (31). We do not expect this to change—in fact, modeling is becoming increasingly important and necessary to refine our understanding of cellular decision making for phage lambda–infected bacteria and many other cellular systems.

2. A BRIEF HISTORY OF PHAGE LAMBDA BIOLOGY: THE EXPERIMENTS

2.1. The Early Days: Discovery of the Phage Lambda Lysis–Lysogeny Decision

Phage lambda is a virus that infects the bacterium *Escherichia coli*. It was discovered around 1949 and reported in 1950 by Esther M. Lederberg (47, p. 5), who mixed regular K12 *E. coli* cells with

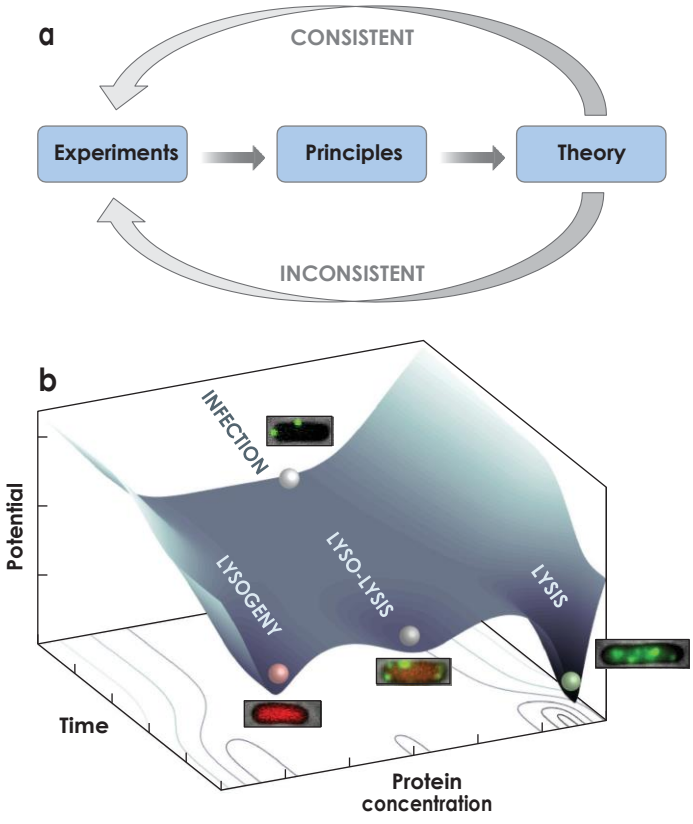

Figure 1

The iterative-collaborative process and cellular decision making. (*a*) Experimental data suggest principles that are convertible into theories. Theories make predictions that prompt repeated or new experiments. (*b*) Schematic illustration of the decision-making landscape of an *Escherichia coli* cell infected by phage lambda. Among lysis, lysogeny, and lyso-lysis, lysis is the default outcome, with stochastic outcomes biased by the multiplicity of infection and host cell properties. Representative cells were infected by phages labeled with a two-color system (8): gpD-mNeongreen (*green*) marks infecting phages and the lytic pathway, while *cI*-mKate2 (*red*) marks the lysogenic pathway. Figure adapted with permission from Reference 7.

UV-treated *E. coli* cells and noticed that the latter had "growth that was nibbled and plaqued." Subsequent research demonstrated that the K12 *E. coli* strain is lysogenic, meaning that it carries a phage lambda DNA integrated into its chromosome. Its interactions with nonlysogenic, lambda-free *E. coli* cells can reveal a remarkable cellular decision-making process (48). When two such strains are cocultured, phage lambda can infect UV-treated cells. Upon infection, although the genomes and environments are identical, two fates are possible: lysis or lysogeny. On the one hand, during lysis, the virus hijacks the host cell's transcriptional and translational machinery to replicate, destroys the cell membrane and cell wall, and releases approximately 100 new phages (79). The released phages can then proceed to infect other susceptible host cells. On the other hand, during lysogeny, the infected cell survives with the viral DNA integrated into the host chromosome. The integrated viral DNA becomes a prophage, while the host cell becomes a lysogen immune to further infections (90). As the name prophage suggests, the prophage can excise itself out of the host's chromosome and reinitiate the lytic program through stress-triggered or spontaneous prophage induction. For example, UV irradiation leads to prophage induction, which is why

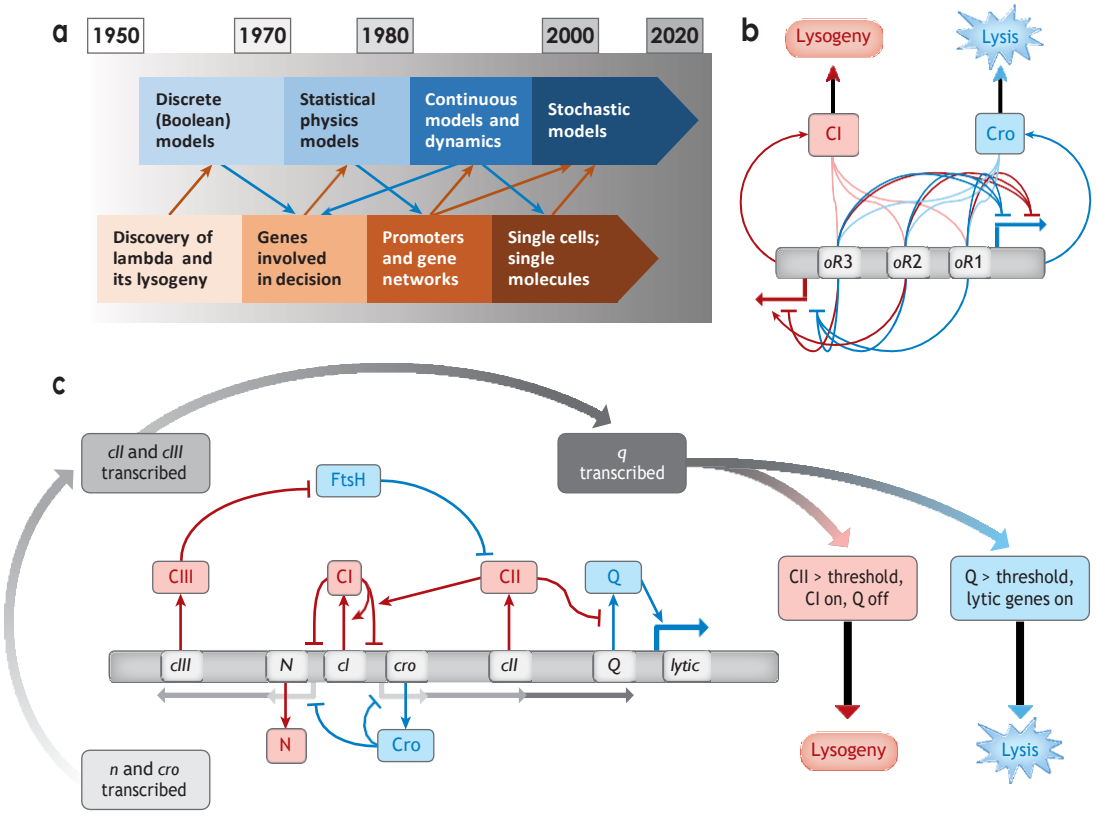

Figure 2

Classical and current views of the phage lambda gene regulatory network underlying cell fate decision. (*a*) Timeline of phage lambda research progression. (*b*) According to the classical view, CI and Cro compete to bind three shared operator sites (*oR*1, *oR*2, *oR*3) on two divergent promoters (*pRM* and *pR*). CI repressor binding to *oR*1 has a negligible effect on *cI* transcription, while CI binding to *oR*2 activates, and CI binding to *oR*3 represses *cI* transcription. CI binding to any operator site represses *cro* transcription. Cro represses both its own and *cI* expression but has a stronger effect on *cI*. Panel adapted with permission from Reference 7. (*c*) Current view of lambda decision making, based on CII and Q threshold crossing.

the UV-treated cells in the Lederberg experiments were phage free. These discoveries introduced a major scientific problem that is still not completely solved and laid the foundation for cellular decision-making research. Phage lambda decision making has become an archetype model system for studies of gene regulatory mechanisms, stochasticity, mutational processes, evolutionary dynamics, and much more.

2.2. The Next Two Decades (1950s and 1960s): Discovery of Decision-Making Genes

In the couple of decades after the discovery of phage lambda (32), experimentalists began mapping the genes involved in the lysis–lysogeny decision and its external triggers, as well as the optimal experimental conditions to work with this phage–host system (36, 39, 40, 93). Concurrently, during these heroic years, great leaps of intuition led to the discovery of operons, transcription factors, and gene regulation in *E. coli* (35), as wonderfully recounted by François Jacob (34). Regarding

phage lambda, the frequency of lysogenic events was found to increase at low temperatures, in old or starved cells, and in cases of high numbers of infecting phages [or high multiplicity of infection (MOI)]. Moreover, the discovery of mutations affecting the chance of lysogeny revealed the importance of the genetic background (21, 25, 50). Toward the end of the 1960s, the CI–Cro pair of repressor genes encoded from divergent promoters (*pRM* and *pR*, respectively) with three shared operator sites (*oR*1, *oR*2, *oR*3) to which either CI or Cro dimers can bind became a focus of research. Mark Ptashne (68) suggested that this overall mutually repressing gene pair (**Figure 2*a***) orchestrates lysis–lysogeny decision making. Simplification of this natural gene circuit to its core interactions may have inspired the design of the toggle switch gene circuit (27), which marked the beginning of the revolutionary field of synthetic biology in 2000.

2.3. The 1980s: A Network View Emerges

By the early 1970s, a comprehensive narrative, synthesizing the results of genetic studies, emerged of how various key phage genes such as *cI*, *cro*, *cII*, *N*, *O*, *P*, *Q*, *int*, and *xis* contribute to the phage lambda decision and to the maintenance of lysogeny (21). The detailed mechanisms of action for these genes remained unclear, but knowing their general functions allowed a fairly accurate description of how they promote lysogeny or lysis (**Figure 2*b***). Central to this narrative was the temporal sequence of gene activity: Early genes turn on immediately upon infection and subsequently control the expression of late genes as the cell–phage system reaches its decision. This temporal order of gene regulation seemed to be crucial to understanding the cell fate bifurcation between lysis and lysogeny. For example, N promotes early expression of CII and CIII proteins, which then act simultaneously to upregulate CI levels to establish lysogeny. Elevated CI maintains the lysogenic state by repressing early genes, some of which would drive expression of the Q activator of late lytic genes. Cro counteracts CI's function by competing to bind the same DNA sites for gene regulation (38). Despite these discoveries, many details of the cell fate bifurcation remained mysterious. Notably, some cells lysed while others lysogenized in the same experiment, indicating that the decision may be at least partly random. A simple 1973 assay by Kourilsky (44) for estimating the lysogenization frequency in bulk revealed that lysogeny requires at least two infecting phages. These findings suggested that higher numbers of infecting phages promote higher CII and then CI levels, thus causing the lysogenic state (21). By using Kourilsky's assay, researchers could further dissect the decision-making process to discover how mutations and environmental conditions affect the frequency of lysogens, leading to an increasingly detailed understanding of genetic and nongenetic factors biasing the cellular decision one way or the other.

Throughout the rest of the 1970s, further mechanisms of viral gene action emerged, such as the antitermination activities of N and Q controlling early and late gene expression, respectively, and the CII-dependence of *pRE*, *pI*, and *paQ* promoters. In addition, antagonistic effects of host FtsH and lambda CIII on the stability of CII were uncovered. These discoveries solidified and refined the decision-making narrative by 1980, exposing a new, CII-centric view (33). Further refinements of our understanding of the mechanisms of action for viral gene network function continued throughout the 1990s (69) and are still ongoing. Subsequent studies then gradually led to the current view (65) of how the phage lambda gene regulatory network drives the cellular decision (**Figure 2*c***).

2.4. The Twenty-First Century: High-Resolution Experiments and Stochasticity

In the 2000s, experimental methods in the study of the lysis–lysogeny decision achieved increasingly higher resolution. Fusing GFP to key promoters activating CII and Q allowed monitoring

of their expression dynamics right after infection (42). Interestingly, CII expression was found to be bell-shaped in time, while Q expression remains low during lysogenic development. CII and Q expression patterns in multiple phage mutants revealed further details of these gene expression trajectories at high temporal resolution. Specifically, in CII mutants, Q activity appeared sooner, implying that CII delays and inhibits Q, which can activate late lytic genes once its level crosses a threshold. Overall, the findings revealed that CII must reach a threshold sufficiently soon to establish lysogeny, or else Q has enough time to cross its own threshold to initiate lysis. Using the same reporter fusions later revealed that FtsH and CIII also influence the CII–Q dynamics (43).

Fluorescent tagging of a late lytic (packaging) gene in single cells (81) allowed monitoring of lytic fate choice in single GFP-expressing cells under the microscope, whereas lack of GFP and resumed cell division indicated lysogenic fate choice. Such phages revealed how infected cell fate depends on host cell size. Interestingly, larger cells had higher probabilities of lysis, explaining away some of the apparent stochasticity in the decision. Moreover, such phages allowed researchers to test, in single cells, earlier accounts that MOI promotes lysogeny. Tracking single cell decisions using a CII-reporter fusion and fluorescent capsid decoration protein fusion (96) not only confirmed the effect of cell size on the decision, but also revealed a viral voting mechanism, whereby individual phages make individual decisions, and lysogeny is only possible if all phages uniformly vote for it. These discoveries revealed additional determinism in phage lambda decision making but still confirmed that both lysogeny and lysis are possible at any MOI and cell volume. Thus, although MOI and cell volume can push the probability of lysogeny toward 1 or 0, it still remains distinct from 1 or 0 for any number of phages infecting any cell, supporting a strong role for stochasticity in phage lambda decision making.

These high-resolution, single-cell studies continued in the 2010s, focusing on the CI–Cro decision-making module reconstructed in the *E. coli* genome, to facilitate studies of this influential genetic module in isolation from other phage lambda molecular processes. Measuring *cI* and *cro* mRNA counts expressed from *pRM* and *pR* in single *E. coli* cells (97) revealed that the rate at which high CI–low Cro lysogen-fate cells switched to low CI–high Cro lytic-fate cells depended exponentially on *cI* gene activity, in agreement with previous bulk estimates (51). More recently, combining CI and Cro protein level measurements at the single-molecule level with potential landscape modeling revealed two new states with low CI, low Cro and high CI, high Cro, in addition to the canonical lytic and lysogenic states of the CI–Cro switch (22). Other high-resolution experiments showed that the isolated CI–Cro genetic switch meets bistability criteria, such as the coexistence of two gene expression states and hysteresis (10). Cells with high CI, which in the stable steady state mimic lysogeny, required a higher perturbation to push them into lysis than did naïve cells, and vice versa. These results deepened the understanding of the CI–Cro genetic switch, but their relevance to decision making by real infecting phages is unclear.

In addition to observing single cells, new opportunities emerged to control them. Introducing time-controlled Q expression from a plasmid (82) at the moment of infection always led to lysis, whereas delaying it from 8 to 50 min biased the decision toward lysogeny (from approximately 5% to approximately 35%), highlighting the importance of Q expression timing in phage lambda decision making. Tracking individual phage integration events revealed lyso-lysis (**Figure 1*b***), a new cell fate involving viral DNA integration into the host's chromosome even in cells that eventually lyse (74). This supports the idea that individual viral DNAs can behave differently in the same cell, as proposed in the phage voting model (96). Moreover, a computational model and single cell–level observations of coinfecting phages during the decision process (88) revealed that individual phages compete for limited host resources during lysis, and that phages infecting earlier tend to win the race. In contrast, during lysogeny, the phages appear to assist one another to lysogenize the host cell, possibly through a common pool of diffusible

transcription factors. This might explain the voting model, since the strategy during lysogeny resembles evolutionary cooperation, requiring all phages to cooperate. Any defectors will take all the resources and cause the opposite outcome. More details of high-resolution experimental studies up to 2018 were summarized recently (75). Individual phage lysis–lysogeny decisions also indicate a potentially important role of recently observed subcellular, spatial organization of phage and host biomolecules (87), where phage DNA recruits the host resource, DnaB, to its vicinity for DNA replication and establishes separate subcellular compartments within cells during the lytic pathway. These individual phage compartments are heterogeneous in size and apparently separated by the *E. coli* nucleoid. In addition, different key lysis–lysogeny decision transcripts were found to be located in different areas inside the cell, presumably leading to different cell-fate decisions. It will be interesting to witness the new challenges and solutions that will emerge as experimental resolution continues to increase.

3. A BRIEF HISTORY OF PHAGE LAMBDA THEORY: THE MODELS

3.1. Mathematical Beginnings in the 1970s: Boolean Models

By the early 1970s, many genes important for lysogenization had been discovered, and genetic narratives of the lysis–lysogeny decision became increasingly consistent with experimental observations. Still, many open questions remained about how cells achieve lysis or lysogeny, such as (a) why subpopulations of genetically identical cells in the same experimental conditions develop lytically or lysogenically; (*b*) how genetic changes and environmental variables such as phage versus cell density, temperature, and host cell physiology modulate lysogenization frequency (percentage of lysogenized cells); and (*c*) whether there are quantitative rules and principles corresponding to molecular events causing cells to select lysogeny or lysis that underlie the decision-making process.

Answering these questions required new concepts and methods to analyze gene networks rather than single genes, and drove the development of Boolean networks, the earliest mathematical models of gene regulation (84). Boolean models (**Figure 3**) consider the genes and their promoters jointly to be either ON or OFF, in a binary fashion (1 or 0). Environmental variables such as temperature can be incorporated as additional nodes with HIGH and LOW states (1 or 0), as applicable for the *cI857* phage lambda mutant with a temperature-sensitive CI protein. The ON–OFF state of each gene or promoter depends on Boolean functions of other gene and environmental inputs. Considering lysogenization frequency as a Boolean, HIGH or LOW variable (1 or 0), these models reproduced existing qualitative observations for various mutants and made predictions for phage strains with multiple mutations that were then confirmed experimentally. Despite being qualitative, these models demonstrated that the decision to lysogenize could be formalized rigorously using a mathematical approach. This provided a way to integrate experimental and genetic factors into a comprehensive and formal theory with predictive capabilities. Although lacking many biological details, Boolean models still provide useful biological explanations and predictions (3).

3.2. Modeling Equilibria: Statistical Physics Approaches in the Early 1980s

The main limitation of Boolean models is their discrete and highly qualitative nature. They treat the genes and promoters jointly as the basic ON–OFF units from which everything else follows, ignoring the mechanistic details of molecular interactions. This makes it difficult to integrate them with molecular biology experiments. In reality, genes encode proteins that interact with other proteins and/or DNA, enabling controllable transcription over a range of rates, rather than just being ON or OFF. Intuitively, modeling these molecular details could shed further light on

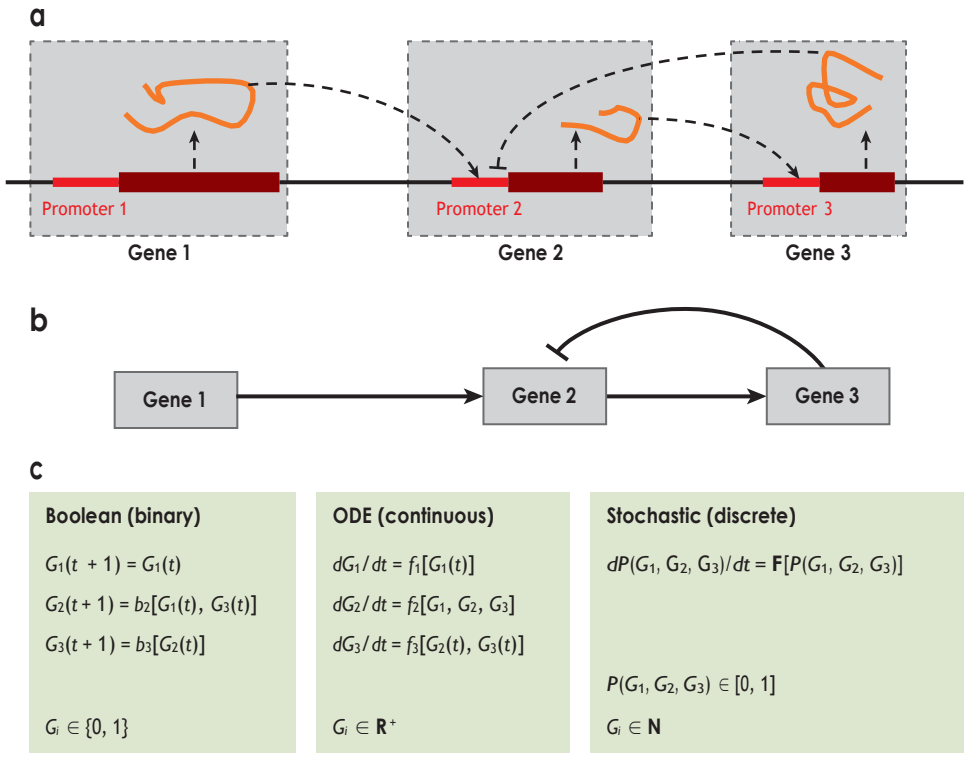

Figure 3

Quantitative modeling methodologies. (*a*) Schematic illustration of gene regulatory interactions involving promoters and transcription factors. (*b*) Gene regulatory network obtained by simplifying the regulatory interactions within the gray boxes. (*c*) Three modeling methodologies for the gene regulatory network. In the three modeling approaches, G_i represents the levels of the protein encoded by gene *i* as a binary, continuous, and discrete variable, respectively; *P* is a distribution; and *t* is time. Abbreviation: ODE, ordinary differential equation.

the molecular mechanisms of cell fate bifurcation and the effect of environmental variables on lysogenization.

In the 1980s, as experiments uncovered higher levels of molecular detail on phage lambda decision making, a need for more detailed mechanistic models became evident. Accordingly, Ackers and colleagues (1) developed mechanistic models of prophage induction using a statistical-thermodynamic approach to account for chemical equilibria of CI dimers binding to the three *oR* operator sites. By considering each possible binding configuration of operators and CI dimers, they could mathematically describe the relative abundance of each *oR*-CI bound state through equilibrium statistical thermodynamics. They parameterized the model using Gibbs free energy estimates of binding from prior studies, and then predicted the repression efficiency of both *pR* and *pRM* as a function of CI dimer concentration. Consistent with prior knowledge about prophage induction, they showed that prophage CI levels suffice to establish near complete repression of *pR*, while *pRM* should remain active. Then, by varying the cooperative binding energies of CI dimers to *oR* operator sites and investigating the change of repression curves, they found that cooperativity steepens the response of *pR* repression to CI dimer concentration. They concluded that cooperativity (*a*) enables switch-like behavior between lysogeny and lysis and (*b*) causes the

lysogenic state to be more stable. These initial observations about combinatorial control and cooperativity, a specific form of ultrasensitivity, led to many further investigations, revealing their important roles across biology (4, 16, 24, 70).

3.3. Modeling Bulk Averages: Ordinary Differential Equations from the Mid-1980s

Classical statistical thermodynamics is excellent for analyzing systems in thermodynamic equilibria, but the processes following infection with phage lambda are far from equilibrium, at least until lysogeny. Therefore, the initial thermodynamic approaches of Ackers and colleagues (1) were insufficient. Three years later, Shea & Ackers (76) followed this work up with a more detailed model, including Cro dimer binding at the same *oR* operators and RecA-mediated CI degradation in ordinary differential equations (ODEs) (**Figure 3**). They simulated the expression of CI and Cro proteins depending on *oR* operator binding states, once again using statistical thermodynamics. In effect, these models used timescale separation, assuming fast DNA–protein interactions that reached quasi-equilibrium compared to the timescale of gene expression. With this more detailed model, Shea & Ackers confirmed that Cro expression from pR responds sharply to CI concentration with a near-complete repression, while CI expression from pRM rises gradually to a peak, after which it plateaus.

Nonlinear dynamics is a natural approach to broadly studying the nature of solutions of ODEs. At the end of the 1980s, Reinitz & Vaisnys (71) investigated prophage induction with the Shea-Ackers model and found bistability for specific CI and Cro transcription rates. However, they noted that the updated model's predictions failed to match important experimental measurements such as the level of CI in stable lysogens. They discussed the possible sources of this disagreement, including (*a*) a mismatch between in vitro and in vivo parameter estimates, (*b*) the use of continuous rather than discrete variables to model protein levels, and (*c*) regulatory links or functions missing from the model. These observations of model deficiencies promoted major advances in modeling GRNs across biology, far beyond phage lambda.

While the CI–Cro module is an attractive target to reveal mechanisms underlying the lysis–lysogeny decision, its direct relevance following host infection is questionable. Specifically, CI is expressed much later than Cro, so the two sides of the switch cannot function biologically, as implied by bistable models or experiments with isolated CI–Cro modules. Around the 1990s, more complex models emerged that incorporated more genetic factors involved in the lysis–lysogeny postinfection decision. In 1995, McAdams & Shapiro (57) developed a complex model of the postinfection decision using an electronic-circuit framework. Their model was more comprehensive, including many known genetic interactions, such as (*a*) the genes *xis*, *int*, *cIII*, and *N* on the left operon and pL's dependence on CI and Cro; (*b*) CI and the *pRM* operon; (*c*) *cro*, *cII*, *O*, *P*, and *Q* on the right operon; and (*d*) the CI–Cro combined effect on *pR* and *pRM*. The model combined previous Boolean and continuous modeling methods and showed that signal or concentration timing delays accumulate throughout the network to cause cell-fate bifurcation between lysis and lysogeny at different MOI.

Overall, these modeling studies demonstrated how discrepancies between models and experiments revealed deficiencies of theories and basic assumptions underlying the models. This paved the way for new experiments to further test and refine existing theories.

3.4. Modeling Single Cells: Stochastic Simulations in the 1990s

ODEs are powerful methods to analyze deterministic processes continuous in time. Their solutions tacitly assume that a typical cell or a bulk average represents all the cells in a population

apart from some random noise that can be ignored. However, there is no representative average capturing cells that lyse and others that lysogenize under the same experimental conditions. At the beginning of the 1990s, it was still unclear why this happens, indicating that there are nondeterministic, stochastic lysis–lysogeny decisions in single cells. In 1997, McAdams & Arkin (56) revisited earlier models to examine how variability in signal timing can cause cell-fate bifurcation. At that time, there were no well-established methods for modeling such biological outcomes as the puzzling randomness of phage lambda decision making. Seeking an approach to simulate stochastic chemical kinetics (**Figure 3**), McAdams & Arkin rediscovered Daniel T. Gillespie's (28–30) algorithm, a partly forgotten 1970s method for exact stochastic simulations of chemical reactions, which was rarely used before single-cell observations of gene expression but became extremely popular afterward. Using it to simulate the behavior of genetically coupled links, in which one promoter's gene product activates or represses expression of another gene's promoter, McAdams & Arkin demonstrated that biochemical stochasticity can cause even simple regulatory systems to behave quite differently on repeated simulation runs, providing a mechanism for stochastic cell-fate bifurcation. In 1998, Arkin and colleagues (5) revisited the electronic-circuit model of the postinfection decision as a coupled set of stochastic biochemical reactions. The major insight they gained was that, in a system as complex as the lysis–lysogeny decision, stochastic fluctuations in gene expression levels can randomize the occurrence of key regulatory events, making stochastic cell-fate decisions possible. The modeling results matched bulk lysogenization data consistently with prior narratives (21) on the sequence of events necessary for lysogeny over lysis. The inclusion of stochasticity solved the puzzle of probabilistically occurring lysogeny or lysis in genetically identical cells infected in identical environmental conditions. Accumulating evidence for nondeterministic components in regulatory processes of both prokaryotes and eukaryotes (13, 17, 95) further bolstered these insights.

3.5. Refining Principles: Thresholds and Steady States

As the models became more complicated, their results became more difficult to interpret, and principles became more difficult to extract. Nonetheless, McAdams & Arkin (56) proposed heuristic decision-making criteria to characterize the simulation outcome to be lysogenic if (*a*) CII's time-integrated concentration exceeds a threshold, indicating high *pI*, *pRE*, and *paQ* activity, and (b) CI dimer concentration exceeds Cro dimer concentration. These decision-making criteria agree with experimental facts about lysogenic development requiring, i.e., high CI levels, low Cro levels, and sufficient CII activity on the promoters *pRE*, *pI*, and *paQ*. While such criteria could separate repeated simulation runs into lysogenic and lytic outcomes, they were still ad hoc and seemed less justified near the decision-making boundary. Could decision-making thresholds be more rigorously defined?

A natural way to assign thresholds and cell fates is by analyzing bifurcations and assigning thresholds to attractor boundaries in bistable systems (71). However, newer findings based on simple CI–Cro models using literature-based parameter estimates indicated that only a single, lysogenic steady state exists (85). Thus, the system appeared monostable without additional feedback loops. Consistently and independently, another model including more details such as *oL*, *oR* operator sites, and CI octamerization also indicated a single lysogenic steady state (72), except when CI's degradation rate increased due to cleavage by RecA. However, a few years later, a simple and elegant deterministic model including CII in addition to CI and Cro produced bistability (91). Unlike prior studies using a statistical-thermodynamic approach to model three operator sites in the *oR* region, Weitz and colleagues (91) used only chemical kinetics and approximated the *oR* region by a single operator site competitively bound by CI and Cro dimers. They found

that, as MOI increases from 1 to ≥3, the system undergoes a bifurcation from a single lytic steady state to having two coexisting stable steady states and then back to a single lysogenic steady state (**Figure 4*c***). Interestingly, the lysogenic state required CII to cross a naturally emerging concentration threshold, suggesting that threshold-crossing events may trigger cell-fate decisions in bistable (or multistable) systems. Considering the importance of thresholds, a related model only used threshold crossing to define lysis and lysogeny (37). Since it takes time to reach the steady states representing the ultimate outcome, this work proposed seeking an earlier molecular or regulatory event that tips the system in the direction of lysis or lysogeny (**Figure 4**). From this perspective, steady states serve as a confirmation of the chosen fate but do not indicate the actual nonequilibrium decision-making event that triggers the realization of one steady state over the other. Concurrently, work in *Bacillus subtilus* indicated that the decision between sporulation and competence depends on the winner of the race between two independently developing pathways (45), representing another example of a cell-fate decision emerging from earlier, nonequilibrium, transient dynamics.

3.6. Predicting Single-Cell Gene Expression Distributions and Decision Statistics

While existing models explained qualitatively the increasing lysogeny trend at higher MOIs, GRN-based models matching the new single-cell, single-phage data were lacking (96). New work synthesizing previous models to include CI, Cro, CII, Q, cell volume growth, and viral DNA replication filled this gap, reproducing experimentally measured single-cell probabilities of lysogeny for various MOIs and cell volumes (19). These models showed a strong association between CI's and CII's threshold crossings, replacing the classical CI with CII as the key lysogenic factor within early decision making GRNs, even if these decisions are dominated by stochasticity. This model also predicted that infection delays lower the chance of lysogeny by effectively lowering CII levels, so late-infecting phages matter less, which was confirmed experimentally.

Despite this progress, it remained unclear how viral replication affects protein levels and, thus, the decision-making process. After all, as the number of gene copies increases, the corresponding transcripts and proteins should follow suit. Surprisingly, a study of mRNA transcript counts from *pR* and *pRE* in single cells found that *cII* mRNA levels remained relatively constant over 18 min in the wild-type phage relative to a DNA replication–deficient mutant phage (73), as opposed to replication-sensitive CI. To solve this puzzle, a model matching CI and CII mRNA time courses identified Cro negative feedback as being responsible for the insensitivity of CII levels to DNA replication. This recapitulates the stabilizing role of negative feedback, which improves the robustness of decision making to fluctuations (9, 64).

3.7. Evolution and Optimality of the Lysis–Lysogeny Decision

While many studies have focused on how the phage lambda lysis–lysogeny decision is made, an emerging question is why it happens the way that it does. Addressing this question requires studying decision making from an evolutionary perspective. Evolutionary studies can benefit from modeling, as realistic, natural evolutionary scenarios are difficult to recreate in the laboratory. Among the first studies to address such a question modeled the population dynamics of temperate and virulent phages in a mixture of susceptible, lysogenic, and resistant bacterial cells (80), seeking to determine the conditions allowing the temperate phages to exist stably in competition with virulent phages. The models predicted that temperate phages can coexist with strictly virulent phages over a broad range of conditions, and that temperateness is beneficial in uncertain environmental

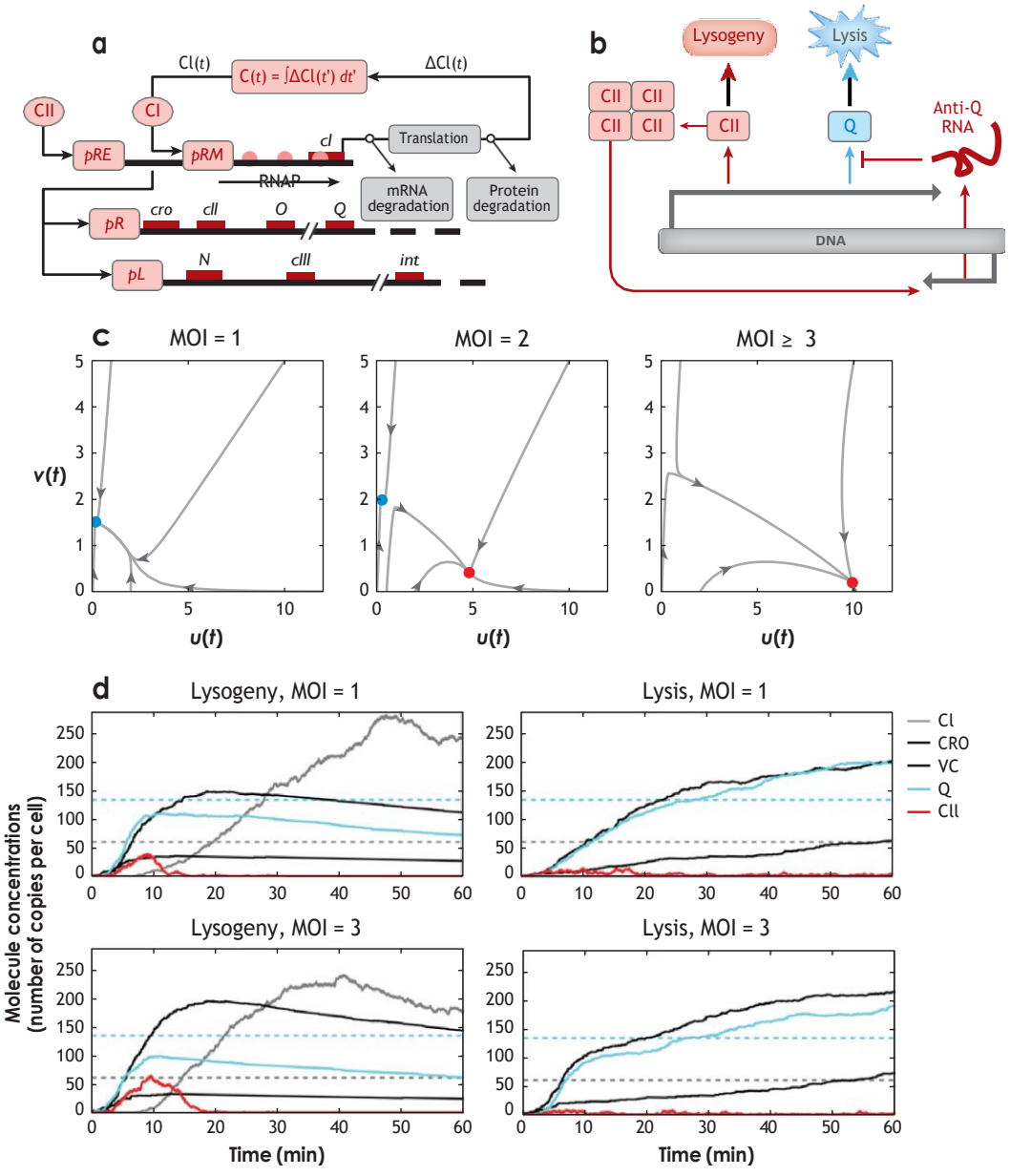

Figure 4

From complicated to simple, to extract principles. (*a*) Modeling schematic for McAdams & Shapiro's (57) simulations. Panel reproduced with permission from Reference 57. (*b*) Cortes and colleagues' (19) 2017 threshold-crossing model. Panel adapted with permission from Reference 19. (*c*) Weitz and colleagues' (91) 2008 results showing a single lytic steady state for MOI = 1, lysis–lysogeny bistability at MOI = 2, and single lysogenic steady state at MOI ≥ 3. The filled circles indicate stable steady states, while the variables *u* and *v* denote CI and Cro concentrations, respectively. Panel adapted with permission from Reference 91. (*d*) Decision-making time courses from Cortes and colleagues' (19) 2017 threshold-crossing model, portrayed as a race between CI and Q toward their respective thresholds. Decisions represent nonequilibrium path properties rather than steady states. Panel adapted with permission from Reference 19. Abbreviations: MOI, multiplicity of infection; VC, viral concentration.

conditions. Within the last decade, similar questions attracted growing interest, leading to game theory–based models showing that, to maximize long-term growth, the postinfection lysogenic probability should evolve to approximately equal the probability of lytic population collapse (54). Therefore, strictly lytic phages have uncertain lifetimes: After depleting a population of susceptible hosts, they must encounter another bacterial population before they degrade.

Other modeling efforts followed by evolutionary experiments with competing wild-type (temperate) and lysogeny-deficient *cI*857 mutant phage lambda confirmed these conclusions. The models predicted that the lysis-preferring virulent phage had an early advantage in outnumbering the tempered phage, but after susceptible host density dropped to low levels, the latter phage gained the advantage in the long run (11). Chemostat experiments confirmed these predictions (11) and subsequently showed that spatial structure could impact the competition by restricting the phages' ability to infect cells to a local region, lowering the effective reach of phages and making lysogenization a more beneficial strategy (12). Further theoretical analysis showed that the lytic strategy is optimal only when the number of hosts is very large (26). Otherwise, the phages reduce host counts, rendering the lytic strategy no longer optimal. Instead, replicating passively as a prophage maximizes fitness when hosts are rare. As MOI signals the phage-to-host ratio, these findings corroborate the MOI-dependent increase of lysogenic tendency. Accordingly, evolutionary game theory of successively competing phage strains showed that the experimentally measured MOI-dependent probabilities of lysogeny provide the highest evolutionary fitness (79). Revisiting and extending the earliest population dynamics models (80) indicated that phage strains with higher postinfection probabilities of lysogeny are stable against invasion from more virulent strains as long as the pool of susceptible host cells is not unlimited (89).

Considering that lysogens are very stable without external perturbations, lysogens and their nonlysogenized counterparts can also compete (63). Prior experimental studies indicated that lysogens often outcompeted their nonlysogen counterparts when grown together (14, 67). Via spontaneous phage induction (SPI), lysogens could release free phage into the mixture, which could then lyse or lysogenize susceptible hosts. Recent work investigating competing lysogens and nonlysogens while keeping the postinfection probability of lysogeny fixed found an optimal, low SPI rate matching experimental estimates (18). Overall, in these evolutionary studies of the lysis–lysogeny decision, modeling and experiments strengthened each other: Either (*a*) follow-up experiments validated modeling predictions or (*b*) the modeling results quantitatively captured experimentally measured outcomes of natural phage evolution.

4. CONCLUSIONS

Since the 1950s, modeling and experiments have been applied progressively and iteratively to understand how the phage lambda lysis–lysogeny decision is made and why lysogeny would be advantageous over lysis in certain contexts. Modeling has been used as a theory-testing procedure to confirm if assumptions can capture experimental data, or as a predictive tool to investigate scenarios that are difficult to explore experimentally or that can only be indirectly studied. Perhaps its most useful application is to infer the intracellular processes underlying the lysis–lysogeny decision and the fitness effects of lysis–lysogeny decisions in various contexts. In the future, new models capturing multicellular, subcellular, spatial, and other processes will be needed. It will be fruitful to consolidate current and future models of various aspects of lysis–lysogeny decision making, including lyso-lysis and lysis time variation (78), deepening the understanding of this fascinating model system and, thereby, of other decision-making processes. Besides increasingly higher-resolution investigations of the phage lambda lysis–lysogeny decision, decision making in other phages; the role of temperate, lytic, chronic phages in the microbiome (55); the role of clustered, regularly

interspaced, short palindromic repeats (CRISPR) immunity in phage infection outcomes (15); and cellular decision making in phage therapy (52) will be other fascinating areas to investigate.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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