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The Ups and Downs of Modeling the Cell Cycle

Review

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We discuss the impact of mathematical modeling on our understanding of the cell cycle. Although existing, detailed models confirm that the known interactions in the cell cycle can produce oscillations and predict behaviors such as hysteresis, they contain many parameters and are poorly constrained by data which are almost all qualitative. Questions about the basic architecture of the oscillator may be more amenable to modeling approaches that ignore molecular details. These include asking how the various elaborations of the basic oscillator affect the robustness of the system and how cells monitor their size and use this information to control the cell cycle.

Introduction

The cell theory implied that cells grew in size and divided to form more cells. Most cells divide at the same rate that they double in mass, thus preserving an average cell mass that is constant over many cell cycles. The rates of cell division and cell growth (often used as a misnomer for cell proliferation) and the coordination between them are regulated by events within cells, signals from other cells in the same organism, and the external environment; regulating cell growth and division in time and space is a crucial factor in shaping the development of plants and animals. Formal studies on cell growth and proliferation and their regulation date back over a century, and the results of these studies have been analyzed mathematically for at least 30 years [1–3]. Notable early examples were the attempt to analyze the cell cycle of mammalian cells as consisting of distinct phases, with the progression from one phase to the next occurring with a probability that was under the control of the cell's environment [4], and modeling the coordination between growth and cell division in fission (*Schizosaccharomyces pombe*) [5] and budding (*Saccharomyces cerevisiae*) yeasts [6] and the slime mold *Physarum polycephalum* [3] as a result of the requirement that cells reach a critical mass before certain cell cycle events could occur.

These early efforts regarded the machinery that controlled the cell division cycle as a black box, and attempted to produce a mathematical description of the rules it obeyed. The landscape changed dramatically in the 1980s, as work on yeasts and the rapid cell divisions of fertilized eggs produced a molecular description of the cell cycle oscillator as a fluctuation in the activity of cyclin-dependent kinases (Cdks) that is driven by a combination of phosphorylation reactions and the periodic destruction of the cyclins that activate Cdks (reviewed in [7]). Experimentally, this oscillation can depend on a single Cdk — Cdk1, also known as Cdc2 or, in budding yeast, as Cdc28 — and a single cyclin, cyclin B.

These discoveries quickly inspired a new type of mathematical analysis, one which attempted to represent the molecular details of the cell cycle oscillator and the pathways that it interacted with as differential equations [8–10]. We discuss mathematical models of the cell cycle that were undertaken to understand its oscillation as well as to study its distinctive features. These efforts have several different goals: to demonstrate that current models of the cell cycle can produce oscillations; to quantify the robustness of the oscillator; and to predict or explain novel behaviors. We also discuss open questions about the cell cycle which, though they do not relate to the oscillatory behavior *per se*, seem like areas where mathematical approaches could complement existing experimental studies and direct further research.

Mathematical and Genetic Modeling

Work on oscillators in physics and engineering, as well as in biological systems such as the cAMP waves in *Dictyostelium* (reviewed in [11]), suggested that mathematical methods would be powerful tools in understanding the cell cycle. While the cell cycle machinery clearly oscillates, it differs from many other oscillatory systems. Its function is to ensure the orderly progression through the steps needed to replicate the cell. Cell growth, which is continuous, must be properly coupled with cell proliferation, which involves replication of discrete structures such as chromosomes and centrosomes, and the division of the cell itself. The cell cycle thus proceeds through a repeating sequence of discrete states; transitions between these states are regulated by external signals as well as by checkpoints, which are dispensable for normal cell cycles but which monitor processes such as DNA replication and chromosome alignment, and block cell cycle progression if these are not properly completed.

The abrupt and highly regulated transitions of the cell cycle contrast with the behaviour of many other oscillators. For instance, in the mammalian circadian clock, protein levels, gene expression and neuronal firing all vary continuously, rather than switching abruptly from day to night phases [12]. Furthermore, while the phase can be gradually shifted by external cues, there is nothing equivalent to a checkpoint mediated by some regulated process — circadian rhythms do not wait for us if we do not sleep. This difference is likely to reflect the function of the different cycles, rather than the principles that underlie them. With the unfortunate exception of those who use modern aviation, organisms have every right to expect a diurnal variation in light and temperature whose duration is constant, whereas the factors that determine the rate of cell growth and the

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ability to complete discrete steps in cell replication are highly variable.

One commonly cited goal of mathematical modeling, of the cell cycle or any other process, is to verify that known interactions in some system can produce the observed qualitative behavior. Typically, these models employ ordinary differential equations in which concentrations of different molecules are dynamic variables. Levels of regulatory molecules at some point in time determine the rates of change in the concentrations of other molecules according to genetically or biochemically determined interactions. For instance, a transcriptional activator will increase the rate of accumulation of an mRNA (or its protein product, depending on the level of detail employed in the model), while a kinase would cause a decrease in levels of unphosphorylated substrate and a commensurate increase in levels of the phosphorylated form.

The exact dependence of reaction rates on concentration is rarely available for these modeling efforts, and even quantification of protein levels is uncommon. The missing data matter. Biological networks with the same connectivity can display many different sorts of behavior, depending on these kinds of detail [13]. Data on cell cycle interactions can be converted into rough models based on ordinary differential equations, which can then be checked for proper oscillatory behavior with plausible-seeming parameters. Dynamical systems theory can go beyond this to enumerate the possible qualitative behaviors of the network — different oscillations and stable states, and how the system might switch between them — using only the general structure of the network [14–17].

Genetic reasoning may be as powerful as dynamical systems theory in addressing this kind of qualitative question. Geneticists iteratively collect information about ordered pathways of positive and negative interactions to understand the behavior of regulatory systems. Their logical methods have been developed to deal with exactly the kinds of data produced by genetic experiments and used in the mathematical modeling efforts discussed above. The purpose of this approach is to pick the model that best explains a particular behavior, rather than to exhaustively list all possible behaviors of a network. For example, the oscillatory behavior of the cell cycle is well known, so the principle question for a qualitative model is whether its interactions can produce oscillations.

The key observations for understanding the cell cycle were that cyclins are necessary to activate the Cdk, but active Cdk causes proteolysis of cyclins. These interactions were immediately seen to be capable of oscillation: cyclin would alternately accumulate when Cdk is inactive and vanish when Cdk becomes active. Even the importance of nonlinearities in generating oscillations in the system was recognized without recourse to differential equations [7] (though Goldbeter [10] presented an alternative model that produced oscillations without nonlinearities). Mathematical models of the cell cycle based on these interactions produce oscillations, demonstrating that they are sufficient to recapitulate the known behavior of the cell cycle [18].

In the absence of quantitative measurements of the reactions in the cell cycle, however, it was not possible to draw further conclusions. For instance, while ranges of parameter values were found where the system could oscillate, it was not possible to determine whether the known interactions in the cell cycle represent a system in this oscillatory domain. Modeling work is also silent on questions of molecular mechanism. Interactions can be direct or involve intermediate steps without changing the form of the equations [17]. In the absence of knowledge about the kinetic constants that describe components, we argue that it is better to make the simplest model that describes the overall topology of a model, rather than the one that represents the largest possible fraction of known components.

Similarities between Different Clocks

Blindness to molecular mechanism can be an advantage in revealing formal similarities between systems involving very different kinds of molecular components and interactions. Despite the significant differences between the cell cycle oscillator and the circadian clock, there are similarities in the pattern of interactions that produce oscillation. In both systems, an activator stimulates its own accumulation as well as the production of a repressor, which destroys the activator (Figure 1A). In the minimal cell cycle, the appropriately modified complex of Cdk1 and cyclin B — referred to as maturation promoting factor (MPF) in the early literature — is the activator; it stimulates its own activation as well as that of the anaphase promoting complex (APC). Phosphorylated APC, however, targets cyclin for degradation, thus inactivating MPF (Figure 1A).

The circadian clock operates at the level of transcription as well as phosphorylation. Clk and Bmal1 are activators which stimulate their own production as well as driving the accumulation of Per and Cry repressors, which block this activation (Figure 1A). Figure 1B shows the minimal mathematical description needed to produce this sort of oscillator and constrasts with Figure 1C, which shows the reaction scheme needed for a model that represents many (but not all of) the known molecular components of the cell cycle oscillator.

A similar architecture is even seen in oscillations of pacemaker cells, such as those in the sino-atrial node. These cells use radically different molecular components to produce regularly spaced action potentials at about 1 Hz. The voltage- and time-dependent behavior of sodium and calcium channels provide for self-reinforcing activation. When the pacemaker cell is fully polarized, an inward sodium current slowly depolarizes the cell. When it reaches a threshold level, voltage-sensitive calcium channels open to allow complete depolarization during an action potential. Voltage-sensitive potassium channels then open to restore the membrane potential. With the membrane potential restored, the slow sodium current begins again. In this cycle, the slow sodium current is analogous to the synthesis of cyclin and the voltage-sensitive calcium current corresponds to MPF activation of preMPF. The potassium channels are analogous to the APC, as they are activated when the

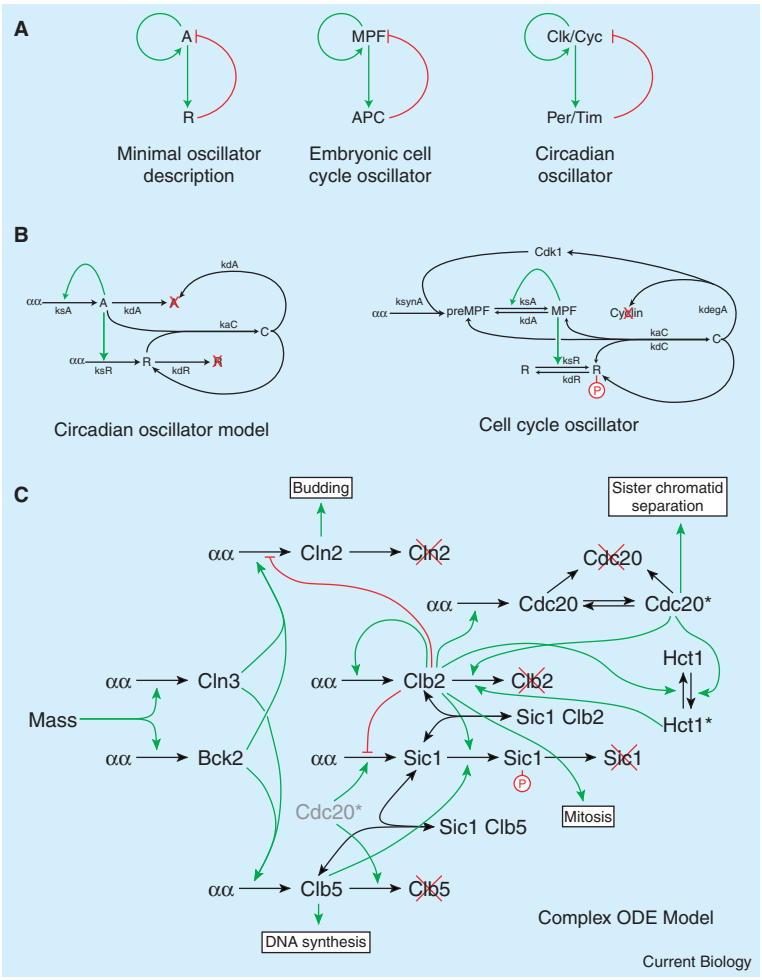

Figure 1. Different levels of modeling.
(A) Minimal logical representations of three oscillators. From left to right, a generic oscillator, the embryonic cell cycle, and a circadian clock. (B) The reaction schemes of two simple models: Barkai and Leibler's [26] reaction schemes for a circadian oscillator, and a minimal cell cycle. The kinetic parameters of the models are listed by the corresponding equations, and an individual reaction can in reality be the sum of several reactions. Both of these models produce limit cycles over a wide range of parameter values. (C) A biochemically realistic representation of the cell cycle oscillator from [26]. This scheme requires 72 parameters used in ordinary differential equations (ODEs), but even at this level of complexity omits details of the reaction scheme, such as the role of at least seven proteins — Esp1, Cdc5, Cdc14, Cdc15, Tem1, Lte1, Spo12 — as intermediates between Cdc20 and the activation of Hct1.

oscillator fires and serve to drive it back to its initial state [19–21].

While there are other general network structures capable of producing oscillations, such as a circle of three repressors where each component inhibits expression of the next [22], this two component architecture appears in the cell cycle and circadian oscillators. The evolutionary histories of these systems are not well understood. One possible explanation for their similarity is that both evolved from systems that fulfilled many of the same functions but were not capable of free-running oscillation. While checkpoints are dispensable for an unperturbed cell cycle, a primitive eukaryote may have relied exclusively on checkpoint-like inhibition to order the steps of the cell cycle. The Cdk and cyclins may have been transcriptional regulators in this earlier system. Some of the closest paralogues of the Cdk are kinases which modify RNA polymerase II in order to control initiation and elongation [23], and cyclins are structurally similar to the basal transcription factor TFIIB as well as the retinoblastoma transcriptional regulator [24,25]. Similarly, an ancestral circadian regulatory system might have induced changes in gene expression in direct response to changes in light intensity, without being capable of sustained oscillations in the absence of light–dark cycles.

In both cases, the hypothetical ancestral system responds in a straightforward manner to internal or external signals that happen to be cyclic. Changes that produced a light-entrained oscillator allowed cells to improve their efficiency by predicting the sun's rise rather than having to wait for it, and a second mechanism of ordering events in the cell cycle reduced the chance of mistakes in chromosome replication and transmission. Thus, the emergence of truly oscillatory systems from these similar ancestral states may explain the analogous mechanisms seen in modern organisms.

Another possibility is that the fundamental architecture seen in these two oscillators is the one most likely to be produced and maintained over the course of evolution, rather than being an accidental consequence of the particular evolutionary history of these two systems. Mathematical modeling could be particularly suited to addressing this possibility. A cell cycle oscillator that was not robust against intrinsic fluctuations and environmental disturbances would clearly be at a substantial disadvantage. In other systems, including the circadian clock, modeling can quantify the robustness of particular systems, and thus could be used to compare different architectures [26–28].

Experiments may also provide insight into robustness. The designed oscillator ('repressilator') of Elowitz

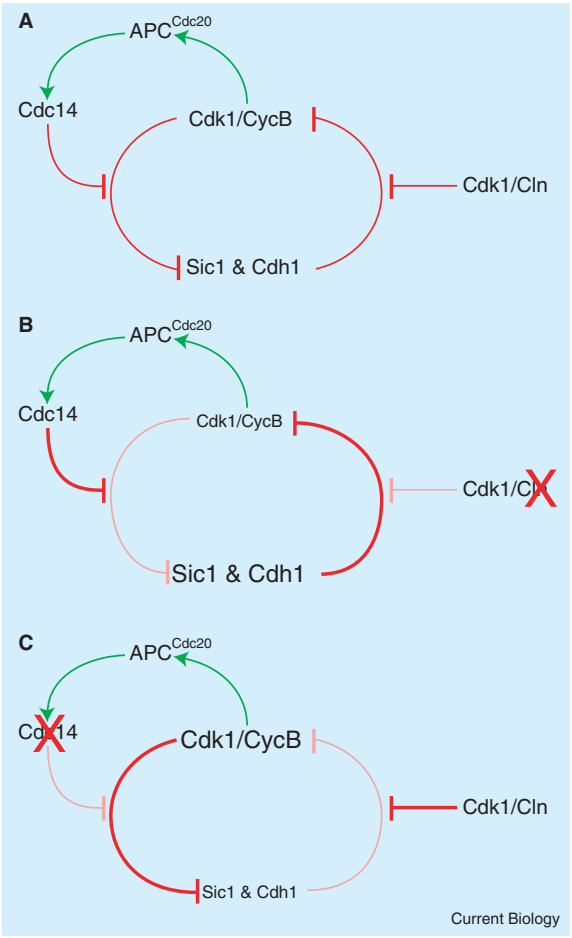

Figure 2. Hysteresis in the cell cycle oscillator.
(A) A simplified scheme showing the mutual antagonism between active Cdk1–cyclinB complexes (MPF) and Sic1, which binds to this complex stoichiometrically and inhibits it, and Hct1 an activator of the APC, which induces the ubiquitination and ultimate destruction of cyclin B. The system is switched between states by G1 cyclins (Cln) which activate Cdk1 to phosphorylate Sic1 and Cdh1, leading to the destruction of Sic1 and inhibition of Hct1, and by the activation of Cdc14, which removes these inhibitory phosphate groups. (B) In the absence of the Clns, the system is locked in a state where Sic1 and Cdh1 are active and Cdk1 is not. (C) Conversely, when Cdc14 is absent, the system is locked in the opposite state, where Cdk1 is active, and Sic and Cdh1 are not.

et al. [22] was much less robust than the cell cycle or circadian oscillators. This may be a consequence of the structure of this oscillator, which lacks the hysteretic switch seen in the cell cycle machinery, or it may simply reflect the extent to which the cell cycle has been refined over the course of evolution. The absence of natural repressilator-like oscillators supports the idea that they are intrinsically less robust.

Systems which are robust to stochastic and environmental fluctuations are also less sensitive to changes in quantitative parameters such as affinities and reaction rates. These parameters are typically consequences of protein structure and are thus genetically encoded. When a wide range of parameters is capable of producing oscillatory behavior, it will be easier for random mutations to generate an oscillatory system than if fine tuning of parameters is necessary. Mathematical tests of robustness are thus useful for understanding what systems can be more easily discovered by evolution as well as which can robustly provide an essential function such as the cell cycle.

Bistability and Hysteresis

Modeling can also be a powerful approach when it is able to discover novel qualitative behavior in the system under study. While the oscillatory behavior of the cell cycle is well established, more detailed aspects of the oscillations are less well understood. Bistability in the cell cycle oscillator, which yields abrupt and irreversible transitions between different phases of the cell cycle, has been a major focus of recent modeling efforts. The teleological importance of this kind of transition in the cell cycle is clear. DNA replication and mitotic chromosome condensation, for instance, must not occur at the same time, and the cell must complete mitosis once sister chromatids have separated.

Abrupt transitions between qualitatively different cell states can result from bistability, in which the oscillator has two possible stable states and can switch between them. A bistable system will display history dependence, or hysteresis — in some situations, either state would be stable, and the system will remain in whichever state it was most recently placed. Hysteretic behavior was first explicitly discussed by Novak and Tyson [29], although the proposal that the cell cycle had different stable states had been made earlier and without reference to mathematical modeling [7]. This mechanism has subsequently been explored in mathematical models of the budding yeast cell cycle, where the mitotic cyclin–Cdk1 complex is known as Clb–Cdc28p [30]. Newly produced Clb–Cdk1 complex is inactivated, but the inactivating proteins are themselves blocked by active Clb–Cdk1, which in turn allows the activation of more Clb–Cdk1 complex (Figure 2). Thus, both low and high levels of Clb–Cdk1 activity are self-perpetuating situations. External perturbations can switch the system between these two states, and they will persist after the perturbation is removed. The switch between the two states will be rapid, and there is no possibility of remaining at an intermediate level of Clb–Cdk1 activity.

Using the modeling results as a guide, this hysteresis has been demonstrated experimentally. Cross *et al.* [31] used conditional alleles of the key cell cycle genes in budding yeast, *CLN3* and *CDC14*, to show that they represent separable systems that activate or inactivate Clb–Cdk1. The authors found that, in the absence of either gene product, Clb–Cdk1 could remain low or high, depending on its activity at the time when cells were moved to restrictive conditions.

Models of early embryonic cell cycles also show hysteresis in the activation of the homologous cyclin B–Cdk1 complex [29]. This work predicts that a higher threshold level of cyclin B activity will be required for mitotic entry than for maintenance of mitosis, which is a consequence of hysteresis. The models also predict a dramatic slowing of mitotic entry when levels of cyclin B are just above the threshold level needed for mitotic

entry, which is a manifestation of a general phenomenon known as critical slowing-down. These qualitative predictions have been confirmed experimentally in *Xenopus* egg extracts, supporting the proposed mechanism as well as demonstrating that this is a highly conserved aspect of the cell cycle [32,33].

Coupling Growth and Cell Division

The cell cycle is governed by a size threshold which couples the continuous process of cell growth with discrete processes of duplication and segregation. There is likely to be a quantitatively interesting mechanism by which cells reliably determine whether they have achieved a threshold size. Early studies of this mechanism [34,35], relying on experimental studies of *Physarum*, proposed the existence of an unstable activator which is synthesized at a rate proportional to cell volume and sequestered by nuclear sites such that a threshold level of activator is set directly by DNA content. Related models [34,36] suggested that the activator is degraded at a rate proportional to its nuclear concentration. At this early stage, mathematical models were used to rule out several alternative mechanisms for cell size determination. It was also recognized that this activator would need to control an 'ultrasensitive trigger', because the change in activator concentration through the cell cycle was relatively small; this was a particular concern in the large, multinucleate *Physarum* plasmodia, which undergo a synchronous mitosis [34].

After the discovery of cyclins and Cdks, cyclins were recognized as good candidates for being the unstable activator whose accumulation triggers cell cycle transitions. Genetic studies in both budding and fission yeast have implicated the cyclins, along with other proteins that affect cyclin–Cdk activation, in the cell size threshold. Current mathematical models of the budding yeast cell cycle, such as that of Chen *et al.* [30], incorporate these components, but do not focus on the mechanism of size determination. In these models, the levels of the G1 cyclin Cln3p as well as Bck2p, a poorly understood protein that is partly redundant with Cln3p, are controlled by cell size. The rate of protein synthesis is proportional to cell mass at all points in the cell cycle, at least in budding yeast [37], so this is a plausible mechanism for size measurement. Furthermore, altered gene dosage of *CLN3*, which would be expected to affect the rate of Cln3p accumulation, alters the cell size threshold in budding yeast, though the alteration is less than is predicted by the model [31].

In fission yeast, cell size is regulated primarily at mitotic onset rather than entry into S phase. In models of the fission yeast cell cycle, the B-type cyclin Cdc13p accumulates at a rate proportional to cell size and localizes in the nucleus, the volume of which is a function of DNA content, so steady-state levels of Cdc13p directly report on the ratio between cell size and nucleus size [38]. There is a threshold level of Cdc13p needed to begin mitosis, set by the mutually antagonistic interactions of Wee1p and the Cdc2p–Cdc13p complex. These interactions ensure that the relatively small change in the synthesis of the unstable activator Cdc13p is transduced into a large change in Cdc2p–Cdc13p activity. This model is clearly wrong in some respects, as mild over- or underexpression of *Cdc13* has little effect on cell size, whereas altering the ratio of the doses of *Cdc25* and *Wee1* has profound effects [39,40].

The fission and budding yeast models both suppress many of the interesting questions about cell size regulation. Cell size is measured by the rate of synthesis of an unstable protein. Brief treatment of fission yeast with the translation inhibitor cycloheximide causes a delay in mitotic entry, and this delay is longest immediately before the commitment to mitosis [41]; a similar effect observed in *Physarum* was the initial basis of the unstable activator model [35]. The cell size threshold cannot simply measure protein synthesis capacity, however. Growth of budding yeast in the continuous presence of cycloheximide, which significantly reduces the protein synthesis capacity per unit mass, can dramatically lengthen the G1 phase of the cell cycle, but does not significantly alter the cell size threshold for start [42,43].

Cell size determination is robust against such changes, but responsive to physiological stimuli such as nutrition. When growth rate is reduced by limitation of carbon or nitrogen sources, the size thresholds are reduced in both budding and fission yeast [44,45]. The cell size threshold seems to respond directly to the rate of metabolism, as cells entering S phase are larger at higher rates of glucose consumption [46]. However, recent studies [47] indicate that transcription of *CLN3* and *BCK2* is induced by glucose, which should decrease the size threshold by raising the amount of Cln3p per unit of protein synthesis capacity. Cell size measurement clearly involves more than the simple accumulation of Cln3p, and it has been suggested that nutritional status is regulating the level of Far1p, an inhibitory protein that binds to and inhibits Cln3p (L. Alberghina, personal communication); a full understanding may require a quantitative accounting for cell growth and protein synthesis.

Integrating Regulatory Signals

Cell size is not the only factor that regulates the timing of division. Even in unicellular organisms, cell cycle progression can be affected by intercellular signals, such as mating pheromones in yeast; in animal cells, it is regulated by a bewildering array of growth factors. Intracellular signals, such as checkpoints or nutrient availability, can also influence the cell cycle. DNA replication and mitosis are intrinsically risky, as is a long-term arrest in G2, which can lead to a second round of DNA replication in a single cell cycle. These factors may explain why cells use G1 to assess whether they have the nutrient stores and appropriate internal state — not in the middle of the stress response — to successfully commit to both events. This assessment requires them to integrate multiple signals to make a single discrete decision about whether they should proceed through the cell cycle. While commitment to the cell cycle is a binary decision for each cell, the integration of various signals might control a probability of cell cycle entry independently for each cell. This stochastic process could yield a graded response at the level of the popu-

lation. In multicellular organisms, this would allow a tissue to produce a varying response to growth factors.

Interaction between different signals has been shown in fission yeast. This organism has a threshold size for exit from G1, though it is usually obscured because cells large enough to enter mitosis will produce daughters that are already larger than this threshold. Cells that would normally be big enough to leave G1 can be kept from doing so by exposing them to mating pheromone. Once cells reach a threshold size, however, they overcome pheromone-induced arrest, so that pheromone has the effect of increasing the threshold size for cell division rather than completely inhibiting G1 exit [48]. This is a non-trivial interaction between two cell division regulators — neither signal entirely overwhelms the other. In *Xenopus* egg extracts, the threshold level of cyclin B needed to enter mitosis is increased by the presence of unreplicated DNA [32]. Cell cycle regulation that is transduced by the alteration of cyclin B levels might interact with this unreplicated DNA checkpoint.

Many organisms adapt to stimuli. Budding yeast cells that cannot repair DNA damage arrest for many hours in G2 but eventually divide [49]. Such adaptation could be due to alterations in the signaling pathway that gradually reduce the output produced by a constant signal, or a constant output from the pathway that is eventually overwhelmed by a continually increasing parameter such as the total amount of cyclin B in the cell. Modeling these two alternatives could inspire experiments to distinguish between them, but has so far been restricted to showing that one of these alternatives can reproduce the phenotype of known mutants [50].

One general question about such a situation is whether the interaction between the two stimuli is physiologically relevant to the organism. If the combination of signals was not important in the evolutionary history of the organism, then the response would be an accidental feature of the mechanism by which the individual signals affected the cell cycle, rather than a selectively advantageous response to the cell's situation. Even in the former case, though, the interaction can illuminate the means by which the signals affect the cell cycle. The impact of unreplicated DNA on the threshold level of cyclin B needed for mitotic entry is consistent with the fact that the checkpoint kinase negatively regulates the Cdk-activating phosphatase Cdc25p and positively regulates the Cdk-inactivating kinase Wee1p. These proteins control the threshold of the hysteretic switch for mitotic entry, so modifying their activity should change the threshold.

Final Thoughts

How much has modeling changed our understanding of the cell cycle? At one level, the answer is surprisingly little. It has served as a consistency test, made the importance of properties such as non-linearity explicit, and suggested the existence of phenomena like hysteresis, although both non-linearity and hysteresis had been discussed by biologists with a naive, but intuitive grasp of dynamical systems. But, even at this level, there are important consequences of modeling. The most important is forcing ideas and assumptions to be stated explicitly and precisely, thus distinguishing what we know from what we think or would like to know. A second advantage is that modeling quickly connects observation to theory. For example, a model that predicts bistability will also predict phenomena such as hysteresis and critical slowing down. However, these other aspects of a bistable system might have been similarly understood, albeit in an *ad hoc* manner, if they were necessary to explain biological observations.

To be successful, modeling must stimulate experiments. For the last 25 years, cell cycle models have been successively updated to reflect the results of experiments, instead of inducing experiments designed to test their predictions. There are signs of change. For example, modeling led to the notion that there are two oscillators that can drive the cell cycle, one of which acts by degrading cyclins and the other by degrading covalent inhibitors of cyclin–Cdk complexes. Normally these work as tightly linked components of a single robust oscillator, but with the appropriate mutations, experiments reveal that either will suffice to drive the cell cycle. [51].

There are important reservations about models that attempt to include all of the molecules known to regulate the cell cycle. The ability of such models to predict the results of some experiments is taken as support for the model, as opposed to the failure to meet some predictions being taken as proof that the model is wrong. This unusual stance appears to be justified by two appeals. The first is that there are components we have yet to discover and whose incorporation into the model would lead to a better match between theory and experiment. The second is that, as the number of experimentally unknown parameters in a model rises, the fraction of all possible parameter combinations that can be explored by simulation falls dramatically. Thus it becomes harder to exclude the possibility that there is a set of parameters for which the model makes all of the correct predictions.

The obvious danger is that if the models are infinitely adjustable, they can no longer be proved false. Our opinion is that theorists and experimentalists must decide whether they are willing to explore the biochemistry and the phenomenological properties of the cell cycle in the detail that has been lavished on bacterial chemotaxis. Some have already begun such work [31], and to the extent that they are successful, their data will constrain detailed models to the point they can be proven false and force them to make quantitative rather than qualitative predictions.

Modeling may also be able to shed light on questions that are experimentally difficult — for example, by explaining why the cell cycle looks the way it does. We have argued that the cell cycle oscillator and circadian clock share a common organization. One possibility is that this structure had three advantages, the ability to make an oscillator out of as few as two proteins, the ability to derive these molecules by the duplication and divergence of existing genes encoding kinases and transcription factors, and the ability of a wide range of parameter sets to support oscillation. Can simulation and analysis support these arguments? Can modeling

help us to explain what was wrong with other patterns of molecular interactions that could produce oscillations, and can they explain the detailed differences between the topologies of the cell cycle and circadian clocks? Can the themes and variations in the eukaryotic cell cycle be rationalized? For example, the cell divisions of within an individual embryo occur with remarkable synchrony, but adjacent embryos may show considerable asynchrony. Does this simply reflect cells whose size makes stochastic variations in protein concentration negligible or is something more interesting afoot? Only time, equations, algorithms, and experiments that are designed to test them will tell.

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