Mechanistic modeling of mitosis: Insights from three collaborative case studies

Jing Chen[1,2,3,4*] and Daniela Cimini[1,2,4*]

[1]Department of Biological Sciences, Virginia Tech

[2]Fralin Life Sciences Institute, Virginia Tech

[3]Center for Soft Matter and Biological Physics, Virginia Tech

[4]Center for the Mathematics of Biosystems, Virginia Tech

* Corresponding authors: chenjing@vt.edu (J.C.), cimini@vt.edu (D.C.)

Abstract

Mechanistic mathematical modeling has become an essential tool in modern biological research due to its powerful ability to integrate diverse data, generate hypotheses, and guide experimental design. It is particularly valuable for studying complex cellular mechanisms involving numerous interacting components. While the full dynamics of such systems usually elude direct experimental observation, modeling provides a means to integrate fragmented data with reasonable and/or informed assumptions into coherent mechanistic frameworks, simulate system behavior, and identify promising directions for further experimentation. When closely integrated with experiments, modeling can greatly accelerate progress in cell biology. However, the value of modeling is not automatic—it must be earned through careful model construction, critical interpretation of results, and thoughtful design of follow-up experiments. To demystify this process, we review three of our collaborative projects in mitosis, drawing on our experiences as a modeler and an experimentalist. We describe how the projects were initiated, why specific modeling approaches were chosen, how models were developed and refined, how model predictions guided new experiments, and how integrated modeling and experimentation led to deeper mechanistic insights. Finally, we emphasize that at the heart of every successful collaboration lies human connection. Productive cross-disciplinary communication is fundamental to bridging experimental and modeling perspectives and fully realizing the potential of integrative approaches in modern cell biology.

Keywords

Mathematical modeling, mechanistic modeling, biophysical modeling, mitosis, spindle assembly

Introduction

Mechanistic mathematical modeling has become an increasingly powerful tool in biological research. Carefully constructed models integrate diverse datasets, offer a coherent framework to depict complex biological systems, formalize new hypotheses, and guide experimental design [1-4]. Close integration of experimental studies with modeling expedites quantitative research of complex biological mechanisms. A typical modeling workflow involves several key steps: identifying a biology question suitable for modeling, defining knowns and unknowns, formulating mathematical equations, parametrizing and calibrating the model using experimental data, making predictions for experimental testing, and importantly, revising the model in light of the discrepancy between model predictions and experimental data. An iterative cycle of modeling and experimentation is usually the best way to devise the most predictive and useful model.

Several misconceptions about mechanistic modeling persist. Von Neumann's famous remark, "With four parameters I can fit an elephant, and with five I can make it wiggle its trunk" [5], is often cited to suggest that overly flexible models can be made to fit any dataset. However, this remark should not be taken to mean that any model including more than a handful of parameters inevitably overfits. In practice, even models with many more parameters can fail to fit biological data. This issue, known as model misspecification, arises when a model lacks essential components needed to capture the system's true behavior [6-9]. From a mathematical perspective, a model defines a constrained subspace of possible outcomes; if empirical data fall outside this subspace, no parameter tuning will achieve a good fit. While this may seem like a failure, such cases often present exciting opportunities to discover unknown biological mechanisms, serving as a catalyst for deeper experimental and theoretical investigations. A related issue is lack of robustness—when a model fits only within an extremely narrow parameter range. Given that biological systems exhibit noise and variations, such fragility suggests that the model may be missing critical components that ensure robustness of the biological mechanism and/or function [10-16].

On the other end of the spectrum lies the model identifiability problem [17-21]: the data may be fitted equally well by multiple model structures or by a model through multiple parameter sets, making it unclear which model structure or which parameterization best represents the biological system under study. The situation in which multiple model structures can fit the data is also known as model degeneracy [21-24]. Identifiability issues with either model structure or parameters suggest insufficient information compared to the unknowns to be determined. Resolving the issue, therefore, requires additional data or proper model reduction. Several common practices include: (i) seeking more data or incorporating more existing knowledge to further constrain the model, (ii) using nondimensionalization to

consolidate functionally related model parameters and reduce the number of free parameters, and (iii) performing parameter sensitivity analysis to identify and eliminate model components or parameters that contribute little to the model's behavior and/or fitting to data. More complex methods for evaluating and coping with identifiability problems were discussed in prior work [17, 18, 20, 22, 25].

Although the above issues are well known in the field of modeling, there are no standard recipes for solving them. Trial and error is indispensable *en route* to a final solution. In this article, we will review a few collaborative projects on mitosis to showcase the real-world thought process behind mechanistic modeling. Through these discussions, we aim to offer insights into the best practices for constructing and interpreting mechanistic models in biological research.

Mitosis, namely the cell division process in eukaryotic cells, is the foundation of eukaryotic life. Malfunctioning of mitosis underlies diseases like cancer and developmental disorders; hence, mitosis has attracted intensive studies from the beginning of modern biology. Mitosis is an exemplary complex biological system: to successfully divide and pass along its genetic material, the cell exploits hundreds of structural, mechanical, and signaling molecules that interact in a complex and dynamic fashion. Although decades of experimental studies have revealed the roster of molecular players and the basic functions of most mitotic molecules, it remains unclear how the dynamic interactions among them are orchestrated to achieve the mitotic process in normal and aberrant scenarios. These dynamic interactions are difficult to study using experimental approaches alone because it is impossible to simultaneously track the activities of every molecular species involved. Experimental data are often limited to fragmented snapshots of the system, and as such, they do not provide comprehensive mechanistic insights. This issue can find remedies in mathematical modeling, where fragmented data are cemented together by appropriate assumptions based on existing knowledge in physics, chemistry, and biology, and a coherent mechanistic picture of the complex dynamics is formulated. Nevertheless, caution must be exercised in the process of developing models, interpreting model results, and inferring information for future experimental design.

The following sections will elaborate on three major projects we have undertaken in recent years: (i) centrosome number evolution in newly-formed tetraploid cell populations [26], (ii) centrosome clustering and bipolar mitotic spindle formation in cells with supernumerary centrosomes [27], and (iii) mitotic spindle assembly checkpoint (SAC) signaling and its relation to cell size, spindle size, and spindle architecture [28]. We will specifically describe how each project started, why specific types of modeling were considered, how models were designed and evolved, what we learned from the models, how models suggested new experiments, and how modeling and experimentation together elucidated complex mitotic mechanisms. Notably, unlike some biology areas that involve big data and benefit from data science

approaches like machine learning, most of our projects involve experimental data sets that are not very large but vary in nature. In our modeling efforts, we have to combine quantitative data and qualitative observations with fundamental laws of physics and chemistry. Through these discussions, we hope to demystify the process of developing useful mechanistic models to address questions that arise from experiments, and ultimately, how to take advantage of mechanistic modeling in modern cell biology research.

Modeling evolution of newly-formed tetraploid cells

Tetraploidization, the process through which a cell acquires a duplicated genome, is strongly implicated in cancer and is believed to occur at some point during progression of ~30% of all human cancers [29, 30]. The following causative link between tetraploidization and cancer had been speculated: tetraploidization is most often caused by cell cycle dysfunctions (e.g., cytokinesis failure, endoreplication, mitotic slippage) that not only double the genome but also double the number of organelles in the cell, including the centrosomes that become the mitotic spindle poles in dividing cells; subsequently, tetraploid cells assemble multipolar mitotic spindles, which often leads to chromosome segregation errors and aneuploidy in the daughter cells, even if the multipolarity is only transient [31, 32]; because aneuploidy and chromosomal instability are known to play a key role in cancer progression, the excess centrosome numbers were postulated to be a major driver of tetraploidy-linked tumorigenesis. Contrary to this theory, however, several experimental groups, including the Cimini lab, observed loss of extra centrosomes in cultured tetraploid cell clones [26, 32-36]. Moreover, it was reported that multipolar mitoses often do not produce viable progeny [32, 37]. The Cimini lab specifically reported a gradual decrease in the fraction of cells with extra centrosomes over the course of 12 days after experimentally-induced tetraploidization (Figure 1A) [26]. They further found that bipolar cell division achieved through centrosome clustering is much more likely to produce viable progeny than multipolar division, and depending on the cell line, 30-40% of the bipolar divisions underwent asymmetric centrosome clustering (i.e., three centrosomes at one pole and one centrosome at the other) [26]. We hence hypothesized that asymmetric centrosome clustering allows some of the daughter cells to inherit the normal centrosome number (one centrosome during interphase and two during mitosis) while maintaining a near-tetraploid chromosome number, and these cells may eventually take over the majority of the cell population.

Because it is difficult to continuously track cell lineages over many generations in the experiment, to test her hypothesis, Cimini sought help from Chen to build mathematical models for the evolution of centrosome numbers in the cell population (Figure 1B). Chen chose a model type that was best suited for the experimental data from the Cimini group. As the numbers of cells in the cell cultures were large (typically ~10^6), Chen ignored random effects and constructed a deterministic ordinary differential

equation (ODE) model for the population dynamics in a cell culture. Because the experiment tracked only the fractions of cells with normal vs. supernumerary centrosomes in continuously growing cell populations, the variables of the ODE model were also chosen to be the fractions, instead of the absolute numbers, of cells with various centrosome counts. Additional assumptions were introduced based on experimental observations. For example, for simplicity the model only considered cells with 2, 4 or 6 centrosomes during mitosis (Figure 1B). This assumption was supported by the rare observation of cells with very high centrosome numbers and the speculation that these cells were either incapable of mitosis or produced inviable progeny. Finally, the model was parametrized as much as possible using experimental data. In fact, all parameters were either directly measured or believed to vary within a narrow range, as suggested by other experiments (for example, the cell division rate was chosen within 20% of $1d^{-1}$). This allowed a highly constrained fitting of the model to the experimental data.

What did we find from the model? First of all, the model confirmed the initial hypothesis. Indeed, it predicted a gradually diminishing fraction of cells with extra centrosomes in the growing cell population (Figure 1C), because cells inheriting one centrosome from asymmetric centrosome clustering of bipolar divisions were more successful in the following mitoses and hence had a selective advantage over cells with extra centrosomes. However, the quantitative fit was off. The model predicted a much faster decrease and a much lower final steady-state fraction of cells with extra centrosomes than experimentally observed (Figure 1C). This discrepancy suggested unresolved subtleties in the population dynamics. Through a parameter sensitivity analysis, Chen found that the final steady-state fraction of cells with extra centrosomes is strongly constrained by the probability of spontaneous cytokinesis failure during cell division. This was expected: since spontaneous cytokinesis failure was the only source of new cells with extra centrosomes in the model (Figure 1B), the steady-state fraction of cells with extra centrosomes should be governed by the generation rate of this cell subpopulation. However, the spontaneous cytokinesis failure had been experimentally measured to be ~2.5% [38], and hence it was a constrained parameter in the model. Any value within the reasonable range of measurement error around 2.5% could not resolve the discrepancy. Therefore, we deduced that some additional mechanism(s) must contribute to maintaining the elevated population of cells with extra centrosomes.

The quantitative discrepancy between the model and experimental data motivated the next hypothesis: some cells with extra centrosomes may be extra efficient at clustering their centrosomes symmetrically, which would maintain a more stable line of progeny with extra centrosomes (Figure 1D, H). With such a "super-clustering" cell subpopulation added, the model successfully fitted the data (Figure 1E). This refined model also predicted that, by Day 12, ~90% of the cells with extra centrosomes were "super-clustering" cells (Figure 1F). This prediction was verified by experimental results showing that cells with

extra centrosomes taken from the Day 12 population had a much higher rate of bipolar mitotic spindles (90%) than those from the Day 0 population (28%) (Figure 1G).

Now, does the success of the new model assumption truly mean that the cells present two phenotypes with distinct capabilities of centrosome clustering as illustrated in Fig. 1H? The answer is uncertain. What is probably more likely is multiple phenotypes or even a continuous spectrum of phenotypes with different centrosome clustering capabilities. Phenotypic conversion may also happen. These extra subtleties cannot be effectively distinguished using the existing cell tracking data, which only counted centrosome numbers over time. In other words, this presents a model identifiability issue given the available data. Continuous tracking of individual cells [26] may help resolve the subtle mechanism, but the small fraction of super-clustering cells could limit our ability to collect sufficient data.

This project exemplifies how a model can be used to quantitatively test hypotheses and generate insights to motivate new hypotheses. In this example, the model misspecification issue revealed subtle mechanisms that one could not think of without modeling. The rapid success of this project mainly depended on the availability of solid experimental measurements for all parameters in the first iteration of the model, which allowed for a strongly constrained fitting to the data. Otherwise, we would have needed to design experiments to collect new data to constrain the parameters in the model, or we would have had to stop at the qualitative conclusion that cells with a normal centrosome number have selective advantage over cells with extra centrosomes. Furthermore, we discussed how variable interpretations could be associated with the second iteration of the model. When drawing conclusions, one must remain aware of alternative interpretations and avoid taking model predictions in their literal form.

Modeling centrosome clustering and bipolar spindle formation

As discussed in the previous example, forming a bipolar spindle through centrosome clustering is a key cellular process that boosts the viability of multicentrosomal mitosis [39-42] and influences the evolution of centrosome numbers in a population of newly-formed tetraploid cells [26]. As such, centrosome clustering is crucial for tetraploidy-driven tumorigenesis [29, 30]. Given the widespread presence of extra centrosomes in cancer cells, inhibiting centrosome clustering has been proposed as a novel cancer therapeutic strategy [39, 43, 44]. However, centrosome clustering is controlled by numerous cellular and microenvironmental factors [40, 41, 45-51], and more importantly, the factors that mediate centrosome clustering also play essential roles in the spindle assembly of normal cells. A thorough mechanistic understanding of centrosome clustering is therefore necessary for developing effective cancer therapies with minimal side effects, and mathematical modeling is particularly useful for studying such complex dynamics from a systems perspective.

It is tempting to study multicentrosomal spindle assembly and centrosome clustering using agent-based models (ABMs), which resolve individual spindle components such as microtubules, molecular motors, chromosomes, and centrosomes. Indeed, ABMs have been developed for normal spindle assembly to interrogate various aspects of the process, such as centrosome separation [52, 53], microtubule alignment [54-57], microtubule-chromosome attachment [58, 59], and metaphase chromosome oscillation [60, 61]. However, because ABMs incorporate numerous molecular details, they require a large number of unknown or uncertain parameters, and are computationally expensive. As a result, systematically characterizing the behavior of ABMs through parameter sweeping is challenging. Furthermore, the complexity of an ABM can sometimes obscure mechanistic insights—analogous to how a 1:1 city map fails to reveal the connectivity between districts or how to improve traffic flow. Before constructing an ABM with the risk of getting lost in excessive detail, we reasoned that it would be helpful to first establish a foundational understanding of the basic governing principles behind centrosome clustering.

To distill the fundamental principles of centrosome clustering, we developed a parsimonious biophysical model that focuses solely on the centrosomes [62]. In this model, centrosomes move within the cell, driven by interactive potential energies between pairs of centrosomes and a radial potential energy that keeps them within the cell boundary but outside the central region (Figure 2A, B). Strictly speaking, mechanical interactions between cellular structures like centrosomes and chromosomes are not conservative forces and cannot be rigorously depicted by potential energies. Even so, potential energies are often used for convenient phenomenological approximation of the expected mechanical interactions. In our model, the inter-centrosomal potential energy essentially represents the sum of forces acting between centrosomes through molecular motors and/or microtubule dynamics. These forces can be transmitted via various direct and indirect microtubule connections (e.g., via a chromosome) between centrosomes. The inter-centrosomal potential energy is assumed to be a concave function of the inter-centrosomal distance (Figure 2A)—implying that centrosomes attract each other when in close proximity and repel each other when far apart. Meanwhile, the radial potential energy captures additional forces acting on centrosomes that constrain them within the cell while repelling them from the chromosome mass, as typically observed (Figure 2B) [63]. The repulsive component of this radial energy accounts for the combined effects of polar ejection forces, steric repulsion from chromosomes, and dynein-mediated cortical pulling [64, 65].

Our parsimonious model revealed several fundamental principles underlying bipolar spindle formation [62]. Specifically, it predicts biophysical conditions that not only promote centrosome clustering but also enable the recovery of bipolar spindles from monopolarity. First of all, the concave dependence of inter-centrosomal interaction on the inter-centrosomal distance, where long-distance repulsion is slightly

stronger than short-distance attraction (Figure 2A), is essential for promoting centrosome clustering while preventing monopolarity. This property is also necessary for a normal mitotic cell with two centrosomes to successfully separate its centrosomes. Additionally, the model predicts that bipolar spindle formation is favored by a combination of factors, including a balance between inter-centrosomal attractive and repulsive forces, cell rounding, small cell size, fewer centrosomes, and appropriate levels and time scales of random fluctuations in inter-centrosomal forces. These model predictions qualitatively align with many experimental observations [26, 62, 66-80], and provide a unified energetic perspective to explain them (Figure 2C).

Notably, this theoretical framework brings a unique and unexpected perspective on factors beyond inter-centrosomal forces, such as cell rounding and cell size. Because our inter-centrosomal potential energy has a slight bias toward long-distance repulsion (to ensure centrosome separation in normal cells with two centrosomes) (Figure 2A), the naïve energetically favorite state of the system is complete centrosome scattering (Figure 2D, top left). However, in a small space, full scattering at the inter-centrosomal distances favored by the repulsive energy well is not possible (Figure 2D, bottom left). In this case, centrosome clustering rises as the most energetically favorable state (Figure 2D, bottom right). Since cell rounding and small cell size limit the maximum possible scattering of centrosomes, both factors promote centrosome clustering. This theory further suggests that inter-centrosomal mechanical interactions likely need to vary and adapt to accommodate cell types of vastly different sizes. These insights from the parsimonious model provide valuable guidance for selecting appropriate components and parameters in future, more detailed ABMs for spindle assembly, as well as for future experimental and translational studies of centrosome clustering.

In this project, experiments from the Cimini lab provided essential data for both model parameterization and validation. Specifically, measurements of the time required for a normal mitotic cell with two centrosomes to initiate and complete centrosome separation allowed calibration of the inter-centrosomal energy potential [62]. With this parameterization, we found a “sweet spot” for random fluctuations of the inter-centrosome potential energy: stable bipolar spindle formation is most likely when these fluctuations occur on a timescale of tens of seconds. This predicted optimal timescale matches that of the expected fluctuation sources, such as microtubule instability and the binding/unbinding dynamics of microtubule-associated motors, suggesting an important functional role of these dynamics in spindle assembly. Moreover, the Cimini lab examined isogenic tetraploid cell clones of different mean sizes [28], and validated the model prediction that smaller cells are more effective in bipolar centrosome clustering [62]. This experimental system provided a rare opportunity to isolate the effects of cell size; otherwise,

different cell sizes are typically associated with different cell types, which would change other parameters in unknown ways, making it difficult to examine the impact of cell size alone.

This project underscores the importance of selecting an appropriate modeling approach based on the available information about the system of interest. If we had chosen to develop an ABM, it would have been rather difficult to identify proper parameter sets for effective bipolar clustering in the first place, and certainly more difficult to explore through the parameter space to discover the general principles behind bipolar spindle formation. Note that in our parsimonious model, most parameters represent lumped effects and cannot be measured directly. While experimental calibration constrains certain timescales and parameter relationships, extensive parameter sweeps and phase diagrams are necessary to systematically explore the model behavior. Consequently, the most valuable insights from our model are qualitative rather than quantitative. Additionally, this project illustrates that a well-designed experimental system is crucial for providing key data to constrain and validate the model as much as possible.

Modeling spindle assembly checkpoint silencing and its relation to spindle architecture and cell size

Mitotic spindle assembly not only relies on mechanical interactions between spindle components but also on a surveillance mechanism known as the spindle assembly checkpoint (SAC), which ensures proper bipolar attachment of chromosomes to the spindle. SAC signaling is active when one or more chromosomes lack, or have only partial, attachment to spindle microtubules. SAC activity prevents the inactivation of key mitotic kinases, thereby blocking premature mitotic progression and avoiding erroneous chromosome segregation before all chromosomes achieve bipolar attachment [81, 82].

Chen and Cimini became interested in the SAC mechanism from distinct perspectives, and identified a compelling common question for collaboration. Prior to this collaboration, Chen had published several studies on the spatiotemporal regulation of SAC signaling [83-85]. Traditionally, SAC signaling is thought to be mediated solely by kinetochores (the multi-protein structures on chromosomes where spindle microtubules attach), as SAC proteins are concentrated on unattached kinetochores and disappear from attached ones [86-90]. It was believed that unattached kinetochores create an environment that recruits and activates SAC proteins, whereas microtubule attachment to a kinetochore alters this environment and turns off SAC activation [82]. However, SAC proteins have been found to be continuously recruited to attached kinetochores and transported along kinetochore microtubules towards the spindle poles [91-95]. This transport seems unnecessary if SAC silencing is solely mediated by removal of SAC proteins from the kinetochores. What functional role, then, does the poleward transport of SAC proteins play? Furthermore, some experimental evidence shows that inactivation of the SAC signal is initiated at the spindle poles [96-100]. Why are the spindle poles involved in SAC silencing?

To understand how the spatial dynamics affect SAC signaling, Chen developed a mean-field partial differential equation model to recapitulate key experimental observations. Particularly, experimental observations indicate that high phosphorylation at kinetochores promotes recruitment of SAC proteins, and that microtubule attachment and kinetochore tension lower kinetochore phosphorylation [101-105]. Based on these experimental observations, and the fact that poleward transport of SAC proteins originates only from attached kinetochores [91], Chen's model assumed that unattached kinetochores strongly recruit SAC proteins and turn them into a transport-inactive state, whereas attached kinetochores weakly recruit SAC proteins and turn them into a transport-active state (Figure 3A). These coarse-grained assumptions captured the basic observations on the spatial dynamics of SAC proteins.

The model predicted that poleward transport allows SAC proteins to accumulate at the spindle poles in a nonlinear fashion as kinetochore attachments progress, with the final kinetochore attachment causing a sharp increase in SAC protein concentration at the poles [83] (Figure 3B, final attachment occurring at t_0). This behavior arises from the antagonistic but asymmetric influence of attached and unattached kinetochores on the transport activity of SAC proteins. The unattached and attached kinetochores act like two reversed pumps that dynamically convert the SAC proteins in the cell between the transport-active and -inactive states. Before all kinetochores are attached, even a single unattached kinetochore, due to its strong recruitment of SAC proteins, can antagonize multiple attached kinetochores and maintain a low fraction of transport-active SAC proteins. As a result, the proportion of transport-active SAC proteins in the cell stays significantly below 100%. However, once the final kinetochore attaches, the entire SAC protein pool becomes transport-active. Since only the transport-active proteins undergo poleward movement, their accumulation at the spindle poles reflects their global abundance in the cell, which rises sharply following the final kinetochore attachment. If the spindle poles further concentrate SAC-silencing factors that are activated by certain SAC proteins once a threshold level is reached, then the sharp increase would offer a SAC silencing signal with a high signal-to-noise ratio. Taken together, Chen's model proposes that poleward transport of SAC proteins plays a crucial role in robust and timely SAC silencing, and explains why SAC silencing is initiated from the spindle poles.

Meanwhile, as a pioneer in mechanistic studies on spindle assembly errors and aneuploidy [31, 106-108], Cimini had recently focused on the impact of tetraploidization on mitosis and its implications for cancer development [26, 109, 110]. Her lab induced cytokinesis failure to generate tetraploid cells, which were then immediately plated at limiting dilutions to establish single-cell clones. This procedure was applied across several diploid human cell lines. Surprisingly, the resulting tetraploid clones exhibited large inter-clonal variations in cell and nuclear size [28], as well as in mitotic error rates and tumorigenic potential [110]. Interestingly, clones of small cells exhibited longer mitotic durations, primarily due to prolonged

metaphase [28]. Since metaphase duration reflects the time required for SAC silencing after all chromosomes/kinetochores are properly attached to the spindle, classical SAC models would predict that smaller cells silence SAC more rapidly, due to shorter signal diffusion distances from kinetochores throughout the cell. However, Chen's spatiotemporal model for SAC silencing offers an alternative view: the entire spindle is involved in SAC signaling, with the kinetochores encoding their attachment status into the poleward transport intensity and the spindle poles serving as the signal receivers that trigger SAC silencing. Therefore, in addition to cell size, the size and geometry of the spindle must also influence SAC silencing time [83-85].

Indeed, when measurable factors including cell size, spindle length, spindle width, spindle pole size, and spindle microtubule density (Figure 3C) were considered, Chen's model was able to explain most of Cimini's experimental data (Figure 3D, E). For example, in one small-cell clone (S1) with a particularly long metaphase duration, a key contributor was found to be its unusually large spindle poles. According to the model, large spindle poles dilute the polar concentration of SAC proteins, and delay the time required to reach the threshold concentration needed to trigger SAC silencing (Figure 3B). Even though the time for SAC silencing to spread from the spindle poles through the cell is shorter for small cells, the overall time duration between the final kinetochore attachment and complete SAC silencing is much longer (Figure 3D). The model also predicted a broader distribution of metaphase duration for clone S1 (Figure 3B, D), consistent with experimental data (Figure 3E). Overall, the model provides a unified theory that mechanistically links metaphase duration to a combination of cell size and spindle architectural features.

This collaborative project not only underscores the unique perspective offered by Chen's spatial model for SAC, but also provides an indirect validation of the model that had long been sought. Direct experimental testing of the model has been challenging because of the low endogenous levels of SAC proteins. Fluorescent labeling and colocalization of low-level proteins typically require protein overexpression, which can cause ectopic localization and skew quantification. Furthermore, the key signal that triggers SAC silencing at the spindle poles may be mediated by a particular subpopulation of SAC proteins (e.g., a protein in its phosphorylated state), which cannot be detected using standard fluorescent labeling methods. Special labeling methods would require prior knowledge of the precise molecular species to be tracked, which is unknown. Cimini's experimental system circumvents these molecular complexities by comparing metaphase durations among isogenic clonal cell lines, thereby indirectly validating the model. Admittedly, quantitative discrepancies remain between model predictions and experimental data, likely due to unresolved factors in both the model and experiments. Additional experiments and future advances in labeling techniques may be required for further testing of the model.

Moreover, using a larger set of matched cell lines with small and large size [110] can provide more datasets for model validation.

Discussion and concluding remarks

We hope the examples discussed in this review help the readers gain a deeper understanding of how mechanistic models are developed to address complex biological processes, especially when experimental data are scarce and fragmented. Models provide a unique framework to unify disparate data, generate testable hypotheses, and guide future experiments. This is particularly relevant in mechanistic cell biology, where chemical, mechanical, and spatial dynamics are complexly coupled and challenging to track simultaneously in experiments. However, missteps in the modeling process can lead to misleading conclusions. To construct a truly useful model, model assumptions must be carefully crafted to maximally integrate available information, whether through mechanistic or phenomenological representations, and customized towards the specific research questions. Furthermore, interpreting model results requires careful mapping of the model to the biological system, taking into account the model's assumptions and level of abstraction. For example, in our model for centrosome number evolution, variable interpretations could be associated with the "super-clustering" cell in the second version of the model. For another example, the spatial model for SAC only posits the possible effect of the spatial dynamics of SAC, but does not elucidate the specific signaling pathway. As a general principle, it is essential to remain aware of alternative interpretations arising from coarse-grained model assumptions and to avoid accepting model predictions in their literal form without critical thinking.

Modeling contributes most effectively to biological research when tightly integrated with experiments. Successful modeling practices depend on mutual understanding and collaboration between experimentalists and modelers. In our collaboration, we already shared many interests when we first interacted. Cimini performs mitosis experiment from a biophysical perspective, and Chen performs biophysics-oriented modeling of biological systems and had previously worked on SAC spatiotemporal modeling. Cimini understands how to interpret model results and recognizes their limitations. Chen's knowledge in biology enables her to set up biologically sound models, and generate biologically relevant model results. Our shared interests and ability to communicate across disciplines have facilitated productive collaborations and multiple successful projects.

Over time, our collaboration has driven our respective research programs in directions that we would have not taken without the collaboration. Chen's modeling research in mitosis expanded beyond SAC signaling to broader spindle assembly mechanisms. Experimental testing of the SAC spatiotemporal model brought opportunities for further model development. These projects have also necessitated different modeling approaches, enriching the methodological repertoire of the Chen lab. The Cimini lab has collaborated

with mathematical modelers in the past [58, 60, 108] and has, for many years, had an appreciation for what modeling brings to her cell biology research. Over the years, mathematical models have helped her interpret experimental data, find mechanistic support for puzzling experimental observations, and design new experiments. Furthermore, because Chen and Cimini are colleagues in the same department, their collaboration has additional benefits, including co-mentoring students and postdocs, frequent in-person meetings, and spontaneous exchanges of ideas among trainees. Importantly, the collaboration has sparked new research opportunities, such as our ongoing project integrating modeling and experiments to study the role of tetraploid cell evolution in cancer.

The inherent complexity of biological systems certainly presents challenges and calls for novel methods to develop effective models. Advances in experimental techniques also constantly create needs for analyzing and integrating new types of data. Yet at the core of scientific progress are the researchers themselves. Meaningful collaboration requires experimentalists and modelers to communicate across disciplinary boundaries, cultivate genuine interest in each other's work, and develop a shared understanding of what models can offer. By fostering these connections, we can push the boundaries of mechanistic cell biology and fully harness the power of mathematical modeling in biological research.

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Figures

Figure 1

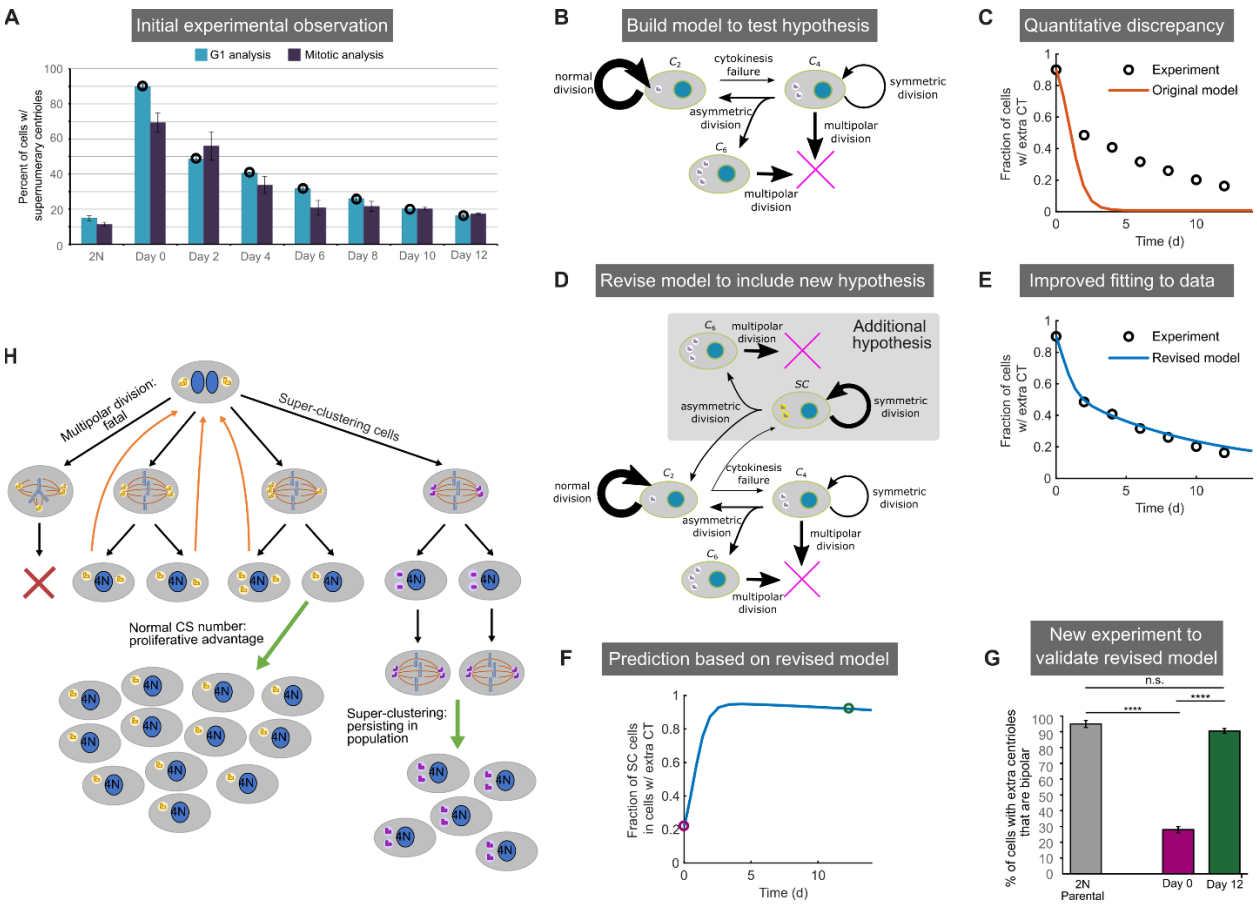

Figure 1: Modeling evolution of newly-formed tetraploid cells. (A) Experimental observation of gradual decrease in the fraction of cells with extra centrosomes following drug-induced tetraploidization. (B) Initial mathematical model for the evolution of centrosome numbers in the cell population. (C) Discrepancy between model prediction and experimental data. Experimental data taken from G1 analysis in (A). (D) Revised mathematical model incorporating an additional hypothesis of a subpopulation of "super-clustering" cells. (E) Revised model fits experimental data. (F) Revised model predicts that in the end most cells with extra centrosomes are "super-clustering". (G) Experimental validation of the new model prediction. The proportion of bipolar divisions in cells with extra centrosomes were much higher at Day 12 than Day 0, aligning with the model prediction that most cells with extra centrosomes were "super-clustering" in the end. (H) Mechanistic picture for evolution of newly-formed tetraploid cells. Daughter cells that inherit a single centrosome through asymmetric bipolar centrosome clustering can stably proliferate and gain a selective advantage. The "super-clustering" subpopulation proliferates more

robustly than other cells with extra centrosomes, eventually comprising the majority of the remaining population with extra centrosomes. Figures adapted from [26].

Figure 2

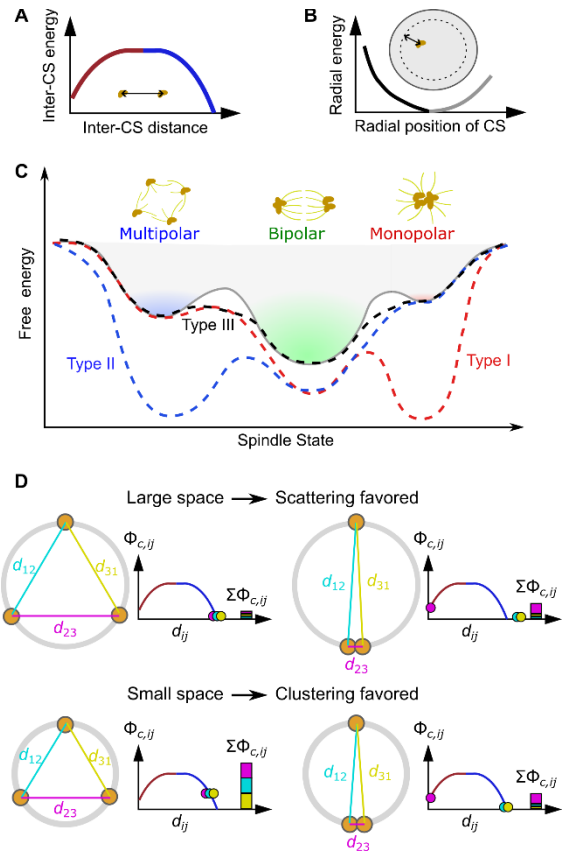

Figure 2: Modeling centrosome clustering and bipolar spindle formation. (A, B) Our parsimonious biophysical model assumes that centrosome movements are driven by pairwise interactive energies between centrosomes (A) and a radial potential energy that keeps each centrosome within the cell but away from the cell center (B). (C) Energy-based unified theoretical framework for various experimental phenomena. Perturbations to the cell can be categorized into three types, favoring monopolar spindle (Type I), multipolar spindle (Type II), and unstable spindle state (Type III), respectively. Detailed examples of each category can be found in [62]. (D) Energetic explanation for the dependence of spindle state on intracellular space, illustrated using examples of cells with three centrosomes. Top row: large space energetically favors centrosome scattering. Bottom row: small space energetically favors centrosome clustering. Colored dots on energy profile indicate the inter-centrosomal energies corresponding to the inter-centrosomal distances shown in the cartoon diagrams (color-coded to match). Stacked colored bars represent the total inter-centrosomal energies in each case. Figures adapted from [62].

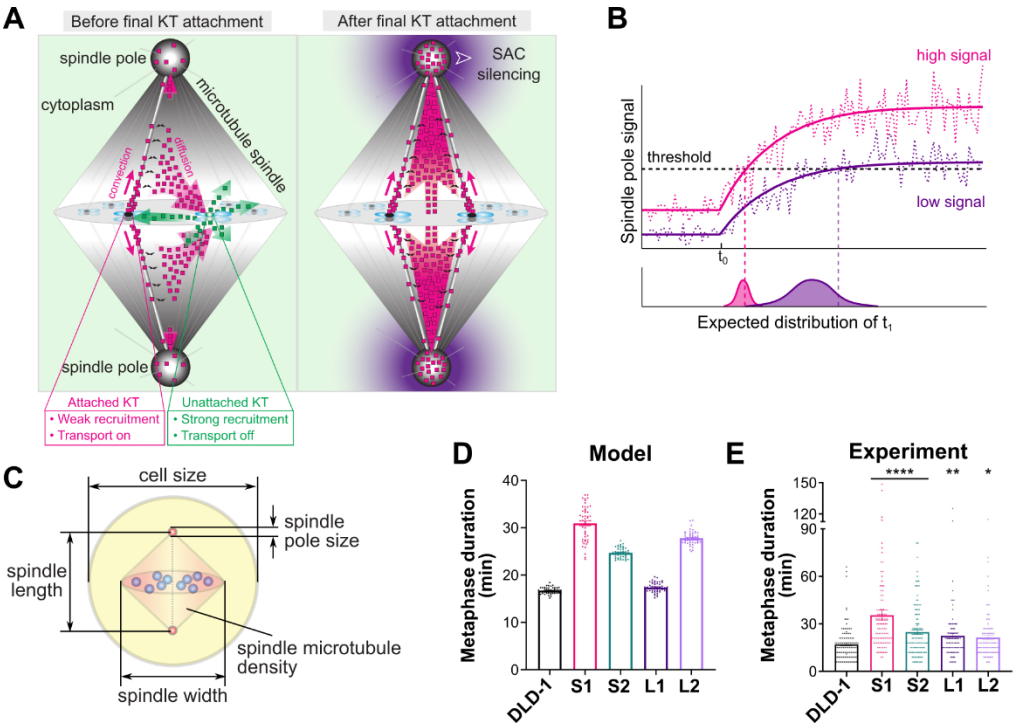

Figure 3: Modeling SAC silencing and its relation to spindle architecture and cell size. (A) Spatiotemporal model for SAC silencing. Left panel: Difference in phosphorylation level at the attached versus unattached kinetochores causes differences in SAC protein recruitment and regulation of their transport activity. Right panel: Once all kinetochores are attached, all SAC proteins become transport-active, resulting in a sharp increase in their poleward transport. The consequent accumulation of SAC proteins at the spindle poles then triggers the SAC silencing pathway. (B) Model-predicted SAC protein concentration at the spindle poles (i.e., spindle pole signal) before and after the final kinetochore attachment, and the resulting timing of SAC silencing. t_0: time of final kinetochore attachment. t_1: time when the spindle pole signal exceeds the threshold and triggers SAC silencing. Solid curves: deterministic simulations. Dashed curves: stochastic simulations. High spindle pole signals result in earlier and more precise timing of SAC silencing (magenta), while low spindle pole signals lead to later and more variable timing of SAC silencing (purple). (C) Spindle architectural features and cell size expected to affect timing of SAC silencing. (D) Model-predicted metaphase durations based on measurements of the features in (C). The metaphase duration is defined as the sum of the "triggering time" required for the spindle pole signal to reach the trigger threshold ($t_1 - t_0$) and the "propagation time" required for SAC silencing to propagate from the spindle pole throughout the cell. The propagation time depends on cell size and is minimally stochastic [28]. (E) Experimentally measured metaphase durations. Figures adapted from [28].