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Review Article

Dynamics of spindle assembly and position checkpoints: Integrating molecular mechanisms with computational models

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ABSTRACT

Mitotic checkpoints orchestrate cell division through intricate molecular networks that ensure genomic stability. While experimental research has uncovered key aspects of checkpoint function, the complexity of protein interactions and spatial dynamics necessitates computational modeling for a deeper, system-level understanding. This review explores mathematical frameworks–from ordinary differential equations to stochastic simulations, which reveal checkpoint dynamics across multiple scales, encompassing models ranging from simple protein interactions to whole-system simulations with thousands of parameters. These approaches have elucidated fundamental properties, including bistable switches driving spindle assembly checkpoint (SAC) activation, spatial organization principles underlying spindle position checkpoint (SPOC) signaling, and critical system-level features ensuring checkpoint robustness. This study evaluates diverse modeling approaches, from rule-based models to chemical organization theory, highlighting their successful application in predicting protein localization patterns and checkpoint response dynamics validated through live-cell imaging. Contemporary challenges persist in integrating spatial and temporal scales, refining parameter estimation, and enhancing spatial modeling fidelity. However, recent advances in single-molecule imaging, data-driven algorithms, and machine learning techniques, particularly deep learning for parameter optimization, present transformative opportunities for improving model accuracy and predictive power. By bridging molecular mechanisms with system-level behaviors through validated computational frameworks, this review offers a comprehensive perspective on the mathematical modeling of cell cycle control, with practical implications for cancer research and therapeutic development.

Contents

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1. Biology of mitotic checkpoints

Accurate chromosome segregation and spindle positioning during cell division are critical for genomic stability, with these processes regulated by the spindle assembly checkpoint (SAC) and spindle positioning checkpoint (SPOC) [1,2]. The SAC ensures that chromosomes are properly attached to the spindle before anaphase, preventing premature separation and aneuploidy [3,4], while the SPOC maintains spindle alignment to ensure accurate orientation of the cell division plane [5]. Dysregulation of these checkpoints is implicated in numerous diseases, particularly cancer, making them important targets for therapeutic intervention [6,7].

Experimental research has greatly advanced our understanding of SAC and SPOC dynamics. However, their inherent complexity remains a challenge due to intricate protein interactions and spatio-temporal requirements within these checkpoints [8,9]. While experimental approaches have provided valuable insights into checkpoint mechanisms, the complexity of protein interactions and spatial dynamics underscores the need for mathematical modeling to fully understand system behavior. These models have helped to reveal emergent properties such as bistability in checkpoint responses and have identified key regulatory nodes that may serve as therapeutic targets. This review primarily focuses on checkpoint mechanisms studied in yeast models, which have been instrumental in elucidating fundamental checkpoint principles. Many core components and mechanisms are conserved between yeast and mammals - for instance, the key SAC proteins Mad2, Mad1, and Bub1 maintain similar functions across species. However, mammals have evolved additional complexity in checkpoint regulation. The mammalian SAC involves additional proteins and regulatory mechanisms not found in yeast, while the SPOC pathway shows both conserved elements and species-specific adaptations. Understanding these evolutionary relationships is particularly relevant when considering how checkpoint dysfunction contributes to human diseases like cancer.

The development of accurate computational models relies heavily on specific types of experimental data that capture both spatial and temporal dynamics of checkpoint proteins. Quantitative mass spectrometry has enabled the precise measurement of checkpoint protein stoichiometry [10,11]. Spatiotemporal dynamics are captured through fluorescence intensity measurements at kinetochores, particularly for studying Mad2 recruitment kinetics, while FRAP (Fluorescence Recovery After Photobleaching) provides crucial data on protein turnover rates [12,13]. Recent advances in lattice light-sheet microscopy have enhanced our ability to track proteins in 3D and 4D [14]. Protein-protein interaction data, including binding affinities (KD values) and association/dissociation rates (kon/koff), are typically obtained through techniques like Bio-Layer Interferometry, which provide real-time binding kinetics [15]. Modern phosphoproteomics approaches have revealed complex post-translational modification landscapes that offer critical insights to inform model parameters [16].

Mathematical and computational models play a crucial role in capturing this complexity by simulating checkpoint behavior at multiple scales [17]. For instance, models have elucidated feedback mechanisms within the SAC [18] and provided insights into the spatial regulation of the SPOC [19]. By integrating experimental findings, mathematical models allow for a deeper exploration of checkpoint dynamics that goes beyond experimental observation alone.

The spindle assembly checkpoint (SAC) is a pivotal regulatory mechanism during mitosis that ensures accurate chromosome segregation. It functions by monitoring the attachment of chromosomes to the spindle apparatus via the kinetochore, a protein structure that forms on each chromosome [20,21]. Recent three-dimensional structural studies have provided detailed insights into how kinetochore-fibers are organized during mitosis [22]. The SAC is activated when one or more kinetochores are not properly attached to spindle microtubules, triggering a signaling cascade that delays the onset of anaphase until all chromosomes are correctly aligned [22,23]. While the SAC and spindle positioning checkpoint (SPOC) represent distinct mitotic checkpoints, they share notable functional similarities. Both pathways monitor physical properties of the spindle and rely on the turnover of inhibitors and activators at specific organelles to broadcast a 'WAIT' signal. The SAC collects information about spindle attachment at each kinetochore, which emits a nucleoplasmic 'WAIT' signal (in budding yeast) until proper attachment is achieved. In a comparable manner, Mad2 and Cdc20 in the SAC have analogous roles to Bfa1 and Tem1 in the SPOC (Fig. 1A). Bfa1's activity is regulated at the spindle pole bodies (SPBs), from where it signals throughout the cytosol to inhibit the downstream pathway activator, Tem1 [19]. Both SAC and SPOC must ensure the reliable transmission of the 'WAIT' signal and also allow its rapid deactivation once the checkpoint is satisfied (Fig. 1B).

Central to the SAC's function are proteins such as Mad2, Cdc20, Bub3, and BubR1, which inhibit the anaphase-promoting complex/cyclosome (APC/C), a critical ubiquitin ligase responsible for targeting key proteins for degradation [24,25]. When the SAC is active, the mitotic checkpoint complex (MCC) containing Mad2, BubR1, Bub3 and Cdc20 binds to APC/C, which already has a bound Cdc20 molecule serving as an APC/C coactivator. This dual-Cdc20 complex formation effectively inhibits APC/C's ability to ubiquitinate its substrates securin and cyclin B [26,27,25].

Research has shown that the SAC is not merely a binary switch but rather exhibits a dynamic response to varying levels of attachment and tension at kinetochores [1,28,29]. The regulation of SAC proteins is influenced by feedback mechanisms that enhance its robustness against disturbances [30]. For instance, once all kinetochores are adequately attached, the dissociation of Mad2 from Cdc20 permits APC/C activation, promoting anaphase onset and demonstrating the SAC's role in maintaining genomic integrity and preventing aneuploidy [3].

Equally important to SAC activation is its silencing mechanism. The AAA+ ATPase TRIP13, working with its adapter p31(comet), actively disassembles the mitotic checkpoint complex (MCC) even during SAC signaling [31,32]. This disassembly promotes the release of Cdc20, enabling the activation of the anaphase-promoting complex/cyclosome (APC/C), which targets securin and cyclin B for degradation, initiating anaphase onset. Concurrently, dynein motors strip SAC proteins such as Mad2, BubR1, and Mad1 from kinetochores, while phosphatases like PP1 dephosphorylate key SAC components, stabilizing kinetochore-microtubule attachments. Proper kinetochore-microtubule attachments also reorganize kinetochore architecture, diminishing SAC signaling. This concurrent operation of activation and silencing mechanisms ensures both checkpoint robustness and its ability to be rapidly reversed once proper kinetochore-microtubule attachments are achieved [33,23]. Together, these interconnected processes ensure accurate chromosome segregation, preventing aneuploidy and maintaining genomic stability.

Defects in the SAC can lead to severe consequences, including the development of cancer. Mutations or dysregulation of SAC components are implicated in various tumor types, underscoring the importance of this checkpoint in cellular health [7]. Understanding the intricacies of the SAC and its regulatory networks is essential for developing therapeutic strategies aimed at targeting mitotic checkpoints in cancer treatment.

The spindle positioning checkpoint (SPOC) ensures the correct positioning of the mitotic spindle during cell division. Proper spindle alignment is crucial for successful mitotic exit and cytokinesis, as it determines the plane of cell division and ensures that sister chromatids

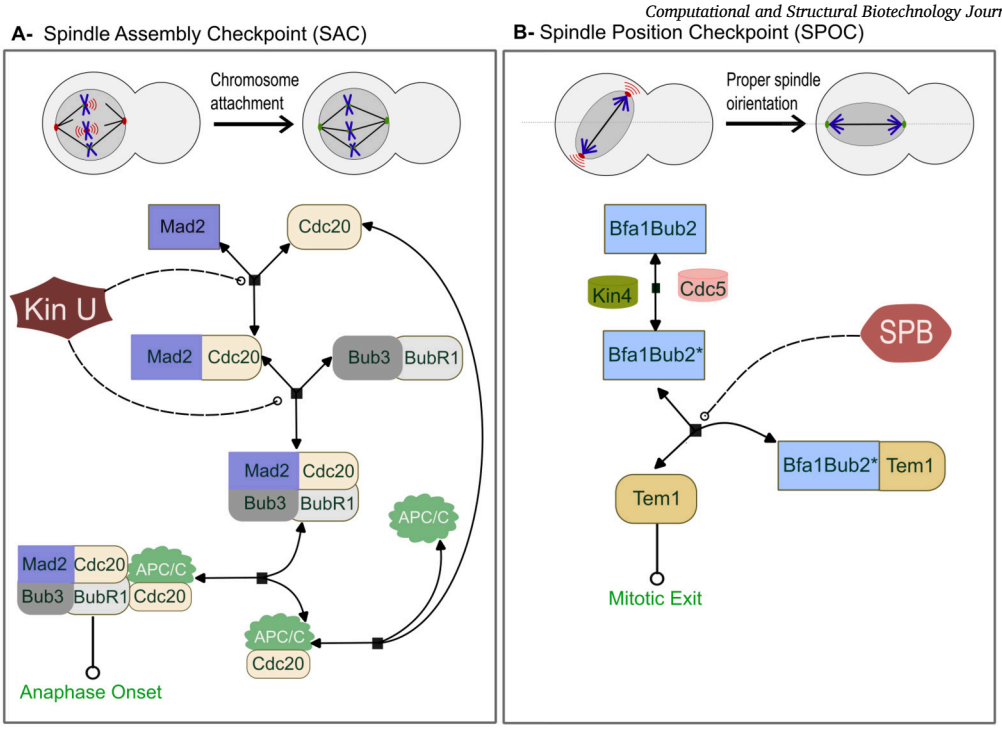

Fig. 1. Visual representation of cell division checkpoints. **(A)** Molecular components and signaling networks of the spindle assembly checkpoint (SAC). Unattached kinetochores catalyze the assembly of the mitotic checkpoint complex (MCC), which consists of Mad2, Cdc20, Bub3, and BubR1. When kinetochores achieve proper amphitelic microtubule attachment, dynein-mediated processes regulate Mad1-Mad2 signaling, modulating MCC assembly. The MCC inhibits the anaphase-promoting complex/cyclosome (APC/C), creating a robust mechanism that halts anaphase onset until all chromosomes are properly attached to the spindle. **(B)**Molecular components and signaling networks of the spindle positioning checkpoint (SPOC). The SPOC monitors spindle orientation through a spatial sensing mechanism at the spindle pole body (SPB). When spindle misalignment occurs, it triggers Kin4 activation, which initiates a phosphorylation cascade involving Cdc5 and Bfa1. This spatially organized signaling network modulates Tem1 activity, creating a robust mechanism that delays mitotic exit until proper spindle alignment is achieved. The coordinated actions of Kin4, Bfa1, and Tem1 at the SPB ensure accurate spindle positioning before cell division proceeds.

are accurately segregated to daughter cells [34,5]. The SPOC operates by monitoring the spatial orientation of the spindle apparatus relative to the cell cortex, ensuring that the spindle is appropriately positioned before the cell proceeds to anaphase. Key components of the SPOC include the Bfa1-Bub2 complex, which functions to inhibit the GTPase Tem1. When spindle misalignment occurs, Bfa1-Bub2 is activated, leading to the inhibition of Tem1 and the prevention of mitotic exit [35–37]. This regulatory mechanism maintains genomic stability by preventing premature exit from mitosis, which could result in unequal chromosome distribution and aneuploidy [5].

Studies have demonstrated that the SPOC employs temporal and compartment specific signals to coordinate mitotic exit with spindle position, using feedback mechanisms that modulate pathway activity [2,19]. Understanding the molecular mechanisms governing the SPOC is important for comprehending how cells maintain genomic stability through proper spindle positioning and accurate chromosome segregation during cell division [38,34,39].

In examining these checkpoints, we analyze mathematical and computational modeling techniques that have advanced our understanding of mitotic checkpoint regulation. Prominent modeling frameworks include ordinary differential equations, partial differential equations, stochastic models, rule-based modeling, and algebraic methods, which can reveal both temporal dynamics (Fig. 2C) and system-level behaviors (Fig. 2D). Here, we discuss their applications, limitations, and key contributions to biological insights.

In this review, we present a comprehensive analysis of mathematical modeling approaches for understanding mitotic checkpoint dynamics. We begin by examining the fundamental biology of the SAC and SPOC systems, highlighting their critical roles in maintaining genomic stability (Section 1). We then systematically explore the primary modeling techniques that have advanced our understanding of these checkpoints, from differential equations to rule-based approaches (Section 3). Each modeling framework is evaluated for its strengths, limitations, and specific applications in checkpoint analysis and how different modeling approaches have revealed key insights into checkpoint mechanisms. In Section 4, we address the current challenges facing checkpoint modeling, including issues of parameter estimation, spatial considerations, and computational complexity. The review concludes with an examination of emerging directions in the field (Section 5), particularly the integration of machine learning with mechanistic models and the application of advanced imaging techniques for model validation. Throughout, we emphasize how these computational approaches complement experimental findings and contribute to our understanding of checkpoint regulation in both normal cell division and disease states.

2. Mathematical modeling of mitotic checkpoints

Mathematical and computational modeling have become indispensable tools for studying mitotic checkpoints. As illustrated in Fig. 3, these approaches form part of an iterative cycle where computational predictions and experimental validation complement each other, allowing researchers to simulate and analyze the intricate dynamics of cellular processes, often revealing insights that experiments alone cannot provide [40,41]. By employing diverse modeling techniques, scientists can explore the behavior of checkpoint proteins, map regulatory networks, and predict cellular responses to perturbations [42–47,17,48]. This integrative approach combining computational and experimental methods is illustrated in Fig. 2.

The spindle assembly checkpoint (SAC) plays a crucial role in ensuring accurate mitotic progression. Even a single misattached kinetochore can halt mitosis [49]. Despite this, questions remain about the molecular mechanisms that enable both robust cell cycle arrest and rapid response,

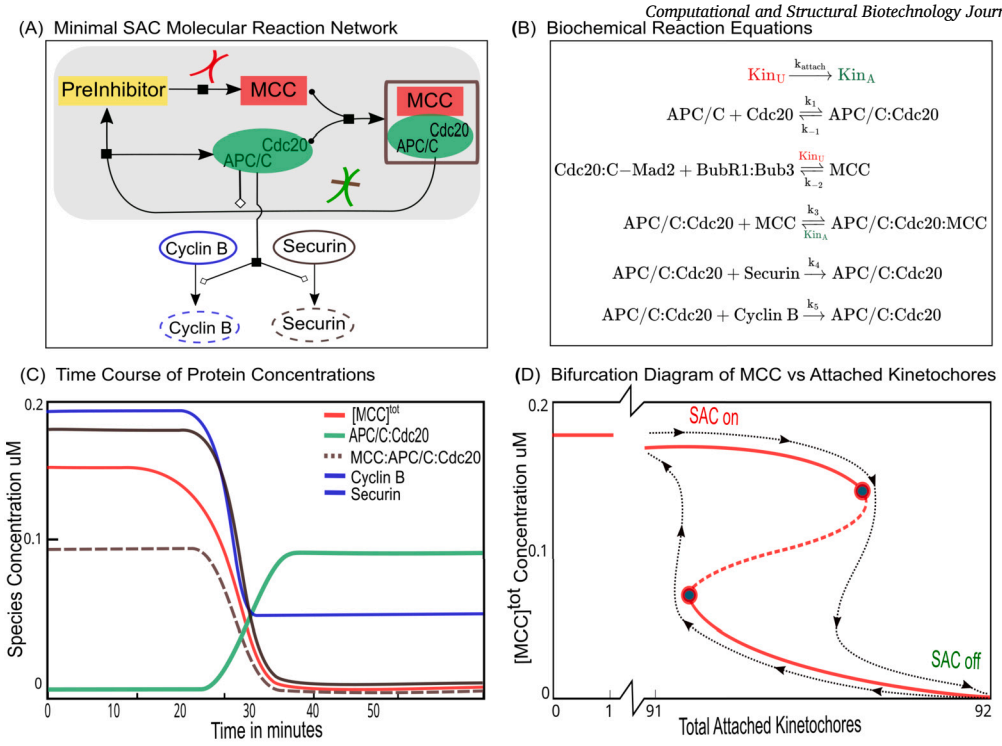

Fig. 2. Example of dynamical modeling of the Spindle Assembly Checkpoint (SAC) system, extended from Henze et al. [52]. (**A**) Simplified minimal SAC Molecular Reaction Network showing key components. Unattached kinetochores enhance the production of the mitotic checkpoint complex (MCC) from preInhibotor(Cdc20:Mad2 and Bub3:BubR1). Eventually, MCC binds tightly to and inhibits the APC/C:Cdc20. Immediately after the last kinetochore attachment to microtubules, the inhibitors dissolve, eventually resulting in active APC/C. This reactivation process is known as SAC silencing, where APC/C:Cd20 plays a role in its feedback loop. APC/C:Cdc20 substrates are Cyclin B and Securin. (**B**) Biochemical Reaction Equations including kinetochore attachment and protein complex formation/dissociation reactions. (**C**) Numerical simulation showing temporal evolution of key protein concentrations during SAC activation and silencing. The switch-like transition occurs around 20 minutes. (**D**) Bifurcation analysis revealing bistable behavior of MCC concentration as a function of attached kinetochores, with distinct 'SAC on' and 'SAC off' states and hysteresis between attachment and detachment pathways (indicated by arrows). Unstable saddle points are shown by dashed lines and stable node points by solid lines. Both stable and unstable states meet at saddle-node bifurcation points shown by solid circles. The SAC checkpoint is released and APC/C activated only when almost all kinetochores are attached (approximately 91.98). As the cell enters anaphase, MCC falls back to zero. The black line indicates how the switch flips from the SAC-active state to the SAC-inactive state as number of attached kinetochores increases.

as illustrated by the minimal reaction network shown in Fig. 2A. The need to link spindle biomechanics to a biochemical signal transduction network 2B introduces spatial challenges that modeling can uniquely address, especially amid ongoing debate about the role of tension sensing in SAC responses [50,51].

In comparison, modeling the spindle positioning checkpoint (SPOC) is particularly advantageous, despite involving relatively few components. These components can exist in various states and localizations, each influencing the other's interactions. Experimentally analyzing these states in living cells poses challenges, but modeling provides accessible insights into these dynamic interactions. For instance, understanding the activity states of Tem1—whether GTP- or GDP-bound—and the phosphorylation states of Bfa1 by Kin4, Cdc5, or in its unphosphorylated form, is critical. Moreover, modeling raises essential questions, such as the potential role of cytosolic pools in the function of SPOC. Through simulation, researchers gain a clearer understanding of how these components interact and identify the necessary conditions for proper SPOC function.

A wide range of mathematical models has been developed to capture the dynamic behaviors of SAC and SPOC. Ordinary differential equations (ODEs) describe time-dependent biochemical interactions, providing insights into protein interactions and cellular dynamics [53,54]. Stochastic models account for the inherent randomness in biological processes, shedding light on the probabilistic nature of cellular events [55,56]. Partial differential equations (PDEs) extend these analyses by modeling spatial dynamics, enabling a closer examination of molecular concentrations across different cellular regions [57,18,58].

Additionally, rule-based simulations offer a structured framework for exploring complex interactions under various conditions through predefined rules [59–63]. Algebraic methods contribute to analyzing system equilibria and understanding stability within checkpoint regulatory networks [55,64]. Having outlined the general importance of mathematical modeling in checkpoint research, we now examine each modeling approach in detail, analyzing their specific strengths, limitations, and applications in understanding checkpoint regulation.

3. Primary modeling techniques of mitotic checkpoints

This section presents widely used modeling techniques for understanding mitotic checkpoints, while acknowledging that alternative methods, such as delay differential equations and fractional differential equations, also offer valuable perspectives on cell cycle dynamics [46,65,44]. Table 1 provides a comprehensive comparison of the primary modeling techniques used in studying mitotic checkpoints, highlighting their strengths, limitations, and typical applications. Although these alternative methods are not detailed here, they hold potential for future research to further enrich our understanding of checkpoint regulation [43,42].

Integrating these modeling approaches with experimental data allows for a deeper exploration of mitotic checkpoint mechanisms and can support advances in strategies for addressing cell cycle regulation challenges [41,55]. The following discussions examine specific modeling techniques, detailing their applications, strengths, and limitations in studying mitotic checkpoint dynamics.

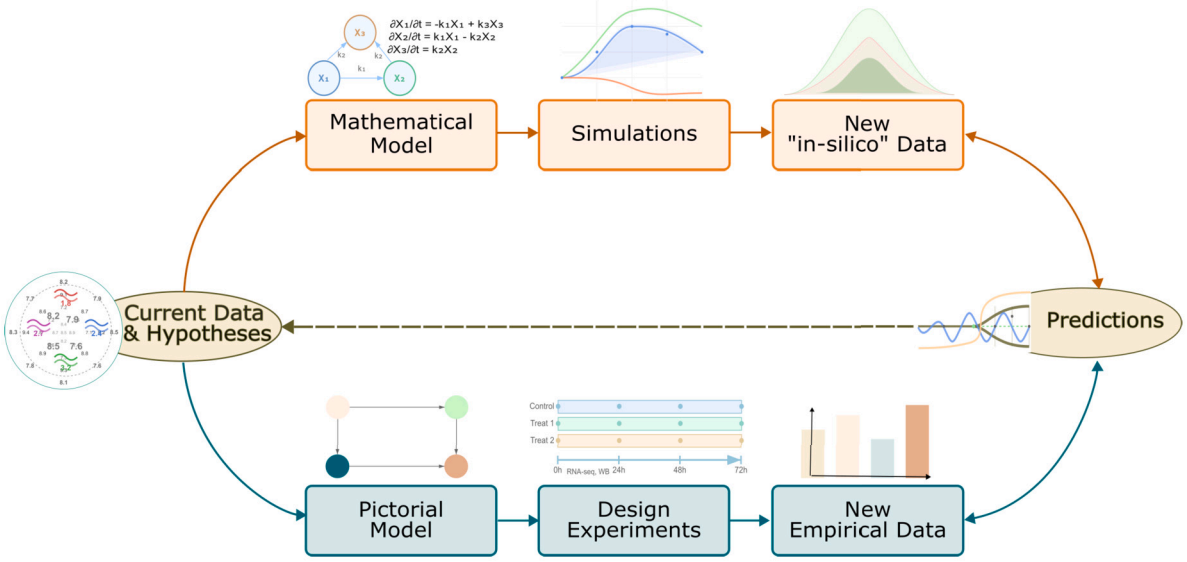

Fig. 3. Integrative systems biology workflow for studying mitotic checkpoints. The cycle illustrates the synergy between computational and experimental approaches in checkpoint research. Top path (orange): mathematical modeling generates predictions through computational simulations and *in-silico* data. Bottom path (teal): experimental design validates predictions through empirical data collection. Central elements show the iterative process of data integration and hypothesis refinement, where current data informs new mathematical models and predictions guide experimental design. Each component represents key methodologies discussed in this review: mathematical modeling (differential equations), simulations (numerical solutions), in-silico predictions, experimental design (time-course studies), and data analysis.

Table 1
Comparison of Modeling Techniques in Cell Cycle Research.

Technique	Strengths	Limitations	Applications
ODEs	- Time dynamics - Scalable - Rich analysis tools	- No spatial data - Fixed outcomes - Parameter sensitive	- Protein signaling - Checkpoint timing - Parameter screening
PDEs	- Space-time dynamics - Models gradients - Multi-compartment	- High computation cost - Many parameters - Boundary conditions	- Protein gradients - Cell polarity - Spindle positioning
Stochastic	- Captures fluctuations - Handles rare events - Models cell variation	- Slow simulation - Needs many runs - Complex statistics	- Switch-like events - Molecular counting - Noise analysis
Rule-Based	- Handles complexity - Easy to modify - Biological intuitive	- Hard to analyze - Memory intensive - Rule conflicts	- Multi-site proteins - Signal cascades - Checkpoint logic
Petri Nets	- Visual modeling - Flow analysis - State verification	- State explosion - Time modeling hard - Size limitations	- Decision points - Cycle analysis - Logic verification
Chemical Organization	- Structure focused - Finds key modules - Predicts stability	- No time evolution - Lacks details - Limited dynamics	- Network stability - Core components - Steady states

3.1. Ordinary differential equations (ODEs)

Ordinary Differential Equations (ODEs) are essential for modeling time-dependent biochemical systems such as the spindle assembly checkpoint (SAC) and spindle positioning checkpoint (SPOC). These models describe the rates of change in molecular concentrations over time, linking them to reaction rates. For SAC and SPOC modeling, ODEs simulate the interactions of proteins and complexes like Mad2, Cdc20, and Bfa1-Bub2, illustrating key checkpoint signaling pathways [42,53,46,19,66,67].

ODEs have addressed key biological questions in checkpoint research. For example, ODE models explain how the SAC achieves switch-like behavior through double-negative feedback loops between Mad2 and Cdc20 [66,67]. They also predicted and explained mutant phenotypes from deletion and overexpression experiments. Similarly, ODE modeling of the SPOC clarified how Bfa1-Bub2 complex regulation achieves spatial sensing of spindle position through phosphorylation cycles mediated by Kin4 and Cdc5 [19] ODE models is also valuable for understanding checkpoint silencing mechanisms [52]. The interplay between TRIP13-dependent MCC disassembly and checkpoint activation requires modeling of competing processes, including TRIP13 ATPase activity, p31(comet)-mediated MCC recognition, and disassembly kinetics. These models must integrate experimental data on TRIP13 enzymatic rates, MCC assembly/disassembly, p31(comet) binding, and silencing factor concentrations, enabling predictions on checkpoint strength and its dynamic reversal once proper kinetochore-microtubule attachments are achieved [31,32,68,23].

A typical ODE model comprises equations that represent temporal changes in system components. For example, a protein's rate of concentration change is given by:

$$\frac{d[\text{Species}]}{dt} = f([\text{Species}], \text{Rate Constants})$$

ODEs require several types of experimental data as inputs for accurate simulations. The primary inputs include:

- Initial protein concentrations for all species (measured through quantitative mass spectrometry, Western blots, or fluorescence microscopy)
- Binding affinities (K_d values) between interacting proteins, which can vary based on post-translational modifications
- Association and dissociation rate constants (k_{on} and k_{off}), often determined through Fluorescence Recovery After Photobleaching (FRAP) or single-molecule tracking
- Enzymatic rate constants for phosphorylation/dephosphorylation events
- Protein degradation and synthesis rates
- When considering compartmentalized models, diffusion coefficients and protein localization data from Fluorescence Correlation Spectroscopy (FCS) or photoactivation experiments may be required

Importantly, these parameters can change dynamically during checkpoint activation and silencing. For example, phosphorylation can alter binding affinities between checkpoint proteins - the interaction between Mad2 and Cdc20 is regulated by phosphorylation states, while BubR1-Bub3 complex formation is modulated by kinetochore-dependent phosphorylation events. These state-dependent changes in interaction parameters must be incorporated into the ODEs for accurate modeling.

These models reveal how checkpoint protein concentrations evolve over time in response to inputs such as unattached kinetochores or spindle misalignment. By adjusting parameters, researchers can simulate various cellular conditions, gaining insights into checkpoint mechanisms.

Notably, some ODE models omit time on the left-hand side, focusing on concentration changes, which could more precisely be termed chemical differential equations (CDEs) [17].

ODEs have addressed several key biological questions in checkpoint research. For example, ODE models helped explain how the SAC achieves switch-like behavior through double-negative feedback loops between Mad2 and Cdc20 [66,67]. These models required experimental data on protein concentrations and binding kinetics measured through biochemical assays and microscopy. The insights revealed how Mad2-Cdc20 interactions create bistability, explaining the checkpoint's ability to maintain a stable metaphase arrest until all kinetochores are properly attached [18]. Similarly, ODE modeling of the SPOC helped understand how Bfa1-Bub2 complex regulation achieves spatial sensing of spindle position [19]. Using experimental measurements of protein localization and activity states, these models demonstrated how phosphorylation cycles of Bfa1 by Kin4 and Cdc5 create a robust checkpoint response. This explained how cells detect and respond to spindle misalignment through spatially-regulated protein modifications.

A wide range of tools is available to solve ODEs and CDEs in checkpoint modeling, each suitable for different biochemical system requirements, including stiff and non-stiff models. Software such as `MATLAB`, `Octave`, `MATHEMATICA`, and `MAPLE` provides accessible platforms, while specialized solvers like `CVODE` from SUNDIALS [69] and `LSODA` [70] handle more complex system needs.

Dynamical systems analysis tools, such as `XPP-AUT` [71], facilitate nonlinear analysis and bifurcation studies, essential for exploring checkpoint behavior. SBML-compatible solvers enhance model sharing across platforms and integration with BioModels databases [72]. Additionally, graphical tools like `CellDesigner` [73] and `COPASI` [74] simplify complex biochemical network simulations.

Parameter sensitivity analysis identifies key parameters that significantly influence checkpoint regulation, offering insights into the robustness of SAC and SPOC responses. Bifurcation analysis complements this by revealing conditions that shift system behavior, such as transitions between steady states or oscillatory patterns, potentially highlighting therapeutic targets in cancer [42,75].

These analyses can be extended with spatial or stochastic elements to more comprehensively capture checkpoint dynamics. Hybrid models incorporate molecular diffusion or localization effects, addressing the spatial organization and randomness critical for checkpoint function [76–79]. Together, these approaches provide a fuller understanding of checkpoint regulation, extending beyond traditional time-dependent models.

In summary, while ODEs are invaluable for SAC and SPOC studies, they are limited in capturing spatial dynamics. This limitation is specifically addressed by Partial Differential Equations (PDEs), which model spatial dynamics in biochemical systems.

Key Concepts: Ordinary Differential Equations

- **Focus:** Temporal evolution of molecular concentrations
- **Core Principles:**
  - Rate equations: $\frac{d[X]}{dt}$ = $\sum$(synthesis/production terms) + $\sum$(activation terms) − $\sum$(degradation terms) − $\sum$(inhibition terms)
  - Common kinetic formulations: Mass action kinetics: $v = k[S]$, Michaelis-Menten kinetics: $v = \frac{V_{max}[S]}{K_m+[S]}$, or Hill kinetics: $v = \frac{V_{max}[S]^n}{K_m^n+[S]^n}$
  - System of nonlinear coupled equations
- **Key Applications:**
  - Protein interaction dynamics
  - Checkpoint activation/deactivation timing
  - Parameter sensitivity analysis
- **Tools:** MATLAB, MATHEMATICA, COPASI, XPP-AUT

3.2. Partial differential equations (PDEs)

Partial Differential Equations (PDEs) model spatial dynamics in biochemical systems, particularly relevant for SAC and SPOC. Unlike ODEs, which focus solely on time changes, PDEs include spatial and temporal dimensions, allowing investigation of molecular concentration variations across the cell [57,18]. PDEs have been instrumental in addressing spatial aspects of checkpoint regulation. PDE models revealed how protein gradients originating from kinetochores create spatial checkpoint signals [18,57,58]. These models demonstrated how cells maintain checkpoint signaling across different cellular compartments, explaining how a single unattached kinetochore can generate a cell-wide checkpoint response.

The diffusion-convection-reaction equation, commonly used in cell biology, can be expressed as:

$$\frac{\partial C(x,t)}{\partial t} = D\nabla^2 C(x,t) - Q\nabla C(x,t) + R(C(x,t),t)$$

Here:

- $C(x,t)$ represents species concentration at position x and time t.
- D is the diffusion coefficient, indicating particle spread due to random motion.
- Q is the convection coefficient, capturing bulk movement's effect on concentration.
- $R(C(x,t),t)$ represents chemical reactions affecting species concentration, based on concentration and time.

Spatial protein distribution and diffusion data can be obtained through several experimental techniques. FRAP experiments at kine-

tochores can reveal Mad2 dynamics in two ways: the recovery curve provides the diffusion coefficient D, while the spatial intensity profile during recovery shows the protein distribution $C(x,t)$. Additionally, FCS measurements at different cellular locations can determine both local protein concentrations and diffusion coefficients. For membrane-bound proteins, Single Particle Tracking (SPT) offers another approach to measure both diffusion and spatial localization. These complementary experimental approaches provide the essential spatial parameters required for PDE model parameterization.

PDEs model spatial heterogeneity, crucial in cell biology, as protein localization can significantly influence interactions and regulatory roles [18,57,80,58,81].

PDEs have been instrumental in addressing spatial aspects of checkpoint regulation. For instance, PDE models revealed how protein gradients emanating from kinetochores create spatial checkpoint signals [18, 57,58]. These models required experimental data on protein diffusion rates and spatial concentration profiles obtained through fluorescence microscopy. The insights demonstrated how cells maintain checkpoint signaling across different cellular compartments, explaining how a single unattached kinetochore can generate a cell-wide checkpoint response.

Advanced numerical methods, such as finite difference, finite element, and finite volume methods, are essential for solving PDEs. These methods discretize the spatial domain, converting continuous PDEs into algebraic equations solvable numerically.

Tools such as `MATLAB`, `MATHEMATICA`, `MAPLE`, `COMSOL Multiphysics`, `FEniCS`, and `OpenFOAM` support these techniques, offering flexibility and advanced numerical schemes [82–84]. Systems biology tools, like `Virtual Cell`, integrate ODEs and PDEs within cellular models [85].

Balancing accuracy and computational demand is critical, as finer spatial meshes increase precision but require more resources.

While PDEs excel at modeling spatial aspects, the deterministic nature of these equations cannot capture the intrinsic randomness inherent in biological systems, particularly when dealing with low molecule numbers. This limitation naturally leads us to consider stochastic approaches.

Key Concepts: Partial Differential Equations

- **Focus:** Spatial-temporal protein distributions
- **Core Principles:**
  - Reaction-diffusion equations: $\frac{\partial C}{\partial t} = D\nabla^2 C - Q\nabla C + R(C,t)$
  - Boundary conditions
  - Spatial gradients
- **Key Applications:**
  - Protein localization patterns
  - Concentration gradients
  - Spatial checkpoint signaling
- **Tools:** COMSOL, FEniCS, Virtual Cell

3.3. Stochastic models

Stochastic models address the intrinsic randomness of biological processes, especially relevant at low molecule counts where fluctuations affect rates and concentrations. Stochastic approaches have revealed crucial insights into checkpoint noise handling. These models explained how cells maintain reliable checkpoint signaling despite fluctuations in protein numbers [52,56]. Using single-molecule tracking data and protein counting from fluorescence microscopy, stochastic models demonstrated how checkpoint mechanisms filter noise while remaining sensitive to genuine signals. For example, studies of Mad2 dynamics showed how cells achieve robust checkpoint activation even with variable protein levels [55].

Stochastic modeling, through Stochastic Differential Equations (SDEs) or simulations, provides unique insights into molecular-level biological dynamics. SDEs add stochastic terms to deterministic ODE or PDE models, accurately representing noise and variability in cellular processes such as gene expression and signaling.

Several mathematical methods are available for modeling stochastic systems. The Gillespie algorithm, for instance, provides an exact method for simulating chemical reactions in small populations by generating reaction events at each time step based on probabilistic rules [86]. Alternative approaches include the tau-leaping method, which approximates Gillespie's exact method and is suitable for systems where multiple reactions occur simultaneously [87].

Hybrid models combine deterministic and stochastic approaches to optimize accuracy and computational efficiency, capturing noise in specific pathways without modeling the entire system stochastically [88]. For large systems, Monte Carlo methods provide flexibility, allowing the study of checkpoint protein interactions under various perturbations [89].

Stochastic models often require considerable computational resources, particularly in multi-component systems where reactions and molecular states multiply. While these models offer valuable insights into noise-influenced checkpoint dynamics, their computational intensity with multiple molecular states creates scaling challenges. Rule-based modeling specifically addresses these challenges by providing a more efficient framework for representing and analyzing complex molecular interactions.

Key Concepts: Stochastic Modeling

- **Focus:** Random fluctuations in molecular processes
- **Core Principles:**
  - Gillespie algorithm
  - Chemical master equations
  - Monte Carlo simulations
- **Key Applications:**
  - Noise in checkpoint signaling
  - Low molecule count dynamics
  - Probabilistic state transitions
- **Tools:** StochKit, Gillespie SSA implementations

3.4. Rule-based modeling

Rule-based modeling (RBM) systems, such as `BioNetGen` and `Kappa`, use rules rather than explicit equations to represent interactions within complex molecular systems [90]. This technique handles combinatorial complexity by defining reaction rules applicable across species or states, allowing the efficient modeling of large biomolecular systems.

Rule-based spatial modeling tools like SRSim extend traditional RBM approaches by incorporating geometric and spatial constraints into molecular interactions [60]. SRSim enables the simulation of complex spatial arrangements and diffusion of molecules, making it particularly valuable for studying checkpoint mechanisms where spatial organization is crucial, such as kinetochore-microtubule attachments in SAC or spindle orientation in SPOC. This spatial rule-based approach provides unique insights into how molecular geometry and spatial distribution affect checkpoint function.

In the context of SAC and SPOC studies, rule-based modeling has been applied to explore how checkpoint proteins interact and how these interactions change in response to different cellular states. The approach simplifies models of multi-state proteins, enabling more extensive simulations under diverse conditions without overwhelming computational resources [91]. These platforms facilitate the modeling of intricate cellular pathways, including feedback loops and post-translational modifications, allowing for a more comprehensive understanding of biological interactions and regulatory mechanisms [92].

Rule-based modeling platforms, such as `BioNetGen`, `Kappa`, and `PySB`, provide versatile interfaces for rule definition, visualization, and analysis. Rule-based modeling has been particularly valuable for understanding complex multi-protein interactions in checkpoints. These models have helped decipher how multiple phosphorylation sites and protein modifications coordinate checkpoint signaling [62]. Using data from proteomic studies and interaction mapping, rule-based models revealed how different protein modifications combine to create checkpoint signals. For instance, studies of kinetochore assembly showed how hierarchical protein recruitment ensures proper checkpoint activation [59]. They employ algorithms that allow for dynamic rule generation and simulating rule-based models, enabling researchers to explore complex systems efficiently, including molecular geometry [60].

However, rule-based models require detailed definitions of interaction rules, which can become complex when modeling large systems with intricate regulatory mechanisms. While powerful, RBM techniques face challenges such as incomplete biological knowledge and complex network representations. Translating rules into biological reality requires careful parameterization and extensive validation to ensure that the model accurately reflects the biological system under study [90]. These ongoing challenges highlight the importance of continual development in RBM as a vital tool in systems biology, enabling innovative strategies to unravel the complexities of biological systems and their dynamics.

Overall, while RBM provides powerful tools for modeling complex molecular interactions, its practical implementation faces several constraints: the need for extensive parameter validation, computational demands for large systems, and limitations in revealing broader system properties. Algebraic methods complement these approaches by offering abstract representations that can illuminate fundamental network properties while requiring fewer parameters.

Key Concepts: Rule-Based Modeling

- **Focus:** Complex multi-state protein interactions and spatial organization
- **Core Principles:**
  - Pattern-based rules
  - Site-specific modifications
  - Combinatorial complexity handling
  - Spatial and geometric constraints
- **Key Applications:**
  - Multi-site protein modifications
  - Complex formation/dissociation
  - Regulatory network analysis
  - Spatial organization of checkpoint components
- **Tools:** BioNetGen, Kappa, PySB, SRSim

3.5. Algebraic methods

Computational modeling aids in understanding complex biological systems like mitotic checkpoints. This section focuses on two prominent algebraic modeling approaches–Petri nets and chemical organization theory. Both methods abstractly represent biochemical interactions, allowing for graphical and qualitative analyses.

Petri nets effectively represent distributed systems and capture biochemical network dynamics. A Petri net consists of places (system states), transitions (events altering states), and tokens (quantities of species). This structure enables graphical representation of interactions, allowing researchers to visualize regulatory networks and comprehend information flow. Petri nets support qualitative analyses that explore properties like reachability, liveness, and boundedness, which are vital for understanding checkpoint proteins during cell division [93].

Chemical organization theory aids in understanding biochemical systems at a higher abstraction level, positing that biological processes are hierarchically organized, where lower-level interactions lead to higher-level behaviors. Chemical organization theory models biochemical systems as being composed of interconnected functional modules, with lower-level interactions between molecular components leading to higher-level system behaviors and functions. By applying this theory, researchers can model relationships between components, identify functional modules, and simplify complex networks for analysis and simulation [94–96]

Both Petri nets and chemical organization theory enhance insights into the dynamic behavior of mitotic checkpoints, contributing to the development of robust models that facilitate therapeutic strategies for cancer treatment. Various tools support the application of algebraic methods in biological modeling. For Petri nets, numerous software options assist in modeling, simulating, and analyzing structures, with surveys highlighting features that guide users in selecting appropriate tools [97].

In chemical organization theory, advancements have produced tools for computing persistent subspaces of reaction-diffusion systems, enhancing the theory's applicability. These tools facilitate the analysis of complex interactions and dynamic behaviors, providing valuable insights into regulatory mechanisms [98,99].

Despite their advantages, algebraic methods present challenges. They require less detailed kinetic information than traditional approaches, which is beneficial when experimental data is limited. However, incorporating spatial properties increases computational demands, especially in larger systems. Additionally, while recommended for high-dimensional systems, the abstraction in algebraic methods may overlook critical dynamic aspects of biological processes. Algebraic approaches, particularly Petri nets and chemical organization theory, have provided unique insights into checkpoint network structure and function. Petri net analysis revealed fundamental control principles in the SAC network, demonstrating how different protein states and transitions maintain checkpoint signaling [64]. These models utilized protein interaction network data and state transition information from biochemical studies, helping identify essential control points in checkpoint regulation [99].

Thus, while powerful for analyzing mitotic checkpoints, researchers must carefully consider the trade-offs in selecting a modeling approach.

Key Concepts: Algebraic Methods

- **Focus:** Network structure and qualitative dynamics
- **Core Principles:**
  - Petri nets: Places and transitions
  - Chemical organization theory
  - Network topology analysis
- **Key Applications:**
  - Checkpoint network structure
  - Steady state analysis
  - System stability assessment
- **Tools:** Snoopy, COT analyzer

These diverse modeling approaches offer complementary insights into checkpoint regulation, with each method addressing different aspects of checkpoint complexity. ODEs provide the temporal framework for understanding reaction dynamics, while PDEs extend this to capture spatial organization crucial for checkpoint signaling. For instance, while ODE models revealed the bistable switching mechanism of the SAC [52], PDE approaches showed how this switch operates across cellular space [18]. Similarly, stochastic methods complement these deterministic approaches by explaining how checkpoints maintain reliability despite molecular noise [55], which is particularly relevant at kinetochores where protein numbers are low. Rule-based modeling handles the combinatorial complexity of multi-protein interactions [62], while algebraic methods like chemical organization theory reveal the hierarchical organization of these interactions [96]. This complementarity is

exemplified in SPOC studies, where ODEs model temporal dynamics of Bfa1-Bub2 regulation [19], PDEs capture spatial aspects of spindle positioning, and rule-based approaches describe complex phosphorylation patterns. Together, these approaches provide a multi-scale understanding of checkpoint function, from molecular interactions to cellular-level organization. The integration of these modeling approaches, supported by experimental data, has enhanced our understanding of checkpoint mechanisms beyond what any single method could achieve. Future developments in checkpoint modeling will likely require increased integration of these approaches, particularly as new experimental techniques provide more detailed data across multiple scales.

While these diverse modeling approaches have significantly advanced our understanding of checkpoint regulation, their implementation and integration face several important challenges. The complexity of checkpoint mechanisms, combined with technical limitations in both experimental and computational methods, creates obstacles that must be addressed to further advance the field. Understanding these challenges is crucial for developing more comprehensive and accurate models of checkpoint function.

4. Challenges in modeling mitotic checkpoints

The integration of multiple modeling approaches in checkpoint research has revealed several fundamental challenges that need to be addressed. These challenges span from biological complexity to technical limitations in both experimental data collection and computational implementation. At the biological level, the intricate nature of mitotic checkpoint networks, particularly the SAC and SPOC, involves numerous proteins interacting through nonlinear feedback loops, many of which remain not fully understood [100,101]. This complexity is further amplified when considering the context-dependent behavior of checkpoint components, such as their specific cellular locations and various modification states. The technical challenges in experimental data collection and computational implementation directly impact model development and validation. For instance, measuring the localized abundance of checkpoint proteins at specific cellular structures like kinetochores or spindle poles remains technically demanding. Similarly, tracking the dynamic changes in protein modifications and interactions in living cells poses significant experimental challenges. These limitations in data acquisition directly affect our ability to develop and validate comprehensive models that can reliably predict cellular behavior across varying conditions.

Parameter estimation and data availability are also limiting factors [102]. Detailed kinetic data, including reaction rates and binding affinities, are often scarce or incomplete, making it challenging to accurately parameterize models [55,43,19]. Assumptions or inferred data are sometimes used to fill gaps, but this can introduce biases and affect the models' predictive power.

Incorporating spatial dynamics adds another layer of difficulty. Molecular events within mitotic checkpoints are often spatially localized, such as at spindle poles or kinetochores. Accounting for this spatial heterogeneity increases the complexity of models. Techniques like partial differential equations (PDEs) or spatial rule-based simulations are employed to address this challenge, but they also raise computational costs, limiting the temporal and spatial scales that can be practically simulated [18,62].

Model validation presents its own set of challenges. Comparing model predictions with experimental data is essential but complicated by the variability and noise inherent in biological systems and experimental methods. High-quality, reproducible data is crucial for refining and validating models, yet such data is often difficult to obtain.

Addressing parameter estimation and spatial modeling challenges requires more comprehensive kinetic and localization data on checkpoint proteins and their interactions. Single-molecule fluorescence techniques could provide this data at finer spatiotemporal scales. For example:

- Förster resonance energy transfer (FRET) and FLIM-FRET (FLIM: Fluorescence Lifetime Imaging) can quantify interaction kinetics and binding affinities in vivo at the single-molecule level [103], constraining intricate feedback mechanisms in kinetic models.
- Photoactivated localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM), and MINFLUX nanoscopy enable nanometer-scale imaging of protein interactions, with MINFLUX achieving resolution down to 1 nm even in living specimens [104], providing spatial distributions and flux dynamics for spatial models.
- Total internal reflection fluorescence (TIRF) microscopy [105] selectively visualizes protein complexes at specific subcellular structures, capturing how localization influences checkpoint function.
- Microfluidics combined with live-cell imaging [106] controls the cellular microenvironment while collecting kinetic response data under varying conditions.

Generating extensive high-resolution spatiotemporal data through such techniques on checkpoint proteins would significantly improve constraints on molecular-scale kinetic parameters and localization rules, reducing uncertainties that impede the development of accurate predictive spatial and stochastic mathematical models.

Addressing the computational challenges of simulating spatial and stochastic checkpoint models requires advances in simulation techniques. Potential areas of focus include:

- Multiscale methods that couple discrete reaction-diffusion models within continuous PDE frameworks, allowing simulation of finer spatial scales within larger domains.
- Agent-based simulations combining discrete and continuum descriptions for enhanced efficiency, where individual proteins could be represented as agents diffusing within coarse-grained spatial regions.
- Parallel and GPU computing implementations of spatial simulations that distribute computations across multiple processing units, enabling simulations of larger and longer-timescale models.
- Rule-based modeling languages and compilers that symbolically represent and simulate reaction-diffusion mechanisms, increasing performance over traditional approaches.
- Approximation methods like coarse-graining, time-scale separation, and temporal multi-resolution that simplify models while preserving important dynamics.

Advancing such computational methods through multidisciplinary efforts in applied math, computer science, and bioengineering would facilitate exploring checkpoint spatiotemporal organization at scales relevant to cellular functioning.

Despite the potential of mathematical and computational models to provide insights into mitotic checkpoints, these challenges underscore the need for improved data acquisition techniques, more efficient computational methods, and further refinement of existing models to better account for the complexity and variability of cellular systems.

Key Challenges in Modeling Mitotic Checkpoints

- Biological complexity: Intricate networks with numerous interacting proteins and nonlinear feedback loops. These components can exist in various states and localizations, each influencing the other's interactions.
- Stochasticity: Inherent randomness and fluctuations in cellular processes due to small molecule counts
- Parameter estimation: Lack of comprehensive kinetic data on checkpoint protein interactions
- Spatial dynamics: Difficulty in accounting for spatially localized molecular events

- Model validation: Variability and noise in experimental data complicate model-data comparison

Potential Solutions:

- Utilize advanced experimental techniques (e.g., single-molecule imaging, microfluidics) to obtain high-resolution spatiotemporal data
- Develop multiscale and hybrid modeling approaches to better integrate spatial and temporal dynamics
- Advance computational methods (e.g., parallel computing, rule-based modeling) to handle increased complexity
- Foster interdisciplinary collaboration to drive innovation in data acquisition and modeling techniques

5. Conclusions and future perspectives

Mitotic checkpoints are essential for maintaining genomic integrity during cell division, underscoring the importance of accurate modeling for understanding biological processes, including cancer progression. Future research should focus on overcoming current limitations and leveraging emerging techniques to deepen insights into the complex regulatory mechanisms governing cell division. The integrative systems biology approach (Fig. 3) will continue to be essential for understanding checkpoint regulation and developing therapeutic strategies. This integrative approach will benefit from emerging techniques and Using advanced approaches to deepen insights into the complex regulatory mechanisms governing cell division.

Future work should prioritize developing multiscale and hybrid modeling approaches that integrate data and models across spatial and temporal scales. This includes agent-based models that couple molecular interactions to cellular behaviors, as well as genome-scale models that link checkpoint dynamics to phenotypic outcomes. These models can better capture the complexity of mitotic checkpoints, providing a more holistic understanding of cell division regulation [107,108].

With the advent of high-throughput experimental platforms, extensive multi-omics datasets are becoming available, offering a valuable opportunity to combine machine learning with mechanistic models. Machine learning can assist in parameter estimation, identification of novel regulatory interactions, and prediction of checkpoint responses, thereby addressing current gaps in experimental data and enhancing model fidelity. Additionally, computational methods must scale to meet the demands of such data complexity, with high-performance computing platforms supporting increasingly sophisticated simulations, especially those that explore spatial dynamics within cellular compartments.

Addressing cellular compartmentalization and spatial organization remains a significant challenge. Advances in spatial rule-based modeling and partial differential equation (PDE) frameworks are promising for simulating checkpoint activity at specific cellular locales, such as kinetochores and spindle poles. Further exploration of these spatial dynamics will provide insights into how compartmentalization contributes to checkpoint regulation, which is critical for understanding checkpoint robustness and fidelity.

Interdisciplinary collaboration among biologists, computer scientists, mathematicians, and physicists will foster innovative computational frameworks and algorithms. Leveraging algebraic methods like chemical organization theory and Petri nets can aid in analyzing complex systems with fewer parameters, facilitating model analysis of large, intricate networks [94,97].

Sophisticated experimental techniques, such as single-molecule imaging and high-resolution live-cell microscopy, will provide richer datasets for validating models. Continued integration of experimental and computational advances through interdisciplinary collaboration will enhance our understanding of checkpoint regulation and accelerate therapeutic discovery, particularly through *in-silico* testing of targeted interventions. Recent experimental advances have provided unprecedented insights into SAC dynamics and molecular mechanisms that await incorporation into computational models. For example, new structural studies of kinetochore-checkpoint protein interactions [13], quantitative measurements of checkpoint protein stoichiometry [10], and detailed characterization of MCC assembly dynamics ([109,23] offer rich datasets for model validation and refinement. Integrating these experimental findings into existing models presents a key opportunity to enhance our understanding of checkpoint regulation.

In summary, the future of mitotic checkpoint research lies in combining advanced computational techniques with high-resolution experimental data and machine learning. These strategies promise to overcome existing challenges, offering a pathway to novel applications in therapeutic development, particularly for diseases linked to cell cycle dysregulation. Ultimately, these efforts will contribute to a more comprehensive understanding of cell division control, guiding innovations in both basic science and medical applications.

CRediT authorship contribution statement

Bashar Ibrahim: Conceptualization, Formal analysis, Visualization, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Not applicable as this is a review article.

References

[1] McAinsh AD, Kops GJ. Principles and dynamics of spindle assembly checkpoint signalling. Nat Rev Mol Cell Biol 2023;24(8):543–59.
[2] Caydasi AK, Khmelinskii A, Duenas-Sanchez R, Kurtulmus B, Knop M, Pereira G. Temporal and compartment-specific signals coordinate mitotic exit with spindle position. Nat Commun 2017;8(1):14129.
[3] Cohen-Sharir Y, McFarland JM, Abdusamad M, Marquis C, Bernhard SV, Kazachkova M, et al. Aneuploidy renders cancer cells vulnerable to mitotic checkpoint inhibition. Nature 2021;590(7846):486–91.
[4] Ben-David U, Amon A. Context is everything: aneuploidy in cancer. Nat Rev Genet 2020;21(1):44–62.
[5] Caydasi AK, Ibrahim B, Pereira G. Monitoring spindle orientation: spindle position checkpoint in charge. Cell Div 2010;5.
[6] Bai Z, et al. Perspectives and mechanisms for targeting mitotic catastrophe in cancer treatment. Biochim Biophys Acta 2023:188965.
[7] Maiato H, Silva S. Double-checking chromosome segregation. J Cell Biol 2023;222(5):e202301106.
[8] Huttlin EL, Bruckner RJ, Paulo JA, Cannon JR, Ting L, Baltier K, et al. Architecture of the human interactome defines protein communities and disease networks. Nature 2017;545(7655):505–9.
[9] Kops GJ, Snel B, Tromer EC. Evolutionary dynamics of the spindle assembly checkpoint in eukaryotes. Curr Biol 2020;30(10):R589–602.
[10] Polley S, Müschenborn H, Terbeck M, De Antoni A, Vetter IR, Dogterom M, et al. Stable kinetochore-microtubule attachment requires loop-dependent ndc80-ndc80 binding. EMBO J 2023;42(13):e112504.
[11] Martinez-Chacin RC, Bodrug T, Bolhuis DL, Kedziora KM, Bonacci T, Ordureau A, et al. Ubiquitin chain-elongating enzyme ube2s activates the ring e3 ligase apc/c for substrate priming. Nat Struct Mol Biol 2020;27(6):550–60.
[12] Vink M, Simonetta M, Transidico P, Ferrari K, Mapelli M, De Antoni A, et al. In vitro frap identifies the minimal requirements for mad2 kinetochore dynamics. Curr Biol 2006;16(8):755–66.
[13] Chen C, Piano V, Alex A, Han SJ, Huis in't Veld PJ, Roy B, et al. The structural flexibility of mad1 facilitates the assembly of the mitotic checkpoint complex. Nat Commun 2023;14(1):1529.
[14] Mimori-Kiyosue Y. Imaging mitotic processes in three dimensions with lattice light-sheet microscopy. Chromosom Res 2021;29(1):37–50.
[15] Xiao M, Zhang S, Liu Z, Mo Y, Wang H, Zhao X, et al. Dual-functional significance of atm-mediated phosphorylation of spindle assembly checkpoint component bub3 in mitosis and the dna damage response. J Biol Chem 2022;298(3).

[16] Olsen JV, Vermeulen M, Santamaria A, Kumar C, Miller ML, Jensen LJ, et al. Quantitative phosphoproteomics reveals widespread full phosphorylation site occupancy during mitosis. Sci Signal 2010;3(104) ra3–ra3.
[17] Ibrahim B. Toward a systems-level view of mitotic checkpoints. Prog Biophys Mol Biol 2015;117(2–3):217–24.
[18] Doncic A, Ben-Jacob E, Barkai N. Evaluating putative mechanisms of the mitotic spindle checkpoint. Proc Natl Acad Sci USA 2005;102(18):6332–7.
[19] Caydasi AK, Lohel M, Grünert G, Dittrich P, Pereira G, Ibrahim B. A dynamical model of the spindle position checkpoint. Mol Syst Biol 2012;8.
[20] McAinsh AD, Marston AL. The four causes: the functional architecture of centromeres and kinetochores. Annu Rev Genet 2022;56(1):279–314.
[21] Pleuger R, Cozma C, Hohoff S, Denkhaus C, Dudziak A, Kaschani F, et al. Microtubule end-on attachment maturation regulates mps1 association with its kinetochore receptor. Curr Biol 2024;34(11):2279–93.
[22] Kiewisz R, Fabig G, Conway W, Baum D, Needleman D, Müller-Reichert T. Three-dimensional structure of kinetochore-fibers in human mitotic spindles. eLife 2022;11:e75459.
[23] Lara-Gonzalez P, Pines J, Desai A. Spindle assembly checkpoint activation and silencing at kinetochores. Seminars in cell & developmental biology, vol. 117. Elsevier; 2021. p. 86–98.
[24] Peters J-M. The anaphase-promoting complex: proteolysis in mitosis and beyond. Mol Cell 2002;9(5):931–43.
[25] Barford D. Structural interconversions of the anaphase-promoting complex/cyclosome (apc/c) regulate cell cycle transitions. Curr Opin Struct Biol 2020;61:86–97.
[26] May KM, Paldi F, Hardwick KG. Fission yeast apc15 stabilizes mcc-cdc20-apc/c complexes, ensuring efficient cdc20 ubiquitination and checkpoint arrest. Curr Biol 2017;27(8):1221–8.
[27] Izawa D, Pines J. The mitotic checkpoint complex binds a second cdc20 to inhibit active apc/c. Nature 2015;517(7536):631–4.
[28] Kim S, Yu H. Multiple assembly mechanisms anchor the kmn spindle checkpoint platform at human mitotic kinetochores. J Cell Biol 2015;208(2):181–96.
[29] Nezi L, Musacchio A. Sister chromatid tension and the spindle assembly checkpoint. Curr Opin Cell Biol 2009;21(6):785–95.
[30] Musacchio A. Spindle assembly checkpoint: the third decade. Philos Trans - R Soc B, Biol Sci 2011;366(1584):3595–604.
[31] Eytan E, Wang K, Miniowitz-Shemtov S, Sitry-Shevah D, Kaisari S, Yen TJ, et al. Disassembly of mitotic checkpoint complexes by the joint action of the aaa-atpase trip13 and p31comet. Proc Natl Acad Sci 2014;111(33):12019–24.
[32] Miniowitz-Shemtov S, Eytan E, Kaisari S, Sitry-Shevah D, Hershko A. Mode of interaction of trip13 aaa-atpase with the mad2-binding protein p31comet and with mitotic checkpoint complexes. Proc Natl Acad Sci 2015;112(37):11536–40.
[33] Brulotte ML, Jeong B-C, Li F, Li B, Yu EB, Wu Q, et al. Mechanistic insight into trip13-catalyzed mad2 structural transition and spindle checkpoint silencing. Nat Commun 2017;8(1):1956.
[34] Caydasi A, Pereira G. Spoc alert-when chromosomes get the wrong direction. Exp Cell Res 2012;318(12):1421–7.
[35] Geymonat M, Spanos A, Walker P, Johnston L, Sedgwick S. In vitro regulation of budding yeast bfa1/bub2 gap activity by cdc5. J Biol Chem 2003;278(17):14591–4.
[36] Caydasi A, Micoogullari Y, Kurtulmus B, Palani S, Pereira G. The 14-3-3 protein bmh1 functions in the spindle position checkpoint by breaking bfa1 asymmetry at yeast centrosomes. Mol Biol Cell 2014;25(14):2143–51.
[37] Hu F, Wang Y, Liu D, Li Y, Qin J, Elledge SJ. Regulation of the bub2/bfa1 gap complex by cdc5 and cell cycle checkpoints. Cell 2001;107(5):655–65.
[38] Morrison SJ, Kimble J. Asymmetric and symmetric stem-cell divisions in development and cancer. Nature 2006;441(7097):1068–74.
[39] Bradner JE, Hnisz D, Young RA. Transcriptional addiction in cancer. Cell 2017;168(4):629–43.
[40] Sapoval N, Aghazadeh A, Nute MG, Antunes DA, Balaji A, Baraniuk R, et al. Current progress and open challenges for applying deep learning across the biosciences. Nat Commun 2022;13(1):1728.
[41] Aderem A. Systems biology: its practice and challenges. Cell 2005;121(4):511–3.
[42] Chen KC, Calzone L, Csikasz-Nagy A, Cross FR, Novak B, Tyson JJ. Integrative analysis of cell cycle control in budding yeast. Mol Biol Cell 2004;15(8):3841–62.
[43] Aguda BD. A quantitative analysis of the kinetics of the g2 dna damage checkpoint system. Proc Natl Acad Sci 1999;96(20):11352–7.
[44] Kitano H. Systems biology: a brief overview. Science 2002;295(5560):1662–4.
[45] He E, Kapuy O, Oliveira R, Uhlmann F, Tyson J, Novák B. System-level feedbacks make the anaphase switch irreversible. Proc Natl Acad Sci USA 2011;108(24):10016–21.
[46] Tyson JJ, Chen K, Novak B. Network dynamics and cell physiology. Nat Rev Mol Cell Biol 2001;2(12):908–16.
[47] Sible JC, Tyson JJ. Mathematical modeling as a tool for investigating cell cycle control networks. Methods 2007;41(2):238–47.
[48] Ma C, Gurkan-Cavusoglu E. A comprehensive review of computational cell cycle models in guiding cancer treatment strategies. npj Syst Biol Appl 2024;10(1):71.
[49] Musacchio A, Salmon E. The spindle-assembly checkpoint in space and time. Nat Rev Mol Cell Biol 2007;8(5):379–93.
[50] Kukreja AA, Kavuri S, Joglekar AP. Microtubule attachment and centromeric tension shape the protein architecture of the human kinetochore. Curr Biol 2020;30(24):4869–81.
[51] Roscioli E, Germanova TE, Smith CA, Embacher PA, Erent M, Thompson AI, et al. Ensemble-level organization of human kinetochores and evidence for distinct tension and attachment sensors. Cell Rep 2020;31(4).
[52] Henze R, Dittrich P, Ibrahim B. A dynamical model for activating and silencing the mitotic checkpoint. Sci Rep 2017;7(1).
[53] Novák B, Tyson JJ. Design principles of biochemical oscillators. Nat Rev Mol Cell Biol 2008;9(12):981–91.
[54] Ibrahim B, Diekmann S, Schmitt E, Dittrich P. In-silico modeling of the mitotic spindle assembly checkpoint. PLoS ONE 2008;3(2).
[55] Henze R, Mu C, Puljiz M, Kamaleson N, Huwald J, Haslegrave J, et al. Multi-scale stochastic organization-oriented coarse-graining exemplified on the human mitotic checkpoint. Sci Rep 2019;9(1).
[56] Kreyssig P, Wozar C, Peter S, Veloz T, Ibrahim B, Dittrich P. Effects of small particle numbers on long-term behaviour in discrete biochemical systems. Bioinformatics 2014;30(17) i475–i481.
[57] Sear RP, Howard M. Modeling dual pathways for the metazoan spindle assembly checkpoint. Proc Natl Acad Sci USA 2006;103(45):16758–63.
[58] Lohel M, Ibrahim B, Diekmann S, Dittrich P. The role of localization in the operation of the mitotic spindle assembly checkpoint. Cell Cycle 2009;8(16):2650–60.
[59] Tschernyschkow S, Herda S, Gruenert G, Döring V, Görlich D, Hofmeister A, et al. Rule-based modeling and simulations of the inner kinetochore structure. Prog Biophys Mol Biol 2013;113(1):33–45.
[60] Gruenert G, Ibrahim B, Lenser T, Lohel M, Hinze T, Dittrich P. Rule-based spatial modeling with diffusing, geometrically constrained molecules. BMC Bioinform 2010;11.
[61] Henze R, Huwald J, Mostajo N, Dittrich P, Ibrahim B. Structural analysis of in silico mutant experiments of human inner-kinetochore structure. Biosystems 2015;127:47–59.
[62] Ibrahim B, Henze R, Gruenert G, Egbert M, Huwald J, Dittrich P. Spatial rule-based modeling: a method and its application to the human mitotic kinetochore. Cells 2013;2(3):506–44.
[63] Henze R, Grünert G, Ibrahim B, Dittrich P. Spatial rule-based simulations: the srsim software. Methods Mol Biol 2019;1945:231–49.
[64] Kreyssig P, Escuela G, Reynaert B, Veloz T, Ibrahim B, Dittrich P. Cycles and the qualitative evolution of chemical systems. PLoS ONE 2012;7(10).
[65] Novak B, Tyson JJ. Modeling the dynamics of the cell cycle. Cell 2008;135(1):113–23.
[66] Ibrahim B, Schmitt E, Dittrich P, Diekmann S. In silico study of kinetochore control, amplification, and inhibition effects in mcc assembly. Biosystems 2009;95(1):35–50.
[67] Simonetta M, Manzoni R, Mosca R, Mapelli M, Massimiliano L, Vink M, et al. The influence of catalysis on Mad2 activation dynamics. PLoS Biol 2009;7(1):e10.
[68] Piano V, Alex A, Stege P, Maffini S, Stoppiello GA, Huis in't Veld PJ, et al. Cdc20 assists its catalytic incorporation in the mitotic checkpoint complex. Science 2021;371(6524):67–71.
[69] Hindmarsh AC, Brown PN, Grant KE, Lee SL, Serban R, Shumaker DE, et al. Sundials: suite of nonlinear and differential/algebraic equation solvers. ACM Trans Math Softw 2005;31(3):363–96.
[70] Petzold LR. Automatic selection of methods for solving stiff and nonstiff systems of ordinary differential equations. SIAM J Sci Stat Comput 1983;4(1):136–48.
[71] Ermentrout B. Xpp-aut phase plane analyzing software. https://sites.pitt.edu/%7Ephase/bard/bardware/xpp/xpp.html, 2002. [Accessed 12 October 2024].
[72] Wolfinger MT, Ewald JC, Flamm C. Sbmltoolbox: an sbml toolbox for Matlab users. Bioinformatics 2008;24(6):871–3.
[73] Kitano H, Funahashi A, Matsuoka Y, Oda K. Celldesigner: a process diagram editor for gene-regulatory and biochemical networks. Biosilico 2005;1(5):159–62.
[74] Hoops S, Sahle S, Gauges R, Lee C, Pahle J, Simus N, et al. Copasi–a complex pathway simulator. Bioinformatics 2006;22(24):3067–74.
[75] Ghosh S, Tyson JJ. Bifurcation analysis of cell cycle regulation models. J Theor Biol 2009;257(2):222–30.
[76] Cheeseman I, Desai A. Molecular architecture of the kinetochore-microtubule interface. Nat Rev Mol Cell Biol 2008;9(1):33–46.
[77] Bardini R, Politano G, Benso A, Di Carlo S. Multi-level and hybrid modelling approaches for systems biology. Comput Struct Biotechnol J 2017;15:396–402.
[78] Hagan R, Manak M, Buch H, Meier M, Meraldi P, Shah J, et al. p31 comet acts to ensure timely spindle checkpoint silencing subsequent to kinetochore attachment. Mol Biol Cell 2011;22(22):4236–46.
[79] Kulukian A, Han J, Cleveland D. Unattached kinetochores catalyze production of an anaphase inhibitor that requires a mad2 template to prime cdc20 for bubr1 binding. Dev Cell 2009;16(1):105–17.
[80] Mistry HB, MacCallum DE, Jackson RC, Chaplain MA, Davidson FA. Modeling the temporal evolution of the spindle assembly checkpoint and role of aurora b kinase. Proc Natl Acad Sci 2008;105(51):20215–20.
[81] Ibrahim B. Spindle assembly checkpoint is sufficient for complete cdc20 sequestering in mitotic control. Comput Struct Biotechnol J 2015;13:320–8.
[82] COMSOL. COMSOL multiphysics: PDE module. https://www.comsol.com/partial-differential-equation-module, 2023.
[83] FEniCS. FEniCS project: finite element solver. https://fenicsproject.org/, 2023.
[84] OpenFOAM. OpenFOAM documentation: PDE solvers. https://www.openfoam.com/, 2023.

[85] Team VC. Virtual cell: computational modeling and simulation software. https://vcell.org/, 2023.
[86] Gillespie DT. Exact stochastic simulation of coupled chemical reactions. J Phys Chem 1977;81(25):2340–61.
[87] Frost SD. Gillespie. jl: stochastic simulation algorithm in Julia. J Open Sour Softw 2016;1(3):42.
[88] Haseltine EL, Rawlings JB. Approximate simulation of coupled fast and slow reactions for stochastic chemical kinetics. J Chem Phys 2002;117(15):6959–69.
[89] Press WH. Numerical recipes. In: The art of scientific computing. 3rd edition. Cambridge University Press; 2007.
[90] Chylek LA, Harris LA, Tung C-S, Faeder JR, Lopez CF, Hlavacek WS. Rule-based modeling: a computational approach for studying biomolecular site dynamics in cell signaling systems. Wiley Interdiscip Rev, Syst Biol Med 2014;6(1):13–36.
[91] Faeder JR, Blinov ML, Goldstein B, Hlavacek WS. Rule-based modeling of biochemical networks. Complexity 2005;10(4):22–41.
[92] Burke PE, Campos CBdL, Costa LdF, Quiles MG. A biochemical network modeling of a whole-cell. Sci Rep 2020;10(1):13303.
[93] Murata T. Petri nets: properties, analysis and applications. Proc IEEE 1989;77(4):541–80.
[94] Dittrich P, Di Fenizio PS. Chemical organisation theory. Bull Math Biol 2007;69:1199–231.
[95] Peter S, Ghanim F, Dittrich P, Ibrahim B. Organizations in reaction-diffusion systems: effects of diffusion and boundary conditions. Ecol Complex 2020;43:100855.
[96] Peter S, Dittrich P, Ibrahim B. Structure and hierarchy of sars-cov-2 infection dynamics models revealed by reaction network analysis. Viruses 2021;13(1).
[97] Thong WJ, Ameedeen M. A survey of Petri net tools. In: Advanced computer and communication engineering technology: proceedings of the 1st international conference on communication and computer engineering. Springer; 2015. p. 537–51.
[98] Peter S, Woitke L, Dittrich P, Ibrahim B. Computing all persistent subspaces of a reaction-diffusion system. Sci Rep 2023;13(1).
[99] Peter S, Ibrahim B, Dittrich P. Linking network structure and dynamics to describe the set of persistent species in reaction diffusion systems. SIAM J Appl Dyn Syst 2021;20(4):2037–76.
[100] Musacchio A. The molecular biology of spindle assembly checkpoint signaling dynamics. Curr Biol 2015;25(20):R1002–18.
[101] Zupanic A, Bernstein HC, Heiland I. Systems biology: current status and challenges. Cell Mol Life Sci 2020;77(3):379–80.
[102] Lenser T, Hinze T, Ibrahim B, Dittrich P. Towards evolutionary network reconstruction tools for systems biology. In: Lecture notes in computer science (including subseries lecture notes in artificial intelligence and lecture notes in bioinformatics) vol. 4447; 2007. p. 132–42.
[103] Sarkar M, Maliekal TT, et al. Finding the partner: fret and beyond. Exp Cell Res 2024:114166.
[104] Sahl SJ, Hell SW. High-resolution 3d light microscopy with sted and resolft, high resolution imaging in microscopy and ophthalmology. New Front Biomed Opt 2019:3–32.
[105] Fish KN. Total internal reflection fluorescence (tirf) microscopy. Curr Protoc 2022;2(8):e517.
[106] Souza A, Nobrega G, Neves LB, Barbosa F, Ribeiro J, Ferrera C, et al. Recent advances of pdms in vitro biomodels for flow visualizations and measurements: from macro to nanoscale applications. Micromachines 2024;15(11):1317.
[107] Sebastian AM, Peter D. Artificial intelligence in cancer research: trends, challenges and future directions. Life 2022;12(12):1991.
[108] Huang R-H, Hong Y-K, Du H, Ke W-Q, Lin B-B, Li Y-L. A machine learning framework develops a dna replication stress model for predicting clinical outcomes and therapeutic vulnerability in primary prostate cancer. J Transl Med 2023;21(1):20.
[109] Sitry-Shevah D, Miniowitz-Shemtov S, Liburkin Dan T, Hershko A. The mitotic checkpoint complex controls the association of cdc20 regulatory protein with the ubiquitin ligase apc/c in mitosis. Proc Natl Acad Sci 2024;121(37):e2413089121.