

Modeling the temporal evolution of the spindle assembly checkpoint and role of Aurora B kinase

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Faithful separation of chromosomes prior to cell division at mitosis is a highly regulated process. One family of serine/threonine kinases that plays a central role in regulation is the Aurora family. Aurora B plays a role in the spindle assembly checkpoint, in part, by destabilizing the localization of BubR1 and Mad2 at centrosomes and responds to changes in tension caused by aberrant microtubule kinetochore attachments. Aurora B is overexpressed in a subset of cancers and is required for mitosis, making it an attractive anticancer target. Here, we use mathematical modeling to extend a current model of the spindle assembly checkpoint to incorporate all signaling kinetochores within a cell rather than just one and the role of Aurora B within the resulting model. We find that the current model of the spindle assembly checkpoint is robust to variation in its key diffusion-limited parameters. Furthermore, when Aurora B inhibition is considered within the model, for a certain range of inhibitor concentrations, a prolonged prometaphase/metaphase is observed. This level of inhibitor concentrations has not yet been studied experimentally, to the authors' best knowledge. Therefore, experimental verification of the results discussed here could provide a deeper understanding of how kinetochores and Aurora B cooperate in the spindle assembly checkpoint.

mathematical | mitosis | kinetochore | metazoan | cell cycle

The faithful separation of chromosomes prior to cell division at mitosis is essential for maintaining genomic integrity. Failure to do so correctly may lead to genomic instability, aneuploidy, and cancer (1–3). Chromosome segregation requires the formation of a microtubule network that connects the spindle poles located at either end of the cell, originating in centrosomes, with kinetochores (protein structures located at the centromeres of each chromosome) (4). This is a highly regulated process involving the interactions between multiple protein complexes and signaling pathways (3, 5). One family of serine/threonine kinases that plays a central role in regulation is the Aurora family consisting of 3 forms in metazoans: Aurora A, Aurora B, and Aurora C. In baker's yeast and budding yeast only one homologue is found (Ark1 and Ipl1, respectively) (6–8). Aurora C is only expressed in germ cells, where as Aurora A and Aurora B are found in all somatic cells (6). Significantly, all 3 kinases are overexpressed in a variety of cancers, suggesting that a growth advantage is gained by deregulating Aurora kinases (5, 9). Conversely, severe inhibition of Aurora kinase activity leads to a fatally flawed mitosis (7, 10–16). Hence, this form of inhibition provides a possible mechanism for selective removal of replicating cells and thus has led to the development of Aurora kinase inhibitors as possible anticancer drugs (13–17).

Aurora B is active throughout mitosis with protein levels peaking at G₂/M phase of the cell cycle (6). Aurora B forms a complex with INCENP and survivin that regulate its activity and localization throughout mitosis (18–20). Proteins of the Aurora B complex are “chromosome passengers” localized to the centromeres from prophase until the metaphase–anaphase transition where Aurora B relocates to the spindle midzone and equatorial cell cortex as well as the microtubules (19). During telophase Aurora B localizes to the midbody throughout cytokinesis (6–8). Aurora B regulates chromosome congression, segregation, and cytokinesis (6, 21).

In budding yeast Ipl1 can promote correct spindle assembly by destabilizing syntelic attachments (22, 23). This has also been demonstrated in mammalian cells for syntelic attachments (14, 24) and merotelic attachments (25). Aurora B promotes turnover of microtubules at the kinetochores (21) possibly by regulating Hec 1 (26). Aurora B is also required for cytokinesis where it phosphorylates and regulates several substrates (7, 8). Characterization of Aurora B inhibitors has suggested that Aurora B plays a role in the spindle assembly checkpoint, in part, by destabilizing the localization of BubR1, Mad2, and Cenp-E at centrosomes (14, 27) and responds to changes in tension to promote biorientation (14, 22, 28, 29). Treatment of cells with Aurora B inhibitors cause chromosome alignment problems, spindle checkpoint override, and cytokinesis failure, broadly consistent with data generated by other methods (14–16).

The effects of Aurora B inhibition on the spindle assembly checkpoint are of particular interest because this checkpoint is crucial to prevent onset of anaphase without proper alignment of chromosomes and correctly attached spindles (30). A wait signal is generated by kinetochores that inhibits the activation of the anaphase-promoting complex (APC/C). Even one unattached kinetochore is thought to be enough to prevent the onset of anaphase (31). How this process occurs has yet to be fully defined, although a number of models have been proposed (30, 32, 33).

Recently, mathematical modeling of the spindle assembly checkpoint, both in open and closed mitosis, has provided some mechanistic understanding of how the checkpoint could operate (34, 35). Doncic *et al.* (34) considered various simplified models of how a single unattached kinetochore could generate a diffusive signal that could prevent the onset of anaphase in yeast. These models were based on cells that undergo closed mitosis, where the nuclear envelope is still intact even at the onset of anaphase. Therefore, the diffusive signal is only required to propagate throughout the nuclear volume. However, metazoan cells undergo open mitosis, where the nuclear envelope is no longer intact while the spindle assembly checkpoint is active. Therefore, the diffusive signal generated at a single unattached kinetochore now has to propagate throughout the entire cell rather than just the nucleus. One possible mechanism describing how this could be achieved was recently proposed by Sear and Howard (35). They considered a 2-step mechanism in which a stimulant (Cdc20) of the APC/C is inhibited: step 1, Cdc20 is assumed to interact with an unattached kinetochore to form an inhibited complex (candidates include C-Mad2-Cdc20 and BubR1-Cdc20, where C-Mad2 represents the closed form of Mad2); step 2, these inhibited complexes are assumed to be able to catalyze the production of further inhibited complexes of Cdc20, which are different in that

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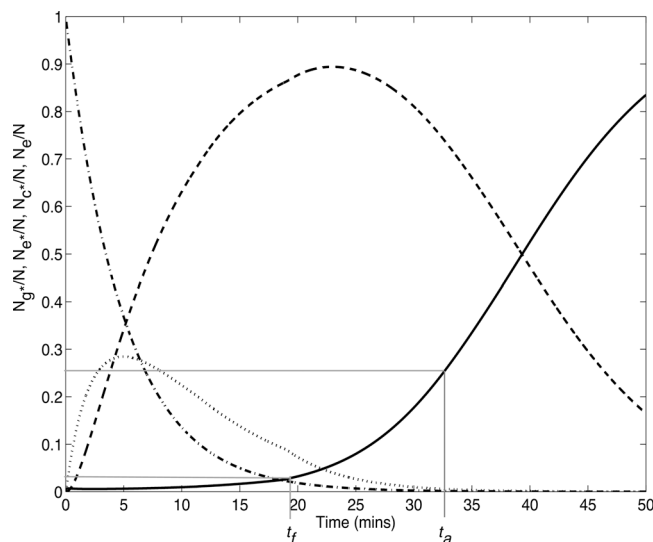


Fig. 2. Plot showing the temporal evolution of the fraction of molecules N_e/N (solid line), N_{e^*}/N (dotted line), N_{c^*}/N (dashed line), and N_{g^*}/N (dash-dotted line). See text for definition of t_f and t_a .

in ref. 35, we model the dynamics of the spindle assembly checkpoint as system Eq. 2 combined with the following system of ODEs,

$$\frac{dN_{e^*}}{dt} = \frac{k_k(B^s)}{v_c} (\langle X_U(t) \rangle + \langle X_S(t) \rangle) N_e - \alpha_e N_{e^*}, \quad [7a]$$

$$\frac{dN_{c^*}}{dt} = \frac{k}{v_c} N_{e^*} N_e - \alpha N_{c^*}, \quad [7b]$$

$$\frac{dN_{g^*}}{dt} = -\alpha_g N_{g^*}, \quad [7c]$$

where, $v_c = 6,000 \mu\text{m}^3$ is the cytoplasmic volume (35).

Recall each unit of type i comprises 2 kinetochores with the expected value $\langle X_i \rangle \in [0, 46]$. However, if $\langle X_i \rangle < 0.5$, then this expected value corresponds to the presence of less than a single kinetochore. Therefore, in system Eq. 7 we assume that, if $\langle X_i \rangle < 0.5$, then $\langle X_i \rangle \equiv 0$. This leads to the definition $t_f := \inf\{t \geq 0 | \langle X_S(t) \rangle < 0.5 \ \& \ \langle X_U(t) \rangle < 0.5\}$ which best represents the time at which the last kinetochore is sensed by system Eq. 7. (This corresponds to $t = 0$ in ref. 35.) Furthermore, we define the time to anaphase (length of prometaphase/metaphase) as $t_a := \sup\{t \geq 0 | N_e(t) > 200,000\}$ (35).

Results

We first analyze the temporal evolution of the wait signal generated by all kinetochore units, described by systems Eq. 2 and Eq. 7. Subsequently, the effects of Aurora B inhibition are studied.

Temporal Evolution of the Wait Signal. The temporal evolution of N_e , N_{e^*} , N_{c^*} , and N_{g^*} , illustrated in Fig. 2, can be split into 2 distinct phases: For $0 \leq t < t_f$, e molecules are under tight inhibition ($N_e/N \leq 0.05$); at $t = t_f$, inhibition is lifted and N_e increases rapidly. These distinct phases can be explained as follows. In the first phase, recall that $\langle X_U(0) \rangle = 46$ and initially all of these units contribute to the production of e^* molecules (see Eq. 7a). Subsequently, $\langle X_U(t) \rangle$ decreases, $\langle X_S(t) \rangle$ increases, but $\langle X_U \rangle + \langle X_S \rangle$ decreases (data not shown). However, throughout this phase the production of e^* dominates the production of e resulting from the decay of g^* , c^* , and e^* . At $t = t_f$ the production of e^* is set to zero (see above discussion). Subsequently, the rapid decay rates (short lifetimes) of g^* , e^* , and c^* ensure a rapid increase in N_e .

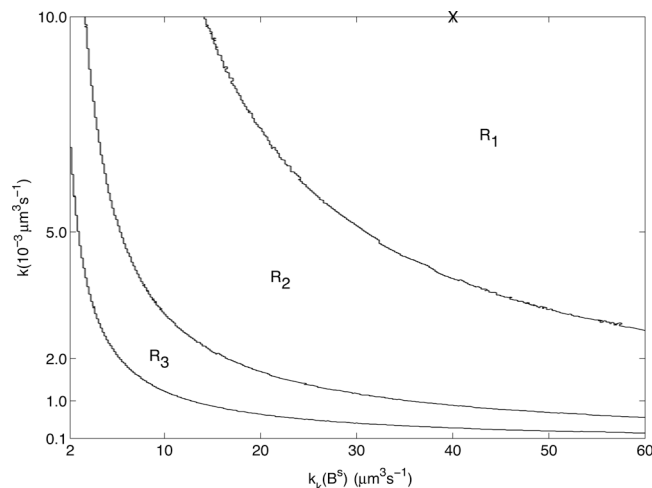


Fig. 3. Plot showing the level of inhibition, defined by, $\max(N_e(t)/N \forall t \in [0, t_f])$, for a range of $k_k(B^s)$ and k values. The regions R_1 , $R_1 + R_2$, and $R_1 + R_2 + R_3$ correspond to the regions where, at most, only 5, 10, or 15% of the molecules are in the uninhibited e state, respectively. X marks the values of $k_k(B^s)$ and k implemented by Sear and Howard (35).

Sear and Howard (35) focused on the signal generated by a single unattached kinetochore. Essentially, they considered this to be the only signal source for a time period in our $[0, t_f]$. Doing so allowed them to compute “steady state” values for N_e , N_{e^*} , and N_{c^*} , which were subsequently used as initial conditions (at a time corresponding to our $t = t_f$) for the second-phase dynamics discussed above. The authors in ref. 35 were concerned that to work effectively, their model required certain system parameters to be chosen at the upper end of their expected range. This has implications for the robustness of the model in application.

The model introduced here not only provides a more complete description of the wait signal dynamics, but also provides greater robustness to variations in the key diffusion-limited parameters, k and $k_k(B^s)$ (see Fig. 3). The model maintains tight inhibition of e molecules for $t < t_f$ for a large range of biological realistic values for k and $k_k(B^s)$, i.e., $k \approx 10^{-3}$ to $10^{-2} \mu\text{m}^3 \text{s}^{-1}$ and $k_k(B^s) \approx 6$ to $60 \mu\text{m}^3 \text{s}^{-1}$. Remarkably, this parameter variation does not significantly alter the necessary rapid release of inhibition in the second phase (data not shown).

We were concerned that the attachment transition (and associated signaling) process detailed above would be too crude to detect the subtle differences between the alternative signaling models proposed by and investigated in refs. 34 and 35. However, on replacing Eq. 6 with systems corresponding to the *direct inhibition* and *emitted inhibition* models, respectively, and embedding them appropriately in Eq. 7, it transpired that the attachment state transition model system is indeed able to differentiate these models. With either of these replacements, sufficiently tight inhibition of N_e was not observed for $t \in [0, t_f]$ (see *SI Appendix*).

Aurora B Inhibition. Aurora B plays a central role in many processes involved in mitosis. As a first step toward understanding this complex role, we assume that the concentration of Aurora B affects the 2 main components of our model in the following way. The role Aurora B plays in releasing aberrant attachments for subsequent repair (14, 24) is modeled by reasonably assuming that the rate constants k_4 and k_5 in Eq. 2 are positively correlated to the value of B^s . In lieu of further experimental evidence we assume this relationship to be linear, i.e., $k_4(B^s) = k'_4 B^s \text{min}^{-1}$, $k_5(B^s) = k'_5 B^s \text{min}^{-1}$, for constants k'_4 and k'_5 . The role of Aurora B in facilitating the recruitment of key checkpoint proteins (14, 27) is modeled similarly by allowing $k_k(B^s) = k'_k B^s \mu\text{m}^3 \text{s}^{-1}$ in Eq. 7,

could be found by titrating the inhibitor and recording the corresponding time to anaphase and/or counting aberrant attachments at anaphase onset.

Clearly, there are many additions and improvements that could be made to the model proposed here, from a more detailed description of the attachment process to a better representation of the role of Aurora B. For example spatial distribution of Aurora B within the cell is ignored in the present model but may play a role in the processes described here. Furthermore, although attempts have been made to include the stochasticity of the search and capture process of the kinetochores, it is assumed here that the spindle checkpoint mechanism is deterministic and it may be that both of these processes have stochastic elements. Finally, the process of search and capture of the kinetochores is very complex, and detailed modeling of these events may provide further refinement of the signaling mechanism modeled here. However, these improvements constitute significant further work, not least because of the lack of experimental evidence that could afford an accurate parameterization of more complex models.

Finally, given the complex nature of the role of Aurora kinase activity within the cell cycle, it seems unlikely that a straightforward PK/PD model with the PD component being of standard response-function type, will yield significant practical information. Developing mechanistic models of the type proposed here may provide a step toward a better understanding of the action of Aurora kinase inhibitors. The strength of these models is their ability to (theoretically) link drug concentration to intracellular response (as is done here) and through further modeling processes relate cell response to cell fate, cell fate to measurable biomarkers, and thus potentially predict drug efficacy. Experimentally verified models could lead to improved dosing and scheduling in clinical trials, and ultimately models may form part of new clinical tools for patient-specific drug treatment.

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