Evaluation of the Effect of Cell Parameters on the Number of Microtubule Merotelic Attachments in Metaphase Using a Three-Dimensional Computer Model

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Abstract. Even chromosome segregation between daughter cells during mitosis is crucial for genome integrity and is mostly regulated by proper attachments of spindle microtubules (MTs) to kinetochores. Abnormalities in this process can lead to chromosome mis-segregation and potentially result in severe developmental disorders, including aneuploidy and cancer. Merotelic attachments when tubulin MTs captured by kinetochore of one chromatid originate from both spindle poles are considered as one of the key molecular processes that cause such abnormalities. Here we present the first comprehensive three-dimensional model of metaphase, the key stage of mitosis in the context of proper chromosome segregation, and the results of its application to supercomputer simulation of kinetochore-MT attachments in metaphase. It appears that large values of the kinetochore crown angle lead to the preservation of merotelic attachments while the size of the cell and the probability of MT detachments affect only the rate of their suppression but do not interfere with the process of suppression itself. It has been demonstrated that the structure and the set of parameters of the model of mitosis have a severe impact on the results of simulations. We also compare the results of supercomputer 3D modeling of mitosis with outcomes of existing two-dimensional models.

Keywords: Mitosis · Metaphase · Chromosomes · Microtubules · Kinetochore · Merotelic attachment · Computer simulation

1 Introduction

An equal segregation of chromosomes between daughter cells is a key yet non-trivial task during mitosis. Such an outcome depends dramatically on the proper kinetochore-microtubule attachments. Merotelic attachments (MAs) correspond to the scenario when tubulin microtubules (MTs) captured by kinetochore of one chromatid originate from both spindle poles. Such erroneous attachments regularly take place at the early stages

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of mitosis. However, if they remain in anaphase, this can lead to mis-segregation of chromosomes and severe developmental abnormalities, including aneuploidy and cancer [1, 2].

Cellular mechanisms that suppress MAs at various stages of mitosis can be divided into two groups, namely, those that prevent the emergence of new MAs and those that reduce the number of existing ones [3, 4]. While protein-protein interactions play a substantial role in both cases, for the mechanisms of the first group, the structure of individual cellular organelles, especially kinetochore, is also of importance. Due to geometric restrictions, it is more difficult for the kinetochore to capture MTs growing from the opposite spindle pole than those growing from a nearby spindle pole.

The influence of geometric factors on MAs is considered in [5]. Using a two-dimensional computer model of the cell, the authors showed the importance of such parameter as the size and thickness of the kinetochore crown, the region that MTs can attach to. In particular, a significant deviation from the values that correspond to the "average" human cell, results in an increase in the number of MAs.

Here, we present the results of mitosis simulation based on a three-dimensional model of the cell that has much more parameters than the model in [5]. The paper has the following structure. In Sect. 2 we provide a brief overview of existing mathematical models of MT–chromosome interactions. Section 3 contains a description of the computer model we developed. Section 4 describes the methodology of numerical experiments as well as the parameters of virtual cells. Finally, in Sect. 5 we present and discuss the results of supercomputer simulations.

2 Similar Works

A detailed analysis of existing mathematical models of a dividing cell and their components can be found in [6, 7]. Below we briefly analyze the works that are closest to our three-dimensional model.

The main mechanism that allows MTs to find chromosomes in the space of a cell is called "search-and-capture" [8]. It is assumed that in metaphase, MTs have random directions and, due to their instability, constantly switch from polymerization to depolymerization state (the so-called catastrophe) and vice versa (see Fig. 1). When the length of the MT turns out to be zero, its direction changes randomly, thus, the search for chromosomes is carried out "blindly". It was shown that if the catastrophe is considered as a probabilistic event, the virtual cell begins to correspond to a living cell in such a parameter as the average length of MTs in time [9]. On the other hand, if the probabilities of these events are set to be constant, the time of detection of the first chromosome is several orders of magnitude greater than the times known from experimental studies [10]. This problem can be solved if the probability of catastrophes is determined through the gradient function, which corresponds to the "fight" of MTs for free tubulin proteins [11]. According to an alternative approach, these times become commensurate with expected ones if kinetochore is "allowed" to interact with MT as a whole, rather than just with its plus-end [12].

Another issue is the formalization of the mechanism of chromosome intracellular motion. The dominating concept of such a mechanism, called the "balance of forces",

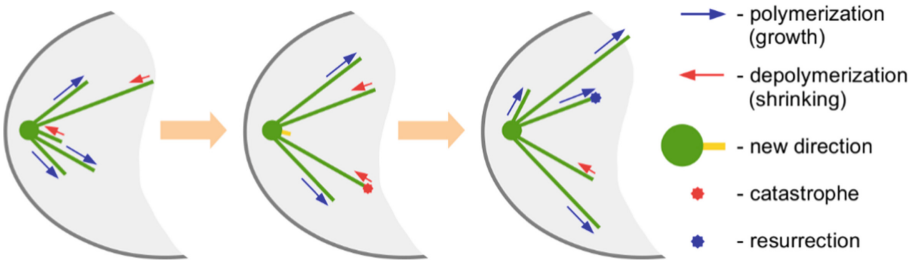

Fig. 1. Illustration of the search-and-capture concept used to describe the instability of MTs.

assumes that each pair of chromosomes moves in such a way that the sum of all forces exerted on them is zero [13]. Initially, three types of forces were analyzed, specifically, (i) the forces exerted on MTs from the spindle poles, (ii) the forces of attraction that arise at the plus-ends of MTs captured by kinetochore, and (iii) the force that counteracts centromere stretching. This approach was further developed by breaking down the forces of the second type into two independent categories, as well as by introducing an additional friction force to account for the viscosity of the cytoplasm [14]. A similar idea of the "balance of forces" is used in many other works. For example, when describing the interaction of MTs with chromosome arms, the repulsive force between the proteins of chromokinesin and tubulin was added [15].

Among recently proposed computer models, it is worth mentioning the representation of the kinetochore in the form of a flexible polymer structure, which, in particular, made it possible to evaluate the effect of thermal effects on its shape [16]. In [17], the growth of small auxiliary MTs directly on the kinetochore was reproduced. These MTs could bind to MTs from the spindle pole thus increasing the efficiency of the search-and-capture mechanism.

3 Computer Model

A detailed description of the computer model proposed by the authors, which is the development of the two-dimensional model [5], as well as its software implementation is provided elsewhere [18]. This model can describe any eukaryotic cell with metacentric chromosomes.

The spindle poles are represented by two material points diverging in the diametrically opposite sides of the cell in the first 180 s (see Fig. 2). MTs represented by lines of zero thickness are growing from the spindle poles in random directions within solid angle of π radians. Similarly to [9], MTs growth dynamics is described by four parameters: the rates of polymerization and depolymerization, V_{pol} and V_{depol}, and the probabilities of catastrophe, f_{cat}, and resurrection, f_{res}. MTs do not interact with each other, but respond to the following events:

– overriding the cell membrane that triggers the transition to the state of depolymerization,
– achieving zero length that leads to choosing a new direction of growth,

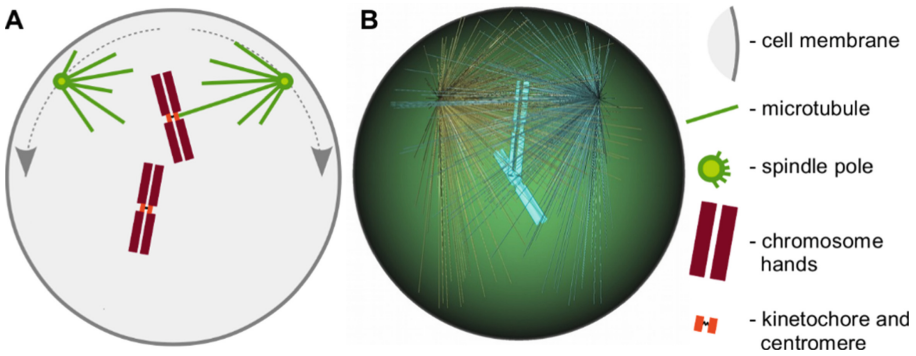

Fig. 2. Divergence of the spindle poles according to the used cell model: (A) schematic representation of model objects; (B) three-dimensional visualization of the model (1500 MTs, 2 pairs of chromosomes).

– collision with the arms of the chromosome that results in the MT "break off" or its transition to the state of depolymerization depending on the model parameter,
– collision with kinetochore that also results in "breaking off" and transition to the state of depolymerization,
– collision with the kinetochore crown that brings about the attachment to the kinetochore with probability K_{on}.

MT is attached to kinetochore by its plus-end and then moves with it. Probability K_{off} corresponds to its detachment and transition to a free state.

A pair of sister chromatids is modeled as a construction of six half-cylinders (see Fig. 3A), the dimensions of which are determined by the following parameters: the lengths of the chromosome, L_{chr}, and of the kinetochore, L_{kin}, the diameter of the arms of the chromosome, $D_{\text{chr}} = 2R_{\text{chr}}$, and of the kinetochore, $D_{\text{kin}} = 2R_{\text{kin}}$, the length of the centromere, S_{L}, the angle α_{kin}, which determines the size of the kinetochore crown. The centromere, in turn, is modeled either as a rod or as an extensible Hookean spring with the elastic coefficient S_{K}. Thus, each pair of sister chromatids has six or seven degrees of freedom, i.e. three spatial coordinates, three rotation angles and the length of the centromere (provided the latter is represented as a spring).

To simulate the chromosomes motion, the principle of the "balance of forces" [13] is written for the center of each chromosome pair in the form of equations of the sum of forces $\sum\limits_k \overrightarrow{F_1^k} + \overrightarrow{F_2} + \overrightarrow{F_3} + \overrightarrow{F_4} = 0$ and angular momenta $\sum\limits_k \overrightarrow{M_1^k} + \overrightarrow{M_3} + \overrightarrow{M_4} = 0$ (see Fig. 3B). Denoting the scalar and vector products by (,) and [,], the forces can be represented as follows:

– $\overrightarrow{F_1^k} = \overrightarrow{R^k}\left(a - b \cdot \left(\overrightarrow{R^k}, \vec{V} + \left[\vec{\omega}, \overrightarrow{r^k}\right]\right)\right)$ and $\overrightarrow{M_1^k} = \left[\overrightarrow{r_1^k}, \overrightarrow{F_1^k}\right]$ define the force and torque exerted by the kth MT attached to the kinetochore. The vectors $\vec{V}$ and $\vec{\omega}$ specify the linear and angular velocities of the pair of chromosomes, the constants a and b characterize the maximum force and its extinction coefficient, respectively. The unit

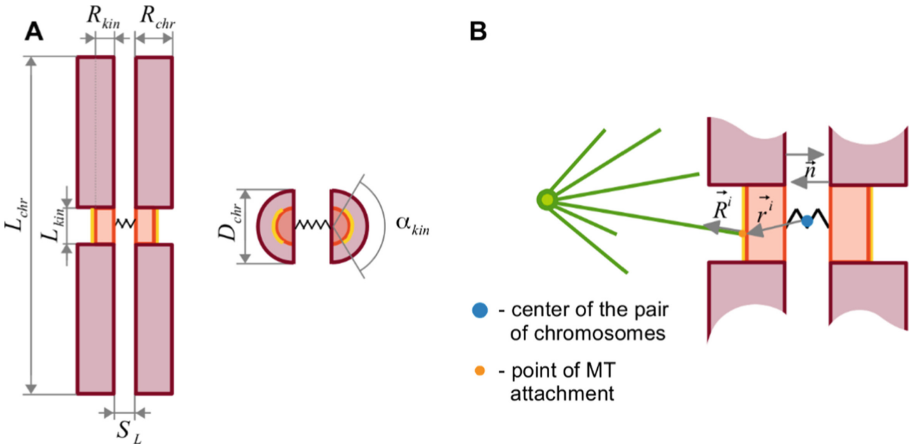

Fig. 3. The meaning of the key parameters of the model: (A) setting the size of the pair of chromosomes; (B) the directions of vectors of forces and points of their application.

vector $\overrightarrow{r^k}$ is directed from the center of the pair of chromosomes to the point of MT attachment, and $\overrightarrow{R^k}$ is directed from the point of attachment to the spindle pole.

- $\overrightarrow{F_2} = \vec{n} \cdot (S - S_L) \cdot S_K$ defines the force that arises when the centromere represented as a Hookean spring is stretched and that is exerted on each chromosome. The unit vector $\vec{n}$ is directed to the center of the sister chromatid, and the scalar S determines the current extension.
- $\overrightarrow{F_3} = \gamma \vec{V}$ and $\overrightarrow{M_3} = \eta \vec{\omega}$ define the friction force and momentum arising due to the viscosity of the cytoplasm. The coefficients γ and η are constant parameters of the model, vectors $\vec{V}$ and $\vec{\omega}$ specify the linear and angular velocities of the pair of chromosomes.
- $\overrightarrow{F_4}(t)$ and $\overrightarrow{M_4}(t)$ correspond to the noise term of the Langevin equation, which statistically reproduces the effect of the Brownian motion of cytoplasm molecules. The rates of translational and rotational motion are characterized by constants D_{trans} and D_{rot}. Random variables are modeled by normal distribution.

For an unambiguous description of the cell, the following parameters were added. The numbers N_{MTs} and N_{chrs} specify the total number of MTs growing from one spindle pole and the number of pairs of chromosomes, respectively. The geometrical dimensions of the cell are determined by its radius, R_{cell}, and by the distance between the spindle poles, L_{poles}.

4 Numerical Experiments Technique

For numerical experiments, we used the open source software package MiCoSi (Mitosis Computer Simulator, https://github.com/m-krivov/MiCoSi) developed by the authors that implements the proposed mathematical model of mitosis. Its accuracy and consistency was verified in two ways. First, MiCoSi software contains automatic tests that simulate trivial scenarios of cell division and track the transition of a simple virtual cell

to the expected state. Second, within each experiment, the evolution of one cell (from a group of identical cells) was tracked in manual mode using the built-in visualizer.

The setup of simulated scenarios and the export of results are carried out by compiling and running an auxiliary program in C#. The conclusions below were obtained using a solver codenamed Experimental, which contains the latest version of the model. As for the implementations of those parts of the algorithm that allow of some alternatives, the choice was made as follows:

– spatial coordinates of chromosomes were "frozen" in the equatorial plane to level out a possible side effect of their oscillations. The validity of such a "freeze" requires a separate detailed study and is not discussed in this paper;
– in the case of rotation of a pair of chromosomes, the attached MTs were not allowed to pass through the kinetochore. Instead, they "wound" round it like threads. While this choice did not lead to noticeable differences in simulation results, we consider it as more consistent with reality;
– MT can attach to the kinetochore not only with its plus-end, but also with any of its points. When passing through the chromosome arm, MT switches to the state of depolymerization rather than "breaks off".

For each case under consideration, the simulations were performed on an ensemble of 100 cells with a time step of 0.1 s, afterwards the results were averaged. The parameters of the model were chosen as corresponding to a human cell (Table 1) but only for modeling one pair of chromosomes. Each numerical experiment consisted in varying one selected parameter and, unless otherwise indicated, in measuring two quantities - the total number of attached MTs and the number of MAs.

The calculations were partially performed on ten nodes of the Lomonosov-2 supercomputer equipped with an Intel Xeon E5 - 2697 v3 series CPU. In total, 100 cores were used, and parallelization between them was performed using MPI and OpenMP technologies. Due to the independence of the calculations, almost linear scalability was observed [18].

Table 1. The values of cell parameters used in computer simulations by default.

Parameter	R_{cell}	L_{poles}	L_{chr}	D_{chr}	L_{kin}	D_{kin}	S_L	S_K	α_{kin}	N_{MTs}	N_{chrs}	D_{trans}
Value	6	10	3	0.5	0.5	0.3	0.1	1300	135	1500	1	0.01
Units of measure	μm							$\frac{pN}{\mu m}$	°	units	units	$\frac{\mu m^2}{s}$

Parameter	V_{pol}	V_{dep}	K_{on}	K_{off}	f_{cat}	f_{res}	a	b	γ	η	D_{rot}
Value	12.8	14.1	1.0	0.002	0.058	0.045	45	3.516	0.006	5700	0.0015
Units of measure	$\frac{\mu m}{min}$	$\frac{\mu m}{min}$	-	-	-	-	pN	$\frac{pN \cdot min}{\mu m}$	$\frac{pN \cdot s}{nm}$	$\frac{pN \cdot s}{rad}$	$\frac{\mu m^2}{s^{-1}}$

5 Results and Discussion

The features of the mathematical model have too significant impact on the results. On Fig. 4, the results of three-dimensional modeling of the beginning of metaphase are compared with similar numerical experiments from [5] conducted on a fairly similar but two-dimensional model. In both cases, the virtual cells were in the same initial states and had similar values of biophysical parameters. The main difference in the results is the sharp increase in the total number of MT attachments between 20 and 50 s after the start of the metaphase (see Fig. 4D) in 3D model, which is primarily due to the possibility of lateral attachments of MTs. After reaching the peak, this number begins to monotonously decrease and eventually stabilizes at a certain level that depends on the cell parameters, while in [5] an opposite conclusion was made about the monotonous growth of the number of MT attachments that reach a "plateau" only by 10–20 min of the metaphase.

This tendency also resulted in noticeable differences in the distribution of kinetochores by the types of attachments (see Fig. 4A). Our 3D model predicts that during the first minute, there should be a fairly sharp transition of all pairs of kinetochores from the "No KMTs" state to the "Merotelic" one (see Fig. 4B), which means that each of them has at least one MA with MT. In the model from [5], this process takes 2 min (see Fig. 4C), and by the time it is completed, about 20% of the kinetochore pairs lose their merotelic attachments or do not have them at all.

At the same time, it should be recognized that the key mitosis patterns known from experimental work are reproduced within the framework of both models [19]. For example, there is a characteristic increase in the number of MAs at the beginning of the metaphase and they are almost completely suppressed towards its end. The values for the total number of attachments are close to the expected ones. Thus, we can conclude that the issue of validating the entire variety of mathematical models of mitosis is becoming more and more relevant, especially if new conclusions about the nature of mitosis are made on their basis.

Large values of kinetochore crown angle lead to the preservation of MAs, the size of virtual cell and the probability of MT detachments affect the rate of their suppression. In [5], it was concluded that the initial position and orientation of a pair of chromosomes have a significant impact on the MAs dynamics. Our calculations confirmed this statement [18], showing that for some configurations, the pair of chromosomes can be rotated by 70°–90° by the end of the metaphase, and this position is stable. To reduce the possible impact of the initial cell configuration on the process under study, in this numerical experiment, a pair of chromosomes was positioned in the center of the cell so that kinetochores were equally accessible to MTs growing from each of the poles, rather than being shielded by chromosomes' arms (see Fig. 5).

If the radius of the cell, r_{cell} (see Fig. 5C), and the probability of the detaching events, k_{off} (see Fig. 5B), are varied, there is a similar change in the total number of MT attachments. At the same time, the time required for the cell to completely suppress MAs increases or decreases by dozens of minutes. This suggests that these two parameters implicitly determine the duration of the metaphase.

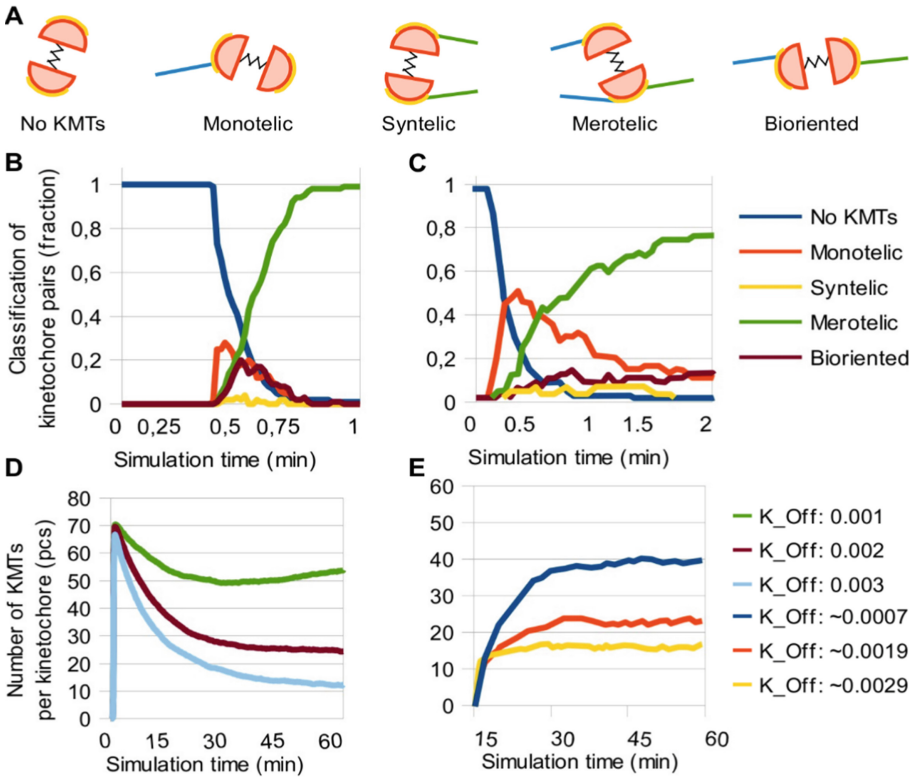

Fig. 4. Transition from 2D to 3D: reproducing the results of modeling the beginning of the metaphase, taken from [5], on the basis of the package developed by the authors: (A) types of kinetochores depending on the nature of MT attachments; (B) classification of kinetochores based on the 3D model, the cell parameters correspond to Table 1; (C) classification of kinetochores from [5] (2D model); (D) the dependence of the average number of MT attachments per kinetochore in 3D model when the probabilities of events are varied; (E) the dependence of the average number of MT attachments per kinetochore from [5] (2D model), the probabilities of detachments are estimated according to [13].

Finally, our calculations confirmed the conclusion [5] that the size of the kinetochore crown, set by α_{kin}, is indeed a key element of the geometric mechanism for suppressing MAs. Large values of this angle lead not only to a slowdown in the rate of MAs suppression, but also to their preservation at the end of the metaphase.

The diameter of the kinetochore does not have a significant effect on MAs at all. If we consider the influence of the kinetochore diameter, D_{kin} (see Fig. 5A), it limits only the total number of MT attachments (from ~20 attachments for $D_{kin} = 2$ μm and ~60 attachments for $D_{kin} = 0.5$ μm), but does not affect the percentage of MAs. Additionally, it was found that with a kinetochore diameter of about 1 μm, by the end of the metaphase, the pair of chromosomes almost completely loses all MT attachments.

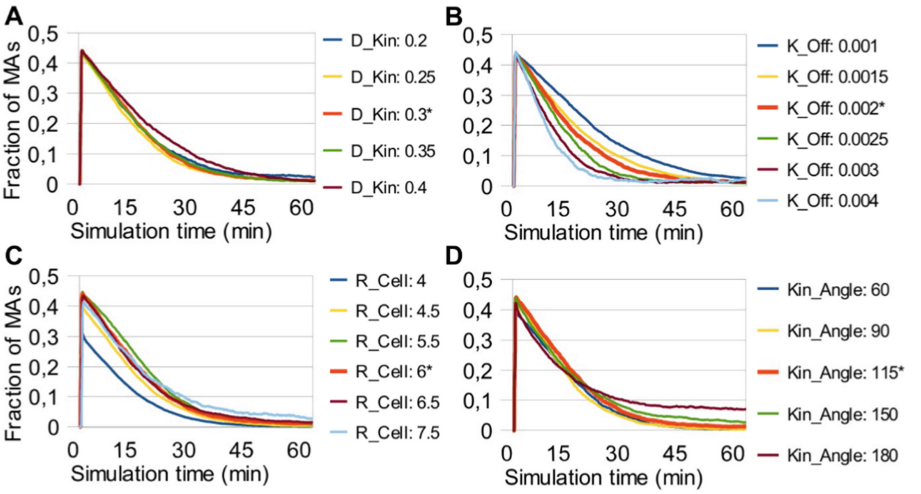

Fig. 5. The efficiency of suppressing MAs depending on the biophysical parameters of virtual cell during the first hour of the metaphase. Values marked with * correspond to the configuration in Table 1: (A) variation in the size of the kinetochore (μm); (B) variation of the probability of MT detachment from the kinetochore (s^{-1}); (C) variation of cell radius (μm) and the distance between the spindle poles (proportional to the radius); (D) variation of the angle of the kinetochore crown (degrees).

It should be emphasized that this conclusion, obtained by mathematical simulations, contradicts some experimentally established facts. When studying cells of female Indian muntjac deer [20], which have only 6 pairs of chromosomes, it was observed that chromosomes with larger kinetochore have more MAs, including the percentage ratio (7.0% vs. 1.6%). As a consequence the authors claimed that the size of the kinetochore is extremely important for suppressing MAs and for erroneous chromosome divergence in anaphase, i.e. chromosomes 'missegregate during anaphase'.

The reason for this discrepancy may be both the features of the proposed mathematical model and differences in the properties of the studied cells. As already noted, the initial position of the chromosomes has a certain influence on the dynamics of MAs, so a similar simulation conducted with other model settings can recalibrate our conclusions. A certain effect may be also observed from further modifications of the model such as a transition to a more complex representation of the kinetochore and account of repulsive forces arising from the interaction of microtubules with chromosomes' arms. Thus, it should be emphasized once again that the choice of the mathematical model of mitosis, unfortunately, has a noticeable impact on the outcomes.

Summing up the results of the numerical simulation it can be argued that large values of the kinetochore crown angle lead to the preservation of MAs at the end of the metaphase. As for the size of the cell and the probability of MT detaching events, they only affect the rate of Mas suppression, but do not interfere with such suppression itself. The diameter of kinetochore does not have a significant effect on MAs at all.

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