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A computational model for the formation of lamin-B mitotic spindle envelope and matrix

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Recent reports show that, after nuclear envelope breakdown, lamin-B, a component of the nuclear lamina in interphase, localizes around the mitotic spindle as a membranous network. How this process occurs, however, and how it influences mitotic spindle morphogenesis is unclear. Here, we develop a computational model based on a continuum description to represent the abundance and location of various molecular species involved during mitosis, and use the model to test a number of hypotheses regarding the formation of the mitotic matrix. Our model illustrates that freely diffusible nuclear proteins can be captured and transported to the spindle poles by minus-end-directed microtubule (MT) motors. Moreover, simulations show that these proteins can be used to build a shell-like region that envelopes the mitotic spindle, which helps to improve the focusing of the mitotic spindle by spatially restricting MT polymerization and limiting the effective diffusion of the free MTs. Simulations also confirm that spatially dependent regulation of the spindle network through the Ran system improves spindle focusing and morphology. Our results agree with experimental observations that lamin-B reorganizes around the spindle and helps to maintain spindle morphology.

1. Introduction

The formation of the mitotic spindle represents one of nature's most fascinating examples of self-assembly, requiring the concerted action of microtubules (MTs), mitotic motors and associated proteins [1]. A long-standing hypothesis is that a static, non-MT structure might support the assembly of the mitotic spindle by functioning as a scaffold to both restrict diffusion and counteract motor forces [2,3]. Until recently, the existence, much less the nature and molecular identity, of this spindle matrix was uncertain. However, experiments have now provided support for the formation of a mitotic membranous network by some nuclear proteins including the nuclear lamina protein lamin-B. Tsai *et al.* [4] showed that the intermediate filament protein lamin-B assembles during mitosis into matrix-like and membrane-containing networks in a RanGTP-dependent manner in *Xenopus* egg extracts. This lamin-B network appears to both permeate and ensheath spindles assembled in egg extracts. Subsequent experiments have demonstrated that the matrix contains a number of spindle assembly factors, including dynein and Nudel [5,6]. Interestingly, *Drosophila* embryos, which are known to perform partially 'open mitosis' with a fenestrated nuclear envelope and in which nuclear lamina surround the pro-metaphase and metaphase spindles, assemble matrix-containing nuclear proteins Skeletor, Megator and Chromator that permeate mitotic spindles [7]. Phenotypic analyses of embryos depleted of Skeletor, Megator or Chromator indicate that these proteins are required for proper the spindle morphogenesis or function.

How these proteins assemble into the spindle matrix remains unclear. Depletion of lamin-B in *Xenopus* egg extracts or *Drosophila* early embryos leads to defects in spindle morphology. Lamin-B co-immunoprecipitates with both dynein and Eg5, the plus- and minus-end-directed motors, respectively, in *Xenopus*

egg extracts [8]. Additionally, lamin-B counteracts the Eg5 forces to help maintain spindle length, width and pole focusing [8]. As, upon nuclear envelope breakdown, lamin-B-containing nuclear envelope remnants are observed to be transported towards the minus ends of spindle MTs by dynein [9–11], the assembly of lamin-B-containing mitotic spindle envelope and matrix may require both dynein and MTs. Consistent with this, depolymerizing MTs or inhibiting dynein in egg extracts severely inhibited lamin-B assembly [4,5].

Although the above experiments suggest that nuclear proteins could use MTs and motors to assemble into the spindle envelope and matrix, how such assembly occurs in conjunction with spindle formation is unclear [12]. Based on the known interactions between lamin-B and motors, we develop a computational model to describe the formation of the lamin-B spindle envelope and matrix and use the model to consider a number of open questions. In the absence of any other structures, are MT dynamics sufficient for a spindle to form, or are additional structures necessary? How is the morphology of the spindle affected by having spatially dependent regulation of the polymerization/depolymerization rates, as might arise because of the RanGTP gradient that develops during mitosis? Can freely diffusible lamin, present throughout the cell after nuclear envelope breakdown, be captured and used to build a network around the spindle? Moreover, what effect does this matrix have on spindle morphology? What possible role does the mitotic matrix play in helping to establish the spindle?

In general, modelling can be used to test hypotheses and make predictions to guide the experimental direction. Given the multitude of possible regulatory models governing spindle formation and the difficulty of isolating them experimentally, computational models are a particularly useful means of testing their respective effects. Mitosis, and, in particular, spindle formation, has received considerable attention of mathematical and computational models [13–15]. Computational models of spindle assembly come in varying forms [16]. They may include individual elements (e.g. MTs and MT motors) and, using first principles from physics, describe the discrete interactions in Monte Carlo simulations [17–19]. Alternatively, continuum models do not attempt to track the interaction of discrete elements. Instead, the filament network is described by a system of coupled differential equations whose solution describes the concentration of the key components over both time and space. As such, they provide information about macroscopic properties of the system. They may be more powerful as they enable consideration of larger systems over longer time scales [20]. Because of the meshwork nature of the spindle matrix, which is likely to include numerous interacting discrete filaments, we use a continuum description of the relevant components.

2. Material and methods

We developed a computational model based on a continuum description to represent the abundance and location of the various molecular species involved during mitosis. Specifically, we tracked the dynamics of free and polymerized tubulin, MTs, the GTPase Ran in both GDP- and GTP-bound forms and the intermediate filament lamin. These species are described by continuous concentrations within a two-dimensional spherical cell model. Simulations were carried out for 900 s using COMSOL MULTIPHYSICS (v. 3.4, COMSOL, Inc., Burlington, MA, USA).

Figure 1. Reactions included in the model. (a) Tubulin (T) attaches to existing MTs (M) in a reversible reaction. Lamin attached to plus-end-directed motors (L_p) increases the rate of polymerization. (b) RanGTP promotes MT polymerization and inhibits depolymerization by decreasing and increasing the corresponding rates (k_{TM} and k_{MT} , respectively). RanGDP acts in a complementary manner. (c) After nuclear envelope breakdown, free lamin (L) is captured by minus-end-directed motors moving along MTs—this lamin is labelled L_m . For this lamin, we consider two models. In the default model, L_m is stabilized by the matrix (denoted L_s). This can then be captured by plus-end-directed motors (denoted L_p). (d) In the second model, lamin that is captured by minus-end-directed motors can then be passed on to plus-end-directed motors. The latter can be stabilized by the matrix. In both cases, L_p inhibits MT diffusion.

2.1. Molecular species

Free tubulin (T) diffuses freely throughout the cell. It binds to and dissociates from existing MTs (figure 1a). Initially, tubulin is uniformly distributed throughout the cell at $12\ \mu\text{M}$ concentration [21].

MTs describe polymerized tubulin. Initially, all MTs are found concentrated at two $1\ \mu\text{m}$ diameter discs, representing asters, $4\ \mu\text{m}$ apart (figure 2a), with initial concentration $30\ \mu\text{M}$ [21]—this is in addition to the concentration of free tubulin. This concentration is not fixed, but is allowed to vary as MT polymerizes/depolymerizes. The centres of these asters are used as markers for directing the movement of plus- or minus-end motors or motor-associated proteins. To this end, the model tracks whether MTs are associated with one or the other aster. For simplicity, we do not include explicitly the effect of poleward MT flux in our model [22]. This flux, which appears to be a conserved process, is coupled to the depolymerization at the minus ends and is a primary driver of chromosome segregation, though it may also play a role in determining spindle size [23]. In our model, MT growth is restricted by depolymerization.

We include the effects of RanGTP on MT polymerization [24]. During mitosis, RanGTP is found preferentially around the chromosomes because RCC1, the guanine nucleotide exchange factor responsible for nucleotide exchange, is chromosome bound [25]. This leads to a high RanGTP to RanGDP ratio near the chromosomes and a low ratio far away [26]. In our model, we restrict production of RanGTP to an ellipsoidal region between the MT asters corresponding to the approximate location of chromosomes during metaphase (figure 2a). RanGTP can diffuse from this region throughout the cytoplasm, where it is hydrolysed. Initially, all Ran is in the GDP-bound form at a concentration

Figure 2. Geometric description of the model. (a) The cell is represented by an impenetrable circle, 20 μm in diameter. Lamin and MTs are initialized to one and two circles that are 4 and 1 μm in diameter, respectively. These species are free to diffuse, however. The ellipse marks the region where RanGDP is converted to RanGTP and where hydrolysis of RanGTP cannot take place. The dotted line shows a cross section used to plot concentration in subsequent figures. (b) Schematic of the vector field used to describe the movement of motor-associated lamin. Minus-end-directed lamin moves along the vector which points to the minus end it attaches ($v_L(x, y)$ and $v_R(x, y)$). Plus-end-directed lamin (L_p) moves in the opposite direction. (c) Distribution of MTs showing resultant vector field.

of 2 μM uniformly distributed around the cell. RanGTP stimulates MT assembly by increasing the rescue frequency of MTs [24]. In our model, RanGTP increases MT polymerization and decreases depolymerization rates; RanGDP functions in a complementary manner (figure 1b).

We include a lamin mitotic matrix in our model. Lamin-B assembles into a matrix-like network during mitosis [4]. Lamin-B is assumed to exist in one of several states: free, unbound lamin (L), lamin that is attached to minus- (L_m) or

plus-end (L_p)-directed motors moving along MTs and stable lamin bound to and forming the spindle matrix (L_s). Initially, all lamin is in the free state, and concentrated at 12 μM within a 4 μm diameter circle that represents the site of the nucleus just before nuclear envelope breakdown (figure 2a). We assume that the lamin that is bound to plus-end-directed motors affects MT dynamics by increasing MT nucleation from tubulin and by stabilizing MTs through reduced diffusion (figure 1c,d). Evidence that lamin may promote MT polymerization comes from the MT assembly promoter XMAP215, which has been shown to associate with the lamin matrix. Lamin may reduce MT diffusion through MT cross-linking proteins, such as NuMA [4] or Rab5 [9], that associate with the matrix.

2.2. Geometry of the cell

Our simulations are not meant to represent any specific cell type, as the information on the mitotic matrix comes from a variety of sources, including *Xenopus* egg extracts and *Drosophila* embryo. We assume that the reactions take place in a circular cell, 20 μm in diameter, surrounded by an impermeable membrane (figure 2a). Moreover, we assume complete breakdown of the nuclear envelope. These intracellular compartments are used solely to locate species at the onset of the simulations. Thereafter, all species diffuse freely within the cell with varying diffusion coefficients (table 1).

2.3. Reactions and interactions

The dynamics of MT growth are highly stochastic, a process known as dynamic instability [31]. However, as we seek a deterministic description of the system, we model MT polymerization and depolymerization using simple first-order reactions, representing mean MT growth.

After breakdown, components of the nuclear envelope translocate towards centrosomes aided by minus-end-directed MT motors [32]. In our model, free lamin can be captured by minus-end-directed motors found on MTs, converting this into minus-end-directed lamin (L_m). These motors pull the lamin towards the aster from which the MT emanates.

After lamin capture by minus-end-directed motors, we consider two putative pathways. The first scheme, used as the default for most of the simulations in the paper, assumes that lamin held by the minus-end-directed motors joins the matrix where it is stabilized—that is, L_m is converted to L_s —at a constant rate. This stable lamin can then be captured by plus-end-directed motors (figure 1c). Our second pathway assumes that lamin attached to minus-end-directed motors is captured by plus-end-directed motors, and it is this lamin that can be stabilized by joining the existing mitotic matrix ($L_m \rightarrow L_p \rightarrow L_s$; figure 1d). As there is no experimental basis for preferring one scheme over the other, we use our simulations to test the effect of the two cases. In both cases, lamin that is attached to motors (L_m or L_p) or the matrix (L_s) can become free lamin (L) at constant rates.

2.4. Mathematical description

The dynamics of MT polymerization are described first. We track whether MTs are attached to the left (M_L) or right (M_R) asters. Because the equations for the two are the same, we write only one

$$\frac{\partial T}{\partial t} = \nabla \cdot (D_T \nabla T) + k'_{MT}(M_L + M_R) - k'_{TM}(M_L + M_R)T - k_{L_p M}(L_{pL} + L_{pR})T$$

$$\text{and } \frac{\partial M_L}{\partial t} = \nabla \cdot (D_{M_{\text{via } L_p}} \nabla M_L) + k'_{TM}M_L - k'_{MT}M_{LT} + k_{L_p M}L_{pL}T - v_L \cdot \nabla M_L.$$

The first terms in both of these equations represent diffusion. Plus-end-directed lamin (L_p) inhibition of MT diffusion is

Table 1. Parameter values.

	symbol	value	units	references
<i>initial conditions</i>				
microtubules	M_L and M_R	30	μM	[21]
free tubulin	T	12	μM	[21]
RanGDP	RanGDP	2	μM	[26]
RanGTP	RanGTP	0	μM	
free lamin	L	12	μM	[4]
<i>kinetic constants</i>				
microtubule depolymerization	k_{TM}	0.06	1 s^{-1}	
tubulin polymerization	k_{MT}	0.002	$1\text{ s}^{-1}\mu\text{M}^{-1}$	
Ran (GTP to GDP)	k_{RanTD}	0.05	1 s^{-1}	
Ran (GDP to GTP)	k_{RanDT}	2.5	1 s^{-1}	
lamin (minus-end directed to free)	k_{Lm2L}	0.05	1 s^{-1}	
lamin (free to minus-end directed)	k_{L2Lm}	0.3	1 s^{-1}	
lamin (minus- to plus-end directed)	k_{Lm2Lp}	0.01	1 s^{-1}	
lamin (plus-end directed to stable)	k_{Lp2Ls}	0.003	1 s^{-1}	
lamin (stable to free)	k_{Ls2L}	0.001	1 s^{-1}	
lamin (plus-end directed to free)	k_{Lp2L}	0.001	1 s^{-1}	
<i>diffusion coefficients</i>				
tubulin	D_T	48	$\mu\text{m}^2\text{ s}^{-1}$	[27]
microtubules	D_M	0.005	$\mu\text{m}^2\text{ s}^{-1}$	
RanGDP	D_{RanGDP}	5.0	$\mu\text{m}^2\text{ s}^{-1}$	[28]
RanGTP	D_{RanGTP}	2.0	$\mu\text{m}^2\text{ s}^{-1}$	[28]
free lamin	D_L	0.1	$\mu\text{m}^2\text{ s}^{-1}$	
lamin captured by minus-end-directed motors	D_{Lm}	0.001	$\mu\text{m}^2\text{ s}^{-1}$	
lamin associated to matrix	D_{Ls}	0.001	$\mu\text{m}^2\text{ s}^{-1}$	
lamin captured by plus-end-directed motors	D_{Lp}	0.001	$\mu\text{m}^2\text{ s}^{-1}$	
<i>other constants</i>				
minus-end motor speed	μ_{Lm}	4	$\mu\text{M m}^{-2}\text{ s}$	[29]
plus-end motor speed	μ_{Lp}	0.5	$\mu\text{M m}^{-2}\text{ s}$	[30]
anti-parallel motor force coefficient	k_f	0.2	$\mu\text{m s}^{-1}\mu\text{M}^{-2}$	
threshold (M effect on minus-end motor)	τ_{MLm}	35	μM	
threshold (M effect on plus-end motor)	τ_{MLp}	35	μM	
threshold (L_p effect on M diffusion)	τ_{LpDm}	1.4	μM	
threshold (L_m effect on L_s)	τ_{LmLs}	0	μM	
threshold (L_s effect on L_p)	τ_{LsLp}	5	μM	

implemented by: $D_{\text{MviaLp}} = (1 - H(L_{\text{pL}}, \tau_{\text{LpDM}}))D_M$, where D_M is the diffusion coefficient for free MTs. The function: $H(x, x_{\text{th}}) = 1/(1 + e^{-2(x-x_{\text{th}})})$ serves as a smooth continuous switch-like function: as x decreases below the threshold x_{th} , the function $H(x, x_{\text{th}})$ approaches zero; as x increases above x_{th} , it approaches one.

Polymerization (k'_{TM}) and depolymerization (k'_{MT}) rates depend on the local concentrations of RanGTP and RanGDP according to

$$k'_{\text{TM}} = k_{\text{TM}} \left(1 + \beta \frac{\text{RanGTP}}{\text{RanGTP}_{\text{max}}} \right) \left(1 - \alpha \frac{\text{RanGDP}}{\text{RanGDP}_{\text{max}}} \right)$$

and

$$k'_{\text{MT}} = k_{\text{MT}} \left(1 - \alpha \frac{\text{RanGTP}}{\text{RanGTP}_{\text{max}}} \right) \left(1 + \beta \frac{\text{RanGDP}}{\text{RanGDP}_{\text{max}}} \right).$$

Here, $\text{RanGTP}_{\text{max}}$ and $\text{RanGDP}_{\text{max}}$ are maximum concentrations obtained within the cell during simulations, and $\alpha = 2$ and $\beta = 1/2$ are constants.

The term $k_{\text{LpM}}(L_{\text{pL}} + L_{\text{pR}})T$ in the first equation represents enhancement of MT polymerization by lamin-B that has been captured by plus-end-directed motors. Finally, the last term $v_L \cdot \nabla M_L$ in the MT equations represents the movement of the asters and is described in more detail below.

We now present the model equations for the lamin states. We do this only for the first model (figure 1c); the changes required for the second (figure 1d) are straightforward:

$$\frac{\partial L}{\partial t} = \nabla \cdot (D_L \nabla L) + k_{\text{Lm2L}} L_{\text{mL}} + k_{\text{Lp2L}} L_{\text{pL}} + k_{\text{Ls2L}} L_{\text{sL}} - k_{\text{L2Lm}} L (H(M_L, \tau_{\text{MLm}}) + H(M_R, \tau_{\text{MLm}})),$$

$$\begin{aligned}
\frac{\partial L_{\text{mL}}}{\partial t} &= \nabla \cdot (D_{\text{Lm}} \nabla L_{\text{mL}}) + k_{\text{L2Lm}} LH(M_{\text{L}}, \tau_{\text{MLm}}) \\
&\quad - k_{\text{Lm2Ls}} L_{\text{mL}} H(L_{\text{mL}}, \tau_{\text{LmLs}}) - k_{\text{Lm2L}} L_{\text{mL}} - v_{\text{LmL}} \cdot \nabla L_{\text{mL}}, \\
\frac{\partial L_{\text{sL}}}{\partial t} &= \nabla \cdot (D_{\text{Ls}} \nabla L_{\text{sL}}) + k_{\text{Lm2Ls}} L_{\text{mL}} H(L_{\text{mL}}, \tau_{\text{LmLs}}) \\
&\quad - k_{\text{Ls2Lp}} L_{\text{sL}} H(L_{\text{sL}}, \tau_{\text{LsLp}}) - k_{\text{Ls2L}} L_{\text{sL}}, \\
\text{and } \frac{\partial L_{\text{pL}}}{\partial t} &= \nabla \cdot (D_{\text{Lp}} \nabla L_{\text{pL}}) + k_{\text{Ls2Lp}} L_{\text{sL}} H(L_{\text{sL}}, \tau_{\text{LsLp}}) \\
&\quad - k_{\text{Lp2L}} L_{\text{pL}} - v_{\text{LpL}} \cdot \nabla L_{\text{pL}}.
\end{aligned}$$

The first terms in these equations all represent diffusion of the respective species. The terms $v_{\text{LmL}} \cdot \nabla L_{\text{mL}}$ and $v_{\text{LpL}} \cdot \nabla L_{\text{pL}}$ are convection terms and model the movement of captured lamin along MTs (directed either to the left or right asters). All other terms describe the transformation between different states of lamin according to the scheme in figure 1.

The conversion between RanGDP and RanGTP is assumed to follow first-order reactions,

$$\begin{aligned}
\frac{\partial \text{RanGTP}}{\partial t} &= \nabla \cdot (D_{\text{RanGTP}} \nabla \text{RanGTP}) + k_{\text{RanDT}} \text{RanGDP} \\
&\quad - k_{\text{RanTD}} \text{RanGTP} \\
\text{and } \frac{\partial \text{RanGDP}}{\partial t} &= \nabla \cdot (D_{\text{RanGDP}} \nabla \text{RanGDP}) - k_{\text{RanDT}} \text{RanGDP} \\
&\quad + k_{\text{RanTD}} \text{RanGTP}.
\end{aligned}$$

The rate constants k_{RanDT} and k_{RanTD} apply only inside and outside the ellipsoidal area around the chromosomes, respectively (figure 2a). However, both species diffuse freely throughout the cell.

2.5. Motor movements and aster separation

Directed MT motors, like kinesins and dynein, move directionally along MTs. In our model, these motors have two functions: they capture lamin and transport it, and they exert force to drive the separation of asters [32].

In our model, the location of the asters is not fixed in space. The force that drives the asters' movement comes from the effect of motors moving along the MTs, which elongates the spindle and separates the asters. Because only motors moving along anti-parallel MTs can exert force [14,15], we assume that this force is proportional to the product of the concentrations of left- and right-associated MTs (i.e. $M_{\text{L}} \times M_{\text{R}}$). Because of geometric symmetry of two half spindles, the vertical forces pushing the asters apart cancel each other. Thus, we only resolve forces in the horizontal direction. Assuming a viscous environment for the cytoplasm, we model aster movement as proportional to the force: $v \propto F = k_f \times M_{\text{L}} \times M_{\text{R}}$. The proportionality constant, k_f , incorporates such variables as motor concentration, binding/unbinding rates, duty cycle and cytoplasmic viscosity.

The movement of motor-associated lamin at a point (x, y) is the sum of the movement of MTs relative to the cell, and that of motors relative to the MTs. For simplicity, we propose that MTs have the same velocity as the aster to which they are attached. If $C_i(x, y)$ ($i = \text{L, R}$, representing the left and right asters, respectively) is the position of the asters' centres, then $W_i = (C_i - P(x, y)) / |C_i - P(x, y)|$ is the unit vector pointing to the aster. Let μ_{Lm} and μ_{Lp} be the velocities of lamin captured by minus- and plus-end-directed motors, respectively. The velocity of the lamin is given by

$$\begin{aligned}
v_{\text{Lm}_i} &= \mu_{\text{Lm}} \cdot W_i H(M_i, \tau_{\text{MLm}}) + v_i \\
\text{and } v_{\text{Lp}_i} &= -\mu_{\text{Lp}} \cdot W_i H(M_i, \tau_{\text{MLp}}) + v_i.
\end{aligned}$$

The switch-like functions $H(M_i, \tau_{\text{MLm}})$ and $H(M_i, \tau_{\text{MLp}})$ ensure that motors move only if the MT concentration is sufficiently high

(above 35 μM) representing the outer extant of the plus end of MTs. The speed of each type of lamin is determined by a mobility parameter. Plus-end-directed lamin moves anywhere from 0.1 to 8 $\mu\text{m s}^{-1}$, while the plus-end-directed motor kinesin has been observed processing at 0.5 $\mu\text{m s}^{-1}$ *in vitro* [30]. Minus-end-directed lamin moves 0.5–8 $\mu\text{m s}^{-1}$ in the simulation, while the minus-end-directed motor dynein was been observed moving MTs at 0.47–6.1 $\mu\text{m s}^{-1}$ and averaging $3.5 \pm 1.3 \mu\text{m s}^{-1}$ [29].

To determine the elongation of the spindle, we measure the distance between the two asters by tracking the position where the peak MT concentration of each aster occurs.

2.6. Simulation parameters

The parameters used in the simulations are shown in table 1. The kinetic parameters of the RanGTP network were selected based on similar published simulations [26,28]. As there is relatively little kinetic data on the lamin network, the values selected were chosen through trial and error. In this case, simulations with altered dynamics (doubling or halving the rates) were carried out and gave qualitatively similar results. The diffusion coefficients for the molecule species were obtained using the Stokes–Einstein equation [33]: $D = k_{\text{B}} T / 6 \pi \eta R$, where $k_{\text{B}} = 1.38 \times 10^{-23} \text{ J K}^{-1}$ is Boltzmann's constant, $T = 300 \text{ K}$ is temperature in Kelvin, $\eta = 5 \times 10^{-3} \text{ Pa s}$ is the viscosity of the cell (assumed to be five times that of water) and R is the Stokes radius of the molecules.

3. Results

3.1. Microtubule and tubulin interactions alone do not generate a focused spindle

To establish a benchmark, we simulated our model under the assumption that the only interacting components are MTs and free tubulin. These simulations showed that, without other regulation, MTs formed two aster-like MT-rich formations around which a steep MT concentration gradient was established (figure 3a). The region where MTs bound to either aster overlap formed an elliptically shaped region with the MT concentration approximately flat (figure 3a,b). At steady state, the free tubulin concentration was uniformly distributed throughout the cell (not shown).

We next tested the effect that motors have on the MTs. The force generated by the motors pushed the two asters away, allowing the spindle region to elongate. This had the effect of separating the two asters as well as reducing the peak concentration of MTs (figure 3c). A plot of the length of the interacting MTs (Material and methods) revealed steady MT growth during the first 100 s (figure 3d). Thereafter, once the MTs from each aster begin to overlap, the force generated by the MT motors pushes the asters apart at a steady rate (figure 3d). No such separation is achieved in the absence of motors.

3.2. Spindle formation and lamin transportation

The previous simulations showed that a spindle region can form without additional components. We next sought to determine the effect that a lamin network could have on the spindle-like region of high MT concentration observed. To this end, we introduced the lamin network into our model (Material and methods). We use the default pathway (figure 1c) to show the effects of lamin. We also included the spatial effects of the RanGTP model. At the onset of these simulations, lamin quickly diffused away from its initial placement, mimicking the release of lamin into the cytoplasm

Figure 3. MT polymerization gives rise to two asters which are pushed away by the MT-based motors. (a) Dynamic of MT concentration during simulation. MTs grow away from the two initial locations. This simulation includes the effect of MT motors which push the two asters away from each other. This force is generated in the region where MTs from both asters overlap (anti-parallel MTs). (b) Concentration profile at steady state of MTs associated with the two asters along the dotted line of figure 2a. (c,d) Comparison of MT profile without and with motors. Though the peak concentration is higher without motors, the asters are not pushed away from each other.

after nuclear envelope breakdown (figure 4a, 20 s). After this initial diffusion, lamin was captured by minus-end-directed motors whereupon it was transported towards the two minus ends (figure 4b). As lamin accumulated near these two asters, it detached from the minus-end-directed motors

and started to bind to the two plus-end-directed motors. Thereafter, lamin was transported outwards along the MTs and distributed forming two ring-like patterns (figure 4c, 500–900 s). At steady state, lamin attached to minus-end motors was found primarily at the minus ends of the asters

Figure 4. The complete model shows that a lamin shell is formed around the MT spindle region. Profiles for the free lamin (a), lamin attached to minus-end-directed (b) and plus-end-directed (c) motors, matrix-associated lamin (d) and MTs (e). (f) MT distribution with and without MT motors. (g) In the complete model, the distance between the asters grows more gradually because of the resistance provided by the lamin network.

(figure 4b). The MTs formed a spindle-like pattern (figure 4e) with higher concentration than in the absence of lamin (peak concentration approx. 55 μM compared with approx. 35 μM in figure 3b). More striking was the lack of MTs outside of the spindle region. Because the lamin matrix inhibits MT growth and diffusion, the initial phase of MT growth was reduced; however, at steady state, the MTs form a more distinct spindle (figure 4f). The asters separated under the action

of the MT-associated motors and the average length of the MTs in the spindle was about 4.3 μm (figure 4g).

3.3. Ran's effect on microtubule dynamics

Previous simulations using agent-based models suggested that spatial differences in the regulation of MT interactions can improve spindle morphology [18]. We first established

Figure 5. Effect of RanGTP on the production of the spindle. (a) Spatial distribution of RanGTP and RanGDP concentrations after 900 s of simulated time. (b,c) MT concentrations without (b) and with (c) the RanGTP network around the cell, and profiles at four different time points.

that a RanGTP gradient was formed with the concentration around the centre of the spindle approximately threefold higher than that away from the spindle, which is similar to that reported [28] (figure 5a). This gradient has the effect of

increasing the MT polymerization rate by up to 21.4% and decreasing the depolymerization rate by up to 20% in the spindle region (Material and methods). To test the effect that this spatial regulation has on our spindle morphology,

Figure 6. Comparison of two putative networks for lamin matrix formation. MT and lamin (L_s) profiles for the scheme of figure 1c (a) and 1d (b).

we carried out simulations without (figure 5b) and with differentiated production of RanGDP and RanGTP (figure 5c) and compared the localization of the MTs. RanGTP's local enhancement of MT polymerization gave rise to a more distinctive spindle region: in the region between the two asters, the concentration of MTs increased from approximately 60 to approximately 90 μM , whereas outside this region, the concentration dropped to nearly zero.

3.4. Comparison of two lamin reaction pathways

Finally, we contrasted the effects of the two proposed mechanisms which determined the transformation after lamin was captured by minus-end-directed MT-associated motors (figure 1c,d). The first scheme assumes that lamin captured by minus-end MT motors will then be captured by plus-end-directed motors, and then attach itself to the developing lamin matrix as the latter is being formed during spindle assembly. The second scheme assumes that minus-end lamin will transition directly to the matrix from where it can be captured by plus-end-directed motors. Both pathways gave rise to similar MT morphologies, though the concentration was somewhat higher in the second scheme (figure 6). However, the effect on the lamin matrix was quite different. In the first scheme, lamin joined the matrix after being transported by the plus-end-directed motors, giving rise to two outer rings which join at the centre of the spindle region (figure 6a). In the second scheme, because lamin joined the matrix immediately after being captured by the minus-end-directed motors, there was a high concentration of lamin matrix at the minus end of the MTs (figure 6b). Two weaker rings were seen as well.

3.5. Parameter sensitivity

By necessity, our model requires a number of parameters that have not been measured experimentally (table 1). We

sought to determine how robust the system is to several of the assumptions made. To this end, we varied the initial concentrations of the various species involved (figure 7). Our results indicate that the formation of a spindle, and its enclosure by a lamin matrix, are robust to changes (± 30 –50%) in the concentrations of the individual components. The robustness of spindle assembly upon varying lamin-B concentration is consistent with the observation that lamin-B depletion in egg extracts does not prevent spindle assembly, but it does lead to changes of spindle morphology. Consistently, changing the total concentration of lamin in our model affects the degree to which the spindle pole is focused. Interestingly, lamin-B has been implicated in limiting tubulin diffusion in mitosis [34]. How this function of lamin-B might affect spindle assembly remains unclear. Our modelling shows that more lamin leads to a bigger spindle. It will be interesting to experimentally vary lamin-B concentration and analyse whether spindle size increases.

Changing the initial MT concentration in the asters had a small effect on the steady-state levels of the MT spindle or lamin matrix. This might be expected as the total tubulin concentration (in MT and free form) is dominated by the free tubulin. As expected, changing this latter value did have a greater effect both on the size of the spindle and on the resultant lamin matrix. When the concentration of free tubulin was increased, there was some interaction between the spindle and the cell membrane that disrupted the shape of the lamin matrix. Although RanGTP is required for assembly of both spindle and lamin-B spindle envelope/matrix in egg extracts [4,35], we found that varying the concentration of Ran had limited effect on either the spindle or lamin matrix.

Although lamin-B is shown to interact with dynein and Eg5, the dynein minus-end transport of lamin-B observed upon nuclear envelope breakdown suggests that

Figure 7. Sensitivity analysis for various model parameters. (a) The initial concentrations of the various components were altered (as noted). Plots show the MT and lamin (L_s) profiles. (b) Change in the force driving aster separation. Shown are the MT and lamin profiles as well as the aster separation as a function of time.

dynein–lamin-B interaction plays a role in assembly of the lamin-B spindle envelope and matrix. We considered the effect of varying the force component between the anti-parallel MTs. Increasing the plus-end-directed motor force,

as might be the effect of having more motors, leads to greater aster separation (figure 7b) and hence a longer spindle. With reduced force, the spindle is smaller, but does have more concentrated MTs.

4. Discussion

The hypothesis that a spindle matrix could aid in spindle formation was proposed decades ago, but relatively little evidence has existed until recently. There is a growing body of experimental evidence for the existence of a MT-independent structure whose absence leads to increased defects in spindle assembly [4,7]. Here, we developed a computational model to investigate the formation of a lamin matrix and to test whether the lamin matrix can function as the proposed spindle matrix.

Simulations that involve only tubulin, MTs and MT-associated motors give rise to a region of high MT concentration between the two asters where a mitotic spindle would be expected to form. MT motors, acting on anti-parallel MTs, helped to push the two asters from each other. The resultant spindle region, however, was relatively unfocused (figure 3c). These simulations show that, though the MT network is sufficient for the formation of a spindle, the tightly focused spindles observed in cells are likely to benefit from other factors.

Introduction of the Ran and Lamin networks into the model improved the morphology of this putative spindle. The Ran network has been extensively studied for its role during mitosis [25,26,28]. RanGTP forms a steep gradient emanating from the location of the chromosomes and that can induce changes in the dynamic instability of the MT network. Previous modelling efforts demonstrated that this spatial regulation alone could give rise to improved spindle morphology [18]. Our model of RanGTP production and hydrolysis, though greatly simplified relative to other models, gave rise to gradients similar to those seen experimentally (figure 5).

After nuclear envelope breakdown, disassembled lamin-B is transported to the spindle poles [5,9–11]. Thereafter, lamin-B surrounds/permeates the spindle and is concentrated at the spindle poles [36–38]. Our simulations showed that lamin initially found far away from the spindle region can be captured and transported effectively by minus-end-directed MT motors (figure 4). This lamin was then induced to form a shell-like network around the high concentration of MTs between the two asters, similar to that seen experimentally (figure 4d). Under lamin regulation, MTs in our simulations formed into a bipolar spindle shape and the increased interaction between the MTs in the spindle region greatly improved the focusing of this spindle (figure 4f). These simulations are consistent with experimental findings in which

disruption of lamin-B led to disorganized spindles [5,8]. Moreover, by helping to focus the spindle which allows more MT motors to act on overlapping MTs, the lamin matrix helps in the consistent separation of asters and MT elongation (figure 4g). The model with lamin and Ran finally generates MT length of the order of 4.5 μm , which is close to the experimental measured length in mitotic egg extracts [39].

Our simulations suggest that lamin-B, captured by MT motors after nuclear envelope breakdown, can act as part of the proposed spindle matrix. There is no direct evidence that lamin is transported along MTs, though kinesin has been implicated in the MT-dependent transport, leading to assembly of other intermediate filament networks [40]. Moreover, kinesins are seen to remain after disassembly of the spindle by removal of MTs, suggesting that they bind to a non-MT structure, which could be the spindle matrix [41,42]. Though lamin-B can interact with both dynein and kinesin Eg5 [8], the role of Eg5–lamin-B interaction in spindle morphogenesis or lamin-B matrix assembly is still unclear. Moreover, dynein is required for formation of the lamin-B matrix [5]. However, as the mechanism by which lamin captured by minus-end-directed motors transitions to the matrix is unclear, we tested two hypothetical pathways. Our simulations suggest that both pathways lead to similar MT spindles (figure 6). The real difference is the shape of the lamin matrix. The scheme in which lamin is captured by plus-end-directed motors before stabilizing to the lamin matrix gives two highly concentrated rings along the spindle poles. This is consistent with experiments aimed at deciphering the mechanical interaction between the matrix and MTs [43]. These experiments, which observed the movement of microneedles during spindle elongation, suggest that there is little role for the presence of an isotropic matrix. However, they support a role for a matrix near the spindle poles.

Previous theoretical studies have suggested that the lamin matrix functions to regulate spindle formation by balancing the force generated by the spindle and, in doing so, focusing the spindle [19,38]. In contrast to these studies, where the effect of the lamin matrix is primarily mechanical, our simulations demonstrate that the matrix spindle, by providing an anchor for spindle assembly factors that regulate MT polymerization and by physically restricting the movement of MTs (through the coupling of the two networks), can also improve spindle focusing.

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