Efficient Chromosome Capture Requires a Bias in the 'Search-and-Capture' Process during Mitotic-Spindle Assembly

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Summary

The mitotic spindle assembles into a bipolar, microtubule-based protein machine during prometaphase. One proposed mechanism for this process is "search-and-capture," in which dynamically unstable microtubules (MTs) search space to capture chromosomes [1]. Although existing theoretical estimates [2, 3] suggest that dynamic instability is efficient enough to allow capture within characteristic mitotic timescales, they are limited in scope and do not address the capture times for realistic numbers of chromosomes. Here we used mathematical modeling to explore this issue. We show that without any bias toward the chromosomes, search-and-capture is not efficient enough to explain the typical observed duration of prometaphase. We further analyze search-and-capture in the presence of a spatial gradient of a stabilizing factor [4–6] that biases MT dynamics toward the chromosomes. We show theoretically that such biased search-and-capture is efficient enough to account for chromosome capture. We also show that additional factors must contribute to accelerate the spindle assembly for cells with large nuclear volumes. We discuss the possibility that a RanGTP gradient introduces a spatial bias into microtubule dynamics and thus improves the efficiency of search-and-capture as a mechanism for spindle assembly.

Results

Optimal Unbiased 'Search and Capture' Is Not Fast Enough to Account for Observed Rates of Spindle Assembly

Before chromatid segregation can occur, a bipolar mitotic spindle consisting of two overlapping microtubules (MTs) arrays must assemble [1]. Some of these MTs attach to the kinetochores. According to the search-and-capture model [1], MTs nucleate in a random direc-

tion and grow and shrink dynamically to probe space and eventually encounter target kinetochores. Theoretical analysis of the search-and-capture model showed that MT dynamic instability is very effective if it is regulated so that the rescue frequency is close to zero (i.e., MTs do not search repeatedly in the "wrong" direction) while the catastrophe frequency is such that a MT grows on average to a length equal to the mean pole-kinetochore distance (i.e., MTs neither undergo premature catastrophe when growing in the "right" direction nor "waste time" growing in the "wrong" direction) [2, 3]. This analysis is consistent with the measured decrease in rescue frequency from 0.175 s^{-1} in interphase to 0.023 s^{-1} in prometaphase [7].

The estimates in [2] do not analyze the capture times for a realistic geometry or number of chromosomes. In the Supplemental Data available with this article online, we describe mathematical analysis and Monte Carlo simulations that estimate the time to capture *for multiple chromosomes*. In short, the computer code places a number of chromosomes at random locations inside a nuclear sphere, 10 μm in radius (Figure 1). Sister kinetochores lie back-to-back—that is, the capture surfaces of partner kinetochores face in opposite directions [8]—so we assume that each kinetochore can be reached by MTs emanating from one pole only. For each kinetochore, we calculate the probability of capture if one assumes that MTs are nucleated in random directions and their dynamic properties are spatially independent (Figure 1C). We then use a random number generator to simulate the number of unsuccessful searches before the chosen kinetochore is captured and find the time to capture. We repeat this procedure for all kinetochores and find the maximal time to capture the final unattached kinetochore. Values of the model parameters are listed in Table S1 in the Supplemental Data.

To test the model, we first examined the simplified case of a single aster containing 250 MTs and searched for a single target at a distance of 10 μm. Our numerical analysis yielded a mean time to capture of 23 min, similar to previous results [2]. We further tested whether the capture of multiple chromosomes can occur in a reasonable biological timescale under the same modeling assumptions. We first calculated the optimal catastrophe frequency (0.0134 s^{-1}) by minimizing the search time and assuming a uniform distribution of chromosomes in the nucleus. Then, using the optimal conditions, we simulated the unbiased model with 46 chromosomes.

Calculated mean times to capture are 511 min and 125 min for 250 and 1000 searching MTs, respectively (Figures 2E and 2F). This time is much longer than the time it takes to capture a single kinetochore because the process of capturing a kinetochore is stochastic, with significant variations. In the case of multiple chromosomes, the process ends only when the last kinetochore is captured, so the most "unsuccessful" and prolonged search determines the time it takes to capture the kinetochore. In fact, the mean time to search

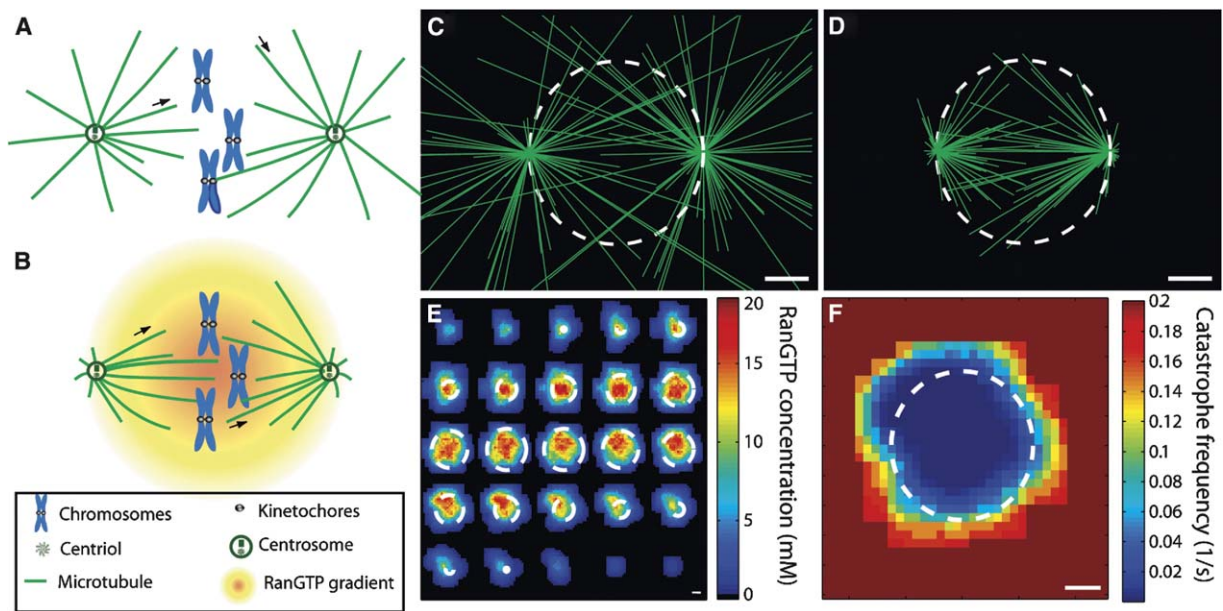*Correspondence: mogilner@math.ucdavis.edu

Figure 1. Biased and Unbiased Search and Capture

Schematic of the unbiased (A) and RanGTP-biased (B) search-and-capture models and graphical representation of stochastic simulations. 2-D projection of 3-D simulation of MT dynamics in the unbiased (C) and biased (D) models. MT distribution for the unbiased model (C) was generated with spatially homogeneous optimal catastrophe frequency. Spatially dependent catastrophe frequency for the biased model (in the middle nuclear cross-section) is shown in panel (F). The catastrophe frequency was calculated based on the assumption that it is an exponentially decaying function of the RanGTP concentration with a chemical scale of 10 μM and a value of 0.2 catastrophes per second in the absence of RanGTP. The 3-D distribution of the RanGTP gradient (serial sections in [E]) was calculated based on the assumption of a uniform distribution of chromosomes in the nucleus and linear superposition of exponentially decaying RanGTP gradients centered at each chromosome. The dashed white line represents the position of the nuclear envelope before NEB. Scale bars represent 5 μm.

is a logarithmic function of the number of the chromosomes (Figure 3B). (See Equation S17 in the Supplemental Data.)

The search time clearly decreases as the number of MTs increases (Figure 2F). Even with 1000 searching MTs, which is an upper limit to the usual estimate of hundreds of MTs, the mean estimated time until capture of 46 chromosomes in the unbiased model is substantially greater than experimental measurements (20–30 min; see Figure S2). Thus, even under optimal conditions, the unbiased model cannot explain the experimental results.

Biased Search and Capture Is Sufficiently Fast to Account for Observed Rates of Mitosis

The MT catastrophe frequency was never measured in the vicinity of chromosomes in vivo, although astral MTs were found to display a catastrophe frequency of 0.075 s^{-1} away from the spindle during prometaphase [7]. This value is 5.6-fold larger than the calculated optimal value of 0.0134 s^{-1}, and simulations show that it would yield an unrealistic mean capture time of 3720 min. This suggests that there is a bias of MT dynamics near the chromosome, such that a MT growing in the "wrong" direction would collapse rapidly, whereas a MT that is close to the target would be allowed to continue its growth. We tested whether such a bias can increase the efficiency of search and capture to reach biologically observed time scales. Although there are several

molecular mechanisms that could plausibly generate such a bias in MT dynamics, here we examine the possibility that a RanGTP gradient could serve to stabilize MTs in the vicinity of chromosomes, as was previously suggested [4, 5, 9–13]. To explore this possibility quantitatively, we simulated the following model.

We calculated the spatial distribution of RanGTP in a gradient decreasing away from the chromosomes (Supplemental Data and Figures 1B and 1E). We made the catastrophe frequency a decreasing function of RanGTP concentration, so that MTs undergo catastrophe very rapidly away from the nucleus and are very stable near the chromosomes. We found that the search durations were minimized under conditions in which RanGTP decreased rapidly away from the nucleus but not rapidly enough to change much between the adjacent chromosomes. In such cases there exists a "stabilizing sphere" of radius similar to that of the nucleus, such that the catastrophe frequency is step-like (Figure 1F) with no catastrophes inside the nucleus and a high frequency of catastrophes outside the nucleus.

We simulated this optimal, simplified, biased model, in which a MT underwent a catastrophe immediately outside the nuclear sphere and did not catastrophe inside it (Figure 1D). (Other than that, the simulations were as described above; see also the Supplemental Data.) The results are shown in Figures 2A and 2B. The mean time until capture became as short as 11–48 min for 1000–250 searching MTs, respectively. This result is
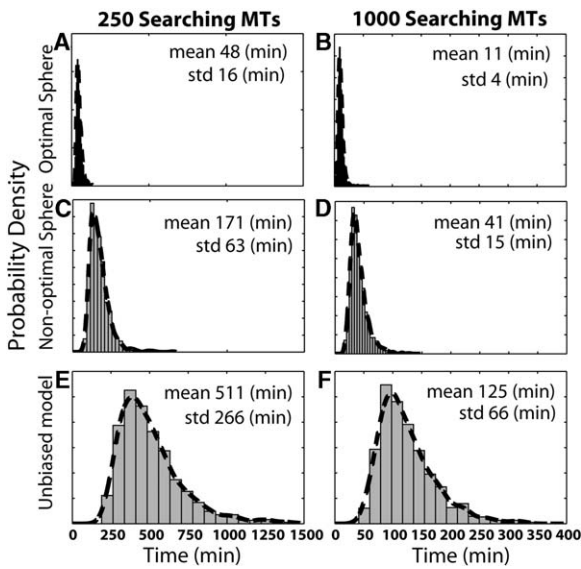
Figure 2. Distribution of Time until Capture

Simulation results summarized as the distribution of time until capture under different assumptions: stabilizing sphere with radius of the nucleus (top: [A and B]), stabilizing sphere with radius of 1.5 times the nuclear radius (middle: [C and D]), and an unbiased model with optimal dynamic instability parameters (bottom: [E and F]). Each model is presented both for 250 searching MTs (left: [A, C, and E]) and 1000 searching MTs (right: [B, D, and F]). Bars are histograms of 1000 simulations, and the dashed line is an estimate of the probability density function obtained from average shifted histograms.

an order of magnitude faster than in the unbiased model because in this case the MTs do not spend time growing in the "wrong" direction and do not grow too long because they are destabilized away from the chromosomes (Supplemental Data). The estimated time compares well with the measured prometaphase duration of 20–30 min (Figure S2), demonstrating that introducing a bias into the catastrophe frequency, with MTs being more stable proximal to the chromosomes and less stable distally, can explain the observed duration of prometaphase.

Our analysis shows that the average search time is inversely proportional to the number of MTs (Equations S17 and S19). Not surprisingly, the cell increases the number of MTs as it enters mitosis. The time also decreases drastically when the size of the kinetochores is increased [7]. We performed simulations for 15 different effective kinetochore radii from 0.08 to 1.2 μm and for 20 different numbers of searching MTs from 100 to 2000. Each set of parameters was averaged from 100 simulations, equating to a total of 30,000 simulations. Figure 3A shows how the search time depends on the kinetochore size and MT number and demonstrates that the biased-search parameters have to be finely tuned to achieve the observed capture time. On the other hand, our analysis predicts that the search time depends weakly, as a logarithmic function, on chromosome number (Figure 3B). Moreover, we predict that the variance of the search time is proportional to the logarithm of the number of chromosomes.

The size of the stabilizing sphere is another important parameter to be regulated. The stabilizing sphere should include all the chromosomes as well as the path between them and the centrosomes, but if it becomes too big, the search-and-capture process loses its efficiency because MTs grow too long and sometimes in the "wrong" direction. We ran the simulation for a sphere with a radius 1.5 times larger than the nuclear radius and observed that the mean time until capture increased 4-fold (Figures 2C and 2D).

An important parameter of our model is spindle size, implemented as the nuclear radius. Previous work [2] showed that the average time of the unbiased search and capture grows exponentially with increasing chromosome-to-pole distance for a single chromosome. Our numerical simulations confirm that, in the unbiased model for multiple chromosomes, the search time is an exponential function of the nuclear size (Figure 3C). In the biased model, according to both analysis and simulations (Figure 3C), the search time increases more slowly as a cubic function of the radius, which makes it orders of magnitude more efficient. However, both models predict an average search time that is larger than characteristic biological time scales for nuclei of 15 μm radius or greater; for the unbiased model, the average predicted time is approximately 10 hr, and for the biased model it is approximately 1 hr.

Prometaphase Is Prolonged by 2- to 3-fold in Hela Cells with Perturbed Levels of Ran

We measured the prometaphase duration approximately 20 min in Hela cells (Supplemental Data). An obvious and testable prediction of our models is that perturbations of the RanGTP gradient should increase both the time to capture and the duration of prometaphase, provided that this gradient affects the MT dynamics as assumed in the model. Our model predicts that a dominant-negative mutant, RanL43E, will reduce the efficiency of any stabilizing gradient and thereby increase prometaphase duration, whereas a constitutively active mutant, RanQ69L, should increase the size of the stabilizing sphere and thereby overstabilize MTs and increase the duration of prometaphase. To test this prediction, we perturbed the RanGTP system in Hela cells constitutively expressing a mitosis biosensor by transfecting them with sequences encoding three different forms of Ran. Specifically, we overexpressed native Ran and introduced a dominant-negative Ran construct as well as a constitutively active one [14]. Both constitutively active and dominant-negative constructs caused a 2- to 3-fold increase in prometaphase duration (Supplemental Data, including Figures S1 and S2), as predicted, indicating that a RanGTP gradient can act as bias generator in the search-and-capture process.

Discussion

Our work demonstrates that without any bias, the search-and-capture mechanism is inefficient except in very small cells. Furthermore, due to the polynomial increase in search time with nuclear size, biased search and capture could not be the sole mechanism for spindle assembly in large cells. This demonstrates the limi-
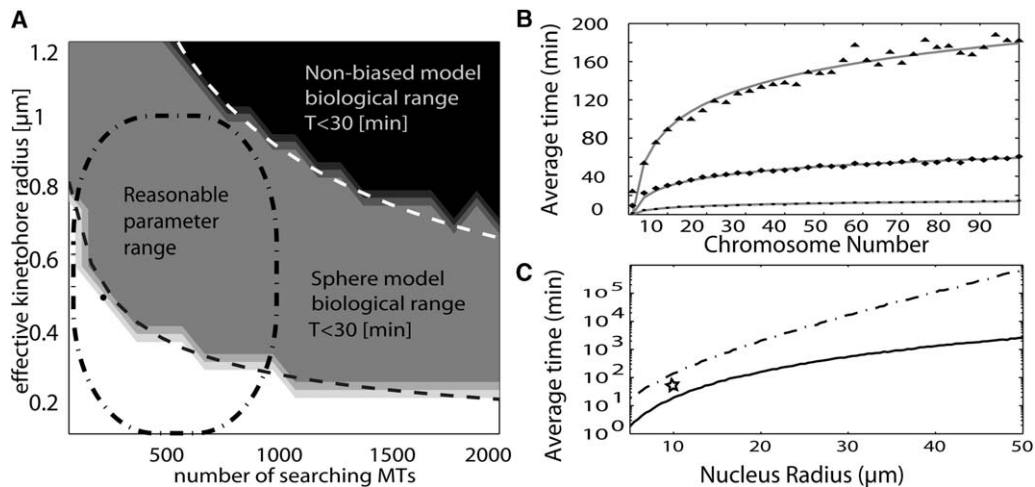
Figure 3. Time-to-Capture Dependence on the Model Parameter Values

(A) Results from a parameter scan of effective kinetochore radius from 0.08 (μm) to 1.2 (μm) and number of searching MTs from 100 to 2000. Black region: The average time until capture for both biased and unbiased models is smaller than 30 min. Gray region: The average time until capture is smaller than 30 min only for the biased model. White region: The average time until capture for both models is greater than 30 min. Dashed lines are analytical (radius is inversely proportional to square root of MT number) fits to the stochastic simulations based on Equation S20 in the Supplemental Data. The dash-and-dot circle represents an order-of-magnitude estimate of reasonable biological range. A black dot marks the parameter choice made by Holy and Leibler [2].

(B) The effect of chromosome number on time until capture. Average time until capture with different numbers of chromosomes under three different models: unbiased model with 1000 searching MTs (triangles), biased model with 250 searching MTs (diamonds), and biased model with 1000 searching MTs (circles). Each data point is the average time until capture from 200 simulations. Gray lines are analytical (logarithmic function) fits to the stochastic simulations based on Equations S17 and S19.

(C) The unbiased (dot-and-dash) model shows exponential increase of the time to capture as a function of nuclear radius. In the biased (solid) model, the time is proportional to the cube of the radius. A typical experimental observation (nuclear radius and prometaphase time), such as that illustrated in the Figure S1, is shown with the star.

tation of the centrosomal assembly pathway and supports experimental evidence that centrosomal and chromosomal spindle assembly pathways are not mutually exclusive [15–17].

Our analysis predicts that the search time depends weakly, as a logarithmic function, on chromosome number. This implies that the time it takes to capture all chromosomes is not sensitive to mutations changing the number of chromosomes. This may have implications for cancer, in which genomic instability often causes an increase in chromosome number [18]. The logarithmic dependency on chromosome number means there is only a 20% increase in the average time it takes to capture chromosomes when the number of chromosomes increases by 10, suggesting that cancer cells pay a very small price for their genomic instability. Moreover, we predict that the stochastic fluctuations (variance/mean) of the search time are independent of the number of chromosomes.

It is tempting to speculate that the cell optimizes not just the rescue and catastrophe frequencies [2] but also the size of the kinetochores [8] and a number of other parameters to decrease the duration of prometaphase. According to our analysis, larger kinetochores reduce the time required for capture, and centrosome-independent kinetochore fiber formation could effectively increase the kinetochore size [19, 20]. In any event, the cell must strike a balance between hiding and exposing the kinetochores, a balance that minimizes kinetochore misorientation, e.g., merotelic or syntelic attachment, and yet permits effective capture.

Our computer models are based on a number of simplifying assumptions that may affect the validity of the results. In the model, any one chromosome-capture event is independent of any other; there is no steric interference between the kinetochores. Such interference would increase the time to capture because some chromosomes would be "hidden" from view until other chromosomes were captured. It is not clear how poleward movements of mono-oriented chromosomes would affect the time it takes to capture the sister chromatid. Also, molecular details of MT-kinetochore or MT-chromosome-arm interactions may affect our estimates if reaching the target does not always lead to kinetochore attachment, or if lateral kinetochore attachments to the wall of the MT polymer lattice are frequent. Our analysis also assumes a purely centrosome-directed spindle-assembly pathway. This may not be the case [20, 21]: MT nucleation near the chromosomes as well as on the centrosomes, and the crosslinking between those differently nucleating MTs, might drastically decrease the duration of bipolar spindle assembly.

Finally, our experimental results are merely an indication that the RanGTP gradient may contribute to the bias. Modeling of the RanGTP gradient [22] suggests that it may not be possible to generate such a gradient in human somatic cells. Moreover, mutations in the Ran effector RCC1 in mammalian tissue culture cells show little change in spindle morphology [23], unlike the response seen in *Xenopus* extract spindles, suggesting that a RanGTP gradient may have mitotic roles in some, but not all systems. Another possibility is that RanGTP

affects MT-kinetochore interactions rather than MT dynamics [24]. Also, chemicals other than Ran [25, 26] could contribute to the bias in search and capture, and there may exist other, as-yet-undiscovered, mechanisms for chromosomes or kinetochores to influence MT dynamics. Further combined experiments and computer simulations of prometaphase in model organisms will lead to an improved understanding of the chemically biased search-and-capture mechanism.

Supplemental Data

Supplemental Data are available with this article online at http://www.current-biology.com/cgi/content/full/15/9/828/DC1/.

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