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Research paper

Uncovering chromatin's contribution to the mitotic spindle: Applications of computational and polymer models

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ABSTRACT

The mitotic spindle is a structure that forms during mitosis to help ensure that each daughter cell receives a full complement of genetic material. In metaphase, the spindle contains microtubules that nucleate inward from two opposing poles. Chromosomes are attached to plus-ends of these microtubules via protein structures called kinetochores. The centromere is the specific region of kinetochore attachment on the chromosome. Chromatin surrounding the centromere (pericentric chromatin) is subject to microtubule-based forces and is commonly modeled as a linear spring, where the force that it exerts is proportional to the distance that it is stretched. We have incorporated physically based models of chromatin to create more accurate and predictive models of the spindle. In addition, using fluorescence microscopy and motion analysis of fluorescently labeled chromatin spots we discovered that pericentric chromatin is restrained relative to free diffusive motion. The characterization of chromatin is crucial to understand mitotic spindle stability and to understand the cell cycle checkpoint regulating anaphase onset.

1. Introduction

The mitotic spindle is an apparatus that forms during the division of eukaryotic cells. Proper function of the mitotic spindle is essential to the accurate segregation of chromosomes, which ensures that daughter cells have all of the genetic information necessary for survival [1]. The structure and organization of the mitotic spindle is highly conserved throughout eukaryotic organisms. Thus, a complete understanding of the structure and function of the mitotic spindle is essential for a complete picture of life and, in the future, may yield many novel drug targets for cancer and other diseases where improper or unregulated cell division is implicated.

At metaphase, the mitotic spindle is a highly symmetric structure, consisting of two microtubule organizing centers (known as spindle poles in the budding yeast *Saccharomyces cerevisiae*, or centrosomes in vertebrate cells), from which two categories of microtubules grow inward. The first of these are the interpolar microtubules, which overlap in the center of the mitotic spindle. These microtubules are held together in an antiparallel fashion through microtubule-based motor proteins, known as kinesins. Microtubule plus-end directed kinesins (Cin8 and Kip1) [2] generate a net outward force on antiparallel microtubule arrays, driving spindle elongation. The second type of microtubules emanating from the spindle poles are kinetochore microtubules that provide physical connection to the replicated sister chromatids through proteinaceous structures known as kinetochores [3]. During metaphase, the sister chromatids are aligned at the center of the mitotic spindle, a region commonly known as the metaphase plate (Fig. 1). In the budding yeast *S. cerevisiae*, one microtubule attaches to each sister chromatid [4] through the kinetochore at a designated DNA sequence known as the centromere [5]. In more complex eukaryotes, such as humans, multiple microtubules associate with a single sister chromatid. The streamlined structure of the yeast mitotic spindle, combined with the ease of genetic manipulations in yeast, make it an ideal organism within which to study the structure and biophysical properties of the mitotic spindle components.

During metaphase this mitotic spindle attains a steady-state length, suggesting that the outward microtubule-based motor force is balanced by an inward force provided by the bi-oriented sister chromatids [6]. Elastic recoil of chromatin DNA has been observed *in vivo* [7], suggesting that microtubules are indeed capable of exerting tensile force on the sister chromatids and that the release of this force can cause the chromatids to recoil poleward. These experiments also demonstrate the highly viscous nature of the environment in the cell. This environment is so viscous that inertia is inconsequential (i.e. low Reynolds number situation).

In many force-balanced based mathematical models of the mitotic spindle, the paired sister chromatids are assumed to exert a force that is linearly proportional to extension length [8]. However, *in vitro* experiments have demonstrated that reconstituted chromatin responds in a complex manner when tensile stresses are applied [9].

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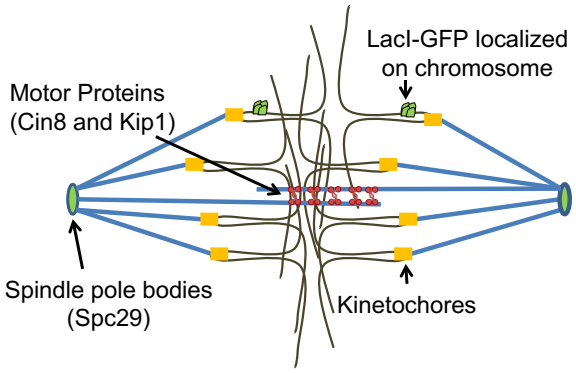

Fig. 1. Schematic of the mitotic spindle in *Saccharomyces cerevisiae*. Microtubules extend inward from the spindle pole bodies. They attach to replicated sister chromatids via kinetochores or are cross-linked by microtubule motor proteins. Multiple arrays of the lactose operator DNA binding site, coupled with a lactose repressor-GFP fusion protein, can be used to visualize chromatin in a living cell. In the cell, sixteen pairs of sister chromatids are organized in a circular fashion around the overlapping interpolar microtubules in the center. Legend: spindle pole bodies (green circles), microtubules (blue lines), chromatin (gray lines), kinetochore (yellow square), kinesins (red ovals), and lacI-GFP (small green circles).

It is impossible to faithfully reconstruct chromatin and the intracellular environment *in vitro*. Thus, even though *in vitro* experiments have been useful in demonstrating that chromatin responds in a complex manner to applied tension, *in vivo* measurements are needed to accurately determine the tensile force that the chromatin can produce. Here, we attempt to explain the behavior of the chromatin as a complex polymer in order to more accurately model its behavior and increase the predictive power of mathematical and computational models of the mitotic spindle.

In depth analyses of biopolymers have been used to determine some of their mechanical properties [10]. These analyses, coupled with super-resolution fluorescence microscopy, provide the tools needed to examine the mechanical properties of the mitotic spindle and chromatin as they are properly assembled *in vivo*. Chromatin near the centromere can be selectively labeled by inserting an array of DNA into the genome and expressing a fluorescent fusion protein that selectively binds to the insert. This can be achieved by inserting a repetitive DNA array containing monomers of the lactose operator (lacO) or tetracycline operator (tetO), into the genome and expressing a fluorescent fusion protein (lactose repressor (lacI) or tetracycline repressor (tetR)) tagged with a fluorophore such as GFP that selectively binds to the insert [11]. Using wide-field epifluorescence microscopy, the diffraction limited spots of the operator bound to the repressor-GFP fusion protein can be observed over extended periods of time. Mean-squared displacement analysis can be applied to characterize the motion of the DNA and other spindle components in order to construct a polymer model.

Previous observations of the lacO spots in the mitotic spindle revealed clear asymmetries in the positioning of the sister chromatids, particularly in chromatin near the centromere. We propose that a more realistic model of pericentric chromatin includes two springs composed of chromatin near the centromere, one on either side of the paired chromatid arms that extend away from the core of the spindle. A drag force may act upon the arms, resulting in asymmetries that would not be predicted with a simple linear spring model.

2. Materials and methods

2.1. Polymer recoil in mitosis

Cells were grown and imaged according to procedures laid out in Harrison, et al., 2009 [12].

2.2. Computational modeling of the mitotic spindle

An initial model computational model of the mitotic spindle was created by Ms. Leandra Vicci (Department of Computer Science, UNC-Chapel Hill) in the Simulink modeling environment (unpublished). This model integrated microtubule dynamics, microtubule protein motor forces, and included compensation for the incredibly viscous intracellular environment. The force-response of chromatin was modeled as a linear spring (Fig. 2A). (Fig. 2 Here)

Since DNA is the major component of chromatin, it follows that the behavior of DNA should serve as the basis for a higher-order model of chromatin. The worm-like chain (WLC) equation is commonly used to describe the mode of motion of polymers like DNA [13].

$$F = \left(k_B{*}\frac{T}{L_P}\right)\left[\frac{1}{4}\left(1-\frac{x}{L_c}\right)^{-2}-\frac{1}{4}+\frac{x}{L_c}\right] \quad (1)$$

In WLC, the amount of force (F) that a polymer exerts per length stretched increases as extension (x) increases. The WLC equation contains two key parameters for characterizing the spring. The first is persistence length (L_p). If a polymer is shorter than L_p , it can be treated as a rigid body. The second parameter is contour length (L_c). This is the total length of the polymer. Boltzmann's constant, k_B, represents the amount of thermal energy in the system when multiplied by temperature, T.

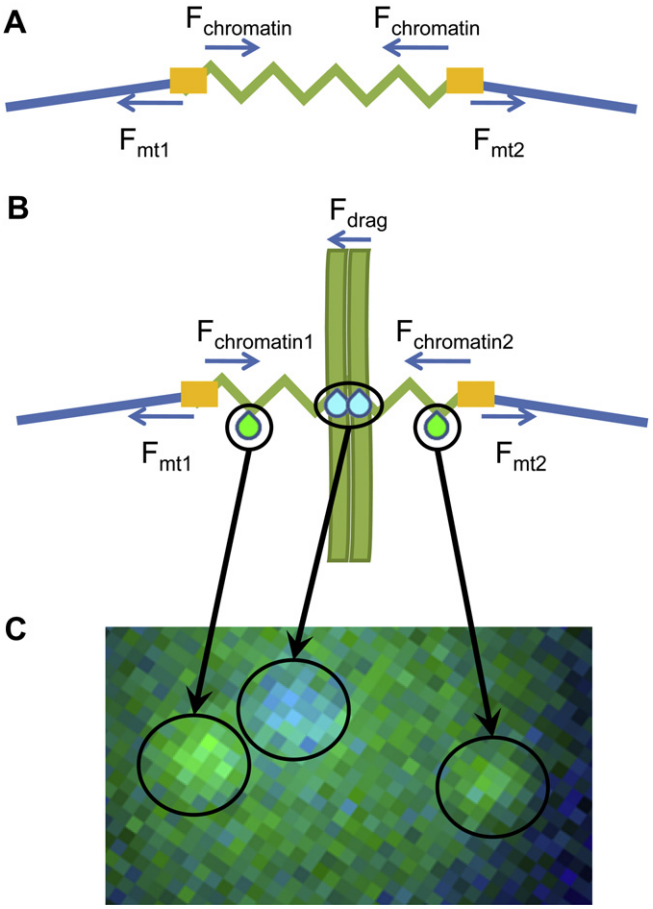

Fig. 2. (A) Classic model of microtubule force and chromosome response. A chromatin spring provides the only resistance to microtubule force. (B) Updated model of microtubule force and chromosome response. Chromatid arms provide a viscous drag force, allowing asymmetrical behavior in pericentric chromatin. (C) Image of asymmetry in pericentric chromatin in *S. cerevisiae*. Green dots represent chromatin near kinetochores while the blue dot represents chromatin at the intersection of the pericentric chromatin and the chromatid arms.