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Electrostatic Mechanism for Depolymerization-Based Poleward Force Generation at Kinetochores

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Abstract

Experiments implicating bound volume positive charge at kinetochores interacting with negative charge at microtubule free ends have prompted our calculation of the force at kinetochores for chromosome poleward motility during mitosis. We present here a corroborating force calculation between positively charged Hec1 tails in kinetochores and negatively charged C-termini at microtubule free ends. Based on experimentally-known charge magnitudes on Hec1 tails and C-termini at microtubule free ends, an *ab initio* calculation of poleward (tension) force per microtubule that falls within the experimental range is demonstrated. Due to the locations of C-termini charges on concave sides of splaying microtubules, this attractive force between subsets of low curvature splaying microtubule protofilaments C-termini eventually fails for subsets of protofilaments with more pronounced curvature, thus generating poleward force as microtubules depolymerize in a dynamic coupling, as observed experimentally. The mechanism by which kinetochores establish and maintain a dynamic coupling to microtubules for force production during the complex motions of mitosis remains elusive, and force generation at kinetochores has emerged as a signature problem in chromosome motility. In agreement with experiment, two separate calculations show that attractive electrostatic interactions over nanometer distances account for poleward chromosome forces at kinetochores.

1. Background

Force generation at kinetochores has emerged as one of the signature problems

in mitotic movements. Consistent with theoretical predictions made over a decade ago [1] [2], electrostatic interactions at kinetochores between negatively charged microtubule plus ends and positive charge at kinetochores have more recently been proposed for chromosome motility during mitosis [3]. A number of currently advanced models involve interactions that are fundamentally electrostatic, including mechanisms for chromosome movements based on protofilament-end splaying. A brief review of current models for force production at kinetochores is given elsewhere [4], where we support the experimental work of Miller *et al.* [3] with an *ab initio* calculation of the force between bound volume positive charge distributions at kinetochores interacting electrostatically with bound negative charge at free ends of microtubules.

Our purpose here is to mathematically corroborate that calculation with one that is based on direct interactions between positively charged unstructured Ndc80Hec1 tails in kinetochores and negatively charged C-termini at the free ends of microtubules, supporting an electrostatic-based model that explains poleward force generation.

Miller *et al.* [3], advances Ndc80/Hec1 as responsible for electrostatics-based force production at kinetochores. They propose that the force-producing interaction is electrostatic since an unstructured positively charged Hec1 tail cannot bind microtubules lacking negatively charged C-termini, concluding that "… the highest affinity interactions between kinetochores and microtubules are ionic attractions between two unstructured domains". Our approach supports the role of Hec1 as bound volume charge distributions—"positively charged Hec1 tails" [3]—at kinetochores, interacting electrostatically with bound negative charge at and near the free ends of microtubules—"ionic attractions between two unstructured domains" [3].

Chromosomes can move toward a proximal pole only when their kinetochores are connected to microtubules coming from that pole [5]. Microtubule polymerization and depolymerization follow a pattern characterized as "dynamic instability." This means that at any given time, some of the microtubules are growing, while others are undergoing rapid breakdown. The rate at which microtubules undergo net assembly, or disassembly (depolymerization), varies with mitotic stage [6]. In the present context, depolymerization-based electrostatic attractions are responsible for poleward force generation at kinetochores; electrostatic interactions for poleward force production at centrosomes are treated elsewhere [7].

The electrostatic properties of tubulin have been well-studied [8] [9] [10] [11]. Large-scale computer calculations have determined the dipole moment to be as large as 1800 Debye [9] [12]. Tubulin has a large overall charge of −20 (electron charges) at pH 7, and up to 40% of the charge resides on C-termini [13]. This large net charge on C-termini is integral to electrostatics-based force production at kinetochores (see below).

2. Results

In the context of force generation for chromosome motility at kinetochores,

Miller *et al.* [3] state that "… our data argue strongly that the Hec1 tail is the critical attachment for deploymerization-coupled movements of chromosomes"; and conclude "… the highest affinity interactions between kinetochores and microtubules are ionic attractions between two unstructured domains." This essentially proposes that bound, oppositely charged distributions are the underlying cause for poleward chromosome motions. As mentioned above, we recently published a force calculation between Hec1 charges, modeled as an experimentally known bound volume positive charge—"unstructured" positive charge—at kinetochores, and experimentally known negative charge at kinetochore microtubule free plus ends that agrees with experimental measurements of the poleward force for chromosome motility [4]. Here we provide a force calculation between positively charged Hec1 tails in kinetochores and negatively charged C-termini at and near microtubule free ends that confirms our previous proposal.

Since the lengths of Hec1 tails are much longer than the location volumes of C-termini charge distributions, Hec1 tails will be modeled as very long linear charges, with a linear charge density λ C/m (Coulombs/meter). A simple application of Gauss's law [14] for an infinitely long line charge distribution gives the electric field magnitude at a distance r from the line charge as

$$E = \lambda/2\pi\varepsilon r \tag{1}$$

where ε ($=k\varepsilon_0$) is the kinetochore permittivity, ε_0 = 8.85 pF/m (picoFarads/meter), and k is the kinetochore dielectric constant. Note that the relatively small contributions from edge effects near the ends of the Hec1 tails are neglected in this calculation.

The N-terminal tail of Hec1 contains an equivalent positive charge Q of 10 (electron charges, *e*) [3], distributed over a distance *l* of 55 nm [15], giving a linear charge density $\lambda = Q/l$ of 10 *e*/55 nm = 29 pC/m (picoCoulombs per meter). The force generating interaction is between positively charged Hec1 tails and negatively charged C-termini on concave sides of splaying protofilaments.

For the force per protofilament, we have:

$$F_{pf} = qE = ne\lambda/2\pi\varepsilon r \tag{2}$$

where $q = ne$ is the charge of n electrons on C-termini of a protofilament interacting with a Hec1 tail. Consistent with their open structures, a cytosol-saturated kinetochore is expected to have a dielectric constant midway between the kinetochore *dry* value and cytoplasmic water [16]. Since most condensed-matter (dry) dielectric constants are between 1 and 5, the value for cytoplasmic water dominates, and a conservative midpoint value k = 45 ((80 + 10)/2) will be assumed [4]. Substituting this value in (2), with λ = 29 pC/m, and the distance of the effective charge centers of C-termini charges, r = 3 nm, we have F_{pf} = 0.6n pN/pf (picoNewtons per protofilament).

Kinetochores generally number at least 8 Hec1 proteins per microtubule [17], and there are 13 protofilaments per microtubule. It will therefore be conserva-

tively assumed that four protofilaments in a microtubule are interacting with a Hec1 tail at any given moment. These subsets would be constantly changing among the microtubules penetrating a kinetochore. Thus, the total force per microtubule $F = 4(0.62)$ $n = 2.5$ n pN/MT (picoNewtons per microtubule). Equating this to the experimental range 1 - 5 pN/MT [18], we find that $n = 0.4$ - 2.5 electron charges. This result, like that of the previous calculation [4], falls well within the observed experimental range [9] [13] [19], and the agreement represents a successful *ab initio* theoretical derivation of this force magnitude.

Since microtubule C-termini are on the concave sides of progressively splaying microtubules, increasing protofilament curvature will lead to a separation of the charges on Hec1 tails and C-termini. Subsets of low curvature splaying protofilaments produce poleward force, while other subsets of protofilaments with more pronounced curvature in later stages of depolymerization fail to bind. Accordingly, poleward forces are generated as microtubules depolymerize, in agreement with observation.

3. Discussion

Electrostatic fields within the cytosol are subject to strong attenuation due to screening by oppositely charged ions (counterion screening), decreasing exponentially to much smaller values over a distance of several *Debye lengths*. The Debye length within cells is typically given to be of order 1 nm [20], and since cells have much larger dimensions, one is tempted to conclude that electrostatic force could not be a major factor in providing the cause for chromosome motility in biological cells. However the presence of microtubules challenges that notion. Microtubules can be thought of as intermediaries that extend the reach of the electrostatic interaction over cellular distances, making the second most powerful force in nature available to cells in spite of their ionic nature.

Cellular electrostatics is also strongly influenced by reduced counterion screening due to layered water adhering to charged molecules. Such water layering – with consequent reduction or elimination of Debye screening – at charged proteins has long been theorized [21] [22], and has been confirmed by experiment [23]. Additionally, water between sufficiently close (up to 3 nm) charged proteins has a dielectric permittivity that is considerably reduced from the *bulk* value far from charged surfaces [24] [25] [26]. The combination of these effects (or conditions)—water layering and reduced dielectric constant—can significantly influence cellular electrostatics in a number of important ways. This is especially true in relation to mitosis [26].

4. Conclusion

Given positive charge at kinetochores and negative charge on plus ends of microtubules, it is difficult to conceptualize there not being an attractive electrostatic poleward-directed force between these structures. A direct calculation of the electrostatic force between positively charged Hec1 tails and negatively

charged C-termini at and near the free ends of microtubules supports an electrostatic force generating mechanism for poleward chromosome motions during mitosis. A singular strength of the present calculation is that the disassembly rate of microtubules at kinetochores is explicitly shown to be correlated with force production at kinetochores. In a broader context, understanding the underlying forces and mechanisms that dictate chromosome movements through mitosis will be critical to the development of approaches to circumvent anomalous cell divisions (e.g., cancer).

Authors' Contributions

LJG conceptualized the theoretical aspects of this article and DHS provided intellectual contributions. Both authors read and approved the final manuscript.

Conflicts of Interest

The authors declare they have no competing interests.

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