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Emergent Mitotic Chromosome Motions from a Changing Intracellular pH

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Abstract

The mechanism by which chromosomes establish and maintain a dynamic coupling to microtubules for force generation during the complex motions of mitosis remains elusive. Equally challenging is an explanation for the timing of poleward, antipoleward, and oscillatory chromosome movements. The molecular cell biology paradigm requires that specific molecules, or molecular geometries, for force generation are necessary for chromosome motions. We propose here that the dynamics of mitotic chromosome motions are an emergent property of a changing intracellular pH in combination with electrostatic forces. We explain this mechanism within the context of Complexity Theory, based on the electrostatic properties of tubulin, known cellular electric charge distributions, and the dynamic instability of microtubules.

Keywords

Mitosis, Ndc80/Hec1, Electrostatics, Chromosome, Motility, Complexity Theory

1. Introduction

In the context of *complexity theory*, we propose that mitotic chromosome motions can be understood as an emergent property of a changing intracellular pH that influences nanoscale electrostatic interactions between 1) microtubules and kinetochores, 2) microtubules and centrosomes, and 3) microtubules and chromosome arms. A chromosome can move toward or away from a pole only when its kinetochore is connected to microtubules emanating from that pole [1]. Importantly, however, microtubule interactions with chromosome arms also contribute to chromosome motion. Thus changes in chromosome motions during mitosis can be attributed to pH_i-dependent changes in microtubule disassembly to assembly (disassembly/assembly) probability ratios at kinetochores, centro-

somes, and chromosome arms.

Microtubule lengthening and shortening follow a pattern of *dynamic instability*: At any given moment some microtubules are polymerizing while others are depolymerizing. Net polymerization and depolymerization vary with mitotic stage [2]. These aspects of microtubule dynamics and electrostatic interactions are fundamental to a complexity theory approach to mitotic chromosome motions. Among the several ways of characterizing complexity theory, we believe most relevant for the present work is that proposed by the eminent physicist Murray Gell-Mann, who describes complexity theory as "surface complexity arising out of deep simplicity."

Electrostatic fields are subject to strong attenuation by counterion screening, decreasing exponentially to negligible levels within a few *Debye lengths*. The Debye length (distance over which a field decreases to 1/e of its previous value) is of the order 1 nm [3], and since eukaryotic cells have much larger dimensions, it has generally been thought that electrostatic force could not be responsible for mitotic chromosome movements. However, the extension of electrostatic force by microtubules, and the combination of water layering and reduced dielectric constant vicinal to charged molecular surfaces, have challenged that notion (see below).

Experiments have revealed that pH_i of many cells rises to a maximum during prophase, decreasing 0.3 to 0.5 pH units during mitosis [4] [5]. Studies have shown that *in vivo* microtubule polymerization is favored by higher pH values [6]. *In vitro* studies on the role of pH in regulating microtubule assembly indicate a pH optimum in the range of 6.3 to 6.7. Disagreement between *in vitro* and *in vivo* studies regarding microtubule polymerization can be attributed to pH_i regulation of the nucleation potential of microtubule organizing centers like centrosomes [7] [8] [9], and ionic strength differences between cells and *in vitro* media [10]. These considerations favor the more complex physiological characteristics of *in vivo* studies to resolve this difference, thus *in vivo* experimental design is more appropriate for observations relating to pH_i conditions affecting microtubule assembly.

The electrostatic properties of tubulin have been well studied [11] [12] [13] [14]. Computer calculations have determined that the electric dipole moment of tubulin is 1800 Debye (D) [12] [15]. Experiments have shown that tubulin net charge varies quite linearly from −12 to −28 (electron charges) between pH 5.5 and 8.0 [16]. This is significant for tubulin-tubulin interactions during mitosis because a number of cell types undergo a decrease of 0.3 to 0.5 pH units from their peak at prophase.

Tubulin exhibits a large overall charge of −20 at pH 7, with up to 40% of the charge residing on C-termini. The C-termini angles with the dimer axis increase as a strong function of pH, extending 4 - 5 nm nearly perpendicularly outward from the microtubule axis at pH 7 [17]. An increased tubulin charge, with an attendant greater negative charge and extension of C-termini, are likely integral to

the higher probability for microtubule assembly during prophase when pH_i is highest.

We propose that the delicate balance of microtubule dynamics "at the edge of chaos" exemplifies a central idea of complexity theory. Specifically, control of microtubule disassembly/assembly probability ratios within microtubule dynamic instability close to the balance point is likely central to mitotic chromosome motions. In particular, we propose that the decrease in pH_i during mitosis controls microtubule disassembly/assembly probability ratios during the phases of mitosis, thus determining the timing and dynamics of chromosome movements through anaphase-A.

Experiments have revealed that mitotic spindles can assemble around DNA-coated beads incubated in *Xenopus* egg extracts [18]. Since the phosphate groups of DNA manifest a net negative charge in this experimental system, centrosomes were proposed to exhibit negative charge [19] [20], and direct measurement has subsequently shown that centrosomes are negatively charged [21].

Given that the electric dipole nature of tubulin dimers likely contributes to the efficiency of aster self-assembly, it follows that microtubule *minus* ends proximal to centrosomes are positively charged with *plus* ends negative. This assignment of charge signs at microtubule free ends agrees with large-scale computer calculations of the electrostatic properties of microtubules [22].

Indirect experimental evidence suggests that pole-facing *plates* of kinetochores exhibit positive charge and interact with negatively charged microtubule free plus ends to provide the motive force at kinetochores for poleward chromosome motions [19] [23]. This has subsequently been supported by experiments implicating positively charged Ndc80/Hec1 kinetochore molecules in establishing a dynamic coupling to negatively charged C-termini at microtubule plus ends during mitosis [24]. Evidence for positive charge at kinetochores also comes from the presence of highly basic molecules in the Dam1 complex [25].

Measurements have shown that pH_i rises to a maximum during prophase and decreases through mitosis. This is consistent with the efficient self-assembly of the spindle during prophase, when—due to the higher pH_i—the greater expression of negative charge on tubulin dimers (notably C-termini) and centrosomes favors microtubule polymerization and microtubule organizing center nucleation [20] [26]. This self-assembly is aided by reduced counterion screening from layered water adhering to the charge of the tubulin subunits. Water layering to charged proteins was predicted long ago [27] [28], and has more recently been observed [29]. Additionally, layered water between sufficiently close (see below) charged molecules has a dielectric constant that is considerably reduced from the *bulk* value away from such surfaces [30], further increasing the potency of an electrostatic component in aster/spindle self-assembly.

The conditions 1) water layering and 2) reduced dielectric constant can significantly strengthen the role of cellular electrostatics in important ways related to cell division. Gaps between charged surfaces within cells that allow these two effects to enhance electrostatic interactions will be designated as *critical dis-*

tances or *critical separations*. These conditions likely increase the efficiency of microtubule self-assembly in asters and spindles by enabling electrostatic interactions over greater distances than counterion screening dictates, and also increasing the strength of these interactions by an order of magnitude due to a corresponding tenfold reduction in the cytosolic dielectric constant between charged protein surfaces separated by critical distances or less [30].

An electrostatic component to the assembly of microtubules in asters/mitotic spindles is consistent with experimental observations of pH effects on microtubule assembly [6], as well as the sensitivity of microtubule stability to calcium ion concentrations [31] [32]. During the high pH_i conditions of prophase, the repulsive electrostatic force between subsets of interacting negatively charged microtubule free plus ends from opposing half-spindles in the growing mitotic spindle could provide the driving force for their poleward migration in the forming spindle [20] [26]. Subsets of interacting microtubules from opposing half-spindles whose free ends are within critical distances may be composed of both growing and shrinking microtubules, but polymerization probabilities will dominate during prophase due to favorable pH_i conditions.

2. Electrostatic Force in Poleward Chromosome Motions

2.1. Electrostatic Microtubule Disassembly Force at Centrosomes

The net charge on free minus ends of microtubules at a negatively-charged centrosome is assumed positive. A negatively-charged centrosome will attract positively-charged ends of microtubules to and into the centrosome, with the electric field gradient vicinal to and within the centrosome matrix destabilizing microtubules approaching and penetrating the centrosome as poleward force is generated (**Figure 1**), in agreement with experimental observations [33].

A brief review of previous publications on polar generation of poleward force, along with an *ab initio* electrostatics-based calculation of the magnitude of force at centrosomes that agrees with experimental data is given elsewhere [34].

2.2. Electrostatic Microtubule Disassembly Force at Kinetochores

Experimental observations on force generation at kinetochores may also be addressed as an electrostatic component within complexity theory. Electron microscope studies have long shown that kinetochore microtubules extend uninterrupted between poles and kinetochores, terminating in poleward-facing plates of kinetochores [35]. This kinetochore-microtubule association has been assumed to be the locus of force generation. Kinetochore-microtubule interactions have been proposed as being based on motor molecules [36], or binding relationships between protofilaments and kinetochore molecules [37] [38]. Indeed, force generation for chromosome motions at kinetochores has emerged as a signature problem in mitotic chromosome motility. Electrostatic interactions between kinetochores and microtubule free ends have been experimentally demonstrated, and proposed for chromosome motility during mitosis [24]. A brief

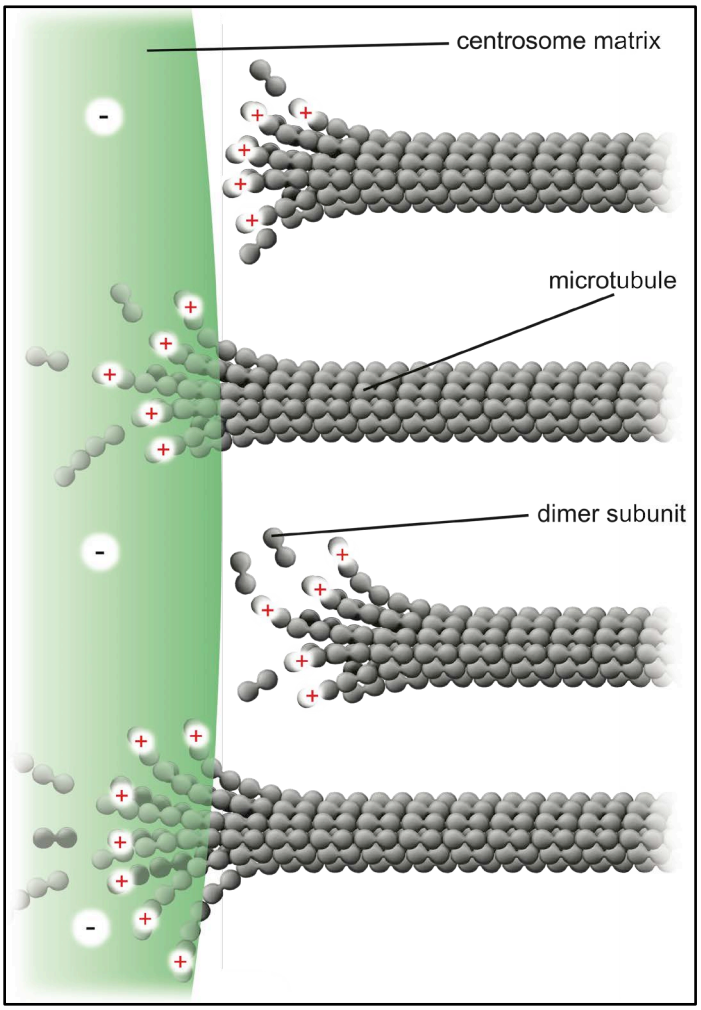

Figure 1. Nanoscale electrostatic disassembly force at a small section of a centrosome. A poleward force results from an electrostatic attraction between positively-charged microtubule free ends and an oppositely-charged centrosome.

review of the voluminous literature on kinetochore force generation as well as an *ab initio* electrostatics-based calculation of the magnitude of poleward force at kinetochores in support of this model is given elsewhere [39]. Since the numerical details of force production at kinetochores are not required in the present work, it will suffice here to outline the broad features of the calculation.

Experiments have recently revealed that kinetochore microtubule dynamics may be dependent on electrostatic interactions between positive charge in the disordered N-terminal 80 amino acid tail domain of Hec1 and negatively charged C-termini at microtubule free ends [24]. We model the kinetochore-microtubule force-producing interaction for penetrating microtubules by assuming the positive charge on Hec1 tails interacts with negatively charged C-termini at the free ends of microtubule protofilaments (**Figure 2**). Thus, poleward force is generated as microtubules disassemble, in agreement with observation. The loss of sufficient attractive force to maintain coupling for a given

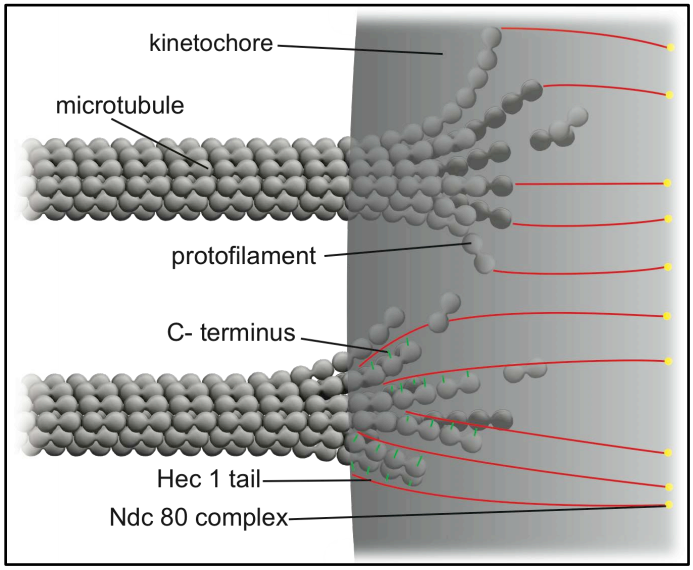

Figure 2. Nanoscale electrostatic disassembly forces acting at a small section of a kinetochore. The bottom microtubule depicts electrostatic poleward force generation between negatively-charged C-termini on gently splaying (curving) protofilaments and positively-charged Hec1 tails. The top microtubule depicts a possible configuration at the point of release between previously interacting C-termini and Hec1 tails.

protofilament is due to the increased distance between negatively charged C-termini and a positively charged Hec1 tail interacting on the concave side of an increasingly splaying (curving) microtubule protofilament. As discussed elsewhere, non-penetrating microtubules may also contribute to poleward force production at both centrosomes [34] and kinetochores [39].

3. Antipoleward Electrostatic Assembly Force

Following the *capture* and end-on attachment of a chromatid pair, negatively charged chromosome arms will be repelled over *critical distances* from negatively charged free ends of astral microtubules in the polar region. As chromatid pairs move farther from poles, tubulin dimer subunits will polymerize in the gaps between subsets of astral microtubule free ends and chromosome arms opened up by electrostatic repulsion at other subsets acting over critical distances.

This mechanism may account for the antipoleward *astral exclusion force*, or *polar wind*, the precise nature of which has been sought since it was first observed in the 1980s [40]. The interaction between astral microtubules and chromosome arms is depicted in **Figure 3**.

As a chromatid pair moves farther from a pole, the electrostatic repulsive force between negatively charged free ends of astral microtubules and chromosomes will decrease with *inverse square* law ($1/r^2$) dependence of the distance (r) from a pole as the microtubules fan radially outward [19] [23] [41].

The above models of poleward force at centrosomes and kinetochores, as well

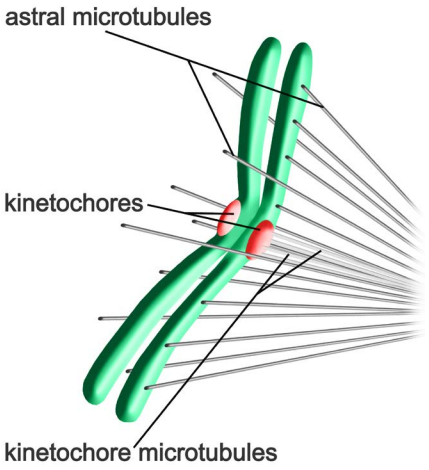

Figure 3. Electrostatic antipoleward force between microtubules and chromosome arms. An antipoleward force results from electrostatic repulsion between negatively-charged plus ends of microtubules and negatively-charged chromosome arms.

as the antipoleward force at chromosome arms, are regarded here as components that function within a complexity theory approach under the overarching control of a decreasing pH_i during mitosis.

4. Complexity Theory Approach to Chromosome Motions

Prometaphase and metaphase are characterized by both poleward and antipoleward chromosome motions, with antipoleward motions dominating during chromosome *congression*. Antipoleward and poleward motions alternate during metaphase oscillations, while poleward motions prevail during anaphase-A. The apparent complexity of these motions has challenged scientific explanation for over 100 years. We propose that a unifying principle can be realized within the framework of complexity theory by attributing the timing of the various post-attachment chromosome motions to pH_i-dependent electrostatic interactions between tubulin dimers controlling microtubule disassembly/assembly probability ratios.

Experiments have shown that during early prometaphase each pair of sister chromatids attaches by a kinetochore to the outside walls of a single microtubule, resulting in a rapid (20 - 50 μm per minute) microtubule sidewall sliding motion toward a pole [42]. A molecular motor-powered microtubule, sidewall sliding model for this prometaphase movement is consistent with known molecular motor behavior [43]. This is followed by end-on *attachment* of a kinetochore to an astral microtubule. However, post-attachment prometaphase, metaphase, and anaphase-A chromosome motions can be understood in terms of a gradual increase in the dominance of nanoscale electrostatic microtubule disassembly forces—operating in dynamic instability *at the edge of chaos*—due to a

steadily decreasing pH_i during mitosis, and also operating in conjunction with inverse square antipoleward microtubule assembly forces. This situation is typical of self-organization in complex dynamical systems characteristic of complexity theory.

Post-Attachment Chromosome Motility through Anaphase-A

Following a *monovalent* (or *mono-oriented*) attachment to one pole, chromosomes are observed to move at speeds of a few μm per minute, in subsequent motions throughout prometaphase [43]. A period of slow motions toward and away from a pole, with antipoleward motions slightly more probable, will ensue until attachment of a sister chromatid results in a *bivalent* (or *bioriented*) attachment to both poles [44]. Statistical fluctuations in the number of disassembling microtubules interacting with kinetochores and centrosomes, and also in the number of assembling microtubules at chromosome arms, results in the observed movements for a monovalent attachment. A pH_i-dependent increased microtubule disassembly/assembly probability ratio is consistent with these motions since pH_i has begun to decrease from higher prophase values. Note that the antipoleward microtubule assembly force will dominate near a proximal pole. Observations that chromosomes can congress to the *metaphase plate* before biorientation [45] [46] are consistent with a continued domination of microtubule assembly forces during prometaphase.

After a bivalent attachment, a chromatid pair will perform a slow (1 - 2 μm per minute) *congressional* motion to the spindle *equator* (midcell) region [44]. This is due to opposing microtubule disassembly forces at the sister kinetochore and distal pole that progressively balance the disassembly forces at the kinetochore and proximal pole as more attachments of sister chromatids to distal poles are made. Antipoleward forces from a proximal pole will dominate until an increasing number of attachments to a distal pole allows the disassembly forces at kinetochores and poles for each of the sister chromatids to balance. This is occurring at the same time that antipoleward forces from a proximal pole are decreasing with an inverse square distance dependence. Motion to the midcell region will therefore also equalize antipoleward assembly forces. Thus both microtubule disassembly and assembly forces will separately tend to equilibrate as a chromatid pair approaches the midcell region. Such balanced pairs of attractive and repulsive forces have previously been postulated for the metaphase alignment of chromatid pairs [47].

An explanation of experimentally observed metaphase midcell oscillations [44] of chromatid pairs also follows directly within the context of the present approach without the need for additional assumptions. An imbalance in the total force on a chromatid pair due to statistical fluctuations in the number of disassembling microtubules at kinetochores and poles and/or the number of microtubules assembling at chromosome arms would result in momentary motion toward a pole in the direction of the instantaneous net force. However, the greater antipoleward inverse square assembly force from a proximal pole will

eventually reverse the direction of motion, resulting in midcell metaphase oscillations. Mid-cell metaphase chromatid pair oscillations are indirect experimental evidence for a continuing increase in the pH_i-dependent disassembly/assembly probability ratios. A continuing increase in the microtubule disassembly/assembly probability ratio will result in parity for microtubule assembly and disassembly probabilities consistent with metaphase midcell oscillations.

At late metaphase, experiments reveal that poleward motions of sister kinetochores stretch centromeric chromatin, producing high kinetochore tensions [48]. These tensions are likely due to the continuing disassembly/assembly probability ratio increase—caused by a further lowering of pH_i—leading to a dominance of poleward microtubule electrostatic disassembly forces at kinetochores and poles tending to pull chromosomes to their respective poles. This is likely integral in causing chromatid separation. Once chromatid (chromosome) separation has occurred, the dominance of poleward microtubule disassembly forces causes anaphase-A chromosome motion.

5. Conclusions

High pH_i during prophase favors spindle assembly. This involves electrostatic attractive forces between oppositely charged regions of tubulin dimers as well as repulsive electrostatic interactions between subsets of growing microtubule free ends driving poleward motion of forming half-spindles. Increases in microtubule disassembly/assembly probability ratios through anaphase-A lead to changes in the motions of chromosomes during mitosis. These changes are attributed to a pH_i-dependent altering of electrostatic interactions between tubulin dimers, thus determining microtubule disassembly/assembly probability ratios.

The timing and dynamics of poleward, antipoleward, and oscillatory chromosome movements during post-attachment prometaphase, metaphase, and anaphase-A are emergent properties of a steadily decreasing pH_i during mitosis acting in conjunction with poleward and antipoleward force production.

At the lowest pH_i during mitosis, the superiority of poleward microtubule disassembly forces at kinetochores and poles over antipoleward microtubule assembly forces at chromosome arms is integral to the initial separation of sister chromatids. Once chromatid separation is effected, anaphase-A chromosome motion results from the predominance of microtubule disassembly forces at kinetochores and poles.

We may thus envision a steadily decreasing intracellular pH as a cell's overarching control over microtubule disassembly to assembly probability ratios—in dynamic instability close to the balance point *at the edge of chaos*—and consequently control over the timing and dynamics of post-attachment chromosome motility through anaphase-A.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

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

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