Continuum Electrostatics in Cell Biology

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Abstract

Recent experiments revealing possible nanoscale electrostatic interactions in force generation at kinetochores for chromosome motions have prompted speculation regarding possible models for interactions between positively charged molecules in kinetochores and negative charge on C-termini near the plus ends of microtubules. A clear picture of how kinetochores establish and maintain a dynamic coupling to microtubules for force generation during the complex motions of mitosis remains elusive. The current paradigm of molecular cell biology requires that specific molecules, or molecular geometries, for force generation be identified. However it is possible to account for mitotic motions within a classical electrostatics approach in terms of experimentally known cellular electric charge interacting over nanometer distances. These charges are modeled as bound surface and volume continuum charge distributions. Electrostatic consequences of intracellular pH changes during mitosis may provide a master clock for the events of mitosis.

Key Words: cell, mitosis, charged molecules, electrostatics

Introduction

Although the molecular biology paradigm has been quite successful in solving a number of problems in the understanding of cell structure and function, important problems remain open. Some of the most notable of these are in the area of cell division. Given the constraints wittingly or unwittingly imposed by the molecular cell biology paradigm, it would seem that models of mitotic motions and events have become more and more complex, and therefore - to this observer at least - more and more unsatisfactory. Insisting on a molecular biology approach to explaining mitotic chromosome motions (and a number of other cellular processes) has striking similarities to the ancient Greeks’ insisting on perfect circles to explain planetary motions. This paper reviews an alternative based on classical electrostatics expressed in terms of stably bound continuum surface and volume charge densities (charge distributions). This sort of approach makes it possible to describe the dynamics, including timing and sequencing, of post-attachment mitotic motions within a comprehensive approach.

The known charge in mitotic chromosome motions is the negative charge on chromosome arms and centrosomes, and positive charge at kinetochores. Negative charge at and near plus ends of microtubules and positive charge at minus ends of microtubules will be assumed. (According to existing convention, one end is designated...
plus because of its more rapid growth, there being no reference to charge in the use of this nomenclature.) Arguments for these two assumptions will be presented; however, they are to be viewed here as the sole postulates within which a comprehensive model for post-attachment mitotic movements and events can be framed.

In 2002 and 2005 papers, I argued that indirect experimental evidence indicates that pole-facing plates of kinetochores exhibit positive charge (Gagliardi, 2002a; 2002b; 2005) and interact with negatively charged microtubule free ends to provide the motive force for poleward force generation at kinetochores. This has subsequently been supported by experiments (Ciferri et al., 2008; Guimaraes et al., 2009; Miller et al., 2008) implicating positively charged molecules at kinetochores in establishing a dynamic coupling to negative charge on microtubules during mitosis.

Assuming a volume positive charge at kinetochores and negative charge at and near the free plus ends of microtubules, it was possible to derive a magnitude of the maximum (tension) force per microtubule for poleward chromosome motions that falls within the experimental range (Gagliardi, 2005). In these three papers, I also proposed that indirect experimental evidence is consistent with a negative charge distribution on centrosomes. Recent direct experimental measurements have confirmed this (Hormeño et al., 2009).

As noted above, a major advantage of focusing on cellular charge distributions is that it appears to offer the possibility of discovering a minimal assumptions model for post-attachment chromosome motions (Gagliardi, 2005; 2009). A model of this sort can point the way to the eventual discovery of specific molecules, and their biochemistries, that are responsible for the various mitotic motions. This is what is now happening. As mentioned above, a number of recent experiments have shown that certain kinetochore molecules that bind with microtubules have a net positive charge, and that poleward force for chromosome motions at kinetochores may be due to electrostatic interactions between these molecules and negative charge on microtubules. These discoveries might have been made sooner if the above mentioned 2002 and 2005 papers had been duly noted.

Some cellular electrostatics

In the cytoplasmic medium (cytosol) within biological cells, it has been generally thought that electrostatic fields are subject to strong attenuation by screening with 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 (Benedek and Villars, 2000), and since cells of interest in the present work (i.e. eukaryotic) can be taken to have much larger dimensions, one would be tempted to conclude that electrostatic force could not be a major factor in providing the cause for mitotic chromosome movements in biological cells. However, the presence of microtubules, as well as other factors to be discussed shortly, change the picture completely.

Microtubules can be thought of as intermediaries that extend the reach of the electrostatic interaction over cellular distances, making the second most potent force in the universe available to cells in spite of their ionic nature.

![Figure 1. A microtubule highlighting a protofilament.](image-url)

Microtubules are 25 nm diameter cylindrical structures comprised of protofilaments, each consisting of tubulin dimer subunits, 8 nm in length, aligned lengthwise parallel to the microtubule axis. The protofilaments are bound laterally to form a sheet that closes to form a cylindrical microtubule. The structure of microtubules is similar in all eukaryotic cells. Cross sections reveal that the wall of a microtubule...
consists of a circle of 4 to 5 nm diameter subunits. The circle typically contains 13 subunits as observed in vivo. Neighboring dimers along protofilaments exhibit a small (B-lattice) offset of 0.92 nm from protofilament to protofilament, as depicted in Figure 1.

Microtubules continually assemble and disassemble, so the turnover of tubulin is ongoing. The characteristics of microtubule lengthening (polymerization) and shortening (depolymerization) follow a pattern known as “dynamic instability”; that is, at any given instant some of the microtubules are growing, while others are undergoing rapid breakdown. In general, the rate at which microtubules undergo net assembly - or disassembly - varies with mitotic stage (cf. Alberts et al., 1994a). Changes in microtubule dynamics are integral to changes in the motions of chromosomes during the stages of mitosis. Poleward and antipoleward chromosome motions occur during prometaphase and metaphase. Antipoleward motions dominate during the congressional movement of chromosomes to the cell equator, and poleward motion prevails during anaphase-A. It is assumed here that poleward chromosome motions are in response to disassembling kinetochore microtubules at kinetochores and poles, and antipoleward chromosome motions are in response to assembling microtubules at chromosome arms. Assembling and disassembling microtubules are depicted in Figure 2.

Experiments have shown that the intracellular pH (pH_i) of many cells rises to a maximum at the onset of mitosis, subsequently falling during later stages (Amirand et al., 2000; Steinhardt and Morisawa, 1982). Studies (Schatten et al., 1985) have shown that in vivo microtubule growth (polymerization) is favored by higher pH values. It should be noted that in vitro studies of the role of pH in regulating microtubule assembly indicate a pH optimum for assembly in the range of 6.3 to 6.4. The disagreement between in vitro and in vivo studies has been analyzed in relation to the nucleation potential of microtubule organizing centers (Kirschner, 1980; De Brabander et al., 1982; Deery and Brinkley, 1983). This favors the more complex physiology characteristic of in vivo studies to resolve this question.

Kinetochore molecules are known to self-assemble onto condensed, negatively charged DNA at centromeres (Alberts et al., 1994b), indicating that kinetochores may exhibit positive charge at their pole-facing plates. This is an example of an important aspect of electrostatic interactions within cells: namely their longer range compared to other intracellular molecular interactions, and the resulting capacity of electrostatic forces to organize molecules and structures within cells. This line of reasoning was the basis for my assuming positive charge on pole-facing “plates” of kinetochores in a previous paper (Gagliardi, 2005). In earlier works I had assumed positive charge at kinetochores for different reasons (Gagliardi, 2002a, b).

Cellular electrostatics is strongly influenced by significantly reduced counterion screening due to layered water adhering to charged molecules. Such water layering - with consequent reduction or elimination of Debye screening - to charged proteins has long been theorized (Jordan-Lloyd and Shore, 1938; Pauling, 1945) and has been confirmed by experiment (Toney et al., 1994).
al., 1994). Additionally, water between sufficiently close (up to 4 nm) charged proteins has a dielectric constant that is considerably reduced from the bulk value far from charged surfaces (Gagliardi, 2005; 2009; 2008). As will be discussed in the next section, this would further increase the tendency for an electrostatic assist to aster and spindle self-assembly.

The combination of these two 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 (Gagliardi, 2009; 2008).

The aster’s pincushion-like appearance is consistent with electrostatics since electric dipole subunits will align radially outward about a central charge with the geometry of the resulting configuration resembling the electric field of a point charge. From this it seems reasonable to assume that the pericentriolar material, the centrosome matrix within which the microtubule dimer dipolar subunits assemble in many cell types to form an aster (Joshi et al., 1992), carries a net charge. This agrees with observations that the microtubules appear to start in the centrosome matrix (cf. Wolfe, 1993a). One may assume that the sign of this charge is negative (Gagliardi, 2002a, b). This assumption is consistent with experiments (Heald et al., 1996) revealing that mitotic spindles can assemble around DNA-coated beads incubated in Xenopus egg extracts. The phosphate groups of the DNA will manifest a net negative charge at the pH of this experimental system. This experimental result was cited in my 2002 and 2005 papers to conclude that centrosomes are negatively charged. As noted above, centrosomes have recently been shown to have a net negative charge by direct measurement (Hormeño et al., 2009).

A number of investigations have focused on the electrostatic properties of microtubule tubulin subunits (Sataric et al., 1993; Brown and Tuszynski, 1997; Baker et al., 2001; Tuszynski et al., 1998). Large scale calculations of the tubulin molecule (Tuszynski et al., 2002) have been carried out using molecular dynamics programs along with protein parameter sets. The dipole moment of tubulin has been calculated to be as large as 1800 Debye (D) (Brown and Tuszynski, 1997; Tuszynski et al., 1995). In experiments carried out at nearly physiological conditions, the dipole moment has been determined to be 36 D (Stracke et al., 2002), corresponding to a dipole charge of approximately 0.1 electron per dimer. Experiments (Tuszynski et al., 1995; Sackett, 1997) have shown that tubulin net charge depends strongly on pH, varying quite linearly from -12 to -28 (electron charges) between pH 5.5 and 8.0. This could be significant for microtubule dynamics during mitosis because, as noted above, many cell types exhibit a decrease of 0.3 to 0.5 pH units from a peak at prophase during mitosis.

It has been determined that tubulin has a large overall negative charge of 20 (electron charges) at pH 7, and that as much as 40% of the charge resides on C-termini. The C-termini can extend perpendicularly outward from the microtubule axis as a function of pHi, extending 4-5 nm at pHi 7 (Tuszynski, 2002). It would therefore seem reasonable to assume that an increased tubulin charge and the resulting greater extension of C-termini may be integral to an increased probability for microtubule assembly during prophase when pHi is highest (Steinhardt and Morisawa, 1982). This will be discussed next.

Intracellular pH as a clock for mitosis

As noted above, in addition to addressing force generation for post-attachment chromosome motions, a continuum electrostatics approach to mitotic motions can also account for the timing and sequencing of the detailed changes in these motions. These changes can be attributed to changes in microtubule dynamics based on a progressively increasing microtubule disassembly to assembly ratio for kinetochore microtubules that is caused by a steadily decreasing pHi during mitosis (Gagliardi, 2002b; 2009).

A higher pHi during prophase is consistent with an enhanced interaction between highly extended C-termini of tubulin dimers and positively charged regions of neighboring dimers. This enhanced interaction is due to their greater...
extension as well as increased expression of negative charge, with both favoring microtubule growth. It would therefore seem reasonable to expect that prophase high pH\textsubscript{i} conditions and the electrostatic nature of tubulin dimer subunits greatly assists in their self-assembly into the microtubules of the asters and spindle. As discussed in the previous section, this self-assembly would be aided by significantly reduced counterion screening due to layered water and the reduced dielectric constant between charged protein surfaces. An electrostatic component to the biochemistry of the microtubules in assembling asters is consistent with experimental observations of pH effects on microtubule assembly (Schatten et al., 1985), as well as the sensitivity of microtubule stability to calcium ion concentrations (Weisenberg, 1972; Borisy and Olmsted, 1972).

The two effects (or conditions) discussed in the last section would be expected to significantly increase the efficiency of microtubule self-assembly in asters and spindles by (1) allowing electrostatic interactions over greater distances than Debye (counter-ion) screening dictates, and (2) increasing the strength of these interactions by an order of magnitude reduction in the cytosolic dielectric constant between charged protein surfaces separated by critical distances or less.

Thus it would seem reasonable to assume that, over distances consistent with the reduced dielectric constant and modified counterion screening discussed above, the electrostatic nature of tubulin dimers would allow tubulin dimer microtubule subunits (1) to be attracted to and align around charge distributions within cells - in particular, as mentioned above, around centrosomes - and (2) to align end to end and laterally, facilitating the formation of asters and mitotic spindles (Gagliardi, 2002a, b; 2009).

Motive force for the migration of asters and assembling spindles during prophase can also be understood in terms of nanoscale electrostatics. As a consequence of the negative charge on microtubules at - and on C-termini near - the plus free ends of microtubules of the forming asters and half-spindles, the asters/half-spindles would be repelled electrostatically from each other and drift apart. Specifically as microtubule assembly proceeds, a subset of the negatively charged microtubule free ends at and near a periphery of one of the growing asters/forming half-spindles would mutually repel a subset of the negatively charged free ends at and near the periphery of the other growing aster/half-spindle, causing them to drift apart as net assembly of microtubules continues and subsets of interacting microtubules are continually replaced (Gagliardi, 2002a, b; 2009). Microtubules disassembling from previously overlapping configurations could also generate repulsive force between asters/half-spindles, but net microtubule assembly will dominate during prophase.

As discussed above, because of significantly reduced counterion screening and the low dielectric constant of layered water adhering to charged tubulin dimers, the necessary attraction and alignment of the dimers during spindle self-assembly would be enhanced by the considerably increased range and strength of the electrostatic attraction between oppositely charged regions of nearest-neighbors. Similarly, the mutually repulsive electrostatic force between subsets of like-charged free plus ends of interacting microtubules from opposite half-spindles in the growing mitotic spindle would be expected to be significantly increased in magnitude and range. Thus mutual electrostatic repulsion of the negatively charged microtubule plus ends distal to centrosomes in assembling asters/half-spindles could provide the driving force for their poleward migration in the forming spindle (Gagliardi, 2002a, b). A subset of interacting microtubules in a small portion of a forming spindle is depicted in Figure 3.

As noted above, it is important to recognize that interacting microtubules can result from either growing or shrinking microtubules but polymerization probabilities will dominate during prophase.
As cited above, experiments have shown that pH$_t$ of many cell types rises to a maximum at the onset of mitosis, subsequently falling steadily through mitosis. Although it is experimentally difficult to resolve the exact starting time for the beginning of the decrease in pH$_t$ during the cell cycle, it appears to decrease 0.3 to 0.5 pH units from the typical peak values of 7.3 to 7.5 measured earlier during prophase. The further decrease in pH$_t$ through metaphase (Steinhardt and Morisawa, 1982) would result in increased instability of the microtubules comprising the spindle fibers. Previously, I noted that in vivo experiments have shown that microtubule stability is related to pH$_t$, with a more basic pH favoring microtubule assembly.

It is important to note that pH in the vicinity of the negatively charged plus ends of microtubules (see discussion of net charge at microtubule free ends below) will be even lower than the bulk pH$_t$ because of the effect of negative charge at the free plus ends of the microtubules. This lowering of pH in the vicinity of negative charge distributions is a general result. Intracellular pH in such limited volumes is often referred to as local pH. As one might expect from classical Boltzmann statistical mechanics, the hydrogen ion concentration at a negatively charged surface can be shown to be the product of the bulk phase concentration and the Boltzmann factor

$$e^{-e\zeta/kT}$$

where $e$ is the electronic charge, $\zeta$ is the (negative) electric potential at the surface, and $k$ is Boltzmann’s constant (Hartley and Roe, 1940). For example, for typical mammalian cell membrane negative charge densities, and therefore typical negative cell membrane potentials, the local pH can be reduced 0.5 to 1.0 pH unit just outside the cell membrane. Because of the negative charge at the plus ends of microtubules, a reduction of pH would be expected in the immediate vicinity of these free ends making the local pH influencing microtubule dynamics considerably lower, and a lower bulk pH$_t$ would be accompanied by an even lower local pH.

A continuum electrostatics model of mitotic events also addresses the dynamics of nuclear envelope fragmentation and reassembly (Gagliardi, 2006). Experimentally observed increases in whole cell sialic acid content (Glick et al., 1971) and intracellular pH during prophase (Amirand et al., 2000; Steinhardt and Morisawa, 1982), followed by an observed release of free calcium from nuclear envelope stores at the onset of nuclear envelope breakdown (Silver et al., 1998; Silver, 1994) could significantly enhance the manifestation of negative charge on the nuclear envelope, providing sufficient electrostatic energy for nuclear envelope fragmentation (Gagliardi, 2006). Experimental observations regarding the mechanical properties of the plasma membrane show that electrostatic stress does manifest itself in ways consistent with this scenario (Weiss, 1968).

Since terminal sialic acids are attached to membrane proteins that are firmly anchored in the lipid bilayer, the observed disassembly of the nuclear envelope is consistent with electrostatic repulsion between membrane continuum charge clusters, which tear apart under the influence of increased electrostatic charge (Gagliardi, 2006). It is difficult to envision a purely biochemical process that would result in the nuclear envelope's breaking into fragments of many molecules each. Models for nuclear envelope breakdown in the current literature do not address this. The observed lowering of both intracellular pH (Amirand et al., 2000; Steinhardt and Morisawa, 1982) and whole cell sialic acid...
content (Glick et al., 1971) during late anaphase and telophase for many cell types is consistent with a decreased manifestation of net negative charge on membrane fragments at that time. These decreases could shift the balance of thermal energy versus electrostatic repulsive energy, allowing the closer approach of membrane fragments necessary for reassembly to occur in nascent daughter cells (Gagliardi, 2006).

An increased probability for microtubule depolymerization, as compared to the prophase predominance of microtubule assembly, is consistent with alternating poleward and antipoleward motions - with antipoleward motions more probable - of monovalently attached chromosomes during prometaphase. As discussed elsewhere (Gagliardi, 2002b, 2005), after a bivalent attachment to both poles, poleward forces toward both poles acting in conjunction with inverse square antipoleward forces exerted between negatively charged microtubule free plus ends and negatively charged chromosome arms could account for chromosome congression. The relative complexity of microtubule disassembly force generation at kinetochores and poles coupled with inverse square antipoleward forces from microtubule assembly at chromosome arms precludes an unequivocal conclusion regarding a possible continuing increase in the microtubule disassembly to assembly (disassembly / assembly) probability ratio during chromosome congression. However, metaphase chromosome midcell oscillations are indirect experimental evidence for a microtubule disassembly/assembly probability ratio approaching unity.

At late metaphase, before anaphase-A, experiments reveal that the poleward motions of sister kinetochores stretch the intervening centromeric chromatin, producing high kinetochore tensions. It is reasonable to attribute these high tensions to a continuing microtubule disassembly / assembly probability ratio increase caused by a further lowering of pH_i. The resulting attendant increase in poleward electrostatic disassembly force on sister chromatids would lead to increased tension. A lower pH_i would also increase the expression of positive charge on sister kinetochores, with the possibility of further increasing the tension due to increased mutual repulsion.

Thus regarding post-attachment chromosome movements through metaphase, it seems reasonable to ascribe an increasing microtubule disassembly/assembly probability ratio, with attendant changes in microtubule dynamics and associated mitotic chromosome motions through metaphase, to an experimentally observed steadily decreasing pH_i. We may then envision the decrease in pH_i from a peak at prophase favoring microtubule assembly, declining through prometaphase as discussed above, and continuing to decline through metaphase when parity between microtubule assembly and disassembly leads to midcell chromatid pair oscillations, culminating in increased microtubule disassembly-associated kinetochore tension close to anaphase-A, as the cell's master clock controlling microtubule dynamics, and consequently the events of mitosis. One might also be tempted to attribute the more complete dominance of microtubule disassembly - with an accompanying predominance of poleward electrostatic disassembly forces at kinetochores and poles - during anaphase-A to a further continuation of a decreasing intracellular pH. However, as discussed elsewhere (Gagliardi, 2002a; 2005; 2008; 2009), any additional possible decreases in pH_i after metaphase may not be the major determinant of anaphase-A and anaphase-B motions.

An explanation of the operation of the so-called spindle assembly checkpoint provides another example of the predictability and minimal assumptions nature of the present approach. A longstanding problem in the cell biology of mitosis involves the operation of the spindle assembly checkpoint, a surveillance mechanism that delays anaphase-A onset until all chromosomes are attached to the spindle and under tension. This may be understood within the context of the present work as the result of an abnormally continuing elevated pH_i beyond prophase. This would have the following effects (1) an increased probability of erroneous attachments due to a diminished efficiency for correcting mistakes (Khodjakov and
Rider, 2009) resulting from a reduced microtubule depolymerization rate, and (2) a decreased kinetochore tension (Nezi and Musacchio, 2009), with both effects due to higher $pH_i$. This is because a higher $pH_i$ would keep the microtubule disassembly/assembly probability ratio from increasing and would also prevent the manifestation of sufficient positive charge on sister kinetochores, with both outcomes serving to curtail the buildup of electrostatic repulsive tension necessary for chromatid separation.

As discussed above, greater kinetochore tension is seen here as resulting from increased electrostatic microtubule disassembly force at kinetochores and poles as well as increased manifestation of positive charge on kinetochores. It is now known that correction mechanisms involve centromere-associated Aurora-B kinase (Liu et al., 2009). This is consistent with the mechanism proposed here since phosphorylation of the N-terminal tail of kinetochore Ndc80 by Aurora-B destabilizes its interaction with microtubules (Cheesman et al., 2006; DeLuca et al., 2006).

Thus elevated kinetochore tension due to an increased disassembly force and increased mutual kinetochore repulsion would initiate chromatid separation and anaphase-A only when intracellular pH decreases sufficiently (Gagliardi 2002b; 2005). As discussed elsewhere (Gagliardi, 2005; 2008; 2009) an elevated calcium concentration at times before and during anaphase - A would further increase the microtubule disassembly/assembly probability ratio consistent with both anaphase-A and anaphase-B motions.

**Continuum electrostatics in mitotic force generation**

Following a *monovalent* attachment to one pole, chromosomes are observed to move at considerably slower speeds, a few µm per minute, in subsequent motions throughout prometaphase (cf. Grancell and Sorger, 1998). In particular, a period of slow motions toward and away from a pole will ensue, until close proximity of the negatively charged end of a microtubule from the opposite pole with the positively charged (sister) kinetochore in the chromatid pair results in a *bivalent* attachment to both poles (Gagliardi, 2002b; 2005). Attachments of additional microtubules from both poles will follow. (There may have been additional attachments to the first pole before any attachment to the second.) After the sister kinetochore becomes attached to microtubules from the opposite pole, chromosomes perform a slow (1-2µm per minute) congressional motion to the spindle equator, culminating in oscillatory motion of chromatid pairs during metaphase.

Chromosome motion during anaphase has two major components, designated as anaphase-A and anaphase-B. Anaphase-A is concerned with the poleward motion of chromosomes, accompanied by the shortening of kinetochore microtubules at kinetochores and/or spindle poles. The second component, anaphase-B, involves the separation of the poles. Both components contribute to the increased separation of chromosomes during mitosis.

Molecular biology explanations of these motions require that specific molecules, and/or molecular geometries, for mitotic chromosome force generation be identified for each motion. As indicated above, electrostatic models within the molecular cell biology paradigm have recently been sought (or advanced) involving positively charged kinetochore molecules interacting with negative charge on microtubules. As in the situation involving models that center partially or wholly on simulations, these molecular biology approaches are quite complex and primarily attempt to address specific mitotic motions, most notably poleward force generation at kinetochores. Critical experimental observations such as the “slip-clutch” mechanism (Maiato et al., 2004), observations of calcium ion concentration on anaphase-A motion (Zhang et al., 1990), and polar generation of poleward force are not addressed. However, it is possible to account for the dynamics of post-attachment mitotic motions in terms of electrostatic interactions between experimentally known, stably bound continuum surface and volume electric charge distributions interacting over nanometer distances. This is the approach that I have taken in a series of papers.
As mentioned above, charge distributions are known to exist at centrosomes, chromosome arms, and kinetochores. Assumptions of negative charge at microtubule plus ends and positive charge at microtubule minus ends - the only assumptions - are sufficient to explain the dynamics, timing, and sequencing of post-attachment chromosome motions. These assumptions will now be discussed.

Excluding possible contributions from microtubule associated proteins, the evidence for a net negative charge at microtubule plus ends and net positive charge at minus ends is as follows: (1) large scale computer calculations of tubulin dimer subunits indicate that 18 positively charged calcium ions are bound within beta monomers with an equal number of negative charges localized at adjacent alpha monomers (Sataric et al., 1993; Brown and Tuszynski, 1997), (2) experiments reveal that microtubule plus ends terminate with a crown of alpha subunits, and minus ends terminate with beta subunits (Song and Mandelkow, 1995), (3) the lower local pH vicinal to a negatively charged centrosome matrix would cause a greater expression of positive charge at microtubule minus ends, (4) the higher pH vicinal to a positively charged kinetochore pole-facing “plate” would cause a greater expression of negative charge at microtubule plus ends, (5) negative charge on centrosome matrices will induce positive charge on microtubule minus ends, and positive charge at pole-facing plates of kinetochores will induce negative charge on microtubule plus ends.

As discussed elsewhere (Gagliardi, 2005; 2009; 2008) force generation from positive charge at the free minus ends of kinetochore microtubules may be responsible for polar generation of poleward force. A calculation of the force per microtubule assuming permanent positive charge at microtubule minus ends falls within the experimental range (Gagliardi, 2005). Although a calculation of induced positive charge on a microtubule minus end from negative charge on a centrosome matrix is difficult because of the complex geometry, a reciprocal calculation of induced negative charge on a centrosome matrix from positive charge at the minus end of a microtubule is relatively straightforward, and agrees with experimental ranges for cellular charge densities and force per microtubule measurements (Gagliardi, 2008).

Similarly, net positive charge at kinetochore pole-facing surfaces would induce negative charge on the plus ends of kinetochore microtubules proximal to kinetochores, and reciprocal calculations at kinetochores similar to those at centrosomes (see previous paragraph) are also in agreement with experimental ranges for cellular charge densities and force per microtubule measurements (Gagliardi, 2008).

The above charge distributions at plus and minus microtubule free ends are also in accord with the common observation that the free ends of an aster/half-spindle’s microtubules distal to centrosomes (the pinheads in a pincushion analogy) are not attracted to the negatively charged outer surface of the nuclear envelope. If this were not the case, the forming half-spindles would not be able to move freely in their migration to the poles of the cell.

As mentioned above, critical experimental observations such as the “slip-clutch” mechanism and calcium ion concentration effects on anaphase-A motion have not been addressed by current models for mitotic chromosome motions. These experiments will now be reviewed along with their natural explanations within the context of a continuum electrostatics approach.

At the high kinetochore tensions prior to anaphase-A mentioned above, coupled microtubule plus ends often switch from a depolymerization state to a polymerization state of dynamic instability. This may be explained by kinetochore microtubule plus or minus free ends taking up the slack by polymerization to sustain attachment and resist further centromeric chromat in stretching. This is known as the slip-clutch mechanism (Maiato et al., 2004).

The slip-clutch mechanism is addressed within the context of the present work as follows: (1) microtubule assembly at a kinetochore or pole is regarded as
operating in passive response to a repulsive robust inverse square electrostatic antipoleward microtubule assembly force acting between the plus ends of astral microtubules and chromosome arms (Gagliardi, 2002b, 2005) and/or an electrostatic microtubule disassembly force at a sister kinetochore or at poles (Gagliardi, 2005); (2) non-contact electrostatic forces acting over a range of protofilament free end distances (up to 4 nm, as discussed above) from bound positive charge - both inside and near “surfaces” - at kinetochores would be effective in maintaining coupling while larger protofilament gaps in the same or other microtubules are passively filled in; (3) the repulsive inverse square electrostatic assembly force acting at the sister chromatid’s arms will provide a positive feedback mechanism to resist detachment. This explanation of the slip-clutch mechanism follows as a direct consequence of the present approach to chromosome motility with no additional assumptions.

There appears to be an optimum calcium ion concentration for maximizing the speed of chromosome motions during anaphase-A. If the [Ca\textsuperscript{2+}] is increased to micromolar levels, anaphase-A chromosome motion is increased two-fold above the control rate; if the concentration is further increased slightly beyond the optimum, the chromosomes will slow down, and possibly stop (Zhang et al., 1990). It has long been recognized that one way elevated [Ca\textsuperscript{2+}] could increase the speed of chromosome motion during anaphase-A is by facilitating microtubule depolymerization (Weisenberg, 1972; Salmon and Segall, 1980; Kiehart, 1981; Cande, 1981; Olmsted and Borisy, 1975), and it has been commonly believed that microtubule depolymerization, if not the motor for chromosome motion, is at least the rate-determining step (Nicklas, 1975; Nicklas, 1987; Salmon, 1975; Salmon, 1989). However, the slowing or stopping of chromosome motion associated with moderate increases beyond the optimum [Ca\textsuperscript{2+}] is more difficult to interpret since the microtubule network of the spindle is virtually intact and uncompromised. Such disruption of the mitotic spindle requires much higher concentrations (Zhang et al., 1990; Wolfe, 1993b).

In terms of the present model, progressively higher concentrations of doubly-charged calcium ions would be expected to first increase the speed due to the greater microtubule disassembly rate associated electrostatic disassembly force, but to eventually shield the negative charge at the plus ends of kinetochore microtubules as well as negative charge at the centrosome matrix, shutting down the poleward-directed nanoscale electrostatic disassembly force.

An experimental test of nonspecific divalent cation effects on anaphase-A chromosome motion by substitution of Mg\textsuperscript{2+} for Ca\textsuperscript{2+} (Zhang et al., 1990) does not offer a definitive test for the possibility of negative charge cancellation by positive ions. This is because high frequency sound absorption studies of substitution rate constants for water molecules in the inner hydration shell of various ions reveal that the inner hydration shell water substitution rate for Mg\textsuperscript{2+} is more than three orders of magnitude slower than that for Ca\textsuperscript{2+} (Diebler et al., 1969), indicating that the positive charge of Mg\textsuperscript{2+} is shielded much more effectively by water than is the case for Ca\textsuperscript{2+}.

Thus, the slowing or stopping of anaphase-A chromosome motion accompanying free calcium concentration increases above the optimum concentration for maximum anaphase-A chromosome speed -- but well below concentration levels that compromise the mitotic apparatus -- is completely consistent with an electrostatic disassembly motor at kinetochores and poles for poleward chromosome motion. This experimental observation has not been addressed by any of the other current models for anaphase-A motion.

Summary

It seems clear that cellular electrostatics involves more than the traditional thinking regarding counterion screening of electric fields and the resulting unimportance within cells of the second most powerful force in nature. The reality may be that the evidence suggests otherwise, and that the resulting enhanced electrostatic interactions are more robust and act over greater distances than previously thought. One aspect of this is the ability of microtubules to extend the reach of electrostatic force over cellular distances;
another lies in the reduced counterion screening and dielectric constant of the cytosol between charged protein surfaces.

High pH\textsubscript{i} during prophase favors spindle assembly. This includes greater electrostatic attractive forces between tubulin dimers as well as increased repulsive electrostatic interactions driving poleward movement of forming half-spindles. Because of significantly reduced counterion screening and the low dielectric constant of layered water adhering to charged free ends of tubulin dimers, the necessary attraction and alignment of tubulin during spindle self-assembly would be enhanced by the considerably increased range and strength of the electrostatic attraction between oppositely charged regions of tubulin dimers. Similarly, the mutually repulsive electrostatic force between a continually changing subset of like-charged plus ends of interacting microtubules from opposite half-spindles in the growing mitotic spindle would be expected to be significantly increased in magnitude and range.

Experimentally observed increases in whole cell sialic acid content and intracellular pH during prophase, followed by an observed release of free calcium from nuclear envelope and endoplasmic reticulum stores, would significantly enhance the expression of negative charge on sialic acid residues of the nuclear envelope, providing sufficient electrostatic energy for nuclear envelope breakdown. Since terminal sialic acids are attached to membrane proteins that are firmly anchored in the lipid bilayer, the observed disassembly of the nuclear envelope into membrane fragments is consistent with electrostatic repulsion between membrane charge clusters that could tear apart under the influence of increased electrostatic charge.

The observed lowering of both intracellular pH and whole cell sialic acid content during late anaphase and telophase is consistent with a decreased manifestation of net negative charge on membrane fragments. This decrease could shift the balance of thermal energy versus electrostatic repulsive energy in favor of thermal energy, allowing the closer approach of membrane fragments necessary for reassembly biochemistry to occur in nascent daughter cells.

Changes in microtubule dynamics are integral to changes in the motions of chromosomes during mitosis. These changes in microtubule dynamics can be attributed to an associated change in intracellular pH (pH\textsubscript{i}) during mitosis. In particular, a decrease in pH\textsubscript{i} - from a peak during prophase - through mitosis may act as a master clock controlling microtubule disassembly[assembly probability ratios by altering the electrostatic interactions of tubulin dimers. This, in turn, could determine the timing and dynamics of post-attachment mitotic chromosome motions through metaphase.

Force generation for the dynamics of post-attachment chromosome motions during prometaphase and metaphase can be explained by statistical fluctuations in nanoscale repulsive electrostatic microtubule antipoleward assembly forces acting between microtubules and chromosome arms in conjunction with similar fluctuations in nanoscale attractive electrostatic microtubule poleward disassembly forces acting at kinetochores and spindle poles. The different motions during prometaphase and metaphase may be understood as an increase in the microtubule disassembly to assembly probability ratio due to a steadily decreasing pH\textsubscript{i}.

Thus it seems reasonable to assume that the shift from the dominance of microtubule growth during prophase, to a lesser extent during prometaphase, and to approximate parity between microtubule polymerization and depolymerization during metaphase chromosome oscillations can be attributed to the gradual downward pH\textsubscript{i} shift during mitosis that is observed in many cell types.

Evidence for a further continuing decrease in pH\textsubscript{i} and an increasing microtubule disassembly to assembly probability ratio is seen in increased kinetochore tension just prior to anaphase. This increased tension has a possible simple interpretation in terms of the greater magnitude of poleward electrostatic disassembly forces at kinetochores and poles relative to antipoleward assembly forces.
between plus ends of microtubules and chromosome arms.

In light of the large body of experimental information regarding mitosis, the complexity and lack of unity of models for the various events and motions gives, at least to this observer, reason to believe that approaching mitosis primarily within the molecular biology paradigm is flawed. This paper reviews the merits of an approach based on continuum electrostatics. Such an approach to mitotic motions based on stably bound charge distributions can be used to frame a minimal assumptions model that incorporates the force production, timing, and sequencing of post-attachment chromosome motions.
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