

# Electrostatic Force in Furrowing of Biological Cells

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**abstract**—Previously, I have proposed that electrostatic repulsion between the negatively charged free ends (FEs) of polar microtubules furnished the driving force for pole separation and cytokinesis in primitive biological cells. The present work shows that given the charge at the FEs of astral microtubules (AMTs) adjacent to the cell equatorial cortex (EC) and layered water associated with the charge it is possible to describe an electrostatic component to the positioning of the cell division plane during cytokinesis. After establishing persistent electrostatic contact with the EC, force exerted by the FEs of AMTs interacting with positive charge induced on the EC could determine the division plane and initiate furrowing as the poles of the cell separate and pull the AMTs poleward.

## I. INTRODUCTION

Primitive eukaryotic cells had to divide prior to the evolution of very many biological mechanisms, and it is reasonable to assume that basic physics and chemistry played dominant roles in both mitosis (nuclear division) and cytokinesis (cytoplasmic division). It is proposed here that electrostatic force played a major role in the dynamics of cytokinesis in primitive cells, and that the fundamental solutions to the problem of cell division that were found by primitive cells may persist in modern eukaryotic cells.

In the cytoplasmic medium (cytosol) electrostatic fields are subject to strong attenuation by screening with oppositely charged ions, and decrease rapidly over a distance of several Debye lengths. The Debye length within cells is typically of order 1 nm [1], and since cells of interest in the present work (i.e. eukaryotic) can be taken to have dimensions between 10–30  $\mu\text{m}$ , one would be tempted to conclude that electrostatic force would not be a major factor in the dynamics of furrow formation in biological cells. However, the presence of microtubules (MTs) changes the picture completely. MTs can be thought of as intermediaries that extend the reach of the electrostatic interaction over cellular distances, making this potent force available to cells in spite of their ionic nature.

Microtubules are 25 nm diameter cylindrical structures comprised generally of 13 *protofilaments*, each consisting of tubulin dimer subunits, 8 nm in length, aligned lengthwise parallel to the MT axis. The protofilaments (PFs) are bound laterally to form a sheet that closes to form a cylindrical MT. Neighboring dimers along PFs exhibit a small offset of approximately 0.92 nm from PF to PF. This offset will be approximated as 1 nm in the calculations in subsequent sections. A number of investigations have focused on the electrostatic properties of MT dimer subunits [2-5]. Studies [6,7] have shown that the net charge depends strongly on pH. Dipole moments have been calculated to be as large as 1800 Debye (D). In experiments carried out at nearly physiological conditions, the dipole moment has been determined to be 36 D [8].

It is reasonable to assume that the electric dipole nature of dimer subunits greatly assists in their self-assembly into MTs. In particular, over the short distances consistent with counter-ion (Debye) shielding, their dipolar nature would allow them to be attracted to, and align around, any net charge distribution within cells. This may account for the efficient self-assembly of the asters during prophase, when MT polymerization and microtubule organizing center nucleation is favored because of the higher intracellular pH ( $\text{pH}_i$ ) at this time [9, 10]. Thus we may envision that electrostatic fields organize and align the electric dipole dimer subunits, thereby facilitating their assembly into the MTs that form the aster [11, 12]. The attraction between oppositely charged ends of the dipolar subunits takes place over the short distances allowed by counter-ion screening. This self-assembly may be further assisted by significantly reduced counter-ion screening due to layered water adhering to the net charge of the dipolar subunits. Such water layering to charged proteins has long been theorized [13, 14], and has been confirmed by experiment [15]. This layering would also operate between charged protofilament free ends (PFEs) and cellular structures.

An electrostatic component to the biochemistry of the MTs in the assembling asters is consistent with experimental observations of pH effects on MT assembly [9], as well as the sensitivity of MT stability to calcium ion concentrations [16, 17]. The mutual electrostatic repulsion of the negatively charged MT free ends distal to the centrosomes in assembling asters could provide the driving force for their poleward migration in the forming spindle [11].

Microtubules continually assemble and disassemble, so the turnover of tubulin is ongoing. The characteristics of MT lengthening (polymerization) and shortening (depolymerization) follow a pattern known as "dynamic instability": that is, at any given instant some of the MTs are growing, while others are undergoing rapid breakdown. In general, the rate at which MTs undergo net assembly – or disassembly – varies with mitotic stage; for example, during prophase the rates of MT polymerization and depolymerization change quite dramatically [18].

The charge on the *plus* free ends of MTs is negative. (According to existing convention, these ends are designated "plus" because of their more rapid growth, there being no reference to charge in the use of this nomenclature.) The negative charge on the plus ends of astral microtubules (AMTs) adjacent to the cell equatorial cortex (EC) would induce positive charge on the EC leading to an attractive force between the free ends of AMTs and the EC. An *ab initio* calculation of the maximum tension force exerted by a MT interacting with induced charge on mitotic structures that agrees with experimental results has been given else-

where [19]. A similar calculation of the magnitude of the maximum (tension) force exerted by AMTs on the EC due to induced charge will be given below.

## II. INDUCED CHARGE ON THE EQUATORIAL CORTEX

As mentioned above, net negative charge at the ends of MTs can induce charge on the cell cortex. The magnitude of the induced charge and the conditions under which charge induction can occur will be discussed next.

A standard derivation from electrostatics [20] shows that a point charge  $q$  at a perpendicular distance  $x$  from a planar boundary between two dielectric materials of permittivity  $\epsilon_1$  and  $\epsilon_2$  will induce a polarization charge density  $\sigma(x, s)$  (C/m<sup>2</sup>) at the interface given by

$$\sigma(x, s) = \frac{qx}{2\pi k_1 (s^2 + x^2)^{3/2}} \left( \frac{\epsilon_1 - \epsilon_2}{\epsilon_1 + \epsilon_2} \right), \quad (1)$$

where  $q$  is embedded in dielectric medium 1 of dielectric constant  $k_1$  and  $\sigma$  is the charge per unit area on the interface at a perpendicular distance  $s$  from a line connecting  $q$  and its image charge in dielectric medium 2. In the context of the present work,  $\epsilon_1$  is the permittivity of the cytosol (essentially cytoplasmic water) at a PFE where a charge  $q$  is located and  $\epsilon_2$  is the permittivity of the dielectric medium (medium 2) within which the image charge is induced, the EC. A nearly planar geometry is assumed for the EC interface because it is much greater in extent than the diameter of a PFE on which the charge  $q$  is located.

Microtubule polymerization occurring at PFEs with distances of 8 to 11 nm from the cortex could add 8 nm electric dipolar tubulin dimers, resulting in PFEs at distances of 0 to 3 nm from the interface between the cytosol and the EC. This range of distances is significant for the present calculation because 1.5 nm may be taken as the thickness of the layered water adsorbed to each charged surface [14, 21]. Thus, as charged surfaces approach within 3 nm, counter-ion screening could be virtually eliminated in the spaces between charged PFEs and the charged EC.

The magnitude of the induced charge density on the EC due to an astral microtubule (AMT) with its nearest PFEs at distances of 1, 2, and 3 nm from the EC will now be calculated. From (1),  $\sigma(x, 0)$  ( $=\sigma(x)$ ) at the interface between the cytosol and a point directly adjacent to the charge at a PFE is

$$\sigma(x) = \frac{q}{2\pi k_1 x^2} \left( \frac{\epsilon_1 - \epsilon_2}{\epsilon_1 + \epsilon_2} \right), \quad (2)$$

where  $\epsilon_1$  is the permittivity of layered cellular water at a PFE and  $\epsilon_2$  is the permittivity of the EC.

It is well established in electrochemistry [22] that the permittivity of the first few water layers outside a charged surface is an order of magnitude smaller than that of the bulk phase. The effective permittivity of water as a function of distance from a charged surface has been determined by atomic force microscopy [23] to increase monotonically from 4–6  $\epsilon_0$  at the interface to 78  $\epsilon_0$  at a distance of 25 nm from the interface. The values of the dielectric constants  $k_1(x)$  at distances of 1, 2, 3, and 4 nm from a charged surface were measured to be 9, 21, 40, and 60, respectively. The experiment was carried out with mica, which is known to have

a surface charge density that varies from 1 to 50 mC/m<sup>2</sup>, in the same range as biological surfaces [24, 25].

The value of the dielectric constant  $k_2$  for the EC has not been established. Consistent with its open structure, a cytosol-saturated EC would be expected to have a dielectric constant that is quite large, roughly midway between the “dry” value and cytoplasmic water [26]. From these considerations,  $k_2$  can conservatively be taken as 30. It is necessary that  $k_2$  be greater than the values of  $k_1$  for charged surface separations of 3 nm or less in order that the net induced charge be opposite in sign to the charge on the PFEs ( $k_1(x) < k_2$  in the above equations). Since the interpolated values of  $k_1(x)$  for separations of up to 3 nm between charged surfaces are less than the experimental values given above for one charged surface, this will be the case here. Calculation will show that PFEs at distances greater than 3 nm from an EC will be very weakly interacting due to the rapid decrease of the induced charge.

It is important to notice that the electrostatic force is repulsive at distances where  $k_1(x) > k_2$ ; however, as we will see, electrostatic interaction energies are small at such distances as a result of the rapid decrease of the induced charge with increasing distance. The onset of counterion screening beyond 3 nm would decrease the interaction energy even further to where it becomes negligible in comparison to thermal energy.

For an AMT with a closest PFE at a distance of 1 nm and a negative charge  $q$  of magnitude one electron charge at each PFE, the maximum positive charge density  $\sigma(x)$  induced on the EC from PFEs at  $x$  distances of 1, 2, and 3 nm is found from (2) to be 2.3, 0.2, and 0.02 mC/m<sup>2</sup> respectively, for a total induced charge density  $\sigma$  of 2.5 mC/m<sup>2</sup>. Similarly, for MTs with closest PFEs at distances of 2 or 3 nm, the total induced charge densities are 0.22 and 0.02 mC/m<sup>2</sup> respectively. An AMT with a closest PFE at 4 nm would induce an extremely small negative charge density, even without including Debye screening.

It is easily verified from (1) that the induced charge density  $\sigma(x, s)$  calculated for the most contributing  $x$  value of 1 nm falls off to 3 % of the maximum value at  $s = 0$  for an  $s$  distance of 3 nm and to 17 % of the maximum for an  $s$  distance of 3 nm if  $x = 2$  nm. Thus, since the radius of a protofilament (PF) is usually taken to be 2.5 nm, much of the image charge is induced over an area “in the shadow” of the cross sectional area of an approaching AMT for the most contributing  $x$  distances. From experimental observations of the spacing between AMTs, this implies that an AMT interacts primarily with the image charge that it induces locally on the EC, and induced charge from other MTs in the approaching AMT bundle is relatively small adjacent to that MT.

### III. ELECTROSTATIC FORCE AT THE CELL EQUATORIAL CORTEX

Microtubules invariably assemble or disassemble at their ends; that is, at some discontinuity in their structure. During anaphase, a subset of AMTs assemble to make persistent contact with the cell cortex. It has long been thought that furrow positioning is determined by some sort of AMT stimulation of the EC. In particular, the EC region of overlap for AMTs from both poles has been implicated in furrow stimulation [27, 28]. However, more recently it has been shown that furrowing and cytokinesis also occur frequently in cells with monopolar spindles

[29]. Interestingly, AMTs that have come into contact with the EC but have subsequently retracted due to dynamic instability were not observed to correlate with furrow ingression [29]. In a recent study of whether or not the stability of AMTs correlates with furrow ingression it was found that the dynamic state of AMTs was not implicated in furrow initiation but persistent contact of the AMTs with the EC was always required [30]. As will be shown below, within the context

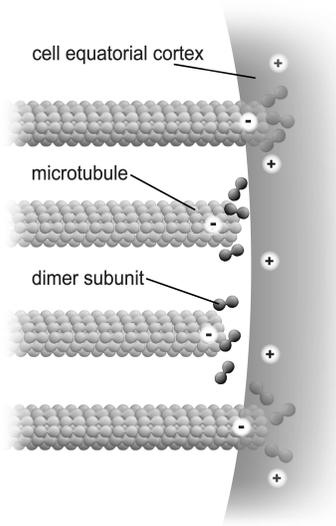


Fig. 1. Nanoscale electrostatic force at a small section of the equatorial cortex. Persistent contact of AMTs with the EC results from an electrostatic attraction between negatively charged AMT free ends and an induced positive charge on the EC

of the present model, induced positive charge on the EC could anchor negatively charged ends of AMTs that approach within 3 nm of the EC to the EC, establishing the persistent contact experimentally determined to be necessary for furrow initiation [30] as depicted in Fig. 1. The range of values of the charge at a PFE will now be determined by comparing the calculated maximum tension force per MT with the experimental range of the maximum tension force per MT for poleward chromosome motions. Integrating (1) to obtain the force between the charge  $q$  on a PFE and the induced image charge at the EC one finds

$$F = \frac{1}{4\pi\epsilon_1} \frac{(\epsilon_1 - \epsilon_2)}{(\epsilon_1 + \epsilon_2)} \frac{q^2}{4x^2}. \quad (3)$$

From the geometry of MTs outlined in the introduction, an AMT with a closest PFE to the EC at a distance of 1 nm could also have PFES at the next closest distances of 2 and 3 nm. A reasonable estimate of the maximum force exerted by the EC on a MT may be obtained from (3) by assuming an AMT with PFES at the closest distances of 1, 2, and 3 nm, and summing the contributions over  $x$  and the experimental values of  $k_1(x)$ . The result is

$$F = 6n^2 \text{ (pN/MT)}, \quad (4)$$

where  $q = ne$ , with  $e$  equal to the charge on an electron and  $n$  the number of electron charges at a PFE. Comparing this with the experimental range 1 – 74 pN/MT for the maximum tension force exerted by a MT [31], we have that  $n = 0.4 - 3.5$  electron charges, well within the experimental range [3, 5, 6, 8].

#### IV. ELECTROSTATIC DETERMINATION OF THE CELL DIVISION PLANE

Within the context of the present model, force generation at the EC for determination of the division plane and furrow initiation for cytokinesis can be attributed to a persistent contact of the EC with charged free ends of AMTs that are anchored at separating poles. As anaphase-B proceeds, the separation of the poles causes the AMTs to pull the EC inward defining the plane of division and initiating furrow formation as depicted in Fig. 2.

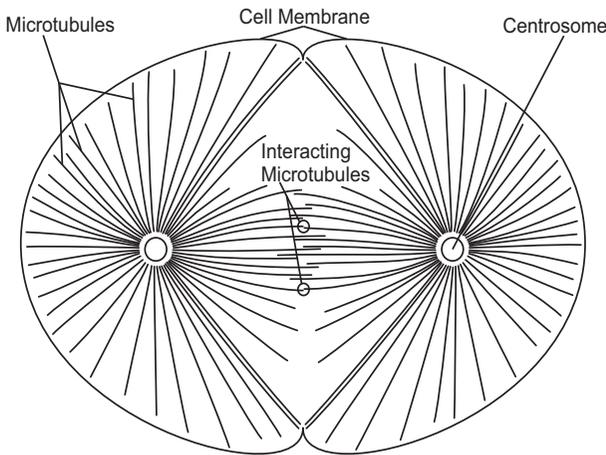


Fig. 2. Electrostatically interacting polar microtubules in the central spindle furnish the force for cell elongation. Astral microtubules in persistent contact with the cell membrane at the cell equatorial cortex determine the division plane and initiate furrow formation

A number of experiments have shown that a free calcium release occurs at the onset of anaphase-A [32, 33, 34]. In addition to destabilizing kinetochore microtubules for anaphase-A motion [35], the free calcium release could also destabilize the free plus ends of polar microtubules (PMTs), causing them to favor depolymerization. Depending on the cell type, it would take some time for the free ends of significant numbers of interacting central spindle PMTs emanating from opposite poles to stochastically depolymerize to within nanometer distances between their respective like-charged free ends (Fig. 2). Therefore, anaphase-B motion can be attributed to an electrostatic repulsion between adjacent negatively charged free ("plus") ends of constantly changing subsets of central spindle PMTs from opposite poles disassembling from a geometry of previously overlapping PMTs [11]. Thus stochastic assembly and disassembly of PMTs – with plus end disassembly favored and minus end assembly at poles favored [36] – would generate a relatively steady repulsive force between the like-charged free ends of PMTs in the mitotic half-spindles. This will result in a steadily decreasing amount of PMT

overlap and anaphase-B pole separation. Anaphase-B motion will cease when sufficient numbers of PMTs in the polar bundles have shortened to the extent that electrostatic interaction distances between their plus ends are too great. The time delay for the onset as well as the duration of anaphase-B motion will then be expected to vary between cell types, as observed experimentally.

## V. DISCUSSION

The model presented in this work encompasses the dynamics and timing of events determining furrow location and initiation. It is proposed that anaphase-B pole separation in combination with persistent contact of astral microtubules (AMTs) with the equatorial cell cortex (EC) can define the division plane of the cell and initiate furrowing. Once furrowing has begun, surface tension force could also play a significant role in completing cell division. The contribution to cytokinesis from the combination of surface tension and anaphase-B pole separation is described elsewhere [37]. It is shown there that the tendency for an elongating cell to separate into two daughter cells scales as the inward directed surface pressure  $2\gamma/R$ , where  $\gamma$  is the cell membrane surface tension and  $R$  is the radius of the cell.

Surface tension considerations for water droplets elongating and separating under gravity at a faucet are appropriate for a cell elongating under anaphase-B cell elongation. Substituting typical experimental values of  $\gamma$  and  $R$  for water or dividing cells into the above equation reveals that the tendency for cell membranes to close around incipient daughter cells is an order of magnitude greater than that for water droplets to pinch off into two droplets, and would have been two orders of magnitude greater for primitive cells with diameters of approximately one-tenth that of a typical modern eukaryotic cell [37].

It is significant that there is also a possible positive feedback mechanism in the surface tension contribution [38, 39, 40]. The inward surface pressure  $2\gamma/R$  increases as  $R$  decreases in the process of forming the incipient daughter cells.

Structural continuity in the polar region of a dividing cell is also an important consideration. The density of AMTs in the polar region, in combination with endoplasmic reticulum forming there during anaphase [41, 42], serves to increase the structural strength of the polar region over that of the equatorial region.

The operation of *contractile rings* is still unknown. Unlike an actin-myosin "purse-string" mechanism, the ring maintains the same cross-sectional thickness as the ring circumference decreases [43]. In addition, the arrangement of actin filaments changes extensively during cytokinesis. This behavior suggests that the ring is being manipulated rather than contracting as in skeletal muscle and that it might simply serve as a structure to strengthen the EC for the exertion of force by AMTs during furrowing.

Thus, in addition to the persistent EC contact with AMTs anchored to separating poles for the initiation of furrowing proposed here, a combination of (1) cell membrane surface tension, (2) polar structural strength, and (3) contractile ring EC structural integrity with persistent AMT contact, each in varying amounts in different cell types, could be responsible for the events of cytokinesis.

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