

**Electrostatic force in prometaphase, metaphase, and anaphase-A chromosome motions**

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(Received 11 December 2001; published 12 July 2002)

Primitive cells had to divide using very few biological mechanisms. This work proposes physicochemical mechanisms, based upon nanoscale electrostatics, which explain and unify the motions of chromosomes during prometaphase, metaphase, and anaphase A. In the cytoplasmic medium that exists in biological cells, electrostatic fields are subject to strong attenuation by ionic screening, and therefore decrease rapidly over a distance equal to several Debye lengths. However, the presence of microtubules within cells completely changes the situation. Microtubule dimer subunits are electric dipolar structures, and can act as intermediaries that extend the reach of the electrostatic interaction over cellular distances. Experimental studies have shown that intracellular pH rises to a peak at mitosis, then decreases through cytokinesis. This result, in conjunction with the electric dipole nature of microtubule subunits, is sufficient to explain the dynamics of the above mitotic motions, including their timing and sequencing. The physicochemical mechanisms utilized by primitive eukaryotic cells could provide important clues regarding our understanding of cell division in modern eukaryotic cells.

DOI: 10.1103/PhysRevE.66.011901

PACS number(s): 87.17.-d, 87.10.+e

**I. INTRODUCTION**

The electromagnetic interaction is primarily responsible for the structure of matter from atoms to objects. Much of physics, all of chemistry, and most of biology are in this size realm. Primitive eukaryotic cells had to divide prior to the evolution of 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 the electrostatic force, a component of the electromagnetic interaction, played a major role in the dynamics of chromosomes during cell division 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.

The mitotic spindle is responsible for the segregation of sister chromatids during cell division. Chromosomes are attached to the spindle with their kinetochores [1] attached to the “plus” ends of polar microtubules [2,3]. Chromosome movement is dependent on kinetochore-microtubule dynamics: a chromosome can move towards a pole only when its kinetochore is connected to microtubules emanating from that pole [4]. Several methodological approaches have been undertaken to obtain information regarding microtubule dynamics, force production, and kinetochore function in mitotic cells. These experiments have revealed that the spindle can produce more force than is actually required to move a chromosome at the observed speeds during anaphase, and that the force for the poleward motion of chromosomes during anaphase A is primarily localized at or near the kinetochore [5–11]. Quite some time ago, Cooper addressed a possible link between endogenous electrostatic fields and the eukaryotic cell cycle [12]. An early review by Jaffe and Nuccitelli [13] focused on the possible influence of relatively steady electric fields on the control of growth and development in cells and tissues.

In the cytoplasmic medium that exists in biological cells, electrostatic fields are subject to strong attenuation by

screening with oppositely charged ions, and decrease rapidly over a distance of several Debye lengths. In most cells the Debye length is typically 1 nm [14], and since cells of interest in the present work (i.e., eukaryotic) can be taken to have dimensions between 10–30  $\mu$ m, one would be tempted to conclude that electrostatic force could not be a major factor in providing the cause for motion within cells. However, the presence of microtubules within cells changes the picture completely. It is proposed here that microtubules 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.

A number of investigations have been related to the electrostatic properties of microtubule dimer subunits [15–18]. The latest studies [19,20] have shown that the net charge depends strongly on pH, with a value of +6 at pH 4.5, varying quite linearly from –12 to –28 between pH 5.5 and 8.0. The dipole moment has just recently been calculated to be between 1200 and 1800 debye [19].

The aster’s pincushionlike appearance is consistent with electrostatics, since electric dipolar 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 quite probable that the pericentriolar material-centriole complex, the centrosome about which the microtubule dimer dipolar subunits assemble to form the aster, carries a net charge. This is consistent with ultramicroscopic observations that the microtubules appear to start in the pericentriolar material region [21], aligning radially outward, with no continuity or connection to the centrioles or anything else.

Since there is no experimental information regarding the sign of this charge, it will be assumed negative. This assumption is made because the free outer ends of the aster’s microtubules (the pinheads in the pincushion analogy) must be negatively charged since they are not attracted to the negatively charged outer surface of the nuclear envelope. If this were not the case, the asters would be unable to move freely

along the periphery of the nuclear envelope in their migration to the poles of the cell. A manifestation of negative charge on centrosomes, due to the higher  $\text{pH}_i$  that exists during prophase [22], could potentiate the nucleation of microtubules at centrosomes to form the asters during this phase of mitosis, in agreement with observation. Experiments [23] have shown 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.

Studies [24] have shown that *in vivo* microtubule assembly (polymerization) is favored by higher  $\text{pH}_i$  values. It should be noted that *in vitro* studies of the role of pH in regulating microtubule assembly indicate a pH optimum for microtubule assembly in the range of 6.3-6.4. The disagreement between *in vitro* and *in vivo* studies regarding microtubule polymerization has been analyzed in relation to the nucleation potential of microtubule organizing centers (MTOCs) [24], and it has been suggested that  $\text{pH}_i$  regulates the nucleation potential of MTOCs [25-27]. This favors the more complex physiology characteristic of *in vivo* studies to resolve this question. It will therefore be assumed in this paper that *in vivo* experimental design is more appropriate for experiments relating to conditions affecting microtubule assembly. As mentioned above, within the context of the present model, increased nucleation follows from the manifestation of negative charge on MTOCs in a higher  $\text{pH}_i$ .

It is reasonable to conclude that the electric dipole nature of dimer subunits greatly assists in their self-assembly into microtubules. In particular, their electric dipolar nature would allow them (over the short distances consistent with Debye shielding) to be attracted to, and align around, any net charge distribution within cells. This may account for the self-assembly of the asters [28] during prophase, when microtubule polymerization and MTOC nucleation is favored because of the higher intracellular pH at this time.

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 breakdown. In general, the rate at which microtubules undergo net assembly, or disassembly, varies with mitotic stage; for example, during prophase the rates of microtubule polymerization and depolymerization change quite dramatically [29].

Thus, we may envision that electrostatic fields organize and align the electric dipole dimer subunits, thereby facilitating their assembly into the microtubules that form the aster. The attraction between oppositely charged ends of the dipolar subunits takes place over the short distances allowed by Debye shielding. An electrostatic component to the biochemistry of the microtubules in the assembling asters is consistent with experimental observations of pH effects on microtubule assembly, as well as the sensitivity of microtubule stability to calcium ion concentrations [30,31]. In addition, the mutual electrostatic repulsion of the negatively charged free ends of microtubules in the assembling asters could provide the driving force for their poleward migration [28]. Ac-

ording to existing convention, these negatively charged microtubule ends are designated "plus" ends because of their more rapid growth, there being no reference to charge in the use of this nomenclature.

## II. ANAPHASE-A CHROMOSOME MOTION

Chromosome motion during anaphase has two components, designated anaphase *A* and anaphase *B*. Anaphase *A* is concerned with the poleward motion of chromosomes, accompanied by the shortening of the microtubules attached to the kinetochores. The second component, referred to as anaphase *B*, involves the separation of the poles. Both components contribute to the increased separation of the chromosomes during mitosis. An electrostatic force mechanism for anaphase-*B* motion in primitive eukaryotic cells within the context of the present work is given elsewhere [28].

Experiments have shown that the intracellular pH of many cells rises to a maximum at the onset of mitosis, subsequently falling during the later stages of cell division [22,32]. Although it is experimentally difficult to resolve the exact starting time for the beginning of the decrease in  $\text{pH}_i$  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 mitosis [22]. With a decrease in  $\text{pH}_i$  through metaphase, the resulting manifestation of positive charge on kinetochores, coupled with their very close proximity and the inverse square nature of the Coulomb electrostatic interaction, could supply sufficient force to effect their initial separation. As of this writing, there is no consensus on a model explaining the initial separation of chromosomes. Thus, both the mechanism and the timing of this separation would appear to be a natural deduction within the framework of the model presented here. This separation heralds the beginning of anaphase *A*.

As mentioned above, intracellular pH ( $\text{pH}_i$ ) is further decreasing at this point in the cell cycle. Another aspect of this reduced pH is seen in the effect that it has on the stability of the microtubules comprising the spindle fibers. Previously, we noted that *in vivo* experiments have shown that microtubule polymerization is related to  $\text{pH}_i$ , with a more basic pH favoring a net assembly (lengthening) of microtubules. The rate at which spindle microtubules assemble and disassemble varies with mitotic phase. A lower  $\text{pH}_i$  during anaphase *A* is consistent with microtubules both assembling and disassembling (shortening), with net disassembly favored.

Experimental studies have revealed that the anaphase-*A* poleward motion of kinetochores, with their attached chromosomes, proceeds by kinetochore microtubule disassembly primarily in the vicinity of kinetochores [5,7], and that approximately 20% of the total disassembly is observed to take place at poles [33]. Disassembly at the poles has also been observed in metaphase cells [33]. Based on experiments centering on observations near kinetochores, it has been proposed that the force to move chromosomes is generated at kinetochores [34].

These observations, including the force at the poles as well as the force at kinetochores, are explained in the context of the present model as follows. Microtubules invariably as-

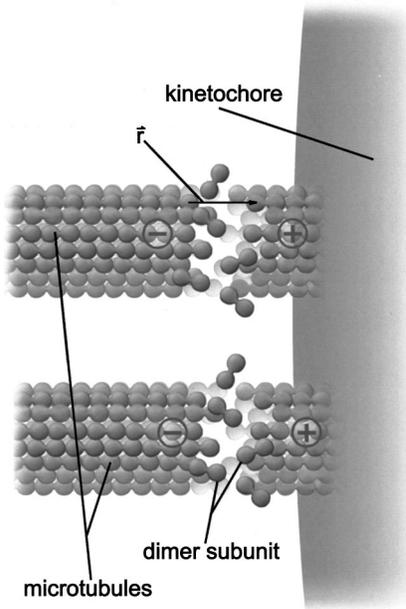


FIG. 1. A small section of a kinetochore during anaphase A. The electrostatic force geometry is depicted for two kinetochore microtubules.

semble or disassemble at their ends; that is, at some discontinuity in their structure. Furthermore, they are known to be in a constant condition of dynamic instability at the balanced state [35]. According to the aster self-assembly model described above, the charge on the ends of the microtubules at a centrosome is positive. Once disassembly commences, the resulting exposed ends of the microtubule stubs that remain attached to the kinetochores will be positively charged, with their negative ends still attached at the kinetochores.

As indicated earlier, experimental studies have shown that disassembly of microtubules at kinetochores accompanies chromosome poleward movement. The motive force for this poleward anaphase-A motion can be attributed to an electrostatic attraction between the positive ends of microtubule stubs and negative ends of the remaining intact kinetochore microtubules. Since a dimer dipolar subunit that has just been lost between these charges is 8 nm in length, the initial electrostatic attraction between these charges will occur over a distance of 8 nm, with the attraction between the two nearest neighbor dimers (one protofilament removed on each side) and the dimer in question in the middle protofilament taking place at distances of 4 and 5.5 nm.

Given that the mitotic spindle consists of a bundle of microtubules, as the microtubules disassemble, a continuously acting force will be provided. The electrostatic disassembly force at a kinetochore is depicted schematically (Fig. 1). The smaller rate of net microtubule disassembly at the poles would also result in force generation by an essentially identical process. Since the process at the poles is fundamentally the same, we will focus on the details of the force producing mechanism at kinetochores.

We now calculate the magnitude of the maximum force produced in this manner by a single microtubule. From the well-known Debye-Hückel result [36] for a spherical charge

distribution of radius  $a$ , we have for the electrostatic potential  $\phi(r)$ ,

$$\phi(r) = \frac{qe^{-(r-a)/D}}{4\pi\epsilon r(1+a/D)}, \quad (1)$$

where  $q$  is the charge magnitude,  $a$  is its radius,  $D$  is the Debye length, and  $r$  is the magnitude of the position vector  $\mathbf{r}$ . To a good approximation, the charged hemispherical caps at the ends of the dimers can be considered spherical, since convex, essentially hemispherical charge distributions are facing each other for the interacting dimers. In addition, Debye shielding increasingly attenuates interactions between virtually all but “nearest neighbor” charge distributions at the dimer ends. For a value of 71 for the cytoplasmic dielectric constant, its permittivity  $\epsilon$  will be taken to be  $71\epsilon_0$ , where  $\epsilon_0$  is the permittivity of free space. The room temperature permittivity value for water is  $80\epsilon_0$ ; the value of  $71\epsilon_0$  incorporates corrections for the temperature and ionic depression of the dielectric constant [37] appropriate to the cytoplasm of mammalian cells.

As indicated earlier, based on the latest calculations, tubulin has a dipole moment between 1200 and 1800 debye. A calculation of the force per microtubule can be carried out based on a charge magnitude  $q$  of six electron charges, consistent with the midrange value of the dipole moment. The computer simulation discussed below reveals that anaphase-A motion is maintained at the experimentally observed velocity over a wide range of values for the charge  $q$ , including values smaller than that used in this calculation.

The electric field  $E(r)$  obtained from the  $r$  component of the negative gradient of the electrostatic potential, multiplied by the magnitude of the charge  $q$  on the end of a dipolar subunit attached to the kinetochore, will give the magnitude of the attractive force  $F(r)$  between the charges of the dipole subunits on the kinetochore and microtubule, respectively. Multiplying the negative gradient of  $\phi(r)$  by the charge  $q$ , we have

$$F(r) = \frac{q^2 e^{-(r-a)/D}}{4\pi\epsilon} \left[ \frac{1}{r^2} + \frac{1}{rD} \right]. \quad (2)$$

For a Debye length of 1 nm, nearest neighbor distances  $r$  of 4 and 5.5 nm, and  $a=2$  nm, we find that the electrostatic disassembly force between the two pairs of tubulin dimer subunits at the nearest neighbor distances in three adjacent protofilaments sums to 5.9 pN. Since there are 13 protofilaments arranged circularly in a microtubule cross section, the computed magnitude of the maximum force per microtubule is approximately 24 pN. This value compares quite favorably to the experimentally measured maximum force per microtubule range of (1–74) pN [10], and represents the only successful *ab initio* theoretical derivation of the magnitude of the force; however, this model calculation is primarily intended to demonstrate that electrostatic interactions are able to produce a force per microtubule within the experimental range. There will be a low probability for microtubule reassembly since the kinetochore and attached microtubule stub

will be moving into the region previously occupied by (now disassembled) dimer subunits, preventing any possible subsequent reassembly.

A computer simulation for anaphase-*A* motion incorporating more completely the geometry of microtubules, various models of microtubule disassembly, and the numerical integration of Newton's second law using the above force function along with typical values of chromosome mass [38] and cytosol viscosity [10], shows that electrostatic force is robust enough to sustain chromosome motion under a variety of simulated conditions. In particular, the simulation shows that the experimentally observed anaphase-*A* chromosome speeds of a few micrometers per minute are determined almost exclusively by the disassembly rate of microtubules over a wide range of disassembly modes and charge values. This software may be downloaded from <http://anaphaseA.tripod.com>

As the distances between the dimers on the interacting microtubules decreases to around 2 nm (see discussion on entropic assembly forces below), short range entropic disassembly force could provide an important contribution to the poleward directed force; however, the geometry precludes a calculation of the magnitude of this contribution, and it would be difficult to experimentally distinguish the entropic disassembly force from electrostatic force. Experimental results for forces associated with growing microtubules are discussed briefly in the section on prometaphase and metaphase chromosome motions.

As discussed above, the lower  $\text{pH}_i$  at this point in the cell cycle is consistent with net microtubule disassembly. In addition, the intracellular pH in the vicinity of the exposed negatively charged microtubule free ends in the kinetochore region will be even lower than the overall  $\text{pH}_i$ , because of the effect of the negative charge at the 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 factor  $e^{-e\zeta/kT}$ , where  $e$  is the electronic charge,  $\zeta$  is the (negative) potential at the surface, and  $k$  is Boltzmann's constant [39]. 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. Therefore, because of the negative charge at the ends of the microtubule dimer subunits in the kinetochore region, a further reduction of  $\text{pH}_i$  would be expected in the immediate vicinity of these free ends. This additional pH reduction would further increase the tendency for net microtubule disassembly.

In contrast, the positively charged free ends of microtubules in the polar region will have a decreased net disassembly rate (as compared to those near kinetochores) because of the increase in local pH over  $\text{pH}_i$  at these ends. This is consistent with the experimental result mentioned earlier that only 20% of the microtubule disassembly during anaphase *A* takes place at poles. Thus, the observed difference in the

disassembly rates between microtubule ends at kinetochores and those at poles follows quite naturally within the present model.

As a result of the decreased intracellular pH during anaphase, the increased positive charge on kinetochores would cause them to be attracted to the negatively charged free ends of microtubules, contributing to the poleward anaphase-*A* force. However, it is not possible to quantify this force since the magnitude of the proposed kinetochore charge is not known.

According to leading molecular motor models of anaphase-*A* motion, the experimentally observed shortening of spindle fibers at a kinetochore is believed to be accompanied by molecular motors that are associated with the kinetochore, and are thought to provide the motive force to move the kinetochore-chromosome assembly. However, there is as yet no consensus on a model that can describe how a molecular motor associated with a kinetochore can be operating while microtubules are disassembling at that kinetochore.

Thus, it is not clear within the context of a molecular motor model why the velocity of the poleward motion during anaphase *A* should be governed by the relatively slow (compared to known molecular motor behavior [40]) shortening rate of microtubules. As indicated above, proponents of these models assume that microtubule disassembly is the rate determining step for the motion, necessitating additional assumptions and models within the framework of the molecular motor models to account for the well-documented chromosome velocities during anaphase *A* and most of prometaphase. No such additional assumptions are needed in the model proposed here.

Anaphase-*B* cell elongation also proceeds at speeds compatible with microtubule disassembly/assembly, necessitating additional assumptions in the leading molecular motor models for anaphase *B*. Anaphase-*B* elongation chromosome speeds follow directly from electrostatic interactions consistent with the model presented in this paper [28]. The various molecular motor models are advanced to explain only one type of mitotic motion (e.g., anaphase-*A* motion), and do not attempt to relate to the other mitotic motions. In addition, there is no attempt to address the timing of anaphase *A* in any of the current models.

It is significant that anaphase *A* has been observed to proceed in isolated spindles in the absence of ATP if conditions in the experimental system are set up to promote microtubule disassembly [41]. These results are difficult to explain within a molecular motor model, but are completely consistent with the present model. In a key experimental study with grasshopper spermatocytes [42], it was found that both anaphase *A* and anaphase *B*, as well as cytokinesis, proceeded independently of chromosomes. The authors of this study concluded that chromosomes, when present, might migrate to the poles by having their kinetochores latch onto the ends of shortening microtubules, a scenario that is completely in accord with the present work. There does not appear to be much discussion in the literature or any consensus on a molecular motor model for the generation of force at the cell poles. Experimental observations regarding the microtubule disassembly force at poles, including the 20% contribution to

microtubule disassembly, are explained consistently within the model presented in this paper by the same electrostatic force mechanism as that operating at kinetochores.

### III. PROMETAPHASE AND METAPHASE MOTIONS

In present terminology *metaphase* usually denotes the relatively brief period during which chromosomes are lined up at the center of the cell and are fully attached to both poles by the microtubules of the spindle. The term *prometaphase* is used to encompass a much wider time period during which most of the complex motions in this stage of mitosis occur.

Two events that are of major significance during prometaphase are (1) the *capture* and *attachment* of chromatid pairs by microtubules, and (2) chromosome movement to, and alignment at, the cell equator. The latter is comprised of several distinguishable motions. Regarding the first event, experiments [43] have shown that each pair of sister chromatids attaches by a kinetochore to the outside walls of a single microtubule, resulting in a rapid microtubule sidewall sliding movement toward a pole. This motion is postulated to be driven by dynein-based molecular motors, since dynein has been found at kinetochores. A molecular motor powered sliding model for this prometaphase movement would appear to be most widely accepted for this motion. In particular, the speed (20-50  $\mu\text{m min}^{-1}$ ) [44] of the kinetochores along the microtubule is consistent with known molecular motor behavior. Consequently, I agree that a molecular motor model for the microtubule sidewall capture motion is supported by the experimental observations. However, I propose that all of the subsequent prometaphase and metaphase motions are based on nanoscale electrostatic force mechanisms.

As indicated earlier, the material of kinetochores is proteinaceous, and could manifest a net positive charge at the lower  $\text{pH}_i$  levels during prometaphase. As a result of the sliding capture motion described above, the approach to the poles will result in the movement of a kinetochore to within several Debye lengths of the ends of other microtubules emanating from the closer pole. The resulting proximity, in conjunction with an electrostatic attraction between positively charged kinetochores and the negatively charged ends of these microtubules, coupled with an electrostatic repulsion between negatively charged chromosomes in the chromatid pair and other microtubule ends, could be a critical step in the orientation and attachment of kinetochores to the free ends of microtubules.

Following this *monovalent* attachment to one pole, chromosomes are observed to move at considerably slower speeds, a few micrometers per minute, in subsequent motions throughout prometaphase [44]. 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 other kinetochore in the chromatid pair results in an attachment to both poles (a *bivalent* attachment). 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 mi-

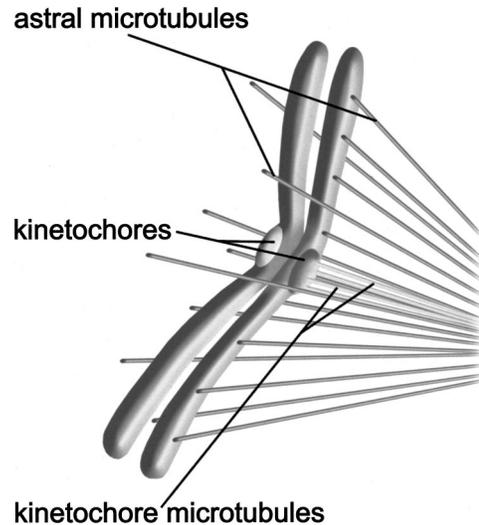


FIG. 2. Electrostatic interactions between microtubules and a chromatid pair during prometaphase.

crotochules from the opposite pole, the chromosomes perform a slow (approximately 2  $\mu\text{m per minute}$ ) but sustained *congressional* motion to the spindle equator, resulting in the well-known metaphase alignment of chromatid pairs. In addition to the mechanism facilitating attachment just discussed, all of the above mentioned experimentally observed postattachment prometaphase motions, as well as the oscillatory metaphase motion, can be understood in terms of electrostatic interactions within the model as follows.

Since chromosomes are negatively charged, following attachment they will be repelled from the negatively charged free ends of the shorter *astral* microtubules in the polar region (Fig. 2). As discussed above, this force will be effective for the short distances allowed by Debye screening. As chromosomes move farther from the poles, there will be a filling-in of dipolar subunits as the microtubules assemble. Polymerization will take place in the gaps as chromosomes drift farther from the poles, and chromosomes will be continuously repelled from the poles. This mechanism may account for the “astral exclusion force,” or “polar wind,” the nature of which has remained unknown since it was first observed [45].

Very short range entropic forces associated with growing microtubules [46] will complement the electrostatic repulsive interaction at small microtubule-chromosome separations, adding to the total astral exclusion force. Although the complex geometry precludes a theoretical calculation of the magnitude of these forces, a model calculation of the repulsive force between two like charged parallel surfaces with an electrolyte in between shows that entropic forces must be included for separations of less than 2 nm; at greater separations electrostatic theory fits the data well [47,48].

The possibility that microtubule polymerization or depolymerization can occur, in combination with this repulsive electrostatic astral exclusion force and the attractive electrostatic poleward directed forces acting at kinetochores (described above in conjunction with anaphase-A motion) is sufficient to explain the observed motion of monovalently

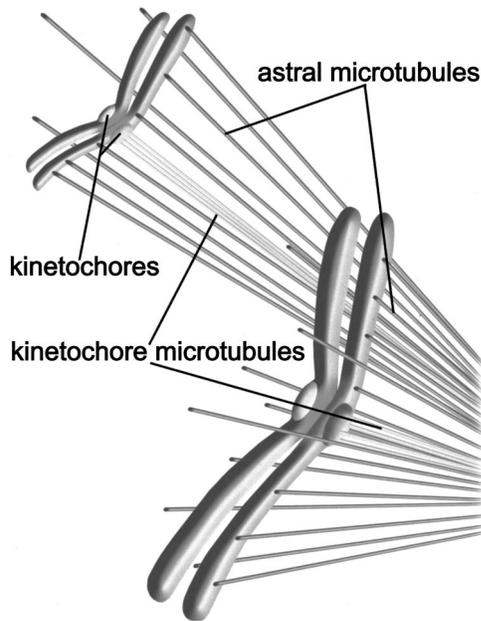


FIG. 3. Two chromatid pairs at differing polar distances depicting the inverse square dependence of the astral exclusion force.

attached chromosomes toward and away from poles. Due to statistical fluctuations in both the number of microtubules interacting with kinetochores and in the number of assembling microtubules responsible for the polar wind, the interaction of these opposing forces could result in a “tug of war,” consistent with the experimentally observed series of movements toward and away from a pole for a monovalently attached chromatid pair.

As the chromatid pair moves farther from a pole, the electrostatic repulsive force between the negatively charged free ends of astral microtubules and chromosomes will decrease as the microtubules fan radially outward (Fig. 3). The charge density at a surface defined by the microtubule ends, and therefore the force, will decrease according to an “inverse square” law as we can see from the following. Given that the repulsive force on a chromosome depends on the total number  $N$  of negatively charged microtubule ends from which it is repelled, we have  $F \sim Nq$ , where  $q$  is the charge at the end of a microtubule. For  $N$  microtubules fanning radially outward from a pole, the total charge  $Nq$  is distributed over an area that increases as  $r^2$ , and  $\sigma$ , the effective charge per unit area at a surface defined by the microtubule ends, decreases as  $r^{-2}$ , resulting in an electrostatic repulsive inverse square law for the astral exclusion force.

After a bivalent attachment has been established, the attractive force to the far pole will be in opposition to the attractive force to the near pole. The inverse square nature of the repulsive astral exclusion force, along with the relatively few initial attachments of kinetochore microtubules to the near pole and at least one attachment to the far pole, would result in a slow but sustained (congressional) motion away from the near pole, as observed. As a chromatid pair moves farther from the nearer pole, there will be a growing number of attachments to both poles. Following additional attachments to both poles, and comparable distances of the chro-

matid pairs from the two poles, the forces exerted by both sets of polar attractive and repulsive forces will tend to equalize. Thus, as a chromatid pair congresses to the midcell region, the number of attachments to both poles will tend to be the same, and equilibrium of poleward directed forces and astral exclusion forces will be approached. Without specifying their nature, such balanced pairs of attractive and repulsive forces have previously been postulated for the metaphase alignment of chromatid pairs [49].

An explanation of the experimentally observed metaphase oscillations about the cell equator just prior to anaphase A provides an additional example of the predictability and minimal assumptions nature of the present model. In agreement with experiment [50], the model predicts that the poleward force at a kinetochore depends on the total number of microtubules interacting with kinetochores. At the metaphase “plate,” the bivalent attachment of chromatid pairs ensures that the poleward directed electrostatic disassembly force at one kinetochore at a given moment could be greater than that at the sister chromatid’s kinetochore (attached to the opposite pole). An imbalance of these forces would result from statistical fluctuations in the number of interacting microtubules at sister kinetochores and at poles. This situation, coupled with similar fluctuations in the number of microtubules responsible for the astral exclusion force, can result in a momentary motion toward a pole in the direction of the instantaneous net electrostatic force. However, because of the inverse square dependence of astral exclusion forces, electrostatic repulsion from the slightly nearer pole will halt further excursion toward this pole, resulting in stable equilibrium midcell metaphase oscillations, as observed experimentally.

In agreement with experiment [51], the model presented in this paper satisfies the requirement that the maximum force per microtubule be the same for all postattachment prometaphase, metaphase, and anaphase-A kinetochore-microtubule interactions.

#### IV. CONCLUSIONS

The nanoscale electrostatic force model presented in this paper encompasses the dynamics, timing, and sequencing of prometaphase, metaphase, and anaphase-A chromosome motions. Electrostatic force could also be integral in the assembly of the aster, the dynamics of prometaphase (kinetochore-microtubule end-on) attachment, and the initial anaphase-A separation of sister chromatids.

Entropic forces complement the electrostatic interactions by providing an additional attractive force for disassembling microtubules, as well as an additional repulsive force for assembling microtubules. Experimental and theoretical considerations for entropic assembly forces indicate that electrostatic force dominates at separations greater than 2 nm. Thermal fluctuation force would also add to the total force; however, the magnitude of this contribution is not expected to exceed 2% of the median measured force per microtubule.

This model also addresses the origin of the force on kinetochore microtubules exerted at poles, as well as the difference in microtubule disassembly rates at poles and kinetochores. The force exerted on microtubules at poles emerges

as the same nanoscale electrostatic microtubule disassembly force as that which acts at kinetochores in anaphase-A, prometaphase, and metaphase chromosome motions.

I agree that molecular motors are probably involved in the sliding microtubule side-wall capture motion of chromatid pairs, and submit that kinetochore dynein may be present for this purpose, but not for the other motions of prometaphase, metaphase, and anaphase A.

The prometaphase astral exclusion force and the dynamics of monovalently and bivalently attached chromosome prometaphase motions are consistently addressed without introducing any additional assumptions or mechanisms.

All experimentally observed postattachment chromosome velocities proceed at the relatively slow rate of a few micrometers per minute, the speed at which microtubules with attached chromosomes lengthen or shorten. These speeds are a direct consequence of the model. By contrast, in molecular motor models for these motions, the speeds would be one to two orders of magnitude greater. Molecular motor models must necessarily invoke microtubule disassembly (or assembly, for some anaphase-B models) to explain the chromosome velocities, but there is as yet no clear mechanism by which a molecular motor associated with kinetochores could be operating at the same time that microtubules are disas-

sembling at that kinetochore. Molecular motor models therefore require additional assumptions and embedded models to account for postattachment chromosome velocities.

The calculated force per microtubule falls within the experimentally measured range, and represents the only successful derivation of the magnitude of this force. As predicted by the model, experimental studies have revealed that this value falls within the range measured for all of the post-attachment chromosome movements of mitosis investigated thus far.

Stable equilibrium metaphase oscillations of chromatid pairs at the metaphase plate just prior to anaphase A are shown to be a logical consequence of the proposed nanoscale electrostatic force mechanisms.

Finally, based on current separate molecular motor models for prometaphase, metaphase and anaphase A, there does not seem to be any possibility to relate their timing and sequencing, a situation that has been remedied by the comprehensive model proposed in this paper.

#### ACKNOWLEDGMENT

The author wishes to acknowledge Patrick Michael West, Jr., for developing the Anaphase-A simulation software.

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