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MICROTUBULES AS ACTIVE TRACKS FOR BI-DIRECTIONAL CELLULAR TRAFFIC OF MOTOR PROTEINS

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The basic cytoskeletal transport in cells is achieved by two oppositely directed processive motor proteins, kinesin and dynein, walking along microtubules. Here, we offer a new view of the mechanism of the transport direction regulation by the intrinsic cell's electric fields that interact with kinks elicited in microtubules.

Keywords: Microtubule; GTP; ATP; kinesin; dynein; kink; ferroelectric; intrinsic cellular electric fields.

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1. Introduction

A living cell possesses a high degree of spatial and temporal order, which is maintained in a state far from equilibrium. This order is mostly sustained by molecular motors that consume the "fuel" molecules such as ATP (adenosin threephosphate) and perform mechanical work within the cell.

Among several classes of motors that carry out different functions in the cell, we pay attention here to cytoskeletal motors which are able to bind to the microtubule filaments and then walk along these filaments in a directed fashion. These classes of motors (kinesin and dynein), (Fig. 1) are responsible for the directed transport of vesicles and other types of cargos across the cell.[1] They are always present at the same cargo. When one motor is active, the other one is turned off. The mechanism for switching between the activities of the two classes of motors is still widely unknown, so that our model offers a plausible scenario in which the cell's intrinsic fields play an essential role.

Microtubules (MTs) are one of the three known types of filaments comprising the cytoskeleton, and they have important roles in many cellular processes such as intracellular transport, cell motility, meiosis and mitosis. In the microtubule, $\alpha\beta$-tubulin heterodimers bind the head to the tail, making protofilaments, and

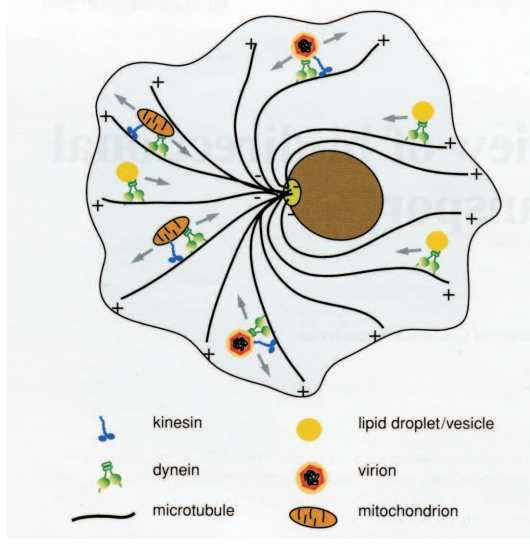


Fig. 1. A diagram of a cell, showing the radial organization of the microtubule cytosceleton, and the related motor proteins kinesin and dynein moving cargos in opposite directions.

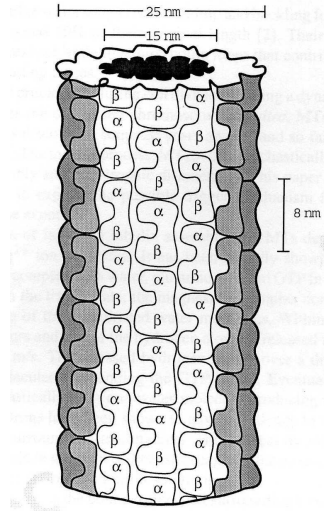
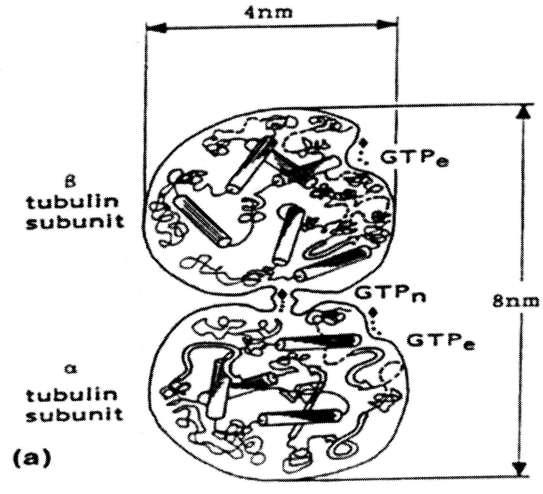
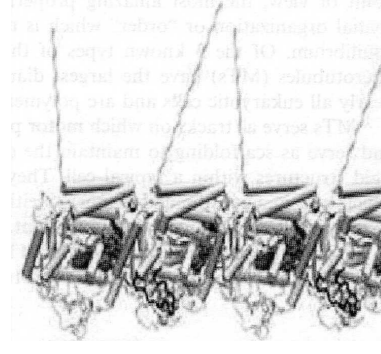


Fig. 2. A diagram of a microtubule holow cylinder indicating the characteristic dimensions.

13 protofilaments associate in parallel, giving rise to a polar cylindrical polymer, (Fig. 2).

The building block of a MT, $\alpha\beta$-tubulin heterodimer, consists of 2 slightly different monomers α and β tubulin.[2] The tubulin monomer contains approximately 450 amino acids comprising some 7000 atoms with a combined mass of 55 kDa.[3]





(b)

Fig. 3. (a) A tubulin dimer with rods indicating α-helices. The sites GTPe are exchangeable nucleotide sites and GTPn are nonexchangeable ones. (b) A single protofilament of tubulin dimers resembling an array of regularly arranged interconnected springs (α-helices). Thin protruding rods are C-termini.

Tubulin's secondary structure is of crucial importance for the dynamical model applied in this article. This structure is dominated by α-helices, polypeptide wound chains of amino acids, represented by rods in Fig. 3(a). These α-helices resemble a system of interconnected nonlinear springs regularly arranged along every protofilament of a MT, [Fig. 3(b)].

Reliable electron crystallography analysis[4] revealed that every tubulin has the protruding rodlike chains of carboxil-terminus domains extending outwards away from the MT into the cytoplasm, (Fig. 4).

This C-terminal domain includes two antiparallel α-helices that define the protofilament crest. Much of the negative charge of the tubulin is concentrated

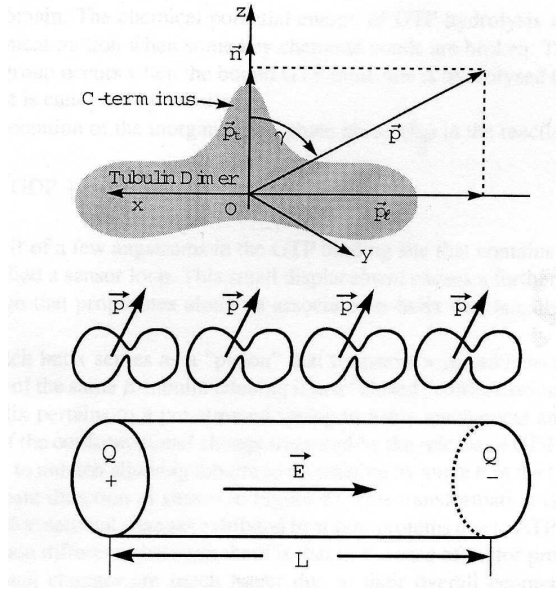


Fig. 4. A map of the surface electric potential on a tubulin dimer with an elongated C-terminal conformation. The "+" and "−" symbols indicate the local field strengths. Figure prepared using MolMol program.

on the C-terminus, (Fig. 4). In the following, this important part of the MT is abbreviated as (CT).

The main dynamical properties of MTs are based on the binding and hydrolysis of GTP (guanosine threephosphate) at the nucleotide exchangeable site GTPe in β-tubulin, [Fig. 3(a)]. Only dimers that have GTP in their GTPe site can polymerize in MTs, but after polymerization, this GTP is hydrolysed. Experiments have revealed that the chemical energy released in GTP hydrolysis causes a complex conformation of the pertinent tubulin dimer, leading to a mechanical tension that can even destabilize a MT, especially at its free tips.

We argue that the reason for this tension lies in the conversion of chemical energy of GTP hydrolysis into mechanical compression of adjacent α-helices within β-monomers.[5] In that respect especially, helix H10 appears to be very flexible. It is located in a very strategic place in the β-monomer, and it is important in both longitudinal and lateral MT interactions.[6] We anticipate that a partial relaxation of the internal tension within a MT takes place in terms of launching localized kink waves, involving significant tilts of CTs along the corresponding protofilaments.

This model is very recently corroborated by our general approach to the problem of conversion of chemical energy, released in the hydrolysis of ATP and GTP molecules, into mechanical movements of strategic α-helices "resembling pistons in mechanical motors".[5]

2. Ferroelectric Properties of MTs can Sustain Kink Excitations of CTs

The secondary structure of tubulin protein has some additional profound implications in our nonlinear model of MT dynamics. Every particular α-helix has an overall dipole moment ranging up to 100 debye, depending on the corresponding lengths. It is generally accepted that this large dipole moment of an α-helix has an important biological role.[7] In as much as every tubulin monomer contains several α-helices that are not oriented randomly, it is not surprising that each tubulin dimer possesses a significant dipole moment p (see arrows in Fig. 5). This dipole moment can be remarkably affected by the properties of a cell solvent, leading to the fact that the counterions could partially screen the intrinsic distribution of charges along the tubulin surface. It was shown[8] that pH can change the CTs from extended to bent conformations.

The idea that MTs have ferroelectric features was proposed some 30 years ago. This was quantitatively elaborated in several stages by Satarić *et al.*[9–11]

The essential result is that a MT, as a whole, represents a giant dipole, (see Fig. 5), creating a constant intrinsic electric field E. The strength of this field depends on the effective charges $\pm Q$ of MT tips, and on its length L, (Fig. 5).

Recent experiments *in vitro* show that MTs seem to be negatively charged cylinders,[12] perhaps due to the overwhelming concentration of negative counterions. We are convinced that the circumstances *in vivo* should favour the ferroelectric polar configuration, especially for MTs that are connected to the cell membrane or other

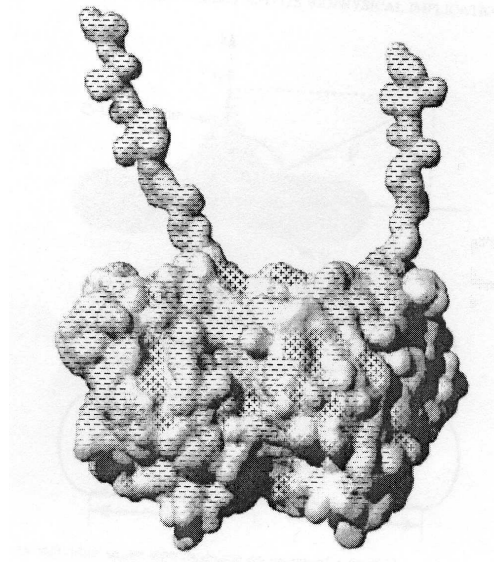


Fig. 5. An individual tubulin dimer with a dipole decomposed; a protofilament with the dipoles of individual dimers and a MT as a giant dipole due to these individual dimer's contributions.

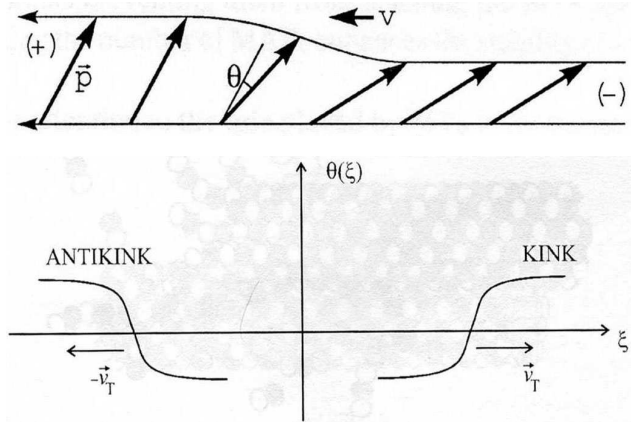


Fig. 6. The antikink's spatial dependence in terms of the local CT orientation; a sketch of antikink and kink excitations (below).

cytoskeletal filaments. The existence of the intrinsic electric field, together with the dimer's tension, bring about conditions for the creation and sustenance of mechano-electric excitations, which involve significant tilts of CTs. We elaborated this chain of nonlinear tilting CT pendula, leading to the kink or anti-kink waves. These excitations resemble the domino-effect, and they travel robustly along protofilaments, (Fig. 6). An easy to grasp analogy is that CTs swing as grass straws when the wind suddenly blows.

The free energy density in the CT dynamics in a MT consists of the following four terms[11,13]:

(a) The rotational kinetic energy density of the tilting motion of a CT is:

$$w_{\text{kin}} = \frac{1}{2} J \left(\frac{\partial \theta}{\partial t} \right)^2 , \tag{1}$$

where J stands for the rotational inertia of a CT rod.

(b) The elastic energy density of the CT-CT interaction within a protofilament expands in terms of the tilt angle θ up to fourth order as:

$$w_{\text{el}} = (-A\theta^2 + B\theta^4) \frac{1}{r^2} , \tag{2}$$

where A and B are the coefficients that are expressed in terms of the cylindrical components of the elasticity tensor, and r is the radial coordinate.

(c) The splay energy density has the form:

$$w_{\text{sp}} = \frac{\kappa}{2} \left(\frac{\partial \theta}{\partial x} \right)^2 , \tag{3}$$

where the coefficient κ denotes the smectic splay elastic modulus. The presence of the splay deformation has been experimentally demonstrated by the bending of MT filaments.[6]

(d) The polarization energy density (see Fig. 4, top sketch) takes the form:

$$w_{\text{pol}} = \left(\frac{p_t^2}{2\chi_t} + \frac{p_l^2}{2\chi_l} \right) - Ep_l - \mu_p p_t \theta \,, \tag{4}$$

where p_t, p_l, χ_t, χ_l stand for the transverse and the longitudinal dipole projections and the corresponding dielectric susceptibilities, respectively. The intrinsic electric field E is directed along the long axis of the MT cylinder (x-axis), and μ_p is a phenomenological constant that depends on the anisotropic polarizability of tubulin and the chirality effect due to CTs.

(e) The presence of viscosity in the medium surrounding the MT (cytosol) can be modeled by including a friction term in the equation of motion in terms of a viscous torque:

$$T_{\text{vis}} = -\Gamma \frac{\partial \theta}{\partial t} \,, \tag{5}$$

where Γ depends on both the viscosity coefficient, which is of the order of 10^{-3} Pas, and the rodlike geometry of a CT, through the Stokes–Einstein formula.

The total free energy functional of coupled nonlinear CT pendula can be written as:

$$F = 2\pi \int_R^{R+d} r dr \int_0^L (w_{\text{kin}} + w_{\text{el}} + w_{\text{sp}} + w_{\text{pol}}) dx \,, \tag{6}$$

where R stands for the radius of a MT ($R = 12.5$ nm), d is the length of a CT ($d = 4.5$ nm) and L is the MT length. Performing a free-energy minimization procedure on Eq. (6) and combining the torque in Eq. (5) with the Euler–Lagrange equation stemming from the free-energy functional, leads to nonlinear differential equation of motion for the angle θ. Using the scaled variables and adopting the traveling wave form, one obtains the following ordinary differential equation:

$$\eta_{\xi\xi} + \gamma \eta_\xi - \eta^3 + \eta + \varepsilon = 0 \tag{7}$$

$$\eta = \theta / \theta_0 \,; \quad \xi = \kappa (x - vt) \,, \tag{8}$$

where η_ξ, $\eta_{\xi\xi}$ are the first and second derivative, respectively, θ_0 is the amplitude of CT tilt, x is the direction of a filament (Fig. 5), v is the kink's velocity and κ is its wave-number. γ is a dimensionless parameter stemming from the viscosity of the solvent, and ε is a constant proportional to the intrinsic electric field E, (Fig. 5).

The important result is that the kink's velocity obeys Ohm's law as follows:

$$v = \mu E \,, \tag{9}$$

where the kink's mobility μ depends on the model parameters, including the ferroelectric properties of CTs, as well as on the viscous dissipation, in a very natural way. This linear response holds even for very strong fields of the order of 10^5 V/m.

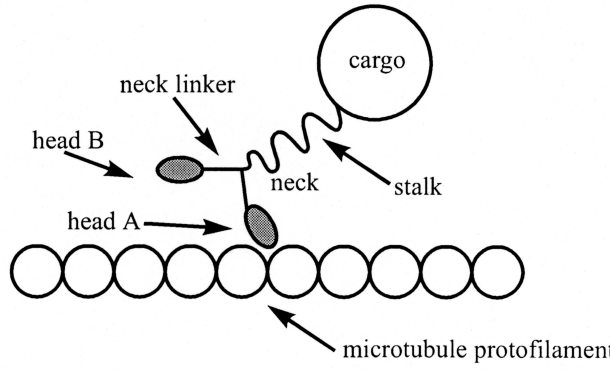


Fig. 7. An enlarged sketch of a kinesin molecule moving with cargo processively along a MT.

3. Movements of the Molecular Motors Along MTs and the Possible Katalitic Role of the Intrinsic Cellular Electric Fields

The kinesin and dynein motors are processive in the sense that they make many directed steps before they detach from the respective MT filaments. Kinesin always goes toward the MT plus tip, while dynein goes in the opposite direction. They walk via discrete steps, the size of which is equal to the repeat distance of the filament that is 8 nm, (Fig. 7). The energy fuel for these motors is the energy of hydrolysis of ATP.

Our straightforward calculations reveal that the kink's velocities along MTs span in the range $(0.2 < v < 2)$ μm/s. It is interesting to note that, depending on the ATP and salt concentrations, and the load placed on the kinesin molecule, it propagates along MTs with velocities ranging between

$$(0 < v_{\rm kin} < 0.9)\ \mu{\rm m/s}\,, \tag{10}$$

i.e., there is a strong overlap in the velocities of the two types of biological motions that may interact with each other.

4. The Role of Intrinsic AC Fields in the Kink's Dynamics

It is well-known that, besides Brownian motion, intracellular oscillating electric fields can drive the transfer of biological molecules.[14] Fraunfelder *et al.*[15] analyzed the forces in biological systems and claimed that "In biological systems, the force is known, and it is the electromagnetic interaction".

Any molecule and any structure with an electric dipole moment can generate an oscillating electromagnetic field with a dominant electrical component in its vicinity. We already stressed the dipolar character of tubulin protein and MTs itself. The strong electrically polar character of biological constituents makes the longitudinal oscillations of the electric field possible, as was first postulated by Fröhlich.[16] The energy supply from metabolic sources can excite these oscillations to be far from

thermodynamic equilibrium. Excitation depends on the amount of energy supplied to the cell. Here, we envisage that the oscillating endogenous electric fields could significantly influence the motion of the aforementioned CT kinks.

It is believed that the ordered (vicinal) water molecules form the electric dipole field, EDF, occuring on either side of a cell membrane. Within the interior of the cell, the water molecules generate the EDF in the vicinity of the cytoskeleton (MTs). Del Guidace *et al.*[17] have proposed that the electromagnetic fields arising from EDF coherent oscillations create the electromagnetic signals comparable in size with the dimensions of MTs. These fields could couple with the already existing dipolar intrinsic field E of a MT, in order to cooperatively control the kink's motion, and consequently regulate the intensive traffic of motor proteins along MTs.

Let us now consider the motion of a kink, Eq. (7) subjected to the harmonic electric force generated by EDF. We assume that the dimensionless driving force has a wavelength that is greater than the typical MT length, and it harmonically depends on time τ:

$$f(\tau) = f_0 \cos(\Omega\tau + \varphi_0)\,. \tag{11}$$

In the following, we will term it the intrinsic alternative current field IACF, where f_0 is the amplitude, Ω the frequency and φ_0 the initial phase of the driving force. By adding Eq. (11) to Eq. (7), we could examine numerically the kink's dynamics in that new regime. Here, we mention some results:

If the intrinsic field E is of the order of a few (V/m), which corresponds to the MTs of typical lengths of a few μm, we obtain the set of parameters $(\beta = 0.001; \varepsilon = 0.0002)$. Choosing $f_0 = 0.01$ and $\Omega = 0.01$, with the initial conditions $\varphi_0 = \pi/2$ and $d\eta/d\tau(0) = 0$, we get an unidirectional slow kink's motion with superimposed oscillations [Fig. 8(a)].

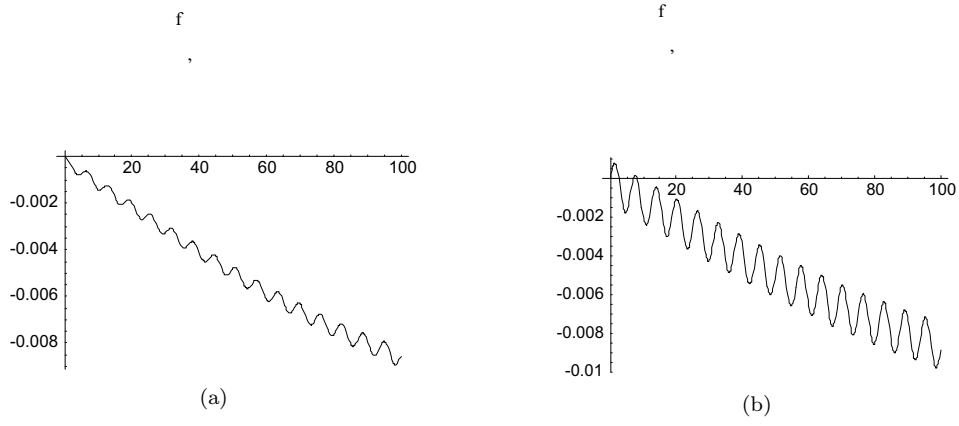


Fig. 8. (a) The time evolution of a kink movement in IACF with the set of parameters: $\beta = 0.001$; $\varepsilon = 0.0002$; $f_0 = 0.01$, $\Omega = 0.01$, $\eta'(0) = 0$ and $\varphi_0 = \pi/2$. (b) The time evolution of a kink with the same parameters as above, except the initial velocity $\eta'(0) = 0.001$.

Under realistic circumstances, it is expected that the kink has a nonzero initial velocity, $d\eta/d\tau$. If we put $(d\eta/d\tau(0) = 0.001)$, under the same conditions, the average kink's velocity is the same, but the oscillations are more conspicuous [Fig. 8(b)].

Eventually, if the frequency of IACF is increased, the velocity of the unidirectional kink's motion is lowered ($\nu = 0.00005$), and the amplitude of the oscillations is increased (Fig. 9).

Our wider considerations show that when the initial phase of $IACF$ is changed from $\pi/2$ to $-\pi/2$, the direction of the corresponding kink's motion is also changed, (Fig. 10).

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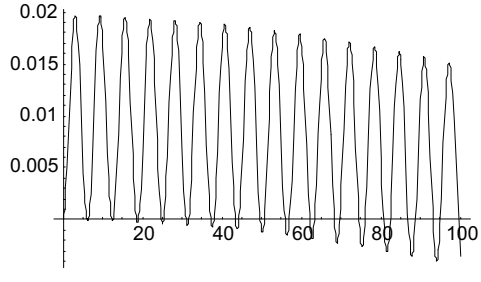

Fig. 9. The time evolution of a kink movement in IACF under the same other conditions and five times increased frequency: $\beta = 0.001$; $\varepsilon = 0.0002$; $f_0 = 0.01$, $\Omega = 0.05$, $\eta'(0) = 0$ and $\varphi_0 = \pi/2$.

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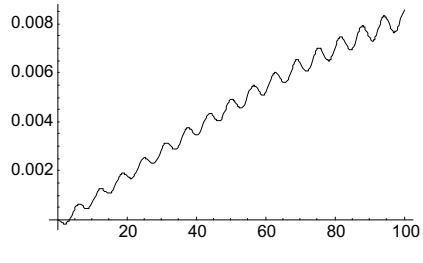

Fig. 10. The time evolution of a kink movement in IACF with the initial phase switched from $\pi/2$ to $-\pi/2$. This change reverses the direction of kink's motion.

The numerical evidence presented here suggests that the combined action of intrinsic field E within a MT and IACF can control the direction, the velocity and the frequency of the superimposed oscillations of the kinks involved. This makes these fields a powerful means for tuning the kink's dynamics, and for determination of the catalytic role in the regulation of cellular traffic sustained by motor proteins.

5. Conclusion and Discussion

How are proteins, with their very diverse functional roles in the cell, able to produce mesoscopic and even macroscopic, movements in a coordinated fashion? The answer to this question is still largely unknown. For example, the formation of the mitotic spindle apparatus and the process of chromosome segregation require concerted efforts of force generation by kinetochore, MTs and a simultaneous action of kinesin and dynein motors.

In a process as important as cell division, mistakes that would frequently occur if random thermal fluctuations were to rule, must necessarily be eliminated.

In this paper, we considered the complex mechanism that controls the traffic of motor proteins walking along the MTs in living cells. In the context of our model, the subtle interplay between the transducted chemical energy of GTP and ATP hydrolysis, dipolar ferroelectric properties of MTs and other related cellular ingredients, leads to the excitation of stable kink waves within CTs.

These waves are envisaged as a control mechanism for switching the kinesin and dynein motors, which are attached to every cargo being a candidate for transport along a MT.

The kink's motion is expected to be tuned by changes in the driving force of IACF regarding amplitude, frequency and its initial phase. It is expected that the local dipolar field carried by a kink would be the switch that turns on one class of motors and turns off the other class.

Within the framework of our model, MTs are not only passive tracks for transport in the cell, but also the signal relays for electrical, mechanical and chemical stimuli that may be transduced over distances comparable to the cell size.

Acknowledgment

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References

1. M. Shiwa, *Molecular Motors* (Wiley-VCH, Weinheim, 2003).
2. E. Nogales and H.-W. Wang, *Current Opinion in Cell Biol.* **18**, 179 (2006).
3. H. A. Schnessler, A. Merskin, A. A. Kolomenski and D. V. Nanopoulos, *J. Mod. Optics* **50**, 2381 (2003).
4. E. Nogales, S. G. Wolf and K. H. Downing, *Nature* **391**, 199 (1998).
5. M. V. Satarić, L. Matsson and J. A. Tuszynski, *Phys. Rev. E* **74**, 051902 (2006).

6. E. Nogales, private communication (2006).
7. W. G. Hol, *Prog. Biophys. Molec. Biol.* **45**, 149 (1985).
8. J. A. Brown and J. A. Tuszynski, From the biochemistry of tubulin to the biophysics of microtubules, *Proc. 1st Workshop on Biological Physics*, eds. V. Sa-Yakanit, L. Matsson and H. Fraunfelder (World Scientific, Singapore, 2000), pp. 100–118.
9. M. V. Satarić, J. A. Tuszynski and R. B. Žakula, *Phys. Rev. E* **48**, 589 (1993).
10. M. V. Satarić and J. A. Tuszynski, *Phys. Rev. E* **67**, 011901 (1-11) (2003).
11. M. V. Satarić and J. A. Tuszynski, *J. Biol. Phys.* **31**, 487 (2005).
12. K. J. Bohm, N. E. Mavromatos, A. Michette, R. Stracke and E. Unger, *Electromag. Biol. and Medic.* **24**, 319 (2005).
13. M. V. Satarić, B. M. Satarić and J. A. Tuszynski, *Electromagnetic Biology and Medicine* **24**, 255 (2005).
14. J. Pokorny, *Electro-Magnetobiol.* **20**, 59 (2001).
15. H. Fraunfelder, P. G. Wolynes and R. H. Austin, *Rev. Mod. Phys. Centenary* **71**(2), 419 (1999).
16. H. Fröhlich, *Advances in Electronics and Electron Phys.* **53**, 85 (1980).
17. D. Guidice, G. Preparata and G. Vitiello, *Phys. Rev. Lett.* **61**, 1085 (1988).