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Model of anaphase chromosome movement based on polymer-guided diffusion

(microtubules/cytoskeleton/mitosis/cell motility/tubulin)

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ABSTRACT **We propose a motility mechanism that may result in the displacement of objects within the cell. The mechanism, which we call polymer-guided diffusion, involves a microscopic cycle of polymer association and dissociation from a lateral binding site. Reassociation occurs at the polymer subunit adjacent to that which has just dissociated, thus generating an apparent sliding movement. The displacement involves only free diffusion and the spontaneous fluctuations of the polymer; the movement thus requires no other energy sources than thermal energy and the energy originally required for the formation of the polymer. In this manner polymer-associated organelles can be guided (inevitably) by diffusional processes toward a final destination. The specific example of the anaphase movement of chromosomes poleward is detailed.**

The molecular mechanism of mitosis in higher eukaryotic cells remains largely unresolved. Microtubules are clearly involved in the process (1), and there is evidence that a dynein-like motive force between antiparallel interpolar microtubules drives the two spindle poles apart in anaphase (2, 3). However, the other major motility events, congression of chromosomes to the metaphase plate and movement of chromosomes poleward in anaphase, are directed by mechanisms that remain to be elucidated.

We focus our attention here on the anaphase movement of chromosomes toward their respective poles, and we offer a mechanistic model for this process. We propose that it is the ordered disassembly at the poles of the kinetochore-to-pole microtubules that creates the movement. The depolymerizing end (or a segment close to it) of these microtubules would attach to a specific high-affinity site that has a fixed position relative to the pole; the coupling between polymer disassembly and macroscopic displacement is achieved through the cycle in which the polymer dissociates from the high-affinity site, diffuses freely by a microscopic distance, and binds again to this site but by the next subunit. What is different about the model is that motion is created without the consumption of energy other than that related to depolymerization at the polymer net-disassembly end. This mechanism, which we call polymer-guided diffusion, will be shown to be quantitatively and qualitatively consistent with many published experimental data. The rationale for offering this model is to address whether it is possible to move an object using only the depolymerization of a microtubule at a given location and to show that the calculated rate of such movement is compatible with actual experimental data. This model does not rule out ATP-driven polymer movements in anaphase, but it demonstrates that such energy-dependent processes are not required.

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The concept of polymer-guided diffusion derives from our discovery that the microtubule-associated protein stable tubule only polypeptide (STOP) (4) can slide along the polymer without measurable dissociation from its surface (5). We have imagined this unusual property could be utilized to direct the movement of microtubules with respect to fixed points in the cell to which such a protein might be bound (6). Here we show, in quantitative terms, that a protein with a high affinity for the polymer may translocate on the microtubule and that this translocation may be coupled with polymer disassembly to create the directed movement of polymer-associated chromosomes. Since the process we describe does not require energy input other than thermal energy (and the energy required for the formation of the polymer on which the movement depends), it has an element of inevitability that is desirable for an event such as anaphase movement whose precision is critical to survival.

The Basic Cycle of Depolymerization–Fluctuation–Diffusion

We present first the model in the case of a linear polymer composed of N identical subunits of unit length l, with one end attached to a large object, A, (a chromosome, for example). To a first approximation, we consider that the polymer is rigid and does not bend so that it has only one degree of freedom. We assume that there exists a fixed structure with a binding site S that does not move and that can bind the polymer end with high affinity. Structure S works as an enzyme with an "exodepolymerase" activity that releases the terminal subunit (Fig. 1).

Initially the polymer is at rest with its free end in the high-affinity site of structure S. The terminal subunit dissociates from the polymer with a first-order rate constant, k_1, through action of the exodepolymerase S. The polymer undergoes rapid fluctuations in length, on a time scale where Brownian motion of object A does not occur; these length fluctuations result in oscillations of the free end around its equilibrium position. Some fluctuations will bring the terminal subunit close to the high-affinity site of structure S and binding of the polymer to site S will take place with an apparent first-order rate constant, k_a, (binding is reversible and dissociation has a first-order rate constant, k_d). The polymer is now "frozen" in a slightly extended configuration since its length is still $N \times l$, although it has only $N - 1$ subunits. The polymer will return to an average length $(N - 1) \times l$; because its free end is now held in a fixed position by structure S, it can shorten only if object A moves over the distance l. Object A can indeed move spontaneously by diffusion (let k_2 be the first-order rate constant for the diffusion of object A over a distance l) to bring the polymer back to the initial state and ready for a new cycle.

Abbreviation: STOP, stable tubule only polypeptide.

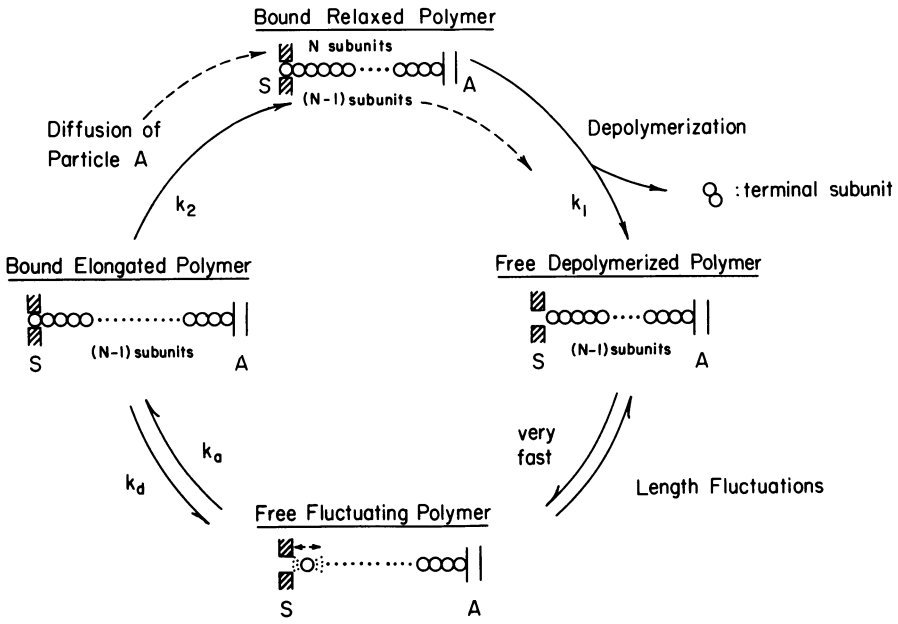

FIG. 1. Depiction of the basic cycle of depolymerization, fluctuation, and diffusion, leading to the guided movement of object A. The polymer links object A to the high-affinity binding site S. Through depolymerization and polymer diffusion, the polymer shortens and rebinds to site S. Object A moves toward site S by guided diffusion through repetitions of this basic cycle. In the model, the polymer does not pull object A; it only guides its normal spontaneous diffusion and thus orients the random movement of object A into a macroscopic displacement.

This sequence of events (depicted in Fig. 1) is the basic process of our model of polymer-guided diffusion. Some of the reactions described above are in fact composed of several steps. For instance, depolymerization involves dissociation of the terminal subunit from the polymer and release of the newly liberated subunit and polymer end from the corresponding site on structure S. The rate constant k_1 corresponds to this overall reaction; it is equivalent to the turn-over rate constant in the case of one substrate–two product reactions. The relationships among the global rate constant k_1 and those for individual steps (such as k_d) cannot be specified unless the order and relative rates of the intermediate steps are known. During review of this paper, it was suggested that the interaction between structure S and the polymer could involve several subunits rather than just the terminal one. Dissociation of the terminal subunit would not abolish the interaction between structure S and the polymer, but only weaken it so as to allow diffusion of the polymer to the next position. We agree that this is certainly a more plausible description of a real system, and indeed this is implicitly admitted below (see Fig. 2). We need not, however, analyze this refinement of our model further, since it leads to the same balance: at each cycle a subunit is removed from one end of the polymer, and the object A has moved by one subunit. The successive steps of the simple cycle given in Fig. 1 explain how a direct coupling between depolymerization and movement can occur. The physical bases of two of these steps are analyzed in more detail below.

Length Fluctuations of the Polymer. Thermodynamic, crystallographic, molecular dynamics, and nuclear magnetic resonance studies have shown that proteins have fluctuations of sizeable amplitude in the pico- to nanosecond time scale; reasonable values for a globular protein of molecular mass 30,000 would be 2 Å and 0.3 aJ for the mean fluctuations in overall dimension and energy, respectively (7–11). We can thus assume that the subunit of our polymer can fluctuate by $\Delta l = \pm 5\%$ of its length (± 2 Å as compared to a diameter of 40 Å taken for a protein of mass 30,000) and that it does so in a nanosecond or less.

Consider a polymer of N subunits with one-dimensional fluctuations; if each subunit fluctuates by $\pm\Delta l$ and if there are no neighbor interactions, then the overall polymer length will fluctuate by $\pm\Delta l\sqrt{N}$. With a large enough N, the average length fluctuations of the polymer will be comparable to the size of a subunit; indeed, if $\Delta l = 0.05l$ as assumed above, it needs only $N = 400$ subunits to obtain $\Delta l\sqrt{N} = 1$. This agrees with the proposal that spontaneous fluctuations of the polymer can make it stretch (or shrink) by 1 subunit. Note that the energy fluctuations are also important: With 400 subunits having a $\Delta E = 0.3$ aJ each, the polymer fluctuation will be ≈ 6 aJ, i.e., $>100kT$. The overall fluctuations of the polymer will be slower than those of a single subunit, but probably still in the nanosecond range.

Diffusion of Object A. The diffusion of a particle A, of equivalent radius R, in an isotropic medium of viscosity η is described by:

$$l^2 = kT \times t/3\pi\eta R,$$

where t is the time needed to diffuse over the distance l (the size of a subunit). The rate constant k_2, in sec^{-1}, is the number of times per second that object A diffuses over the distance l and is given by:

$$k_2 = kT/3\pi\eta R l^2.$$

The apparent linear velocity corresponds to a movement of $|k_2|$ 1-Å steps per sec and is given by:

$$v = k_2 l = kT/3\pi\eta R l.$$

With $l = 40$ Å as above, $R = 2$ μm (an average chromosome), and $\eta = 1$ poise (1 poise = 0.1 Pa·sec) (a medium 100-fold more viscous than water), one finds $k_2 = 125\ \text{sec}^{-1}$; the object A can thus move with a velocity, $v = 0.5$ μm/sec (125 steps of 40 Å/sec). Note that the value of the overall linear velocity v of A depends upon the size of the elementary step 1 of diffusion: the smaller l, the greater the velocity.

The energy involved for the diffusion of the particle A is kT, the thermal energy that is independent of its size and of the distance, and is thus much lower than the energy fluctuations of the polymer attached to object A.

Kinetic Constraints of the Basic Cycle

The Length Fluctuations of the Polymer and the Binding of the Terminal Subunit to Site S Must Take Place Before Object A Can Diffuse Away. The object A must remain motionless on the time scale needed for the free end of the polymer to find the high-affinity site of structure S; therefore, $k_a >> k_d$. Length fluctuations are rapid; binding also can be very fast, all the more since the terminal subunit and the binding site are already close to each other (Fig. 1). This proximity is central because it excludes the possibility that the ends of other polymers compete with the one that has just dissociated for binding to structure S (or similarly that a given polymer end can bind to another structure S than the one from which it has dissociated). The rate constant k_a is thus independent of the concentration of polymer ends or of structures S. Also, assuming that these two components remaining in the vicinity of each other is equivalent to having a flexible link of a given length holding them together, the association between them is an intramolecular reaction with a first-order rate constant in units of sec^{-1} (12). This reaction could occur in a time as short as 0.1 nsec (13) in which object A (radius, 2 μm) would diffuse by <1 Å; binding would thus be faster than the diffusion of object A as required.

The End of the Polymer Must Remain Bound to Structure S Until Object A Has Completed Its Diffusion over the Distance *l*, the Subunit Length. The polymer must relax from its slightly elongated state by waiting for object A to diffuse and not by dissociating from structure S; therefore, $k_2 > k_d$. The rate of dissociation measured in cases of simple protein–protein (14), antigen–antibody (15), or protein·DNA (16) interactions can be as low as 10^{-2}–10^{-4} sec^{-1}; that k_d be lower than the 10^2 sec^{-1} estimated above for k_2 seems a realistic assumption.

Depolymerization of the Terminal Subunit Must Take Place Only After Object A Has Completed Its Diffusion. For the basic cycle to close on itself, the diffusion of object A must be faster than the subsequent depolymerization: $k_2 > k_1$. Depolymerization should not be too fast; in the present numerical example, it should remain slower than removing 10^2 subunits per sec. The so-called exodepolymerase S need not be an enzyme in the strict sense; it could be an organized structure that locally creates conditions that favor depolymerization. The pole of the mitotic spindle could be such a structure.

The condition $k_a >> k_2 > k_1, k_d$ summarizes the kinetic constraints on the basic cycle. The cycle turns at a rate that is controlled by its slowest step, depolymerization (because $k_2 > k_d$, the binding step is not reversible). The movement of object A and the release of the terminal subunit appear to be simultaneous: this mechanism realizes a kinetic coupling between diffusion and depolymerization. The populated state will be the bound relaxed polymer that precedes the rate-limiting step.

This basic cycle corresponds to a process in which the fluctuations having a given orientation are selected among the random ones by their ability to be coupled to depolymerization. This is clearly a nonequilibrium situation in which energy, that of interaction between the subunits in the polymer, is being dissipated. It is because the various steps take place on a microscopic scale that the polymer can guide the diffusion of object A by coupling diffusion to its depolymerization.

Two Observations with Which the Model Is Consistent

Our model of polymer-guided diffusion specifies that the rate of displacement of object A is limited by that of depolymerization, k_1, i.e., does not depend on the size of object A. It has indeed been observed that in anaphase, all the chromosomes move at a velocity independent of size, indicating that their displacement is not limited by their hydrodynamic properties. Also, this displacement is inhibited if the depolymerization is blocked by taxol (17). This is consistent with our model in which slowing down depolymerization results in slowing particle displacement. So polymer-guided diffusion, even in its crudest form, correctly accounts for both the size-independent velocity and its variations with an effector of the rate of depolymerization such as taxol. It is also expected that the movement of object A could be accelerated by an effector that increases k_1 (provided that the limit imposed by diffusion is not reached, or $k_1 < k_2$).

Pull, Push, or Pull–Push

In the basic cycle the movement of object A is coupled to a shortening of the polymer upon depolymerization, and object A is apparently pulled toward structure S. It is obviously possible to propose a cycle in which object A is apparently pushed away from structure S, by coupling the displacement of object A to a lengthening of the polymer upon polymerization, structure S being now another site that acts as a polymerase.

Two such structures could act together, one as a pulling and the other as a pushing center; the polymer between them will show treadmilling, and a particle attached to it will be transported from one center to the other. This transport occurs at a rate controlled by that of polymerization or depolymerization; it is not dependent upon an energy source. It is only the existence of the polymer and the existence and nonrandom orientation of the polymer's net assembly and disassembly ends with respect to structure S, and not the transport itself, that may require energy expenditure in the form of ATP or GTP hydrolysis. In contrast with another model (18), this transport coupled to treadmilling is not associated with any mechanical strain or tension along the polymer (other than the microscopic spontaneous fluctuations).

Adjustments to Real Data

Viscosity of the Medium. Values differing by several orders of magnitude have been reported for the viscosity of an intracellular medium (19). The viscous drag is minimal in the case of displacements limited by free diffusion or slower. The value of 1 poise is used above; it is among the lowest values proposed (20). Our model would still be valid with a much higher value, but the assumption of an isotropic cytoplasm with a defined viscosity is certainly an oversimplification. Therefore, the estimate of the rate of diffusion is only indicative of an order of magnitude for the value.

Velocity of Displacement. During anaphase, chromosomes move at a rate of $\approx$1 μm/min (20). This is lower by one order of magnitude than the upper limit estimated above for a polymer-guided diffusive displacement, 0.5 μm/sec. Values as high as $R = 20$ μm or $\eta = 10$ poise would have given a limit still compatible with the observed rates.

Real Structure of Microtubules. The microtubule structure is more complex than the idealized linear polymer depicted in Fig. 1. With 13 protofilament strands comprising the polymer, the actual distance of displacement of the polymer with respect to structure S in a single cycle need be no greater than 1/13th of the 80-Å length of a subunit within a single protofilament, and k_2 would, therefore, be 50-fold greater than calculated above. This being the case, one could expect the geometry of structure S to be a "tunnel" with multiple polymer binding sites, into which the microtubule would insert. The hollow tube structure of the polymer would also allow greater lateral rigidity than a filled rod of the same mass (21). Therefore, the actual structure of microtubules accounts

better for the lateral rigidity assumed above than does an ideal polymer.

Potential Utility of STOP Protein

STOP protein has been found to be specifically associated with the mitotic spindle in cycling cells (R.L.M. and C. T. Rauch, unpublished results). Its potential utility in the mechanism described here is, therefore, explicit. STOP protein has two properties of interest; it "slides" on the microtubule polymer (5), and it allows dissociation only of those tubulin subunits that become exposed between it and the polymer end (5, 6, 22, 23). STOP protein, whether or not it is active in anaphase as part of an S site, is a useful paradigm for the existence of a site with these properties. Through the sliding of STOP and the thermodynamic barrier to its dissociation from the polymer, sliding and treadmilling will coordinately produce a one-way mechanism in which the polymer must migrate as its subunits dissociate beyond the STOP binding site, or sliding alone can cause the shortening of the polymer in anaphase (Fig. 2).

The precise mechanism of polymer displacement on structure S is not critical to the overall process. We have presented the case of a rapid cycle of association and dissociation of structure S from the polymer. The effect would be the same, but made simpler by the existence of a hypothetical sliding track on the polymer and by the nondissociation of the moving polymer from its binding site.

General Considerations of Anaphase

When chromosomes migrate poleward in anaphase, the microtubules that tether them to centrosomes progressively shorten while remaining firmly attached at their extremities to both kinetochore and centrosome (1, 24). The model we propose here accounts for this activity and for the paradox that the polymer must be both firmly attached and depolymerizing at its end.

The model proposed here may be considered a refinement of a mitotic model offered (25). In that model, the various spindle behaviors were related to an intrinsic treadmilling property of microtubules and to forces generated wherever antiparallel microtubules interact. The model successfully predicted the polarity orientation of microtubules in the spindle (26, 27) and may account for prometaphase congression of chromosomes to the metaphase plate and anaphase separation of the spindle poles. Anaphase movement of chromosomes poleward was imagined to be due to lateral linkages of kinetochore-to-pole microtubules with the interpolar microtubules that generated poleward forces (25).

This scenario remains possible; nonetheless, it is apparent that poleward movement of chromosomes (in anaphase A) can occur in the absence of any apparent linkage to interpolar microtubules and in the absence of a bipolar spindle (28). Further, lysed-cell model studies demonstrate that chromosome-to-pole movement is distinct from pole separation in having no requirement for ATP (2, 3). Our proposal here accounts for the independence of chromosome movement and the absence of an energy requirement.

It is apparent from many studies that microtubule disassembly is the governor of poleward chromosome movement, but it has been deemed unlikely that it may itself generate the motive force (1). Our model accounts, on a molecular scale, for these observations. In the mechanism proposed, microtubule disassembly governs the rate of movement but does not generate movement.

The model also accounts for the observed endwise tethering of kinetochore-to-pole microtubules at centrosomes and the independence of the rate of movement to kinetochore-to-pole microtubule length or to load (29).

Unlike the paradigm systems for cell motility, skeletal muscle and ciliary microtubules, the spindle is an unstable and ephemeral structure, whose destruction in fact appears intimately tied to its function in anaphase. The destruction is that of a highly dynamic microtubule system, whose assembly and disassembly are controlled *in vitro* by a complex array of associated proteins and physiological signals (30). Therefore, one imagines that the nature of the binding site S is such that it could allow the microtubule to stably bind and would couple the onset of net depolymerization to general cellular signals—for example, the calcium release at centrosomes in anaphase (31).

Calmodulin is localized to centrosomes in the mitotic spindle (32), and calcium influx could possibly activate its association with STOP protein (22) and simultaneously augment tubulin subunit dissociation from the polymer. *In vitro*, the STOP protein totally protects interior polymer segments against calcium-induced disassembly (22). Although the molecular detail of STOP protein reactivity on microtubules is not thoroughly elucidated, one can picture a variant cycle as depicted in Fig. 2, as follows: Calcium influx causes rapid disassembly of the polymer up to the STOP protein–calmod-

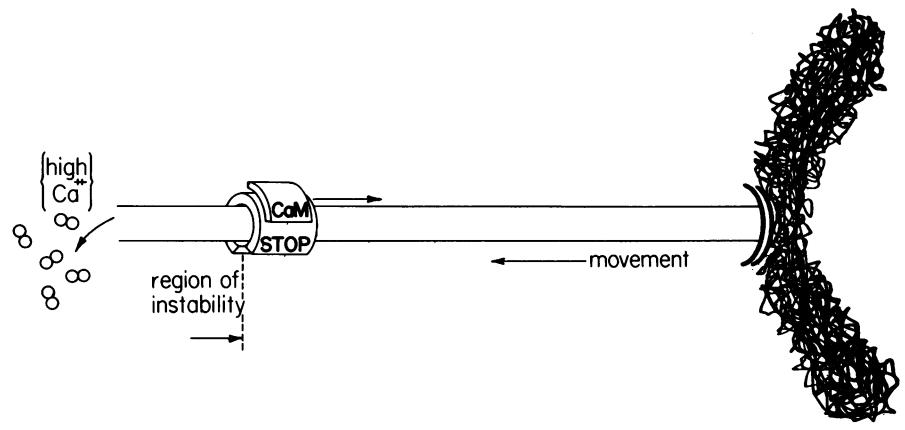

FIG. 2. Depiction of a hypothetical structure for the high-affinity binding site and polymer disassembly end, in the special case that STOP proteins linked to calmodulin (CaM) constitute the high-affinity site (S). STOP slides on a track on the microtubule. Its role is to guide the polymer displacement and to hold the polymer laterally. STOP also stabilizes the polymer region lying between it and the kinetochore so that the microtubule remains polymerized in a metastable manner until beyond the STOP, in a region where its instability increases rapidly due to high local calcium concentrations. Depolymerization is rapid beyond the STOP and, consequently, diffusion of the polymer and the kinetochore is inexorably unidirectional and poleward. The mechanism differs from that shown in Fig. 1 in that the polymer never physically releases from its high-affinity binding site while diffusing past this site.

ulin site, which links the polymer to the centrosome. Each incremental step in sliding is directed unidirectionally toward the chromosome by the large k_1 generated by high local calcium concentrations. The polymer, though shortening, is never measurably released from its STOP binding site. Further, almost all dissociation of STOP from its calmodulin binding site will occur in a time frame when the polymer is relaxed.

Although we have discussed polymer-guided diffusion as it relates to the anaphase movement of chromosomes, it is clear that an inverted mechanism at the kinetochore could account for growth of microtubules from kinetochores in prometaphase (33, 34), and steady-state treadmilling of kinetochore-to-pole microtubules at metaphase between two fixed sites (35–37).

In general, such a polymer-guided diffusional system can induce the displacement of polymers and their attached elements both in mitosis and in interphase. This diffusional system, coupled with treadmilling, can indeed account for the rapid centrosome-directed flow of subunits within microtubules, as described in microinjected interphase cells (38, 39). Clearly, a diffusional system can also account for the microscopic detail of the treadmilling of microtubules from the kinetochore to the centrosome in metaphase (37) in accord with the pull–push mechanism expressed above.

Note Added in Proof. We have described a mechanism of polymer guided diffusion, as exemplified by coupled sliding and polymer disassembly at the centrosome locus. Evidence (37, 40) indicates the possibility of a disassembly mechanism at the centromere in anaphase. In such a case the exodepolymerase activity would be coupled to object A instead of site S. This variation would not violate the principles of the mechanism proposed.

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