A physical model of microtubule sliding in ciliary axonemes

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ABSTRACT Ciliary movement is caused by coordinated sliding interactions between the peripheral doublet microtubules of the axoneme. In demembranated organelles treated with trypsin and ATP, this sliding can be visualized during progressive disintegration. In this paper, microtubule sliding behavior resulting from various patterns of dynein arm activity and elastic link breakage is determined using a simplified model of the axoneme. The model consists of a cylindrical array of microtubules joined, initially, by elastic links, with the possibility of dynein arm interaction between microtubules. If no elastic links are broken, sliding can produce stable distortion of the model, which finds application to straight sections of a motile cilium. If some elastic links break, the model predicts a variety of sliding patterns, some of which match, qualitatively, the observed disintegration behavior of real axonemes. Splitting of the axoneme is most likely to occur between two doublets N and N + 1 when either the arms on doublet N + 1 are active and arms on doublet N are inactive or arms on doublet N − 1 are active while arms on doublet N are inactive. The analysis suggests further experimental studies which, in conjunction with the model, will lead to a more detailed understanding of the sliding mechanism, and will allow the mechanical properties of some axonemal components to be evaluated.

INTRODUCTION

It is well known that the motion of cilia and eukaryotic flagella depends upon the sliding of axonemal microtubules (Satir, 1968). Summers and Gibbons (1971, 1973) demonstrated that sliding could be observed directly with the light microscope in isolated axonemes after treatment with trypsin and appropriate addition of ATP. Presumably, trypsin destroyed or weakened certain links, particularly the radial spokes, that controlled the extent and pattern of sliding within the intact ciliary axoneme, without affecting the mechanochemistry of the dynein arms, the proteins responsible for the force generation during sliding. Sale and Satir (1977) examined the basis of sliding at electron microscope resolution. They showed that the mechanochemical cycle of the arms produced a polarization of motion, such that the active arms attached to any doublet microtubule (N) pushed the adjacent microtubule (N + 1) in a tipwards (+) direction. No exception to this rule was found by them, or by subsequent investigators who have examined the polarity of sliding (Woolley and Brammall, 1987). The axonemal microtubules are arranged in a cylinder, and all nine doublets, with the exception of those connected by a permanent bridge (numbers 5 and 6 in sea urchin or mussel gill cilia), are capable of sliding. The velocity of sliding has been measured (Takahashi et al., 1982); sliding of any two doublets continues at a uniform velocity even as the microtubule overlap zone decreases to almost zero, suggesting that velocity is load-independent.

The detailed physical processes that lead to the extrusion of doublets from an axoneme by sliding have not been considered previously. These will form a basis for an eventual understanding of how sliding is controlled and converted into ciliary beating. A description of these processes is necessary to form a bridge between current knowledge of the mechanochemical cycle of the dynein arm (Satir et al., 1981; Johnson, 1985; Sugrue et al., 1988), and the virtually unknown sliding-bending conversion that occurs during motility. The biophysical requirements for microtubule displacement in particular directions within the axoneme are critical to the interpretation of a large number of experimental papers concerning microtubule sliding. The analysis, which is presented here, will be especially useful in formulating computer simulations of axonemal structure at 4 nm resolution that are being constructed to examine the molecular details of beat generation (Avolio et al., 1986; Sugrue et al., 1988). Several new hypotheses of how beat is generated require such an analysis. In addition, the analysis may apply in some degree to any case of polarized sliding between adjacent microtubules such as may occur in axostylar movements, cell elongation, and possibly in mitotic anaphase.

It is convenient to consider the analysis in a series of problems of increasing complexity, beginning with the distortion, without bending, of the cylindrical bundle of microtubules, followed by considerations of extrusion of a

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doublet and a set of doublets. In this treatment, the known behavior and properties of axonemal structures are utilized as follows:

(*a*) The mechanochemical cycle of dynein arms requires direct bridging between doublets (N) and (N + 1) (Spungin et al., 1987), and active arms contribute to attachment between doublets. (*b*) In ATP, inactive arms are unattached (Spungin et al., 1987); such arms do not contribute to the forces holding the axoneme together. (*c*) Interdoublet links connect doublets. These links are probably of several sorts with a variety of properties (Warner, 1976). This situation is simplified here by considering only the elastic properties of circumferential links between adjacent doublets. Initially it is useful to assume that all attachment between doublets, except those requiring active arms, is through a standardized set of elastic linkages that are equivalent in extent and properties between any two doublets. (*d*) In an isolated axoneme, as considered here, doublet sliding is not restricted by additional constraints, such as those provided by basal structures.

ANALYSIS

A computer-generated model, following the studies of Avolio et al. (1986) and Sugrue et al. (1988), of a complete axoneme is shown in Fig. 1 *a*, and contains the tubulin subunit structure of the outer doublets, the dynein arms, radial spokes, and central complex. While microtubule sliding patterns have implications for the behavior of many axonemal structures, the patterns themselves can be examined on the basis of a simplified model of the axoneme, which does not contain the central complex or the radial spokes. The situation can be simplified further, providing greater clarity and without loss of information, by "breaking" the elastic links between a neighboring pair of doublets and "unrolling" the cylinder so that the microtubules lie in a plane (Fig. 1 *b*). It is convenient to represent each doublet as a single rod, the elastic links as thin lines and active dynein arms as thick bars inclined to the axis of a rod (Fig. 1 *c*); inactive dynein arms are not represented. Because this analysis is concerned only with relative displacements of the microtubules, the model has not been scaled to represent the natural periodicities of the links and arms or the sizes of the microtubules. In Fig. 1 *c*, the rod (N + 4) at one end of the array is shown broken at the other end, so that its two sets of linkages (i.e., to N + 5 and N + 3) can be represented clearly. In Fig. 1 *d* the model has been reduced so that sets of linkages between pairs of doublets are replaced by single linkages of each type; because all linkages of a given type are regarded as equivalent, this reduction is possible with no loss of generality, and leads to simplification of the mathematical analysis and its pictorial interpretation. In general, microtubules will be identified by the numbering shown in Fig. 1, i.e., N, N + 1, N + 2, · · · N − 2, N − 1, N; a microtubule thus has two possible numbers, N + *m* or N + *m* − 9, where *m* may take a value between 0 and 9.

Problem 1: axonemal distortion

In this section, the behavior of the system in response to forces generated by dynein arms interacting between two microtubules is considered, subject to the conditions that none of the elastic linkages is broken, and that the microtubules do not bend. The interaction of the dynein arms between doublets N and N + 1 causes them to slide relative to each other, thereby stretching the links between themselves and their neighboring tubules (N − 1, N + 2), and developing tensions $T_{0,1}$, $T_{0,-1}$, and $T_{1,2}$, respectively in the links joining (N, N + 1), (N, N − 1), and (N + 1, N + 2) (Fig. 2 *a*). For simplicity, N is omitted from the subscripts which identify the various tensions. Tension $T_{0,-1}$ in the link between doublets N and N − 1 will cause doublet N − 1 to slide, and so induce a tension $T_{-1,-2}$ in the link joining microtubules N − 1 and N − 2. A similar argument will obtain for each microtubule. If the system is suspended freely in a liquid, the tensions developed in the links will be symmetric about a line midway between doublets N and N + 1, and parallel to them, so that doublet N + 5 will suffer no displacement relative to the liquid. During sliding, each microtubule is in dynamic equilibrium under the influence of the tensions in the links, the viscous resistance to movement provided by the liquid environment, and, for doublets N and N + 1, the force exerted by the dynein arms. A microtubule sliding with a velocity of v relative to the environment experiences a viscous resistance kv, where k is a constant containing microtubule dimensions and the viscosity of the liquid. k can, in principle, be calculated, but its precise form depends on the shape of the microtubule, including its appendages, and the proximity of neighboring doublets and of the substrate. The equation representing dynamic equilibrium in the sliding direction of a microtubule m not involved in active sliding (i.e., $m \neq 0$ or 1) is

$$T_{m,m+1} \cos \theta_{m,m+1} = T_{m+1,m+2} \cos \theta_{m+1,m+2} + kv_m. \quad (1)$$

For the active microtubule, N, the equilibrium equation is

$$F_D = T_{0,1} \cos (\pi - \theta_{0,1}) + T_{0,-1} \cos \theta_{0,-1} + kv_0, \quad (2)$$

where F_D is the interdoublet force, with a similar expression for microtubule N + 1. By symmetry, $v_5 = 0$, $\theta_{5+p,5+p+1} = \theta_{5-p,5-p-1}$, and $v_{5+p} = v_{5-p}$, where p may take an integer value between 0 and 4.

Assuming the cylindrical arrangement of the axoneme

to be maintained by an appropriate distribution of forces, which will be discussed later, Eqs. 1 and 2 allow the relative longitudinal displacement of each doublet to be determined as a function of time, provided the elastic properties of the links, the forces generated by the dynein arms (F_D) and the viscous coefficient k are known. Although there is insufficient information about these parameters to give the precise form of the axonemal distortion, the general features of the expected pattern are shown in Fig. 2 *a*. The angle θ between the elastic link and the axis of the microtubule increases for successive microtubules between N or N + 1 and N + 5, and as a result, the extension of corresponding successive elastic links decreases.

Active relative sliding of doublets N and N + 1 will cease when the longitudinal components of the tensions in the links attached to these microtubules balance the force generated by the dynein arms. The other microtubules will come to equilibrium under conditions governed by Eq. 1 with $v_m = 0$. A simple set of conditions satisfying this equilibrium situation are

$$T_{m,m+1} = T_{m+1,m+2} \quad \text{and} \quad \cos\theta_{m,m+1} = \cos\theta_{m+1,m+2}, \qquad (3)$$

where $m \neq 0$ or 1. As shown in Fig. 2 *b*, Eq. 3 indicates that all links between inactive doublets are collinear in the unrolled axoneme, develop the same tension, and have the same extension. Other stable equilibrium positions are possible, but require information about the forces maintaining the cylindrical arrangement for their specification. A stable distortion of the axoneme will be maintained while the shearing forces are applied.

Experimental observations demonstrate that a unique break can occur in the axoneme along an interdoublet gap between adjacent microtubules, resulting in "unrolling" of the axoneme (Sale and Satir, 1976). One way in which this could occur would be for the elastic links joining the pair of interacting doublets to break, with simultaneous or subsequent detachment of the dynein arms. The removal of the active shearing force would leave the microtubules influenced only by elastic restoring forces, and the system would return to the equilibrium state modeled by Fig. 1, except that a break would exist between doublets N and N + 1.

The foregoing arguments apply to distortion of a system in which the dynein arms on one microtubule only are active. Similar arguments can be used to discuss situations where the arms on two or more doublets are active. For example, if the arms on doublets N − 2, N − 1, and N are active, the static equilibrium is as shown in Fig. 3, where the elastic links between inactive microtubules are collinear, but those between the active doublets will be so only if the shearing forces and elastic resistances between all the interacting pairs of doublets are the same (a situation indicated by the dotted line in Fig. 3). In this particular example, doublet N + 5 suffers zero displacement in a "free" axoneme.

The equilibrium patterns that arise when arms on several contiguous doublets are active are shown in Fig. 4, with the patterns for no activity (Fig. 4 *a*) and a single active doublet (Fig. 4 *b*) included for comparison. In drawing Fig. 4, it has been assumed that each doublet generates the same active force and that the elastic resistances between all doublets are equal. Consequently, the displacements of adjacent doublets are essentially the same, although the doublets at either end of the active group may be subject to different elastic forces contributed by elastic links associated with the inactive group of doublets, and the links between active and inactive doublets are separately collinear. Notice that the situation where all nine doublets are active (Fig. 4 *f*) gives rise to zero relative displacement of neighboring microtubules because the active force acting on each doublet is zero.

The microtubule patterns expected if a single microtubule becomes attached to the substrate will be as discussed above, except that in reaching the eventual pattern, the attached doublet, rather than the symmetrically positioned doublet, will suffer zero displacement relative to the substrate. If two or more adjacent doublets attach firmly to the substrate, so that relative movement between them is not possible under the action of the applied forces, the disposition of remaining doublets can be found by applying the analysis above.

It is appropriate to indicate here, for later discussion, that the links joining inactive and interacting doublets attach differently to the microtubule in terms of the angle made with the long axis of the doublet. This is easily seen in Fig. 2 *a*, where the angle $\theta_{1,0}$ of a link joining interacting doublets is obtuse, whereas those, $\theta_{0,-1}$, $\theta_{-1,-2}$, etc. between inactive, or passively moved, doublets are acute. Further, where the number of interacting doublets is smaller than the number of inactive doublets, the absolute extensions of each link in the interacting set will, on average, be greater than that in the inactive set.

Summary of axonemal distortion

(*a*) Axonemal distortion can occur when active microtubule sliding is resisted by elastic linkages that remain unbroken.

(*b*) Tension developed in the linkages between inactive doublets leads to passive sliding of those doublets.

(*c*) Considerations of dynamic equilibrium dictate that, during sliding, links between different microtubules are stretched by unequal amounts.

(*d*) Stable equilibrium is achieved when the active forces are balanced by the tension in the elastic links.

(*e*) The links between interacting doublets have distor-

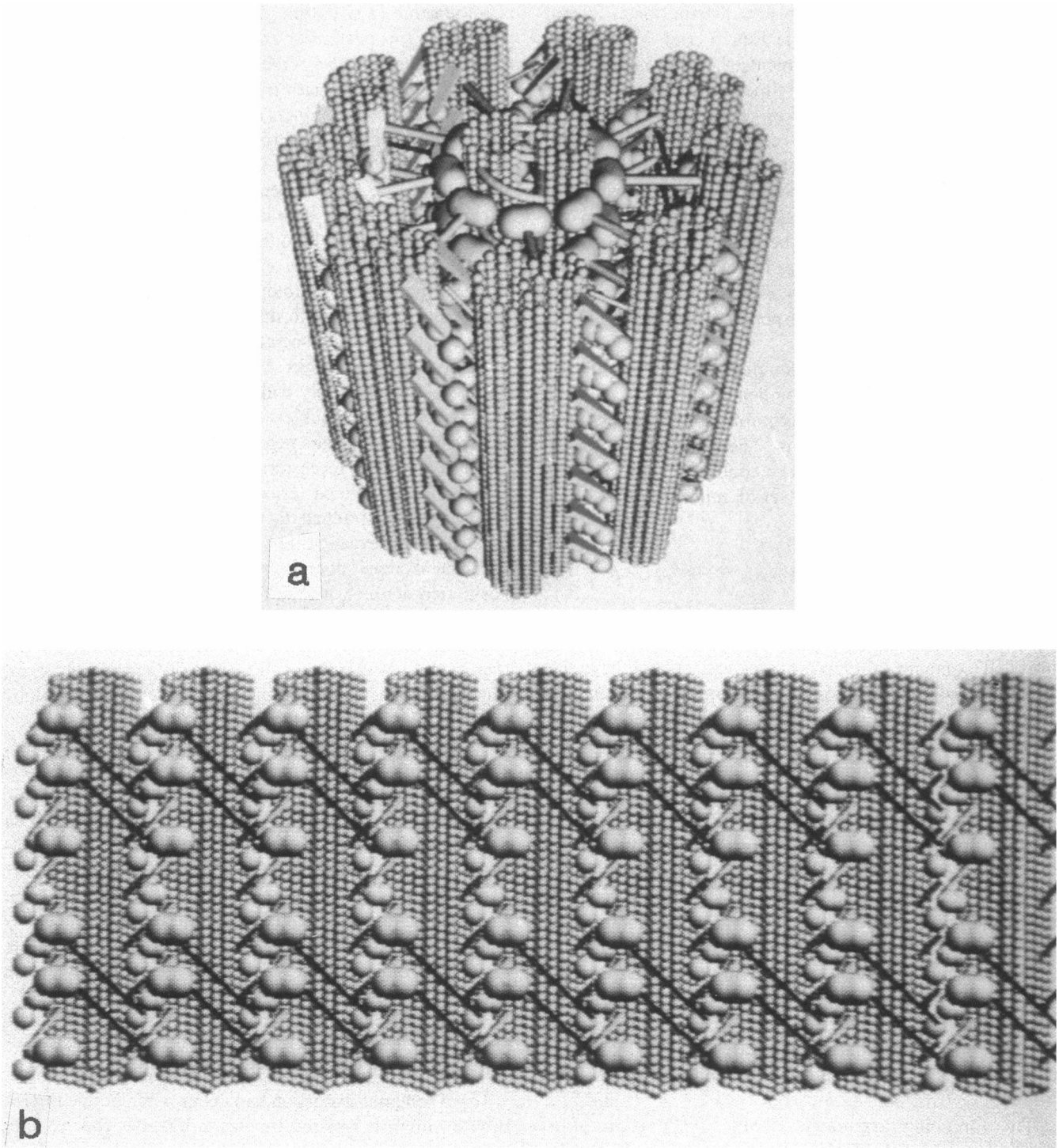

FIGURE 1 (*a*) Computer-generated model of complete axoneme, viewed to give an impression of the three-dimensional character of the structure. (*b*) Computer-generated model of the unrolled axoneme; the central complex has been omitted from the model.

tion characteristics different from those between inactive doublets.

(*f*) A unique break can occur in an axoneme between a pair of interacting doublets if the elastic links between those doublets break and the dynein arms detach. In the absence of shearing forces, elastic restoring tensions will cause the broken axoneme to assume an equilibrium configuration.

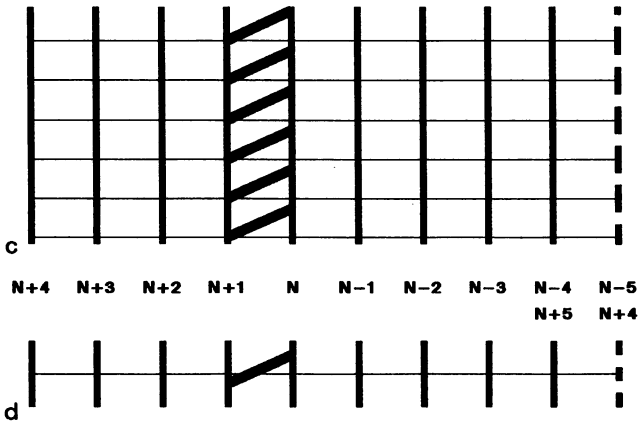

FIGURE 1 (*c*) Simplification of the model: microtubules are represented by thick lines, elastic linkages by thin lines and active dynein arms by thick bars inclined to the microtubule axis. (*d*) Further simplification achieved by representing the microtubules as short lines and the several dynein arms and elastic linkages between neighboring doublets as single links of appropriate type.

Problem 2: extrusion of a doublet

On the basis of the proposed model, the extrusion of a single doublet from an axoneme freely suspended in its environment can occur if one doublet only has active arms, i.e. the situation shown in Fig. 2 for axonemal distortion. If more doublets are active, different patterns of extrusion may occur, as will be discussed later, although under certain conditions a single microtubule only can be extruded. Where the active shearing force is between doublets N and N + 1 alone, it is clear that only one, or possibly both, of these microtubules can be extruded. For a single doublet to be extruded, the elastic links between the interacting doublets must be broken while the dynein arms remain attached; in addition, links between either doublets N and N − 1 or N + 1 and N + 2 have to be broken. On mechanical grounds, links between the interacting doublets are likely to be the first to break, as can be seen from a consideration of Fig. 2 *a*, because they suffer the greatest stretching during axonemal distortion. With these links (between N and N + 1) broken and the dynein arms still attached, microtubule sliding and axonemal distortion continue and the elastic links still attached to doublet N and N + 1, respectively, across interdoublet gaps adjacent to the N, N + 1 gap, experience stretching greater than that of any of the other remaining links (Fig. 2 *a*). In this situation, the links most likely to break next are those which are stretched by the greatest amount, so that doublet N, or N + 1, or, rarely, both of them, may become free of elastic connection to the rest of the axoneme. The interaction of the dynein arms between doublets N and N + 1 induces the extrusion of

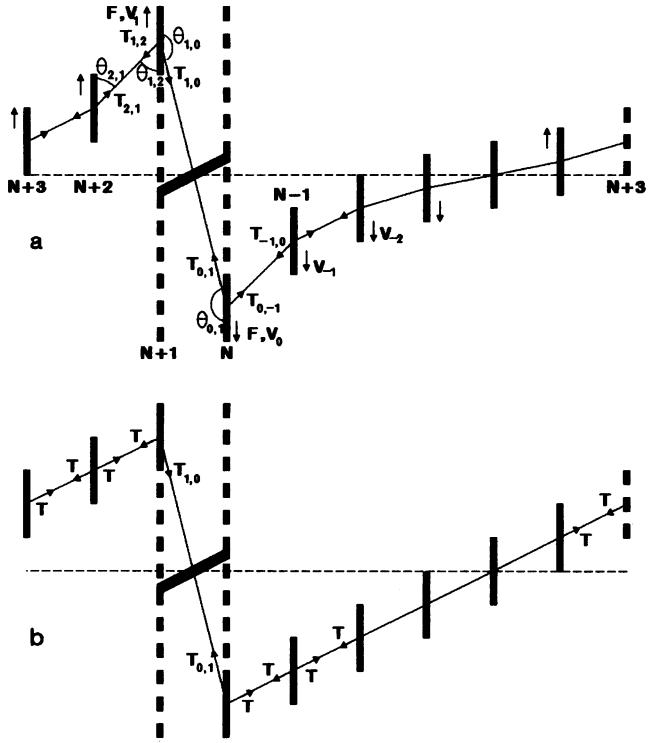

FIGURE 2 Distortion of the axoneme: (*a*) before static equilibrium is reached; (*b*) static equilibrium. For detailed explanation, see text.

one or both of these doublets, while the elastic properties of the remaining intact links cause the remainder of the axoneme to return to a passive equilibrium configuration. If the links between doublets N + 1 and N + 2 are broken first, doublet N + 1 will slide tipwards, i.e., with the (+) end of the microtubule leading (Fig. 5). If the initial breakage is between doublets N and N − 1, the action of

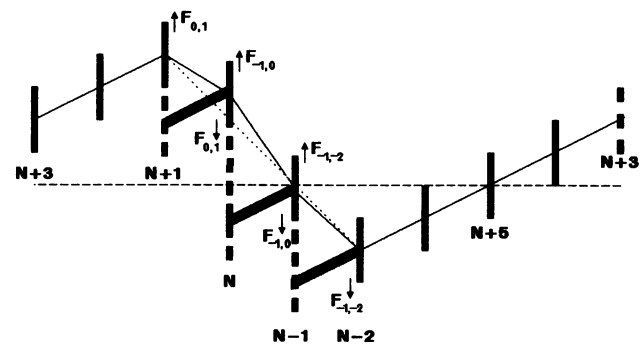

FIGURE 3 Static equilibrium with active dynein arms on three doublets; the interdoublet forces between each pair of interacting doublets is assumed to be different and gives rise to differently angled links. If the interdoublet forces were equal, the links would be collinear, as indicated by the dotted line. For detailed explanation, see text.

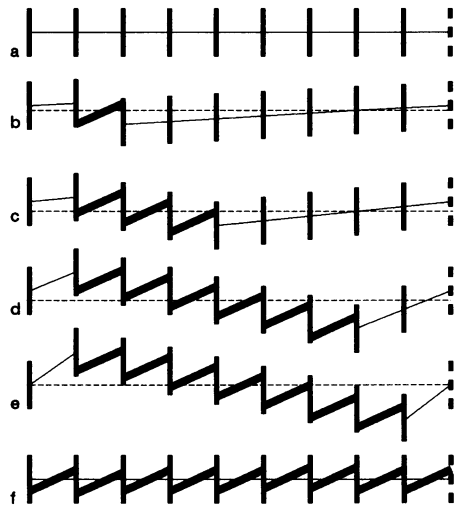

FIGURE 4 Static equilibrium with active arms on (*a*) zero, (*b*) one, (*c*) three, (*d*) six, (*e*) eight, and (*f*) nine doublets.

the dynein arms is to move doublet N baseward, i.e., in the (−) direction.

It is appropriate to consider here the mechanism by which the elastic links might break. There are two general possibilities, first that the links break in a purely random manner, so that an equal probability exists that the two sets of links discussed in the previous paragraph break, and second, that a bias exists, so that one set of links will break in preference to the other. In the first case, of stochastic breakage, a cooperative phenomenon probably takes effect in the axoneme, in the sense that when one link has broken between two microtubules, the remaining links are required to withstand the same shearing force as when all the links were intact; each link will therefore experience a greater loading, and will have a greater tendency to break than before. In a real axoneme, where structural variations are such that the precise symmetry of Fig. 2 *a* is unlikely to exist, initial link breakage will occur first between either of the doublet pairs (N + 1, N + 2) and (N, N − 1); once link breakage has started between a pair, cooperative effects dictate that it will continue until all links are broken and the appropriate doublet is free to slide. Variability in structure also precludes the possibility, except under special circumstances, of the two sets of links being broken at once, leading to the simultaneous extrusion of doublets N and N + 1 in opposite directions.

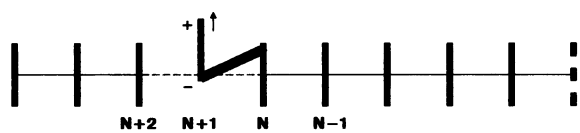

FIGURE 5 Extrusion of doublet N + 1 tipwards, or in the (+) direction. Links between N + 1 and both its neighbors are broken. The (+) and (−) ends of the microtubule are indicated. With the link between N + 1 and N + 2 broken, the remainder of the axoneme returns to an equilibrium configuration under the action of restoring elastic forces.

The case of biased breakage requires that one set of links breaks in preference to the other, so that the extrusion of either doublet N or N + 1 is favored. Such a situation could arise if the two ends of a link have different properties (Fig. 6), and the distortion is such that one end breaks preferentially. It may be seen from Fig. 6 that the two sets of links are asymmetric about the site of dynein arm activity, as noted in Problem 1, so that corresponding ends of links in the two sets may experience different stresses, leading to preferential breakage of one set, and hence the preferential extrusion of either doublet N or N + 1. Because stochastic breakage predicts that half the axonemes will have doublet N + 1 extruded, whereas for the other half it will be doublet N, it is possible, in principle, to distinguish biased from stochastic breakage experimentally.

The analysis of the extrusion of a single doublet has assumed that the axoneme is suspended freely in its environment. If one or more microtubules are attached to the substrate, the sliding pattern observed may vary, and the extrusion of only a single doublet may occur even if the dynein arms on more than one doublet are active.

Summary of extrusion of a single doublet

(*a*) Extrusion of a single doublet can occur when one set of dynein arms is active and both sets of elastic links attached to one of the interacting doublets are broken.

(*b*) Extrusion of a doublet can be toward the (+) or (−) end of the tubule, depending on which set of links is broken in addition to that joining the interacting doublets.

(*c*) Links may break by a stochastic or biased process.

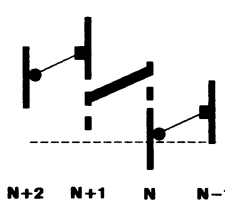

FIGURE 6 Indicating, by ● and ■, that the two ends of a link may have different properties, leading to biased breakage.

Problem 3: extrusion of a set of doublets

Except where the two interacting doublets are extruded in opposite directions, as discussed in Problem 2, extrusion of more than one doublet from the axoneme requires that dynein arms be active on two or more doublets. It is appropriate first to consider the situation where arms are active only on two adjacent doublets, and then to develop the analysis for arm activity on three or more contiguous doublets. As in Problem 2, it will be assumed that links break in the order of decreasing stretching, with links between the interacting doublets, which are stretched by the greatest amount, being the first to break. Activation of the sets of dynein arms on adjacent doublets may occur sequentially or simultaneously, and the two cases can produce different microtubule extrusion patterns, as will be discussed below. Additionally, groups of doublets may be extruded by the action of arms on three or more contiguous doublets, or on two or more noncontiguous doublets. It is convenient to discuss these four possibilities under separate headings.

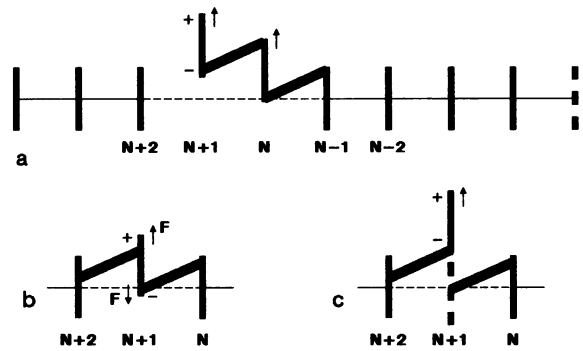

FIGURE 7 Possible microtubule patterns associated with sequential activation of dynein arms on neighboring doublets. (*a*) Doublet N − 1 becomes active after doublet N; links between N + 1 and N + 2 are broken. (*b*) Doublet N + 1 becomes active after doublet N, with the forces, *F*, acting on doublet N + 1 due to its interaction with doublets N and N + 2 being equal and opposite; the motion of doublet N + 1 is arrested, and the remainder of the axoneme may distort in the manner shown in Fig. 2. (*c*) as (*b*), but with the force between doublets N and N + 1 being greater than that between N + 1 and N + 2; doublet N + 1 continues to be extruded in the direction indicated by the arrow. In *a*, *b*, and *c* the links between doublets N + 2 and N + 3 remain unbroken.

Case 1: sequential activation of sets of dynein arms on two adjacent doublets

This case represents a logical development of Problem 2, in which the dynein arms on doublet N were active, and three possible extrusion patterns were demonstrated: (*a*) doublet N + 1 in the (+) direction, (*b*) doublet N in the (−) direction, and, rarely, (*c*) simultaneous movement of doublets N + 1 and N in the (+) and (−) directions, respectively. It is convenient to consider the effect of further dynein arm activity on each of these patterns in turn.

Doublet N + 1 extruded in (+) direction

Because the arms on doublet N are active, the sequential activation of a set of arms on a neighboring doublet could involve that either (*i*) on doublet N − 1 or (*ii*) on the sliding doublet N + 1. (*i*) If the arms on doublet N − 1 become active, doublet N will be moved in the (+) direction to follow doublet N + 1 in the manner of a telescope (Fig. 7 *a*). (*ii*) From Problem 2, extrusion of doublet N + 1 requires inactivity of the arms on doublet N + 1 and breaks in the links between doublets N + 1 and N + 2. On purely physical grounds, sequential activation of the extruding doublet N + 1 appears to be unlikely. However, if the arms on doublet N + 1 could become active, and were able to interact with doublet N + 2, the force generated would be such as to move doublet N + 2 in the (+) direction. Two situations are possible, depending on whether or not the elastic links between doublets N + 2 and N + 3 break. If they do not break, doublet N + 1 will assume an equilibrium configuration determined by the balance of forces generated by its interaction with microtubules N and N + 2. In this case, if the overlap of doublets N + 1 and N + 2 is such that the force developed between them is greater than that between doublets N and N + 1, doublet N + 1 may be pulled back into the axoneme to the equilibrium position (Fig. 7 *b*). If, however, the overlap is such that the shearing force between doublets N + 1 and N + 2 is less than that between doublets N and N + 1, doublet N + 1 will continue to be extruded (Fig. 7 *c*), but at a slower rate than before the activation of the arms on the second doublet.

If the elastic links between doublets N + 2 and N + 3 break under the influence of the dynein arm activity, doublet N + 2 will move in the (+) direction. During the initial stages of doublet N + 2 sliding, doublet N + 1 will project beyond it, as shown in Fig. 8 *a*, but in the later stages, doublet N + 2 will project beyond doublet N + 1 (Fig. 8 *b*) to give a pattern indistinguishable, except in terms of doublet numbers, from the situation shown in Fig. 7 *a*. Fig. 7 *a*, which results from arm activation on doublet N, followed by link breakage and then sequential activation of doublets N − 1, N − 2, etc. is the simplest route for telescoping in a (+) direction. Fig. 8 *a* is never seen in negative-stain electron micrographs because it would result in a scoring of the mechanochemical cycle as

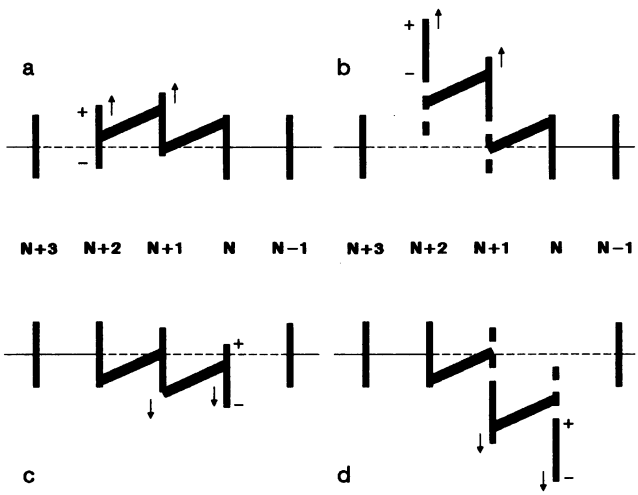

FIGURE 8 Microtubule sliding pattern with sequential activation of arms on doublets N and N + 1: (*a*, *b*) with links between N + 2 and N + 3 broken; (*a*) initial phase of activity; (*b*) later phase of activity. (*c*, *d*) With links between N and N − 1 broken; (*c*) initial phase of activity; (*d*) later phase of activity.

reversed (cf. Sale and Satir, 1977). Negative-stain images suggest that when doublet N + 1 is extruded in the (+) direction, it will be followed by doublet N, then by N − 1, etc., such that in a partially or completely unrolled sliding axoneme there will be a break at subfibre A of doublet N + 1 which will be the tipmost displaced doublet found, with arms unattached to doublet N + 2. Fig. 8 *b* can be thought of as a more complex variation of the Fig. 7 *a* pathway, with a different sliding history.

Doublet N extruded in (−) direction

The analysis of this situation parallels that above, with appropriate modifications of doublet number. From Problem 2, extrusion of doublet N requires inactivity of the arms of doublet N − 1 and breakage of the links between doublets N and N − 1 (Fig. 8 *c*). Physical reasoning suggests that subsequent activation of the arms on doublet N − 1 is improbable. If the arms on doublet N − 1 do become active, and the links between doublets N − 1 and N − 2 break, doublet N would be followed by doublet N − 1, a situation never preserved in negative-stain images. If the links between doublets N − 1 and N − 2 do not break, doublet N may retreat to an equilibrium position or be extruded more slowly, depending on the relative magnitudes of the two sets of forces acting on it. On the other hand, if the arms on doublet N + 1 become active, this doublet will telescope out from the array in the (−) direction behind doublet N (Fig. 8 *d*). Negative-stain images (Fig. 9) strongly suggest that when doublet N is extruded in the (−) direction, it will be followed by doublet N + 1, then by doublet N + 2, etc., such that in a

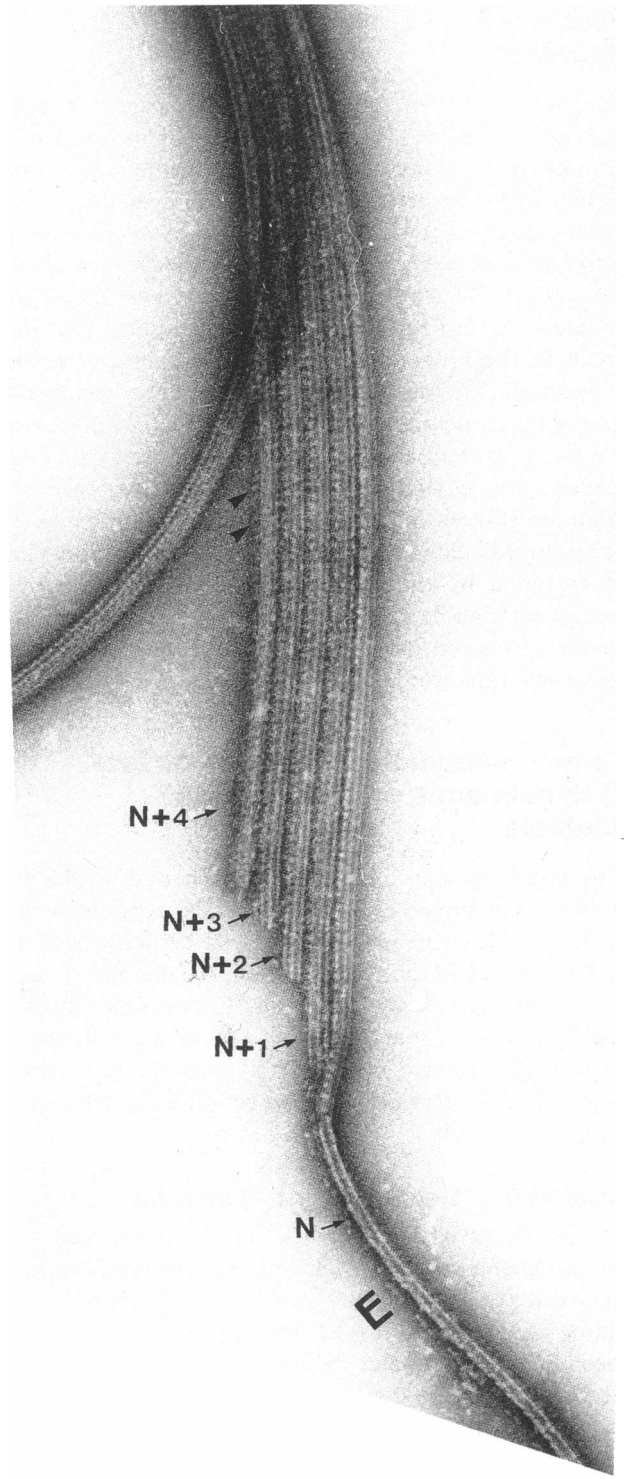

FIGURE 9 Negative-stain image of *Tetrahymena* axoneme after ATP-induced sliding. Several doublets have been extruded from the base of the axoneme. Note that doublet N has telescoped furthest baseward, followed by N + 1, N + 2, etc. as in Fig. 7 *d*. Links between doublet N and N − 1 have been broken and arms on doublet N − 1 are inactive. Bracket indicates spoke group on doublet N; arrowheads indicate unattached dynein arms of doublet N + 4. 57,000×.

partially or completely unrolled sliding axoneme there will be a break at subfibre B of doublet N, which will be the basalmost displaced doublet seen, with no attachments to doublet N − 1. For telescoping doublets as in Fig. 9, one must determine where the unextruded portion of the axoneme lies to be able to distinguish whether sliding occurred in a (+) direction with sequential activation of doublets N, N − 1, N − 2 (Fig. 7 *a*) or in a (−) direction with sequential activation of doublets N, N + 1, N + 2, etc. (Fig. 8 *d*). Without an absolute reference point images will appear to be the same, although their sliding histories are different. In these cases, the direction of propagation of arm activity from one doublet to its neighbor must be determined by which links break first.

Doublets N and N + 1 extruded simultaneously in opposite directions

From Problem 2, this requires simultaneous breakage of the links between doublets N and N − 1 and between doublets N + 1 and N + 2, and inactivity of the arms on doublets N − 1 and N + 2. Activation of the arms on adjacent doublets would then be completely independent of this rare sliding event.

Case 2: simultaneous activation of sets of dynein arms on two adjacent doublets

Consider the behavior of the system subject to simultaneous activation of the arms on doublets N and N − 1. Stretching of the elastic links is such that those between the interacting doublets break, as in the previous analysis, leaving doublet N free to move under the influence of the applied shearing forces. The pattern of sliding is determined by which, if any, of the other links break; as before, the links suffering the greatest stretching, i.e., those joining the interacting doublets, will be assumed to break before the others. It is convenient to discuss the situations where (*a*) no links are broken except those joining the interacting doublets and (*b*) other links are broken.

No additional links break

If the forces generated by the arms on doublets N and N − 1 are equal, the axoneme will be distorted, but no extrusion will occur. The pattern of distortion can be inferred from the analysis in Problem 1. If the forces developed by the arms on the two doublets are not equal, doublet N will be extruded in the (+) or (−) direction, depending on which force is the greater. Once extrusion begins, Problem 2 and negative-stain observations suggest that when doublet N is extruded in the (+) direction its arms will be at the free edge of the axoneme, whereas if it is extruded in the (−) direction, its subfibre B will be at the free edge.

Additional links break

The links which suffer the greatest distortion, and will therefore be assumed to break, are those between doublets N + 1 and N + 2 and between doublets N − 1 and N − 2. If only the links between microtubules N + 1 and N + 2 break, doublets N and N + 1 will telescope out of the array in the (+) direction to give the pattern shown in Fig. 10 *a*. Because doublet N always maintains arm attachments to doublet N + 1, and they start to slide simultaneously, doublet N + 1 will always protrude beyond doublet N during telescoping (Fig. 10 *b*). As before, when the links between an interacting and an inactive doublet are broken, the set of inactive doublets will assume an equilibrium under the influence of the elastic tensions in the links. Breaking only the links between doublets N − 1 and N − 2 will allow doublets N and N − 1 to telescope from the axoneme in the (−) direction (Fig. 10 *c*). Because doublet N − 1 always maintains arm attachment to doublet N during sliding in the (−) direction, and because they start to slide simultaneously, doublet N − 1 will always protrude beyond doublet N (Fig. 10 *d*). Should the elastic links between the two sets of doublets N + 1 and N + 2, and N − 1 and N − 2, break simultaneously, an unlikely event, doublets N + 1 and N − 1 will be extruded in the (+) and (−) directions, respectively, while doublet N may remain in the equilibrium position, although inequalities in the magnitudes of the shearing forces may cause doublet N to move in either direction. Note that Fig. 7 *a* and Fig. 8, *a* and *b* (where activation is sequential) and Fig. 10, *a* and *b* (where arm activation is simultaneous) yield essentially

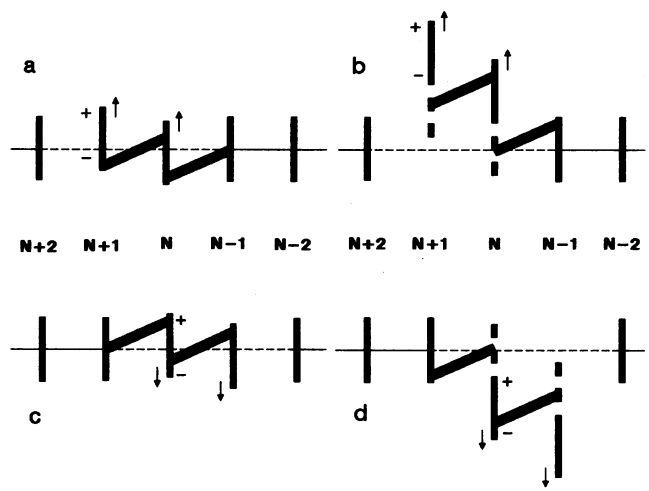

FIGURE 10 Microtubule sliding pattern with simultaneous activation of arms on doublets N and N − 1: (*a*, *b*) with links between N + 1 and N + 2 broken; (*a*) initial phase of activity; (*b*) later phase of activity. (*c*, *d*) With links between N − 1 and N − 2 broken; (*c*) initial phase of activity; (*d*) later phase of activity.

identical end patterns of sliding; the same is true for Fig. 8, *c* and *d* and Fig. 10, *c* and *d*.

Case 3: activation of sets of dynein arms on three or more contiguous doublets

The prediction of the various possible sliding patterns which result from the simultaneous or sequential activation of sets of dynein arms on three or more contiguous doublets follows readily from the discussion of Cases 1 and 2 above. No new situations are encountered in the analysis, other than the involvement of additional interacting doublets, and detailed descriptions of the many possible sliding patterns will therefore not be given. The end patterns of sliding will be, in general, a telescoped extrusion of the active doublets, although patterns intermediate between the onset of sliding and the end pattern may vary according to the precise character of doublet arm activity. A case which may give a different end pattern according to whether doublet activity is simultaneous or sequential is when the dynein arms on all nine doublets are active. If the activity is simultaneous, and each doublet is exerting the same force on its neighbor, no doublet extrusion will occur because an individual doublet will experience equal and opposite forces due to interactions with its two neighbors. If, however, doublet activity is sequential, extrusion of doublets will be induced by the initial activity, and a condition of force balance may not be reached even when all nine doublets are active. It is appropriate to note here that when the number of active doublets exceeds the number of inactive ones, the stretching of links between active doublets is less, on average, than that of links between inactive microtubules. A set of links between inactive tubules may thus break before the sets joining the active doublets, but the latter sets will eventually break because of the continued activity of the dynein arms. This phenomenon will not affect the predicted microtubule sliding pattern, though it will determine the temporal dependence of the activity.

Case 4: activation of sets of dynein arms on two or more noncontiguous doublets

Groups of doublets can be extruded if sets of dynein arms on two or more noncontiguous doublets become active, either simultaneously or sequentially, and appropriate elastic links are broken; if no elastic links break, stable distortion of the array will occur in a manner determined by the interacting doublets. The sliding behavior if linkages break can be predicted on the basis of previous arguments, and although no new situations are encountered, it is instructive to consider an example (Fig. 11), where the arms are active on two doublets, N and N − 5, which are not immediate neighbors. The stable distortion which arises if no elastic links break is shown in Fig. 11 *a*. On the basis of previous arguments (Problem 2), the links most likely to break are those connecting the interacting doublets to their passive neighbors, but if one such set of links breaks, the set at the other end of the same passive group is unlikely to break; thus, in Fig. 11 *b*, the links between doublets N − 4 and N − 3 have broken, allowing the tensions in links joining the noninteracting doublets to relax. The contiguous group of doublets N to N − 3 will slide as a unit, as may the group N − 5, N − 6 through to N + 1 with no further link breakage. The "isolated" doublet N − 4 will be free to slide relative to its active neighbor.

Simultaneous activation of noncontiguous doublets is considered unlikely in an active, intact axoneme, but is possible after protease treatment of the system. In the event that elastic linkages are broken, so that doublets are extruded, the arguments above indicate that the axoneme will split physically into two (or more, depending on the number and distribution of the active doublets) sets of interacting microtubules. In experimental studies of reactivated, sliding axonemes, telescoped groups of fewer than nine microtubules are often seen in negative-stain electron micrographs; while such groups could arise artifactu-

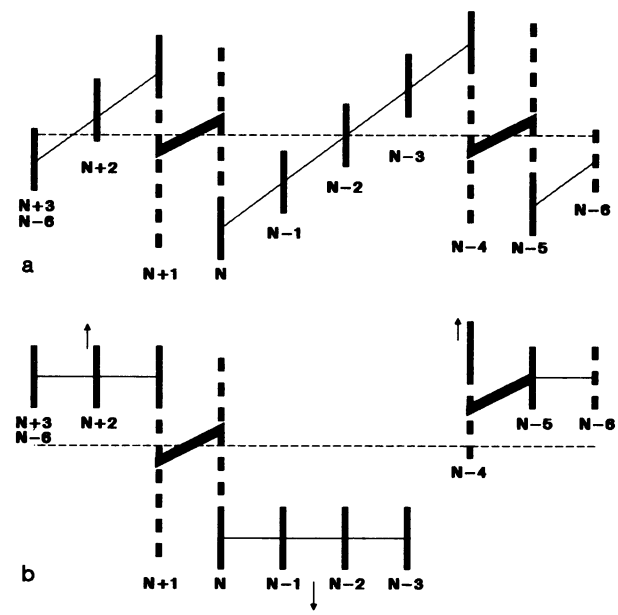

FIGURE 11 Axonemal distortion (*a*) and sliding (*b*) resulting from activity of 2 noncontiguous doublets, N and N − 5. In *b* the links are drawn horizontal to indicate a "relaxed"state; the true configuration would be angled as a result of resistance to movement of the doublets due to the viscosity of the environment.

ally during the EM preparative procedures, and the phenomenon could sometimes be due to the activation of noncontiguous doublets, the most likely explanation is that less than nine contiguous doublets have been activated and at the boundaries between active and inactive doublets extrusion has occurred (e.g., in Fig. 8, *a* and *b* the link between doublets N and N − 1 is broken). In all observations made, the configuration of the doublets corresponds to telescoping as indicated in Fig. 8, *b* and *d* (or Fig. 10, *b* or *d*); no other types of sliding pattern have been recorded.

Summary of extrusion of a set of doublets

(*a*) Extrusion of a set of doublets can occur by (*i*) activation of sets of dynein arms on two or more contiguous doublets, (*ii*) activation of two or more sets of dynein arms on noncontiguous doublets, and (*iii*) activation of the set of dynein arms on a single doublet, with, in each case, appropriate breakage of elastic linkages.

(*b*) When sets of dynein arms on two or more doublets produce extrusion, activation of dynein arms may be simultaneous or sequential on adjacent doublets. Simultaneous or sequential doublet activation produce the same end patterns of sliding.

(*c*) Telescoping of microtubules can occur in the (+) or (−) direction. Telescoping in the (+) direction will occur when the initial breakage of doublet links occurs at the N + *m* side of the active set of doublets, at the first inactive doublet. Doublet N + *m* will protrude furthest from the axoneme in the (+) direction. Telescoping in the (−) direction will occur when the initial breakage of doublet links occurs at the N side of the active set of doublets (N, N + 1, . . . N + *m*), between the active doublets and the first inactive doublet. Doublet N will protrude furthest in the (−) direction. When both sets of links break simultaneously, the active set of doublets will separate from the inactive set, except that doublet N + *m* will go with the active set.

(*d*) In sequential activation of dynein arms, for telescoping in the (−) direction, arm activation must proceed from doublet N to N + 1 to . . . to N + *m*; to telescope in the (+) direction, arm activation must proceed from doublet N to N − 1 to . . . N − *m*. Therefore arm activity determines link breakage. Then link breakage determines telescoping direction and progression of activity around the axoneme, if such occurs.

DISCUSSION

In this paper microtubule sliding behavior resulting from various patterns of dynein arm activity and elastic link breakage is described on the basis of a simplified model of the axoneme. The model consists of a cylindrical array of nine microtubules joined, initially, by elastic links, with the possibility of dynein arm interaction between neighboring pairs of microtubules; central structures of the axoneme are not modeled. The model predicts a variety of sliding patterns, some of which match, qualitatively, the observed disintegration behavior of real axonemes (Summers and Gibbons, 1971, 1973; Takahashi et al., 1982; Sale and Satir, 1977; Woolley and Brammall, 1987; Tanaka and Miki-Noumura, 1988). Our analysis has relied on the observation that electron microscope images of sliding always show one polarity only. Therefore, breakage of links, activation patterns etc. that are not consistent with this polarity have been rigorously excluded. The reported direct experimental observations in light microscopy of microtubule sliding contain some information permitting detailed comparisons with the model predictions. Clearly, sliding in both (+) and (−) directions occurs in a single axoneme during a complete disintegration, but separate sets of doublets disintegrate at different times. "Piggybacking" is also observed. These observations probably represent the more complex pathways, consistent with Problems 2 and 3 and do not provide crucial tests of the model. Whether a single doublet moves back and forth during sliding might be critical to the model, since it would require polarity rule violations, but is so far undetermined to our knowledge. Zanetti and Warner (1982) and Tanaka and Miki-Noumura (1988) have shown that at low, nonphysiological concentrations of ATP, sliding is limited so that only one or two doublets are extruded from the axoneme, while complete disintegration is seen at higher ATP concentrations. Where sliding of all nine doublets from one axoneme is seen in electron micrographs, all sliding interactions have identical polarity, and the only apparent 'violation' of the polarity rule is at the edges of the opened axoneme, where the dynein arms are completely unattached (and therefore presumed inactive at the time of splitting) (Fig. 9; see also Fig. 9.21 of Goodenough and Heuser, 1989). Further experiments may allow more critical evaluations of the model to be made and hence lead to an assessment of the mechanical properties of certain axonemal components. Because the model axoneme is assumed to be suspended freely in a liquid environment, a similar experimental arrangement should be realized; Sato et al. (1988) have shown recently that axonemal disintegration by sliding does occur under these circumstances. Should the axoneme adhere to the substrate, additional restraining forces, which are difficult to specify, will act on the microtubules and may modify the sliding behavior, as noted in earlier sections.

Problem 1 is interesting in connection with studies of interdoublet links in the unrolled straight portions of axonemes (Sale and Satir, 1976; Warner, 1983; Goode-

nough and Heuser, 1989). Presumably, restricted sliding to produce a basal bend has occurred before the axonemes have unrolled. In the unrolled axonemes, the distortions produced in microtubule relationships are preserved. Systematic displacement in interdoublet links that repeat in sets of two at 96 nm is observed from doublet to doublet, especially after the dynein arms have been extracted. The links are sometimes angularly tilted and extended as they would be if active sliding was restricted to only one doublet and static equilibrium achieved (e.g., as our Fig. 2 *b*). However, the tips of unrolled axonemes show that both active and passive sliding between adjacent doublets results in ~0.1-μm displacements, yet the interdoublet links are never stretched to cover this distance; therefore they must detach and re-attach as sliding proceeds, as Warner (1983) also concludes. For these structures, the distortions of Fig. 2, *a* and *b* would seem only to occur as an intermediate stage where sliding was relatively limited. This situation is comparable to an early stage of the disintegration process. A determination of the position of each doublet microtubule during this early phase of sliding, not yet recorded with sufficient temporal or spatial resolution, together with a knowledge of which doublets are active, would allow Eq. 2 to be applied, with subsequent determination of the elastic features of the links. In this regard, it is interesting to consider the work of Takahashi et al. (1982) on microtubule sliding, where, for low concentrations (<150 mmol m^{-3}) of ATP their Fig. 8 shows a microtubule bundle maintaining a constant length (i.e., showing no sliding) for a short period after the addition of substrate; this is followed by sliding disintegration at constant velocity. The immobile period could represent a phase where protease digestion is insufficient to allow sliding disintegration and/or where dynein arm activity is increasing to the stage where the total force generated is sufficient to break the links, after which sliding ensues. If the links were capable of significant extension, a variable sliding velocity would be expected during stretching, followed by a constant velocity after link breakage.

Reports in the literature contain inconsistent descriptions of the links and their properties. Early studies (see Warner, 1976 for references) indicated the existence of two types of link, one joining the A-tubules of adjacent doublets, the other joining the A-tubule of one doublet to the B-tubule of its neighbor. Warner (1983) now suggests that only one type of linkage exists. These are the links preserved in pairs at 96 nm repeat after dynein arm extraction, as discussed above. Interestingly, whereas the angular tilt and logitudinal stretch of the interdoublet links is always limited, the circumferential stretch is not limited because the links still connect doublets at some distance from each other. These links form A–B connections. Whether they also form A–A connections, as Warner suggests, or whether there are additional types of links that have more longitudinal elasticity and are more difficult to preserve, especially after extraction procedures, remains to be determined.

This paper has considered the qualitative pattern of microtubule sliding induced by dynein arm activity on one or more tubules, and resisted by elastic linkages between the doublets. The experimental observations and data available are not sufficiently detailed, for technical reasons, to make a quantitative assessment of the mechanical properties of the various axonemal components, but this analysis points out which properties and structures are relevant to the development of sliding and splitting of the axoneme. One conclusion is that splitting of the axoneme is most probable at the boundary between a group of doublets with active arms and a group of doublets with inactive arms. Splitting occurs between two doublets N and N + 1 when either (*a*) arms on doublet N + 1 are active and arms on doublet N are inactive, or (*b*) arms on doublet N − 1 are active while arms on doublet N are inactive. Splitting probably precedes extrusion of doublet N and influences the direction of arm activation around the axoneme, if activation is not simultaneous in adjacent doublets. The arms and elastic links, perhaps together with the radial spokes, function as a mechanochemical unit in the production of axonemal splitting and sliding.

The analysis also provides the basis for detailed consideration of splitting of doublet subgroups in tethered axonemes (Sale, 1986; Satir and Matsuoka, 1989) and of axonemal bending, which will be developed separately.

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REFERENCES

Avolio, J., A. N. Glazzard, M. E. J. Holwill, and P. Satir. 1986. Structures attached to doublet microtubules of cilia: computer modeling of thin-section and negative-stain stereo images. *Proc. Natl. Acad. Sci. USA.* 83:4804–4808.

Goodenough, U. W., and J. E. Heuser. 1989. Structure of the soluble and in situ ciliary dyneins visualized by quick-freeze deep-etch microscopy. *In* Cell Movement. F. D. Warner, P. Satir, and I. R. Gibbons, editors. Alan R. Liss, Inc., New York. 1:121–140.

Johnson, K. A. 1985. Pathway of the microtubules-dynein ATPase and the structure of dynein: a comparison with actomyosin. *A. Rev. Biophys. Chem. Biophys.* 14:161–188.

Sale, W. 1986. The axonemal axis and Ca^{2+}-induced asymmetry of active microtubule sliding in sea urchin sperm tails. *J. Cell Biol.* 102:2042–2052.

Sale, W., and P. Satir. 1976. Splayed *Tetrahymena* cilia: a system for analyzing sliding and axonemal spoke arrangements. *J. Cell Biol.* 71:589–605.

Sale, W., and P. Satir. 1977. Direction of active sliding of microtubules in *Tetrahymena* cilia. *Proc. Natl. Acad. Sci. USA.* 74:2045–2049.

Satir, P. 1968. Studies on cilia: III. Further studies on the cilium tip and a 'sliding filament' model of ciliary motility. *J. Cell Biol.* 39:77–94.

Satir, P., and T. Matsuoka. 1989. Splitting the ciliary axoneme: implications for a 'switch point' model of dynein arm activity in ciliary motion. *Cell Motil. Cytoskeleton.* 14:345–358.

Satir, P., J. Wais-Steider, S. Lebdusca, A. Nasr, and J. Avolio. 1981. The mechanochemical cycle of the dynein arm. *Cell Motil.* 1:303–327.

Sato, F., Y. Mogami, and S. A. Baba. 1988. Flagellar quiescence and transience of inactivation induced by rapid pH drop. *Cell Motil. Cytoskeleton.* 10:374–379.

Spungin, B., J. Avolio, S. Arden, and P. Satir. 1987. Dynein arm attachment probed with a non-hydrolyzable ATP analog. *J. Mol. Biol.* 197:671–677.

Sugrue, P., J. Avolio, P. Satir, and M. E. J. Holwill. 1988. Computer modelling of *Tetrahymena* axonemes: the mechanochemical cycle of the inner and outer dynein arms. *Cell Motil. Cytoskeleton.* 11:193–194.

Summers, K. E., and I. R. Gibbons. 1971. Adenosine triphosphate-induced sliding of tubules in trypsin-treated flagella of sea-urchin sperm. *Proc. Natl. Acad. Sci. USA.* 68:3092–3096.

Summers, K. E., and I. R. Gibbons. 1973. Effects of trypsin digestion on flagellar structures and their relationship to motility. *J. Cell Biol.* 58:618–629.

Takahashi, K., C. Shingyoji, and S. Kamimura. 1982. Microtubule sliding in reactivated flagella. *In* Prokaryotic and Eukaryotic Flagella. W. B. Amos and J. G. Duckett, editors. Cambridge University Press, Cambridge. 159–177.

Tanaka, M., and T. Miki-Noumura. 1988. Stepwise sliding disintegration of *Tetrahymena* ciliary axonemes at higher concentrations of ATP. *Cell Motil. Cytoskeleton.* 9:191–204.

Warner, F. D. 1976. Ciliary inter-microtubule bridges. *J. Cell Sci.* 20:101–114.

Warner, F. D. 1983. Organization of interdoublet links in *Tetrahymena* cilia. *Cell Motil.* 3:321–332.

Woolley, D. M., and A. Brammall. 1987. Direction of sliding and relative sliding velocities within trypsinized sperm axonemes of *Gallus domesticus*. *J. Cell Sci.* 88:361–371.

Zanetti, N. C., and F. D. Warner. 1982. Evidence for a role of 13S axonemal ATPase in modulation of ciliary microtubule sliding. *Cell Motil.* 2:509–523.