STRUCTURAL CONFORMATION OF CILIARY DYNEIN ARMS AND THE GENERATION OF SLIDING FORCES IN *TETRAHYMENA* CILIA

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ABSTRACT

The sliding tubule model of ciliary motion requires that active sliding of microtubules occur by cyclic cross-bridging of the dynein arms. When isolated, demembranated *Tetrahymena* cilia are allowed to spontaneously disintegrate in the presence of ATP, the structural conformation of the dynein arms can be clearly resolved by negative contrast electron microscopy. The arms consist of three structural subunits that occur in two basic conformations with respect to the adjacent B subfiber. The inactive conformation occurs in the absence of ATP and is characterized by a uniform, 32° base-directed polarity of the arms. Inactive arms are not attached to the B subfiber of adjacent doublets. The bridged conformation occurs strictly in the presence of ATP and is characterized by arms having the same polarity as inactive arms, but the terminal subunit of the arms has become attached to the B subfiber. In most instances the bridged conformation is accompanied by substantial tip-directed sliding displacement of the bridged doublets. Because the base-directed polarity of the bridged arms is opposite to the direction required for force generation in these cilia and because the bridges occur in the presence of ATP, it is suggested that the bridged conformation may represent the initial attachment phase of the dynein cross-bridge cycle. The force-generating phase of the cycle would then require a tip-directed deflection of the arm subunit attached to the B subfiber.

KEY WORDS motility · cilia · microtubules · cross-bridges · dynein · ATP

Several independent studies confirm the sliding tubule mechanism of ciliary and flagellar motion (20, 21, 25); however, we as yet do not understand how the adenosine triphosphatase or dynein arms actually participate in the mechanochemical activity that must be responsible for linear displacement of microtubules. The displacement event clearly involves both the enzyme-mediated hydrolysis of ATP and the formation by the arms of intermittent cross-bridges between adjacent doublets (10, 12, 13). However, the relationship of the chemical events to the structural or molecular events is not understood. It is, of course, necessary that the dynein cross-bridges have the capability to alter their orientation in the bridged condition in order to cause sliding.

The dynein arms were recently found to consist of 3–4 morphologically similar subunits that in isolated form have a mean diameter of 93 Å and electrophorese as dynein 1 (26). The subunits, as a polymer, presumably have both an active site that can bind B subfiber tubulin and a region of conformational instability that will, in the correct

physiological environment, allow a change in the orientation of the dynein cross-bridge and thus move the attached B subfiber one step with respect to the A subfiber.

An important contribution to the continuing development of the sliding tubule model comes from Sale and Satir (19) who observed that active sliding in *Tetrahymena* cilia was a distally or tip-directed phenomenon, the net force being directed away from the proximally directed angle of the dynein arms. In effect, the B subfiber is pushed by the arms of the adjacent A subfiber away from the ciliary base. It can therefore be predicted that the net conformational alteration to the arms that causes this motion will occur in the same direction: towards the ciliary tip.

Measured values of sliding in bent regions of lamellibranch gill cilia have suggested a simple dynein cross-bridge mechanism based on a quantal sliding cycle (22, 23). Quantal sliding presumably reflects a quantal arm cross-bridging event but, because of an absence of prior data on the conformational states of the dynein arms, the model has had limited predictive capability.

In the present report, two conformational states of the arms are described as a function of the presence or absence of ATP. Isolated cilia from *Tetrahymena* were actively disintegrated in the presence of ATP which then permits high-resolution imaging of the dynein arms by negative contrast electron microscopy.

MATERIALS AND METHODS

Axoneme Disintegration

Cilia from the protozoan *Tetrahymena pyriformis* strain B-III were isolated and purified according to the detailed procedures reported previously (26).

In order to visualize the microtubule sliding phenomenon and associated dynein arm conformations, it was first necessary to allow the cilia to actively disintegrate in the presence of ATP (21). Isolated, demembranated cilia were resuspended in a solution consisting of 40 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (HEPES), 0.15 M KCl, 2 mM $MgSO_4$, 0.5 mM EDTA, and 1 mM dithiothreitol (DTT) at pH 7.8 (9). For *Tetrahymena* cilia, active sliding disintegration does not require external proteolysis. Purified cilia disintegrate spontaneously in the presence of 0.1 mM ATP (19, 26), although the amount of disintegration can vary between 20 and 100% in different preparations. Disintegration was monitored by both dark-field and phase-contrast light microscopy and spectrophotometry (350 nm).

Electron Microscopy

For negative contrast electron microscopy, ATP-induced, disintegrating cilia were observed by dark-field or phase-contrast microscopy and applied to a carbon-coated grid at the appropriate time, typically 2–10 min after the initial exposure to ATP. Axonemes were applied to the grid surface by touching it to a drop of the disintegrating cilia. The grid was drained, but not dried, and negatively contrasted with 2% aqueous uranyl acetate at pH 4.5.

Electron micrographs were taken at original magnifications of 60,000–100,000 on a Siemens Elmiskop 101 operated at 80 kV. Micrographs were calibrated by using three reliable internal standards for cilia: the 960-Å radial spoke repeat, the 240-Å dynein arm repeat, and the 160-Å central sheath repeat (4). All micrographs contain at least one and often all three of these periodic structures, and it is probable that the measured data are free from calibration error. Some micrographs were subjected to linear photographic translation in order to enhance the visible arm conformations. All translations were moved two arm periods which superimposes the image of one arm over the images of two adjacent arms.

OBSERVATIONS

Intact cilia from *Tetrahymena* that have not been exposed to ATP exhibit an intricate array of periodicities associated with the dynein arms, radial spokes, and central sheath. Favorable views of arms at the edges of the axoneme (Fig. 1*a*) reveal the uniformly tilted arms described below as the inactive conformation. However, once the axoneme has broken or frayed open, the apparently delicate and precise organization of the arms, spokes and sheath is seriously disturbed (Fig. 1*b*), and only rarely are unambiguous views of arms found in the inactive conformation (Fig. 1*c*). This may partially explain the general inability to preserve the arms for negative contrast electron microscopy. However, the simple addition of 0.1 mM ATP to the cilia appears to have a stabilizing effect on the subsidiary components of the axoneme, and particularly the arms, so that they become routinely visible in disintegrated preparations (cf. Figs. 1*b* and 3).

In most instances, dynein arm conformation was studied by allowing cilia to actively disintegrate in the presence of ATP (21). Although this phenomenon is now well characterized, *Tetrahymena* cilia are unusual in that they do not require the addition of a proteolytic enzyme to disrupt the constraining linkages within the organelle (19, 26). The cilia disintegrate in two ways or, more typically, combinations of both ways. The cilia

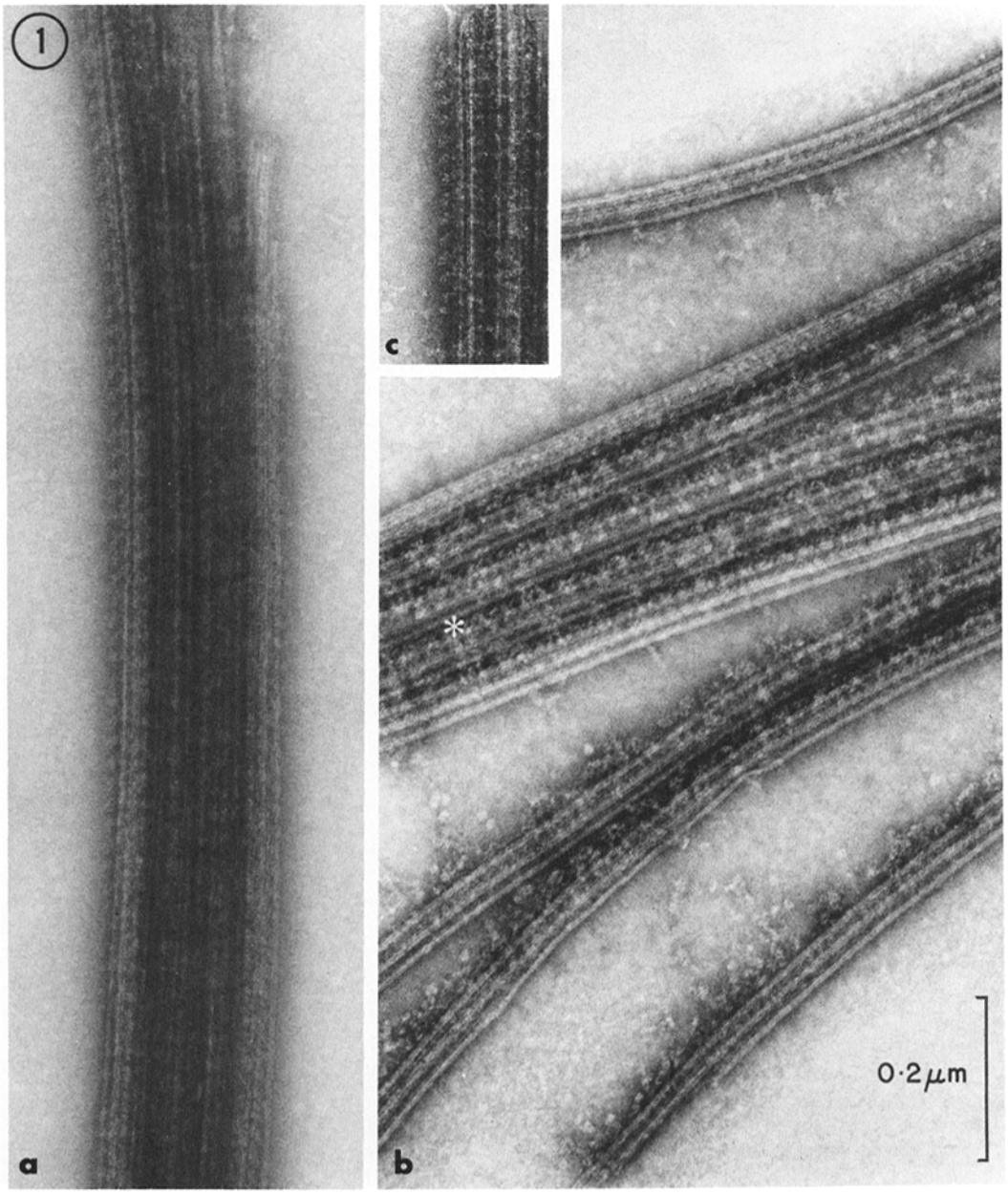

FIGURE 1 Isolated, negatively contrasted cilia from *Tetrahymena* that have not been exposed to ATP but have otherwise been processed in the same way as cilia shown in the remaining figures. Fig. 1 *a* shows part of an intact cilium where the inactive but uniformly tilted outer row dynein arms are visible at both edges of the axoneme. Fig. 1 *b* shows part of a cilium that has frayed open. The arms are visible along the A subfiber of each doublet, but they have a fuzzy, ambiguous appearance (*) that is caused, in part, by both their collapse and the apparent superimposition of the two arm rows. This appearance is characteristic of opened cilia that have not been reactivated with ATP. Fig. 1 *c* shows a doublet from a frayed cilium where the uniformly tilted arms are well resolved. × 115,000.

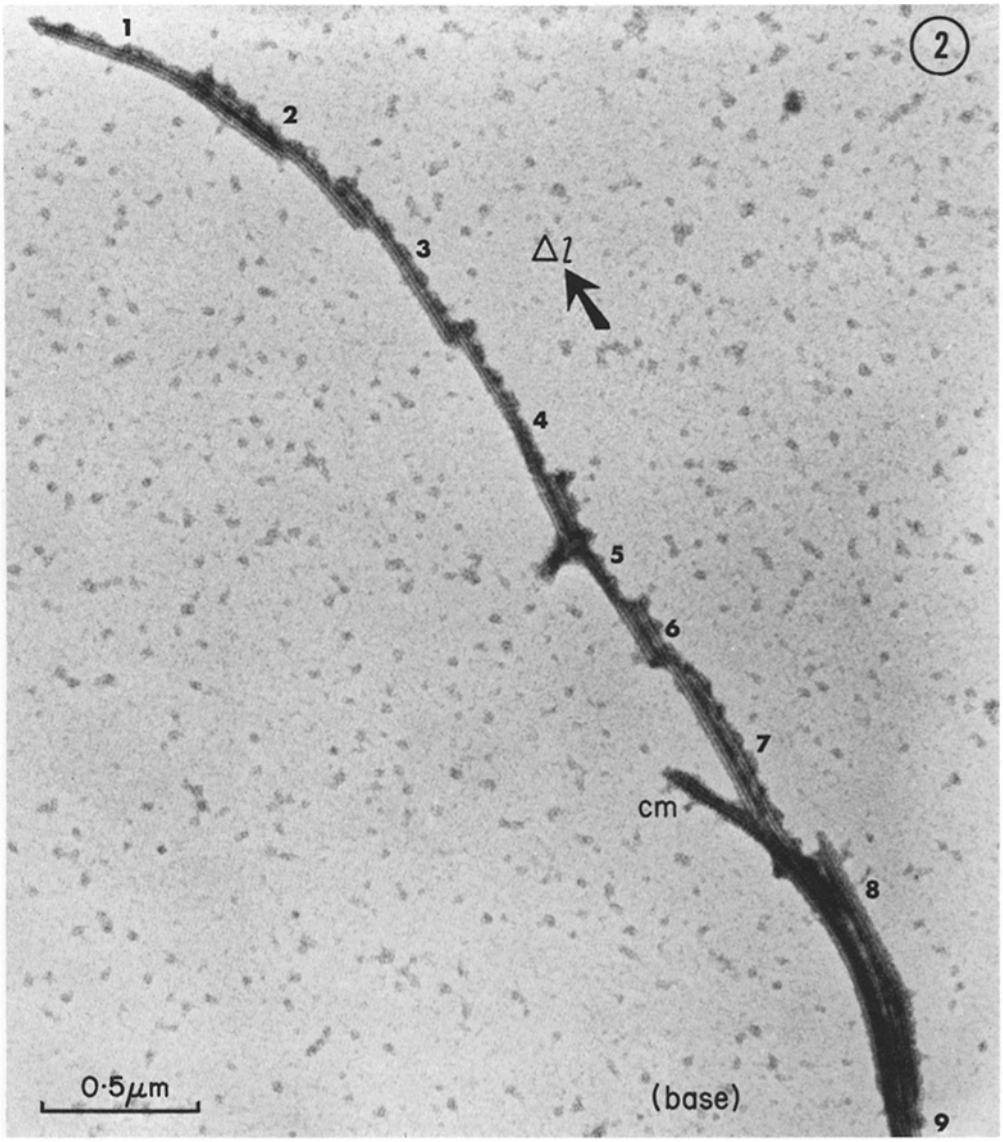

FIGURE 2 A short segment of a *Tetrahymena* cilium that has actively disintegrated (doublets 1–9) in the presence of ATP to about × 7.5 its original length. The sliding (Δl) is tip directed although, subsequent to disintegration, most of the dynein arms have been solubilized from the individual doublets. Note the variable overlap remaining between successive doublets. The central pair microtubules (*cm*) remain near the proximal portion of the axoneme. × 42,000.

can slide apart in the now familiar telescoping phenomenon (Fig. 2) or they can simply fray apart on the grid supporting film (Fig. 3). The amount of disintegration can vary from 20 to 100% in different preparations, although it is not possible to identify the factor causing this variability. Broken segments of cilia, 0.5–2 μm long, are the most useful for demonstrating active sliding and dynein arm conformations. However, once the cilia have disintegrated, the arms can be very labile and sometimes are entirely absent from the doublets. For example, Fig. 2 shows a short segment of an axoneme that has telescoped to about 7.5 times its original length, and yet all arms are missing from the microtubules. Those micrographs where sliding (Δl) has actually oc-

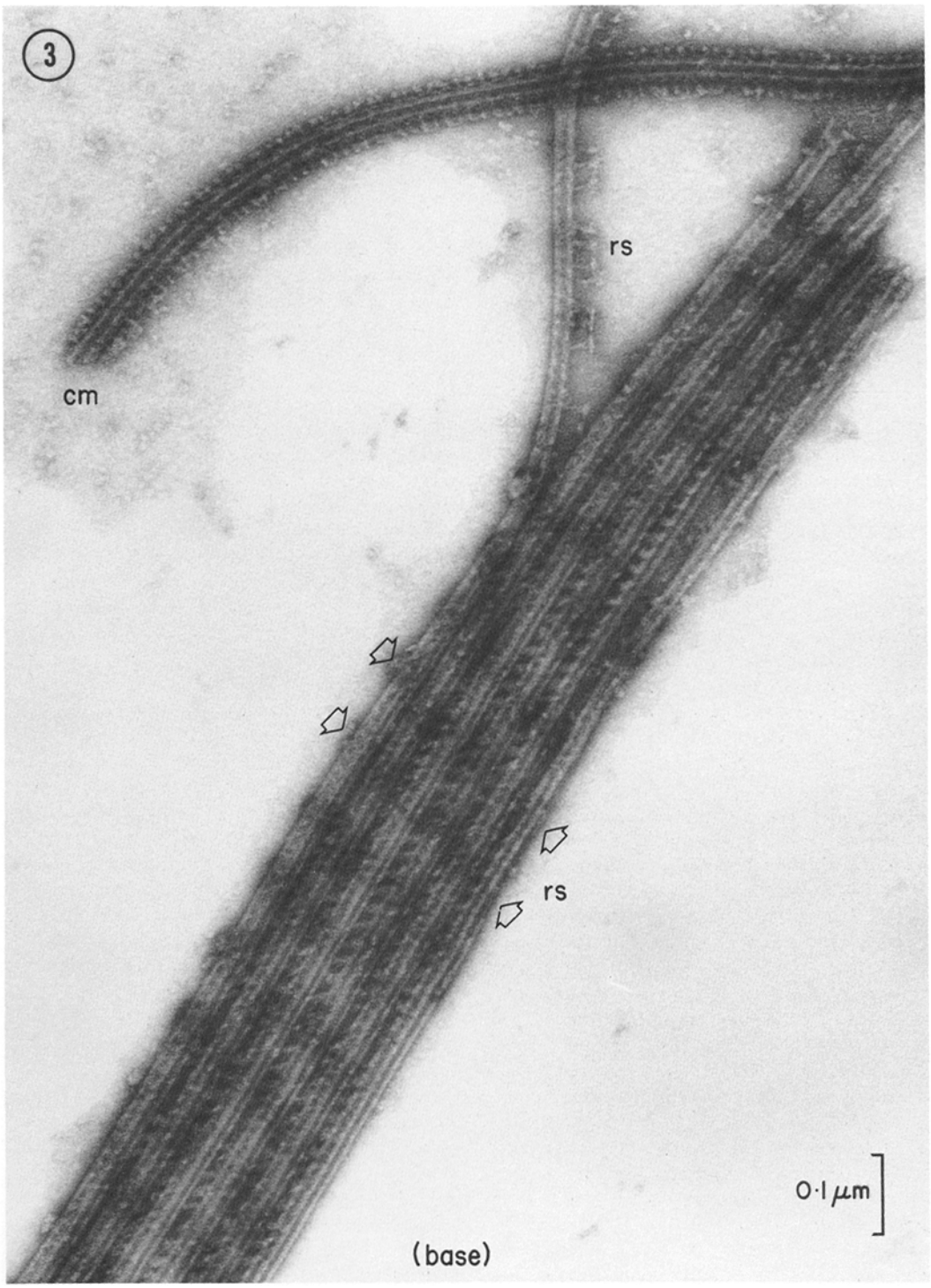

FIGURE 3 A portion of a negatively contrasted *Tetrahymena* cilium that has frayed apart in the presence of ATP. The axoneme base is positioned at the bottom of the figure, as defined by the radial spoke spacing (*rs*) and base-directed polarity of the dynein arms (240 Å period). The arms are predominantly in the bridged conformation. The arrows denote the banding pattern (960 Å period) caused by stain accumulation near the radial spokes. A single microtubule of the central pair (*cm*) and its attendant sheath projections (160 Å period) has fallen away from the doublet microtubules. The position of view is from inside the axoneme looking out (subfiber A and arms to the left side of the doublets). × 125,000.

curred, as defined by Sale and Satir (19), are so-designated on the plate (as in Fig. 2). The remaining micrographs are of axonemes that showed combined sliding and fraying (as in Fig. 3).

By convention, all axonemes in the figures are oriented with the cilium base nearest the bottom of the page and with the position of view being inside the axoneme looking out (arms to the left side of the doublet). The markers for determining this orientation (Fig. 3) (spoke spacing, arm polarity, and enantimorphism) are now well established (19, 25, 26).

Dynein Arms: The Inner and Outer Rows

The dynein arms along doublet microtubules have two different general appearances when viewed by negative contrast electron microscopy. The appearances seem to be associated with the inner and outer rows of arms. The first appearance, described below as the inactive conformational state, is typified by an orderly, unambiguous row of arms, uniformly tilted and consisting of three subunits with a uniform thickness along the arm length (as in Fig. 4*a* and *b*). The second appearance is typified by arms having an ambiguous though uniform tilt and an apparent thickening near the terminal region (Fig. 4*c*) so that the subunit nature of the arm is generally obscured. These arms are often associated with amorphous material that may also obscure their otherwise uniform periodicity along the subfiber.

The two appearances often occur in the same axoneme. For example, Fig. 5 shows a partially telescoped axoneme where arm appearance 1 is found along the top doublets and appearance 2 along the bottom doublets. The two appearances are not necessarily related to the position of view, as Fig. 5 might suggest; but both can be present when arms are viewed from relative positions either inside or outside the axoneme.

Inasmuch as the two appearances occur in both the presence and absence of ATP, in both bridged and unbridged arms, and with approximately equal frequencies, it is suggested that each represents a given row of arms. Furthermore, since the arms that are visible at the edges of intact cilia have appearance 1 (Fig. 1*a*) and since these arms can only be outer row arms, it is concluded that appearance 1 represents the outer rows of arms and that appearance 2 must therefore represent the inner rows of arms. All following observations and data are from arms having appearance 1 (Fig. 4*a* and *b*) even though most micrographs show both appearances. It is specifically assumed that the two appearances do not represent different conformational states associated with a common cross-bridge cycle (see below).

Inactive Conformation

Traditional images of cilia and flagella have shown the dynein arms in the inactive (unbridged) or nonmotile state. This conformation presumably reflects a regulatory inhibition of the potential dynein-subfiber B interaction. Negative contrast preparations of both intact and frayed cilia not exposed to ATP (Fig. 1), as well as ATP-disintegrated cilia, reveal extensive regions along doublets where the unbridged or inactive conformation of the arms is visible. For ATP-disintegrated cilia the unbridged condition is found along single doublets where the arms are not in proximity to another doublet (as in Fig. 4). As described previously (26), the arms repeat at 240 Å along subfiber A and are uniformly tilted towards the base of the cilium. This polarity is characteristic of all nine doublets under all observed conditions as illustrated by Figs. 1, 3, 5, and 6.

The arm angle is uniform over the entire field of view along a particular doublet or between adjacent doublets in the same axoneme (Figs. 3–6). The mean angle of inclination from the perpendicular is 32° ± 4° SD for all groups of arms that have been measured. For regions along individual doublets, however, the mean angle has a range of 27–38° which is interpreted as normal variation resulting from differences in the orientation of doublet collapse on the carbon supporting film. For example, Fig. 7*a* and *b* shows a doublet along which the arms have a mean tilt of 28°, Fig. 7*c* and *d* shows arms that have a mean tilt of 37°, and Fig. 9 shows arms that have a mean tilt of 33°. It is concluded that these variations have no physiological significance.

As described previously (26), the subunit composition of unbridged arms is routinely visible. When viewed in longitudinal doublet profile, single arms can be seen to consist of three morphological subunits (Fig. 7) that have a center-to-center spacing of 78 ± 2 Å SD and lie in a common plane that forms the inclination angle. Individual arms have an overall length of 186 ± 5 Å SD measured at the perpendicular and 238 ± 11 Å SD measured on the incline. Although the three subunits of the arms are generally well-resolved, as illustrated by Fig. 7, we cannot ex-

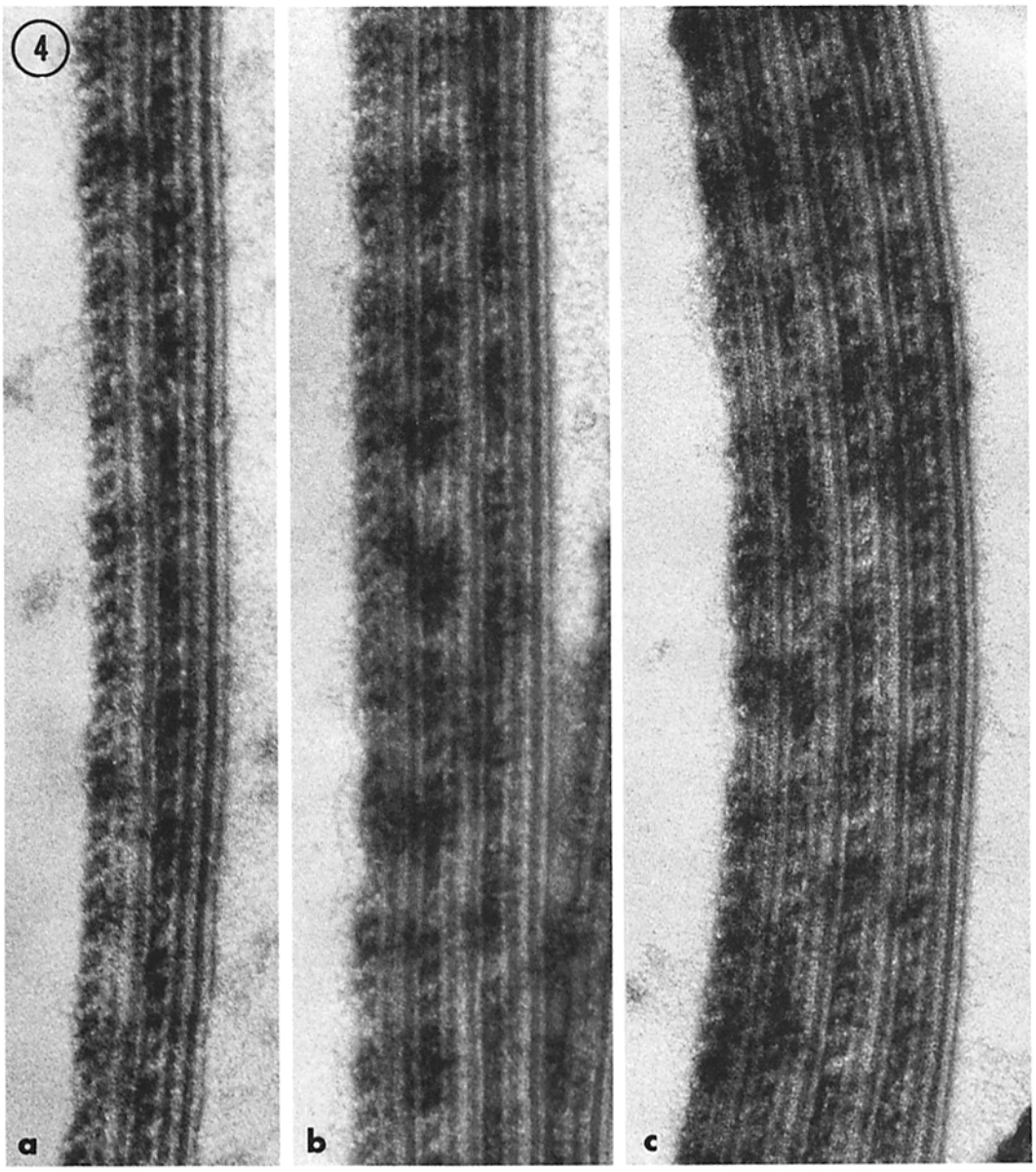

FIGURE 4 Doublet microtubules and dynein arms from regions of ATP-disintegrated cilia. The polarity of the arms is base-directed and those arms along the free doublets (Figs. 4*a* and *b*) are in the inactive conformation and have appearance 1 which is associated with the outer rows of arms. Those arms in Fig. 4*c* have appearance 2 which is associated with the inner rows of arms. The three subunits of the arms are particularly clear in Figs. 4*a* and *b*. × 180,000.

clude the possibility that an additional arm subunit may be concealed by proximity to the A subfiber. It appears that the three arm subunits may represent the three structural segments of the arm that characteristically appear in transverse axoneme sections (2), although when seen in transverse profile the three segments clearly lie in different planes.

In summary, the inactive or unbridged arm conformation occurs in the absence of ATP but can

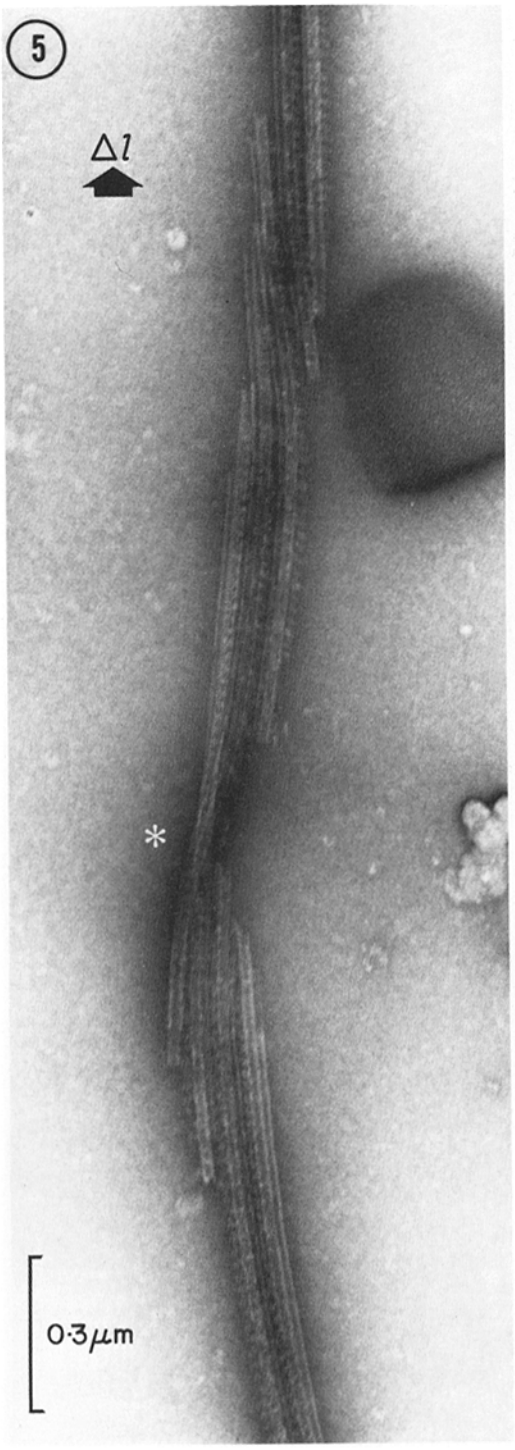

FIGURE 5 A short segment of a cilium that has partially disintegrated (Δl) in the presence of ATP. Note the constant base-directed arm polarity along the seven doublets in the field of view. Because of a twist in the axoneme (*) those doublets at the top of the figure are viewed from outside the axoneme looking in (and arm appearance 1), whereas those doublets at the bottom of the figure are seen from inside the axoneme looking out (and arm appearance 2). × 68,000.

often be found in ATP-disintegrated cilia along random doublets that lie free from potential interaction sites on the B subfiber.

Bridged Conformation

Only a single major structural distinction can be made between the inactive and bridged arm conformations: the arms in the bridged conformation are attached to the adjacent B subfiber when the cilium is allowed to disintegrate in the presence of ATP (Figs. 3, 6, and 8–10). Arm polarity is identical to the inactive conformation. Bridged arms have a mean inclination of 32° ± 3° SD from the perpendicular and show the same restricted range of mean tilt variability as the inactive arms.

In the bridged conformation, the three arm subunits are only occasionally resolved, although this appears to result from excessive stain accumulation in the trough formed by adjacent doublets. The terminal subunit of the arm appears to be attached to protofilament(s) in the B subfiber (Fig. 10). In the bridged condition, the mean interdoublet spacing is 163 ± 5 Å SD and may be as little as 120 Å, remembering that the arms are actually attached off-center. The mean spacing represents a decrease of 14.2% from the interdoublet spacing of 190 ± 4 Å SD found between unbridged doublets of sectioned cilia. This spacing decrease is consistent with the 13.2% decrease in rigor axoneme diameter reported by Gibbons (13). The bridged arms appear to occur for considerable distances along single doublets (Figs. 3 and 8) and they also occur between several successive doublets within a single axoneme (Figs. 6 and 8). It should be kept in mind, however, that only a few of the arms may actually be bridged to the B subfiber, but these arms produce a structural condition that looks as if all arms are bridged.

The following criteria are used to establish that the arms are bridged to the B subfiber rather than simply being in close spacial proximity. (*a*) The doublet microtubules are clearly free to slide apart and typically do so in the presence of ATP (as in Figs. 2, 5, and 6), apparently having lost the constraints that otherwise hold them together.

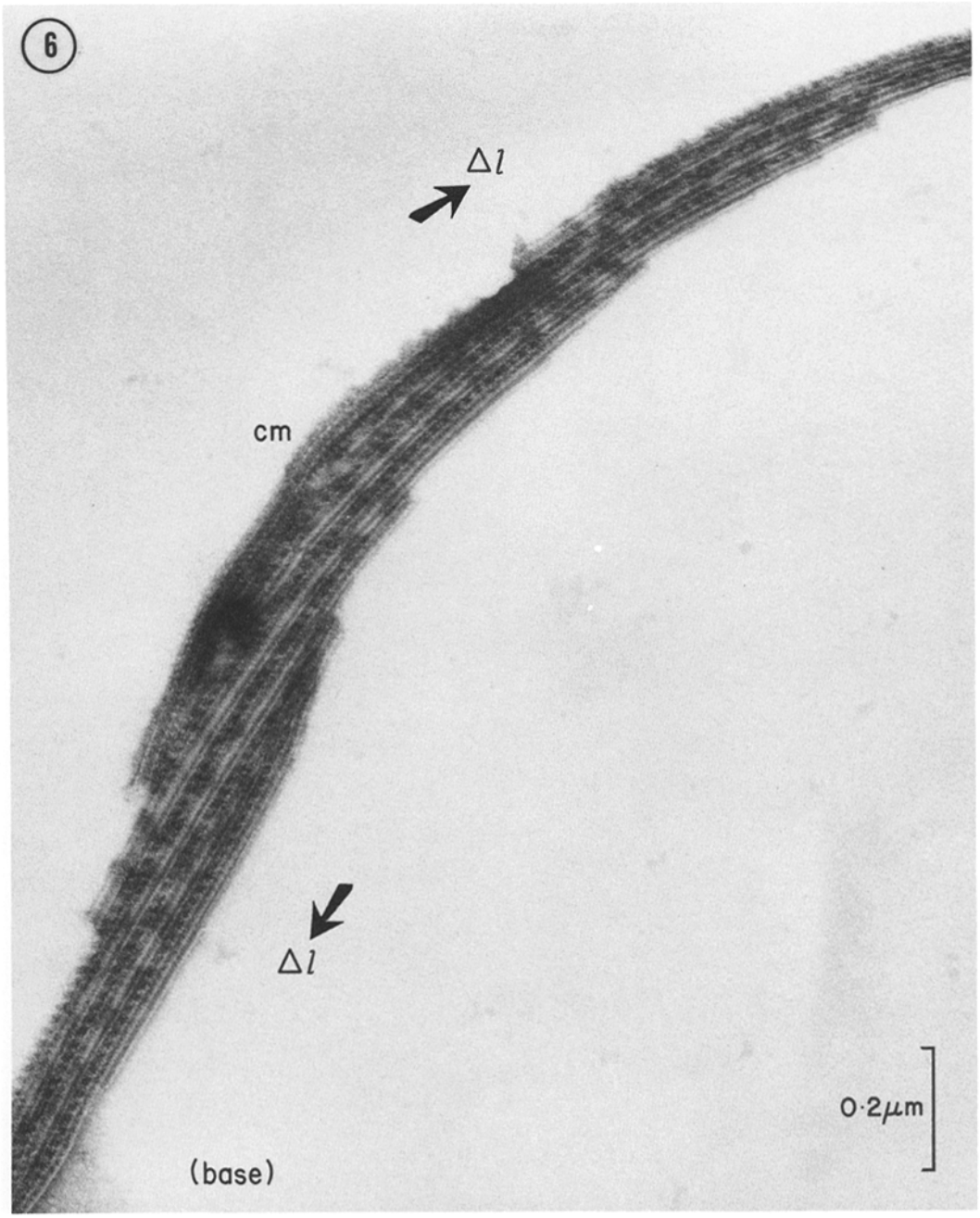

FIGURE 6 A short segment of a cilium that has partially disintegrated (Δl) in the presence of ATP. The arms exhibit a constant base-directed polarity along the seven doublets visible in the field and are predominantly in the bridged conformation. The central pair microtubules (*cm*) are positioned midway along the telescoped axoneme. As marked by the position of the central pair, sliding appears to have occurred in both tip and base directions (see text for explanation). × 83,000.

(*b*) The interdoublet spacing is uniform and does not exceed 163 Å which is somewhat less than the length of the unbridged arms. (*c*) Images are seen where the arms remain attached to protofilaments that have been pulled out of the B subfiber (Fig. 10*b*). (*d*) Arms having the bridged confor-

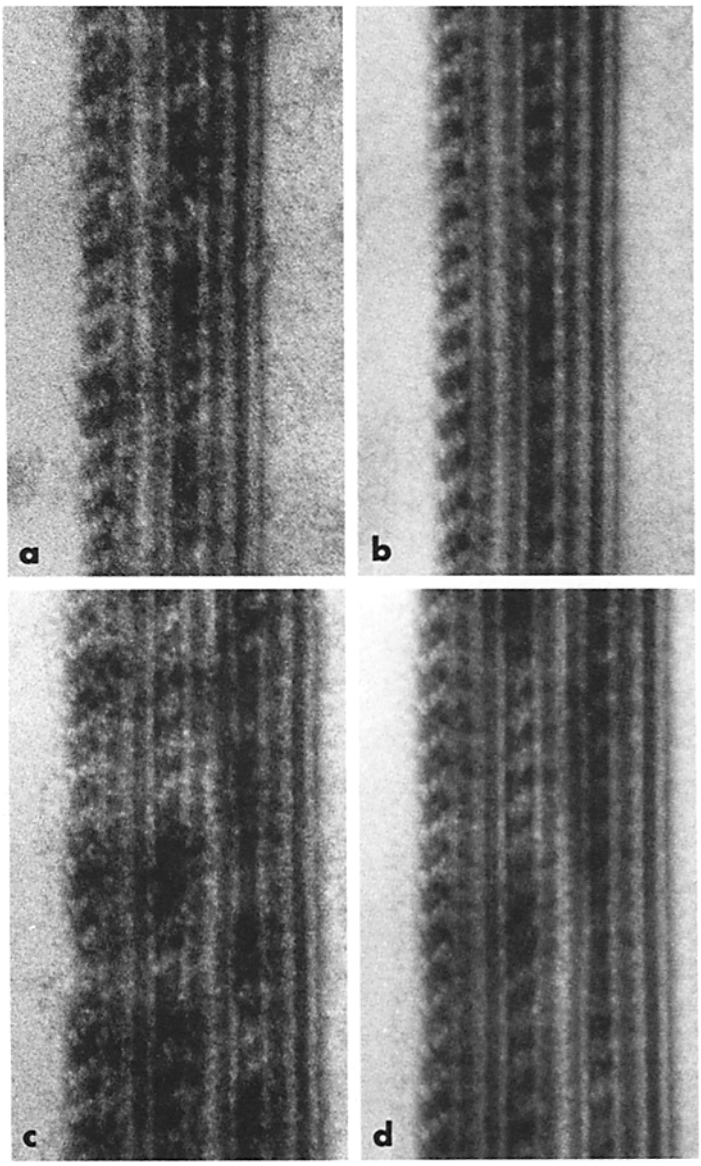

FIGURE 7 Short regions of ATP-disintegrated doublets where the free arms are in the inactive conformation. The three structural subunits of the arms are particularly well-resolved. Fig. 7*b* is a two-step linear translation of Fig. 7*a* while Fig. 7*d* is a translation of Fig. 7*c*. In Fig. 7*a* the mean inclination angle of the arms is 28° from the perpendicular, whereas in Fig. 7*c* the mean angle is 37°. The three arm subunits are spaced at 78 Å center-to-center. × 225,000.

mation are never seen in cilia that have not been exposed to ATP (Fig. 1).

The arms of ATP-disintegrated axonemes, however, are not always bridged to an adjacent B subfiber. In some instances the arms have been solubilized (Fig. 2), in which case the otherwise uniform interdoublet spacing is disrupted. In other infrequent instances, the bridged state appears to have relaxed since a uniform opaque region is present between the end of the arms and the B subfiber (Fig. 5).

It is presently not possible to equate the bridged arm conformation to the bridged arms described by Gibbons (13) as resulting from flagellar rigor

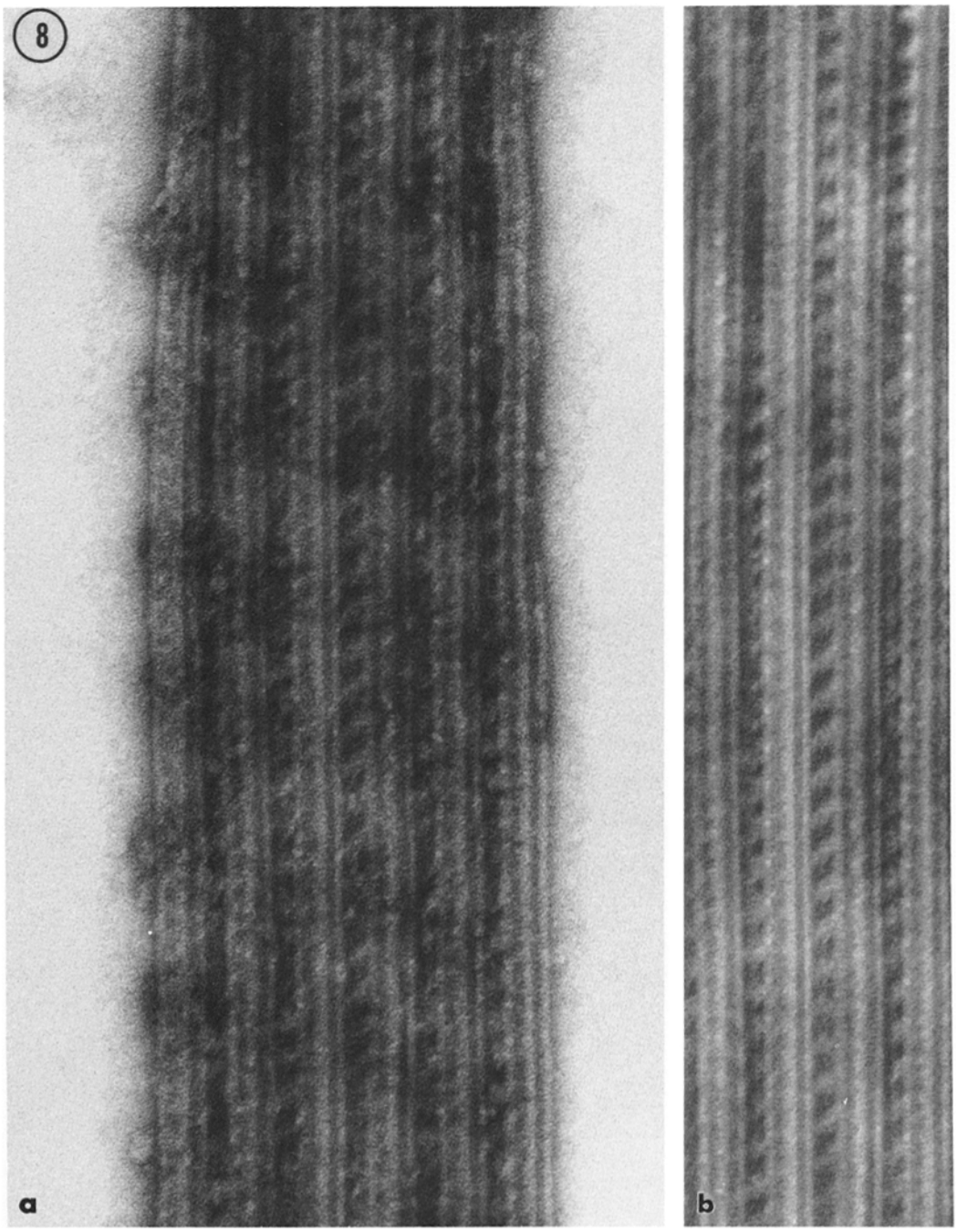

FIGURE 8 A region of an ATP-frayed cilium where the arms are predominantly in the bridged conformation but have both appearances 1 and 2 described in the text. Fig. 8*b* is a two-step translation of Fig. 8*a*. × 225,000.

induced by ATP depletion. ATP is necessary to induce the bridged conformation in *Tetrahymena* cilia, but it has not been intentionally depleted, although this may be the net effect of adding the aqueous negative stain to the disintegrating axonemes. However, use of either the maximal (1

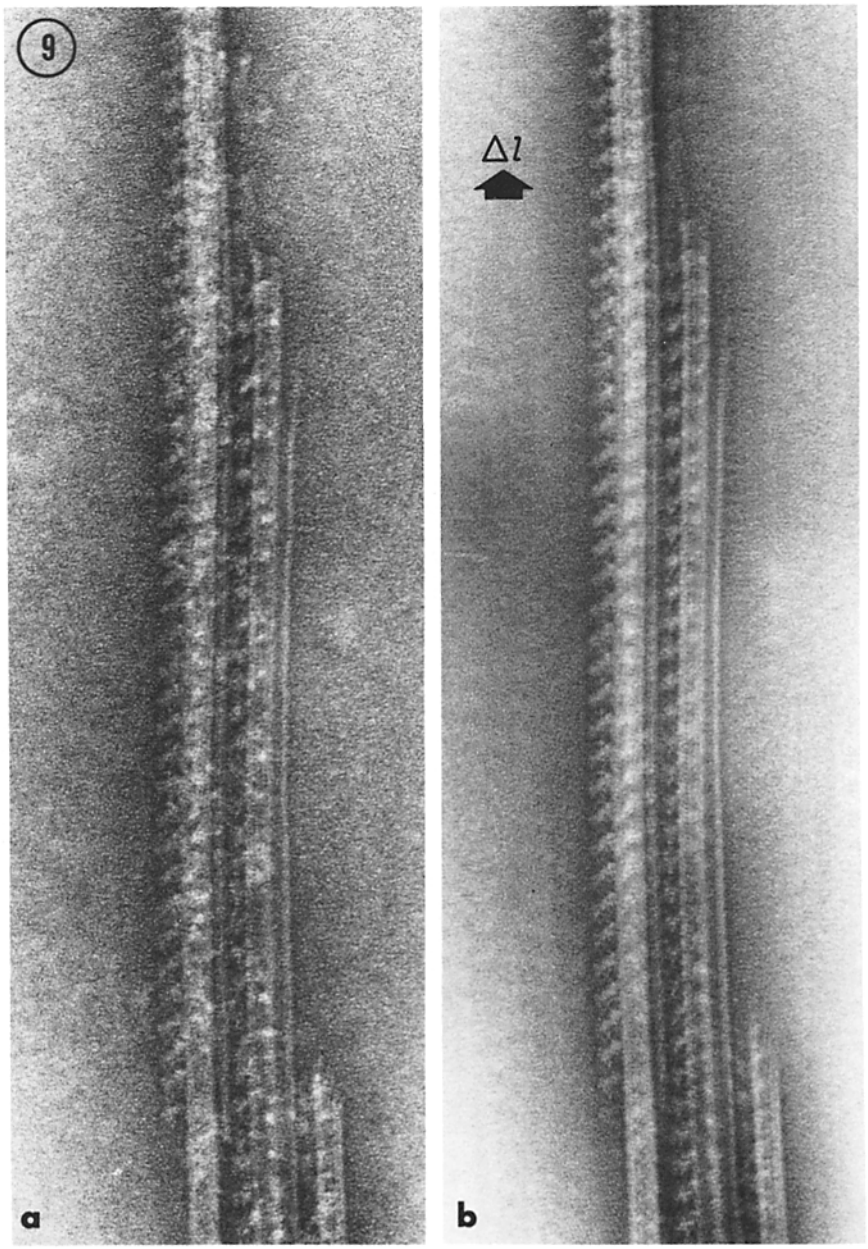

FIGURE 9 The overlap regions of three doublets that have disintegrated (Δl) in the presence of ATP. The free outer row arms are in the inactive confirmation (33° mean inclination angle), whereas the other arms are in the bridged conformation, but have appearance 2 associated with the inner rows of arms. Fig. 9*b* is a two-step translation of Fig. 9*a*. By sighting down the left doublet in Fig. 9*b*, the inner row of arms can be seen superimposed over the A subfiber. × 160,000.

mM) or minimal (0.002 mM) ATP concentrations necessary to disintegrate the axonemes does not affect the observed arm conformations when subsequently diluted by the negative stain.

In conclusion, it should be emphasized that ATP-related cross-bridged arms show no detectable changes in either polarity or inclination angle when compared with inactive or unbridged arms,

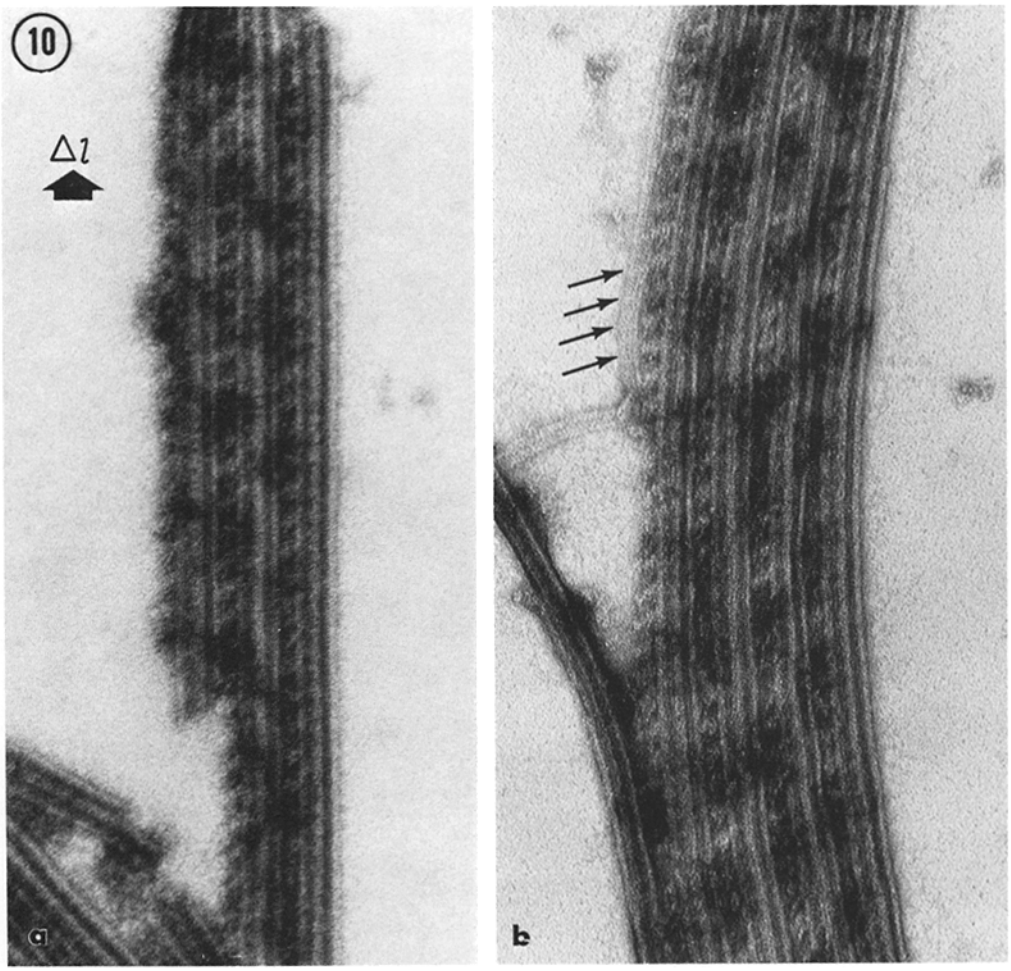

FIGURE 10 Doublet microtubules from ATP-disintegrated (ΔI) cilia. Fig. 10*a* includes an overlap region where the arms along the middle doublet have appearance 1 in the bridged conformation. Fig. 10*b* shows a ribbon of four protofilaments that has been pulled out of the B subfiber but remains attached to the adjacent arms in the bridged conformation (arrows). × 160,000.

although the terminal subunit of the bridged arms has become attached to the B subfiber. The bridged condition has never been observed in arms that have not been exposed to ATP. The two dynein arm conformations are examined below in the general context of the ciliary sliding tubule mechanism and by comparison with the actin-myosin based interactions of striated muscle.

DISCUSSION

Dynein arms from *Tetrahymena* cilia have two detectable conformations when viewed by negative contrast electron microscopy. The conformations appear to be related to the presence or absence of ATP or motion. The inactive or unbridged arm conformation is generally found in nonmotile axonemes but can also occur in the presence of ATP when the arms are not in proximity to binding sites on the B subfiber. Inactive arms are characterized by a uniform, base-directed tilt of 32°. The bridged arm conformation is observed only in the presence of ATP and is also characterized by a 32° base-directed tilt. However, the terminal subunit of the arms has become attached to protofilaments in the B subfiber.

We have suggested that the two observed arm conformations represent structural manifestations of the temporal force-generating mechanism in cilia and thus are part of a mechanochemical

cross-bridge cycle. As such, the arm conformations demonstrate the accessibility of dynein-tubulin interactions to direct investigation by high-resolution optical techniques.

The inactive arm conformation is similar to the brief descriptions of arms that have appeared sporadically in the literature (1, 8, 17, 24), although the detailed structure of both the arms and the dynein molecule has remained an enigma. However, the recent description of the dynein molecule and 3–4 subunit arms (26) demonstrates that dynein is not structurally analogous to the muscle protein myosin. It might also be expected that dynein is not functionally analogous to myosin, apart from the fact that both proteins are ATPases that participate in mechanochemical events.

The muscle term "rigor" has recently been applied to flagella whose active beating and bend form were arrested by depletion of the ATP supply. When examined by thin-section microscopy, the rigor axonemes were found to have their arms in a cross-bridged configuration (11, 13). However, in the context of the present observations, the use of the term rigor as potentially applied to the bridged arm conformation must be questioned. As derived from the interactions of the muscle proteins actin and myosin, the rigor state is defined by its resistance to sliding resulting when myosin cross-bridges are unable to dissociate when the ATP supply is depleted. As such, rigor cross-bridges represent the end result of the force generating phase of the cross-bridge cycle and are the equilibrium state for the attached cross-bridge in muscle. Although the initial attachment phase of the cycle is not detectable by optical techniques, it is presumed that attachment is reflected by a nearly perpendicular orientation of the myosin Sl subunit relative to the actin thin filament. The force generating step then requires a myosin subunit deflection away from the perpendicular. This deflection occurs in the direction of sliding or towards the center of the sarcomere when the myosin thick filament is used as the reference line.

In contrast, however, intrinsic or inactive dynein arm polarity is directed towards the base of the cilium at an angle of 32°. This polarity occurs along both rows of arms and all nine doublets and is opposite to the direction of force generation required for sliding in these cilia (19) when the A subfiber is used as the line of reference. In both the presence and depleted absence of ATP, this polarity is unchanged, although the terminal subunit of the arm is then found attached to the B subfiber. Moreover, the bridged conformation not only occurs in the presence of ATP and sliding, but also is found in regions of ATP-related fraying where no detectable sliding has occurred (as in Fig. 8). These observations suggest that dynein bridges, unlike muscle rigor bridges, are initiated by the presence of ATP and represent the first phase of a cross-bridge cycle. The bridged conformation in these cilia should, therefore, not be equated with rigor. However, since the ATP-disintegrated cilia were not actively beating, it can only be assumed that the bridged conformation in *Tetrahymena* cilia is the same as the "rigor" bridged arm conformation observed by Gibbons (13). Our observations further suggest that the base-tilted dynein bridges represent the equilibrium state for the attached cross-bridge and that there must be a rate-limiting step that converts the bridged arms into a transient energized state which produces force.

At the present time it is difficult to envision how a three-subunit dynein arm can undergo the conformational change necessary to produce force and cause sliding, particularly when all three subunits of the arm appear to electrophorese as dynein 1 (26). It may be that the three-subunit arm is an oversimplified interpretation of arm substructure and that additional, perhaps nonsymmetrical regions of either the arm or the dynein molecule await discovery. However, it is to be expected that the conformational change reflecting the force-generating step of the dynein cross-bridge cycle will occur in a direction that is opposite to the intrinsic polarity of the arms and will involve the arm subunit that is attached to the B subfiber. Active sliding was recently shown to be strictly quantal in bent regions of lamellibranch gill cilia, presumably reflecting a quantal cross-bridge cycle, and yet the incremental value measured was only 14 Å (22, 23). However, the value was strictly dependent upon the standard against which sliding was being measured, which in that instance was the radial spoke period along the A subfiber. Given the nature and periodicities of the tubulin latttice (3), as well as measured values for sliding as related to bending (22), it can be predicted that the dynein cross-bridging event is likely to be in the range of 40–80 Å.

It is difficult to generalize the ATP-induced disintegration phenomenon to the regulated bending of actively beating cilia. It may simply be that

since all extrinsic regulatory mechanisms are apparently nonoperational in disintegrating cilia, in the presence of ATP all arms become activated and sliding begins along that doublet which has the least remaining physical restraints against sliding. This would then be followed by sliding of the next doublet in a counterclockwise number sequence, resulting in tip-directed sliding along the path of minimal structural resistance. Alternatively, if sliding in a beating cilium is actually initiated along a specific doublet, then activation of disintegrating cilia may likewise be initiated at the same site.

It is important to note that dynein bridging, as typified by the bridged conformation, apparently can occur simultaneously between several if not all of the doublets. Within a given region of a frayed or telescoped cilium, all arms can appear to be in the bridged conformation (as in Figs. 3, 6, and 8). This suggests that all arms may become activated in the presence of ATP and that controls to initiate the general sliding phenomenon may be extrinsic to the basic cross-bridge mechanism. This conclusion is consistent with Gibbons's (13) micrographs of rigor flagella since the arms between all nine doublets appear to remain in a bridged configuration. Simultaneous bridging suggests that active sliding may be a two-part phenomenon. The first part would involve the simple formation of the bridge while the second part would involve a regulatory signal to activate the conformational alteration to the bridge necessary to cause sliding. Brokaw (5, 6) has suggested that dynein cross bridges may exhibit self-oscillating behavior. The present observations fit well with an oscillating type of cross-bridge mechanism. An oscillating mechanism could explain why, in the presumed absence of the load or external regulatory activity required to produce oscillation, the bridged conformation is retained in the presence of ATP.

We have previously demonstrated that cumulative, but not necessarily active sliding was restricted to bent regions of lamellibranch gill cilia and that, within the bend, sliding was tip-directed (25). The intrinsic base-directed arm polarity and predominantly tip-directed sliding observed in *Tetrahymena* cilia (19), under all conditions, strongly implies that force generation in these cilia occurs only in one direction: toward the ciliary tip. However, it is then necessary to postulate the existence of extrinsic controls capable of regulating the form, direction, and propagation of the bend, as well as controls regulating the recovery phase of the beat cycle.

Although force generation appears to occur only towards the ciliary tip, the required variability in the direction of overall ciliary motion could be provided by those doublets which are activated to slide or by those doublets which are prevented from sliding by the activation of external structural or regulatory constraints. For example, the direction of the ciliary beat is apparently under external regulatory control in *Paramecium* and *Mytilus* cilia. The application of electrical or chemical (Ca^{++}) stimuli causes the beat cycle of the cilia to reverse direction (18). This implies that different sectors of the axoneme may be capable of responding to a signal to activate sliding independent of other sectors (7), which if true, would provide a simple means for controlling both the direction of the ciliary beat and the effective-recovery phases of the beat cycle.

Although most cilia and flagella propagate a tip-directed wave, flagella from *Crithidia* normally propagate a base-directed wave that can be reversed to a tip-directed wave by application of external physical constraints (14, 15, 16). This phenomenon seems to imply that, at least in this species, force generation can be bipolar. However, it is necessary to distinguish between the absolute direction of force generation as provided by the dynein cross-bridge cycle and the eventual direction of active sliding. Consider two doublets of identical mass, suspended in solution with equal viscous resistance from all directions. Although dynein-mediated force generation may be towards the doublet tip, sliding would be expected to occur equally in both directions away from the midpoint of the doublets if all restrictions against sliding were absent. That is, the eventual direction of sliding, independent of a single direction of force generation, could be controlled by the location of resistance to sliding. In this instance, by restraining the doublet with the free arms, sliding would become base-directed, whereas by restraining the doublet with the bridged arms, sliding would become tip-directed.

In support of this reasoning, Fig. 2 shows a segment of a cilium that has telescoped in the tip direction, with the central pair remaining near the original base of the organelle. Fig. 6, however, shows a segment that appears to have telescoped in both tip and base directions as marked by the midway location of the central pair, implying that no physical restrictions against base-directed

sliding existed in this particular axonemal segment. Alternatively, it may be that both arm polarity and thus the direction of active sliding are normally reversed in some species. For example, Allen and Borisy (1) have indicated that the dynein arms of *Chlamydomonas* flagella have a reverse or tip-directed polarity. In the context of the present observations, it is to be expected that a tip-directed arm polarity would generally result in base-directed active sliding in model flagella having no structural or regulatory restrictions against sliding in that direction. Thus, it is not strictly necessary to postulate the existence of a bipolar force-generating mechanism although it remains to be determined whether such a mechanism might actually occur in some flagella.

In summary, the two observed dynein arm conformations as they relate to tip-directed active sliding in *Tetrahymena* cilia suggest that sliding must result from a conformational change to the otherwise base-directed arms. It is predictable that this change will occur in a direction that is opposite to the intrinsic polarity of the arms, and, since it can be expected to occur when the arms are attached to the B subfiber, the resultant force vector and sliding in this species will be directed towards the ciliary tip.

Our present understanding of the chemistry of motility-coupled dynein activity does not permit a correlation between the structural and chemical events of a potential cross-bridge cycle. It is not known what event initiates the formation of the dynein cross-bridges and what event induces the conformational change that must represent the force-generating phase of the cycle. ATP and cations are clearly related to cross-bridging activity, but the way in which they function during the cycle remains to be determined. Unlike the situation in myosin cross-bridging, however, the presence of ATP appears to be required for preservation of the attachment of the terminal subunit of the dynein arms to the B subfiber. Our observations suggest that dynein-tubulin interactions are very different from myosin-actin interactions, and they may partly explain the general inability to demonstrate spontaneous dynein binding by either the B subfiber or solubilized tubulin.

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