Polarity of Dynein-Microtubule Interactions in Vitro: Cross-bridging between Parallel and Antiparallel Microtubules

F. D. WARNER and D. R. MITCHELL
Department of Biology, Biological Research Laboratories, Syracuse University, Syracuse, New York 13210

ABSTRACT Ciliary doublet microtubules produced by sliding disintegration in 20 μM $MgATP^{2-}$ reassociate in the presence of exogenous 30S dynein and 6 mM $MgSO_4$. The doublets form overlapping arrays, held together by dynein cross-bridges. Dynein arms on both A and B subfibers serve as unambiguous markers of microtubule polarity within the arrays. Doublets reassociate via dynein cross-bridges in both parallel and antiparallel modes, although parallel interactions are favored 2:1. When 20 μM ATP is added to the arrays, the doublets undergo both vanadate-sensitive and insensitive forms of secondary disintegration to reproduce the original population of doublets. The results demonstrate that both parallel and antiparallel doublet cross-bridging is sensitive to dissociation by ATP even though normal ciliary motion depends strictly on dynein interactions between parallel microtubules.

Potential use of ciliary or flagellar dynein as a morphological probe of microtubule polarity (9) has been encouraged by recent studies describing polarized dynein decoration of both ciliary doublet microtubules and neuromicrotubules (8, 16). Microtubules possess intrinsic molecular polarity because of directional distribution of the α and β tubulin subunits (2). Polarity is reflected in the assembly process which occurs preferentially at the distal or plus (+) end of microtubules (1, 3, 4), generally characterized as the end not embedded in the organizing center. Kirschner's (10) analysis of microtubule assembly suggests that all microtubules within the cell may have the same polarity with respect to the organizing site.

Dynein-microtubule organization in cilia is polarized and force generation apparently occurs only in one direction: towards the distal end of the axoneme and away from proximally directed tilt of the dynein arms (15, 17). Because both A and B subfibers of doublet microtubules are assembled at the distal (+) end (5), potential exists for interaction only between microtubules whose polarity has the same (or parallel) orientation, assuming that the assembly end characterizes true molecular polarity of both the A and B subfibers. In contrast, early models for sliding filament mechanisms in the mitotic apparatus required that force producing cross-bridge activity occur between microtubules whose polarity had opposite (or antiparallel) orientation, for example, between kinetochore and centrosomal microtubules of each half-spindle (6, 11, 12). However, recent studies on spindle tubule polarity suggest that tubules of each half-spindle have parallel rather than antiparallel orientation (20, 21).

When considering the use of dynein as a probe of microtubule polarity in nonciliary systems, several features of dynein-microtubule interactions in cilia must be kept in mind. Ciliary microtubules possess two classes of dynein binding sites (13). One site is insensitive to substrate ($MgATP^{2-}$) for the dynein ATPase. It is found predominantly along the A subfibers but also occurs along B subfibers and both central pair microtubules. A second site is sensitive to ATP and it occurs predominantly along B subfibers. Each site apparently interacts with a different end of the dynein arm and, hence, the arm also may have both chemical and morphological polarity. Individual neuromicrotubules also have both ATP-sensitive and insensitive classes of dynein binding sites. Using the morphological polarity provided by flagellar dynein arms recombined with neurotubules, Haimo et al. (8) found that ATP-sensitive dynein cross-bridging occurred but only between parallel tubules, similar to the situation occurring in intact cilia but obviously lacking potential differentiation imparted to cilia by the presence of the A and B subfibers. Because both classes of dynein binding sites apparently can occur along individual microtubules, we must ask whether microtubule polarity has any relationship to the relative polarity of interacting microtubules established by dynein cross-bridging.

Ciliary axonemes isolated from *Tetrahymena* spontaneously slide apart or disintegrate in low concentrations of $MgATP^{2-}$

THE JOURNAL OF CELL BIOLOGY · VOLUME 89 APRIL 1981 35-44

(18). In the presence of excess dynein, the disintegrated doublets reassociate to form overlapping arrays of doublet microtubules, cross-bridged by the dynein arms. When ATP is added to these suspensions, the doublets undergo a secondary disintegration (13). By using this phenomenon, we have examined the question of polarity of dynein binding and cross-bridging

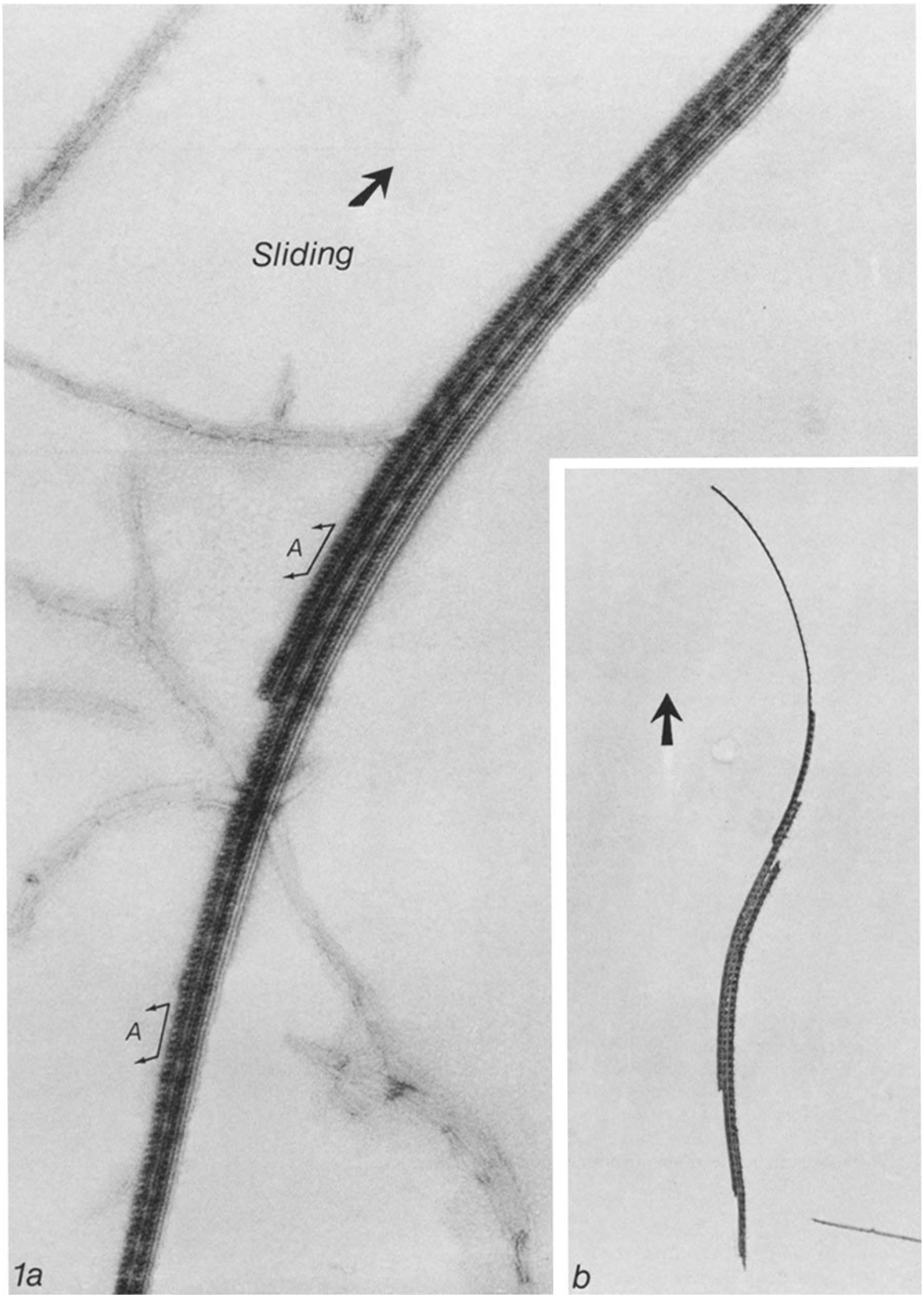

FIGURE 1 Isolated *Tetrahymena* cilia reactivated with 0.1 mM $MgATP^{2-}$ to cause sliding disintegration. Typical sliding figures are recognized as partially overlapping doublets cross-bridged by the dynein arms. Free dynein arms are polarized and tilt uniformly toward the base of the cilium (bracketed arrows) away from the direction of active sliding. *a*, × 51,000; *b*, × 1,200.

between interacting microtubules. We have found that exogenous dynein recognizes the intrinsic molecular polarity of both A and B subfibers as determined by predominantly polarized binding to the subfibers. But, in addition, we have found that native A subfiber-bound dynein will bind B subfiber tubulin, producing both parallel and antiparallel modes of cross-bridging. Both modes of cross-bridging appear to be sensitive to dissociation by ATP.

MATERIALS AND METHODS

Cilia were isolated from *Tetrahymena thermophila* and demembranated in Triton X-100. Details of the isolation procedure, dynein purification and decoration, and the substrate-dependent disintegration response are presented in two recent publications (13, 18). In general, however, isolated axonemes were routinely disintegrated in 2–6 mM $MgSO_4$, 20 μM ATP, and 1–10 mM HEPES at pH 7.4. 30S dynein was isolated by extraction in 0.5 M KCl. Doublets were reassociated under conditions which favor ATP-sensitive dynein binding to the B subfiber (13). Axoneme disintegration and doublet reassociation were monitored by both negative-contrast and thin-section electron microscopy as well as by spectrophotometrically measured changes in suspension turbidity (ΔA 350 nm; reference 18). The kinetics of doublet reassociation have not been studied relative to dynein-tubulin ratios, but we have used the stoichiometry reported in reference 13 which supports substantial reassociation in addition to decoration of the B subfiber.

RESULTS AND DISCUSSION

Cilia isolated from *Tetrahymena* and demembranated in Triton X-100 slide apart when substrate for the dynein ATPase is added to the axonemal suspension. This disintegration reaction can be monitored by both electron microscopy and turbidimetric changes (ΔA 350 nm) of the suspension. By both methods, the reaction is observed to be sensitive to the substrate concentration (18). In <30 μM $MgATP^{2-}$, axonemes disintegrate by sliding, resulting in populations of individual doublet microtubules retaining their normal complement of dynein arms on the A subfibers. As the substrate concentration is elevated to >30 μM, sliding is progressively inhibited until little or no sliding occurs in ~0.5 mM $MgATP^{2-}$.

Sliding disintegration is illustrated in Fig. 1 and is immediately recognized by the partially overlapping arrays of doublet microtubules, cross-bridged by the dynein arms and curved into gentle arcs. In low concentrations of ATP, disintegration is nearly complete (18). In regions of remaining doublet overlap, arm tilt or polarity is generally ambiguous, but the characteristic base-tilted polarity along the A subfibers is uniformly clear in nonoverlap regions. The polarity of the arms in nonoverlap regions serves as an unambiguous marker of microtubule polarity and base-tip orientation in these axonemes (15, 17).

When doublet microtubules produced by axonemal sliding in 20 μM $MgATP^{2-}$ are subsequently incubated in stoichiometric concentrations of isolated 30S dynein and 6 mM $MgSO_4$, exposed B subfiber dynein binding sites decorate with dynein. Simultaneously, the doublets reassociate via the dynein arms to form extensive arrays of cross-bridged doublets (Fig. 2) (13). The arrays consist of as few as two and as many as 10 bridged doublets, and doublets from one group often branch and join with doublets from other groups. The process is complete within ~10 min after the addition of dynein. Within any preparation, the extent of reassociation is related to the proportion of doublets and exogenous dynein in suspension because free dynein and A subfiber-bound dynein compete for the same available B subfiber binding sites. Doublets to which exogenous dynein has not been added also reassociate but at a very slow rate.

Reassociation and decoration are accompanied by a 10–20% increase in suspension turbidity (Fig. 3) depending upon the stoichiometry of added dynein (13). The reassociated doublets retain sensitivity to subsequent addition of ATP. As little as 1 μM ATP dissociates dynein from decorated B subfibers and causes secondary disintegration of the reassociated doublets. These phenomena are accompanied by a drop in suspension turbidity to an absorbance value near or below the value existing before the addition of exogenous dynein. That part of the turbidity decrease related to active (hydrolysis dependent) secondary disintegration can be eliminated by adding 10 μM vanadate to the suspension. This inhibits ATPase activity and, hence, active sliding disintegration, but does not interfere with passive dissociation of dynein from the B subfiber (13).

With this system, we have examined the polarity of the overlapping arrays of reassociated doublets before and after the readdition of ATP to the suspension.

Microtubule Polarity: Parallel and Antiparallel Reassociation

The polarity of microtubules reassociated in 30S dynein and 6 mM $MgSO_4$ is easily determined by both negative-contrast and thin-section electron microscopy. Native (A subfiber) and decorated (B subfiber) dynein arms provide unambiguous markers for plus (+) and minus (−) microtubule orientation in negatively contrasted preparations. Individual subfibers generally can be distinguished on the basis of the teardrop configuration (16) assumed by B-subfiber-bound dynein, as opposed to the extended configuration of native A-subfiber-bound dynein (cf. Figs. 1 and 4) (17). The polarity of doublet microtubule reassociations was determined only for those interactions where arm tilt was unambiguous (~70% of all visible reassociations; as in Fig. 4). Although several preparations were examined with similar results, only one was scored. From a total of 78 reassociated doublets, 53 (or 68%) were parallel interactions with all rows of nonbridged dynein arms (A and B subfiber) inclined in a single direction. The remaining 25 (or 32%) were antiparallel interactions with the free arms (A and B subfiber) on bridged doublets oppositely inclined. Parallel and antiparallel cross-bridging was generally mixed within any single array of doublets. One mode of reassociation apparently does not promote continued reassociation in the same mode beyond the extent of the original 2:1 distribution. Several examples of reassociated doublets are illustrated in Figs. 4 and 5. From the same group of micrographs, there were seven examples of doublet-central pair microtubule reassociation (as in Fig. 4 *c*). Five of these reassociations were parallel and two were antiparallel. The marker for central pair polarity is the terminal cap at the distal end of the tubules (Fig. 4 *c* [7]).

Similar types of reassociation were seen when preparations were examined by thin-section electron microscopy (Fig. 4 *d–f*), although by this method polarity is best visualized by using disintegrated preparations not exposed to exogenous dynein. The enantiomorphic profile of the arms and the location of the radial spokes provide the necessary markers for microtubule orientation and polarity. Both thin-section and negative-contrast preparations suggest that doublet reassociation occurs predominantly by A to B subfiber dynein cross-bridging, although some bridging and dynein binding does occur at atypical sites (Figs. 4, and 6 [13]).

Polarity Determinants for the Dynein-B Subfiber Complex

Dynein decoration of both A and B subfibers of ciliary

doublet microtubules, as well as decoration of single neurotubules, manifests a considerable level of specificity (8, 13, 16). Exogenous dynein binds in a polarized array with the recombined arms (on both subfibers) tilted ~32° from the perpendicular and pointing in a single direction, which, in the case of ciliary doublets, is towards the proximal (−) end of the axo-

FIGURE 2 Doublet microtubules produced by disintegration in 20 μM $MgATP^{2-}$ and subsequently reacted with 6 mM $MgSO_4$ and 30S dynein. The doublets have reassociated via dynein cross-bridges to produce long overlapping arrays. Although not obvious from the limited field of view, the suspension also contains individual doublets fully decorated by dynein. × 21,000.

neme. Native dynein arms on the A subfiber are stable in the presence of ATP and, similarly, dynein rebound to extracted A subfibers forms a stable dynein-tubulin complex in the presence of ATP (13). In contrast, dynein binding to the B subfiber, both *in situ* and in vitro, is intrinsically unstable and the dynein-tubulin complex is readily dissociated by micromolar concentrations of ATP (13). From these observations, we can reasonably conclude that the 30S dynein molecule has two chemically discrete ends which recognize ATP-sensitive and ATP-insensitive binding sites on the tubulin lattice. The only method for distinguishing which end has bound to the lattice is the sensitivity of the resulting dynein-tubulin complex to ATP.

We have used unextracted doublets in our reassociation experiments and a dynein-tubulin stoichiometry which promotes ATP-sensitive dynein binding to the B subfiber (13). Therefore, most B subfiber decoration in our preparations involves the same (free) end of the 30S dynein molecule that the subfiber would see *in situ*. Accordingly, the tilt of the 30S molecule when attached to the B subfiber alone is actually opposite the tilt when it is attached to the A subfiber alone, even though both dynein-A and dynein-B subfiber complexes, by morphological criteria, tilt proximally. Stated more succinctly, ATP-sensitive and ATP-insensitive classes of binding sites have the same relative polarity with respect to intrinsic doublet polarity, but they recognize different ends of the 30S dynein molecule, resulting in proximally directed dynein-B subfiber and dynein-A subfiber complexes. This interpretation is supported by the in vitro binding characteristics of 30S dynein (13) and by the observation of measurably different angles formed by the two complexes (16). Collectively they are consistent with the supposition that the dynein-B subfiber complex is in a thermodynamic state equivalent to myosin S1 decoration of F-actin or rigor in skeletal muscle.

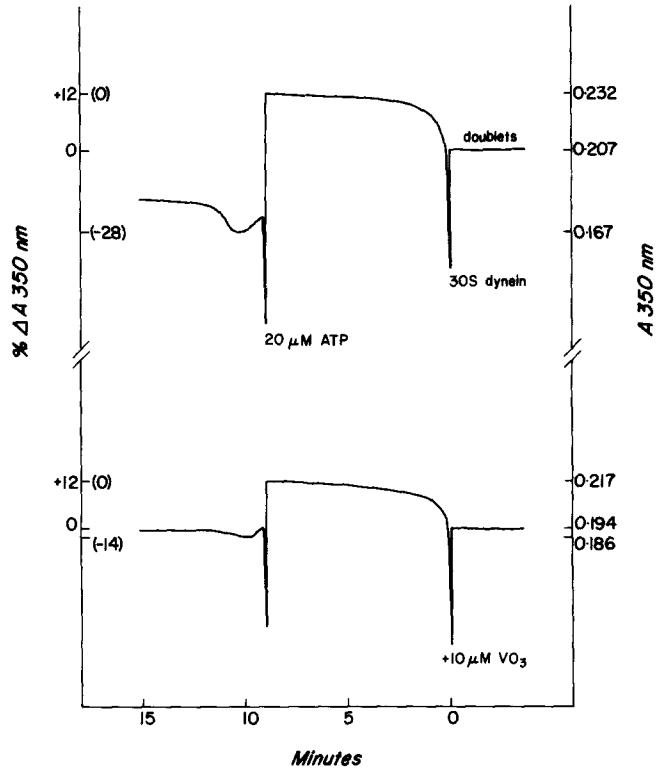

FIGURE 3 Spectrophotometric tracings at 350 nm of isolated doublets suspended in 6 mM $MgSO_4$ and 1 mM HEPES, pH 7.4. The tracings are read from right to left. In the upper tracing, when 30S dynein is added to the suspension the turbidity increases by 12% accompanied by dynein decoration of B subfibers and reassociation of the doublets, as illustrated in Fig. 2. If 20 μM ATP is added to the suspension, the turbidity decreases 28%, accompanied by loss of decorated dynein and secondary (active and passive) disintegration of the doublet arrays, as illustrated in Fig. 7 *a*. The lower tracing records a similar experiment except that 10 μM vanadate was added simultaneously with 30S dynein. Vanadate inhibits dynein ATPase activity and, hence, blocks that part of the ATP-induced absorbance decrease associated with hydrolysis-dependent disintegration. This results in a suspension of passively dissociated doublets, as illustrated in Fig. 7 *b*. The spikes in the tracings are optical artifacts related to the additions of dynein and ATP. Details of the turbidimetric assay are provided in references 13 and 18.

Therefore, it is likely that the proximal direction of dynein tilt on the B subfiber is an accurate reflection of B subfiber and doublet polarity in the reassociated arrays illustrated in Fig. 4. However, independent verification of doublet polarity is provided by the uneven spacing of the triplet radial spokes, where it will be remembered that the wider (32 nm) spacing between spokes one and two of each group is positioned nearest the proximal (−) end of the axoneme. Fig. 5 *a* and *b* illustrate two examples of antiparallel doublets where both radial spoke and arm tilt markers are readily apparent. Similar verification resides in identification of the respective subfibers (Fig. 5 *c*), as described in the previous section.

Dynein cross-bridging between antiparallel doublets is difficult to reconcile with the presumed steric specificity of the protein-protein interactions; it essentially demands free rotation of the dynein molecule with respect to the ATP-sensitive B subfiber binding site. In contrast, cross-bridging between parallel doublets requires only that the bridged arms undergo a conformational change to accommodate the chemical polarity of the arm with respect to the two classes of binding sites on the A and B subfiber lattices. Free rotation of the 30S molecule predicts that a suspension of doublet microtubules and 30S dynein might exhibit low level bipolar decoration of the B subfiber. Bipolar decoration has not been seen in other studies (8, 13, 16) but we have found several examples of either ambiguous decoration or decoration strongly suggestive of bipolar orientation of the 30S dynein-B subfiber complex (Fig. 6).

Reactivation of Reassociated Doublet Microtubules

Preparations of reassociated doublet microtubules retain their sensitivity to subsequent addition of ATP. When 20 μM ATP is added to the reassociated suspensions, the doublet arrays disintegrate into a population of doublets (Fig. 7 *a*) but retain regions of their original overlap, similar to the condition existing before the addition of 30S dynein. As noted above, secondary disintegration is accompanied by a decrease in suspension turbidity (Fig. 3; upper tracing) owing both to active disintegration and to passive dynein release from the B subfiber. The active or hydrolysis-dependent part of the change in turbidity can be blocked by adding 10 μM vanadate to the suspension (Fig. 3, lower tracing and reference 13). Vanadate inhibits dynein ATPase activity and, hence, cross-bridge cycling and sliding but does not interfere with dissociation of B-subfiber-bound dynein by ATP.

Presently, we recognize three types of axoneme disintegration that occur in response to the addition of ATP (14, 18). Two types result from substrate ($MgATP^{2-}$) hydrolysis and

manifest either sliding (Fig. 1) or fraying (18) and both can be inhibited by micromolar vanadate. The third type results from ATP^{4-} binding but not its hydrolysis and, hence, it is insensitive to the inhibitory effects of vanadate. ATP binding results in dissociation of cross-bridged doublets (Fig. 7 and reference 14) caused by release of dynein arms from the B subfiber. Only

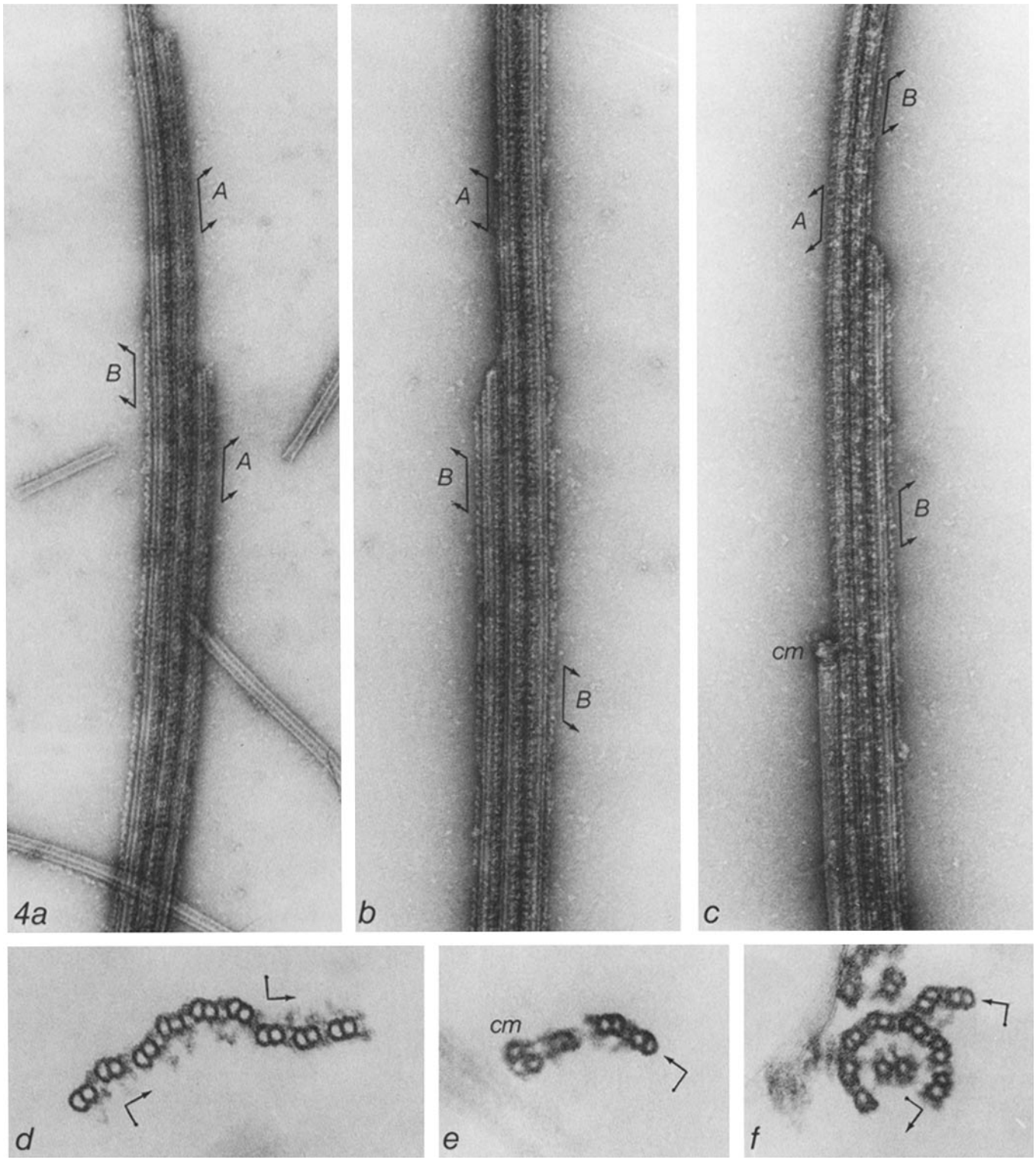

FIGURE 4 *a–c*, Doublet microtubules reassociated in the presence of 30S dynein and 6 mM $MgSO_4$. Doublets are cross-bridged by dynein in both parallel (arms pointing in a common direction) and antiparallel (arms pointing in opposite directions) modes. Dynein arm polarity is designated by the bracketed arrows. The letter within the brackets designates the subfiber (A or B) to which the arms are attached. The distal assembly or plus (+) end of the doublets is positioned away from the direction of arm tilt. × 58,000. *d–f*, Groups of partially disintegrated doublets reassociated in 3 mM $MgSO_4$ but minus exogenous 30S dynein and processed for thin-section electron microscopy. The doublet groups have reassociated in both parallel and antiparallel modes, as indicated by the enantiomorphic doublet-arm profiles and radial spoke positions (right angle brackets). *cm*, Central pair microtubules. × 60,000.

active sliding and passive dissociation of cross-bridged doublets occur in this study because substrate concentrations higher than were used are required for hydrolysis-dependent fraying (18).

Electron microscope examination of secondary disintegration in the absence of vanadate reveals examples of both active sliding and passive dissociation; however, dissociation appears to predominate (Fig. 7 *a*) probably because the interdoublet

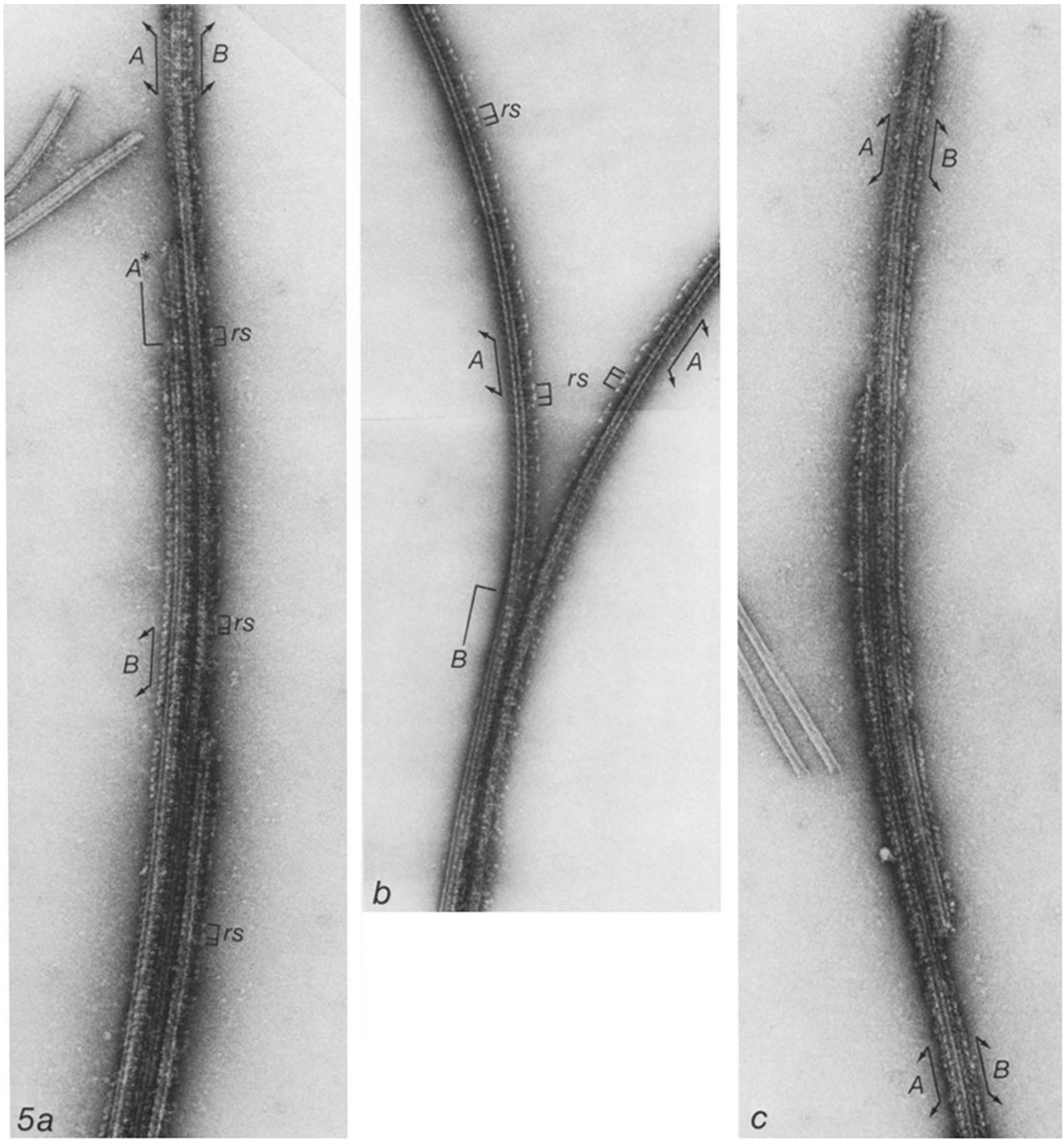

FIGURE 5 Doublet microtubules reassociated in the presence of 30S dynein and 6 mM $MgSO_4$. Three morphological markers provide independent and consistent verification of doublet polarity or proximal-distal orientation in the doublet arrays. In Fig. 5 *a*, the distal (+) end of the left doublet is marked by the A subfiber extension (*) characteristic of this region, as well as by proximally directed 30S dynein which decorates the B subfiber. The proximal (−) end of the middle doublet is marked by the uneven spacing of the triplet radial spokes (*rs*) as well as by the proximally directed arms on the A subfiber. The radial spoke spacings also mark the proximal end of the third doublet on the right. Therefore, this group of doublets clearly manifests one antiparallel and one parallel reassociation. An antiparallel reassociation also is illustrated in Fig. 5 *b* but it is seen after the readdition of 5 μM ATP which dissociates B subfiber-bound dynein. Fig. 5 *c* illustrates a parallel reassociation where polarity is marked by the proximally directed dynein tilt on both the A and B subfibers. *a*–*c*, × 58,000.

links normally holding doublets together are disrupted. Secondary disintegration in the presence of 10 μM vanadate reveals examples only of the passive mode of disintegration (Fig. 7 *b*), similar to the vanadate-insensitive disintegration described by Sale and Gibbons (14). Although the two kinds of preparations are difficult to distinguish from one another (Fig. 7), both are easily distinguished from the reassociated doublets illustrated in Fig. 2. Both parallel and antiparallel microtubule cross-bridging appear to be sensitive to added ATP because suspensions in both the presence and absence of vanadate are nearly completely disintegrated by ATP and no disproportionate amount of antiparallel bridging was detected in those regions of remaining doublet overlap. An example of an antiparallel doublet association remaining after ATP addition is illustrated in Fig. 5 *b*.

Because of obvious limitations to the techniques, we do not

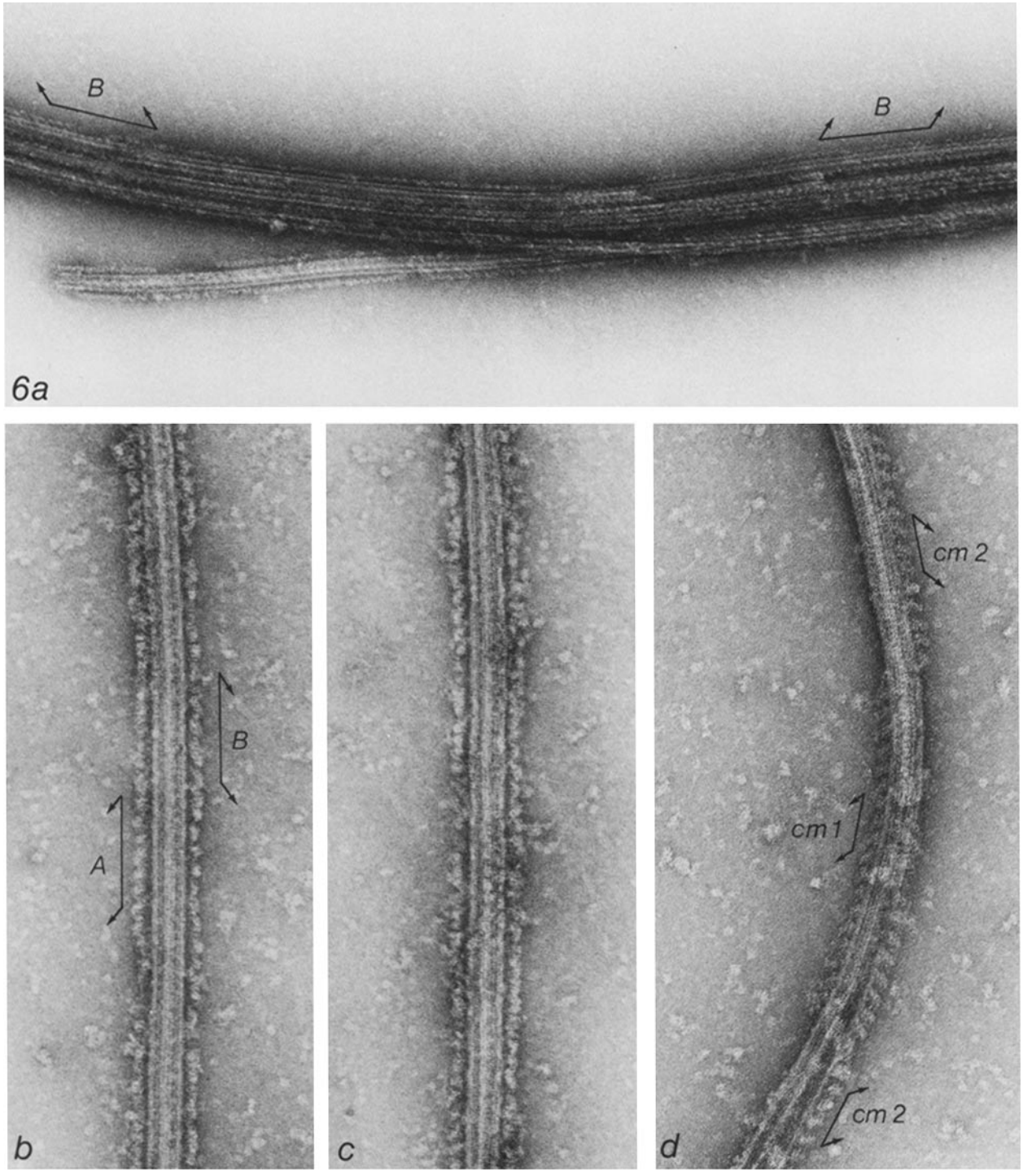

FIGURE 6 Extracted (0.5 M KCl; *b* and *c*) and unextracted (*a* and *d*) microtubules recombined with 30S dynein and 6 mM $MgSO_4$. The polarity of dynein decoration generally is both uniform (bracketed arrows) and unidirectional (*a* and *b* and Fig. 3). However, examples are found where polarity is either ambiguous (*c*) or appears to be bidirectional along a single doublet (*a*) or central pair tubule (*cm 1* or *2*; *d*). *a*, × 63,000; *b* and *c*, × 120,000; *d*, × 129,000.

know whether antiparallel doublets are interacting in a physiologically significant way. Nevertheless, the results suggest that both parallel and antiparallel cross-bridging are sensitive to dissociation by ATP even though cross-bridge cycling in intact cilia occurs strictly between parallel microtubules. Independent of their ATP sensitivity, the ability of antiparallel tubules to be cross-bridged by dynein arms is both unambiguous and inexplicable. The proteins dynein and tubulin are behaving in ways for which currently there is no explanation or precedent to be found in other systems. Dynein clearly recognizes microtubule polarity, as distinguished by predominantly polarized binding to both the A and B subfibers, but, with regard to ATP-sensitive A to B subfiber cross-bridging, dynein may not recognize any functional polarity.

Specificity of Dynein-Microtubule Interactions

Although decoration of microtubules by exogenous dynein appears to be a straightforward reaction limited only by availability of binding sites on the tubulin lattice and the state of the active site on the dynein molecule (13), the chemical nature and specificity of the reaction have not yet been resolved. It simply has been assumed that the binding reaction is specific with respect to the molecular and chemical polarity of the microtubule in the same way that myosin S1 decoration of F-actin manifests a high level of molecular specificity.

In those systems where microtubule interactions are either known or presumed to be responsible for motion, the relationship of microtubule polarity to the interactions is often uncertain. For example, the half-spindle positioning of the centrioles and kinetochores in the mitotic apparatus suggested that potential interactions occurred between antiparallel microtubules (12). Recent studies, however, suggest that the kinetochore is not a microtubule organizing center (21) and hence the major classes of tubules in each half-spindle actually may have parallel orientations (20). In the microtubular axostyle of certain protozoa, the polarity of the microtubules is known to have the same orientation (19) and, hence, potential interactions can occur only between parallel microtubules. Similarly, in the example of reassembled neuromicrotubules, dynein cross-bridging was seen to occur only between parallel microtubules (8). In the case of ciliary and flagellar doublet microtubules, in vivo interactions occur only between parallel microtubules, although it is not clear whether intrinsic lattice differences

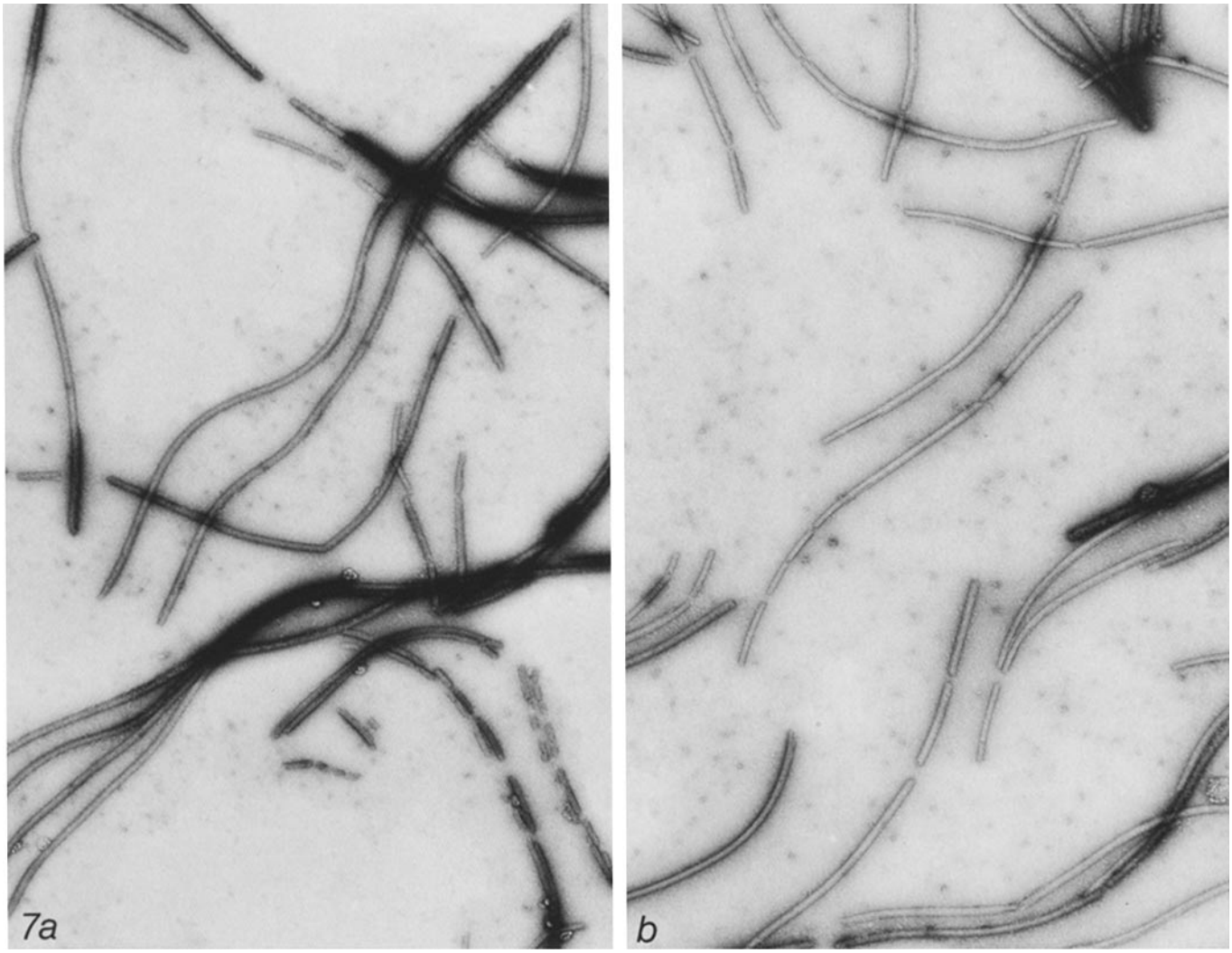

FIGURE 7 Doublet microtubules reassociated in 30S dynein and 6 mM $MgSO_4$ and subsequently exposed to 20 μM ATP to cause secondary disintegration of the doublet arrays (cf. Figs. 7 and 2). Although not visible in this image, disintegration in the absence of vanadate (*a*) has occurred by both sliding and dissociation, whereas in the presence of 10 μM vanadate to inhibit dynein ATPase (*b*), disintegration has occurred solely by dissociation. × 16,500.

between the A and B subfibers (2) influence polarity relationships of the protein-protein interactions.

The present observation that dynein cross-bridging can occur between both parallel and antiparallel microtubules in vitro, coupled with the observation that dynein sometimes will bind to a single microtubule in both polar and bipolar modes, additionally confuses the issue. These observations may be explained either by a lack of specificity in the dynein binding reaction or they may relate to intrinsic properties of the protein-protein interactions. Dynein-microtubule binding clearly has a considerable level of specificity, as seen in the following properties: binding in both parallel and antiparallel modes is strictly dependent on a divalent cation, such as Mg^{2+} (13); binding in both modes occurs with the same characteristic 24-nm repeat (17); binding in both modes can occur at multiple sites on the tubulin lattice (13); and binding in both modes appears to be sensitive to dissociation by ATP. These properties are, of course, thermodynamic properties and, hence, their relationship to kinetic properties of the dynein cross-bridge remains uncertain.

In conclusion, either the specificity of dynein-tubulin interactions in vitro is not absolute or else the proteins are interacting in an unusual way and microtubule polarity is related only nominally to the ability of microtubules to be cross-bridged by dynein. In either event, the observations have important implications if dynein decoration and microtubule polarity are to be used as in vitro probes to investigate potential microtubule interactions in other systems, particularly those in the mitotic apparatus or neural processes.

This study was supported by research grant GM20690 from the National Institutes of Health.

Received for publication 15 September 1980, and in revised form 1 December 1980.

REFERENCES

1. Allen, C., and G. G. Borisy. 1974. Structural polarity and directional growth of microtubules of *Chlamydomonas* flagella. *J. Mol. Biol.* 90:381–402.
2. Amos, L. A., and A. Klug. 1974. Arrangement of subunits in flagellar microtubules. *J. Cell Sci.* 14:523–549.
3. Bergen, L. G., and G. G. Borisy. 1980. Head-to-tail polymerization of microtubules in vitro. Electron microscope analysis of seeded assembly. *J. Cell Biol.* 84:141–150.
4. Bergen, L. G., R. Kuriyama, and G. G. Borisy. 1980. Polarity of microtubules nucleated by centrosomes and chromosomes of Chinese hamster ovary cells in vitro. *J. Cell Biol.* 84: 151–159.
5. Binder, L. I., and J. L. Rosenbaum. 1979. The in vitro assembly of flagellar outer doublet tubulin. *J. Cell Biol.* 79:500–515.
6. Borisy, G. G. 1978. Polarity of microtubules of the mitotic spindle. *J. Mol. Biol.* 124:565–570.
7. Dentler, W. L., and J. L. Rosenbaum. 1977. Flagellar elongation and shortening in *Chamydonomas.* III. Structures attached to the tips of flagellar microtubules and their relationship to the directionality of flagellar microtubule assembly. *J. Cell Biol.* 74:747–759.
8. Haimo, L. T., B. R. Telzer, and J. L. Rosenbaum. 1979. Dynein binds to and cross-bridges cytoplasmic microtubules. *Proc. Natl. Acad. Sci. U. S. A.* 76:5759–5763.
9. Hyams, J. 1980. Polarity of spindle microtubules. *Nature (Lond.).* 284:402–403.
10. Kirschner, M. W. 1980. Implications of treadmilling for the stability and polarity of actin and tubulin polymers in vivo. *J. Cell Biol.* 86:330–334.
11. McDonald, K. L., M. K. Edwards, and J. R. McIntosh. 1979. Cross-sectional structure of the central mitotic spindle of *Diatoma vulgare.* Evidence for specific interactions between antiparallel microtubules. *J. Cell Biol.* 83:443–461.
12. McIntosh, J. R., P. K. Hepler, and D. G. Van Wie. 1969. Model for mitosis. *Nature (Lond.).* 224:659–663.
13. Mitchell, D. R., and F. D. Warner. 1980. Interactions of dynein arms with B subfibers of *Tetrahymena* cilia: quantitation of the effects of magnesium and adenosine triphosphate. *J. Cell Biol.* 87:84–97.
14. Sale, W. S., and I. R. Gibbons. 1979. Study of the mechanism of vanadate inhibition of the dynein cross-bridge cycle in sea urchin sperm flagella. *J. Cell Biol.* 82:291–298.
15. Sale, W. S., and P. Satir. 1977. Direction of active sliding of microtubules in *Tetrahymena* cilia. *Proc. Natl. Acad. Sci. U. S. A.* 74: 2045–2049.
16. Takahashi, M., and Y. Tonomura. 1978. Binding of 30S dynein with the B-tubule of the outer doublet of axonemes from *Tetrahymena pyriformis* and adenosine triphosphate-induced dissociation of the complex. *J. Biochem.* 84:1339–1355.
17. Warner, F. D., and D. R. Mitchell. 1978. Structural conformation of ciliary dynein arms and the generation of sliding forces in *Tetrahymena* cilia. *J. Cell Biol.* 76:261–277.
18. Warner, F. D., and N. C. Zanetti. 1980. Properties of microtubule sliding disintegration in isolated *Tetrahymena* cilia. *J. Cell Biol.* 86:436–445.
19. Woodrum, D. T., and R. W. Linck. 1980. Structural basis of motility in the microtubular axostyle: implications for cytoplasmic microtubule structure and function. *J. Cell Biol.* 87: 404–414.
20. Euteneuer, U., and J. R. McIntosh. 1980. Polarity of midbody and phragmoplast microtubules. *J. Cell Biol.* 87:509–515.
21. Tippit, D. H., J. D. Picket-Heaps, and R. Leslie. 1980. Cell division in two large pennate diatoms *Hantzshia* and *Nitzschia.* III. A new proposal for kinetochore function during prometaphase. *J. Cell Biol.* 86:402–416.