Polarity of Flagellar Assembly in *Chlamydomonas*

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Abstract. During mating of the alga *Chlamydomonas*, two biflagellate cells fuse to form a single quadriflagellate cell that contains two nuclei and a common cytoplasm. We have used this cell fusion during mating to transfer unassembled flagellar components from the cytoplasm of one *Chlamydomonas* cell into that of another in order to study in vivo the polarity of flagellar assembly.

In the first series of experiments, sites of tubulin addition onto elongating flagellar axonemes were determined. Donor cells that had two full-length flagella and were expressing an epitope-tagged α-tubulin construct were mated (fused) with recipient cells that had two half-length flagella. Outgrowth of the shorter pair of flagella followed, using a common pool of precursors that now included epitope-tagged tubulin, resulting in quadriflagellates with four full-length flagella. Immunofluorescence and immunoelectron microscopy using an antiepitope antibody showed that both the outer doublet and central pair microtubules of the recipient cells' flagellar axonemes elongate solely by addition of new subunits at their distal ends.

In a separate series of experiments, the polarity of assembly of a class of axonemal microtubule-associated structures, the radial spokes, was determined. Wild-type donor cells that had two full-length, motile flagella were mated with paralyzed recipient cells that had two full-length, radial spokeless flagella. Within 90 min after cell fusion, the previously paralyzed flagella became motile. Immunofluorescence microscopy using specific antiradial spoke protein antisera showed that radial spoke proteins appeared first at the tips of spokeless axonemes and gradually assembled toward the bases. Together, these results suggest that both tubulin and radial spoke proteins are transported to the tip of the flagellum before their assembly into flagellar structure.

THE flagellum is an ideal organelle for the investigation of the polarized assembly of microtubule and microtubule-associated structures. Flagella extend from a particular region of the cell surface, and contain proteins, synthesized in the cytoplasm, which must be targeted to the flagellar compartment. Once in the flagellum, the proteins must be transported to their proper assembly site(s) on the elongating flagellar axoneme. Earlier in vivo labeling studies, using radioactive protein precursors, suggested that these assembly sites were at the tips of the elongating organelles (Rosenbaum and Child, 1967; Rosenbaum et al., 1969; Witman, 1975).

The initial studies demonstrating polarity of flagellar assembly in vivo were carried out in the flagellated protozoans *Ochromonas* (Rosenbaum and Child, 1967) and *Chlamydomonas* (Rosenbaum et al., 1969). Flagella were experimentally detached, and cells were allowed to regenerate new flagella. When these replacement flagella were about half-length, radiolabeled protein precursors were added until regeneration was completed. The distribution of labeled protein was then determined by light microscope autoradiography; it was shown that 65% of the label was located over the distal half of the flagellum (Rosenbaum and Child, 1967; Rosenbaum et al., 1969). In a refinement of these studies, similarly pulse-labeled flagellar axonemes were isolated from *Chlamydomonas* cells and examined by negative staining and electron microscope autoradiography. 65% of the label incorporated into the axoneme was located over the distal half of the axonemal microtubules (Witman, 1975). Because the microtubules comprise >50% of the axonemal protein mass, these experiments provided strong evidence that the flagellar outer doublet microtubules assemble by the distal addition of tubulin subunits. No conclusion could be drawn regarding the polarity of assembly of the central pair microtubules, as they were solubilized during the preparation of the axonemes for EM autoradiography (Witman, 1975). Moreover, the significance of the presence of $\sim$35% of the radioactivity found over the proximal regions of flagella (Rosenbaum and Child, 1967; Rosenbaum et al., 1969; Witman, 1975), which presumably had been assembled before the addition of labeled precursors, was not clear. This label was hypothesized to be due to turnover of nontubulin components of the flagellum. Finally, since the axoneme is composed of >250 different polypeptides (Piperno et al., 1977), the use of a general protein label made it impossible to draw clear conclusions about the polarity of assembly of specific axonemal structures.

With the isolation of antibodies specific for certain *Chlamydomonas* flagellar proteins, the cloning and sequencing of a number of genes encoding these proteins (Curry et

al., 1992; Mitchell and Kang, 1991; Schloss et al., 1984; Silflow et al., 1985; Williams et al., 1986, 1989), and the ability to reintroduce these genes to form stable transformants (Kindle et al., 1989; Diener et al., 1990), it has become possible to study the polarity of assembly of specific flagellar structures. The general approach that we have taken has been to use cell fusion during mating to transfer a pool of traceable subunits from a donor cell into a recipient, and then cytologically, using specific antibodies, locate these subunits once they have been added into the structure of the recipient cell's flagella. Early studies in *Chlamydomonas* (Lewin, 1954; Randall et al., 1964) had shown that when wild-type cells were mated with certain paralyzed mutants, within an hour the pair of previously paralyzed flagella were actively beating on the quadriflagellate dikaryon zygotes. The interpretation of these cell fusion experiments, known as "dikaryon rescue," is that flagellar protein(s) missing in the mutant was present in unassembled form in the cytoplasm of the motile cell and became available in the common cytoplasm of the dikaryon for assembly in situ onto the mutant axonemes (Luck et al., 1977; Huang et al., 1981; Dutcher et al., 1984).

We have used this procedure to reinvestigate the polarity of assembly of the flagellar microtubules, and, in addition, to study the polarity of assembly of one class of axonemal microtubule-associated structures, the radial spokes. For microtubule assembly studies, transformant cells expressing an epitope-tagged α-tubulin construct (Kozminski, K. G., D. R. Diener, and J. L. Rosenbaum, 1992, submitted for publication) were used to donate a pool of marked tubulin subunits to recipient cells that had half-length flagella. After cell fusion, the half-length flagella grew to full length using a common pool of unassembled subunits that now included tagged α-tubulin. The epitope-tagged α-tubulin was then localized using immunofluorescent and immunogold techniques using an epitope-specific mAb (Field et al., 1988). Similarly, the assembly of radial spokes onto spokeless, but full-length, axonemes during dikaryon rescue of a spokeless mutant was followed by immunofluorescent analysis using antibodies to specific radial spoke proteins (Williams et al., 1986).

The results of these experiments show that both the flagellar outer doublet and central pair microtubules assemble solely at their distal tips. Surprisingly, radial spokes first appear at the tips of spokeless, but full-length, axonemes during dikaryon rescue, and assembly progresses toward the base of the axonemes. These results indicate that both tubulin and radial spoke proteins are transported to the tip of the flagellum before their assembly into flagellar structure.

Materials and Methods

Chlamydomonas Strains and Culture

Wild-type cells (CC124 mt−) and the radial spokeless mutant pf14 (CC1024 mt+) were obtained from the *Chlamydomonas* Genetics Center (Duke University, Durham, NC). The transformed *Chlamydomonas* cell line 4A5, which expresses an epitope-tagged α-tubulin construct, is described elsewhere (Kozminski, K. G., D. R. Diener, and J. L. Rosenbaum, 1992, submitted for publication). Briefly, an oligonucleotide encoding 12 amino acids, including a 9-amino acid epitope from influenza virus hemagglutinin (Field et al., 1988), was inserted near the 3' end of the coding region of a genomic copy of the *Chlamydomonas* α-1 tubulin gene (Silflow et al., 1985). This construct was cotransformed with the *Chlamydomonas* gene encoding nitrate reductase into *nit1-305* mt+ cells (Diener et al., 1990); nitrate reductase–positive cells were screened for expression of epitope-tagged tubulin by Western analysis using the epitope-specific mAb 12CA5 (Field et al., 1988). Immunofluorescent analysis showed that epitope-tagged tubulin was assembled into both the cytoplasmic and axonemal microtubules of expressing cell lines (Kozminski, K. G., D. R. Diener, and J. L. Rosenbaum, 1992, submitted for publication).

Vegetative cells were grown on both minimal and acetate-containing media (Sager and Granick, 1953). Gametes were produced from both liquid cultures and from plates by transferring cells into nitrogen-free liquid medium (Sager and Granick, 1954). Matings were performed by mixing approximately equal numbers of gametes of opposite mating types; the cells rapidly agglutinated and quadriflagellates were observed within minutes. Although many of the gametes fused within 10–15 min of mixing, residual cells mated over a longer time scale; thus, quadriflagellate populations contain both older and younger fusions. The quadriflagellate phase lasts several hours before zygotes proceed into meiosis.

Reagents and chemical stocks, unless otherwise noted, were obtained from Sigma Chemical Co. (St. Louis, MO).

Preparation of Cells with Partially Regenerated Flagella

Pool-depleted cells with partially regenerated flagella were produced by deflagellating wild-type mt− gametes by pH shock (Witman et al., 1972) and allowing them to begin flagellar regeneration. When the new flagella were approximately one-third to one-half length, further protein synthesis was inhibited by the addition of cycloheximide (10 μg/ml) to the medium and the cells were allowed to continue regeneration for 1 h. The flagella of these cells reach about one-half to two-thirds of their final length; assembly ceases as the cells deplete a preexisting cytoplasmic pool of unassembled axonemal components (Rosenbaum et al., 1969; Lefebvre et al., 1978). It has been shown that if similarly treated cells are washed out of cycloheximide, flagellar regeneration resumes as new subunits are synthesized (Lefebvre et al., 1978). Partial regeneration prior to the addition of cycloheximide was necessary as the preexisting pool of unassembled flagellar precursors in gametes is not large enough to produce flagella of sufficient length to allow for efficient mating.

Immunofluorescence Microscopy

Chlamydomonas cells were processed for immunofluorescence following a modification of the procedure of Holmes and Dutcher (1989). Cells were allowed to adhere to poly-L-lysine–coated multiwell slides (Carlson Scientific, Peotone, IL). Cells were washed in microtubule stabilization buffer (MTSB[1]: 30 mM Pipes, 25 mM KCl, 5 mM $MgSO_4$, 5 mM EGTA, and 12% hexylene glycol (Aldrich Chem. Co., Milwaukee, WI; vol/vol), pH 6.8), and then were simultaneously fixed and permeabilized in MTSB + 4% paraformaldehyde (Electron Microscopy Sciences, Fort Washington, PA) + 0.5% NP-40 for 30 min. Unreacted aldehydes were blocked with 10 mg/ml $NaBH_4$, and the slides were washed with distilled water and briefly air dried. The wells were rehydrated in PBS containing 1% BSA (PBSB), and this was replaced by a dilution of primary antibody in PBSB. The anti-epitope mAb 12CA5 (Field et al., 1988) was used at a dilution of 1:10, whereas affinity-purified radial spoke protein polyclonal antisera (Williams et al., 1986) were used at dilutions of 1:100–1:500. After incubation in primary antibody, wells were washed several times with PBSB, and incubated with secondary antibody (fluorescein-conjugated goat anti-rabbit or goat anti-mouse; Zymed Labs. Inc., South San Francisco, CA) diluted 1:200–1:1,000 in PBSB. After washing, slides were mounted in 90% glycerol containing phenylamine diamine (Pringle et al., 1989). Cells were viewed on a Zeiss Universal microscope equipped for epifluorescence and photomicrography; micrographs were taken on TMAX 400 (Eastman Kodak Co., Rochester, NY) and developed according to the manufacturer's directions.

Immunoelectron Microscopy

Axonemes were obtained for immunoelectron microscopy by allowing cells to settle onto poly-L-lysine–coated formvar-covered nickel grids. Flagella were simultaneously separated from the cell bodies and demembranated by inverting the grids onto a solution of HMDEK (30 mM Hepes, 25 mM KCl, 5 mM $MgSO_4$, 1 mM DTT, 0.5 mM EDTA, pH 7.4) + 1% NP-40. The

1. *Abbreviations used in this paper*: MTSB, microtubule stabilization buffer; PBSB, PBS containing 1% BSA.

resulting axonemes were fixed in HMDEK + 1% glutaraldehyde (Electron Microscopy Sciences) and immunogold labeled (Johnson and Rosenbaum, 1990) using 10 nm gold conjugated to goat anti-mouse antibodies (Zymed Labs. Inc.). After immunogold labeling, the grids were washed briefly in distilled water, dried, and viewed in an electron microscope (model EM201; Philips Electronic Instrs. Co., Mahwah, NJ). Although negative staining was omitted to accentuate the immunogold label, the axonemes are sufficiently electron dense to be seen clearly without additional contrast. Electron micrographs were taken using Estar film 4489 (Eastman Kodak Co.) and developed according to the manufacturer's directions.

Results

Sites of Tubulin Addition in Regenerating Axonemes

Cells expressing the epitope-tagged tubulin construct and having full-length flagella were mated with pool-depleted wild-type cells that had partially regenerated flagella of one-half to two-thirds full-length in the continued presence of cycloheximide (10 μg/ml). The donor cells contained unassembled flagellar components, including epitope-tagged α-tubulin, that became available to the regenerating cells after fusion. The recipient cells' pool of unassembled flagellar

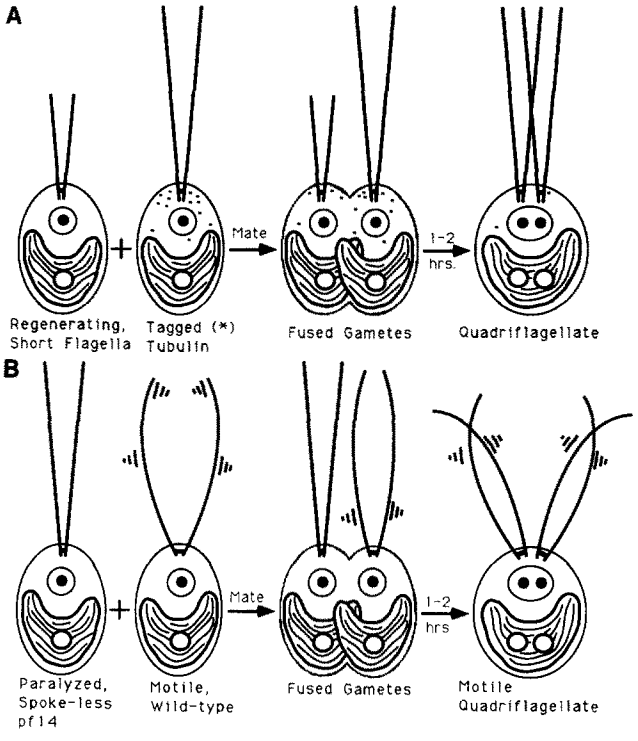

Figure 1. (*A*) Introduction of epitope-tagged tubulin subunits into cells with regenerating flagella. The recipient cells were pool-depleted gametes with one-half to two-thirds full-length flagella. The donor cells were expressing epitope-tagged α-tubulin and had full-length flagella. Within 1–2 h after mating, regenerating of the shorter pair of flagella was completed using epitope-tagged tubulin and other unassembled flagellar components from the donor cell, leaving the quadriflagellate with four equal-length flagella. These flagella were examined for the distribution of tagged tubulin. (*B*) Diagram of dikaryon rescue of the radial spokeless mutant pf14. Radial spokeless, paralyzed gametes were mated with wild-type gametes. Immediately after mating, the quadriflagellates have a pair of paralyzed flagella and a pair of motile ones. Within 1–2 h after mating, all four flagella of the quadriflagellates begin beating. During the time course of rescue, quadriflagellate flagella were examined for the distribution of radial spoke proteins.

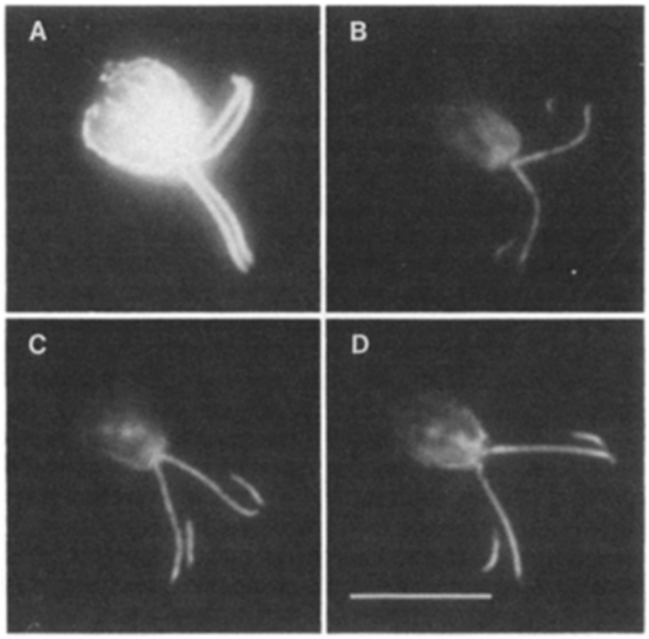

Figure 2. Immunofluorescent localization of tubulin in quadriflagellates. (*A*) Using a polyclonal anti-α-tubulin antiserum, all four axonemes as well as the cytoskeletal microtubules of a dikaryon are labeled. (*B–D*) Using the antiepitope mAb, two of the axonemes of a quadriflagellate are labeled along their full lengths and two axonemes are labeled only along their distal portions. Before mating, the antiepitope antibody labels all microtubules of donor gametes (expressing the epitope-tagged tubulin) but does not label any structures in wild-type recipient gametes (data not shown). Bar, 10 μm.

subunits had been depleted by partial regeneration in the absence of protein synthesis. This reduced the dilution of the tagged tubulin from the donor cell pool after cell fusion.

Immediately after mating, the dikaryons had one pair of full-length flagella and one pair of shorter flagella. Within 1 h after mating, regeneration of the shorter pair of flagella was completed using the pool of unassembled precursors from the donor cell, leaving the dikaryons with four flagella of equal length (Fig. 1 *A*). When these quadriflagellates were examined by immunofluorescence for the distribution of epitope-tagged tubulin, two of the four axonemes were labeled along their full lengths and two were labeled only along their distal portions (Fig. 2 *B–D*). The two fully labeled axonemes were from the donor cell that had been expressing the tagged tubulin construct; the other pair were the recently regenerated axonemes from the recipient cell. The antiepitope mAb reacts specifically with the epitope-tagged gene product and does not recognize proteins in nontransformed wild-type cells (Kozminski, K. G., D. R. Diener, and J. L. Rosenbaum, submitted for publication; and data not shown). Epitope-tagged tubulin was present only in the distal portions of the latter pair of axonemes; no signal was present over their proximal portions.

When the sets of four axonemes from quadriflagellates were examined by immunoelectron microscopy, one pair was labeled along their full lengths and the other pair was labeled only along their distal portions (Fig. 3). As before, the two that were labeled along their full lengths were from the donor cell that had been expressing epitope-tagged tubulin, whereas the partially labeled pair was from the recipient cell. Epitope-tagged tubulin subunits only became available for

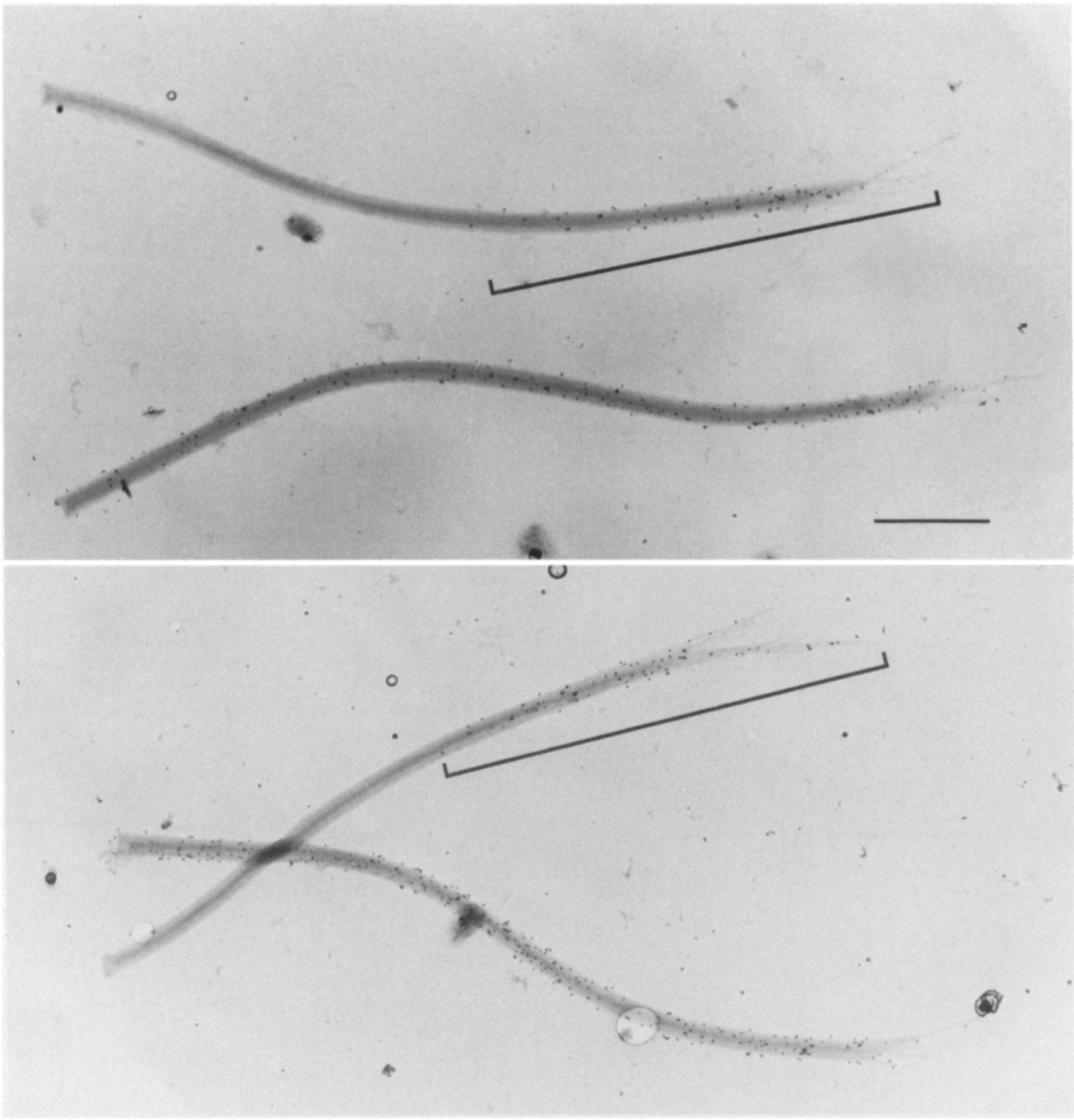

Figure 3. Immunogold localization of epitope-tagged tubulin in quadriflagellate axonemes. The four axonemes shown are from a single quadriflagellate cell. In this whole mount, the axonemal bases (where they have been detached from the cell) are oriented to the left and the axonemal tips to the right. Of the four axonemes of a quadriflagellate, two are labeled along their full lengths and two are labeled only at their distal ends (*brackets*). Bar, 1 μm.

incorporation into the recipient axonemes after cell fusion during mating. Note that no labeling occurs along the proximal portions of the microtubules of the partially labeled pair. When these axonemes splay apart at their distal ends, as they sometimes do during the labeling procedure, it is clear that, during the later stages of regeneration, tagged tubulin had been incorporated into the distal ends of both the outer doublet and the central pair microtubules (Fig. 4).

Pattern of Radial Spoke Addition during Dikaryon Rescue

The mutant pf14 has paralyzed, full-length flagella and cannot swim because of the complete absence of radial spokes (Witman et al., 1978; Piperno et al., 1981). In wild-type cells, radial spokes are attached in a row along the A-tubules of each of the outer doublet microtubules and extend toward

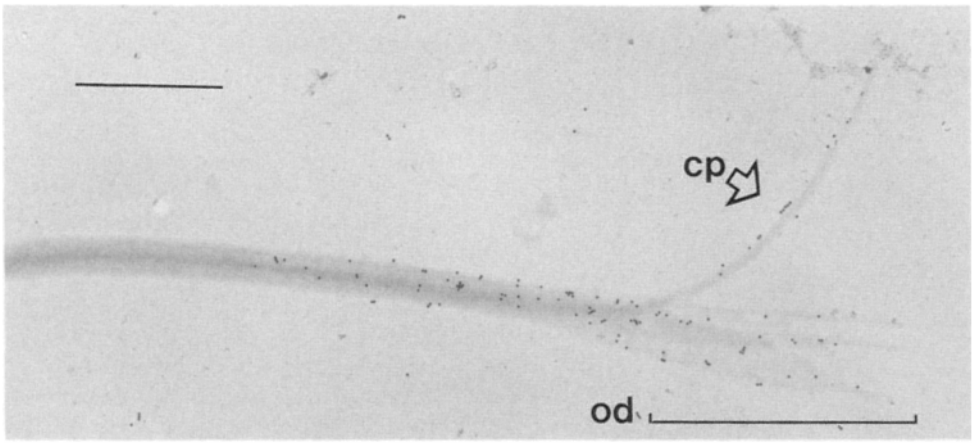

Figure 4. Immunogold localization of epitope-tagged tubulin on a splayed, regenerated axoneme. The longer central pair microtubules (*cp*, *arrow*) curve out of the shorter outer doublet microtubules (*od*, *brackets*), as is characteristic of negative stain whole-mounts (data not shown). This close-up shows that the distal-end labeling is distributed over both the central pair and outer doublet microtubules. No label is present on the proximal regions of this axoneme (extending to the left). Bar, 1 μm.

the central pair microtubules (Hopkins, 1970; Warner, 1970). In the mutant pf14, no radial spokes, nor the 17 different radial spoke proteins that comprise these structures, are present in the flagella. When paralyzed pf14 cells are mated with wild-type cells, the resulting quadriflagellate initially has one pair of paralyzed flagella and one pair of motile ones (Fig. 1 *B*). However, within 90 min after mating, all four flagella of the quadriflagellate are motile (Piperno et al., 1981). The pool of unassembled axonemal components provided by the wild-type cytoplasm includes the radial spoke protein 3 (rsp3) that had been missing in the mutant. Radial spokes are assembled onto the previously spokeless axonemes, restoring motility (Piperno et al., 1981). Using antibodies against radial spoke proteins (Williams et al., 1986), we have examined cells during the the process of dikaryon rescue to establish the temporal pattern of radial spoke protein appearance on the previously spokeless axonemes.

Immunofluorescent analysis, using an affinity-purified polyclonal antiserum to rsp3, of dikaryons fixed at different times after mating is shown in Fig. 5. Each dikaryon initially has a pair of flagella from the wild-type parent that contained radial spokes (appearing fluorescent along their full lengths) and a pair of flagella from the pf14 parent that lacked radial spokes (appearing dark by immunofluorescence, data not shown). In cells fixed at ~15 min after mating, some fluorescence appeared over the tips of some of the otherwise dark, spokeless axonemes (*arrows*, Fig. 5); at subsequent time points, the fluorescent signal brightened and extended proximally. The border between the fluorescent and nonfluores-

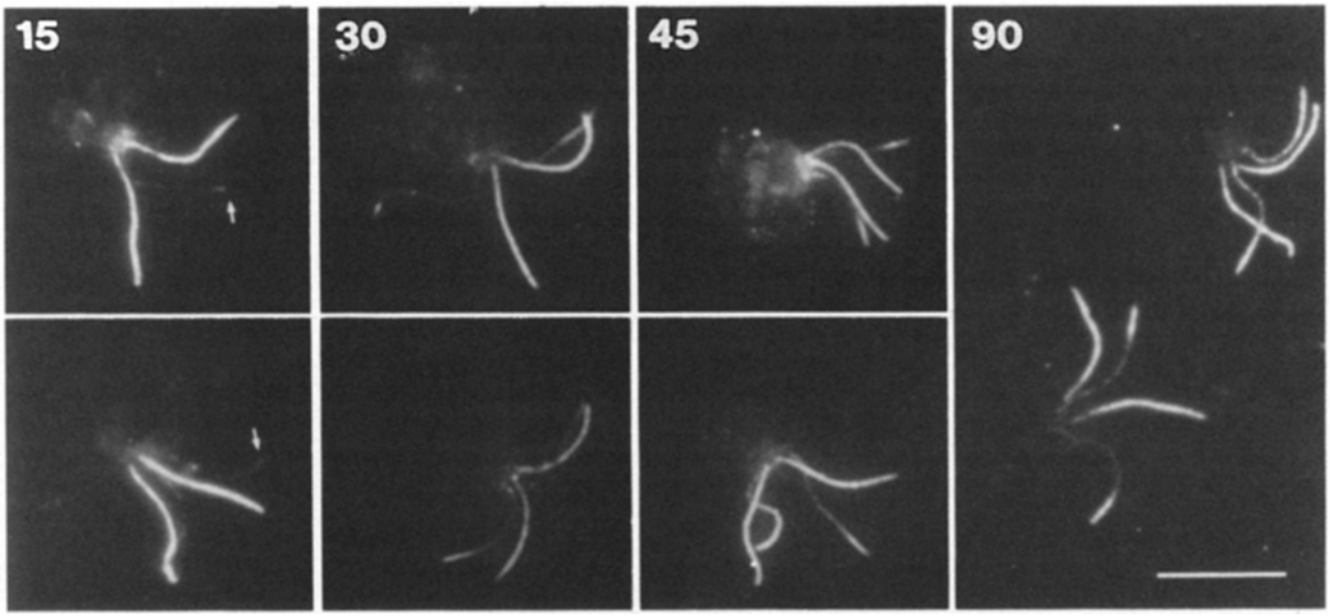

Figure 5. Immunofluorescent localization of radial spoke protein 3 (rsp3) in quadriflagellates at various times during dikaryon rescue. Two examples are shown for each of the 15-, 30-, 45-, and 90-min time points. At 15 min after mating, the two wild-type axonemes are fluorescent along their full lengths (they have radial spokes), whereas the two mutant axonemes are not fluorescent (they lack radial spokes and radial spoke proteins). Minor fluorescence is apparent at some of the tips of these otherwise dark axonemes (*small arrows*). By 30 min, this fluorescence has brightened and spread toward the bases of the mutant axonemes. By 45 min, the zone of fluorescence has expanded further. By 90 min, after mating some of the quadriflagellates have four flagella that are fluorescent along their full lengths (upper cell of the pair). Bar, 10 μm.

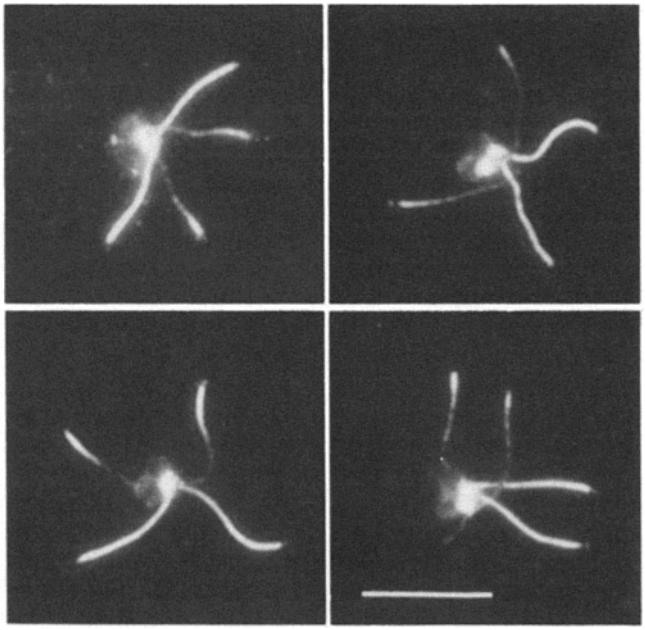

Figure 6. Immunofluorescent localization of radial spoke protein 2 (rsp2) in quadriflagellates at 45 min after mating. rsp2 distributions are similar to those observed for rsp3 in Fig. 5 (45-min samples). Bar, 10 μm.

cent zones on these flagella is not very distinct. Low levels of fluorescence over the basal regions may represent either unassembled radial spoke proteins moving out along the length of the flagellum or a low level of spoke assembly occurring along the lengths of the outer doublets during the dikaryon rescue. In populations of cells fixed at 90 min after mating, quadriflagellates were seen that have four flagella that are fluorescent along their full lengths. Also seen at these later time points were patterns of earlier stages, representing younger dikaryons from later matings. Restoration of spoke proteins in a tip-to-base direction was also seen when an affinity-purified polyclonal antiserum to another spoke protein (rsp2) was used (Fig. 6).

Discussion

The results of experiments examining the in vivo incorporation of epitope-marked tubulin onto axonemes after cell fusion show that both the outer doublet and central pair microtubules are assembled from their distal ends. Although the distal assembly of flagellar outer doublet microtubules might have been inferred from earlier pulse labeling and autoradiographic studies in *Ochromonas* (Rosenbaum and Child, 1967) and *Chlamydomonas* (Rosenbaum et al., 1969; Witman, 1975), this is the first study to show directly that the microtubules themselves are assembled distally. These experiments also show, for the first time, that the central pair microtubules assemble distally during flagellar growth. It had been proposed that the central pair microtubules might assemble through addition to their bases (Dentler and Rosenbaum, 1977) since their proximal ends are not continuous with the basal body microtubules and do not appear to be structurally blocked. Moreover, the 35% of the label that was localized proximally in the original autoradiographic studies could have been due, in part, to proximal addition of tubulin to the central pair microtubules. In contrast to these earlier autoradiographic studies (Rosenbaum and Child, 1967; Rosenbaum et al., 1969; Witman, 1975), the patterns of both immunofluorescent and immunogold localization of epitope-tagged tubulin observed in the present study show labeling only over the distal portions of the microtubules. These results also indicate that, within the limits of sensitivity of the techniques used, there is no turnover of the flagellar outer doublet or central pair microtubules along their lengths, or some epitope-tagged tubulin would have been localized in the proximal portions of the flagella of the recipient cells (Figs. 2 and 3).

The results of the assembly of radial spoke proteins onto spokeless, full-length flagella were more surprising. The flagella were not elongating and radial spoke sites presumably were available for assembly along the full lengths of the outer doublet microtubules of the axonemes. However, radial spoke proteins first appeared near the distal ends and then in a zone that extended proximally. Consistent with these data, the flagella of the paralyzed radial spokeless mutant first recover motility at the flagellar tips at ~30 min after fusion with a wild-type cell (K. A. Johnson, unpublished observations).

Distal assembly, not only of the outer doublet and central pair microtubules, but also of the radial spokes, raises questions concerning the targeting of proteins to the flagellar compartment and the mechanism(s) by which they are transported to the distal tips of flagella. It is possible that the assembly process has more than one component, because earlier pulse-labeling studies (Rosenbaum and Child, 1967; Rosenbaum et al., 1969; Witman, 1975) demonstrated that some newly synthesized proteins are incorporated into the basal half of the axoneme late in regeneration. The identities of these proteins remain unknown, although from the present study it is clear that they are neither tubulin nor radial spoke proteins. Because the genes for several flagellar proteins have now been cloned and sequenced (Curry et al., 1992; Mitchell and Kang, 1991; Schloss et al., 1984; Silflow et al., 1985; Williams et al., 1986, 1989), problems of targeting, transport, and assembly now can be approached directly through deletion analysis and site-directed mutation of these genes followed by their reintroduction to cells by transformation (Ang, L. H., D. R. Diener, and J. L. Rosenbaum, manuscript in preparation).

The results presented in this study suggest two alternative mechanism(s) by which flagellar precursors may move to the tip of the flagellum before their assembly. It is possible that flagellar precursors enter the flagellar compartment at its base and diffuse passively through the space between the flagellar membrane and the outer doublets of the axoneme to tip assembly sites. The observed tip-to-base pattern of radial spoke restoration onto full-length but previously spokeless axonemes might be a consequence of the ranks of dynein arms that closely interconnect adjacent outer doublets. Radial spoke proteins may have to travel the length of the axoneme, past the distal ends of the outer doublet microtubules, before gaining access to assembly sites on the inner faces of the outer doublets. An alternative hypothesis is that precursors are transported in a more active, directed process. The presence of putative carriers associated with the flagellar membrane and/or axoneme can be inferred from

(*a*) descriptions of the rapid movements of latex beads up and down the external surface of the flagellar membrane (Bloodgood, 1977), (*b*) observations of particle movement underneath the flagellar membrane when viewed using high resolution differential interference contrast microscopy (Kozminski, K. G., K. A. Johnson, P. Forscher, and J. L. Rosenbaum, 1992, submitted for publication), and (*c*) reports of molecular motors, other than flagellar dynein, in the flagellum (Kozminski, K. G., K. A. Johnson, P. Forscher, and J. L. Rosenbaum, 1992, submitted for publication; Sale, W. S., L. A. Fox, K. E. Sawin, and J. Heuser, submitted for publication). Once unassembled axonemal proteins arrive at the flagellar tips, they may be targeted to capping structures that link the ends of the axonemal microtubules to the flagellar membrane (Dentler and Rosenbaum, 1977; Dentler, 1980). At this point, the precursors may be released from their carriers and become available for assembly into axonemal structure.

We would like to thank Dennis Diener and Keith Kozminski for the transformed *Chlamydomonas* cell line 4A5.

Work in the authors' laboratory has been supported by grants from the National Institutes of Health (GM-14642) and the American Cancer Society (ACS NP-723). K. A. Johnson has been supported by a National Institutes of Health postdoctoral fellowship (GM-13758).

Received for publication 23 June 1992 and in revised form 25 August 1992.

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