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Association of cytoplasmic dynein with manchette microtubules and spermatid nuclear envelope during spermiogenesis in rats

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SUMMARY

During spermiogenesis, the shape of the spermatid nucleus, which is spherical, changes and it becomes the sperm head. A microtubular structure called a manchette is thought to be involved in this morphogenetic process. In this report, we demonstrate the localization of cytoplasmic dynein and manchette development by a double immunofluorescence technique using anti-bovine brain MAP 1C and anti-tubulin. Before step 6 of the Leblond and Clermont staging, the microtubules showed a fine reticular network, and the dynein staining was homogeneous. In step 6, the microtubular network was concentrated around the nucleus. The manchette developed in step 7 spermatids, and was fully formed, with a skirt-like appearance, covering the nuclear surface in step 8. Dynein fluorescence was associated with the microtubular manchette in steps 7-10. During these steps, the nucleus was protruded from the cytoplasm. In steps 11-13, the most active stages in nuclear shaping, the dynein was densely localized at the nuclear surface covered by the manchette. As the nucleus acquired a shape similar to the mature spermatozoon at step 14, the dynein fluorescence was localized only at the concave side of the nuclear caudal edge. The manchette became narrower and elongated. In step 15, the manchette extended into the elongated cytoplasm, diminishing during steps 16-18. The localization of the dynein was limited to the ventral aspect of the caudal head in these steps. There was little dynein fluorescence in mature spermatozoa. Immunoelectron microscopy showed positive reactions in the nuclear envelope and the inner region of the microtubular manchette. These observations suggest that cytoplasmic dynein, possibly bound to the nuclear envelope, and manchette microtubules are involved in the protrusion of the spermatid nucleus from the cytoplasm.

Key words: cytoplasmic dynein, spermatid, spermiogenesis, immunohistochemistry, immunoelectron microscopy, microtubule-based motility

INTRODUCTION

The spermatid head is dramatically changed during the period of spermatogenesis when spermatids are transformed into spermatozoa, i.e. during spermiogenesis (Leblond and Clermont, 1952; Oakberg, 1956; Fawcett et al., 1971). The mechanisms involved in the shaping that occurs during this period have not yet been established. Special characteristics of the spermatid and Sertoli cells, such as microtubular manchettes and ectoplasmic specialization, as well as the pattern of chromatin condensation that is inherent in a species, are thought to be involved in nuclear shaping (reviewed by Fawcett et al., 1971). The manchette is a distinct structure, which consists of a parallel array of microtubules extending from the nuclear ring of the transforming spermatid nucleus through the cytoplasm (reviewed by Fawcett et al., 1971). The structure forms at the beginning of nuclear shaping, becomes larger and more prominent during transformation, and diminishes as the spermatid becomes mature. Since the manchette appears concurrently with nuclear shaping, it is conceivable that it may function to transform the nucleus into the sperm head. Many observations of spermiogenesis in both mutant and chemically treated mice indicate that the manchette is involved in the shaping of the spermatid nucleus (Dooher and Bennett, 1977; Cherry and Hsu, 1984; Cole et al., 1988; Meistrich et al., 1990; Russell et al., 1991), while it has also been emphasized that chromatin condensation and ectoplasmic specialization may be more important in this process (Fawcett et al., 1971). A recent electron microscopic study has clearly demonstrated the linkage of manchette microtubules to the nuclear envelope (Russell et al., 1991). This linkage is considered to be the morphological basis for the transfer of forces and for deforming the nucleus during nuclear shaping. And the structural features suggest involvement of microtubule-based motility in the transformation of the spermatid into the spermatozoon.

Cytoplasmic dynein is a motor protein responsible for the intracellular microtubule-based motility in various tissues; the testis is particularly rich in cytoplasmic dynein. Collins and Vallee (1989) demonstrated that the content of the dynein in the testis was almost three times higher than that in the brain.

Although Sertoli cells are a source of cytoplasmic dynein (Neely and Boekelheide, 1988; Neely et al., 1990), it seems that this relatively small component of the tissue could not account for the large amount of the dynein in the testis. The larger fraction may originate from other testicular cells. Our immunohistochemical study, using anti-bovine brain cytoplasmic dynein (MAP 1C), demonstrated that cytoplasmic dynein was localized predominantly in spermatids, while Sertoli cells were labeled only moderately (Yoshida et al., 1992).

In this study, we examined the subcellular localization of cytoplasmic dynein in spermatids that were transforming into spermatozoa. We used antibodies specific to tubulin and cytoplasmic dynein in the immunohistochemical study, and demonstrated the association of the dynein with the microtubular manchette and the adjacent envelope of the transforming nucleus. The dynein was expressed exclusively during the period when the nucleus was reshaping. These observations indicate that, during spermiogenesis, the microtubular manchette and the nucleus might assemble a mechanical structure that is driven by cytoplasmic dynein.

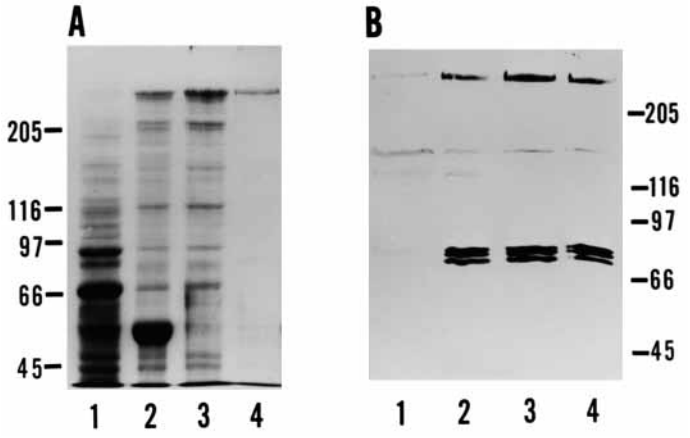

Fig. 1. Immunoblot analysis of cytoplasmic dynein prepared from rat testes. (A) Coomassie Brilliant Blue staining. (B) Immunoblot staining with an antibody against bovine MAP 1C, brain cytoplasmic dynein. Microtubules were assembled from rat testicular cytosolic extract (lane 1) and sedimented to obtain a microtubule fraction (lane 2). The microtubules were extracted with 10 mM ATP solution and the supernatant was obtained by centrifugation (lane 3). The supernatant was fractionated by sucrose density gradient centrifugation (lane 4). The anti-cytoplasmic dynein antibody reacted with the high molecular mass polypeptides, and 150 kDa and 74 kDa subunits, throughout the purification steps. Another 130 kDa band is visible in lanes 1 and 2 of (B).

MATERIALS AND METHODS

Antibodies

MAP 1C, brain cytoplasmic dynein, was purified from the microtubules by the method of Pascal et al. (1987), with slight modifications (Yoshida et al., 1990, 1992). An antibody was prepared by immunizing a rabbit with MAP 1C. The antibody was purified by absorption with a CNBr-Sepharose 4B column conjugated with microtubule-associated protein (MAP) 1C and then by elution with 0.2 M glycine-HCl buffer (pH 2.5). As the purified antibody reacted with both MAP 1C and MAP 2, the antibody was absorbed with purified MAP 2, as described previously (Yoshida et al., 1990). Mouse anti-α-tubulin antibody was purchased from Cedarlane Laboratory Ltd (Ontario, Canada). An antibody against flagellar 21 S dynein purified from sea urchin spermatozoa was raised in a rabbit and affinity-purified. This antibody was referred to as AD1 in our previous study (Yoshida et al., 1989).

Purification of rat testicular cytoplasmic dynein

Cytoplasmic dynein was prepared from testes according to the method of Collins and Vallee (1989). One-year-old male rats were deeply anesthetized and the testes were dissected. Twenty rat testes (30 g) were homogenized in 2 volumes of PEM buffer (0.1 M PIPES buffer, pH 6.6, 1 mM EGTA, 1 mM $MgSO_4$, 2 mM PMSF, 50 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM dithiothreitol) containing 0.25 M sucrose, using a Polytron homogenizer, and then by 2 passes in a Teflon homogenizer. The homogenate was centrifuged at 100,000 ***g*** for 1 hour. The supernatant was recovered, taxol was added to 20 μM, and the sample was warmed to 37°C for 15 minutes. The microtubules were sedimented at 45,000 ***g*** at 20°C for 30 minutes, and washed by resuspension in 6 ml of Tris/NaCl buffer (20 mM Tris-HCl, pH 7.6, 50 mM NaCl, 5 mM $MgCl_2$, and 0.5 mM EDTA) containing 5 μM taxol, followed by centrifugation. Cytoplasmic dynein was extracted from the microtubules with Tris/NaCl buffer containing 10 mM Mg-ATP. The extracted protein was fractionated by sucrose density gradient centrifugation at 31,000 ***g*** for 16.5 hours in a RPS-27 rotor (Hitachi, Japan).

NTPase activity

NTPase activity was assayed in Tris/NaCl buffer at 30°C (Yoshida et al., 1989). The assay was initiated by the addition of ATP or CTP solution to a final nucleotide concentration of 1 mM. After a 30 minute incubation, the extent of NTP hydrolysis was determined by measuring the amount of inorganic phosphate using the colorimetric procedure of Lin and Morales (1977). The assay of ATPase activity stimulated by microtubules was performed in the presence of purified bovine brain tubulin (0.5 mg/ml).

Electrophoresis and immunoblots

Electrophoresis was performed on 7% polyacrylamide gels by the method of Laemmli (1970). The proteins were electrophoretically transferred to an Immobilon membrane (Millipore, Bedford, MA) by the method of Towbin et al. (1979), with slight modifications (Yoshida et al., 1992). After blocking in 0.5% skimmed milk solution, the membrane was treated with anti-MAP 1C (1 μg/ml) overnight. After several washes, it was incubated in goat anti-rabbit IgG Fab′ (200-fold diluted, MBL, Nagoya, Japan) for 2 hours. The immunoreactive protein was developed with diaminobenzidine-H_2O_2 solution.

The sperm proteins were extracted from spermatozoa collected from the epididymis, as described previously (Yoshida et al., 1989). The samples were electrophoresed and analyzed by immunoblotting.

Immunofluorescence staining of rat spermatids

The seminiferous tubules of the testis were cut into several pieces. Imprint smears of germinal cells were then prepared on poly-L-lysine-coated glass slides. The smears were fixed immediately with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (P_i buffer, pH 7.4) for 20 minutes and were permeabilized for 30 minutes by treatment with 0.1% Triton X-100 in P_i buffer containing 4% paraformaldehyde. Following several washes in 10 mM phosphate-buffered saline (PBS, pH 7.2) and treatment with 10% normal goat serum for 30 minutes, they were incubated with anti-MAP 1C antibody (10 μg/ml). After being washed in PBS, the cells were incubated with FITC-labeled goat anti-rabbit IgG (MBL, 100× dilution) for 1 hour at room temperature, and again washed in PBS. For double immunofluorescence, they were incubated with anti-α-tubulin mouse monoclonal antibody (200× dilution) for 2 hours at

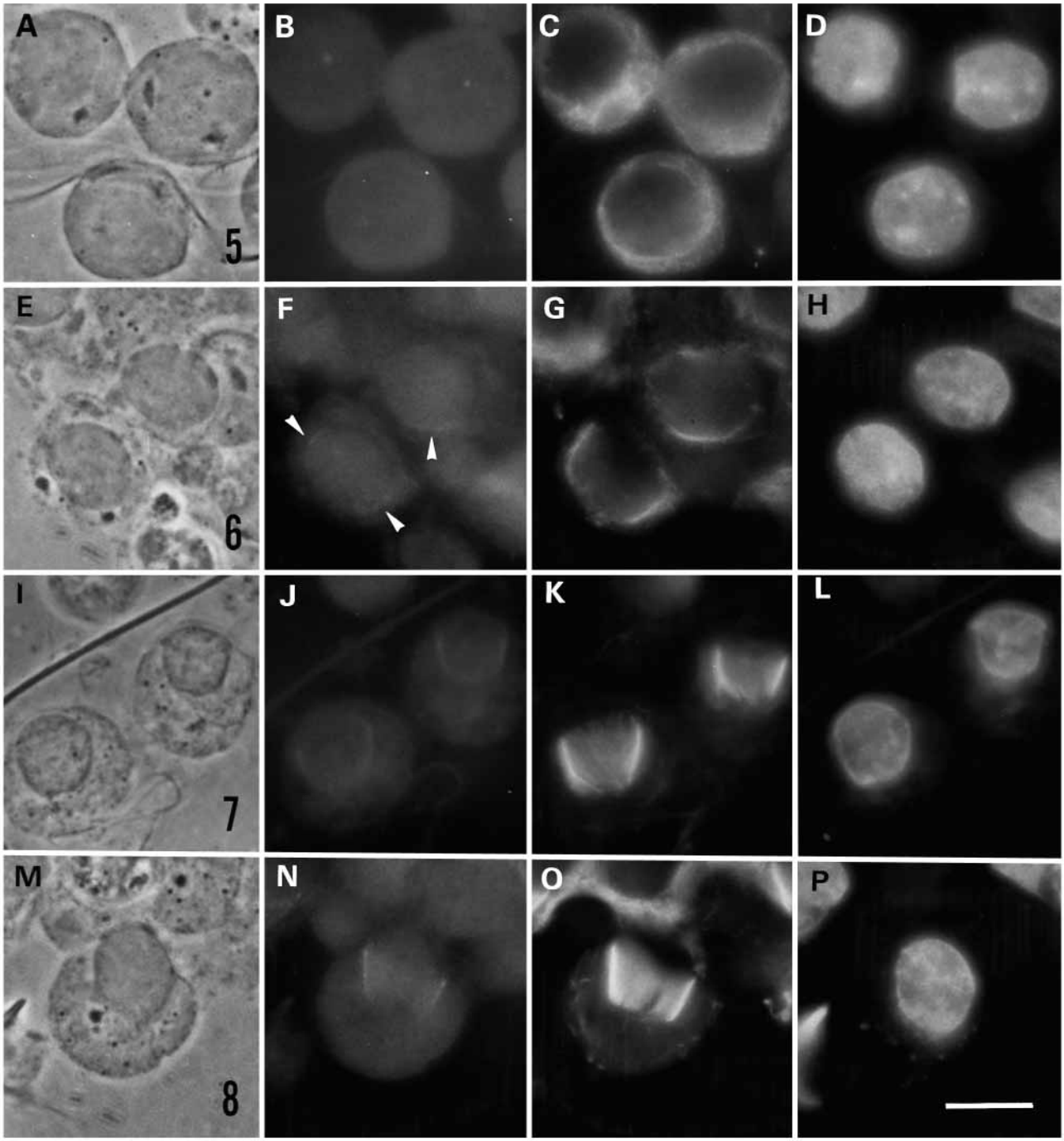

Fig. 2. Immunolocalization of cytoplasmic dynein in step 5-8 spermatids. The spermatids were observed by phase-contrast microscopy (A,E,I and M). The numbers in the images indicate the spermatid step in each row. The spermatids were stained immunologically with anti-dynein antibody (B,F,J and N) and anti-tubulin antibody (C,G,K and O), and counter-stained with DAPI for nuclei (D,H,L and P). Step 5 spermatids show a reticular pattern stained with anti-tubulin, but a homogeneous staining for anti-dynein. In step 6, cytoplasmic microtubules are condensed in the perinuclear region, and cytoplasmic dynein is beginning to be concentrated in the region (arrowheads in F). Microtubular manchettes are observed in step 7-8 spermatids (K and O), accompanied by dynein staining (J and N). Bar in (P), 10 μm.

room temperature, and then with rhodamine-B-labeled goat anti-mouse IgG (100× dilution, Tago) for 1 hour at room temperature. And they were washed three times in PBS. The nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI, 5 μg/ml) in the second washing solution. The cells were mounted and viewed through a Zeiss fluorescence microscope equipped with appropriate filter systems for the double immunofluorescence technique.

Immunoelectron microscopy

Anesthetized rats were perfused with phosphate-buffered saline (pH 7.2, PBS) and then with 4% formaldehyde/0.1% glutaraldehyde in Pi buffer through a catheter placed in the heart. The testes were dissected, trimmed, and fixed in 4% formaldehyde/0.1% glutaraldehyde at 4°C for 3 hours. The specimens were then refixed in paraformaldehyde-lysine-periodate solution at 4°C for 4 hours. They

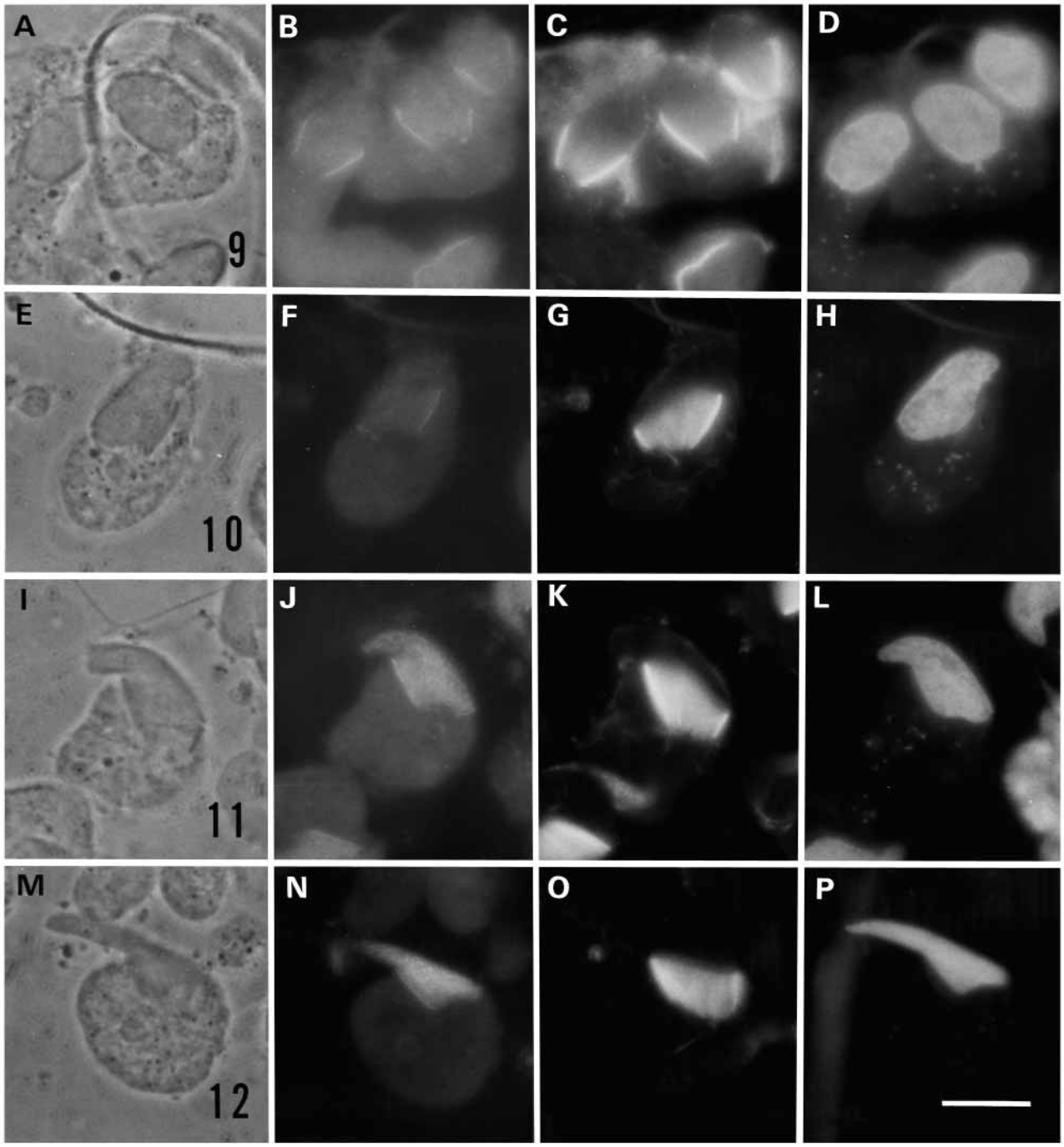

Fig. 3. Immunolocalization of cytoplasmic dynein in step 9-12 spermatids. The spermatids were observed by phase-contrast microscopy (A,E,I and M). The numbers in the images indicate the spermatid step in each row. The spermatids were stained immunologically with anti-dynein antibody (B,F,J and N) and anti-tubulin antibody (C,G,K and O), and counter-stained with DAPI for nuclei (D,H,L and P). The nuclei in steps 9 and 10 have become elongated and curved (D and H). The manchettes change to asymmetrical shapes in steps 10-13 (G,K and O). The dynein fluorescence appears to be associated with the manchette, but is thinner than the manchette fluorescence (compare F and G), indicating the immediate localization of the dynein on the nuclear envelope. In steps 11-12, the nuclear surface is covered with the manchette and the apical surface beyond the perinuclear ring is labeled by the dynein antibody (J and N). Bar in (P), 10 μm.

were sequentially treated with PBS containing 10%, 15%, 20% sucrose and 5% glycerol/20% sucrose. They were then embedded in OCT compound (Miles, IN, USA) and frozen in dry ice/acetone. Sections (6 to 10 μm) were cut using a cryostat and placed on glass slides coated with poly-L-lysine. After being dried, the sections were washed in PBS with 10% sucrose, and were treated with 10% normal goat serum. The sections were then incubated overnight at 4°C with anti-MAP 1C (1 μg/ml), and were washed extensively, followed by overnight incubation at 4°C with peroxidase-conjugated goat anti-rabbit IgG Fab′ (200-fold diluted, MBL, Nagoya, Japan). After extensive washes, the sections were refixed in 0.5% glutaraldehyde, and peroxidase activity was developed with diaminobenzidine-H_2O_2

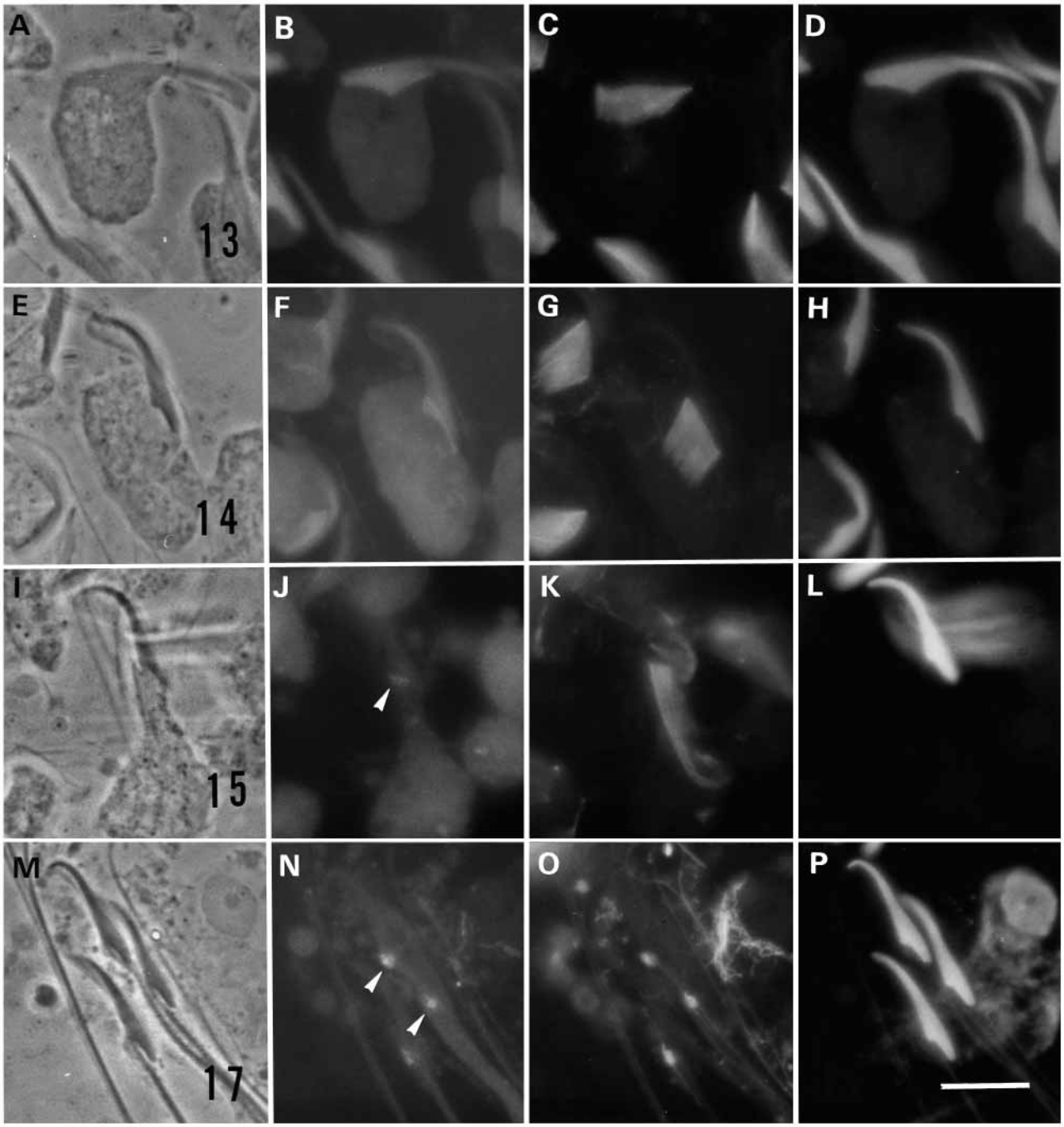

Fig. 4. Immunolocalization of cytoplasmic dynein in step 13-17 spermatids. The spermatids were observed by phase-contrast microscopy (A,E,I and M). The numbers in the images indicate the spermatid step in each row. The spermatids were stained immunologically with anti-dynein antibody (B,F,J and N) and anti-tubulin antibody (C,G,K and O), and counter-stained with DAPI for nuclei (D,H,L and P). The fluorescent part of the dynein has decreased gradually in a step 13 spermatid (B). As the perinuclear ring reaches the nuclear caudal edge (G) and the nucleus appears mature in step 14 (E and H), the dynein is localized only at the concave side of the nuclear caudal edge (F). The fluorescence has become more diminished (arrowhead in J) and is eventually seen as dot-like structures (arrowheads in N). Bar in (P), 10 μm.

solution. After further washes, the sections were incubated with 2% OsO_4 for 1 hour and washed with water. And they were run through a graded series of ethanol and embedded in epoxy resin. Seminiferous tubules, including appropriate stages of germ cells, were selected under a light microscope and trimmed. The serial sections were cut with Ultracut E, picked up on copper grids and examined at a 70 kV in Hitachi 700 electron microscope without lead or uranyl staining.

RESULTS

Cytoplasmic dynein in rat testes and characterization of antibody

Cytoplasmic dynein was purified from the microtubular fraction of rat testes, as described by Collins and Vallee

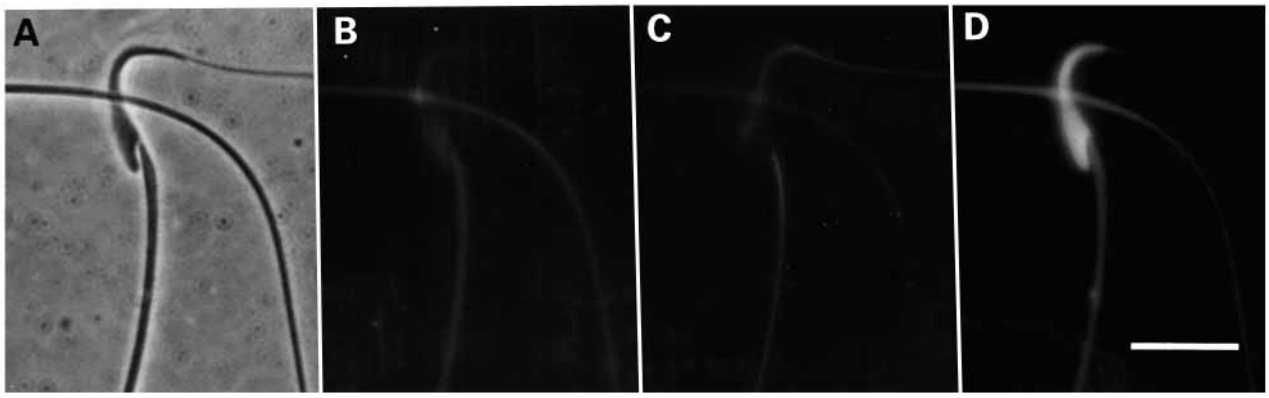

Fig. 5. Immunolocalization of cytoplasmic dynein in a mature spermatozoon isolated from the epididymis. (A) Phase-contrast microscopy; (B) anti-dynein staining; (C) anti-tubulin staining; (D) DAPI staining. There was little cytoplasmic dynein fluorescence. Bar in (D), 10 μm.

(1989). The dynein was extracted with 10 mM ATP from the microtubules. Prominent bands at the position of kinesin (120 kDa and 90 kDa) were detected in the supernatant after ATP extraction (lane 3, Fig. 1A). The dynein was further purified by sucrose gradient centrifugation (lane 4, Fig. 1A). The purified dynein showed both CTPase and ATPase activity, the CTPase activity being approximately 5-fold higher than the ATPase activity. The ATPase activity was stimulated 1.4-fold by the addition of microtubules (data not shown). Throughout the purification steps, anti-bovine MAP-1C antibody reacted with heavy chains and bands of 150 kDa and 74 kDa. Bands in the region of 74 kDa were shown as doublets or triplets. Before ATP release, a band at 130 kDa was sometimes visible in the samples. This polypeptide might be a type of 150 kDa species.

The extracted proteins from rat spermatozoa were also analyzed by immunoblotting using antibodies specific to cytoplasmic dynein and to flagellar dynein. Anti-cytoplasmic dynein did not react with any sperm proteins, indicating the absence of cytoplasmic dynein in spermatozoa and no cross-reactivity of anti-cytoplasmic dynein with the flagellar dynein of rat sperm (not shown).

Immunological localization of cytoplasmic dynein in rat spermatids

The spermatids at various stages during spermiogenesis were stained by immunofluorescence, using the anti-MAP 1C antibody. The localization of cytoplasmic dynein changed dramatically in spermatids transforming into spermatozoa. Before step 6 of the Leblond and Clermont (1952) staging, the microtubules had a fine reticular network in the round spermatid. The dynein staining revealed homogeneous cytoplasm during these steps (Fig. 2A-D). In step 6, the nucleus, with an enlarged head cap, was spherical and then became eccentric (Fig. 2E). As the microtubular network was rearranged and concentrated around the nucleus (Fig. 2G), weak dynein fluorescence was detected in the perinuclear region where the microtubules were dense (Fig. 2F). The nucleus protruded from the cytoplasm in steps 7-10. A microtubular manchette stained with anti-tubulin antibody was developed in step 7 spermatids (Fig. 2K). In step 8, the manchette was fully formed, had a skirt-like appearance, and covered the caudal half of the nucleus (Fig. 2O). The nuclear changes in steps 9 and 10 were elongation and curvature (Fig. 3D,H). The manchette became angular in step 10 (Fig. 3G). In steps 7-10, the dynein fluorescence was associated with the manchette (Figs 2J,N, and 3B,F). However, the dynein fluorescence was thinner than that of tubulin and the dynein staining seemed to be localized in the immediate vicinity of the nuclear envelope. Steps 11-13 were the most active stages in the nuclear shaping (Figs 3I-P and 4A-D). The chromatin became more condensed, and the perinuclear ring and the manchette moved to the caudal part of the nucleus. During these stages, the dynein was densely localized at the nuclear surface, which was covered with the manchette, and at the apical surface beyond the perinuclear ring (Figs 3J,N and 4B). At step 14 (Fig. 4E-H), when the nucleus acquired a shape similar to that of the mature spermatozoon, the dynein fluorescence was visible only at the concave side of the nuclear caudal edge (Fig. 4F). The manchette became narrowed and elongated (Fig. 4G). In step 15, the manchette, which then had a comet-like appearance, extended into the elongated cytoplasm, diminishing during steps 15-17 (Fig. 4K,O). The localization of the dynein was limited to the ventral aspect of the caudal head during these steps. In mature spermatozoa isolated from epididymis, there was little cytoplasmic dynein fluorescence (Fig. 5B).

Double immunofluorescence, using anti-flagellar dynein and anti-tubulin, showed that the staining pattern with anti-flagellar dynein was identical to that of anti-tubulin during steps 9 to 17. In addition, the flagella of spermatids after step 17, as well as the flagella of mature spermatozoa from the epididymis, were labeled with anti-flagellar dynein (not shown).

Immunoperoxidase electron microscopy using the antibody revealed the subcellular localization of cytoplasmic dynein. The positive reaction was strong in the nuclear envelope covered by the manchette (Fig. 6A), and in the region between the nuclear envelope and the innermost microtubules (Fig. 6B). Distances between the outer membrane of the nuclear envelope and the closest microtubules were in the range of 45-65 nm. The perinuclear ring also showed a positive reaction in the cytoplasmic membrane and in the area between the microtubule and the plasma membrane, as well as in the vicinity of the nuclear membrane (Fig. 6B). However, the manchette elongated into the cytoplasm was not labeled (Fig. 6A). Replacing anti-cytoplasmic dynein antibody with normal rabbit IgG eliminated the positive reaction produced by the antibody (Fig. 6C).

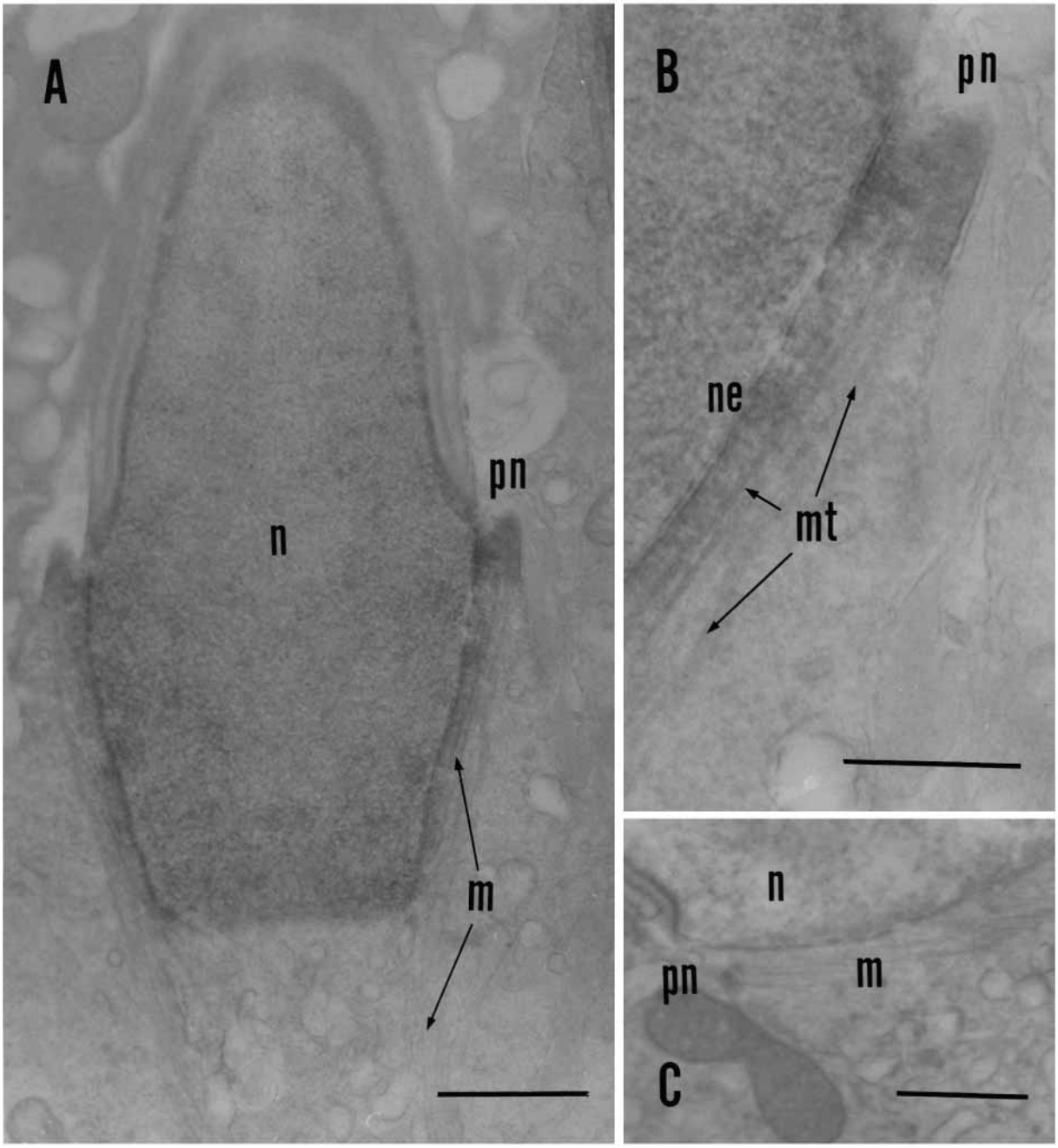

Fig. 6. Immunoelectron microscopic localization of cytoplasmic dynein in spermatids. (A) A possible step 12 spermatid labeled with anti-cytoplasmic dynein by the immunoperoxidase method. The positive reaction is seen at the lateral surface of the nucleus (n) covered by the manchette (m), whereas the manchette elongated into the cytoplasm is not labeled. (B) High magnification of the right perinuclear ring of A. The positive reaction is seen in the region between the nuclear envelope (ne) and the innermost microtubules (mt). The perinuclear ring (pn) also shows a positive reaction in the cytoplasmic membrane and in the area between the microtubule and the plasma membrane, as well as in the vicinity of the nuclear membrane. (C) A perinuclear area with the manchette in a possible step 9 spermatid that was labeled with normal rabbit IgG for a control experiment. Replacing anti-cytoplasmic dynein antibody with the normal IgG eliminated the positive reaction. Bars: (A and C), 1 μm; (B) 0.5 μm.

DISCUSSION

The microtubular manchette of the spermatid has been studied extensively in previous electron microscopic studies (Fawcett et al., 1971; Rattner and Brinkley, 1972; Cole et al., 1988; Meistrich et al., 1990; Russell et al., 1991). The manchette, an organelle consisting of laterally associated microtubules, appears transiently during spermiogenesis. The microtubules are arranged in a skirt-like structure originating in a dense perinuclear ring and extending through the cytoplasm. Linkages between the microtubules were demonstrated in these studies, suggesting that they act in a coordi-

nated manner. Further, the microtubules were shown to have links with the membranes of associated vesicles in the manchette (Fawcett et al., 1971) and with the nuclear membrane (Rattner and Brinkley, 1972; Russell et al., 1991). The links between the microtubules and the nucleus were found in an earlier study (Rattner and Brinkley, 1972). Recently, Russell et al. (1991) clearly visualized the microtubule-nuclear membrane linkages as rod-like structures, about 10 nm in diameter and 40-70 nm in length, in both routine and cytoskeletal preparations of the testes. The structures were observed to connect the innermost microtubules with the outer leaflet of the nuclear envelope in step 8 through step 11 spermatids. The structures were also visible between the perinuclear ring and the plasma membrane. It is speculated that the linkers consist of microtubule-associated proteins and/or microtubule motors, including cytoplasmic dynein (Russell et al., 1991). In this study, we demonstrated immunocytochemically that the dynein was associated with the microtubular manchette and the nuclear envelope in step 7 through step 14 spermatids, with strong expression being shown in steps 8 to 12. Our immunoelectron microscopy demonstrated that the dynein was concentrated in the region between the nuclear envelope and the closest microtubules. The distance between the envelope and the innermost microtubule were 45-65 nm. Russell et al. (1991) reported earlier that the linkages were 40 to 70 nm long. The size of the dynein molecule is ~50 nm from the base to the top of the head, as determined by scanning transmission electron microscopy (Vallee et al., 1988). The dynein molecule could be the structural element that links the envelope with the microtubule.

Cytoplasmic dynein has been identified as the motor protein responsible for retrograde (or minus-end directed) transport in membranous organelles (Paschal and Vallee, 1987; Schnapp and Reese, 1989; Schroer et al., 1989; Vallee, 1991). Recent biochemical and immunohistochemical studies indicate a high affinity of cytoplasmic dynein to vesicular membranes in neural cells (Hirokawa et al., 1990; Lacey and Haimo, 1992; Yu et al., 1992). In other types of cells also, the dynein is thought to be generally associated with membranous organelles, such as the Golgi apparatus (Corthésy-Theulaz et al., 1992), the endoplasmic reticulum (ER; Toyoshima et al., 1992; Kihira et al., unpublished observation), lysosomes (Lin and Collins, 1992) and endosomes (Goltz et al., 1992). Since the nuclear envelope is a compartment of the ER (Watson, 1955; Sitia and Meldolesi, 1992), it is feasible that cytoplasmic dynein could be attached to the envelope. The nucleus is positioned stably near the centriole, the organizing center of the cytoplasmic microtubular array. The pericentriolar positioning of the nucleus may be provided by the dynein molecules binding to the envelope. In spermatids, our study demonstrated that the dynein was concentrated at the nuclear surface, which was associated with the microtubular manchette during the period of nuclear shaping. The concentrated distribution of the mechanochemical molecules on the nuclear envelope might generate the strong force that was exerted on the nucleus.

Russell et al. (1991) discussed how the manchette could change the shape of the nucleus. Their hypothesis was that the manchette could progressively 'zipper' down the nuclear envelope and that the controlled zippering and unzippering of the manchette to the nuclear envelope could take place during nuclear shaping in rodent spermatids. It is also surmised that the nuclear ring and entire manchette move on the spermatid nucleus toward the caudal region, in concert with acrosome expansion. The dynein molecules that bind to the nuclear envelope could slide on the microtubules, which could be responsible for the movement of the manchette relative to the nucleus. The polarity of the manchette microtubules is not yet known. However, an electron microscopic study has demonstrated periodic densities in the nuclear ring, the probable microtubule organizing center (Russell et al., 1991). Further, we found that microtubular changes at the cytoplasmic free ends seemed more dynamic those at the ring ends. It is thus conceivable that the perinuclear ring is the minus end of the microtubule and the cytoplasmic end is the plus. Therefore, the dynein molecules bound to the nuclear envelope could slide on the microtubules in the direction of the nuclear ring, the possible minus end. This sliding could account for the movement of the manchette toward the caudal nucleus, and this movement could yield the protrusion of the nucleus from the cytoplasm, concomitant with the translocation of the cytoplasm behind the nucleus. Thus, producing the protrusion of the head is possibly one of the roles played by the mechanical structure, which consists of the microtubular manchette and the nuclear envelope, driven by cytoplasmic dynein.

A recent study using a monoclonal antibody against intermediate chains of chick brain cytoplasmic dynein (clone 70.1) also demonstrated the localization of the cytoplasmic dynein in the manchette of step 15-17 spermatids (Hall et al., 1992). The fluorescent pattern seen during these steps was identical to our findings. However, Hall et al. did not detect the localization in other spermatid steps. There is a discrepancy between their immunohistochemical results for step 7-14 spermatids and ours. This monoclonal antibody reacted with a single 72 kDa band in the immunoblots of rat testes, whereas our polyclonal antibody detected multiple 74 kDa bands. Recently, Paschal et al. (1992) found at least three isoforms of the 74 kDa cytoplasmic dynein subunit by PCR analysis performed on rat brain cDNA. These results indicate that the monoclonal antibody may react with only one of the 74 kDa isoforms localized in step 15-17 spermatids. Conversely, a different isoform might be localized on the nuclear envelope in step 7-14 spermatids. This isoform diversity might be related to specifying the functions of cytoplasmic dynein and targeting to organelles during spermiogenesis; cytoplasmic dynein could play many roles in the dynamic process of spermiogenesis. Therefore, the microtubular manchette could be an interesting target for research, in terms of exploring microtubule-based motility, as well as investigating spermiogenesis.

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