INTRODUCTION

Mammalian spermatogenesis begins with numerous mitoses of spermatogonia (stem cells) which eventually give rise to primary spermatocytes. Successively, meiotic divisions of spermatocytes give rise to spermatids. The metamorphosis of these haploid germ cells, or spermiogenesis, culminates in the release of testicular spermatozoa. The process of spermatid differentiation into spermatozoa takes two to three weeks depending on the species and can be subdivided into three successive phases of equal duration (Clermont, 1972). Early spermatids are round cells with an organisation similar to that of any undifferentiated somatic cell. In particular they contain a microtubule organising centre (MTOC), the centrosome, made of two centrioles with associated \( \gamma \)-tubulin and randomly distributed microtubules (Fouquet et al., 1998; Manandhar et al., 1998). However, by the end of the round phase, spermatids have secured a polarity. The Golgi apparatus and the associated acrosome, a giant lysosome, are located at the cell anterior pole whereas the centrioles are positioned at the cell caudal pole. In addition, a naked flagellum is growing from the distal centriole and a centriolar adjunct (mini flagellum) is developing from the proximal centriole (de Kretser and Kerr, 1994). The second phase of spermatid differentiation, i.e. elongation phase, is characterised by the development of a cone-shaped bundle of microtubules, the manchette, encasing the nuclear posterior pole up to a nuclear ring of undetermined material, at the acrosome boundary. The fully developed manchette is not actually associated to the MTOC, yet, the prominent accumulation of \( \gamma \)-tubulin in the pericentriolar material of both centrioles, particularly around the centriolar adjunct (Fouquet et al., 1998), suggests that these structures may primarily nucleate the manchette microtubules. During the third phase of spermiogenesis, i.e. maturation of spermatids, the manchette slips backwards and eventually depolymerises. Then, the future spermatozoon loses practically all the spermatid cytoplasm and organelles as a residual body, except for most of the mitochondria, which assemble at the base of the flagellum to form the middle piece. The manchette is a unique set of parallel cytoplasmic microtubules, that encircles the nucleus of elongating spermatids not only in mammals but also in many other zoological groups including sauropsidae, amphibiaans, fishes, insects and annelids (Courtens and Loir, 1981). The role of the manchette microtubules has not been ascertained, but they seem to be involved in organelle translocation and in the process of spermatid elongation, both at the nuclear and cytoplasmic level. Most authors believe indeed that the manchette is involved in the nuclear shaping of spermatids (Russell et al., 1991). Considering the possible functions of the manchette, it seems probable that motor proteins will be revealed as key actors. Motor proteins are members either of the dynein family, minus-end directed motors, or of the kinesin superfamily, plus-end directed motors. Both intermediate and

SUMMARY

Actin related protein of vertebrate, Arp1, is a major component of the dynactin complex. To characterise and localise Arp1 during mammalian spermatogenesis, polyclonal antibodies were raised against a human recombinant Arp1. Anti-Arp1 antibodies were used for western-immunoblotting, indirect immunofluorescence and immunoelectron microscopy. In round spermatids, Arp1 was detected at the centrosome and at the Golgi apparatus. In elongated spermatids, Arp1 was predominantly found along microtubules of the manchette and at their site of attachment to the nuclear envelope. In maturing spermatids, Arp1 was still present in the pericentriolar material, but in testicular spermatozoa it was not detectable. These various localisations of Arp1 and their changes during spermatid differentiation suggest that the dynactin complex in association with dynein might contribute to several activities: the functional organisation of the centrosome and of the Golgi apparatus and the shaping of the nucleus by manchette microtubules.

Key words: Spermatozoon differentiation, Actin-related protein, Centrosome, Golgi, Microtubule, Sperm head shaping
heavy chain of cytoplasmic dynein and heavy chain of kinesin are found in rat testis extracts (Hall et al., 1992; Criswell and Asai, 1998). Cytoplasmic dynein is detected by indirect immunofluorescence at the manchette (Hall et al., 1992; Yoshida et al., 1994) and in association with the nuclear envelope in rat elongating spermatids (Yoshida et al., 1994). To translocate organelles or other cargoes along microtubules, cytoplasmic dynein requires interacting with dynactin, an accessory protein complex. This multi-subunit complex contains a major component, a short F-actin-like filament originally called actin-RPV (Lees-Miller et al., 1992) or centrinactin (Clark and Meyer, 1992) now referred to as Arp1 (Frankel and Mooseker, 1996).

To get more information about the function of spermatid manchette during mammalian spermatogenesis we have raised polyclonal antibodies against human Arp1 and found that Arp1 is associated with manchette microtubules and may be part of the connection between them and the nuclear envelope. Arp1 is also found in association with the centrosome and the Golgi apparatus.

**MATERIALS AND METHODS**

**Biological samples**

Testes and epididymides of sexually mature active mice (*Swiss*), rat (*Sprague-Dawley*), rabbits, pig or monkey (*Macaca fascicularis*) were removed under anaesthesia. Samples were used for western immunoblotting, indirect-immunofluorescence (IIF) and/or immunochemistry (IEM). Testicular biopsies and ejaculated spermatozoa from human donors were used only for IEM. HeLa cells were grown to 75% confluence in Dulbecco’s modified Eagle’s medium and used for western immunoblotting.

**Isolation of germ cells**

Testes were placed in isolation medium (TIM, 104 mM NaCl, 45 mM KCl, 2.4 mM MgSO₄, 6.0 mM Na₂HPO₄, 0.7 mM KH₂PO₄, 5.6 mM glucose, pH 7.2, 1.2 mM CaCl₂; Dietrich et al., 1983). The tunica albuginea was removed and seminiferous tubules were dispersed mechanically in TIM. Epididymides were cut into pieces and placed in TIM under agitation, to free mature spermatozoa. Cell suspensions, from testes or epididymides, were removed under anaesthesia. Samples were used for western immunoblotting.

**Antibodies against human Arp1**

Human actin-RPV was expressed in *E. coli* BL21 (DE3) using pET vectors and purified from insoluble inclusion bodies as previously described (Melki et al., 1993). Human actin-RPV was solubilised at 90°C in 50 mM Tris-HCl, pH 6.8, 4% SDS, 2% β-mercaptoethanol, 12% glycerol, 0.01% Bromophenol blue (Laemmli, 1970) and subjected to PAGE. The gels were stained (Hager and Burgess, 1980) and the band corresponding to recombinant human actin-RPV probed with the anti-human actin-RPV serum and revealed by an enzyme linked chemiluminescence reaction according to the manufacturer’s recommendations (Renaissance western blot chemiluminescence reagent plus, NEN Life Science Products, Inc., Boston, MA).

The specificity of the antiserum was tested by the use of various cell extracts. Cells were lysed by sonication in 0.1 M MES, pH 6.8, 1 mM MgCl₂, 1 mM EGTA, 1 mM DTT and 0.05% Triton X-100. The protein extract thus obtained was denatured at 90°C in PAGE-sample buffer (Laemmli, 1970), subjected to PAGE, western blotted and detected by an enzyme linked chemiluminescence reaction.

The antiserum immunoglobulin-G fraction was immunopurified by use of a Protein G Sepharose 4 Fast Flow column (Amersham Pharmacia Biotech, Uppsala, Sweden). The antiserum was incubated with affinity chromatography gel overnight at 4°C. Then the resin was washed in 20 mM phosphate buffer, pH 7.0, 100 mM NaCl and the IgG was eluted in 0.2 M glycine, pH 2.6. After adjusting the pH to 7.5 by addition of Tris-HCl pH 8.5, the concentration of the IgG was determined (Bradford, 1976) and the fractions divided into aliquots and stored at −20°C.

Affinity purified antibody was obtained by the use of human actin-RPV immobilized on Affi-gel 15 column (Bio-Rad corp.). Human actin-RPV (10 mg) in 0.1 M MES, pH 6.8, 0.5 M GuHCl was incubated for 4 hours under rotatory shaking with 0.5 ml of Affi-gel 15 equilibrated in 0.1 M MES, pH 6.8. The coupling reaction was terminated by addition of 25 mM Tris, 200 mM glycine, pH 8.3, and the resin equilibrated in 20 mM phosphate buffer, pH 7.0, 100 mM NaCl. The antiserum was incubated with affinity chromatography gel overnight at 4°C. Then the resin was washed with equilibration buffer and the IgG was eluted using 0.2 M glycine, pH 2.2. After adjusting the pH to 7.5 by addition of Tris-HCl pH 8.5, the concentration of the affinity purified anti-human actin-RPV antibody was determined (Bradford, 1976) and the fractions divided into aliquots and stored at −20°C.

**Indirect immunofluorescence**

A sufficient amount of cell suspension, from testes or epididymides, were laid on 8-wells slides, previously coated with poly-L-lysine (0.1%). Cells were fixed and permeabilised at −20°C in methanol for 5 minutes and in acetone for 30 seconds. Anti-Arp1 antibody, diluted 1:100 in phosphate-buffered saline containing 0.1% bovine serum albumin, was added to cells and incubation was pursued either overnight at 12°C or for 2 hours at room temperature. Labelling was developed with a secondary antibody, FITC-labelled anti-rabbit (F-9887; Sigma, St Louis, MO, USA). Slides were mounted in Mowiol plus Fluoromount-G™ (Southern Biotechnology Associates Inc, Birmingham, AL, USA).

**Immunoelectron microscopy**

All samples were routinely fixed for 1 hour in 0.1 M cacodylate buffer, pH 7.3, containing 1% glutaraldehyde and embedded in Lowicryl K4M. Thin sections collected on 200 mesh uncoated nickel grids were incubated for 2 hours either with immunopurified IgG or with affinity purified antibody directed against Arp1. then with immunopurified antibody conjugated to 10 nm gold particles (G-1021; Sigma/Aldrich Chimie, France). Antibodies were diluted in Tris buffered saline (TBS), pH 7.9, containing 0.2% bovine serum albumin (Fraction V, IBF). Grids were contrasted with saturated aqueous uranyl acetate before observation. Several control experiments were performed, either omitting the primary antibody, or pre-absorbing the primary antibody with the immunogene (recombinant human actin-RPV) at a molar ratio of 1:10 overnight at 4°C.

**RESULTS**

**Specificity of the antibody directed against Arp1**

The specificity of the polyclonal antibody directed against actin-RPV was evaluated by western blot. The antibody we generated recognised a single band that had a molecular mass...
observation prompted us to examine, in detail, both by differentiation and/or maturation of spermatozoa. This reorganisation of the cellular content of actin-RPV during epididymal spermatozoa. This strongly suggests a bulk spermatids while it was undetectable in protein extracts of proteins present in HeLa cell or testis extracts. 

that it does not cross react with endogenous actin or with other polyclonal antibody we generated is specific of actin-RPV and chains (Fig. 1). We conclude from these results that the antiserum was found not to recognise actins are over 50% identical and around 90% similar. The cytoskeletal proteins. Indeed, human actin-RPV and we next tested whether the antibody recognised these major Arp1 in HeLa cells and germ cells from different species. (A) Immunodetection by western blot in extracts made of HeLa cells, mice (m) and pig (p) testis, isolated mice spermatids (spt) and spermatozoa (spz). (B) Coomassie blue staining of the corresponding 8.5% polyacrylamide gel. α- and β-actin were included in order to check the specificity of the anti-Arp1 antiserum. Molecular mass markers are indicated at the right.

of 42 kDa, consistent with that of the calculated mass of full-length actin-RPV (42613 Da), in extracts of HeLa cells and of mice or pig testes (Fig. 1). Because actin-RPV and α- or β-actin share a significant degree of homology (Herman, 1993), we next tested whether the antibody recognised these major cytoskeletal proteins. Indeed, human actin-RPV and α or β-actins are over 50% identical and around 90% similar. The antiserum was found not to recognise α- or β-actin polypeptide chains (Fig. 1). We conclude from these results that the polyclonal antibody we generated is specific of actin-RPV and that it does not cross react with endogenous actin or with other proteins present in HeLa cell or testis extracts.

Actin-RPV was detected in protein extracts of testicular spermatids while it was undetectable in protein extracts of epididymal spermatozoa. This strongly suggests a bulk reorganisation of the cellular content of actin-RPV during differentiation and/or maturation of spermatozoa. This observation prompted us to examine, in detail, both by immunofluorescence and immunoelectron microscopy, the fate of actin-RPV during spermiogenesis.

**Detection of Arp1 by indirect immunofluorescence during mouse and rat spermiogenesis**

Localisation of Arp1 was investigated by indirect immunofluorescence in both mouse and rat isolated germ cells. At the beginning of mouse spermiogenesis, i.e. in most early round spermatids, Arp1 associated fluorescence was detected as a single bright spot of about 0.5 μm in width, very close to the nucleus (Fig. 2A,A'). Most probably, this fluorescence signal corresponded to the Arp1 localisation at the centrosome, as already described (Clark and Meyer, 1992). In late round spermatids the fluorescent spot was less frequently observed than in younger cells. In contrast, all late round spermatids exhibited a light fluorescence signal outlining the nucleus hemisphere opposite to the acrosome (Fig. 2B,B'). During the second period of spermiogenesis, i.e. in elongating spermatids, the perinuclear labelling was persistent (Fig. 2C,C'), but a lighter labelling was also detected at the manchette (Fig. 2D,D'). At the beginning of the third period of spermiogenesis, i.e. the maturation phase, there was a decrease in labelling signal, both at the perinuclear area and at the manchette, concomitant with the backward movement of the latter. It was noticeable that in most maturing spermatids a small bright spot resumed the centriole area at the base of the spermatid head (Fig. 2E). However, in testicular spermatozoa with a fully differentiated flagellum or in residual bodies Arp1 was not detectable. Consistently, Arp1 was not detected by western immunoblotting in mature spermatozoa isolated from epididymis. That Arp1 was not always detectable at the centrosome in spermatids exhibiting the manchette could result from an actual change in the protein cellular distribution. However, we cannot exclude that Arp1 localisation at the centrosome was masked by the broad fluorescence signal associated to the manchette. Strikingly, large fluorescent spots of 1.5 to 2 μm width were detected in some round spermatids and spermatocytes (Fig. 2E). This distinctive signal suggested that Arp1 might be associated not only to the centrosome, but also to a nearby structure. In fact, the Golgi apparatus is usually associated with the centrosome. Therefore, one possibility is that Arp1 is also associated with the Golgi apparatus.

The distribution of Arp1 during spermiogenesis in rat is illustrated in Fig. 3. Except for a more discrete labelling of the centriole and/or Golgi area, the labelling sequence of the nucleus and manchette according to the differentiation stage was similar to that reported above for the mouse. Thus perinuclear labelling occurred at first in late round spermatids (Fig. 3A,A'). Then the fluorescence signal intensified and extended to the manchette of elongating spermatids (Fig. 3B,B',C,C'). Finally Arp1 fluorescence both around the nucleus and in the manchette disappeared progressively from the beginning of the maturation phase of spermatids so that no signal could be detected in testicular spermatozoa (Fig. 3C,C').

**Localisation of Arp1 by immunogold during spermiogenesis**

Arp1 is associated with manchette microtubules and nuclear envelope

To determine more accurately the cellular localisation of Arp1 we examined mouse and rat spermatids by immunoelectron
microscopy. In young spermatids, Arp1 was actually associated with the microtubules at the centrosome (Fig. 4A). At this stage of differentiation, cytoplasmic microtubules are practically undetectable; accordingly, Arp1 labelling was not discernible far off the centrioles. In late round spermatids the spherical nucleus is covered by the acrosome at the anterior hemisphere and by some short microtubules at the posterior hemisphere that will subsequently develop into the manchette structure. At this stage, both microtubules and nuclear envelope were labelled with anti-Arp1 antibody (Fig. 4B). At the beginning of the elongation phase, the manchette has acquired the classical cone-shaped organisation with numerous parallel microtubules linked together and connected by fuzzy material

---

Fig. 2. Indirect immunofluorescence (IIF) of mouse isolated germ cells with anti-Arp1 IgG. (A-E) Fluorescence; (A',D') phase contrast. (A) Two early round spermatids with a single bright spot (●); and above these two cells an elongating spermatid (►) with a similar spot is also visible. (B) Late round spermatids with a semicircular labelling along the nucleus. (C,D) Elongating spermatids showing both labelling around the nucleus and in the manchette (m). (E) Small fluorescent spots in elongating (►) and maturing spermatids (=) and larger spots in some spermatocytes (→) and round spermatids (●). Bars, 10 μm.

---

Fig. 3. Indirect immunofluorescence (IIF) of rat isolated germ cells with anti-Arp1 IgG. (A-C) Fluorescence; (A'-C'), phase contrast. (A) Labelling on the border of the nucleus in a late round spermatid. (B-C) Labelling around the nucleus and in the manchette (m) in elongating spermatids; no labelling in a testicular spermatozoon; Bar, 10 μm.
Fig. 4. Arp1 immunogold detection with anti-Arp1 IgG in the centrioles and manchette of the rat (A,C,E) and mouse (B,D,F) spermatids. (A) Labelling of the proximal centriole in a round spermatid. (B) Labelling of the nuclear envelope (●) and of few associated microtubules (m) in a late round spermatid. (C,D) Longitudinal and cross sections of elongating spermatids: gold particles associated with manchette microtubules (m) and along the nucleus (▶) (nr, nuclear ring; E 3, nuclear envelope). (E,F) Maturing spermatids: gold particles associated with manchette remnant (m), and the centriolar adjunct (★) and its pericentriolar material. n, nucleus. Bars, 0.2 μm.
to the nuclear envelope (Russell et al., 1991). At this time, gold particles were observed in association with manchette microtubules, but also contiguous to the fuzzy material sandwiched between the manchette and the nuclear envelope (Fig. 4C,D). Irrespective of the orientation of the section, Arp1 labelling was hardly found at the centrioles and never detected...
precisely localised along the compartment. The most striking results were obtained with Arp1 labelling at Golgi stacks, preferentially in the cis-medial Golgi cisterna. The association with Golgi stacks remained at later stages of spermiogenesis (Fig. 6E), and was already discernible in primary spermatocytes (Fig. 6F). In control sections treated with antibodies (IgG fraction or affinity purified antibody) pre-adsorbed with the recombinant human actin-RPV, no labelling could be detected (Fig. 6D).

**DISCUSSION**

Arp1 was first identified in vertebrate cells by Lees-Miller et al. (1992) as a major component of the dynactin complex, which activates dynein-mediated transport of organelles along microtubules. The same year Clarks and Meyer (1992) identified the same actin homologue and called it centrinactin since it was preferentially localised at the centrosome. In the present work, we confirm that Arp1 primarily localises at the centrosomes with Arp1 cellular distribution is regulated during the differentiation of male germ cells. Arp1 localised mainly at the centriolar material when the cells were practically devoid of manchette and other cytoplasmic microtubules. In contrast, when the manchette was present, Arp1 was associated with these microtubules rather than the centrosome. Although the presence of Arp1 at the centrosome is known for certain, its potential implication in centrosomal architecture and/or function is unclear (Zimmerman et al., 1999). In fact, it appears that nucleation of microtubules is not the sole function of the centrosome as it recruits also proteasomal machinery and heat shock proteins (Wigley et al., 1999). Therefore, we propose that the centrosome could play a role of reservoir of Arp1 or other MAP and/or that it might be a station for quality control of such proteins.

Our results show that Arp1 is present also at the cis-Golgi network, up to the medial compartment, of male germ cells. The role of microtubules in the organisation and function of the Golgi apparatus has been suspected for many years mainly on the basis of the effects of anti-microtubular drugs (for review see Thyberg and Moskalewski, 1999). The role of microtubules associated motor proteins in the transport in and out of the Golgi complex was later demonstrated using immunocytochemical analysis after antibody injection and subcellular fractionation (for review see Hirokawa, 1998; Lippincott-Schwartz, 1998). In particular the vesicular transport from endoplasmic reticulum to Golgi is believed to require dynein in association with the dynactin complex. Holleran et al. (1996) and Lippincott-Schwartz (1998) proposed that in somatic cells a meshwork of spectrin and ankyrin interact with Arp1 polymers to connect the cargo membrane on one side and microtubule-bound dynein/dynactin complex on the other side. Therefore, the presence of Arp1 in the Golgi/pre-Golgi structures of spermatids as demonstrated here by immunoelectron microscopy corroborates this model.

The manchette is a unique set of cytoplasmic microtubules characterised by such high organisation. First it appears as a cone-shaped sheath, then evolve into a flat cylinder. Besides, this structure is rather stable and lasts five to seven days, depending on the species. This implies that the manchette is made of stabilised cytoplasmic microtubules. Indeed, these microtubules are made of detyrosynated α-tubulin (Fouquet et

at the nuclear ring. At the end of the elongation phase, the nucleus is flat and elongated, and the manchette appears as a flattened cylinder. Then at the beginning of the maturation phase the whole manchette and nuclear ring move backward. During this period the perinuclear fuzzy material was not decorated with gold particles, whereas the manchette remained labelled with anti-Arp1 antibody (Fig. 4E). Notably, gold particles were observed anew in association with the centriolar material (Fig. 4E,F). The centriolar labelling lasted until the middle piece started to form. Finally, gold particles could not be detected in testicular spermatozoa or in residual bodies.

To generalise the above results the expression of Arp1 was then investigated during rabbit, monkey and human spermiogenesis. The examination of human spermatids seemed indeed necessary since the anti-Arp1 antibody had been raised against human recombinant protein. In the three species Arp1 was associated to centriole microtubules of round spermatids (Fig. 5A,B). The labelling along the nuclear envelope and the associated microtubules of the manchette was observed from late round spermatids to late elongating spermatids (Fig. 5B-F). In addition the fuzzy material connecting the manchette to the nucleus was labelled also with anti-Arp1 antibody (Fig. 5F). As in rodents, during the maturation phase of spermatids Arp1 transiently accumulated at the centriolar adjunct and pericentriolar material after the disassembling of the manchette. Finally, Arp1 was not detected in mature spermatozoa. Pre-adsorption of the primary antibody, either the IgG fraction or the affinity purified one, with the immunogene, recombinant human actin-RPV, abolished Arp1 labelling (Fig. 5D).

**Arp1 is associated with the Golgi apparatus of spermatids**

Observation by IIF with anti-Arp1 antibody, showed unusually large fluorescent spots in spermatids and spermatocytes that could reflect Arp1 association not only to the centrosome but also to the Golgi apparatus. In round spermatids (Fig. 6A-D) the Golgi derived vesicles coalesce to form the acrosome which bind the anterior pole of the nucleus. In these cells Golgi stacks assume the classical polarity, as previously reported by Martínez-Menárguez et al. (1993), with the cis-Golgi network surrounded by the rough endoplasmic reticulum, and the trans-Golgi network with clathrin coated vesicles facing the acrosome. In mouse round spermatids (Fig. 6A) Arp1 labelling was detected at Golgi stacks, preferentially in the cis-medial compartment. The most striking results were obtained with rabbit round spermatids (Fig. 6B,C), as gold particles were precisely localised along the cis-Golgi network, the cis- and medial-Golgi cisterna. The association with Golgi stacks remained at later stages of spermiogenesis (Fig. 6E), and was already discernible in primary spermatocytes (Fig. 6F). In control sections treated with antibodies (IgG fraction or affinity purified antibody) pre-adsorbed with the recombinant human actin-RPV, no labelling could be detected (Fig. 6D).
abolished by pre-adsorbing the affinity purified antibody with the antigen (D). G, Golgi, er, endoplasmic reticulum; a, acrosome. Bars, 0.2 μm.

al., 1997) a stable subpopulation of cytoplasmic microtubules (Gundersen et al., 1984). However, manchette microtubules contain neither acetylated α tubulin nor glutamylated or glycylated α /β tubulin which characterise the most stable microtubules found in axonemes (Fouquet et al., 1994, 1997; Kann et al., 1998). The stability and organisation of the manchette might be influenced by various microtubule associated proteins (MAP) such as Tau, a neuronal protein that is also detected at the manchette (Ashman et al., 1992). Moreover the spectrin, which is able to bundle microtubules in vitro, is present in the manchette (Kann and Fouquet, 1993). Finally, motor proteins could play such a role in manchette organisation. In particular, kinesin that alters the arrangement of microtubules in vitro (Nédélec et al., 1997) is associated with the manchette (Hall et al., 1992).

The polarity of the manchette microtubules has not been ascertained. Yet, the presence of γ-tubulin exclusively at the spermatid centrosome suggests that their minus-end is nucleated at this location while their plus-end will be at the nuclear ring (Fouquet et al., 1998). The presence of kinesin and dynein in the manchette (Hall et al., 1992; Yoshida et al., 1994) is consistent with a role of these microtubules in the bidirectional translocation of organelles in spermatid cytoplasm. Indeed, smooth vesicles linked to microtubules on the outer and inner sides of the manchette have been observed (Rattner and Brinkley, 1972; Dym and Cavicchia, 1978). That Arp1 is associated with the manchette microtubules suggest that dynactin complex might interact with dynein to translocate cytoplasmic material along the manchette. However as noted by Russell et al. (1991) the spermatid cytoplasm extends two to three times the length of the manchette so that other mechanisms must be operative to explain cytoplasm elongation and organelle redistribution during spermatid differentiation. Finally, the late localisation of Arp1 at microtubule minus ends, in the vicinity of the centrioles, could result from a dynein mediated minus end transport of the protein coupled to the entrapment of Arp1 in the pericentriolar material.

Our most striking observation is the actual association of Arp1 with the fuzzy material that links manchette microtubules to the nuclear envelope. This specific distribution of Arp1 is in agreement with the localisation of dynein evidenced by IIF in the same cell type by Yoshida et al. (1994). Thus, in higher eukaryotes cytoplasmic microtubules can interact with the nucleus through the dynactin/dynein complex, as it was described in yeast and filamentous fungi (for review see Karki and Holzbaur, 1999). Although dynein/dynactin is implicated in nuclear migration in lower eukaryotes it may be involved in nuclear modelling, i.e. elongation and flattening, in elongating spermatids. The presence of Arp1 at the boundary of manchette microtubules and nuclear envelope, substantiate the most popular hypothesis of the role of the manchette in nuclear shaping (Russell et al., 1991). One can imagine that the manchette could act like a corset by constraining the nucleus through the dynein/dynactin complex. During the elongation phase, before the transition from histone to protamine (Hecht, 1990), the nucleus appears to be malleable and susceptible of shape change. At this stage the spermatid head begins to acquire its species-specific shape. When the manchette has fulfilled its task and disassemble, the nucleus will condense and rigidify while nuclear proteins are replaced (Fouquet and Kann, 1994). Nuclear shape change of spermatids results in restriction of nuclear pores to the nucleus caudal part in the so-called redundant nuclear envelope. This redistribution of nuclear pores could be the consequence either of the attachment of the manchette to the nucleus by dynein/ dynactin complex or of the slipping backward of the manchette.

Finally, Arp1 could not be detected in the nuclear ring itself. Accordingly, the presence of dynein was not ascertained at this precise location (Hall et al., 1992; Yoshida et al., 1994). Therefore these results do not support an active role of the nuclear ring in nuclear shaping, although it should be an important structural element. The nuclear ring was first proposed to be the MTOC of the manchette (Brinkley, 1985) but γ-tubulin is found exclusively in the spermatid centrosome (Fouquet et al., 1998). Therefore, we propose that the nuclear ring might act as giant kinetochore by capturing the plus end of the manchette microtubules primarily nucleated at the centrosome. Yet, any hypothesis with regard to the function of the nuclear ring cannot be validated as long as its molecular composition will be unknown.

To conclude, the presence of Arp1 in the centrosome and in the Golgi apparatus of mammalian spermatids support the role of the dynactin/dynein complex in the functional organisation of these organelles. The association of Arp1 both to manchette microtubules and to links between these microtubules and the nuclear envelope are consistent with a role of the manchette in organelle translocation and nuclear shaping during spermiogenesis.

The authors thank Eugenio Prieto for technical assistance in photographic work. This work was supported by a grant from the Association pour la Recherche sur le Cancer (to R.M.) and by the MENRT to EA2508 (to J.-P.F. and M.-L.K.).

REFERENCES


