INTRODUCTION

Cytoplasmic cleavage in animal cells involves the formation of a transient structure known as the contractile ring (Kuriyama, 1992; Marsland and Landau, 1954), which is composed of circumferentially aligned microfilaments that encircle the equatorial region of cell constriction (Schroeder, 1968, 1972). It is now widely accepted that the interactions between actin filaments and myosin-II in the contractile ring generate contractile forces that constrict and divide the cell in two during cytokinesis (for review see Conrad and Schroeder, 1990; Mabuchi, 1986; Satterwhite et al., 1992). The rapid assembly of the contractile ring occurs as anaphase progresses, and it is completely disassembled by the end of cleavage, when daughter cells remain attached only by a narrow connection or bridge containing the midbody (Schroeder, 1972). The mechanisms that induce cleavage furrowing are still poorly understood and little is known about the molecular control of cytokinesis.

It is generally believed that the formation of the spindle is temporarily and spatially coupled with furrowing. Moreover, it has been proposed that furrowing stimulation is mediated by still unknown signals that are transported from the mitotic apparatus to the cortical layer via astral microtubules at a specific stage of cell division (Rappaport, 1971, 1986). In addition, signals generated at the spindle poles are thought to determine the position of the contractile ring between the two poles. In the case of nematodes, where asymmetrical early cleavage divisions occur, this is known to be determined by the position of the spindle in the dividing blastomeres (Albertson, 1984). The formation and positioning of the spindle is in turn dependent on the centrosomes as the major microtubule organizing centers, so we could consider centrosomes as the origins of signals leading to the most crucial events involved in spindle positioning and cytokinesis.

Since the use of antibodies from different species has indicated that common antigenic sites exist at the spindle...
poles of cells of varied origins (reviewed by Tousson et al., 1991), we decided to study the localization in the nematode *Parascaris univalens* of centrosomal antigens using two centrosome-associated antibodies from *Drosophila melanogaster*, Bx63 and Rb188. Monoclonal Bx63 antibody was raised against *Drosophila* embryonic and nuclear extracts (Frasch et al., 1986) and it identifies two proteins of 185 kDa and 66 kDa on *Drosophila* western blots (Whitfield et al., 1988). The gene coding for the 185 kDa protein has been cloned and antibodies have been obtained using a purified fusion protein as immunogen (Whitfield et al., 1988). The polyclonal antibody Rb188 recognizes the 185 kDa but not the 66 kDa band. In this report we show that Rb188 and Bx63 antigens are conserved in both evolutionarily distant species. We have also found, that in *Parascaris* embryonic cells Bx63 reveals, in addition to centrosomes, novel midzone structures. Since the cytoplasmic structures revealed by Bx63 display a cell cycle-dependent organization, we have considered whether they may somehow participate in the process of cytokinesis. To this end, we have investigated their dependence on spindle microtubules and/or microfilaments. Furrowing inhibition by cytochalasin-B does not affect the distribution of Bx63 antigen. Moreover, comparison of the immunolocalization of Bx63 antigen in the midzone structures of the cell with the contractile ring assembly using anti-actin and anti-myosin antibodies reveals that both structures do not simultaneously coincide. We propose that the new midzone structures we have discovered in *Parascaris* are specifically involved in the spindle-mediated formation of the midbody during cytokinesis. Moreover, our results show that the determination of the position of the midbody in the blastomere precedes the mechanical act of furrowing. We show that the midbodies persist attached to sister cells at least up to larval morphogenesis, and we discuss the possible meaning of this persistence in relation to the establishment of cell to cell communication in nematodes during early development.

**MATERIALS AND METHODS**

Live specimens of *Parascaris univalens* (2m=2) were collected at the local abattoir along with horse intestinal contents. The worms were washed and maintained in a balanced salt solution (0.7% NaCl) at 37°C. As soon as possible after collection, the animals were dissected to remove gonads. Fertilized eggs were extracted from the uteri and kept at 4°C. To obtain *Parascaris* embryos in different developmental stages, eggs were first dechorionated and then incubated overnight at 4°C in 10% FCS in PBS (pH 7.4) and rinsed in PBS. Filters incubation with Rb188 antiserum was performed for 2 hours at room temperature. Rb188 antiserum was diluted 1:400 in 10% FCS in PBS. Filters were washed in TCBS (20 mM sodium citrate, 500 mM NaCl, 0.005% Tween-20, pH 5.5) three times for 15 minutes. They were then incubated with Protein G-HRP conjugate solution (0.033% in TCBS, 1% gelatin), (Bio-Rad) for 2 hours. The washing cycle was repeated in TBS (20 mM Tris, 500 mM NaCl, pH 7.5) three times for 15 minutes before developing the staining with a 0.2% filtered solution of o-dianisidine (Serva) in 1 M Tris-HCl, pH 7.4, 1 M imidazole (Serva) and 0.001 vol of H2O2. Filter incubation with Bx63 mAb was performed for 2 hours at room temperature. Bx63 mAb was diluted 1:5 in 10% FCS in PBS. Filters were washed for 15 minutes in: (1) 1% NP40 (Sigma) in PBS; (2) 0.1% NP40 in PBS; and (3) PBS alone. They were then incubated in peroxidase-conjugated anti-mouse antibody (Dakopatts) at room temperature for 2 hours. The washing cycle was repeated, including an extra wash in PBS before developing the staining as describe above. Filters incubated only in second antibodies were used as controls.

**Indirect immunofluorescence**

Fixation of male meiotic divisions

Slides of gonadal meiotic divisions were obtained by dissecting small pieces of testis in a drop of 0.7% NaCl at 37°C. After squashing, slides were frozen in liquid N2 to remove cover slips and immediately fixed in 100% methanol for 10 minutes at −20°C, washed three times in PBS for 10 minutes at room temperature and permeabilized in 1% Triton X-100 (Merck) in PBS for 10 minutes. They were subsequently washed in PBS three times and incubated for 30 minutes at room temperature with 2% nonfat dried milk in PBS, and then incubated with antibodies as described below.

**Fixation of mitotic embryonic divisions**

Embryos were fixed either in 100% methanol at −20°C for 10 minutes, or 4% paraformaldehyde in PBS for 10 minutes at room temperature and washed three times in PBS for 5 minutes. In both cases they were treated with acetone at −20°C for 1 minute, washed three times in PBS for 5 minutes, treated with 1% Triton X-100 in PBS for 10 minutes, and subsequently washed in PBS three times for 5 minutes at room temperature. Before incubation with antibodies embryos were treated with 1% BSA (bovine serum albumin; Sigma) in PBS at room temperature for 30 minutes.

**Antibody incubation**

Primary and secondary antibodies were diluted in 3% BSA in PBS and used as follows: (a) Rb188 antiserum (1:50) followed by FITC-conjugated anti-rabbit (1:50) (Boehringer), (b) Bx63 (1:5) followed by FITC-conjugated anti-mouse (1:100) (Tago), or TRITC-conjugated anti-mouse (1:50) (Dakopatts). (c) Anti-tubulin YL1/2 (1:100) (Sera-Lab) followed by FITC-conjugated anti-rabbit (1:100) (Sigma). (d) Anti-myosin (1:10) (Sigma) followed by FITC-conjugated anti-mouse (1:100). (e) Rhodamine-phalloidin (1:80) (Molecular Probes Inc.). Slides of gonadal meiotic divisions and embryo suspension were incubated with primary antibody for 1 hour at room temperature, washed three times in 1% BSA in PBS for 7 minutes each, and incubated with secondary antibody for 1 hour at room temperature. Slides and embryo suspension were then washed three times in 1% BSA in PBS for 7 minutes, washed in PBS for 10 minutes and, finally, PBS was replaced by staining solution. To perform double staining with a second primary antibody the process was repeated before staining. The staining solution consisted of 20 parts 1 mg/ml p-phenylenediamine in a 1:10 (v/v) mixture of PBS: glycerol; 1 part
2 µg/ml Hoechst 33258 in distilled H2O. Slides were stained for 10 minutes, a coverslip was placed over the sample and the preparations were sealed. Embryos were stained for 10 minutes; a drop of the embryo suspension was placed on a slide and the embryos were allowed to stick. Shortly afterwards, a coverslip was deposited on the top, gently pressed, and the preparations were sealed.

Fluorescence microscopy observation and photography
All observations were made under a microscope equipped with epifluorescence optics (C. Zeiss, Inc., Thornwood, NY). Photomicrographs were taken with Tri X film and Ektachrome 160T (Eastman Kodak Co., Rochester, NY). Western blot filters were photographed with Microfilm (Kodak).

Cold treatment
Dechorionated fertilized eggs in M9 buffer, pH 7. (Brenner, 1974) were transferred to Petri dishes, incubated at 37°C for 1 hour, and then incubated on ice for 1 hour (in medium prechilled to 4°C). Embryos were fixed and processed for immunofluorescence as described above.

Nocodazole treatment
Nocodazole (Sigma) was stored as a 2 mg/ml stock solution in DMSO at 4°C and diluted in M9 before use. Dechorionated fertilized eggs in M9 buffer were transferred to Petri dishes, incubated at 37°C for 2 hours to obtain embryos with two interphase blastомерes. Nocodazole was added to 10 µg/ml culture medium, and embryos were incubated for another 6 hours at 37°C. Embryos were fixed and processed for immunofluorescence as described above.

Cytochalasin B treatment
Dihidrocytochalasin B (Sigma) was stored as a 1 mg/ml stock solution in DMSO at 4°C and diluted in embryonic culture medium immediately before use. Dechorionated fertilized eggs in M9 buffer were transferred to Petri dishes and incubated at 37°C for 30 minutes. Cytochalasin B was added to 8 µg/ml culture medium during 2 hours, 4 hours and 6 hours at 37°C. Furrowing inhibition was determined by phase-contrast microscopy and rhodamine (Rh)-phalloidin staining. Embryos were fixed and processed for immunofluorescence as described above.

RESULTS

Rb188 and Bx63 antibodies identify in Parascaris the same proteins as in Drosophila on western blots
Polyclonal Rb188 (Whitfield et al., 1988) and monoclonal Bx63 (Frasch et al., 1986) antibodies recognize in Drosophila a common antigen of 185 kDa that is a centrosomal component during the entire cell cycle. Bx63 also recognizes an additional 66 kDa antigen that is not encoded by the 185 kDa gene and whose location in Drosophila cells remains uncertain (Whitfield et al., 1988). Fig. 1A shows that in Parascaris embryonic extracts polyclonal Rb188 antibody identifies a single 185 kDa component and monoclonal Bx63 antibody recognizes two polypeptides with molecular masses of 185 kDa and 66 kDa. These antigens correspond to similar molecular mass bands that Rb188 and Bx63 antibodies recognize in Drosophila melanogaster embryonic extracts (Fig. 1B).

Rb188 staining is restricted to centrosomes during Parascaris meiotic and mitotic divisions
The cytological analysis of Rb188 staining, in Parascaris meiotic male cells (Fig. 2A) and early mitotic embryo cells (Fig. 2B), showed that, in both cell types, Rb188 decorates the spindle poles from prometaphase to late telophase. Depending on the intensity of the staining, we have often observed that the central region of the centrosomes, the centriolar domain, is almost devoid of staining (insert in Fig. 2B). Therefore Rb188 antibody most probably associates preferentially to the pericentriolar material. No Rb188 staining is found either in interphase cells or in pronuclear embryonic stages (data not shown).

Bx63 reveals a new cell cycle-dependent structure in Parascaris embryos
The cytological analysis of Bx63 antibody staining in early mitotic embryonic cells showed that the antibody decorates the spindle poles mostly at anaphase and telophase (Fig. 3C,D and E). However, the staining is usually weak, at times diffuse and its pattern variable between embryos. These more or less erratic results were independent of the fixation procedure and the antibody concentration employed.

To our surprise, we found that starting at the transition from metaphase to early anaphase, Bx63 antibody also recognizes a cytoplasmic antigen that concentrates on fibrillar-like structures at the equatorial plane of the blastomeres (Fig. 3). These structures look at first like loops that apparently extend from, and beyond, the equatorial plane into both cell poles (Fig. 3A). As chromosome segregation proceeds from early anaphase to late anaphase, Bx63 signal becomes progressively accumulated in, and restricted to, the
entire midzone region of the blastomeres (Fig. 3B,C and D). At this stage, when embryos were viewed obliquely to the equatorial plane, a ring structure was clearly detected with Bx63 antibody (Fig. 3E). Double-labeling observations with Rb188 and Bx63 antibodies confirmed that the detected ring-like structure is recognized by Bx63 antibody but not by Rb188 (Fig. 3E).

To study the distribution of Bx63 antigen relative to mitotic spindle microtubules we analyzed double-stained
Midzone and midbody antigens in the nematode *Parascaris*

Embryos with Bx63 and anti-tubulin antibodies from anaphase up to cytokinesis. Superimposition of anti-tubulin fluorescence and Bx63 signal at the equatorial region of the blastomere is seen (arrows). Bar, 10 µm.

As telophase proceeds, and furrowing cleavage takes place, Bx63 signal persists into the midbody. As can be seen in Fig. 6A, after the first blastomere division Bx63 staining is localized as a single fluorescent body lying at the boundary between the two interphasic daughter cells. The cytological observations of the first blastomere division suggest that Bx63 recognizes cytoplasmic structures that display a cell cycle-dependent distribution and organization. This was further reinforced when we examined embryos undergoing the next divisions, which in *Parascaris* are asynchronous (Goday et al., 1992). Fig. 6B shows a three blastomere embryo stained with Bx63 and Hoechst. In it, a fluorescent body shared by two interphasic sister cells can be seen, as well as the typical Bx63 fluorescent pattern in the equator of the third blastomere, that is still in early anaphase. Fig. 6C shows an embryo with four interphasic nuclei containing three Bx63 fluorescent bodies, which arose from the previous three blastomere divisions. In more advanced embryos we always found that the Bx63 midbody signal persisted between cells (Fig. 6D), up to the initiation of larval morphogenesis.

We want to point out that Bx63 and tubulin staining were often detected in the polar bodies in those instances when they were still closely attached to the blastomeres (Figs 3D,D’ and 7).

**Bx63 is found in the equatorial region before actin and myosin intervene in the formation of the equatorial contractile ring**

Immunofluorescence analysis of early *Parascaris* embryos using the F-actin probe phalloidin showed that a high concentration of actin is present in the contractile ring site at telophase (Figs 8,9). The same results were obtained with respect to myosin when anti-myosin antibodies were employed (data not shown). It may be interesting to point out that actin and myosin staining were frequently observed in those polar bodies that remained attached to the somatic cells (Fig. 8).

Again, the antibody Bx63 recognizes an antigen that transiently concentrates and locates into a ring-like structure at the equatorial zone of the cell. As shown above, the Bx63 signal is reduced, just before cytokinesis, to the precursor of the midbody, seemingly associated to spindle microtubules (Fig. 5). These results lead us to investigate if the Bx63 antigen was somehow involved in cell furrowing. Using Bx63/phalloidin double immunofluorescence, we

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**Fig. 4.** Double-immunofluorescence labeling with Bx63, anti-tubulin and Hoechst 33258 chromatin staining of a *P. univalens* embryo in first anaphase. Superimposition of anti-tubulin fluorescence and Bx63 signal at the equatorial region of the blastomere is seen (arrows). Bar, 10 µm.

**Fig. 5.** Double-immunofluorescence labeling with Bx63, anti-tubulin and Hoechst 33258 chromatin staining of a *P. univalens* embryo in first anaphase. Bx63 signal associates with polar microtubules into the forming midbody at the equatorial midplane of the cell. Bar, 5 µm.
reexamined the distribution of Bx63 signal in cells in anaphase and telophase relative to the assembly of the contractile ring (Figs 8,9). No simultaneous location of the ring structure (stained by Bx63) and the contractile ring (stained with either phalloidin or anti-myosin) was observed. Moreover, in embryos undergoing their first division we found that the progressive concentration and positioning of Bx63 signal in the center of the equatorial plane (precursor of the midbody) occurs while the contractile ring is still assembling, that is, before the constriction leading to cytokinesis is formed (Figs 8,9).

**The distribution of Bx63 antigen does not depend on the actin-microfilament system**

To analyze the distribution of Bx63 and its microfilament dependence we examined the distribution of Bx63 signal in cytochalasin B-treated embryos (Fig. 10). This microfilament inhibitor prevents furrowing in the nematode *C. elegans* by disruption of actin microfilaments (Hill and Strome, 1988; Strome and Wood, 1983). Similarly, furrowing is also inhibited and cytokinesis does not occur in *Parascaris* embryos grown in the presence of the drug. This was easily followed by phase-contrast microscopy of treated eggs displaying the characteristic altered morphology and blebbing (Krishan, 1972; and Fig. 10) and confirmed with Rh-phalloidin staining (data not shown). Alterations in the typical spindle pattern position and in the orientation between spindles were evident when spindles were analyzed with anti-tubulin antibodies in treated embryos that had undergone a few early divisions (data not shown). Embryos at the one blastomere stage, before the onset of first division, were grown in the presence of cytochalasin B, fixed at different nuclear stages, and double-stained with Bx63 and anti-tubulin antibodies. Fig. 10A shows that by the end of the first division, just before the first spindle totally disassembles, Bx63 decorates a midbody-like structure still associated with microtubule remnants. In more advanced embryos, like the one depicted in Fig. 10B, the antibody decorates three fluorescent bodies, the number expected for untreated embryos of similar age: always one less than the number of blastomeres or, in other terms, the exact number of finished mitosis. In most of the early embryos treated with cytochalasin B the position of the Bx63 signals differs from controls, as expected if alterations in the orientation and positioning of the spindles had occurred due to treatment with the drug.

All these results support the suggestion that the presence and distribution of the Bx63 antigen does not depend on the actin-microfilament system in *Parascaris*.

**Fig. 6.** Indirect immunofluorescence staining with Bx63 (A-D) and Hoechst 33258 chromatin staining (I) of *P. univalens* embryos at different developmental stages. (A) A Bx63 single fluorescent body between the two cells (arrow) with nuclei in interphase (arrows in I). (B) A three-cell embryo showing a Bx63 fluorescent body (thin arrow) corresponding to the two interphasic cells (thin arrows in I) and Bx63 midzone signal (thick arrow) between the chromosomes of the third cell still in early anaphase (thick arrow in I). (C) A four-cell embryo showing interphase nuclei and three Bx63 fluorescent bodies corresponding to three cell divisions. (D) A more advanced embryo where several Bx63 fluorescent bodies are seen at the plane of focusing. Bars, 5 µm.
The localization of Bx63 antigen is dependent on non-kinetochore spindle microtubules

To learn about the dependence on microtubules of the midzone antigens revealed by Bx63, we treated Parascaris embryos with nocodazole, a microtubule-destabilizing drug. The embryos were dechorionated, grown up to the stage of two blastomeres and transferred to drug-containing medium for up to 6 hours, a time when normally four blastomere embryos are found. Fig. 11 shows a treated embryo with two binucleate cells. Besides the expected midbody between cells, a remnant of the first blastomeric division, no trace of Bx63 staining is found, suggesting that the positioning of the Bx63 antigen is dependent on microtubules.

To further test the association of Bx63 with microtubules we studied cold-treated embryos. As in other organisms, cold treatment of Parascaris embryos does not disrupt kinetochores microtubules; instead (as shown in the anaphase depicted in Fig. 12), it results in the absence of only polar and midbody-associated microtubules. In these chilled embryos we never found Bx63 signal in the cell cytoplasm (data not shown).

All the data presented above lead us to conclude that the formation of the novel midzone structures we have described in Parascaris, is only dependent on non-kinetochore microtubules, not on microfilaments or kinetochore microtubules.

DISCUSSION

Parascaris centrosomes contain an antigen common to Drosophila centrosomes

In this report we have shown that the centrosome-associated protein recognized in Drosophila by polyclonal Rb188 and monoclonal Bx63 antibodies is also present in the centrosomes of the nematode Parascaris.

In western blots of total embryonic extracts from Drosophila melanogaster both antibodies identify a 185 kDa protein associated with the centrosome and the nucleus and encoded by a single-copy gene (Frasch et al., 1986; Whitfield et al., 1988). In addition, the Bx63 antibody recognizes in both species a 66 kDa component that does not

Fig. 7. Bx63 staining of a polar body in a P. univalens embryo at first metaphase. Double immunofluorescence labeling with Bx63, anti-tubulin and Hoechst 33258 chromatin staining. Bx63 signal is seen at the polar body in coincidence with specific tubulin staining (thick arrows). Note the Bx63 light staining of centrosomes (thin arrows). Bar, 5 μm.

Fig. 8. Immunofluorescence labeling with Rh-phalloidin, Bx63 and Hoechst 33258 chromatin staining of a P. univalens embryo at first telophase. A high concentration of actin is present at the contractile ring site (thick arrow) and at the attached polar body (small arrows). At this stage Bx63 staining is already restricted to a single body at the equatorial zone of the cell (thin arrow). Note that the location of the contractile ring is slightly asymmetrical in the dividing blastomere. Bar, 10 μm.
seem to be closely related to the 185 kDa component and is of uncertain cellular location in Drosophila cells (Frasch et al., 1986; Whitfield et al., 1988).

From our western blot and immunofluorescence analyses, it is clear that in Parascaris, as in Drosophila, the 185 kDa antigen recognized by Rb188 associates to the spindle poles in meiotic and mitotic cells. Rb188 staining of centrosomes in Parascaris cells was only detected from prometaphase to late telophase and not during interphase. This may indicate that either the 185 kDa antigen is modified or too diffuse to be recognized by the antibody during interphase or, alternatively, that the antigen is specific for spindle poles during Parascaris cell division.

We have also seen that Bx63 antibody in Parascaris, as in Drosophila, identifies 185 kDa and 66 kDa antigens on western blots. However, the cytology with Bx63 antibody in Parascaris cells showed a fluorescence pattern in spindle poles that was weaker and less reproducible than that of Drosophila. For this reason, centrosomal staining with Bx63 was less consistent than in fruit flies, and mostly obtained in anaphasic and telophasic embryonic cells (Fig. 3). In this respect, we think that the centrosomal staining differences between Rb188 and Bx63 in Parascaris could be due to the fact that the monoclonal Bx63 antibody may recognize an epitope of the 185 kDa antigen that is less available by immunofluorescence staining. Nevertheless, our data, coupled with data obtained from Drosophila, led us to conclude that in Parascaris the 185 kDa antigen is responsible for centrosomal staining during cell division. Preliminary results by western blot and cytological analysis from our laboratory indicate that the same antigens are recognized by both antibodies in Caenorhabditis elegans embryos.

In Drosophila the 66 kDa component recognized exclusively by Bx63 antibody has not been related to any particular cellular structure during cell division. In the case of Parascaris embryos, Bx63 revealed specific cytoplasmic structures other than centrosomes. We think that the 66 kDa antigen constitutes a specific component of these particular structures in blastomeres of Parascaris. Obviously, this does not exclude the possibility that the 66 kDa antigen could also be a component of the centrosomes of Parascaris. We have started a search for specific antibodies against the 66 kDa band to investigate this point.

**Cytokinesis and Bx63 antigens in Parascaris embryos**

The role of the mitotic spindle in cleavage has been intensively investigated, specially in echinoderm and amphibian eggs after elimination of spindles using colchicine (Hamasaguchi, 1975; Sawai and Yomota, 1990) or mechanical methods (Hiramoto, 1956). From these experiments it emerged that the spindle somehow ‘informs’ the cell about the plane of cleavage up to the onset of anaphase. Once anaphase is turned on the spindle is dispensable for furrowing.

Our cytological analysis using the antibody Bx63 revealed that specific cytoplasmic structures located in the cell midzone of Parascaris dividing eggs are redistributed in a cell cycle-dependent way. An interesting aspect to note is that Bx63 signal appears starting from the transition of metaphase/anaphase. It is first seen as expanded fibrillar-looking loops that, as chromosome segregation proceeds, concentrate in the midzone and turn into a ring-like structure that spans the entire equatorial region of the blastomeres. By the end of anaphase the antigens participate in the organization of the future midbody.

The midzone, or spindle interzone, of a cell is defined as the region between segregating chromosome sets where overlapping interpolar plus-end microtubules incorporate subunits and the midbody begins to form. In the last years an increasing number of mitosis-dependent antigens has been found associated in different cell types with the midzone region during anaphase, and with the midbody at telophase. Many of these antigens are also found in spindle-related cellular structures such as centrosomes, chromosomes or both. Presumably, they are transported to the midzone region associated with spindle microtubules at the same time as chromosome segregation. In fact, many of them are seen to colocalize with interzonal microtubules (for review see Andreassen et al., 1991; Cooke et al., 1987; Sato et al., 1991; Sellito and Kuriyama, 1988; Tousson et al., 1991).

The data obtained from chilled and nocodazole-treated eggs, where no Bx63 staining was detected, suggest a specific dependence of the cellular Bx63 antigen organization on polar non-kinetochore microtubules. Cytologically, the association between Bx63 antigen and the plus end of microtubules is seen during the progression of anaphase, when, as microtubules bunch, Bx63 antigen congregates into the forming midbody (Fig. 5). Prior to this,
it is difficult to discern at the cytological level if microtubules are already associated with the expanded midzone structures revealed by Bx63. However, superimposition of equatorial tubulin staining and Bx63 signal was often seen prior to the formation of the midbody (Fig. 4). Therefore, it is possible that the Bx63 ring-like structures may be permanently associated with the plus end of polar microtubules. This would explain the simultaneous congression of Bx63 antigen into the midbody with the bunching of polar microtubules that is observed in anaphase B. In addition, specific Bx63 and tubulin staining of polar bodies was also correlated. In *Parascaris*, the extruded first polar body often remains attached to the embryo and initiates division after the formation of a complete small spindle. When this occurs, chromatid segregation is generally arrested at the metaphase/anaphase transition. This, we think, explains the maintenance of a visible Bx63 signal only in the polar bodies that have developed small spindles.

While the completion of anaphase B finalizes karyokinesis, cytokinesis is concluded only with the final separation of the daughter cells. Cytokinesis involves first furrowing and, secondly, the ‘sealing’ or cutting of the intercellular bridge, which connects the daughter cells at a final point (the midbody), and that contains non-kinetochore microtubules (Mullins and Biesele, 1973, 1977). A certain number of cellular proteins, and in particular proteins that associate with the mitotic apparatus, have been found to localize within the intercellular bridge in the midbody and/or adjacent regions. Recently, Rattner (1992) mapped the mammalian intercellular bridge into distinct zones characterized by both fine structure and protein composition. Among the proteins reported to end at the midbody (for references see Rattner, 1992; Tousson et al., 1991), a mitosis-specific antigen of 6×10^4 M_r (TD-60), detected by an autoimmune serum revealed a new continuous disc structure (‘telophase disc’), that forms and bisects the late anaphasic cell at the spindle equator (Andreassen et al., 1991). As reported, no other midzone protein has been previously demonstrated to span the entire equator of the cell.

**Fig. 10.** Indirect immunofluorescence staining with Bx63 of *P. univalens* embryos grown in the presence of cytochalasin B. (A) Phase-contrast and double-immunofluorescence staining with Bx63 and anti-tubulin of an embryo at the end of the first division. The characteristic blebbing resulting from cytochalasin B treatment is observed (arrows) (left). Bx63 decorates a midbody-like signal associated with microtubule remnants (arrows) (center and right). (B) Phase-contrast, Bx63 and Hoechst 33258 staining of a four-interphasic-nuclei embryo. Note the slightly altered morphology of the embryo and the absence of cleavage (left). Bx63 decorates three fluorescent bodies (arrows) that correspond to the three nuclear divisions that have occurred (center). Arrows point to the four interphasic nuclei (right). Bars, 5 µm.
at anaphase well beyond the boundaries of the anaphase spindle. The telophase disc appears to contain myosin but not actin, and to survive disassembly of interpolar microtubules. Since it becomes constricted at the exact position of the cleavage furrow during telophase, it has been proposed to have a possible role in mammalian cell cytokinesis. In particular, to interact with cortical actin and to constrict during furrowing (Andreassen et al., 1991). Here we show a new equatorial structure revealed by the antibody Bx63 with location in the midzone, ring pattern, and ultimate participation in the midbody. The double-staining of anaphasic and telophasic embryos with Bx63 and Rh-phalloidin probe has provided evidence that Bx63 and actin contractile ring structures do not localize simultaneously in *Parascaris*. In fact, the contractile ring is fully assembled while Bx63 signal is already restricted to a discrete site located in the center of the cell equatorial plane (Figs 10, 11). These results strongly suggest that the structures revealed by Bx63 are not directly involved in the contractile ring assembly. This is further reinforced by the fact that Bx63 signal does survive the disassembly of the microfilament system by cytochalasin B in growing embryos. Moreover, in cytochalasin-treated embryos the Bx63 signal was always present where changes in the position of the spindles occurred. This latter observation is in accordance with data on *C. elegans* cytochalasin-treated embryos and with the evidence that astral microtubules and cytoplasmic actin are implicated in maintaining proper spindle orientation in dividing early blastomeres in nematodes (Hill and Strome, 1988; Hyman and White, 1987; Strome and Wood, 1983).

Since the appearance of the Bx63 antigen during embryonic divisions does not depend on interactions with microfilaments, it is difficult to consider its active participation in the mechanical process of furrowing constriction. Instead, it is more likely that Bx63 antigen has a spindle-dependent role in cytokinesis. In this respect, we think that it could be part of a complex and as yet unknown group of signals generated by the spindle at the onset of anaphase and aimed to ensure a correct cell cleavage.

From our data on *Parascaris* Bx63 antigens, it is tempting to speculate whether a particular subset of proteins interacting with the microtubules plus end is specifically involved in the organization of the midbody. That is, involved in the process of polar microtubule bunching, during anaphase B, into a specific site at the midplane of the cell. Moreover, according to our results, the final position of the midbody in the cell is prefixed before actual furrowing takes place. This may be of importance in the linking that exists between nuclear division and cytokinesis.

The availability of specific antibodies against the 66 kDa *Parascaris* antigen will permit us to further characterize it and to experimentally approach the functional role of these proteins during cytokinesis. In spite of its more difficult cytology, the extensive knowledge about its genetics makes the use of *C. elegans* specially interesting, since similar antigens are present in *C. elegans* developing embryos (own unpublished observations).

**Fig. 11.** Indirect immunofluorescence staining of a nocodazole-treated *P. univalens* embryo with Bx63 (left) and Hoechst 33258 chromatin staining (center). Phase-contrast image (right). A four-nuclei embryo (arrows) was obtained by incubating a two-cell embryo in nocodazole medium for 6 hours. The single Bx63 body resulting from first blastomere division is seen (arrow). Bar, 10 µm.

**Fig. 12.** Indirect immunofluorescence staining with anti-tubulin and Hoechst 33258 chromosome staining of a cold-treated *P. univalens* embryo. After a 1 hour treatment, only kinetochore microtubules are present (arrows). Chromosomes are in anaphase. The small Hoechst signal at the bottom lights up the polar body (arrowhead). Bar, 5 µm.
Persistence of the midbody in *Parascaris* embryos

From our observations, the Bx63 midbody signals located between sister blastomeres in the midbody persist in the developing embryos from their appearance after the first division until larval morphogenesis prior to hatching. As discussed above, it is generally accepted that cytokinesis concludes totally with the ‘sealing’ of the intercellular bridge that contains the midbody structures. The persistence of midbody structures between daughter cells after cleavage furrow contraction has been reported in different cell types and organisms since classical studies (for review see Wilson, 1987). An interesting question is whether the persistence of a midbody implies that cytoplasmic communication via the intercellular bridge channel can occur during the period of time before the actual sealing takes place. Recently, experiments involving microinjecting fluorescent dyes into cultured individual cells and growing embryos have demonstrated that intercellular bridges or midbody junctions permit the passage or diffusion of molecules between connected cells. Thus, using Lucifer Yellow dyes, sealing of midbodies in PtK2 cells takes up to 4 hours, in contrast to early sister blastomeres of the sea urchin *Arabia punctulata* that take only 15 minutes (Sanger et al., 1985). Moreover, an EM structural survey of intercellular bridges in the embryo of the squid *Loligo pealei* has suggested that closing, by systems of transverse membranous cistermae, and re-opening of intercellular bridges can occur in relation to the cell cycle (Cartwright and Arnold, 1980).

Nematodes have been classically considered as an example of cell-autonomous development from the early studies on developing embryos of Ascarids (Boveri, 1910; Stevens, 1909). However, in the nematode *C. elegans* the existence of early cell-cell interactions that are involved in the determination of embryonic cells has been demonstrated recently (Pries and Thomson, 1987; Schierenberg, 1987; Wood, 1991). Among the possible pathways of signal transduction between cells, transfer of signal molecules through communication channels such as gap junctions and cytoplasmic bridges has been proposed (reviewed by Slack, 1991). The pattern of cell to cell communication in *C. elegans* embryos has been recently investigated by microinjecting tracer dyes into cultured individual cells and growing embryos (for review see Bossinger and Schierenberg, 1992). Intercellular communication via gap junctions (or communication channels) and via cytoplasmic bridges was distinguished by microinjecting Lucifer Yellow and rhodamine-labeled dextrane (RD), respectively. The results indicated that RD dye remained restricted to the injected cells and its descendants and it was confirmed that cytoplasmic bridges are never formed between non-sister blastomeres.

In view of these data we think that the persistence of the Bx63 midbody signals between interphasic blastomeres may indicate that sister blastomeres are still connected in *Parascaris* early developing embryos. However, dye-tracer experiments are necessary to know whether the Bx63 midbody staining can be correlated to the presence of a potentially ‘open’ intercellular bridge in *Parascaris*. This would be specially interesting since preliminary observations indicated a progressive and localized disappearance of fluorescent Bx63 spots at the beginning of larval morphogenesis. Alternatively, if midbody persistence does not necessarily mean a site of intercellular communication, it is also thinkable that these cellular structures could play a certain role in the maintenance of the cellular architecture of the early embryo. This could, in turn, facilitate other means of cell to cell communication during early development.

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