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The distribution of cytoplasmic bacteria in the early *Drosophila* embryo is mediated by astral microtubules

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SUMMARY

Maternally inherited cytoplasmic bacteria have occasionally been observed in embryos and adults of different strains of several *Drosophila* species. While there is a considerable body of data on the relationship between bacteria and embryo viability, little is known about the behavior of these bacteria during the early development of *Drosophila*. In eggs laid by infected *Drosophila melanogaster* females we showed that cytoplasmic bacteria were initially concentrated in a thin cortical layer and scattered in the yolk region. During the following syncytial blastoderm mitoses the bacteria mainly accumulated towards the poles of the mitotic spindles, suggesting that astral microtubules play a role in localizing bacteria. This is supported by the observation that treatment of the infected embryos with the microtubule-disrupting drug colchicine led to the partial dissociation of the bacteria from the spindle poles, whereas cytochalasin treatment left almost all the bacterial clusters intact. Moreover, bacteria were not found near the polar bodies and yolk nuclei, which were without astral microtubules. In mitosis-defective embryos, with centrosomes dissociated from the nuclei, the bacteria were concentrated in association with the isolated astral microtubules, and in cold-treated embryos, in which microtubules regrew from isolated centrosomes after recovering, the bacteria clustered around the newly formed asters. These observations, also supported by electron microscope analysis, indicate a close relationship between cytoplasmic bacteria and astral microtubules, and suggest that the latter were able to build discrete cytoplasmic domains ensuring the proper distribution of cytoplasmic components during the blastoderm mitoses, despite the lack of cell membranes.

Key words: Proteobacteria, microtubule, syncytial blastoderm, *Drosophila melanogaster*

INTRODUCTION

A number of studies on several species of insects have shown that cross-mating between conspecific populations of different geographical origin is either sterile or produces very few offspring (for a review, see Stevens and Wade, 1990). This incompatibility, which may be unidirectional or bidirectional when non-viable eggs are obtained in one or both crosses, is maternally inherited (Barr, 1980, 1982; Subbarao, 1982; Hoffmann et al., 1986; Hoffmann and Turelli, 1988; Breeuwer and Werren, 1990, 1993; Montchamp-Moreau et al., 1991). Light and transmission electron microscope analysis have revealed in most cases that cytoplasmic incompatibility in insects is associated with the presence of cytoplasmic bacteria (Wolstenholme, 1965; Breeuwer and Werren, 1990; Wright and Barr, 1981; Kellen et al., 1981; Louis and Nigro, 1989; Binnington and Hoffmann, 1989; O'Neill and Karr, 1990). This hypothesis has been supported by the finding of bacteria in the gonads of infected strains of *Drosophila simulans* (Binnington and Hoffmann, 1989; Louis and Nigro, 1989; Bressac and Rousset, 1993) and by experiments in which compatibility was restored by culturing infected strains in media containing antibiotics (Yen and Barr, 1973; Kellen et al., 1981; Wade and Stevens, 1985; Trips et al., 1981; Wright and Barr, 1981; Hsiao and Hsiao, 1985; Richardson et al., 1987; Hoffmann and Turelli, 1988; Breeuwer and Werren, 1990; O'Neill and Karr, 1990). Analysis of 16 S ribosomal RNA has revealed that these bacteria belong to the alpha-subdivision of the Proteobacteria, and are closely related to the the genus *Wolbachia* (Breeuwer et al., 1992; O'Neill et al., 1992; Rousset et al., 1992).

It has been reported that cytoplasmic bacteria are aligned with the spindle axis in *Drosophila simulans* embryo (O'Neill and Karr, 1990) and are clustered around free centrosomes in *Drosophila melanogaster* embryos in which nuclear division is blocked by mutation (Glover et al., 1990), but no evidence about the mechanism of their movements through the embryo cytoplasm was provided.

The experiments reported in this paper were designed to provide more information on the association of bacteria with the spindle microtubules in order to investigate the role of the cytoskeleton in the segregation of the bacteria during the syncytial blastoderm mitoses of the early *Drosophila melanogaster* embryo, and to contribute to knowledge of the behavior of these bacteria during early development. Moreover, the nuclear cycle-related distribution of discrete cytoplasmic components will be useful for understanding how the cytoskeleton builds defined spatial domains around the blastodermic nuclei of *Drosophila*, even though they are not yet separated by the plasma membrane.

MATERIALS AND METHODS

Fly stocks

Drosophila melanogaster stocks used in this study were Oregon-R, which served as a control strain, and two lines of flies that carried cytoplasmic bacteria. Infected stocks were kindly provided by the laboratory of M. Gatti (University of Rome, Italy) from a P-element-induced maternal-effect lethal collection. The infected embryos used in this study were derived from normal females and homozygous females carrying P-element-induced female-sterile mutations. The embryos obtained from homozygous mutant females have several mitotic defects and die during the first mitoses or during the syncytial blastoderm stage, whereas the embryos derived from normal females do not have developmental defects.

Collection and staging of the embryos

Eggs were collected from 4- to 5-day-old females at 25˚C on agar plates, dechorionated in a 50% commercial bleach solution and washed with distilled water. The age of the embryos was determined, according to the method of Campos-Ortega and Hartenstein (1985), by direct observation with interference contrast microscopy or by counting the somatic nuclei. Following removal of excess liquid by blotting on tissue paper, the embryos were processed for different procedures.

Drug treatment

Selected embryos were collected and transferred onto filter paper, and then permeabilized with heptane according to the method of Limbourg and Zalokar (1973). After 3-4 minutes the eggs were dried, covered with a small square of tissue paper (Kleenex) and treated with cytochalasin B (Sigma, St Louis, Mo) and colchicine (Sigma). Cytochalasin B and colchicine were dissolved in dimethylsulfoxide and diluted to a concentration of 40 μg/ml with D20 culture medium (Echalier and Ohanessian, 1970). The eggs were incubated at 25˚C for 15 minutes.

Cold treatment

Embryos at appropriate stages were collected on a plastic film (Parafilm) and allowed to develop on ice in a cold room for 30 minutes.

Fluorescence microscopy

The dechorionated embryos were fixed and their vitelline membrane was removed essentially as described by Warn and Warn (1986), except for a final fixation with acetone for 5 minutes at –20˚C. The embryos were then washed in phosphate buffered saline (PBS) and incubated for 1 hour in PBS containing 0.1% bovine serum albumin to block nonspecific staining. The samples were then washed with PBS and incubated for 1 hour at room temperature in 1:200 mouse monoclonal antibody against α-tubulin (Amersham, Bukinghamshire, UK). After rinsing in PBS the embryos were incubated for 1 hour in the secondary antibody (goat anti-mouse fluorescein-conjugated IgG; Cappel, West Chester, PA). For double labeling the embryos were incubated for 4-5 hours at room temperature with Rb188 antiserum, which specifically recognizes an antigen associated with the centrosome of *Drosophila* embryos (Whitfield et al., 1988). These embryos were washed and treated with rhodamine-conjugated goat anti-rabbit IgG (Cappel). For nuclear envelope identification the embryos were incubated overnight at 4˚C with a monoclonal antibody raised against human fibroblast vimentin (Sclavo, Siena). To stain the nuclei, the embryos were incubated for 3 minutes with 1 μg/ml of the DNA-specific dye Hoechst 33258 in PBS. The embryos were then washed in PBS and mounted in 90% glycerol containing 2.5% *n*-propyl gallate (Giloh and Sedat, 1982).

Fluorescence observations were carried out with a Leitz Aristoplan microscope equipped with fluorescein and UV filters. Photomicrographs were taken with Kodak Tri-X Pan film and developed in Kodak HC 110 developer for 7 minutes at 20˚C.

Electron microscopy

Two different fixation protocols were used. Some embryos were fixed in the trialdehyde solution of Kalt and Tandler (1971) for 2 hours. After rinsing in 0.1 M cacodylate buffer, pH 7.2, the embryos were postfixed in 1% osmium tetroxide for 2 hours. Some were fixed according to Dallai and Afzelius (1990) to improve the visualization of microtubules. After fixation the material was dehydrated in a graded series of ethanol, treated with propylene oxide, embedded in Polarbed (BioRad, Polaron, Cambridge, MA) and polymerized at 60˚C for 48 hours. Random and serial sections cut using an LKB Nova ultramicrotome and a diamond knife (Diatome Ltd, Switzerland) were collected on copper grids and stained with uranyl acetate and lead citrate. Sections were observed and photographed with a Philips CM10 electron microscope.

RESULTS

Bacteria in preblastoderm embryos

Hoechst staining showed that the eggs laid by infected *Drosophila melanogaster* females contained a large number of DNA-positive bodies. Previous studies in *Drosophila simulans* suggest that these bodies are cytoplasmic bacteria, on the basis of light microscopy coupled with tetracycline treatment (O'Neill and Karr, 1990) and transmission electron microscope observations (Binnington and Hoffman, 1989; Louis and Nigro, 1989). The bacteria, more abundant in eggs laid by younger females, were concentrated in a thin layer in the cortical cytoplasm and diffused throughout the yolk region. When the zygotic nucleus entered mitosis many bacteria accumulated near the poles of the mitotic apparatus. Fig. 1A,B shows Hoechst staining of the surface and yolk region of a third nuclear division cycle embryo. The cytoplasm surrounding the dividing nuclei was almost devoid of bacteria, despite the large accumulation of these microorganisms in the cortical cytoplasm (Fig. 1A) and at the spindle poles (Fig. 1B). Several bacteria were free in the cytoplasm, far from the mitotic spindles. As mitosis progressed most of the intravitelline bacteria migrated towards the nuclear regions and a few isolated bacteria were still observed free in the yolk region (Fig. 1C). Hoechst stain failed to reveal clusters of bacteria in control embryos obtained from Oregon-R flies (Fig. 1D).

Behavior of bacteria in syncytial blastoderm embryos

The distribution of bacteria in the periplasm of the embryos was unchanged during the first nine mitoses. When the first nuclei reach the embryo surface during telophase of nuclear cycle 9 the bacteria localized in the cortex accumulated in the spindle poles. During interphase of nuclear cycle 10, the microtubules that form the mitotic spindles nucleated from two widely separated foci localized over the nucleus, and radiated towards the plasma membrane and along the apical side of the nuclear envelope. Bacteria were scattered in the cytoplasm over the nuclei (Fig. 1E,F) with a feeble concentration at the microtubule nucleating sites during the following prophase (Fig. 2A,B). At metaphase, the mitotic spindles were well formed, with clearly visible astral microtubules, whereas the

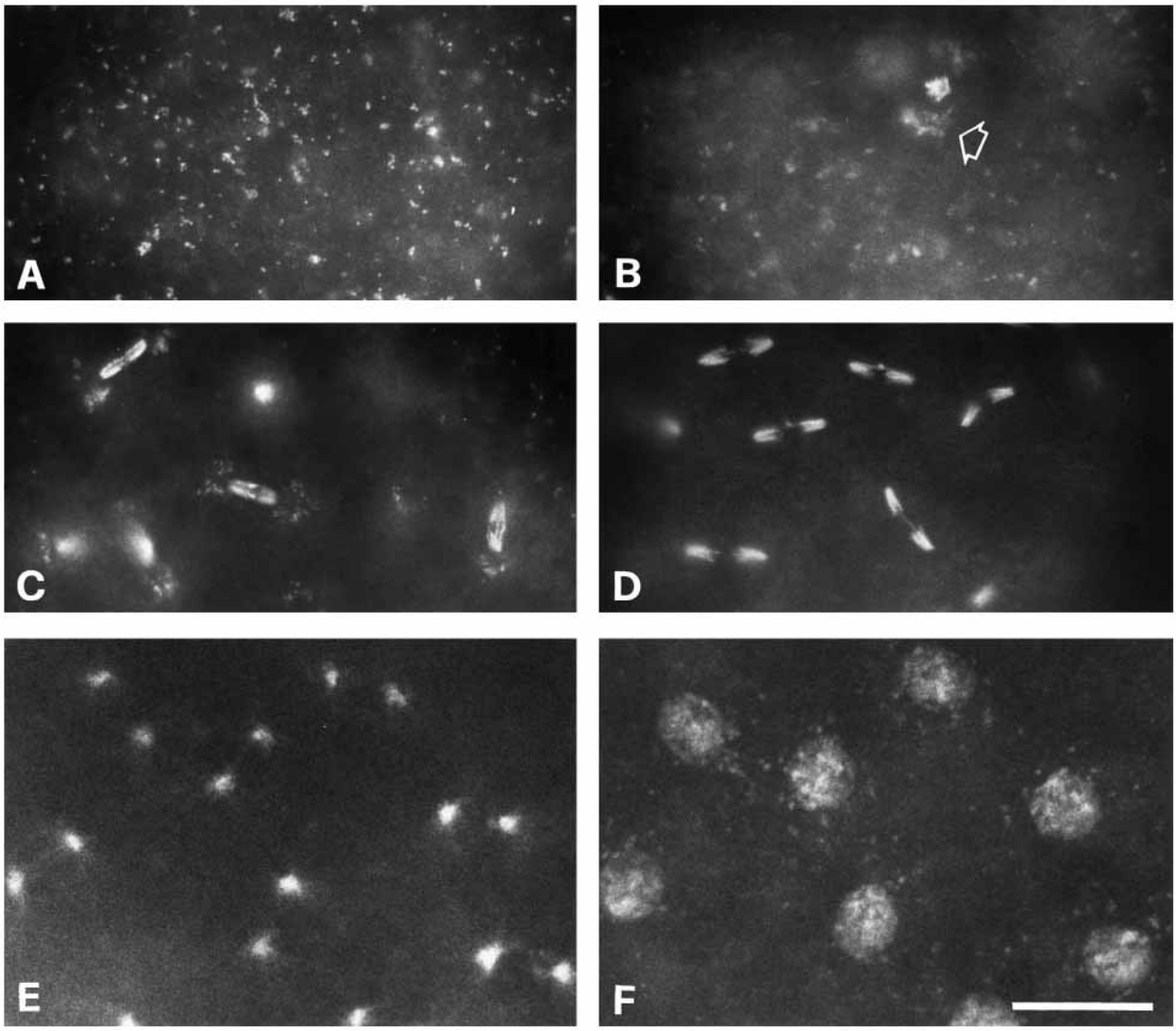

Fig. 1. (A) Surface and (B) deep views of an infected *Drosophila melanogaster* embryo stained with Hoechst 33258 during the third intravitelline mitosis. Bacteria are observed in the cortical cytoplasm and in the yolk region as small bright dots. Several bacteria are concentrated near the nuclear regions (arrow). (C) Detail of an infected embryo during early anaphase of the sixth nuclear mitosis. Hoechst stain shows that most of the bacteria are clustered near the pole of the mitotic spindles. (D) Control embryo at late anaphase of the seventh nuclear cycle. Hoechst stain does not reveal bacteria in the cytoplasm or around the nuclear regions. (E,F) Distribution of bacteria during interphase of a tenth nuclear cycle embryo, double stained for tubulin (E) and DNA (F). Bars: (A,B,C,D) 35 μm; (E,F) 20 μm.

embryo cytoplasm between the spindles was apparently devoid of microtubules (Fig. 2C). Bacteria accumulated towards the poles of the mitotic apparatus and formed large flat clusters aligned with the spindle axis (Fig. 2D). Few bacteria were observed in the cytoplasm among the spindles. At anaphase, the astral microtubules elongated further (Fig. 2E) and the polar clusters of DNA-positive bodies were slightly more expanded than during metaphase (Fig. 2F). As anaphase progressed, the bacteria surrounded the extremities of the nuclear envelope. Telophase was characterized by a reduction of interzonal microtubules, whereas the astral ones were well developed (Fig. 3A). At this stage, the bacteria formed loose aggregates on opposite sides of the spindle axis, around the reforming daughter nuclei (Fig. 3C).

Bacteria colocalized with the poles of the mitotic apparatus, not with spindle microtubules

Triple fluorescence with an antibody against α-tubulin, Hoechst dye and Rb188 serum, which specifically recognizes a centrosomal associated protein from *Drosophila* embryos (Whitfield et al., 1988), allowed us to compare the positions of microtubules, bacteria and centrosomes (Fig. 3A,B,C). Our observations revealed that bacteria were concentrated around the centrosomal region along the astral microtubules, but were absent from the spindle region. Double labeling with Hoechst dye and an antibody raised agaist human fibroblast vimentin, which has been shown to stain the periphery of *Drosophila* syncytial nuclei (Callaini and Riparbelli, 1991), confirmed that the microorganisms were concentrated at opposite poles in

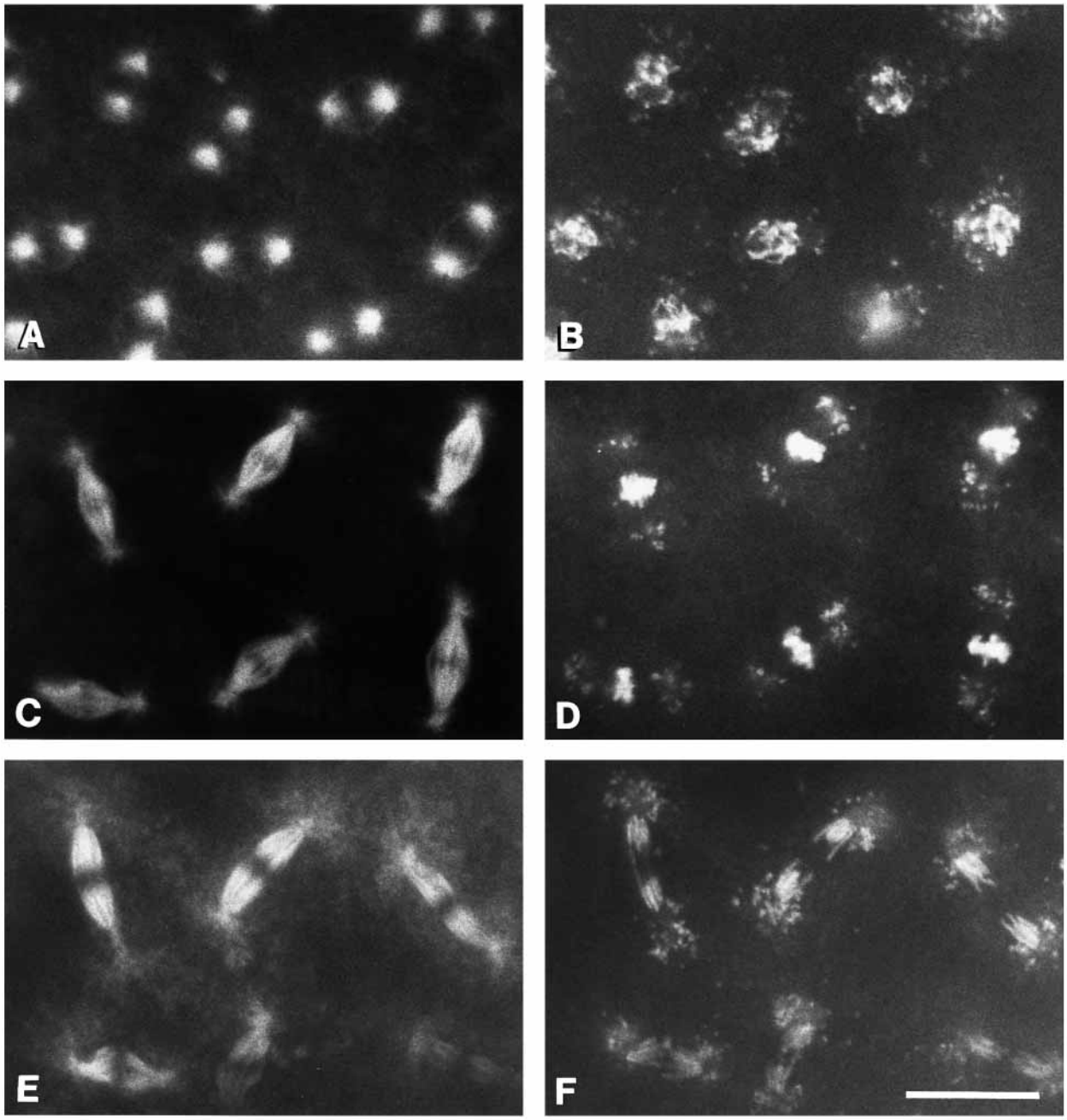

Fig. 2. Distribution of bacteria during mitosis of a tenth nuclear cycle embryo double stained for tubulin (A,C,E) and DNA (B,D,F). (A,B) Prophase; (C,D) metaphase; (E,F) anaphase. Bar, 20 μm.

dividing nuclei, and absent from the nuclear region (Fig. 3D,E).

To verify the relationship between spindle poles and microorganism clustering, we performed two experiments that allowed us to obtain centrosomes isolated from the spindle microtubules. First we examined the distribution of the bacteria in mitosis-defective embryos. These embryos showed abnormal spindles and irregular chromosome condensation; the centrosomes moved away from the spindles but retained their capacity to nucleate microtubules. Double labeling with Hoechst dye and an anti-tubulin antibody revealed that the bacteria were not found near spindles lacking centrosomes, but clustered in association with the asters nucleated by the isolated centrosomes (Fig. 4A,B). The second experimental system utilized cold-treated early embryos. It was found that low temperature cause the depolymerization of the astral microtubules and the dissociation of the centrosomes from the spindle regions. Short astral microtubules grow back from isolated spindle poles after recovery (Callaini and Marchini, 1989). Therefore, we obtain abnormal embryos with high numbers of isolated centrosomes, still able to nucleate short microtubules. The bacteria accumulated towards the remnant

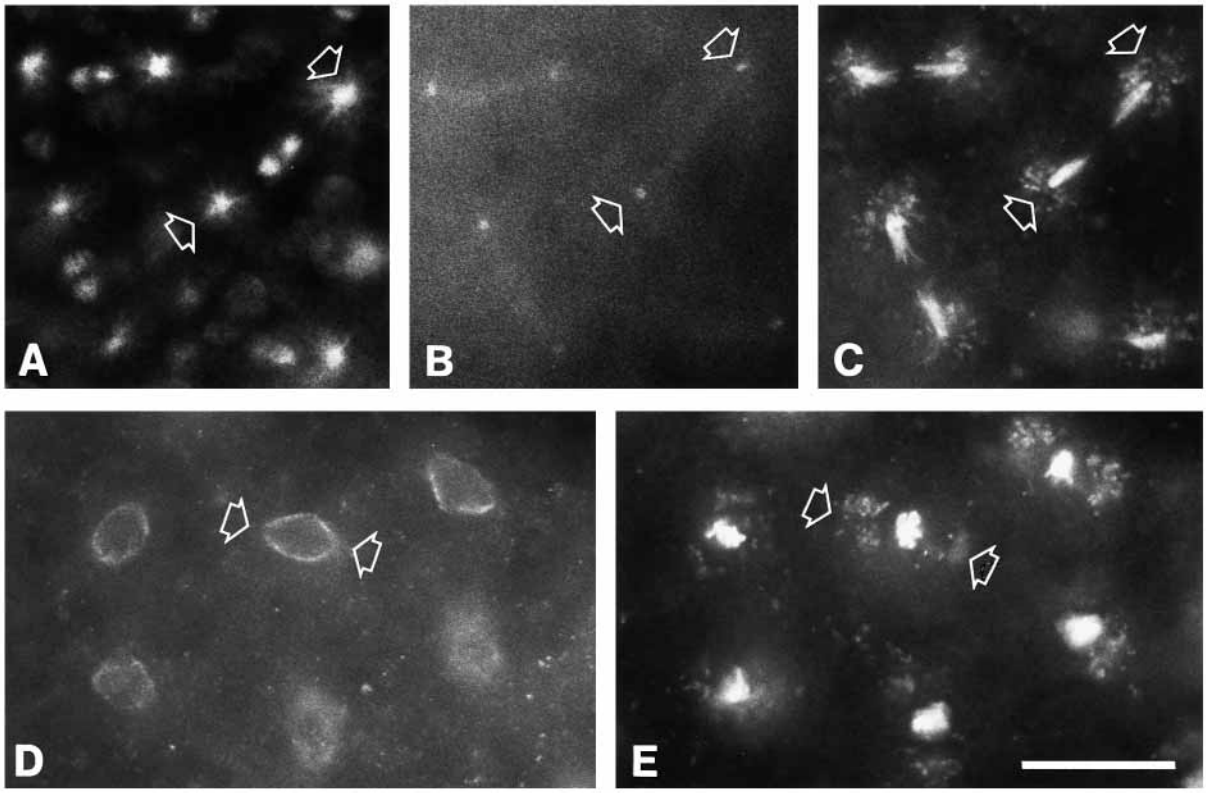

Fig. 3. Detail of an embryo triple stained for microtubules (A), centrosomes (B), and DNA (C) during early telophase of nuclear cycle 10. Arrows point to astral microtubules in (A), centrosomes in (B), and clusters of bacteria in (C). (D,E) Distribution of bacteria during metaphase of nuclear cycle 10 with respect to the nuclear envelope. Arrows point to opposite poles of the nuclear envelope in (D), and bacteria in (E). Bar, 20 μm.

poles of the mitotic spindles and also formed small irregular aggregates near the isolated spindle poles (Fig. 4C,D). A few bacteria were scattered in the cortical cytoplasm.

A second stock of lethal embryos shows the duplication of the centrosomes whithout full separation of the sister spindles (Fig. 5A,B). Irregular spindles lose their centrosomes, which migrated to just below the plasma membrane (Fig. 5D,E). Bacteria were initially accumulated in association with the multiple spindle poles (Fig. 5C), but diffused among the spindles when the centrosomes moved towards the embryo surface (Fig. 5F). Bacteria were never associated with the cortical centrosomes, which were apparently devoid of astral microtubules.

The position of the bacteria was perturbed by colchicine but not cytochalasin

When the embryos were incubated with the microtubule inhibitor colchicine, the mitotic spindles were destroyed and chromosomes attained a metaphase-like configuration. Microorganism clustering was less than in normally developing embryos. Bacteria were arranged in irregular aggregates near the chromosomes or dispersed in the cytoplasm between the metaphase plates (Fig. 6A). Short incubation (15 minutes) with 40 μg/ml of cytochalasin was found to be sufficient to cause alterations to the cortical microfilament network (Callaini et al., 1992). Under these conditions nuclear division continued but mitotic spindles showed some abnormalities due to the partial fusion of adjacent spindles (Fig. 6B). The accumulation of bacteria in association with the poles of mitotic spindles persisted in cytochalasin-treated embryos, although some abnormal clusters were observed in association with adjacent fused spindles (Fig. 6C).

Bacteria were not associated with polar bodies and yolk nuclei

Polar body remnants were still visible in the periplasm of tenth nuclear cycle embryos as typical monopolar spindles containing star-like chromatids. Despite the large number of bacteria associated with the poles of the mitotic spindles, DNA-positive bodies were not found near the polar body domains (Fig. 7A). Rb188 failed to reveal centrosomal material associated with polar body spindles (not shown), and astral microtubules were probably absent.

When the somatic nuclei reached the embryo surface during the tenth nuclear division cycle, the stationary nuclei within the interior of the egg still divided and clusters of bacteria were found at their spindle poles. However, these nuclei ceased dividing after the eleventh mitosis and became polyploid. The centrosomes moved away from the yolk nuclei and continued the replication cycle, despite the fact that the yolk nuclei had ceased dividing (Callaini and Dallai, 1991). Hoechst staining failed to reveal clusters of DNA-positive bodies associated with the yolk nuclei. Only a few scattered bright dots, associated with isolated centrosomes, were observed (Fig. 7B,C). Most of the yolk centrosomes lack bacteria.

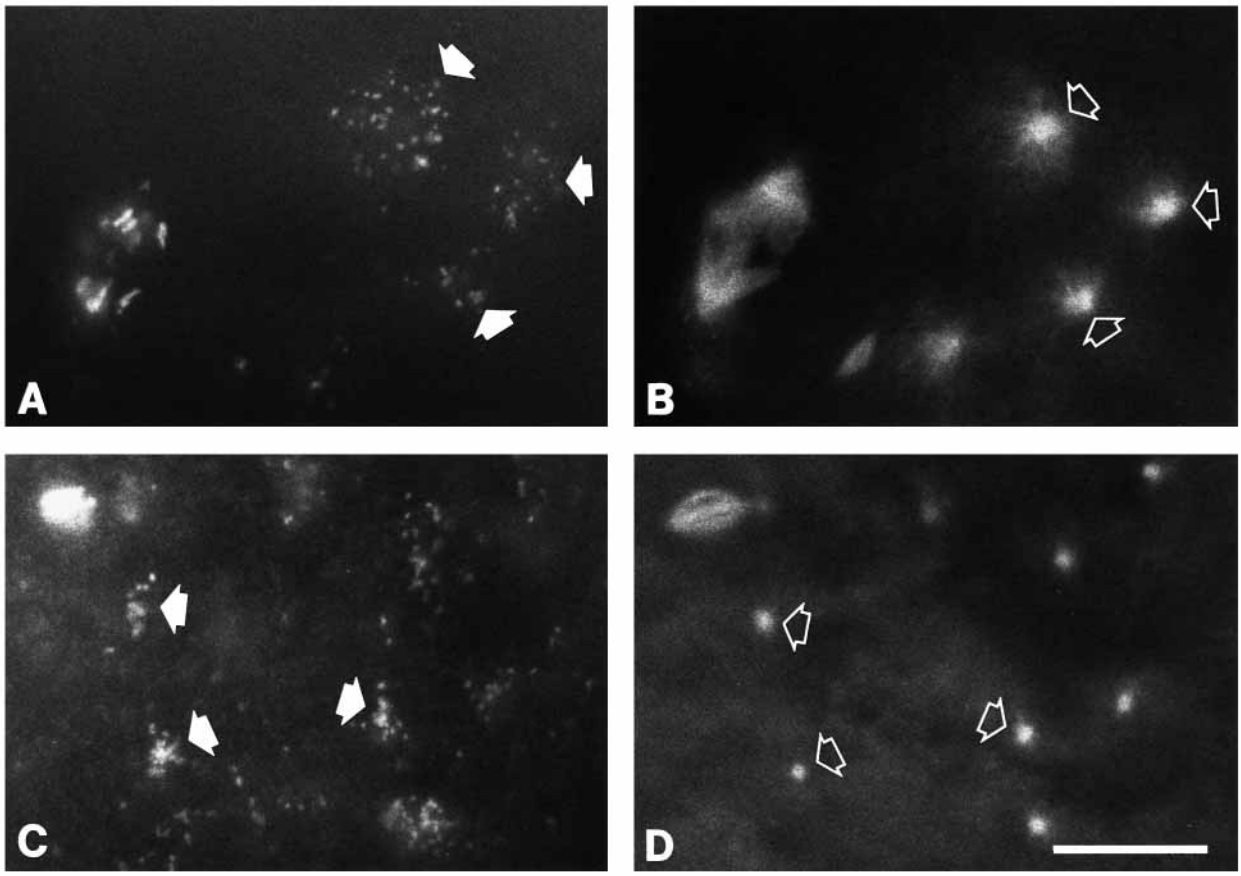

Fig. 4. Distribution of microtubules and bacteria in mitosis-defective embryos (A,B) and in embryos exposed to low temperature (C,D). Clusters of bacteria (filled arrows) are visible near the isolated spindle poles (open arrows). A,C, Hoechst staining; B,D, tubulin staining. Bar, 20 μm.

Bacteria were closely associated with astral microtubules

Trasmission electron microscope observations confirmed the presence of clusters of bacteria near the poles of the mitotic spindles in syncytial embryos. These bacteria, contained in a cytoplasmic vacuole is typical for *Drosophila* rickettsias, have a double-layered envelope (Szollosi and Debec, 1980; Fig. 7D). Most of the bacteria were aligned along astral microtubules with their long axis directed toward the centrosomal region. Observation of many serial sections of glutaraldehyde/paraformaldehyde-fixed embryos, and of material treated with a glutaraldehyde/tannic acid fixative, frequently revealed the close association between astral microtubules and cytoplasmic vacuoles containing the bacteria (Fig. 7D). This association was apparently mediated by short electron-dense bridges that cross-link microtubules and vacuolar membranes (Fig. 7D, inset).

DISCUSSION

Astral not spindle microtubules are required for the bacteria to cluster around the syncytial nuclei

In accordance with previous reports on *Drosophila simulans* (O'Neill and Karr, 1990) and *Drosophila melanogaster* (Glover et al., 1990), we have found that bacteria are concentrated around the syncytial nuclei in the early *Drosophila melanogaster* embryo. Double labeling with the DNA-specific Hoechst dye and an anti-tubulin antibody showed that the highest concentration of bacteria was at the poles of the mitotic spindles, suggesting that the bacteria are associated with the microtubules of the asters. Several findings support this conclusion: (a) during the intravitelline mitoses, the bacteria were first scattered in the cytoplasm, then they mainly accumulated toward the spindle poles, leaving large cytoplasmic areas free. (b) The spatial reorganization of the bacterial clusters seemed to follow a distinct pattern that is highly dependent upon astral microtubule disposition during mitosis. (c) Study of mitotic-defective embryos revealed that the bacteria accumulated around the isolated spindle poles, as previously reported in other *Drosophila* mutants (Glover et al., 1990), but not near spindles devoid of poles. (d) In cold-treated early *Drosophila* embryos, where microtubules regrow from sparse foci after a short recovery at 24°C (Callaini and Marchini, 1989), an altered distribution of the bacteria was to be expected. Indeed, the bacteria do not form regular clusters close to the nuclear regions but concentrate around newly formed asters far from the nuclei. (e) Bacteria were not found around the yolk nuclei during later nuclear cycles. From the tenth mitosis the yolk nuclei ceased dividing (Foe and Alberts, 1983) and the cen-

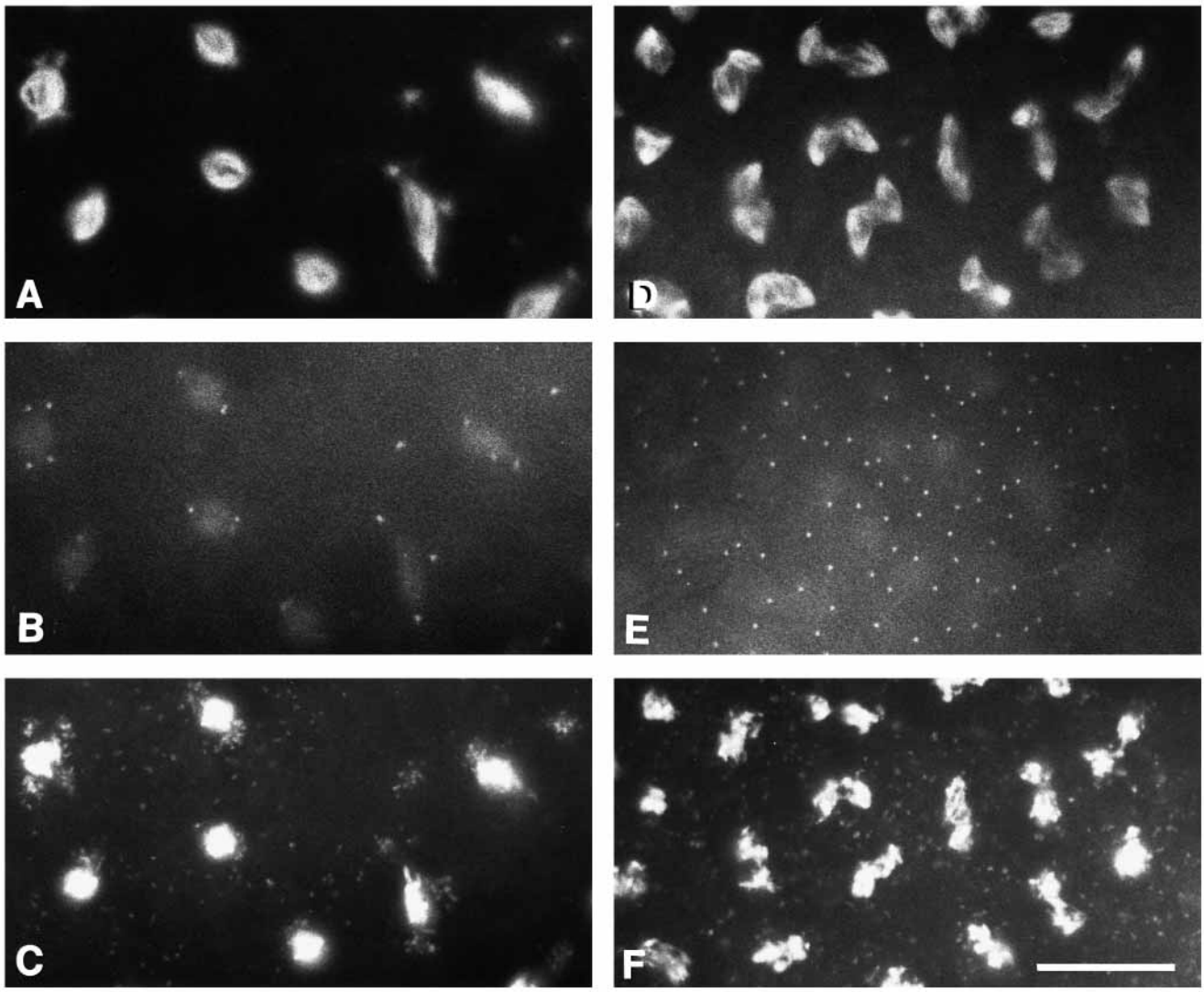

Fig. 5. Distribution of bacteria in mitosis-defective embryos triple stained for microtubules (A,D), centrosomes (B,E), and DNA (C,F). Pictures in (A,B,C,D,F) were taken at the level of the nuclei; picture in (E) represents a surface view just below the plasma membrane. The spindles in (A) have their centrosomes and bacteria are associated with spindle poles. The spindles in (D) are devoid of centrosomes, which have migrated to the embryo surface; bacteria are diffuse in the cytoplasm among the nuclei. Bar, 20 μm.

trosomes moved away into the surrounding cytoplasm (Callaini and Dallai, 1991). (f) When the embryos were treated with the cytoskeletal drug colchicine the polar clusters of bacteria were broken up and dispersed in the periplasm. When treated with cytochalasin, only slight changes in microorganism localization occurred, mainly due to the fusion of adjacent spindles and the resulting deformation of the mitotic poles.

The concentration of bacteria near the poles of the spindles and their reorganization during mitosis reflects the ability of the microtubules of the mitotic apparatus to capture bacteria. This agrees with previous data showing that the asters of the mitotic apparatus accumulate cytoplasmic components such as mitochondria in ascidian (Sawada and Schatten, 1989), pigment granules in amphibian (Kobayakawa, 1988) and endoplasmic reticulum in echinoderm (Harris, 1975; Henson et al., 1989) embryos. However, although the above observations suggest that astral microtubules are responsible for the localization of the bacteria, they are not sufficient for us to conclude that bacteria interact directly with microtubules. Unequivocal evidence is difficult to provide. Nevertheless, in syncytial embryos fixed with a glutaraldehyde/tannic acid mixture, known to give better visualization of microtubular structures (Dallai and Afzelius, 1990), we often found that the bacteria were linked to microtubules by short electron-dense bridges. This observation, together with the dynamic behavior of bacteria during mitosis (present data), probably reflect the ability of the asters of the mitotic apparatus to bind bacteria, presumably with the aid of specific accessory proteins that cross-link microtubules and the cytoplasmic vacuoles containing the bacteria. In other systems several lines of evidence support the main role of microtubules for transporting intracellular components (Vale, 1987; Waterman-Storer et al., 1993), a process that is mediated by cytoplasmic motor proteins associated with the membranes (Schroer, 1991). Cyto-

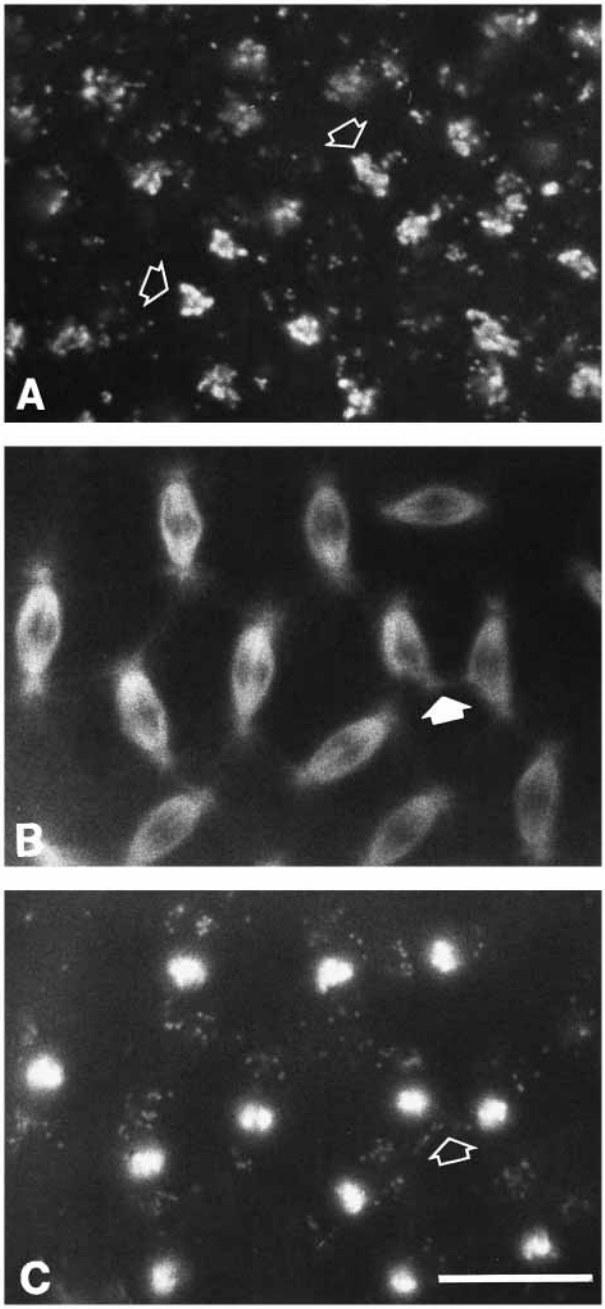

Fig. 6. (A) Colchicine-treated embryo arrested at metaphase of nuclear cycle 11. The bacteria form irregular clusters between the nuclear regions (open arrows). (B,C) Metaphase of a cytochalasin-treated nuclear cycle 11 embryo. Bacteria are clustered at the poles of the spindles, as in normally developing embryos. The cluster of bacteria is slightly deformed (open arrow) where adjacent spindles are fused together (filled arrow). Hoechst (A,C) and tubulin (B) labelings. Bar, 20 μm.

plasmic dynein may be implicated in the accumulation of membrane-bounded bacteria arround the spindle poles, since dynein-mediated capture of Golgi membranes and their further accumulation towards the centrosome have been demonstrated in Chinese hamster ovary cells (Corthesy-Theulaz and al., 1992).

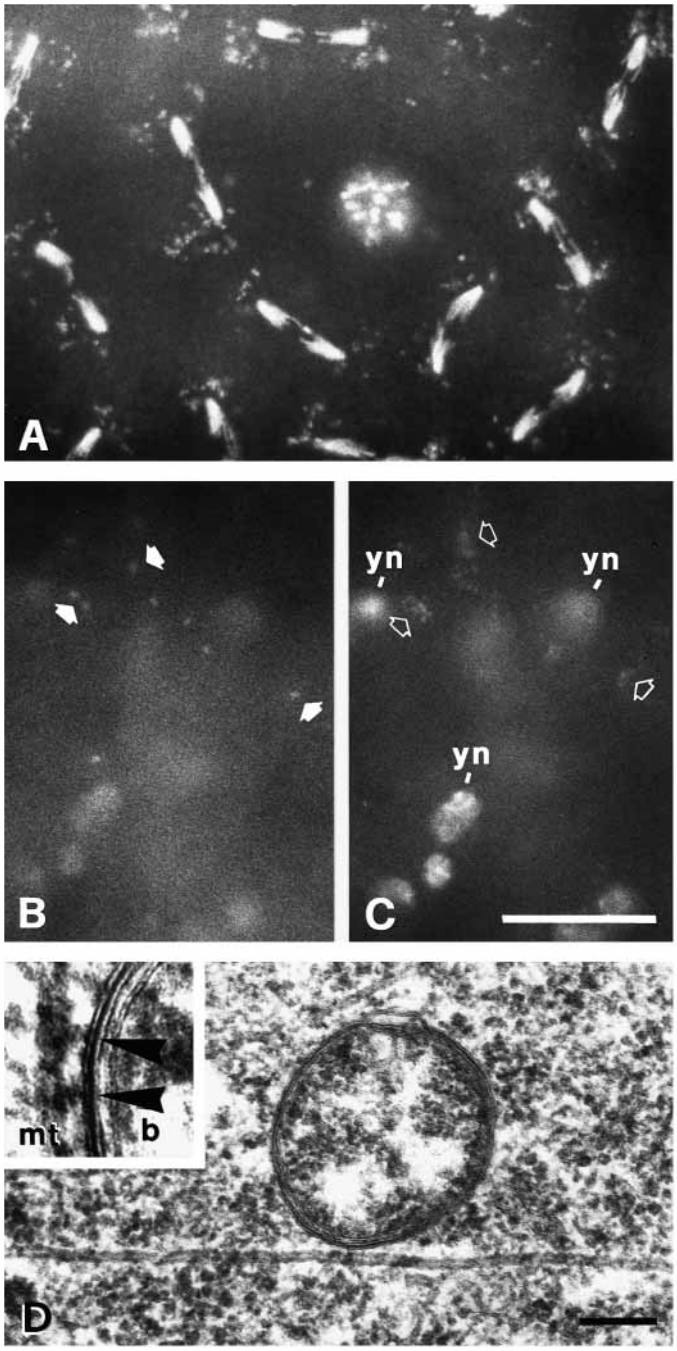

Fig. 7. (A) Hoechst labeling of an embryo during late anaphase of the eleventh nuclear division cycle. Bacteria are clustered around the somatic nuclei, but not near the polar body. (B,C) Deep view at the level of yolk nuclei (yn) of a twelfth nuclear cycle embryo double stained for centrosomes (B) and DNA (C). Small clusters of bacteria (open arrows) are associated with isolated centrosomes (filled arrows). (D) Detail of bacteria associated with astral microtubules. Arrowheads in the inset point to electron-dense bridges between a microtubule (mt) and a cytoplasmic vacuole containing bacteria (b). Bars: (A,B,C) 20 μm; (D) 0.2 μm; inset, 0.1 μm.

If the association of bacteria with microtubules may have been fortuitous, then we should consider alternative mechanisms to explain bacterial traffic in the embryo cytoplasm. We

can speculate that the astral microtubules, perhaps in association with other cytoskeletal elements, may form a highly structured cytoplasmic domain, able to trap nearby particles, which are also passively redistributed throughout the cytoplasm during mitosis. The microtubules of the asters are known to play an important role in rearranging the embryo cytoplasm (Elinson, 1990). The segregation of the pole plasm in *Tubifex* (Shimizu, 1989) and the reorganization of the germ plasm in amphibian (Ressom and Dixon, 1988) embryos require the presence of astral microtubules. By virtue of their ability to sequester cytoplasmic components, as confirmed by the nuclear cycle-dependent distribution of the bacteria, astral microtubules might play an important role in concentrating and storing cytoplasmic components, and perhaps molecular information, around the syncytial nuclei of the early *Drosophila* embryo. Moreover, the microtubules of the asters, in association with other cytoskeletal elements, contribute significantly to the building of cytoplasmic islands in which the nuclei divide without mutual interaction, also providing the proper distribution of cytoplasmic components for sister cytoplasmic islands in the absence of cleavage furrows. The importance of microtubules in the compartmentalization of the periplasm is also suggested by the observation that the apical microtubular network appears to be a major component in localization of the *fushi-tarazu* transcripts in cellularizing embryos (Edgar et al., 1987).

Is there a direct interaction between bacteria and the *Drosophila* embryo?

The distribution of bacteria in most *Drosophila* tissues (for review see Dimitriadis and Tartas, 1992) suggests a symbiotic relationship. However, there are indications that the presence of bacteria is associated with the reproductive isolation of different geographical strains of *Drosophila* species (see Boyle et al., 1993). In the incompatible cross, mating and oviposition are normal, but the eggs fail to hatch (Hoffmann et al., 1986). Despite the large mass of data, available on *Drosophila* and other insect species, the actual role of bacteria in such cytoplasmic incompatibility is not yet understood, and how the bacteria spread through the host tissues is unknown. In closely related species of parasitic wasps, the bacteria are apparently associated with abnormal condensation of chromosomes, which leads to failure of the first mitotic cleavage (Breeuwer and Werren, 1990) and in *Drosophila simulans* strains egg non-viability is associated with early developmental mitotic defects (O'Neill and Karr, 1990). It is unlikely that cytoplasmic incompatibility arises only from structural and functional alterations of the sperm cell, caused by interaction with bacteria, since males of infected strains can successfully reproduce with infected females. Moreover, bacteria have been found in oocytes and spermatocytes, but not in spermatozoa, of infected flies (Binnington and Hoffmann, 1989; Bressac and Rousset, 1993). Males of uninfected strains produce normal progeny when mated with infected females. These observations suggest that the sperm of infected males carry an unknown factor that affects fertilization or early development in incompatible crosses. The action of this factor may be limited in its interaction with the infected egg cytoplasm, which probably contains a bacterially-induced complementary factor. Alternatively, the presence of bacteria in male tissues may result in the loss of a fertilization factor in the male germ cells, soon restored by the infected egg cytoplasm. Barr (1981) suggests that bacteria can modify the sperm cell in a such way that a foreign body is retained when it enters an uninfected egg. We do not known whether the bacteria we have observed cause similar effects in *Drosophila melanogaster*. However, bacterial symbionts have been found in *Drosophila melanogaster* (Glover et al., 1990; Holden et al., 1993) and a partial cytoplasmic incompatibility has been reported between Australian populations of this species (Hoffmann, 1988). The numbers of bacteria that we have observed in the egg cytoplasm of *Drosophila melanogaster* are apparently lower when compared with the pictures representing *Drosophila simulans* embryos (O'Neill and Karr, 1990). The different density of bacteria in the egg cytoplasm of these *Drosophila* species may be responsible for the different levels of cytoplasmic incompatibility. A correlation between bacterial levels in the embryo and the degree of cytoplasmic incompatibility has been observed in strains of *Drosophila simulans* (Hoffmann et al., 1990; Montchamp-Moreau et al., 1991) and in transinfected *Drosophila melanogaster* lines (Boyle et al., 1993).

It is not clear how the bacteria are transferred from the egg cytoplasm to the adult tissues. The high concentration of the bacteria at the posterior pole of the egg, and their incorporation into pole cells, which typically develop into germ-line tissues, suggest a vertical transmission (O'Neill and Karr, 1990). The localization of bacteria in the spidle regions may be due to trapping by a specialized cytoplasmic domain surrounding the spindle poles, which could ensure the proper distribution of these bacteria around the dividing nuclei. Wright and Barr (1980) found that bacteria were associated with the spindle apparatus of dividing cystocytes of the mosquito *Aedes scutellaris*, and suggested that this relationship may have significance in successful transmission of the infection to each generation of germinal cells by a small number of bacteria. Bacteria are also found in blastoderm cells and have been observed during gastrulation, in interphase cells and associated with the poles of the spindles when the cells enter post-blastodermic mitoses (data not shown). Because of the limited number of bacteria in blastoderm cells, the redistribution of bacteria during post-blastodermic mitoses was not uniform and several daughter cells were eventually devoid of bacteria. This observation can explain the findings that bacteria were not uniformly distributed in adult tissues (Dimitriadis and Tartas, 1992).

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