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Cytoskeleton-dependent transport of cytoplasmic particles in previtellogenic to mid-vitellogenic ovarian follicles of *Drosophila*: time-lapse analysis using video-enhanced contrast microscopy

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SUMMARY

In *Drosophila* oogenesis, several morphogenetic determinants and other developmental factors synthesized in the nurse cells have been shown to accumulate in the oocyte during pre- to mid-vitellogenic stages. However, the mechanisms of the involved intercellular transport processes that seem to be rather selective have not been revealed so far. We have investigated in vitro, by means of video-enhanced contrast time-lapse microscopy, the transport of cytoplasmic particles from the nurse cells through ring canals into the oocyte during oogenesis stages 6-10A. At stage 7, we first observed single particles moving into the previtellogenic oocyte. The particle transfer was strictly unidirectional and seemed to be selective, since only some individual particles moved whereas other particles lying in the vicinity of the ring canals were not transported. The observed transport processes were inhibitable with 2,4-dinitrophenol, cytochalasin B or N-ethylmaleimide, but not with microtubule inhibitors. At the beginning of vitellogenesis (stage 8), the selective translocation of particles through the ring canals became faster (up to 130 nm/second) and more frequent (about 1 particle/minute), whereas during mid-vitellogenesis (stages 9-10A) the velocity and the frequency of particle transport decreased again. Following their more or less rectilinear passage through the ring canals, the particles joined a circular stream of cytoplasmic particles in the oocyte. This ooplasmic particle streaming started at stage 6/7 with velocities of about 80 nm/second and some reversals of direction at the beginning. The particle stream in the oocyte was sensitive to colchicine and vinblastine, but not to cytochalasin B, and we presume that it reflects the rearrangement of ooplasmic microtubules described recently by other authors. We propose that during stages 7-10A, a selective transport of particles into the oocyte occurs through the ring canal along a polarized scaffold of cytoskeletal elements in which microfilaments are involved. This transport might be driven by a myosin-like motor molecule. Either attached to, or organized into, such larger particles or organelles, specific mRNAs and proteins might become selectively transported into the oocyte.

Key words: VEC microscopy, oogenesis, microfilament, microtubule, intercellular transport

INTRODUCTION

Oogenesis of insects with meroistic ovaries is characterized by the supply of the growing oocyte with organelles and macromolecules from synthetically highly active nurse cells (Telfer, 1975). In the polytrophic follicle of *Drosophila*, a cluster of 15 germ-line derived nurse cells is connected with the oocyte by way of cytoplasmic bridges or ring canals (for reviews of *Drosophila* oogenesis, see King, 1970; Mahowald and Kambysellis, 1980; Spradling, 1993). Recently, it has been demonstrated that ooplasmic determinants needed for the first steps of embryonic development are produced in the nurse cells (for examples, see Sander and Lehmann, 1988; St Johnston et al., 1989; Hay et al., 1990; Lasko and Ashburner, 1990; Ephrussi et al., 1991; Kim-Ha et al. 1991; Suter and Steward, 1991; Cheung et al., 1992).

During late vitellogenesis (stages 10B-12), the nurse-cell cytoplasm has been observed to stream rapidly into the oocyte (Gutzeit and Koppa, 1982; Bohrmann and Sander, 1987). This bulk transfer of cytoplasm, leading to the ultimate degeneration of the nurse cells, depends on cytoskeletal elements, especially on the functioning of actin filaments (Gutzeit, 1986a). Presumably, cortical nurse-cell microfilaments contract and force the cytoplasm to flow into the oocyte (Cooley et al., 1992). On the other hand, the distribution and localization of organelles and macromolecules within the oocyte depends on the integrity of microtubules (Gutzeit, 1986b; Pokrywka and Stevenson, 1991; Theurkauf et al., 1992).

So far, the mechanisms of the intercellular transport processes that, on indirect evidence, must occur during previtellogenesis (up to stage 7) as well as during early and mid-vitellogenesis (stages 8-10A), have remained elusive (for a review, see Gutzeit, 1986c). A previously postulated ‘electrophoretic’ transport of charged molecules (Woodruff et al.,

1988; Woodruff, 1989) does not seem to play an important role during *Drosophila* oogenesis (Bohrmann et al., 1986a,b; Bohrmann and Gutzeit, 1987; Bohrmann, 1991a,b; Sun and Wyman, 1989, 1993). However, microtubules are likely to be involved in intercellular transport processes during the previtellogenic stages 1-6 (Koch and Spitzer, 1983; Theurkauf et al., 1992).

We report here the direct visualization of intercellular movements, as well as their inhibition, of individual cytoplasmic particles from the nurse cells through ring canals into the oocyte during previtellogenic to mid-vitellogenic stages 7-10A. The extremely low-contrast images generated by these minute particles were improved by video-enhanced contrast (VEC) microscopy (for a review, see Shotton, 1988). With this method, the particles were clearly seen with high contrast, permitting their intra- and intercellular movements to be recorded in time lapse, although their size might be one order of magnitude smaller than the calculated resolution limit of the light microscope. The observed transport processes of individual particles through the ring canals are sensitive to inhibition of actin filaments, while the coordinated movements of particles within the ooplasm apparently depend on intact microtubules.

MATERIALS AND METHODS

Preparation of follicles

Drosophila melanogaster Oregon R flies were reared at room temperature (about 23˚C) on standard food with additional fresh yeast. Individual females 2-4 days old were killed by crushing the thorax with tweezers without previous etherization or chilling. Previtellogenic follicles of stages 6-7 or vitellogenic follicles of stages 8-10A (see Figs 1 and 2) were carefully dissected out of the epithelial sheath of the ovariole with tungsten needles in R-14 medium (Robb, 1969; Bohrmann, 1991a). Follicles lacking any signs of injury were washed in R-14 and immediately transferred to a medium-filled chamber consisting of a microscopic glass slide and a large cover glass which was supported by small pieces of thin paper and held in place with vaseline. During normal incubation periods (see below), the evaporation of the medium was negligible.

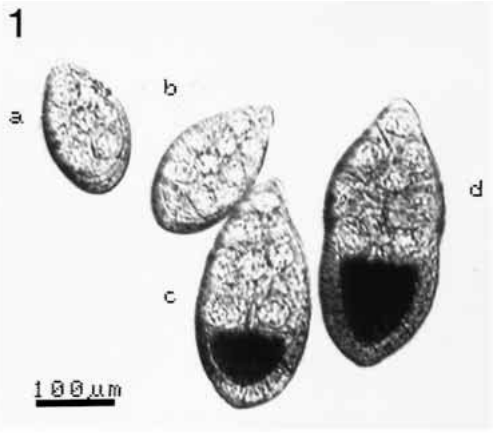

Fig. 1. Video prints using bright-field optics, to show living whole mounts (kept in R-14 medium) of previtellogenic follicles (a and b, stages 6 and 7) and mid-vitellogenic follicles (c and d, stages 9 and 10A) of *Drosophila melanogaster*. The follicles consist of an oocyte (bottom) and 15 nurse cells (top), surrounded by a layer of follicle cells.

Video-enhanced contrast (VEC) microscopy

The chamber containing the follicles was transferred to a Zeiss Axioskop microscope equipped with Nomarski Differential-Interference-Contrast (DIC) optics, a tungsten-halogen lamp, and a system for video microscopy (for review, see Inoué, 1986). We used a SIT-video camera C-2400 (Hamamatsu Photonics, Japan), a digital image processor Argus-10 (Hamamatsu), a time-lapse S-VHS video-tape recorder AG-6720 (Panasonic, Japan) and a b/w-TV monitor WV-5410 (Panasonic). With this system, we obtained high-contrast images with good resolution (see Fig. 2), even when microscope-light intensity was reduced considerably, thereby minimizing specimen damage during time-lapse recordings. The principles of VEC microscopy have been described in detail previously (e.g. Allen et al., 1981; for reviews, see Weiss, 1986; Shotton, 1988). We used a ×100/1.3 NA objective, a ×1 to ×1.6 magnification changer, and a ×10 eyepiece, resulting in a video monitor screen of maximum magnification ×8,000. Light intensity, Wollaston-prism setting and camera sensitivity were optimized, and analogue brightness and contrast settings of the camera adjusted for optimal contrast and resolution. When appropriate, the image contrast was further enhanced digitally. Digital image averaging was performed over 128 video frames to enhance the signal-to-noise ratio.

Time-lapse video-tape recordings (at 1-6 frames per second) of cytoplasmic particle movements occurring within as well as between nurse cells and oocyte (see Figs 3 and 4) started at about 15 minutes after dissection and lasted usually for another 10-30 minutes. However, particle transfer was still observable and had not changed after about 1 hour of in vitro culture. We do not believe that we observed in vitro artifacts since *Drosophila* follicles younger than stage 10, which were incubated for 30-45 minutes in R-14 medium, have been shown to develop normally when transplanted into host flies (Gutzeit and Koppa, 1982).

Distance as well as velocity measurements of moving particles were performed on tape recordings using the Argus-10 image processor and a video recorder with jog-shuttle function (NV-F70HQ, Panasonic). Statistical significance of differences between mean values was tested with the *t*-test. Video prints were produced on a video-copy processor P66E (Mitsubishi, Japan).

Inhibitor studies

Directly after dissection, the follicles were incubated for 15 minutes in the presence of one of the following inhibitors dissolved in R-14 medium: (a) the inhibitor of oxidative phosphorylation 2,4-dinitrophenol (Serva, Heidelberg, Germany; 1 mM; Bohrmann, 1991a); (b) the microtubule inhibitor colchicine (Sigma, Deisenhofen, Germany; 20 µg/ml with 0.1% dimethylsulphoxide; Gutzeit, 1986b); (c) the microtubule inhibitor vinblastine (Sigma; 100 µg/ml with 1% methanol; Huebner and Anderson, 1970), which is pharmacologically distinct from colchicine; (d) the microfilament inhibitor cytochalasin B (Sigma; 10 µg/ml with 0.2% ethanol; Gutzeit, 1986a); (e) the sulfhydryl reagent *N*-ethylmaleimide, an inhibitor of myosin-driven motility (Sigma; 1 mM; Sheetz and Spudich, 1983). The respective inhibitor was also present during subsequent video recordings lasting 10-30 minutes.

Cytochalasin B-treated follicles and control follicles were fixed and stained with rhodaminylphalloidin as described previously (Warn et al., 1985; Gutzeit, 1986a; Bohrmann et al., 1992), and the structures of F-actin rings surrounding the cytoplasmic bridges were analysed using fluorescence microscopy.

Electron microscopy

Follicles were dissected as described above, fixed for 30 minutes at 4˚C with 2% formaldehyde/0.1% glutaraldehyde/1% OsO_4 dissolved in 100 mM sodium cacodylate buffer, pH 7.3. The follicles were

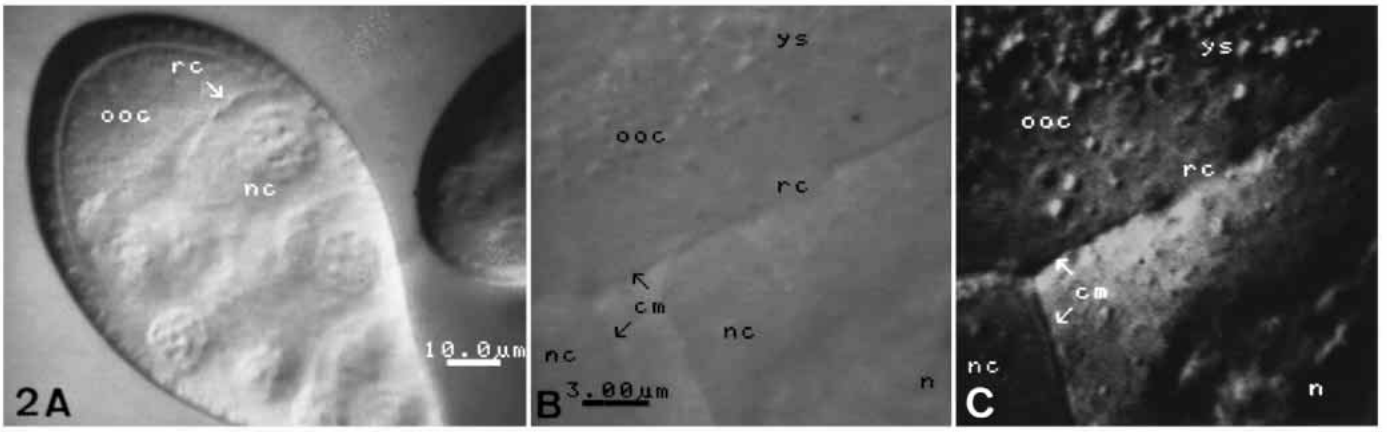

Fig. 2. Video prints, using Nomarski-DIC optics, of an early vitellogenic stage-8 follicle. (A) Overview showing the location of a ring canal (rc) connecting a nurse cell (nc) with the oocyte (ooc). (B) The same ring canal seen at higher magnification without contrast enhancement. (C) Same area as in (B), seen with video-enhancement of contrast (VEC). Minute particles in the nurse-cell cytoplasm which are not detectable in (B) are clearly resolved as a relief in (C); cm, cellular membranes; n, nurse-cell nucleus; ys, yolk spheres (larger particles in the oocyte).

washed 3 times for 10 minutes each in the same buffer, dehydrated in a graded acetone series and embedded in glycidether 100 (Serva). Ultrathin sections were viewed without any conventional staining procedures in a Zeiss CEM 902 energy-filtering electron microscope using electron spectroscopic imaging (ESI; for details, see Bauer, 1988). The ESI method uses inelastically scattered electrons of certain energy losses ΔE to create images with high contrast depending on the different densities and element compositions of the cellular structures.

RESULTS

Particle movements within the nurse cells

With VEC microscopy, the nurse-cell cytoplasm appeared to contain countless minute particles, possessing apparent diameters of 100-500 nm (Figs 2 and 4), which were seen oscillating and moving randomly over small distances during all developmental stages analysed (stages 6-10A; see Figs 1 and 2). Because of the small field of view at the high magnification used in the present study, we concentrated our observations to the nurse cells bordering the oocyte. Here we detected, in several cases already during stage 6, single cytoplasmic particles that moved over distances of about 5 µm towards a ring canal leading into the oocyte, while the large nuclei were seen to oscillate and slowly rotate. During stages 9 and 10A, Gutzeit and Koppa (1982), using time-lapse cinematography and bright-field optics, have observed characteristic back-and-forth movements of nurse-cell cytoplasm which, however, did not result in any lasting translocation of cytoplasm.

During all analysed stages, the nurse-cell cytoplasm in an area of 3-5 μm^2 next to a ring canal appeared to be specialized when compared with other cytoplasmic areas: this cytoplasm seemed to have a higher viscosity, and the density of particles appeared to be higher here. In these areas, the shifting of particles was evidently restricted, in that more particles were observed to move back and forth perpendicular to the plane of the ring canal than parallel to it.

Particle movements within the oocyte

With our VEC method, we detected coordinated circular movements of cytoplasmic particles in the oocyte as early as stage 6/7 (Fig. 3; Table 1). In 6 out of 8 analysed oocytes of this stage, the direction of particle streaming was seen to reverse during the period of observation. In 3 of these oocytes, such a reversal occurred even two times, with an interval of 10-14 minutes in between. At stage 7, reversal of circular streaming was observed only in 4 out of 9 oocytes, and no reversal was found in oocytes of older stages (Table 1). The velocity of this ooplasmic particle streaming was in the order of 80-100 nm/second during previtellogenic to early vitellogenic stages. During mid- to late vitellogenic stages 10A-13, Gutzeit and Koppa (1982) have observed a more massive streaming of the ooplasm showing velocities of 200-500 nm/second; this process might be also qualitatively different from the streaming of ooplasmic particles that we observed during earlier stages using VEC microscopy (see Discussion).

The particle stream never affected the oocyte nucleus and its direct ooplasmic vicinity; this is presumably due to the dense microtubule network existing in this region (Gutzeit, 1986b; Theurkauf et al., 1992). Moreover, during stages 6-10A, a 3-5 µm wide cortex area of the oocyte did not participate in streaming. During stages 10B-13, this stationary oocyte cortex

Table 1. Ooplasmic particle streaming and particle transport from nurse cells through ring canals into the oocyte during stages 6 to 10A

Stage	Number of analysed follicles	Ooplasmic particle streaming	Particle transport through ring canals
6	6	–	–
6/7	8	+*	–
7	9	+*	±
7/8	8	+	±
8	19	+	+
9	12	+	+
10A	9	+	+

+ Observed in (almost) all tested follicles.
± Observed in about half of the tested follicles.
– Never or seldom observed.
*Reversal of direction observed in some oocytes.

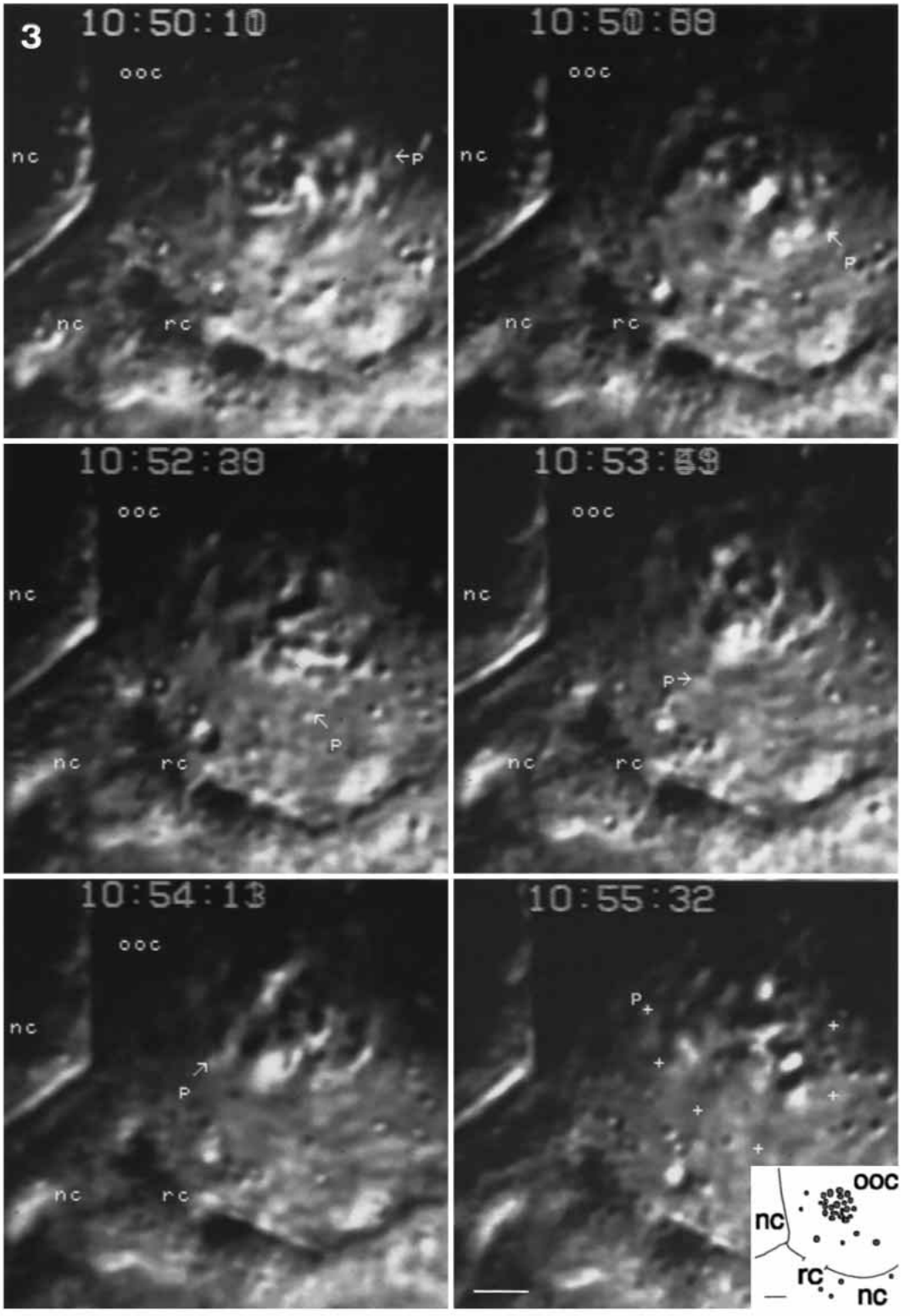

Fig. 3. Sequence of six video frames showing particle streaming in the oocyte (ooc) of a previtellogenic stage-6/7 follicle (inset shows locations of cellular membranes). Distance between frames is about 1 minute (see hour:minute:second at top). One particle (p) in the stream was selected and its movement was followed; the respective positions of the particle are indicated by arrows. Time-lapse recording (1 frame per second) and digital image averaging (over 128 frames) revealed that the ooplasmic particles streamed in a whirl that is also visible on the prints. The stream rotated clockwise, and the selected particle moved with a velocity of 86 nm/second. In the last frame, the positions of the particle in the six frames are again indicated with crosses to show the actual route of the particle, its last position being marked with a p. No particles were found to be transported from a nurse cell (nc) through a ring canal (rc) into the oocyte at this stage. Bar, 3 μm.

becomes 7-15 μm thick (Gutzeit and Koppa, 1982). Contrary to the situation in the nurse cells, in the oocyte the cytoplasmic vicinity of the ring canals did not appear to be of higher viscosity than other ooplasmic areas: particles entering the oocyte from a nurse cell (see below) rapidly joined the ooplasmic particle stream and could no longer be tracked.

Transport of particles through ring canals

Using VEC microscopy, we observed cytoplasmic particles moving from the nurse cells into the previtellogenic oocyte through about 5 μm wide ring canals as early as stage 7. The transfer of particles seemed to be rather selective, since only some individual particles moved into the oocyte, whereas

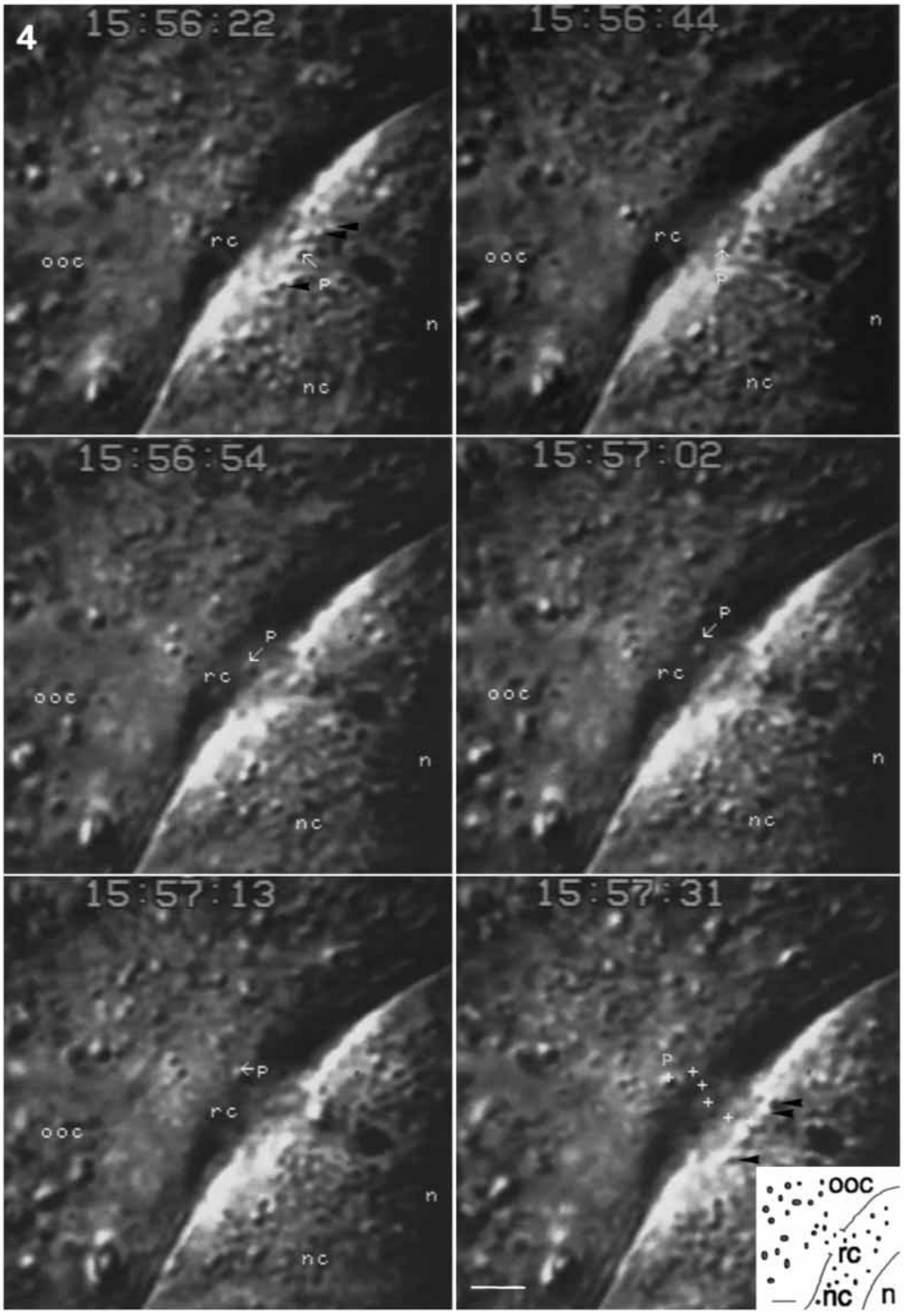

Fig. 4. Sequence of six video frames showing an individual particle (p) being transported from a nurse cell (nc) through a ring canal (rc) into the oocyte (ooc) of a stage-8 follicle (inset shows locations of cellular membranes). The respective positions of the particle at intervals of about 10-20 seconds (see hour:minute:second at top) are indicated by arrows. The particle was seen to be selectively transported through the ring canal over a more or less rectilinear distance of about 5 μm, with a velocity of 70 nm/second, before it joined the particle stream in the oocyte (time-lapse recording at 6 frames per second using 128 frames averaging). In the last frame, the positions of the particle in the six frames are again indicated with crosses to show the actual route of the particle, its last position being marked with a p. Several similar particles visible in the nurse-cell cytoplasm near the ring canal (three particles are marked with arrowheads on the first and last frame) remained more or less stationary during the period of observation; n, nurse-cell nucleus. Bar, 3 μm.

similar particles lying next to them were not transported (Fig. 4). Moreover, particle transport through the ring canals was strictly unidirectional, interrupted only by short stops or slight deflections from the rectilinear direction of movement. We concentrated our analysis on those ring canals that connected the nurse cells directly with the oocyte, but we observed similar transport processes in ring canals connecting two nurse cells: particle movements were always directed towards the nurse cell located closer to the oocyte. In a few cases (only 3 times during a total observation period of about 35 hours), we registrated very rapid backward movements into the nurse-cell cytoplasm (about 600 nm/second) of single particles that had been already on their way through the ring canal.

During stage 6, no particle transport could be detected with

Table 2. Comparison of particle transport from nurse cells through ring canals into the oocyte during stages 8 to 10A

	Stage 8	Stage 9	Stage 10A
Number of analysed follicles	19	12	9
Cumulative time of observation in minutes	244	261	232
Total number of particles seen to be transported	259	74	66
Mean number per minute of transported particles	1.1	0.3	0.3
Observed distance of particle transport in µm (±s.d.)	4.63±1.80 (n=29)	3.26±0.98* (n=26)	3.85±0.75 (n=10)
Velocity of observed particles in nm/second (±s.d.)	60±40	25±16*	27±12*

*Value significantly different from value at stage 8 (P<0.05).
n, Number of particles analysed for distance measurements and calculations of velocities.

our VEC method (Table 1; Fig. 3) and, therefore, we refrained from analysing follicles younger than stage 6. At the beginning of vitellogenesis (stage 8), transfer of particles from the nurse cells into the oocyte became more prominent (Fig. 4), whereas during previtellogenic stages such movements occurred rather seldomly and were slow and difficult to analyse in detail (Table 1). Even when compared to mid-vitellogenic stages 9 and 10A, during stage 8 the transfer of particles through the ring canals showed the highest frequency as well as velocity (Table 2; Fig. 5). Moreover, at stage 8 the analysed particles were selectively transported through the ring canals over a larger distance than at later stages (Table 2). In the plane of observation, which was usually a median optical section through a ring canal, individual cytoplasmic particles appeared among many other oscillating particles about 1-3 µm in front of the ring canal and disappeared from the plane of observation at a similar distance behind the ring canal. This selective transport of single cytoplasmic particles through the ring canals was both quantitatively and qualitatively different from the bulk transfer of nurse-cell cytoplasm into the oocyte which was reported by Gutzeit and Koppa (1982) to start late in vitellogenesis, during stage 10B, and to end during stage 12.

Inhibition of particle movements

In order to obtain closer insights into the mechanisms underlying the observed intra- and intercellular transport processes, we treated the follicles with several inhibitors. Because of the prominent particle transfer occurring in stage 8, mainly follicles of this stage were analysed (Tables 3, 4; Fig. 6). However, the results did not differ when follicles of younger and older stages were treated equally. When 2,4-dinitrophenol, an inhibitor of oxidative phosphorylation and thus of (nearly) all energy-dependent cellular processes was used, almost all movements of particles within as well as between the cells stopped within 15 minutes of incubation (Table 3). When the

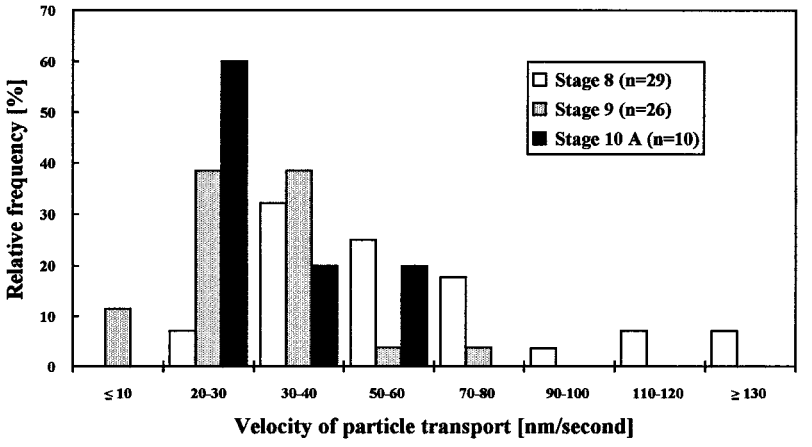

Fig. 5. Comparison of velocities of particle transport from the nurse cells through ring canals into the oocyte as observed during stages 8-10A. The transport velocities of individual particles (n, number of analysed particles per stage) were determined, and the relative frequencies of velocities plotted for each stage. Short interruptions of transport or slight changes in direction were not considered. At stage 8, more particles were found to be transported with higher velocities (50-130 nm/second) than at stages 9 and 10A.

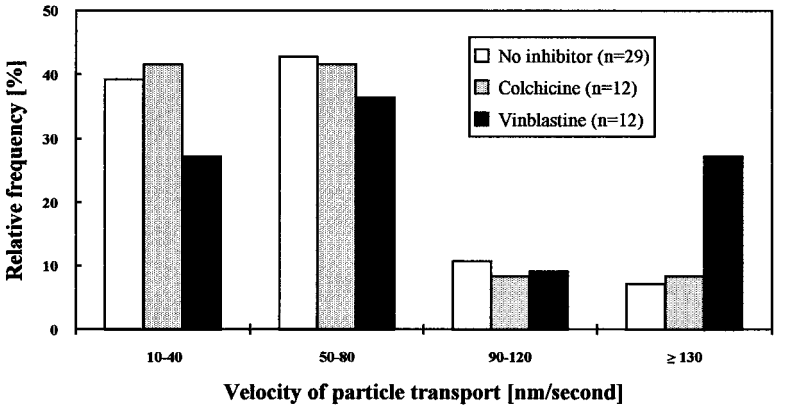

Fig. 6. Comparison of velocities of particle transport from the nurse cells through ring canals into the oocyte in the presence of microtubule inhibitors as observed during stage 8. The transport velocities of single particles (n, number of analysed particles per inhibitor) were determined, and the relative frequencies of velocities plotted for each inhibitor. Short interruptions of transport or slight changes in direction were not considered. Both microtubule inhibitors were found to block ooplasmic particle streaming but did not reduce the velocities of particles transferred through ring canals.

Table 3. Inhibition of ooplasmic particle streaming and particle transport from nurse cells through ring canals into the oocyte in follicles of stage 8

Inhibitor	Number of analysed follicles	Ooplasmic particle streaming	Particle transport through ring canals
Controls (no inhibitor)	19	+	+
2,4-Dinitrophenol	4	–	–
Colchicine	9	–	+
Vinblastine	4	–	+
Cytochalasin B	4	+	–
N-Ethylmaleimide	5	–	–

+ Observed in (almost) all tested follicles.
– Never or seldom observed.

Table 4. Comparison of observed distances and velocities of particle transport from nurse cells through ring canals into the oocyte in the presence of microtubule inhibitors during stage 8

Inhibitor	Number of analysed particles	Distance of transport in µm (±s.d.)	Velocity in nm/second (±s.d.)
Controls (no inhibitor)	29	4.63±1.80	60±40
Colchicine	12	4.44±0.92*	60±30*
Vinblastine	12	4.35±1.44*	80±50*

*Value *not* significantly different (P>0.05) from control value (no inhibitor).

microtubule inhibitor colchicine was applied, particle streaming within the oocyte as well as most of the movements of particles and nuclei in the nurse cells came to an end (Table 3), whereas transport processes through the ring canals were not affected. Neither the frequency nor the velocities and observed distances of particle transport changed significantly (Table 4; Fig. 6). Similar results were obtained with vinblastine; however, cytoplasmic movements within the nurse cells continued in three out of four tested follicles in an almost normal way, and several particles were transported with higher velocities (>130 nm/second) through the ring canals in the presence of this microtubule depolymerizing drug (Fig. 6).

When the microfilament inhibitor cytochalasin B was applied, the transfer of particles through ring canals was almost completely inhibited (Table 3). During a cumulative observation period of 96 minutes (four follicles), only five particles were seen moving into the oocyte. In contrast, particle streaming within the oocyte as well as motions of particles and nuclei in the nurse cells were not affected. Only in follicles of stages 6-7, in the presence of cytochalasin B, the ooplasmic streaming became more irregular and the oocyte lost its normal shape while the nurse cells started bulging some distance into the oocyte.

Using rhodaminylphalloidin staining, we did not detect any differences between the F-actin rings surrounding the canals of cytochalasin B-treated and of untreated control follicles, which means that the inhibition of particle transfer is not due to ring canal collapse and closure. As reported previously, cytochalasin B does only depolymerize thin microfilament bundles in *Drosophila* follicles but does not visibly affect thicker F-actin structures (Gutzeit, 1986a).

When *N*-ethylmaleimide, an inhibitor of myosin-driven motility (e.g. Sheetz and Spudich, 1983), was applied to the follicles, all movements within the cytoplasm stopped (Table 3); with this inhibitor even fewer movements of particles were detectable than with 2,4-dinitrophenol. Thus, we presume that cytoplasmic myosin plays a decisive role in particle movements during oogenesis.

Presumed nature of transported particles

Using VEC microscopy, it is not possible to determine the nature of the particles observed. Their shape and size might give some hints, although the apparent particle size is likely to be increased due to diffraction (see e.g. Shotton, 1988). As judged from our electron-microscopic ESI analysis of ring canals (Fig. 7) and from in vivo experiments using the mitochondria-specific fluorescent dye rhodamine 123 (unpublished observations), we suspect that small mitochondria were among the particles seen to be transported into the oocyte during stages 7-10A. In ultrathin sections, also several lipid droplets were detected near the ring canals (Fig. 7); therefore, part of the particles seen to be transported might have been lipid droplets. The countless ribosomes present (Fig. 7) are too small to be detectable with VEC microscopy. On the other hand, since the transport processes observed during previtellogenic to mid-vitellogenic stages seemed to be rather selective, further organelles and supramolecular assemblies which are produced in minor quantities by the nurse cells are likely to be among the particles seen to be transported (Fig. 7; see Discussion).

DISCUSSION

From the specific effects of depolymerizing drugs we infer the involvement of cytoskeletal elements in the observed particle movements, although neither actin filaments nor microtubules have been directly observed in our time-lapse study. Recently, in *Drosophila* follicles, microtubule bundles have been described to extend from the oocyte through ring canals into the nurse cells during previtellogenic stages 1-6; however, from stage 7 on, the stage when we first observed transport of individual particles from nurse cells to oocyte, microtubule bundles were no longer detectable within the ring canals (Theurkauf et al., 1992).

In nurse cells of stage-8 follicles, we observed, in several cases, short strings of particles extending towards a ring canal; thus, the cytoplasmic area next to the ring canals seemed to have a filamentous structure. Microfilament bundles associated with the F-actin rings surrounding the canals were reported to extend for some distance into the nurse-cell cytoplasm (Gutzeit, 1986a); however, within ring canals, microfilament bundles have not been found so far (Warn et al., 1985; Gutzeit, 1986a; Yue and Spradling, 1992; our own observations).

The unidirectional particle transport that we observed during stages 7-10A, occurring through the ring canals along invisible tracks, required the functional integrity of microfilaments. However, the structure of the F-actin rings surrounding the canals was not visibly altered by cytochalasin B. Thus, we presume that the selective particle transfer into the oocyte is due to a scaffold of actin and/or actin-associated cytoskeletal

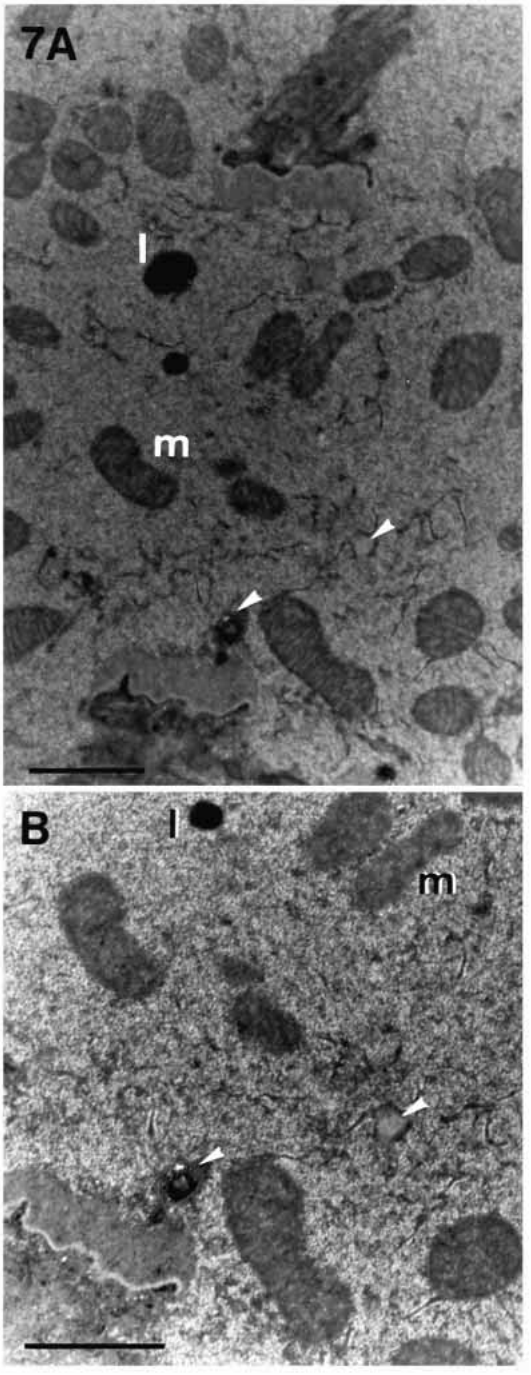

Fig. 7. ESI micrographs of an ultrathin section through a ring canal connecting a nurse cell (to the left) with the oocyte (to the right) of a mid-vitellogenic stage-9 follicle. Images generated by inelastically scattered electrons at ΔE = 66 eV (A) and 190 eV (B), respectively, showing good contrast without any additional staining. Several mitochondria (m) and lipid droplets (l) as well as copious ribosomes can be seen near and in the ring canal; arrowheads, unknown organelles. Bars, 1 μm.

elements extending through the ring canals. This assumption is further supported by the observed stage-specific distance of rectilinear particle transport which was not affected by microtubule inhibitors (see Table 4). Moreover, as seen in the VEC time-lapse recordings, the inferred cytoskeletal scaffold seemed to restrict the unspecific flow of cytoplasmic particles into the oocyte until late vitellogenic stage 10B. Whether intermediate filaments do exist in *Drosophila* and thus might be involved in transport processes is still a matter of discussion (see Fyrberg and Goldstein, 1990).

According to our results, a possible candidate for a motor protein of the observed selective particle transport might be cytoplasmic myosin. In other systems, short myosin filaments or non-filamentous myosin are known to play a role in cytoplasmic streaming and intracellular translocations of supramolecular assemblies and organelles (e.g. Sheetz and Spudich, 1983; for reviews, see Citi and Kendrick-Jones, 1987; Fyrberg and Goldstein, 1990). An experimental inhibition of cytoplasmic myosin function by means of injected antibodies would help to clarify whether myosin is a motor of intercellular particle transport in *Drosophila* follicles. The protein encoded by the *Bicaudal-D* gene, which is required for the formation of an oocyte, has similarities to regions of the myosin heavy chain and seems to play a role in intrafollicular transport (Suter and Steward, 1991). Another gene, *hu-li tai shao*, which encodes a homolog of adductin, has been shown to be required for the correct association of actin filaments with the ring canals as well as for the stabilization of ring canals (Yue and Spradling, 1992).

Recently, in squid axoplasm, a type of unidirectional organelle movement that is ATP-dependent, actin-dependent, and probably generated by a myosin-like motor molecule has been described by Kuznetsov et al. (1992). Moreover, these authors have observed individual particles to move consecutively along both actin filaments and microtubules. In pre- to mid-vitellogenic *Drosophila* follicles, this might be the case, too, since microfilament-dependent particle transfer through the ring canal was followed by microtubule-dependent particle movement within the oocyte.

Comparable to our results, during late vitellogenic stages 10B-12, Gutzeit (1986a,b) observed a differential effect of microfilament and microtubule inhibitors on the bulk streaming of nurse-cell cytoplasm into the oocyte and on rapid ooplasmic streaming, respectively. However, contrary to the transport processes of individual particles during pre- to mid-vitellogenic stages, the fast bulk transfer of nurse-cell cytoplasm (>2 μm/second; Gutzeit and Koppa, 1982) is not particle-selective and seems to be due to pressure flow depending on the contraction of microfilaments (Gutzeit, 1986a; Cooley et al., 1992). It is not very likely that such a pressure-flow mechanism is also responsible for the transport of single particles as observed in our study.

The rapid ooplasmic streaming during late vitellogenic stages (up to 500 nm/second) which, due to large yolk spheres, could be easily observed using time-lapse cinematography and bright-field optics (Gutzeit and Koppa, 1982; Bohrmann and Sander, 1987), is obviously different from the streaming of minute ooplasmic particles only detectable with VEC microscopy during the earlier stages (up to 100 nm/second). Theurkauf et al. (1992) reported a rearrangement of ooplasmic microtubules beginning in stage 6: the bulk of microtubules is shifted from the posterior pole to the anterior cortex of the oocyte. During stage 6/7, we first detected coordinated movements and often circular streaming of ooplasmic particles. Therefore, we believe that we observed indications

of the beginning of microtubule rearrangement. During stages 7-10A, an anterior-to-posterior microtubule density gradient forms within the oocyte (Theurkauf et al., 1992). This process, which appears to be necessary for the correct localization of the anterior determinant *bicoid* (Pokrywka and Stephenson, 1991), coincides with a continuous ooplasmic particle streaming that we observed during the respective stages. A further redistribution of microtubules takes place during stage 10: microtubules assemble in a dense subcortical network 5-10 μm below the oocyte surface (Theurkauf et al., 1992). This network is likely to mediate rapid ooplasmic streaming up to stage 13 (Gutzeit and Koppa, 1982), which seems to be necessary for the distribution of the entering bulk of nurse-cell cytoplasm in the oocyte.

In ovarian trophic cords of the hemipteran *Dysdercus intermedius*, transport of mitochondria along microtubules towards previtellogenic oocytes (with velocities of 20-500 nm/second) has been observed using VEC microscopy (Dittmann et al., 1987). In telotrophic ovaries, the oocyte becomes disconnected from the tropharium at the beginning of vitellogenesis and, therefore, organelles and macromolecules produced by the nurse cells are delivered into the oocyte only during previtellogenesis. In *Drosophila*, the bulk of organelles and macromolecules necessary for subsequent embryogenesis reaches the oocyte during late-vitellogenic nurse-cell regression (stages 10B-12). However, since such organelles were also found in ring canals and in the oocyte during stages 7-10A, they might be needed already during oogenesis. Occasionally, nuage-like material (components of polar granules) and some more unusual organelles (e.g. symbiotic prokaryotes) have been observed in ring canals (for reviews, see King, 1970; Mahowald and Kambysellis, 1980).

In recent years, it has become evident that several specific mRNAs and proteins, in particular morphogenetic determinants, which are synthesized in the nurse cells, accumulate in the *Drosophila* oocyte before nurse-cell regression (for reviews, see e.g. Lasko, 1992; St Johnston and Nüsslein-Volhard, 1992). We suspect that, during stages 7-10A, such mRNAs and proteins might become transported into the oocyte and localized therein either attached to, or organized into, larger particles or organelles which we observed moving due to a cytoskeleton-dependent process through the ring canals. Further VEC-microscopic analyses of intercellular transport processes in follicles of suitable mutants (see e.g. Schüpbach and Wieschaus, 1991), as well as further studies on the transport and localization of morphogenetic determinants in the presence of cytoskeletal inhibitors, would help to test this assumption.

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