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Drosophila unconventional myosin VI is involved in intra- and intercellular transport during oogenesis

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Abstract. During mid-oogenesis of *Drosophila*, cytoplasmic particles are transported within the nurse cells and through ring canals (cytoplasmic bridges) into the oocyte by means of a microfilament-dependent mechanism. Video-intensified fluorescence timelapse microscopy, in combination with microinjections of antibodies directed against *Drosophila* 95F myosin, have revealed that this unconventional myosin of class VI is involved in the transport processes. The results indicate that certain cytoplasmic particles in the nurse cells move along microfilaments due to their direct association with myosin VI motors. Additional myosin-VI molecules located at the rim of the ring canals seem to be involved in particle transport into the oocyte. Microinjected mitochondria-specific dyes have revealed that some of these particles are mitochondria.

Key words. 95F myosin; cytoskeleton; microfilament; ring canal; mitochondria.

In the ovarian follicle of *Drosophila melanogaster*, a cluster of 15 nurse cells is connected to the oocyte by intercellular bridges or ring canals (for reviews see [1, 2]). The synthetically highly active nurse cells supply the growing oocyte with a wealth of macromolecules and organelles. In particular, ooplasmic determinants necessary for embryonic pattern formation are produced by the nurse cells and become transported into the oocyte [3, 4]. The mechanisms of the various intra- and intercellular transport processes that occur during different phases of oogenesis are only partly understood and are currently a matter of intense investigation (for reviews, see [5, 6]).

Several studies have shown that cytoskeletal elements play a role in specific as well as nonspecific transport processes within the nurse cell/oocyte syncytium of *Drosophila*. While the bulk transfer of nurse cell cytoplasm during late vitellogenesis (stages 10B–12) depends on the functioning of actin filaments [7–9], it has been found that microtubules are involved in the distribution and localization of various macromolecules within the oocyte, especially during previtellogenic stages 1–6 ([10]; for reviews see [11, 12]). Intercellular transport driven by an electrical potential gradient [13] does not however seem to play an important role during *Drosophila* oogenesis [14–16].

Recently, we have demonstrated by video-enhanced contrast (VEC) timelapse microscopy that, during previtellogenic to midvitellogenic stages 7–10A, a possibly selective and strictly unidirectional transport of cytoplasmic particles occurs from the nurse cells through the ring canals into the oocyte [17] (fig. 1). During these stages, microtubules extending through the ring canals have not been detected [18]. The observed particles or organelles, which might contain specific mRNAs and proteins needed for the first steps of embryonic development, move by means of a microfilament-dependent process, and we have proposed that it might be driven by a myosin-like motor molecule [17]. In several systems, unconventional myosins have been found to be involved in the translocation of cytoplasmic particles or organelles (for reviews, see [19–21]).

In order to learn more details about the mechanisms of cytoplasmic transport in *Drosophila* follicles, video-

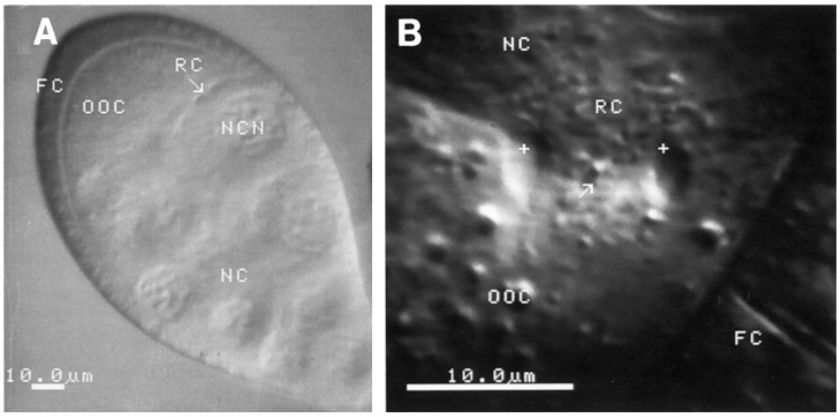

Figure 1. Video prints (Nomarski-DIC optics) of a stage-8 *Drosophila* follicle consisting of an oocyte (OOC) and 15 nurse cells (NC) surrounded by a layer of somatic follicle cells (FC). (*A*) In this overview, a ring canal (RC, shown in optical cross section) that connects the oocyte with a nurse cell is marked; NCN, one of the polyploid nurse cell nuclei. (*B*) Ring canal (+ +) at higher magnification (still frame of a timelapse recording using VEC microscopy). Cytoplasmic particles or organelles (the arrow points to a large one) are passing unidirectionally from the nurse cell through the ring canal into the oocyte (for more details, see [17]).

intensified fluorescence (VIF) timelapse microscopy in combination with a microinjected rhodamine-labeled anti-*Drosophila*-95F-myosin monoclonal antibody [22] was used in the present study. This method revealed, within the nurse cell cytoplasm, moving fluorescent particles that were associated with 95F myosin, a class VI unconventional myosin [23, 24]. In addition, 95F myosin was detected at the rim of the ring canals. Inhibition experiments support the hypothesis that these ring canal-associated myosin VI molecules participate in the possibly selective transport of cytoplasmic particles or organelles into the oocyte. Further experimental evidence confirms our earlier assumption [17] that some of these organelles are mitochondria. Thus, the present study indicates that myosin VI is involved in intra- and intercellular transport processes during stages 8–10A of *Drosophila* oogenesis.

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. Vitellogenic ovarian follicles of stages 8–10A (for stages, see [1]) were carefully dissected out of the epithelial sheath of the ovariole with tungsten needles in R-14 medium [25, 26]. Follicles lacking any signs of injury were washed in R-14 and immediately transferred to the microinjection chamber.

Microinjection procedure. The microinjection chamber (an inverted type of the chamber described in [27]) was constructed from microscopic glass slides cut to the appropriate size and glued together. A removable cover glass forming the bottom of the chamber was held in place with vaseline. An injection pipette and a suction pipette passed from opposite sides through thin films of paraffin oil covering the flanks of the medium-filled chamber to prevent evaporation. Microinjections were carried out using two Zeiss micromanipulators, a piezo translator (Märzhäuser, Wetzlar, Germany) and an inverted microscope (Zeiss Axiovert 135) equipped with epifluorescence and Nomarski Differential-Interference-Contrast (DIC) optics. For pressure injections, the in-

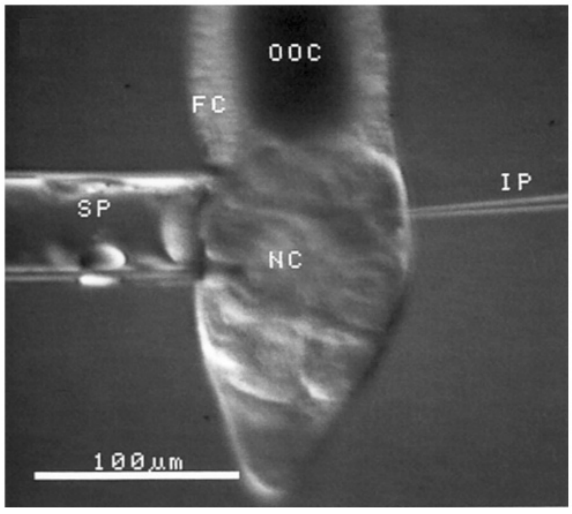

Figure 2. Low magnification overview (oblique illumination) of the microinjection procedure. A stage-10A follicle is held in place with a suction pipette (SP) while a nurse cell (NC) is impaled with an injection pipette (IP), FC = follicle cells, OOC = oocyte.

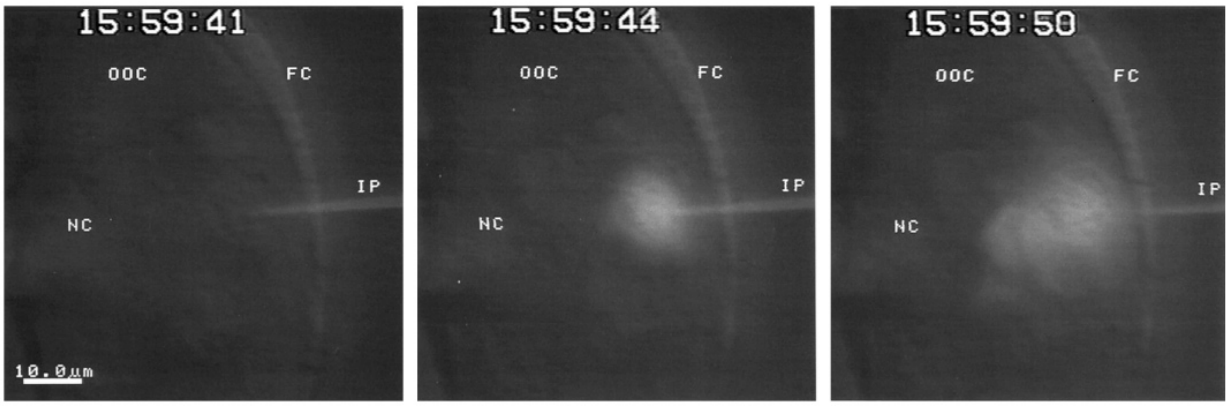

Figure 3. Sequence of three video frames showing the time course (hour:minute:second at top) of the injection of Rd-mAB into a nurse cell at stage 8 (fluorescence microscopy). Within seconds, the Rd-mAB spreads throughout the cell, and the fluorescence is still diffuse. For abbreviations, see figure 2.

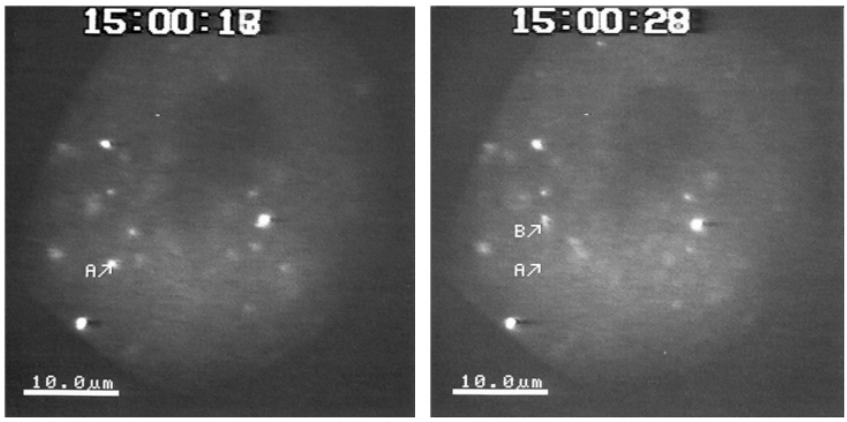

Figure 4. Sequence of two video frames showing the time course (hour:minute:second at top) of the movement of a fluorescent particle in the nurse cell cortex about 15 minutes following the injection of Rd-mAB (stage 10A; VIF microscopy). According to the timelapse recording, the particle moved linearly within 11 seconds from position A to position B.

jection pipette (tip diameter 1–2 µm) was coupled to a microinjector 5242 (Eppendorf, Hamburg, Germany). Micropipettes were pulled from 1 mm glass capillaries with a filament (Clark, Pangbourne, UK) on a horizontal pipette puller (Campden, London, UK). The injection pipette was filled from the back with 2–4 µl of the respective injection solution using a plastic syringe drawn out into a fine tip. The recipient follicle was held in place with a suction pipette (tip diameter 30–50 µm; see fig. 2) which was coupled to a screw-adjustable syringe (Hamilton, Reno, NV, USA) filled with paraffin oil. In order to avoid possible artefacts, the volume injected into each nurse cell (using a pressure of about 300 hPa) was in the order of only 1–2 pl, which is less than 5% of the volume of a nurse cell at stages 8–9 (calculated according to [28]). Iontophoretic injections were carried out using a current source (max. 16 V, 2.5 A; Stabizet, Siemens, Germany). The injection was stopped as soon as fluorescence was clearly detectable in the injected nurse cell with a SIT-video camera (fig. 3).

Injected solutions. A rhodamine-labeled anti-95F-myosin monoclonal antibody (Rd-mAB 3C7) and an affinity-purified anti-95F-myosin polyclonal antiserum (pAB), which were both kindly provided by Dr Kathryn G. Miller (St. Louis, MO, USA), were pressure injected. A rabbit nonimmune serum (NIS) served as a control [29]. The following dilutions in phosphate-buffered saline (PBS) were used: Rd-mAB 1:5 or 1:10, pAB and NIS 1:2 or 1:5. Thus, in the injected nurse cell, the dilution factor was in the range of 1:100–1:200 for Rd-mAB, and in the range of 1:40–1:100 for pAB and NIS, respectively. For the pAB (original concentration 12 mg/ml), the injected volume resulted in a final concentration of about 100–300 µg pAB per ml of nurse cell cytoplasm.

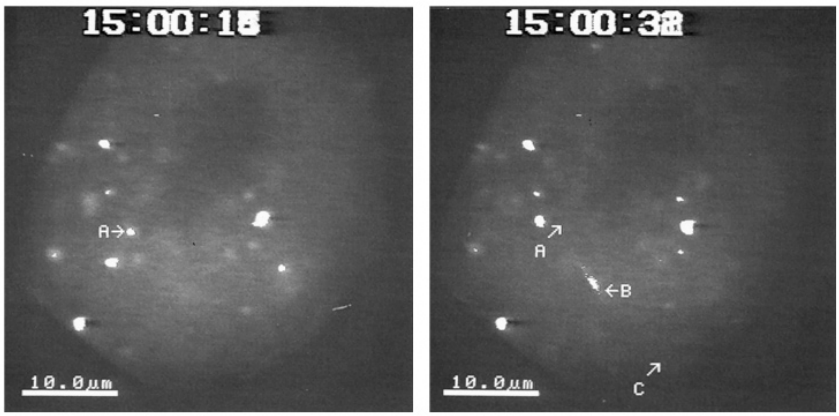

Figure 5. Similar sequence as in figure 4 (same nurse cell), showing the time course of the very far and fast movement of a fluorescent particle (about 15 minutes following Rd-mAB injection; VIF microscopy; focal plane near cell cortex). The particle moved linearly within 27 seconds from position A to position C. To show the 'comet's tail' of the particle after 17 seconds at position B with minimal background fluorescence, the image contrast was extremely enhanced ('slice' function of the image processor).

Table 1. Distances and velocities of linear movements of fluorescent and unlabeled particles in the nurse cells as well as from nurse cells through ring canals into the oocyte (stages 8–10A; n = number of analysed particles).

	Linear particle movements	
	in the nurse cells	through ring canals into the oocyte
a) Fluorescent particles (VIF microscopy)		
Distance in µm (±SD)	6.4 ± 3.3 (n = 21)	3.2 ± 0.9 (n = 8)
Velocity in nm/second (±SD)	296 ± 207 (n = 21)	75 ± 28 (n = 8)
b) Unlabeled particles (VEC microscopy)		
Distance in µm (±SD)	4.4 ± 1.5 (n = 16)	3.7 ± 0.8 (n = 16)
Velocity in nm/second (±SD)	104 ± 37 (n = 16)	76 ± 35 (n = 16)

In order to stain mitochondria, the positively charged fluorescent dyes 2-(4-dimethylaminostyryl)-1-ethylpyridinium iodide (DASPEI, Sigma; 5–10 µg/ml with 0.5–1% dimethylsulphoxide (DMSO) [30]) and rhodamine 123 (Rd123, Kodak; 5 µg/ml with 0.5% DMSO [31]) were injected either by pressure or iontophoretically.

Video-intensified fluorescence (VIF) microscopy. During as well as after the injections (i.e. 5–15 min following the onset of dissection), the follicles were viewed using VIF microscopy (for reviews see [32, 33]). The low light level video system consisted of a SIT-video camera C-2400 (Hamamatsu Photonics, Japan), a digital image processor Argus-10 (Hamamatsu), a timelapse S-VHS video tape recorder AG-6720 (Panasonic, Japan) and a monochrome TV monitor WV-5410 (Panasonic). The image processor was used for noise reduction ('frame averaging') and contrast enhancement ('stretching'). With this system, even weak levels of fluorescence were detectable, thereby permitting the amount of the injected fluorescent probe to be minimized, which reduces background fluorescence. Using a 50 W mercury arc lamp, the appropriate filter combinations for green or blue excitation light, a ×63/1.25 NA Plan-Neofluar objective and a ×16 eyepiece, clear images of the fluorescent particles (figs 4, 5) were obtained at a maximum magnification of ×4000 on the video monitor screen. Timelapse recordings (at two to three frames per second) of individual follicles were carried out with intermissions for up to two hours. In order to reduce photobleaching as well as specimen damage, each recording period lasted only a few minutes. Distance and velocity measurements of moving particles were performed on tape recordings using the Argus-10 image processor. Video prints were produced on a video copy processor P66E (Mitsubishi, Japan).

Video-enhanced contrast (VEC) microscopy. The injected follicles were viewed using Nomarski Differential-Interference-Contrast (DIC) optics, a 100 W tungsten halogen lamp, and the video system described above. With this setup, high contrast images with good resolution were obtained (fig. 1, [17]). The principles of VEC microscopy have been described in detail previously (for reviews see [32, 33]). I used a ×100/1.3 NA Plan-Neofluar objective and a ×16 eyepiece, resulting in a maximum magnification of ×6500 on the video

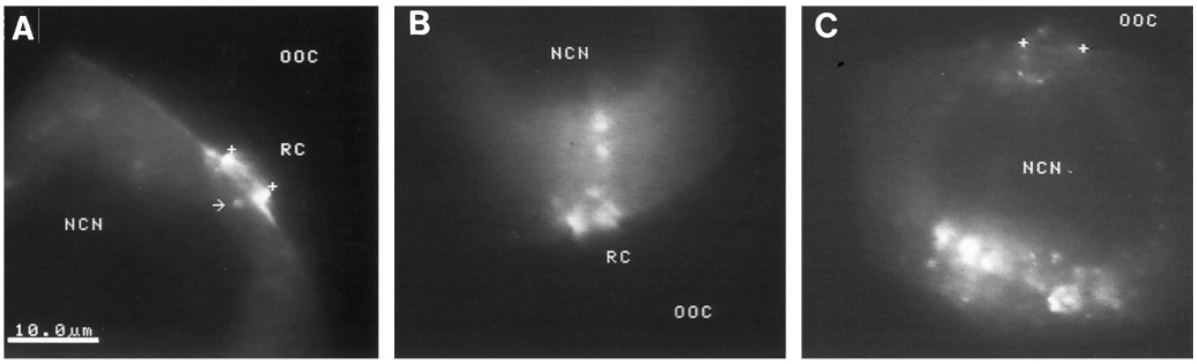

Figure 6. Examples of fluorescent particles (arrow in (*A*)) accumulating in nurse cells near ring canals shown in optical cross sections (10–20 minutes following Rd-mAB injections; VIF microscopy). In (*A*) and (*B*), very small amounts of Rd-mAB were injected close to the respective ring canal, whereas in (*C*) a larger amount was injected on the opposite side of the nurse cell nucleus. For abbreviations, see figure 1.

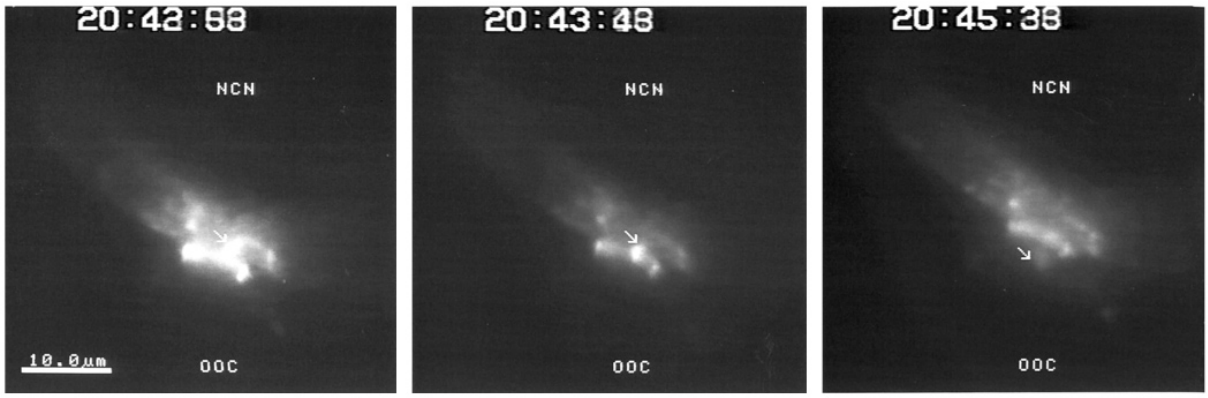

Figure 7. Sequence of three video frames showing the time course (hour:minute:second at top) of the ring canal passage of a fluorescent particle (arrow) from the nurse cell into the oocyte (about 15 minutes following Rd-mAB injection; VIF microscopy; optical cross section). In the last frame, the particle identified by continuous timelapse recording has left the focal plane. For abbreviations, see figure 1.

monitor screen. Light intensity, Wollaston prism setting and camera sensitivity were optimized, and analogue brightness and contrast settings of the camera were adjusted for optimal contrast and resolution. Digital image averaging was performed over 128 video frames to enhance the signal-to-noise ratio. Timelapse recordings (at two to three frames per second) started at about 5 minutes after injection and lasted with intermissions for up to two hours.

Inhibitor studies. Directly after dissection, the follicles were incubated with one of the following inhibitors dissolved in R-14 medium [8, 17, 26, 34]: (a) the inhibitor of oxidative phosphorylation 2,4-dinitrophenol (DNP, Serva; 1 mM), (b) the microtubule inhibitor colchicine (Sigma; 20 µg/ml with 0.1% DMSO), and (c) the microfilament inhibitor cytochalasin B (Sigma; 10 µg/ml with 0.2% ethanol). The application of cytochalasin D [8] gave comparable results (unpublished observations). The respective inhibitor was present during injections and during subsequent video recordings.

Results

Movements of cytoplasmic particles in the nurse cells. A rhodamine-labeled monoclonal antibody directed against *Drosophila* 95F myosin (Rd-mAB) was microinjected into individual nurse cells during vitellogenic stages 8–10A of oogenesis (fig. 3). Within 10–20 minutes following the injection, a subset of the cytoplasmic particles seen with VEC microscopy (fig. 1, [17]) became fluorescently labeled and were now detectable with VIF microscopy (fig. 4). In accordance with Mermall et al. [22], who used the same antibody to analyse movements of cytoplasmic particles in *Drosophila* embryos in vivo, I propose that the fluorescent particles seen in the nurse cells are directly associated with 95F myosin (myosin

Table 2. Incubation of follicles with various inhibitors. Inhibition of linear movements of unlabeled particles in the nurse cells as well as from nurse cells through ring canals into the oocyte (within 10–20 minutes following incubation, stages 8–10A, VEC microscopy).

Inhibitor	Number of analysed follicles	% Of follicles in which linear particle movements were	
		unaffected	inhibited
Controls (no inhibitor)	40	100	0
2,4-Dinitrophenol	19	0	100
Cytochalasin B	22	0	100
Colchicine	21	100	0

Table 3. Injections of various sera into nurse cells. Inhibition of linear movements of fluorescent and unlabeled particles in the nurse cells as well as from nurse cells through ring canals into the oocyte (within 10–25 minutes following injection; stages 8–10A).

Injected sera	Number of injected follicles	% Of follicles in which linear particle movements were		
		unaffected	reduced	inhibited
a) Fluorescent particles (VIF microscopy)				
Rd-mAB	23	100	0	0
Rd-mAB + NIS	6	100	0	0
Rd-mAB + pAB	12	0	8	92
b) Unlabeled particles (VEC microscopy)				
Rd-mAB	15	100	0	0
NIS	11	100	0	0
pAB	41	0	32	68

VI). In earlier studies, we microinjected various fluorescent probes into *Drosophila* nurse cells, including different antibodies ([14, 16] and unpublished observations), but we have never observed such particles. Obviously, the particulate staining described here is specific for the microinjected Rd-mAB.

Fluorescent (VIF) as well as unlabeled (VEC) particles performed oscillations (random movements) or were seen in linear motion over distances between 3 and 17 μm in the nurse cell cytoplasm. Such movements were still observable in follicles that were kept for up to two hours in R-14 medium. Several earlier studies have proved the suitability of R-14 for various types of in vitro experiments with *Drosophila* follicles (e.g. [14, 16, 17, 26–29]). In addition, *Drosophila* follicles younger than stage 10, incubated for about one hour in R-14, developed normally when transplanted into host flies ([7] and our own unpublished observations). Therefore, it is rather unlikely that the observed particle movements are in vitro artefacts.

The fluorescent particles seen with VIF microscopy were also identifiable with VEC microscopy. Based on the frequency of linear movements as observed with both types of microscopy, it is very likely that all VEC particles performing linear movements correspond to VIF particles and were myosin VI associated particles. Such movements, which are presumed to represent active transport along microfilaments, occurred usually in the nurse cell cortex and showed velocities between 65 and 750 nm/second. Examples of linear movements of VIF particles are shown in figures 4 and 5. Table 1 lists the mean values of distances and velocities of linear particle movements measured using either VIF or VEC microscopy. When compared with the mean values for VIF particles, the respective values for VEC particles seemed to be smaller. Due to the small focal depth and the wealth of particles seen with VEC microscopy [17], they could be reliably traced only over relatively short distances. It was not possible to determine exactly the values for some particles that were moving very far as well as fast, especially during stage 10A.

Movements of cytoplasmic particles from the nurse cells through ring canals into the oocyte. In general, the fluorescent particles accumulated in front of ring canals leading into the oocyte (fig. 6) while some of them were seen passing through the canals (fig. 7). These probably selective particle movements through the ring canals were unidirectional and more or less linear over distances between 2 and 5 μm showing velocities between 35 and 150 nm/second.

For both microscopical methods, the calculated mean values of distances and velocities of particle movements through ring canals did not differ significantly (table 1). However, when compared with the velocities of linear movements within the nurse cell cytoplasm, the movements through ring canals were considerably slower. A comparison of the frequencies of ring canal passages, as observed with both microscopical methods, indicated that only some of the particles that moved through a respective ring canal were labeled by the Rd-mAB and, thus, seemed to be directly associated with myosin VI. This result suggests that the

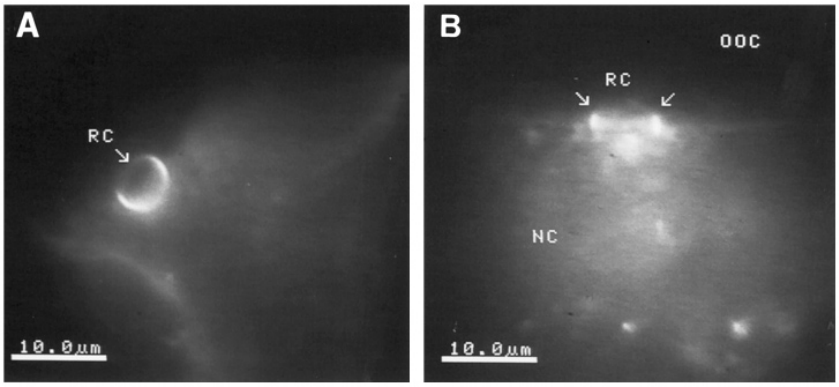

Figure 8. Video prints showing different aspects of fluorescently labeled ring canals (arrows) 10–20 minutes after injections of Rd-mAB (VIF microscopy). In (*A*), the ring canal is shown in oblique orientation with extremely enhanced contrast and reduced brightness in order to demonstrate clearly the fluorescence of the rim. In (*B*), the ring canal is shown in optical cross section, and brightness and contrast are adjusted so that fluorescent particles are visible. This labeling indicates that myosin VI is located at the rim of the ring canals. For abbreviations, see figure 1.

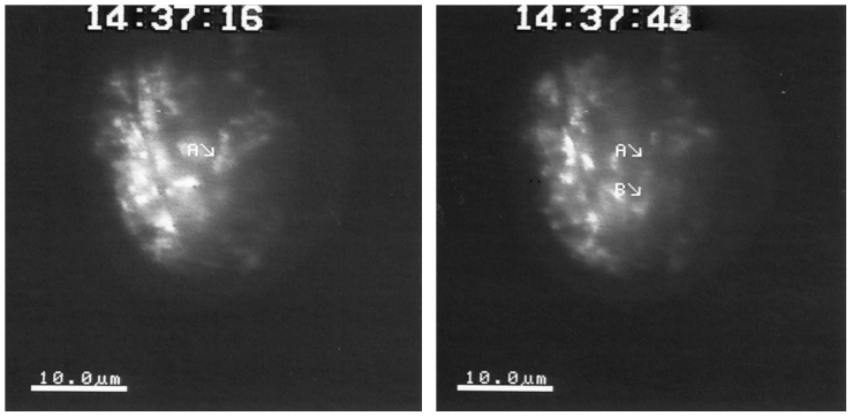

Figure 9. Sequence of two video frames showing the time course (hour:minute:second at top) of the movement of a fluorescent mitochondrion in the nurse cell cytoplasm about 10 minutes following the injection of DASPEI (VIF microscopy; focal plane near cell cortex). According to the timelapse recording, the mitochondrion moved linearly within 28 seconds from position A to position B. Countless mitochondria are labeled which results in conspicuous background fluorescence.

mechanism of transport through the ring canals is different from the mechanism of transport within the nurse cell cytoplasm.

It has to be considered that, due to the relatively small amounts of injected Rd-mAB, not every myosin VI associated particle moving through a ring canal might have become sufficiently labeled to be detectable. However, the sensitivity of VIF microscopy is very high and, in many experiments, the Rd-mAB was injected close to a ring canal leading into the oocyte. Thus, it is very likely that the amount of antibody was sufficient to label most if not all myosin VI associated particles in the vicinity of this ring canal. Consequently, the unlabeled particles passing through the canal should not be directly associated with myosin VI.

Inhibition of linear particle movements. All linear particle movements observable with VIF and VEC microscopy during stages 8–10A within the nurse cells and through the ring canals into the oocyte were inhibitable with 2,4-dinitrophenol and with cytochalasin B (or cytochalasin D) but not with colchicine (table 2). These results indicate that the movements depend on metabolic energy as well as on intact microfilaments.

The injected concentrations of Rd-mAB did not inhibit linear particle movements during VIF or VEC microscopy (table 3). However, when an affinity-purified anti-95F-myosin polyclonal antiserum (pAB, [22]) was injected about 15 minutes following the Rd-mAB injection, the linear movement of fluorescent particles in the nurse cells was reduced considerably or ceased totally within 10–25 minutes, and particle transport through the ring canals was no longer observed (table 3a). As a

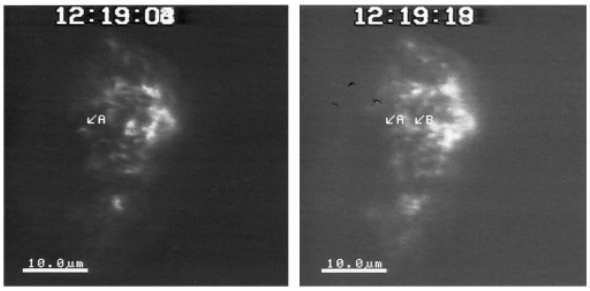

Figure 10. Similar sequence as in figure 9 showing the time course of the movement of a fluorescent mitochondrion in the nurse cell cytoplasm about 10 minutes following the injection of Rd123 (VIF microscopy; focal plane near cell cortex). According to the timelapse recording, the mitochondrion moved linearly within 15 seconds from position A to position B. With Rd123, photobleaching was faster than with DASPEI.

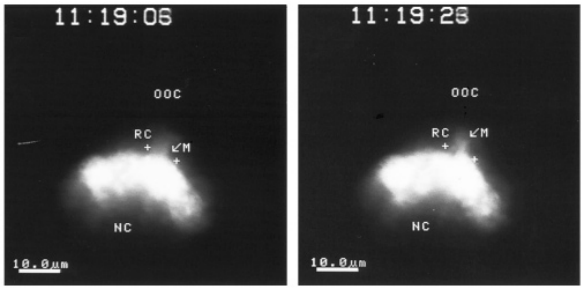

Figure 11. Sequence of two video frames showing the ring canal (++) passage of several fluorescent mitochondria (M; 'comet's tail' due to frame averaging) from the nurse cell into the oocyte (about 15 minutes following DASPEI injection; VIF microscopy; optical cross section; hour:minute:second at top). Due to the bright background fluorescence of the large number of mitochondria present in the nurse cell, single mitochondria are difficult to visualize in such frames, especially when focused on the ring canal (focal plane deep in the cell). However, the movements were reliably identified by continuous timelapse recording. For further abbreviations, see figure 1.

control, a rabbit nonimmune serum (NIS) was injected and had no inhibitory effects.

In addition, in most of the pAB-injected follicles, VEC particles were no longer seen passing through the ring canals into the oocyte, and the number of linear movements in the injected nurse cells was reduced considerably, tending towards zero within 10–25 minutes (table 3b). Due to the wide range of observed velocities (table 1), it was not possible to measure exactly the reduction of velocity occurring with time. The pAB was injected in different concentrations, but only the 1:2 dilution consistently inhibited the particle movements. It is assumed that, contrary to the Rd-mAB, the inhibitory effects of the pAB are due to its binding to several myosin VI epitopes that are necessary for particle transport [22].

In the described experiments, only linear particle movements were influenced, while other movements (oscillations or random movements) were not affected. Also, particle streaming in the oocyte (which is microtubule based; see [17]) was not influenced when a nurse cell was injected. Therefore, I suspect that most (if not all) linear particle movements described – within the nurse cell cytoplasm as well as through the ring canals – depended on interactions between microfilaments and myosin VI.

Since the Rd-mAB labeled only some of the particles that were transported via an actomyosin-dependent process through the ring canals into the oocyte, many of these particles seemed not to be directly associated with myosin VI motor molecules. However, in addition to particles, the Rd-mAB always labeled the rim of the ring canals of the injected nurse cells (best seen in figs 7 and 8) which indicates that myosin VI is a component of the ring canal structure itself.

Movements of mitochondria in the nurse cells and through ring canals into the oocyte. Electronmicroscopy suggests that mitochondria are among the particles and organelles seen to be transported through the ring canals into the oocyte [17]. Thus, individual nurse cells (stages 8–10A) were injected with the positively charged fluorescent dyes DASPEI (number of follicles: $n = 37$) and Rd123 ($n = 17$). These dyes are known to stain active mitochondria specifically [30, 31].

Within 10–20 minutes following injection, countless mitochondria became fluorescently labeled in the respective nurse cell. These mitochondria were seen moving either randomly or linearly over similar distances and with similar velocities as the myosin VI associated particles described above. Examples of linear movements of fluorescent mitochondria are shown in figures 9 and 10.

Compared with the movements of myosin VI associated fluorescent particles, the movements of mitochondria were more difficult to analyse, since the number of fluorescent mitochondria was very high, resulting in bright background fluorescence. Moreover, although DASPEI was more stable than Rd123, both dyes were subject to conspicuous photobleaching, and the practicable observation times were much shorter than for the rhodamine-labeled antimyosin mAB (only about 5–15 minutes). Given these constraints, single fluorescent mitochondria were rather difficult to visualize on their passage through a ring canal (focal plane deep in the cell). However, passages hardly detectable on still frames such as in figure 11 were reliably documented in many timelapse video tape recordings.

In accordance with the movements of myosin-VI associated particles, the linear movements of fluorescent mitochondria that occurred within nurse cells and also

through ring canals into the oocyte were inhibitable with 2,4-dinitrophenol (n = 13) and with cytochalasin B (n = 9) but not with colchicine (n = 10). Moreover, such movements were never observed following injections of the anti-95F-myosin pAB. Therefore, the transport of a certain population of mitochondria within the nurse-cell/oocyte syncytium should depend on interactions between microfilaments and myosin VI.

Taken together, the results described support the hypothesis that cytoplasmic particles or organelles are transported within the nurse cells along microfilaments by means of a myosin VI motor. Further myosin VI molecules, associated with microfilaments located at the rim of the ring canals, seem to be involved in the unidirectional and possibly selective transport of such particles from the nurse cells through the ring canals into the oocyte. Presumably, these myosin VI molecules associate only transiently with the particles during their ring canal passage.

Discussion

Our knowledge about the *Drosophila* myosin superfamily has grown considerably (reviewed in ref. 35), and a few members of this family have already been detected in the ovary. Nonmuscle myosin II, a conventional myosin involved in several morphogenetic events during embryogenesis [36, 37], has recently been shown to play a role in the bulk transfer of nurse cell cytoplasm into the oocyte during stages 10B–12 [38] as well as during several types of cell shape changes during oogenesis [39]. Moreover, 95F myosin, an unconventional myosin VI involved in the organization of the syncytial blastoderm [40], has been found in the ovary using immunoblots [23]. Unambiguous in vivo evidence suggests that this myosin participates in particle transport during embryogenesis [22], and it seemed promising to test whether myosin VI is also involved in intra- and intercellular particle transport as observed during stages 7–10A of oogenesis [17].

Using microinjections of a rhodamine-labeled monoclonal antibody (Rd-mAB, [22]), the present study revealed linear movements of particles that were obviously associated with myosin VI, within the nurse cell cytoplasm. These particles might merely represent maternal stores of inactive myosin VI for later use during embryogenesis. However, inhibitor studies as well as double injection experiments using an affinity-purified anti-95F-myosin polyclonal antiserum (pAB) which inhibits myosin VI function [22] indicate that the fluorescent particles or organelles were moved by means of an ATP-, microfilament- and myosin VI-dependent mechanism.

Most of the linear movements of myosin VI associated particles were observed in the nurse cell cortex which is known to contain a high density of F-actin [8, 41]. In addition, many myosin VI associated particles were seen passing unidirectionally and presumably selectively through the ring canals into the oocyte. The ring canal transport was also found to depend on ATP, microfilaments and myosin VI. Within the canals, microfilament bundles have not been observed so far [42, 43]. Thus, we suspected that a polarized microfilament-containing scaffold which might not be detectable using standard fluorescence or electronmicroscopic techniques extends through the ring canals [17]. Recently, using laser scanning confocal microscopy, a basket of actin filaments has been detected that is associated with the side of the ring canal facing the nurse cell cytoplasm [43]. Although the polarity of these filaments is not yet known, they could be involved in the observed ring canal transport.

Manseau et al. [44] have presented evidence that cytochalasin D induces microtubule bundling and abnormal microtubule-based cytoplasmic streaming within the oocyte. However, neither Gutzeit [8, 34] nor Manseau et al. [44], using similar inhibitor concentrations as in the present study, have reported an influence of cytochalasins on the pattern of microtubules in the nurse cells. Several further studies (reviewed in ref. 44) indicate that the effect on the microtubule array in the *Drosophila* oocyte by microfilament inhibitors is rather exceptional. Thus, in the nurse cells, cytochalasins most likely affect only those particle movements that depend on microfilaments.

Several proteins have been reported to be components of the ring canal structure: a phosphotyrosine protein, F-actin, hu-li tai shao protein, kelch protein and nonmuscle myosin II [39, 45, 46]. The present study indicates that myosin VI is an additional component of the ring canal structure [47]. Since many of the particles that moved by an F-actin- and myosin VI-dependent mechanism through the ring canals were not labeled by the Rd-mAB, they seemed not to be supplied with their own myosin VI motors. Thus, I propose that the ring canal associated myosin VI molecules mediate the transport of cytoplasmic particles from the nurse cells into the oocyte during stages 8–10A of oogenesis by associating transiently with these particles during their ring canal passage.

Although the experiments described strongly support the hypothesis that myosin VI plays a direct role in linear particle movements, other interpretations of the data cannot be totally ruled out. For example, it has to be considered that the injected affinity-purified pAB might have bound in rather high amounts to the ring canal associated myosin VI molecules, so that other possible transport mechanisms might have become hampered. Also, possible crosslinking by the pAB of inactive myosin VI molecules on the particles might have prevented the functioning of other motor molecules due to steric hindrance. These possibilities seem however to be less likely.

In *Drosophila* oogenesis, most of the macromolecules and organelles necessary for subsequent embryogenesis reach the oocyte during late vitellogenic nurse cell regression (stages 10B–12). However, many specific components, in particular morphogenetic determinants produced in the nurse cells, have been shown to accumulate in the oocyte before this phase (for a review, see ref. 4). The present study indicates that mitochondria are transported into the oocyte by an actomyosin-dependent mechanism. It has long been known that (selected?) mitochondria become enriched in the developing germ plasm [48], and recent studies have shown that mitochondrial large ribosomal RNA is required for pole cell development [49, 50].

In other systems, movements of mitochondria along both microtubules and microfilaments have been demonstrated [51, 52], and there is growing evidence for a more general inter-relationship between microtubule and microfilament-dependent organelle transport [53, 54]. It is assumed that microtubules provide the tracks for movements over longer distances while microfilaments are responsible for movements within local regions of the cytoplasm, especially within the cell cortex [55].

Recently, it has been found that microinjected fluorescently labeled mRNA forms granules that become distributed within cultured cells in a microtubule and, presumably, also microfilament-dependent manner ([56]; for reviews, see refs. 57, 58). Specifically in *Drosophila* oogenesis, there is good evidence that not only microtubules, but also microfilaments are involved in mRNA transport and localization, since tropomyosin (which is presumed to stabilize microfilaments and to regulate the movement of myosin motors in muscle cells) is required for the localization of *oskar* mRNA [59]. The results of the present study suggest that specific mRNAs (and presumably also specific proteins) become associated with larger particles or organelles that are transported within the nurse cells and through the ring canals into the oocyte by means of a microfilament- and myosin VI-dependent mechanism.

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