Int. J. Dev. Biol. 41: 499-507 (1997)

Original Article

Cytoplasmic transport in *Drosophila* ovarian follicles: the migration of microinjected fluorescent probes through intercellular bridges depends neither on electrical charge nor on external osmolarity

JOHANNES BOHRMANN* and STEFAN SCHILL

Institut für Biologie I (Zoologie), Universität Freiburg, Germany

ABSTRACT Using video-intensified fluorescence microscopy and a pseudocolor display of fluorescence intensity, we analyzed the distribution of microinjected molecules within the nurse-cell/oocyte syncytium of *Drosophila* ovarian follicles. We varied the composition and the osmolarity of the culture solution as well as the electrical charge and the molecular mass of the microinjected fluorescent probe. As culture solutions, we used four simple salines (IMADS) and a complex tissue-culture medium (R-14) that matched the osmolarity of adult hemolymph. Small amounts of two anionic dyes (Lucifer Yellow CH and Lucifer Yellow dextran) as well as of two cationic dyes (rhodamine 6G and tetramethylrhodamine dextran-lysine) were iontophoretically microinjected either into a nurse cell or into the oocyte of stage-10 follicles. In the tissue-culture medium, within a few seconds following microinjection, all tested dyes passed through the intercellular bridges in both the anterior direction (to the nurse cells) and the posterior direction (to the oocyte), independent of their electrical charge or molecular mass. In all simple salines, irrespective of their osmolarity, Lucifer Yellow CH was found to preferentially migrate in the posterior direction and to accumulate in the oocyte due to progressive binding to yolk spheres. Thus, with this sensitive method, no correlation was detectable between the external osmolarity, the electrical charge and the preferential direction of migration of a microinjected probe. Our results indicate that the electrical gradient described by other authors does not exert significant influence on the migration of charged molecules through intercellular bridges *in situ*.

KEY WORDS: *electrical gradient, in vitro culture, microinjection, ring canal, video microscopy*

Introduction

In the meroistic ovarian follicle of *Drosophila*, a syncytium of germ-line cells consisting of one oocyte and a cluster of 15 nurse cells is surrounded by a layer of somatic follicle cells. Cytoplasmic continuity between nurse cells and oocyte is maintained by way of intercellular bridges or ring canals (for reviews, see King, 1970; Mahowald and Kambysellis, 1980). Via these structures, the polyploid and synthetically highly active nurse cells supply the growing oocyte with a wealth of organelles and macromolecules. In particular, various cytoplasmic determinants necessary for embryonic pattern formation are known to enter the oocyte in this way during different phases of oogenesis (reviewed in Spradling, 1993; St Johnston, 1995).

Despite an increasing number of investigations in this field, the mechanisms that are involved in intra- and intercellular transport within the nurse-cell/oocyte syncytium of *Drosophila* are not yet fully understood (for reviews, see Gutzeit, 1986a; Mahajan-Miklos and Cooley, 1994). While several studies have shown that cytoskeletal elements play a role in some specific as well as some unspecific transport processes (reviewed in Cooley and Theurkauf, 1994; Pokrywka, 1995), it is still controversial whether an electrical potential gradient, as first proposed for *Hyalophora* follicles by Woodruff and Telfer (1980), is involved in cytoplasmic transport during *Drosophila* oogenesis.

Some investigators (Woodruff *et al.*, 1988; Woodruff, 1989) have found an electrical gradient of several millivolts across the intercellular bridges connecting the nurse cells (cathode) with the oocyte (anode). In contrast, others did not observe any significant potential difference between both cell types (Bohrmann *et al.*,

Abbreviations used in this paper: IMADS, ionically matched adult *Drosophila* saline; LY-CH, Lucifer Yellow CH; LY-dex, Lucifer Yellow dextran; R-14, Robb's tissue-culture medium; Rh-dex-lys, tetramethylrhodamine dextran-lysine; Rh-6G, rhodamine 6G; SIT, silicon-intensified target; VIF, video-intensified fluorescence.

***Address for reprints:** Institut für Biologie I (Zoologie), Universität Freiburg, Hauptstr. 1, D-79104 Freiburg, Germany. FAX: 761.2032596.
e-mail: bohrmjoh@ruf.uni-freiburg.de

0214-6282/97/$05.00

Printed in Spain

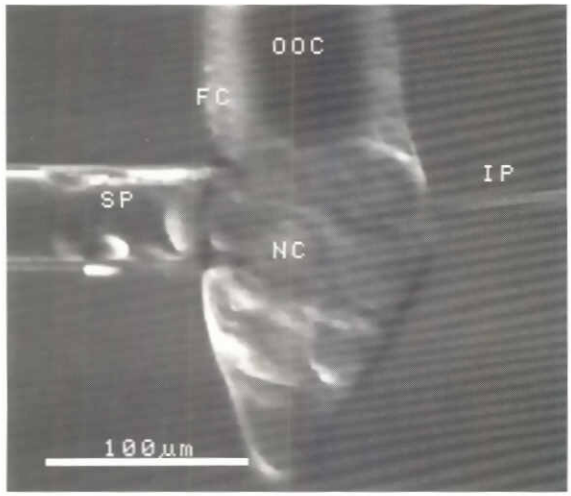

Fig. 1. Video print (oblique illumination) showing the microinjection procedure. *A Drosophila stage-10A follicle consisting of an oocyte (OOC) and 15 nurse cells (NC) surrounded by a layer of somatic follicle cells (FC) is hold in place with the suction pipette (SP) while a nurse cell is impaled with the injection pipette (IP).*

1986a; Sun and Wyman, 1993). The electrical gradient has been supposed to exert influence on the distribution of charged molecules by intercellular electrophoresis and, recently, it has been reported that changes in external osmolarity may affect the polarity of the gradient as well as the distribution of the anionic dye Lucifer Yellow CH (Singleton and Woodruff, 1994).

In order to test for this hypothesis in greater detail, we analyzed the rate of migration of various types of microinjected probes using video-intensified fluorescence (VIF) microscopy (for reviews, see Inoué, 1986; Shotton, 1988). By means of a highly sensitive SIT-camera and a digital image processor with pseudocolor and measuring functions, very low levels and minimal differences of fluorescence intensity were detectable. We varied the composition and the osmolarity of the culture solution as well as the electrical charge and the molecular mass of the fluorescent probe. In contrast to some studies (Woodruff *et al.*, 1988; Woodruff, 1989; Singleton and Woodruff, 1994), but in accordance with other studies (Bohrmann and Gutzeit, 1987; Verachtert and De Loof, 1989), we did not detect any significant correlation between the electrical charge and the preferential direction of migration of a microinjected probe. Thus, the distribution of charged molecules within the nurse-cell/oocyte syncytium of *Drosophila in situ* might not be expected to be significantly influenced by an electrical potential gradient.

Results

Migration of Lucifer Yellow CH in follicles incubated in solutions of different osmolarity and composition

As culture solutions, we used four simple salines named IMADS ("Ionically matched adult *Drosophila* saline"; Singleton and Woodruff, 1994) and the complex *Drosophila* tissue-culture medium R-14 (Robb, 1969). In order to test whether the osmolarity of the culture solution may significantly affect the migration of charged molecules, the osmolarity of IMADS was varied between 240 and 410 mOsm (IMADS 240, IMADS 270, IMADS 300 and IMADS 410). Although we have measured a 15 mOsm higher value than Singleton and Woodruff (1994) for IMADS of the same composition (see Materials and Methods), the osmolarity range tested by us matched the range tested by these investigators (255, 275, 300, 400 mOsm). In order to investigate whether the composition of the culture solution may affect the migration of charged molecules, we compared the data obtained using different IMADS with those obtained using R-14. The osmolarity of our R-14 (260 mOsm) was close to that reported for adult *Drosophila* hemolymph (251 mOsm; Singleton and Woodruff, 1994), and R-14 has been previously shown to be superior than simple salines for various types of *in vitro* experiments with *Drosophila* follicles (Bohrmann, 1991).

For microinjections, we used vitellogenic follicles of stage 10A (King, 1970). At this stage, dye migration is not yet affected by cytoplasmic bulk flow from the nurse cells into the oocyte which starts at stage 10B. After carefully puncturing the respective follicle by means of a piezo translator, we iontophoretically microinjected very small amounts of the negatively charged fluorescent dye Lucifer Yellow CH (LY-CH) into a nurse cell or into the oocyte (Fig. 1). In two of the salines, the follicles changed their morphology after only a few minutes of incubation: they were found to either swell (in IMADS 240) or shrink (in IMADS 410) considerably. Due to their soft condition, shrunken follicles were more difficult to microinject than normal follicles.

With VIF-microscopy, extremely low levels of fluorescence intensity are detectable, thereby permitting the amount of the microinjected dye to be minimized. The extent of migration of the dye from the microinjected cell into a neighboring cell was evaluated by analyzing video recordings of the follicles using a pseudocolor display of fluorescence intensity.

In all five culture solutions, within a few seconds following microinjection, LY-CH passed through the intercellular bridges in both the anterior direction (to the nurse cells, e.g. Fig. 2) and the posterior direction (to the oocyte, e.g. Fig. 3). In order to obtain quantitative data, we used two methods for the evaluation of dye migration: we determined the time that a pseudocolor front needed to cover a distance of 30 µm (Fig. 4) as well as the distance that the front moved within 20 sec. Both methods are very sensitive, and they gave comparable results.

The results indicate that the rate of dye migration depends to some extent on the osmolarity of the culture solution; however, a consistent influence of osmotic strength was not revealed. In all IMADS solutions, irrespective of their osmolarity, LY-CH was found to preferentially migrate in the posterior direction (Fig. 5; $P<0.05$) and to accumulate within about 10 min in the oocyte (e.g. Fig. 3). In contrast to Singleton and Woodruff (1994), we observed no reversal of the preferential direction of dye migration when solutions with high instead of low osmolarity were used (Fig. 5).

When the data obtained using IMADS are compared with those obtained using R-14 (Fig. 5), it becomes evident that the composition of the culture solution exerts significant influence on the movement of the dye through the cytoplasmic bridges. In R-14, the rate of dye migration in both directions did not differ significantly ($P>0.05$), and preferential accumulation of LY-CH in the oocyte was only found after about 30 min of incubation.

Previous studies have shown that, in various cell types, microinjected LY-CH became increasingly bound to cytoplasmic organelles (see Discussion). While Woodruff (1989) and Singleton

and Woodruff (1994) supposed this phenomenon to occur only in follicles maintained *in vitro* for more than 30 min, we observed granular staining of LY-CH in the oocyte as rapidly as 10 to 15 min following microinjection.

In order to identify the LY-CH-binding organelles, we incubated cryosections of follicles in IMADS containing LY-CH. These experiments revealed that the dye strongly binds to the yolk spheres in the oocyte whereas cytoplasmic organelles in the nurse cells and in the follicle cells became only minimally stained (Fig. 6). The staining of yolk spheres was recognized by comparing the autofluorescence of the yolk spheres (visible under ultraviolet illumination) with the LY-CH fluorescence (visible under blue illumination and still detectable after several hours of washing). Figure 7 shows a time course of the progressive binding of LY-CH to yolk spheres following increasing times of incubation in dye-containing saline. Since fluorescent yolk spheres were visible in all focal planes and not only near the surfaces of the cryosections, LY-CH most likely binds to the intact yolk spheres rather than to the yolk proteins in the interior of these membrane-bound organelles. Gel-electrophoresis and Western blotting of ovary proteins confirmed this notion since binding of LY-CH to yolk proteins was found to be minimal (our unpublished observations). It is possible, however, that LY-CH is able to enter the yolk spheres *in vivo* via an organic anion transporter (cf. Steinberg *et al.*, 1987; O'Driscoll *et al.*, 1991).

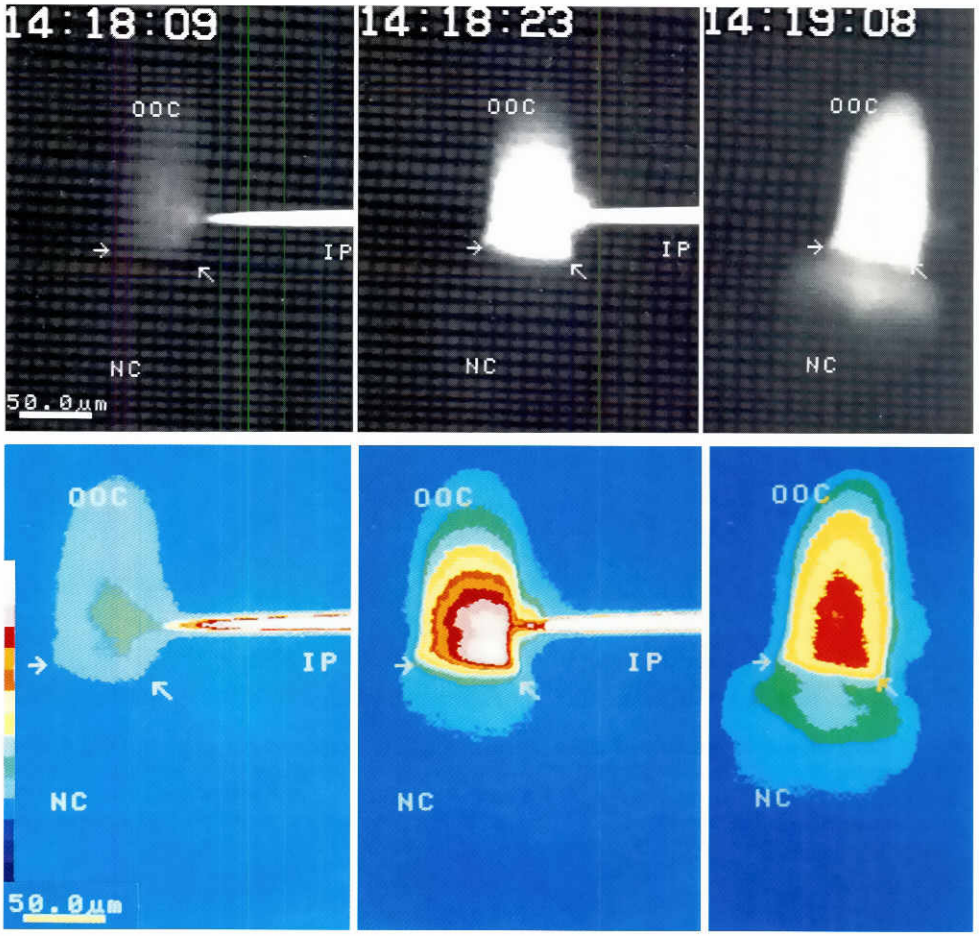

Fig. 2. Sequence of three video frames displayed in b/w (top row) and pseudocolors (bottom row) of a time-lapse recording using VIF-microscopy. *The sequence shows the time course (hour:minute:second at top) of a microinjection of LY-CH into an oocyte and the migration of the dye into the neighboring nurse cells (culture solution: IMADS 270). The arrows mark the nurse-cell/oocyte border. The pseudocolor scale indicates the 15 different levels of fluorescence intensity used for analysis (maximum: white, minimum: dark blue). For abbreviations see Figure 1.*

Clearly, the demonstrated binding of the dye to the yolk spheres is an important component of the observed rapid accumulation of LY-CH in the oocyte of microinjected follicles incubated in IMADS. Indeed, it may prove to be the most important factor responsible for this phenomenon. Another tested anionic Lucifer dye, LY-VS, was found to bind even more rapidly than LY-CH to these (and presumably other) cytoplasmic organelles (our unpublished observations; see also Bossinger and Schierenberg, 1992a).

In order to test whether the irradiation applied during time-lapse recording might exert some negative influence on the viability of the cells, we microinjected stage-10B follicles as a control. Such follicles have been previously shown to develop in R-14 up to stage 14 more perfectly than stage-10A follicles (see Discussion). When irradiated for even longer than 30 min, these follicles were found to develop *in vitro* with normal morphology up to the end of oogenesis (stage 14). On the other hand, irrespective of osmotic strength and irradiation, stage-10B follicles were not capable of developing any further in IMADS (data not shown). Thus, there is strong evidence that R-14 reflects more perfectly than IMADS the conditions *in situ*, and we only used R-14 during the following experiments.

Migration of fluorescent probes with different electrical charge and molecular mass

We further investigated whether microinjected molecules possessing different electrical charges and molecular masses would behave differently in the nurse-cell/oocyte syncytium of *Drosophila*. We microinjected, besides LY-CH (anionic, 457 M_r), the fluorescent probes rhodamine 6G (Rh-6G, cationic, 479 M_r), Lucifer Yellow dextran (LY-dex, anionic, 10,000 M_r), and tetramethylrhodamine dextran-lysine (Rh-dex-lys, cationic, 10,000 M_r) into a nurse cell or into the oocyte of stage-10A follicles incubated in R-14.

Within a few seconds following the microinjections, all tested dyes passed through the intercellular bridges in both the anterior direction (to the nurse cells) and the posterior direction (to the oocyte). Using either anionic or cationic dyes, no significant differences were detectable between both directions of migration (Fig.

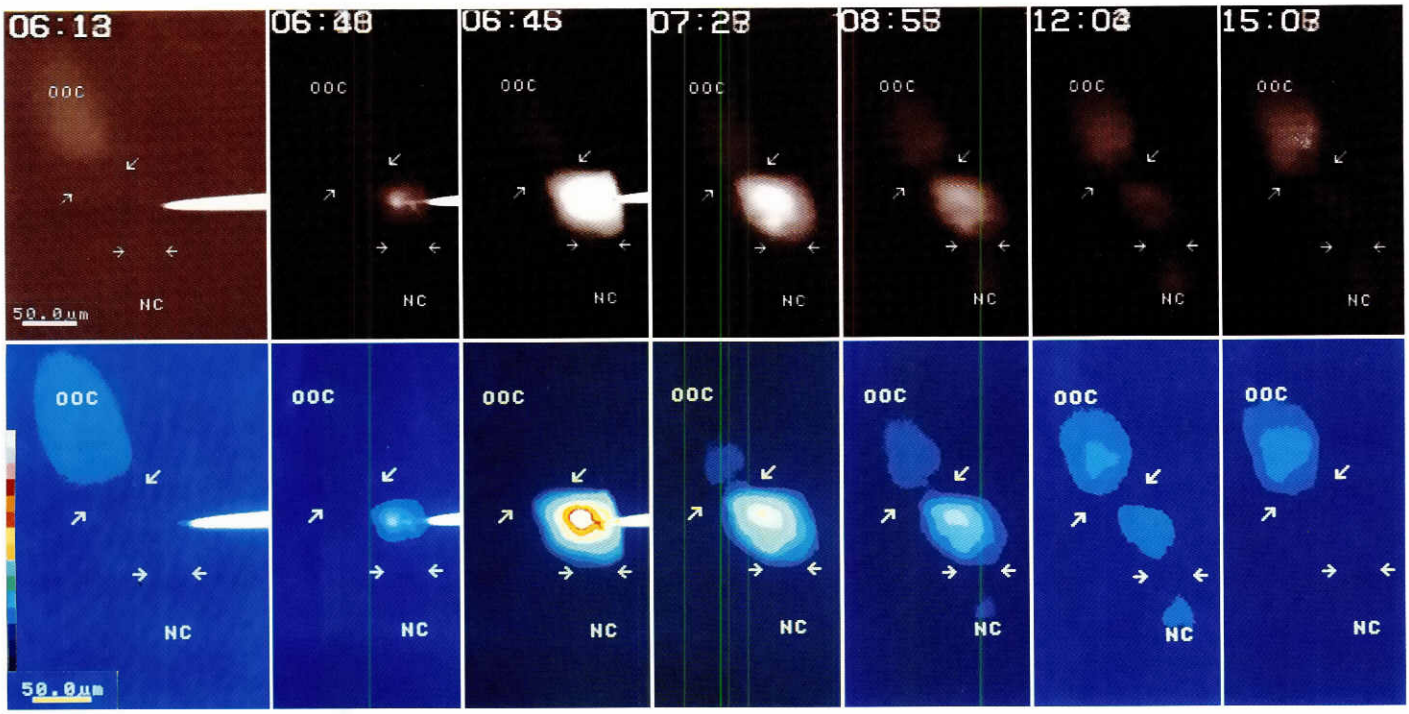

Fig. 3. Sequence of seven video frames displayed in b/w (top row) and pseudocolors (bottom row) of a time-lapse recording using VIF-microscopy. *The sequence shows the time course (minute:second at top) of a microinjection of LY-CH into a nurse cell and the migration of the dye into the oocyte as well as into a nurse cell lying anterior to the microinjected cell (culture solution: IMADS 240). The first frame was taken before the compensation for autofluorescence in the oocyte. The arrows mark the borders between the oocyte and the nurse cells as well as between the two nurse cells. For abbreviations and pseudocolor scale see Figures 1 and 2, respectively.*

8; P>0.05). Moreover, small molecules did not consistently migrate faster than much larger molecules. While our results indicate that the rate of migration of the anionic dyes depended significantly on the respective molecular mass (Fig. 8; P<0.05), this was not true for the cationic dyes (P>0.05). Accumulation of LY-dex in the oocyte was only observed when the follicles were incubated *in vitro* for much longer periods (>1 hour) than LY-CH-injected follicles (see also Bossinger and Schierenberg, 1996). The cationic dyes, however, migrated rather slowly in either direction and did not accumulate in the oocyte. These dyes bind strongly to various types of negatively charged cytoplasmic constituents, and Rh-6G, in particular, is known to enter active mitochondria (Chen, 1989). Thus, these experiments indicate that the microinjected molecules, as long as they do not bind to elements of the cytoplasm, spread within the *Drosophila* nurse-cell/oocyte syncytium by free diffusion and are not significantly influenced by electrical phenomena.

Discussion

In order to clarify the issue, whether or not an electrical potential gradient between the nurse cells and the oocyte might be involved in cytoplasmic transport through intercellular bridges during *Drosophila* oogenesis, we incubated stage-10A follicles in various culture solutions and microinjected various types of fluorescent probes. For analysis, we used a highly sensitive VIF-microscopy set-up and a pseudocolor display, which allowed us to iontophoretically microinject the smallest amounts and to evaluate exactly the intercellular migration of the probes under optimal *in vitro* conditions (R-14 medium, short incubation periods). Previous investigators have either used pressure injections of fluorescent proteins and a SIT-video camera for the qualitative analysis of equilibrium concentrations (Woodruff *et al.*, 1988), or iontophoretic injections of LY-CH (which adsorbs less rapidly to cytoplasmic constituents than the fluorescent proteins) and a less sensitive video set-up (Woodruff, 1989; Singleton and Woodruff, 1994).

Lucifer Yellow binds to yolk spheres

Contrary to Singleton and Woodruff (1994), who reported that the external osmolarity may affect the polarity of the electrical gradient, and thus also the distribution of charged molecules, we did not detect any differences in the preferential directions of dye migration when solutions with different osmotic strengths were compared. Irrespective of osmolarity, the anionic dye LY-CH was always found to first migrate in both directions and then to accumulate in the oocyte. The reasons for these different results are not yet fully understood. However, since cryosections revealed that LY-CH preferably binds to yolk spheres, it seems plausible that the accumulation of the dye in the oocyte (reported by Singleton and Woodruff to occur only in 255-mOsm IMADS) results from free diffusion through the ring canals combined with some sort of "affinity chromatography" in the oocyte.

This hypothesis is further supported by previous studies which have shown that, also in other systems, LY-CH binds to cytoplasmic components (De Laat *et al.*, 1980) and, particularly, to yolk spheres (Bossinger and Schierenberg, 1992b). Moreover, during the investigation of gap-junctional communication in *Drosophila*

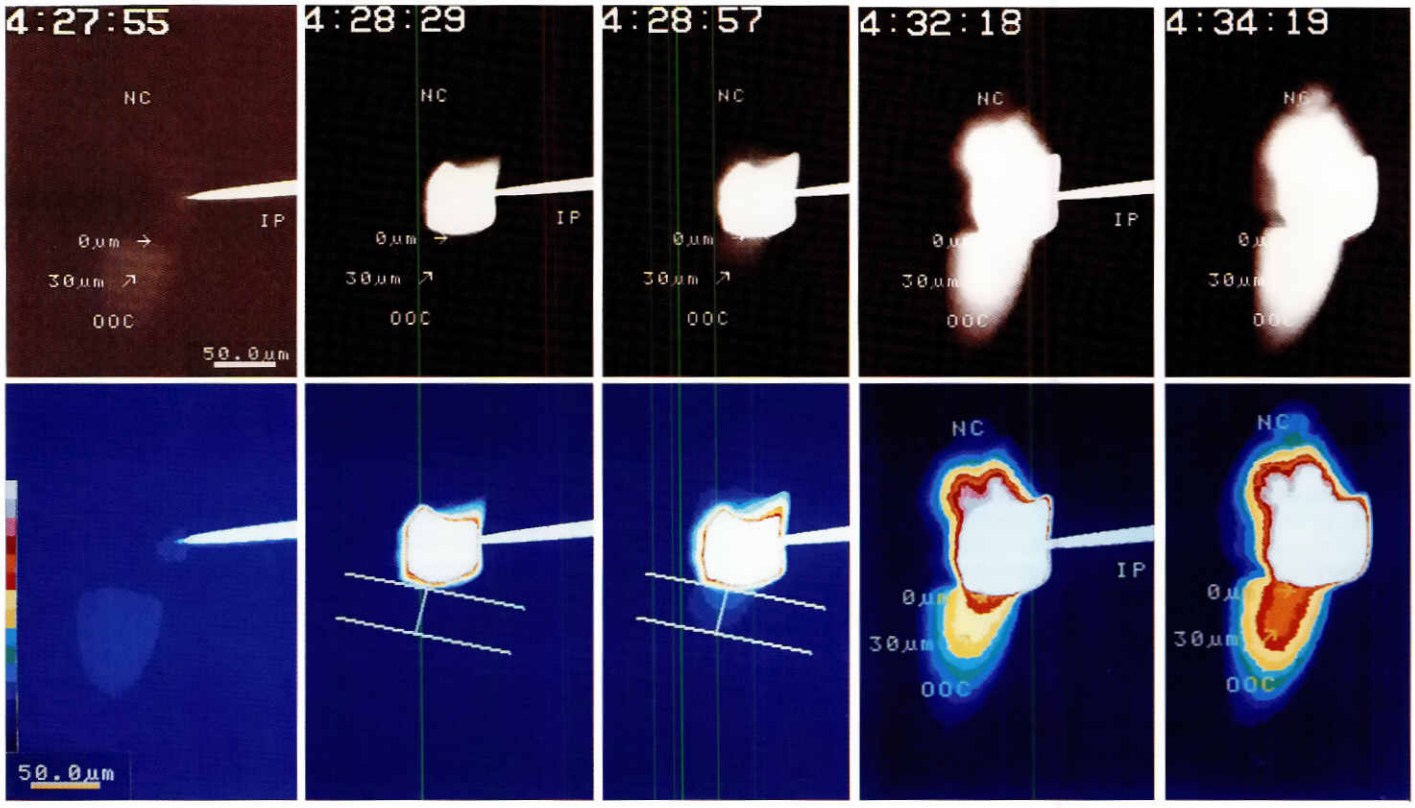

Fig. 4. Sequence of five video frames displayed in b/w (top row) and pseudocolors (bottom row) of a time-lapse recording using VIF-microscopy. *The sequence shows one of the two complementing methods used for the evaluation of dye migration: determination of the time (hour:minute:second at top) required for a change to the next "brighter" pseudocolor in the oocyte at a 30 µm distance (between bars) from the microinjected nurse cell. The first frame was taken before the compensation for autofluorescence in the oocyte. The follicle was microinjected with LY-CH and incubated in R-14. Our second method implied the determination of the distance that a pseudocolor front moved within 20 sec (see Figs. 5 and 8). For abbreviations and pseudocolor scale see Figures 1 and 2, respectively.*

follicles, a similar phenomenon has been observed when dye-coupling of LY-CH between oocyte and follicle cells was analyzed (Bohrmann and Haas-Assenbaum, 1993). In these experiments, due to the larger amounts of dye that were microinjected into the oocyte by pressure, the follicular epithelium became strongly fluorescent. However, within several minutes of incubation, LY-CH was always found to disappear from the follicle cells and to accumulate in the oocyte. In the present study, presumably due to the very small amounts of dye that were iontophoretically microinjected, dye-coupling between germ-line cells and follicle cells was never observed. Since we have found that dye-coupling can reliably be detected only after short preincubation periods under optimal *in vitro* conditions using follicles from young ovaries and pressure injections of LY-CH (Bohrmann, 1993; Bohrmann and Haas-Assenbaum, 1993), we suspect that it was due to these restrictions that other investigators did not observe dye-coupling in *Drosophila* follicles (Verachtert and De Loof, 1989; Woodruff, 1989; Singleton and Woodruff, 1994).

In vitro conditions

In order to provide a reliable basis for *in vitro* experiments, it is necessary to use a culture solution in which the metabolism of the cells approaches the *in vivo* situation as closely as possible. The crucial test of a culture solution for ovarian follicles is to evaluate the developmental capacities of the follicles maintained *in vitro* (Bohrmann, 1991). Although IMADS matches the main ionic concentrations of adult *Drosophila* hemolymph (Singleton and Woodruff, 1994), control follicles of stage 10B were not capable of developing any further in IMADS of different osmotic strengths. The ionic concentrations of R-14 (Robb, 1969) are, in some respects, more closely related to the concentrations measured for larval hemolymph (Croghan and Lockwood, 1960; Begg and Cruickshank, 1963) than to that measured for adult hemolymph (Van der Meer and Jaffe, 1983). However, in R-14, stage-10B follicles (and also a certain percentage of stage-10A follicles) develop with normal morphology up to the end of oogenesis (Petri *et al.*, 1979; Bohrmann and Sander, 1987; Bohrmann, 1991; present study). Moreover, follicles younger than stage 10 that had been incubated for up to one hour in R-14, were found to develop normally when transplanted into host flies (Gutzeit and Koppa, 1982; our own unpublished observations). Several further studies have also proved the suitability of R-14 for various types of *in vitro* experiments with *Drosophila* follicles (e.g. Mahowald *et al.*, 1983; Overall and Jaffe, 1985; Bohrmann *et al.*, 1986a,b; Bohrmann and Gutzeit, 1987; Bohrmann, 1993, 1996; Bohrmann and Haas-Assenbaum, 1993; Bohrmann and Biber, 1994). It is thus rather unlikely that the phenomena observed in the present study using R-14 are *in vitro* artifacts.

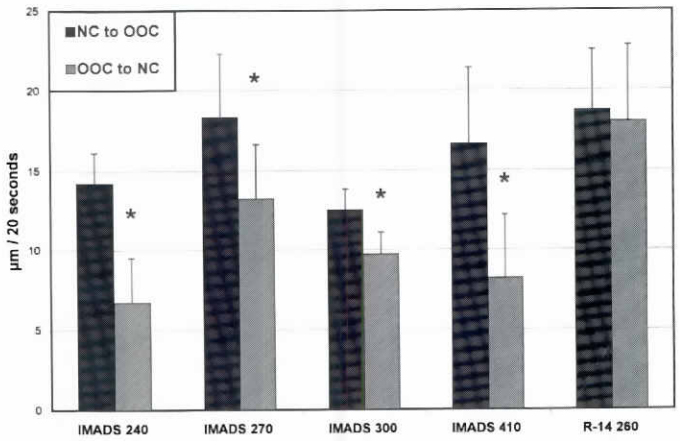

Fig. 5. Migration of LY-CH in follicles incubated in solutions of different osmolarity (in mOsm) and composition (IMADS vs R-14). *Displayed is the distance (in µm) that a pseudocolor front moved within 20 sec into a neighboring cell when either a nurse cell (NC to OOC, black columns) or the oocyte (OOC to NC, hatched columns) was microinjected. Number of follicles per column: n= 4-9; bars indicate standard deviation; *difference between corresponding values (same culture solution) is significant (P<0.05).*

Cytoplasmic transport via intercellular electrophoresis

When evaluating the relevance of an electrical gradient for cytoplasmic transport in *Drosophila* follicles *in situ*, some further points, in addition to the suitability of the culture solution, are worth considering. An electrical gradient would only exert influence on the distribution of molecules that can freely diffuse through the intercellular bridges. In *Drosophila* follicles of stage 10A, the intercellular bridges are 5-10 µm in diameter and only 1-2 µm in length. It is rather difficult to see how the migration of charged molecules could significantly be influenced by an electrical "gradient" acting through a relatively wide pore over such a small distance.

There is good evidence that the fluid phase of the cytoplasm is extremely crowded with macromolecules, a factor that would hamper free diffusion due to collision with other molecules (reviewed in Luby-Phelps, 1994; Luby-Phelps and Weisiger, 1996). Thus, for macromolecules, free diffusion would be a rather slow and inefficient mode of cytoplasmic transport. Moreover, the vast majority of RNAs and proteins in the *Drosophila* follicle are, most likely, not free to diffuse since they are associated with the cytoskeleton (for reviews, see Mahajan-Miklos and Cooley, 1994; Pokrywka, 1995).

We microinjected into the follicles very small amounts of various fluorescent probes and always observed migration in both directions through the ring canals. Using such small amounts, a possible effect of an electrical gradient on the intercellular movement of a charged molecule should have been detectable. While Woodruff *et al.* (1988), Woodruff (1989) and Singleton and Woodruff (1994) were concerned with equilibrium concentrations rather than rates of movement of microinjected molecules, our results indicate that both phenomena are strongly related: in all salines, LY-CH was found both to migrate faster in the posterior direction and to accumulate in the oocyte within only a few minutes of incubation.

The osmolarity of our R-14 was almost identical to that reported for adult *Drosophila* hemolymph (Singleton and Woodruff, 1994). However, in this medium we did not detect any significant correlation between the electrical charge and the preferential direction of migration of different microinjected molecules. Since R-14 is best suited for *in vitro* experiments with *Drosophila* follicles, an electrical gradient, which was presumed to appear at this particular osmotic strength (Singleton and Woodruff, 1994), might not be expected to exert significant influence on the distribution of charged molecules between nurse cells and oocyte *in situ* (see also Bohrmann and Gutzeit, 1987).

Cytoplasmic transport via the cytoskeleton

Several previous studies have presented evidence that, in different phases of *Drosophila* oogenesis, cytoskeletal elements are involved in cytoplasmic transport processes (for reviews, see Cooley and Theurkauf, 1994; Pokrywka, 1995). During previtellogenic stages 1-6, microtubules play a role in the distribution and localization of various organelles and macromolecules within the oocyte (e.g. Theurkauf *et al.*, 1992; Pokrywka and Stephenson, 1995). Later on, during stages 7-10A, cytoplasmic particles and organelles are unidirectionally and obviously selectively transported from the nurse cells into the oocyte by means of a microfilament- and myosin-VI-dependent process (Bohrmann and Biber, 1994; Bohrmann, 1996). The unspecific bulk transfer of nurse-cell cytoplasm into the oocyte during subsequent stages 10B-12 depends on the contraction of microfilaments (Gutzeit and Koppa, 1982; Gutzeit, 1986b; Cooley *et al.*, 1992). Thus, there is strong and growing evidence that the cytoskeleton and its molecular motors, rather than an electrical gradient, is responsible for most (if not all) of the cytoplasmic transport processes that occur in the nurse-cell/oocyte syncytium of *Drosophila*.

Materials and Methods

Preparation of follicles

Drosophila melanogaster Oregon R flies were reared at room temperature on standard food with additional fresh yeast. Individual females 2-4 days-old were killed by crushing the thorax with tweezers without previous

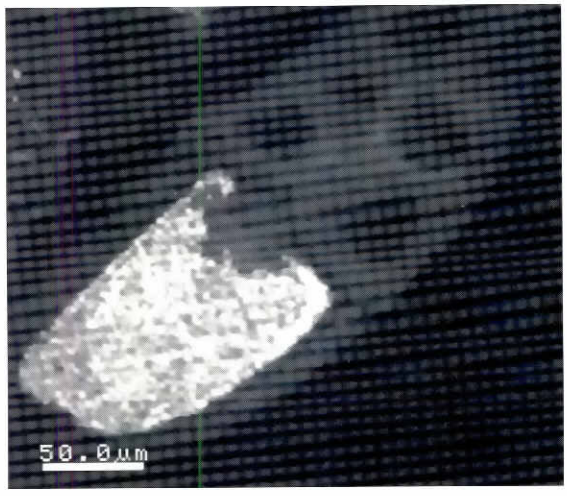

Fig. 6. Video print of a cryosection through a stage-10 follicle (incubated in IMADS containing LY-CH) showing the preferential binding of LY-CH to the yolk spheres in the oocyte (bottom). *At the anterior end of the oocyte, a delta of yolk-sphere free cytoplasm showing no fluorescence is visible.*

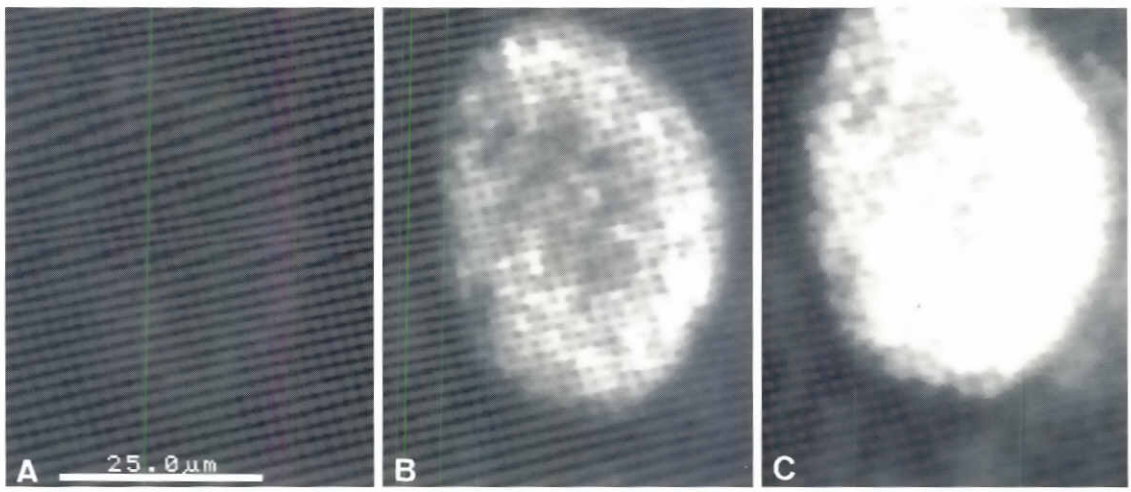

Fig. 7. Time course of the progressive binding of LY-CH to the yolk spheres in the oocyte. *The sequence of video prints shows the same cryosection (cross-section) through an oocyte incubated for* **(A)** *5 min,* **(B)** *10 min and* **(C)** *15 min, respectively, in IMADS containing LY-CH. After each incubation step, the section was washed for 15 min. In* **(C)** *the follicle cells surrounding the oocyte are slightly stained. It is very likely that the rapid accumulation of LY-CH within the oocyte, as observed during the microinjection experiments using IMADS (e.g. Fig. 3), is a result of the progressive binding of the dye to yolk spheres.*

etherization or chilling. Follicles were carefully dissected out of the epithelial sheath of the ovariole with tungsten needles in the respective culture solution (see below). Vitellogenic follicles of stage 10A (for stages, see King, 1970) lacking any signs of injury were washed in the culture solution and immediately transferred to the microinjection chamber.

Microinjection procedure

The microinjection chamber (an inverted type of the chamber described by Bohrmann and Haas-Assenbaum, 1993) was constructed from several 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, a suction pipette and a silver-wire electrode passed from both sides through thin films of paraffin oil covering the flanks of the chamber to prevent evaporation of the culture solution. Iontophoretic microinjections were carried out using a motorized micromanipulator (Zeiss), a piezo translator (Märzhäuser PM 20), an inverted microscope (Zeiss Axiovert 135, equipped with epifluorescence optics) and a constant current source (Siemens Stabizet).

Micropipettes were pulled from 1 mm glass capillaries with a filament (Clark) on a pipette puller (Campden). The injection pipette (tip diameter 1-2 µm) was filled from the back with 2-4 µl of the respective injection solution (see below), then filled up with bidistilled H_2O and fixed in a pipette holder containing a silver-wire electrode. The recipient follicle was held in place with a suction pipette (tip diameter 30-50 µm) mounted on a micromanipulator and coupled to a screw-adjustable syringe (Hamilton) filled with paraffin oil. By means of the piezo translator, we were able to puncture a nurse cell or the oocyte of the follicles very carefully. Thus, enzymatic digestion of the basement membrane, as performed e.g. by Singleton and Woodruff (1994), was not necessary. Follicles showing any signs of injury following microinjection were not analyzed further.

Polarity and voltage were chosen according to the electrical charge and molecular mass of the respective probe. The microinjected amount was standardized using a pseudocolor-video display (see below) of fluorescence intensity. After having adjusted brightness and contrast of the video image to compensate for background fluorescence, the current was applied until a maximum level of fluorescence intensity (i.e. pseudocolor "white") was reached at the site of microinjection (usually after 3-8 sec at 5-10 V, depending on the respective probe).

Culture solutions

As culture solutions, we used four simple salines having osmolarities of 240, 270, 300 and 410 mOsm (IMADS; Singleton and Woodruff, 1994) and a *Drosophila* tissue-culture medium of 260 mOsm (R-14; Robb, 1969). The osmolarities of the solutions, as measured using a freezing-point depression technique (Knauer Halbmikro-Osmometer), were adjusted by varying the content of glucose. In order to obtain IMADS of 240 mOsm, we reduced the content of sodium glutamate from 100 mM to 75 mM. Contrary to Singleton and Woodruff (1994), who reported 255 mOsm as the lowest osmolarity of their IMADS, we repeatedly determined for IMADS without glucose an osmolarity of 270 mOsm. Thus, due to the different freezing-point-depression osmometers that were used in the two studies, the measurements differed by about 15 mOsm. Singleton and Woodruff (1994) measured 255, 275, 300 and 400 mOsm for their IMADS. The other ion concentrations in IMADS were as follows: 25 mM KCl, 15 mM $MgCl_2$, 5 mM $CaSO_4$, 2 mM sodium phosphate buffer (pH 6.9).

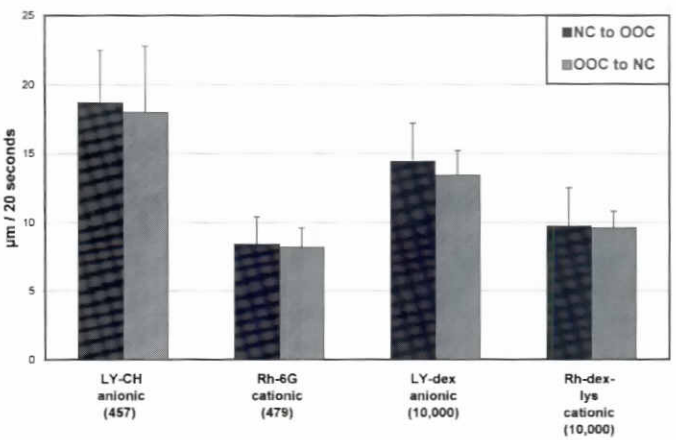

Fig. 8. Migration of fluorescent probes with different electrical charge and molecular mass (M_r) in follicles incubated in R-14. *Displayed is the distance (in µm) that a pseudocolor front moved within 20 sec into a neighboring cell when either a nurse cell (NC to OOC, black columns) or the oocyte (OOC to NC, hatched columns) was microinjected. While the small anionic LY-CH migrated significantly faster than the large anionic LY-dex ($P<0.05$), no significant difference was found for small and large cationic dyes ($P>0.05$). Number of follicles per column: n= 4-9; bars indicate standard deviation; all differences between corresponding values (same fluorescent probe) are not significant ($P>0.05$).*

Microinjected molecules

We microinjected one of the following fluorescent probes having different electrical charges as well as different molecular masses M_r: (1) Lucifer Yellow CH (LY-CH, anionic, 457 M_r; Sigma; stock solution 2% w/v in bidistilled H_2O), (2) rhodamine 6G (Rh-6G, cationic, 479 M_r; Sigma; stock solution 1% w/v in ethanol), (3) Lucifer Yellow dextran (LY-dex, anionic, 10,000 M_r; Molecular Probes; stock solution 3% w/v in bidistilled H_2O), and (4) tetramethylrhodamine dextran-lysine (Rh-dex-lys, cationic, 10,000 M_r; Molecular Probes; stock solution 3% w/v in bidistilled H_2O).

Video-intensified fluorescence (VIF) microscopy

During, as well as after, the iontophoretic microinjections (i.e. 5-10 min following the onset of dissection), the follicles were viewed using VIF-microscopy. The low-light-level video system consisted of a highly sensitive SIT-video camera (Hamamatsu C-2400), a digital image processor (Hamamatsu Argus-10), a time-lapse S-VHS video-tape recorder (Panasonic AG-6720), a b/w-TV monitor (Panasonic WV-5410) and a color-TV monitor (Sony PVM-1440QM). The image processor was used for noise reduction, contrast enhancement and measurements (see below). With this system, very weak levels of fluorescence were detectable, thereby permitting the amount of the microinjected fluorescent probe to be minimized. Using a 50 W mercury-arc lamp, the appropriate filter combinations for green (BP546/FT580/LP590) or blue (BP485/FT510/LP520) excitation light, a x10 objective and a x16 eyepiece, we obtained a magnification of x650 for measurements on the video-monitor screen.

Video prints were produced on a b/w video-copy processor (Mitsubishi P66E). Color photographs were taken directly from the screen in the freeze-frame mode of the image processor using a Nikon camera and 100 ASA film. Video recordings (at 6-50 frames per second) of individual follicles were carried out for 10-30 min. In order to reduce photobleaching as well as specimen damage during observation periods lasting longer than 10 min, the irradiation was repeatedly interrupted for several minutes.

Measurements

Using a pseudocolor display of fluorescence intensity, the migration of a fluorescent probe from the microinjected cell into a neighboring cell was evaluated with the image processor by analyzing video recordings of microinjected follicles. We used two complementing methods for this evaluation: by means of the time/date generator and the measuring function of the image processor, the distance that a pseudocolor front moved within 20 sec as well as the time that the front needed to cover a distance of 30 µm was determined for each follicle. For the data obtained with both methods, mean values (±S.D.) were calculated and compared. Statistical significance of differences between mean values was established at the α=0.05 level using the *t* test. Both methods gave comparable results.

Since the 256 gradations of brightness were expressed as 15 pseudocolors, there was a 7% difference of brightness between two colors (17 gradations per color). However, by compensating for background and autofluorescence and by adjusting brightness and contrast close to a color change, minimal differences of fluorescence intensity were detectable.

Cryosections

Whole ovaries were dissected out of flies in R-14 medium and fixed for 30 min at 4°C in 4% formaldehyde dissolved in 66 mM Sörensen buffer pH 7.4. After washing, they were embedded in a cryosectioning medium (Hartmann, 1984), frozen in dry ice/acetone and cut on a cryotome into 10 µm sections which were collected on glycerol/gelatine-coated slides as described by Grau and Gutzeit (1990). The sections were then incubated with LY-CH in IMADS (stock solutions diluted 1:1000) for 5-15 min, washed for another 15 min (up to several hours), and video prints were performed as described above.

Acknowledgments

We thank Olaf Bossinger for the gift of fluorescent dextrans, Barbara Braun for help with the cryosections, Roswitha Koppa for technical assistance, and Richard Woodruff for critical comments on the manuscript. The Deutsche Forschungsgemeinschaft gave financial support.

References

BEGG, M. and CRUICKSHANK, W.J. (1963). A partial analysis of *Drosophila* larval haemolymph. *Proc. Roy. Soc. Edinburgh Sect. Biol. 68:* 215-236.

BOHRMANN, J. (1991). *In vitro* culture of *Drosophila* ovarian follicles: the influence of different media on development, RNA synthesis, protein synthesis and potassium uptake. *Roux Arch. Dev. Biol. 199:* 315-326.

BOHRMANN, J. (1993). Antisera against a channel-forming 16 kDa protein inhibit dye-coupling and bind to cell membranes in *Drosophila* ovarian follicles. *J. Cell Sci. 105:* 513-518.

BOHRMANN, J. (1996). Unconventional myosin VI is involved in cytoplasmic particle transport during *Drosophila* oogenesis. *Eur. J. Cell Biol. 42 (Suppl.):* 307.

BOHRMANN, J. and BIBER, K. (1994). Cytoskeleton-dependent transport of cytoplasmic particles in previtellogenic to mid-vitellogenic ovarian follicles of *Drosophila*: time-lapse analysis using video-enhanced contrast microscopy. *J. Cell Sci. 107:* 849-858.

BOHRMANN, J. and GUTZEIT, H.O. (1987). Evidence against electrophoresis as the principal mode of protein transport in vitellogenic ovarian follicles of *Drosophila*. *Development 101:* 279-288.

BOHRMANN, J. and HAAS-ASSENBAUM, A. (1993). Gap junctions in ovarian follicles of *Drosophila melanogaster*: inhibition and promotion of dye-coupling between oocyte and follicle cells. *Cell Tissue Res. 273:* 163-173.

BOHRMANN, J. and SANDER, K. (1987). Aberrant oogenesis in the patterning mutant dicephalic of *Drosophila melanogaster*: time-lapse recordings and volumetry *in vitro*. *Roux Arch. Dev. Biol. 196:* 279-285.

BOHRMANN, J., DORN, A., SANDER, K. and GUTZEIT, H.O. (1986a). The extracellular electrical current pattern and its variability in vitellogenic *Drosophila* follicles. *J. Cell Sci. 81:* 189-206.

BOHRMANN, J., HUEBNER, E., SANDER, K. and GUTZEIT, H.O. (1986b). Intracellular electrical potential measurements in *Drosophila* follicles. *J. Cell Sci. 81:* 207-221.

BOSSINGER, O. and SCHIERENBERG, E. (1992a). Transfer and tissue specific accumulation of cytoplasmic components in embryos of *Caenorhabditis elegans* and *Rhabditis dolichura: in vivo* analysis with a low-cost signal enhancement device. *Development 114:* 317-330.

BOSSINGER, O. and SCHIERENBERG, E. (1992b). Cell-cell communication in the embryo of *Caenorhabditis elegans*. *Dev. Biol. 151:* 401-409.

BOSSINGER, O. and SCHIERENBERG, E. (1996). Cell-cell communication in nematode embryos: differences between *Cephalobus spec.* and *Caenorhabditis elegans*. *Dev. Genes Evol. 206:* 25-34.

CHEN, L.B. (1989). Fluorescent labeling of mitochondria. *Methods Cell Biol. 29:* 103-123.

COOLEY, L. and THEURKAUF, W.E. (1994). Cytoskeletal functions during *Drosophila* oogenesis. *Science 266:* 590-596.

COOLEY, L., VERHEYEN, E. and AYERS, K. (1992). chickadee encodes a profilin required for intercellular cytoplasm transport during *Drosophila* oogenesis. *Cell 69:* 173-184.

CROGHAN, P.C. and LOCKWOOD, A.P.M. (1960). The composition of the haemolymph of the larva of *Drosophila melanogaster*. *J. Exp. Biol. 37:* 339-343.

DE LAAT, S.W., TERTOOLEN, L.G.J., DORRENSTEIJN, A.W.C. and VAN DEN BIGGELAAR, J.A.M. (1980). Intercellular communication patterns are involved in cell determination in early molluscan development. *Nature 287:* 546-548.

GRAU, V. and GUTZEIT, H.O. (1990). Asymmetrically distributed ecdysteroid-related antigens in follicles and young embryos of *Drosophila melanogaster*. *Roux Arch. Dev. Biol. 198:* 295-302.

GUTZEIT, H.O. (1986a). Transport of molecules and organelles in meroistic ovarioles of insects. *Differentiation 31:* 155-165.

GUTZEIT, H.O. (1986b). The role of microfilaments in cytoplasmic streaming in *Drosophila* follicles. *J. Cell Sci. 80:* 159-169.

GUTZEIT, H.O. and KOPPA, R. (1982). Time-lapse film analysis of cytoplasmic streaming during late oogenesis of *Drosophila*. *J. Embryol. Exp. Morphol. 67:* 101-111.

HARTMANN, R. (1984). A new embedding medium for cryosectioning eggs of high yolk and lipid content. *Eur. J. Cell Biol. 34:* 206-211.

INOUÉ, S. (1986). *Video Microscopy.* Plenum Press, New York.

KING, R.C. (1970). *Ovarian Development in Drosophila melanogaster.* Academic Press, New York.

LUBY-PHELPS, K. (1994). Physical properties of cytoplasm. *Curr. Opin. Cell Biol. 6:* 3-9.

LUBY-PHELPS, K. and WEISIGER, R.A. (1996). Role of cytoarchitecture in cytoplasmic transport. *Comp. Biochem. Physiol. 115B:* 295-306.

MAHAJAN-MIKLOS, S. and COOLEY, L. (1994). Intercellular cytoplasm transport during *Drosophila* oogenesis. *Dev. Biol. 165:* 336-351.

MAHOWALD, A.P. and KAMBYSELLIS, M.P. (1980). Oogenesis. In *Genetics and Biology of Drosophila,* Vol. 2d (Eds. M. Ashburner and T.R.F. Wright). Academic Press, New York, pp. 141-224.

MAHOWALD, A.P., GORALSKI, T.J. and CAULTON, J.H. (1983). *In vitro* activation of *Drosophila* eggs. *Dev. Biol. 98:* 437-445.

O'DRISCOLL, D., WILSON, G. and STEER, M.W. (1991). Lucifer Yellow and fluorescein isothiocyanate uptake by cells of *Morinda citrifolia* in suspension cultures is not confined to the endocytotic pathway. *J. Cell Sci. 100:* 237-241.

OVERALL, R. and JAFFE, L.F. (1985). Patterns of ionic current through *Drosophila* follicles and eggs. *Dev. Biol. 108:* 102-119.

PETRI, W.H., MINDRINOS, M.N., LOMBARD, M.F. and MARGARITIS, L.H. (1979). *In vitro* development of the *Drosophila* chorion in a chemically defined organ culture medium. *W. Roux Arch. Entw.Mech. Org. 186:* 351-362.

POKRYWKA, N.J. (1995). RNA localization and the cytoskeleton in *Drosophila* oocytes. *Curr. Top. Dev. Biol. 31:* 139-166.

POKRYWKA, N.J. and STEPHENSON, E.C. (1995). Microtubules are a general component of mRNA localization systems in *Drosophila* oocytes. *Dev. Biol. 167:* 363-370.

ROBB, J.A. (1969). Maintenance of imaginal discs of *Drosophila melanogaster* in chemically defined media. *J. Cell Biol. 41:* 876-885.

SHOTTON, D.M. (1988). Review: video-enhanced light microscopy and its applications in cell biology. *J. Cell Sci. 89:* 129-150.

SINGLETON, K. and WOODRUFF, R.I. (1994). The osmolarity of adult *Drosophila* hemolymph and its effect on oocyte-nurse cell electrical polarity. *Dev. Biol. 161:* 154-167.

SPRADLING, A. (1993). Developmental genetics of oogenesis. In *The Development of Drosophila melanogaster* (Eds. M. Bate and A. Martínez Arias). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 1-70.

St JOHNSTON, D. (1995). The intracellular localization of messenger RNAs. *Cell 81:* 161-170.

STEINBERG, T.H., NEWMAN, A.S., SWANSON, J.A. and SILVERSTEIN, S.C. (1987). Macrophages possess probenecid-inhibitable organic anion transporters that remove fluorescent dyes from the cytoplasmic matrix. *J. Cell Biol. 105:* 2695-2702.

SUN, Y-A. and WYMAN, R.J. (1993). Re-evaluation of electrophoresis in the *Drosophila* egg chamber. *Dev. Biol. 155:* 206-215.

THEURKAUF, W.E., SMILEY, S., WONG, M.L. and ALBERTS, B.M. (1992). Reorganization of the cytoskeleton during *Drosophila* oogenesis: implications for axis specification and intercellular transport. *Development 115:* 923-936.

VAN DER MEER, J.M. and JAFFE, L.F. (1983). Elemental composition of the perivitelline fluid in early *Drosophila* embryos. *Dev. Biol. 95:* 249-252.

VERACHTERT, B. and DE LOOF, A. (1989). Intra- and extracellular electrical fields of vitellogenic polytrophic insect follicles. *Biol. Bull. 176 (Suppl.):* 91-95.

WOODRUFF, R.I. (1989). Charge-dependent molecular movement through intercellular bridges in *Drosophila* follicles. *Biol. Bull. 176 (Suppl.):* 71-78.

WOODRUFF, R.I. and TELFER, W.H. (1980). Electrophoresis of proteins in intercellular bridges. *Nature 286:* 84-86.

WOODRUFF, R.I., KULP, J.H. and LA GACCIA, E.D. (1988). Electrically mediated protein movement in *Drosophila* follicles. *Roux Arch. Dev. Biol. 197:* 231-238.

Received: December 1996
Accepted for publication: March 1997