THE ROLE OF MICROFILAMENTS IN CYTOPLASMIC STREAMING IN DROSOPHILA FOLLICLES

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SUMMARY

During the last phase of oogenesis in Drosophila, nurse cell cytoplasm can be seen to be streaming into the growing oocyte when visualized in time-lapse films. This process can be reversibly inhibited by cytochalasins. The distribution of F-actin filaments in the nurse cells has been studied by staining with rhodamine-conjugated phalloidin. At the beginning of cytoplasmic streaming (stage 10B) increasingly thick bundles of microfilaments formed, many of which spanned the nurse cell cytoplasm from the cell membrane to the nuclear membrane. The association of F-actin with the nuclear membrane persisted when nurse cell nuclei were isolated mechanically. The experimental evidence suggests that microfilament contraction in the nurse cells leads to cytoplasmic streaming by pressure flow.

INTRODUCTION

Ovarian follicles of Drosophila consist of 16 germ line cells: 15 nurse cells and the oocyte form a syncytium; all cells are directly or indirectly connected with each other by cytoplasmic bridges (ring canals), which are mechanically strengthened by non-contractile rings of F-actin (Warn, Gutzeit, Smith & Warn, 1985). During the final phase of vitellogenesis, the 15 nurse cells rapidly decrease in size and contribute their cytoplasm to the growing oocyte (Fig. 1). This process has been studied previously by time-lapse films (Gutzeit & Koppa, 1982). During stages 10B to 12 (for stages, see King, 1970), nurse cell cytoplasm flows through the ring canals into the oocyte (nurse cell streaming) where due to cytoplasmic streaming in the oocyte (ooplasmic streaming) it rapidly becomes mixed with the ooplasm. This process is interesting for at least two reasons: firstly, the system is ideally suited for studying the control of cytoplasmic streaming and intrafollicular transport; secondly, cytoplasmic streaming may be developmentally significant since circumstantial evidence from mutant dicephalic follicles is compatible with the notion that the oocyte needs to be supplied with critical quantities of 'anterior factors' from the nurse cells for the correct specification of the anteroposterior axis of the embryo (Lohs-Schardin, 1982; Frey, Sander & Gutzeit, 1984).

The present study addresses the question of the mechanism of cytoplasmic streaming and it will be shown that cytoskeletal elements, in particular microfilaments (MF), play an essential role in this process.

Key words: microfilaments, cytoplasmic streaming, Drosophila oogenesis.
Fig. 1. Nurse cell regression. Nurse cell chambers of stage 10B (A) and stage 12 (B) follicles photographed by differential interference contrast. At stage 10B nurse cell cytoplasm begins to stream into the oocyte (ooc). This process is completed at stage 12 (B) when only the shrunken nurse cell nuclei (ncn) remain in the degenerate nurse cell chamber. At the anterior egg pole the follicle cells differentiate the chorionic appendages (open arrowhead) and the micropyle (arrowhead), fc, somatic follicle cells. Bar, 50 μm.

MATERIALS AND METHODS

Time-lapse filming

Cytoplasmic streaming has been recorded by time-lapse cinematography as described previously (Gutzeit & Koppa, 1982). In order to ensure fast and homogeneous exchange of culture medium during filming, a newly designed flow-through chamber was used (for details, see Gutzeit & Kaltenbach, 1985). Isolated follicles of stage 10B or 11 were placed in the chamber and cultured in Robb's R 14 medium (Robb, 1969), which was pumped through the chamber at a rate of 0.26 ml min⁻¹. After recording the normal pattern of streaming for about 15 min (50–90 frames min⁻¹), the culture medium was replaced by medium containing the inhibitor to be tested. Cytochalasin B and D (Sigma) were dissolved in dimethylsulphoxide (DMSO) or ethanol (Uvasol, Merck). The stock solutions of the inhibitors contained 10 mg ml⁻¹ and 2 mg ml⁻¹, respectively. These solutions were diluted at least 1:1000 with culture medium (see Table 1 for details). Control follicles were cultured in medium containing only the solvent (ethanol or DMSO) at 1:1000 dilution, but not the inhibitor. Under these conditions there was no effect on cytoplasmic streaming.

Medium with and without the inhibitor was exchanged by means of a two-way valve (Pharmacia). Since the flow-through chamber ensures uniform and reliable replacement of different media, it was possible to estimate the time when the follicle became exposed to the inhibitor. From tests with Trypan Blue dye we estimated the accuracy to be of the order of ±30 s. For details concerning the concentrations of applied inhibitor see Table 1. The effect of the drug on cytoplasmic streaming was only followed for up to 2 h of culture in vitro, since by about that time cytoplasmic streaming is arrested in control follicles not exposed to the drug (Gutzeit & Koppa, 1982).

Whenever the effect of the drug was studied for longer than 30 min, we filmed short sequences at 15- or 30-min intervals in order to avoid possible artefacts that may be caused by constant illumination of the follicles with the microscope lamp.
**Microfilaments in cytoplasmic streaming**

Drawings from time-lapse films were made by projecting the film by a system of mirrors onto drawing-paper and the direction of streaming was marked wherever it could be seen clearly.

**Fluorescence microscopy of F-actin in nurse cells**

Staining of MF with rhodaminyl phalloidin (RH-phalloidin) was carried out as described previously (Warn et al. 1985, their method 2). Rh-phalloidin was a generous gift from Professor Th. Wieland, Heidelberg (FRG). The preparations were viewed in a Leitz microscope using a ×63 oil immersion objective and photographs were taken on Ilford HPS high-speed film. In some cases we teased nurse cell chambers apart with tungsten needles after fixation. The preferred fracture plane was usually along the nurse cell membranes so that single cells or a small number of nurse cells could be isolated. Owing to the much reduced thickness of such preparations, their MF pattern was usually easier to analyse as compared to *in toto* stained follicles.

Nurse cell nuclei were isolated by cutting through the nurse cell cluster with tungsten needles (before fixation), thereby rupturing the cell membranes. The large nuclei can easily be recognized in the stereomicroscope under transmitted light. The nuclei were washed by sucking them up several times in a micropipette pulled from a 200-μl measuring capillary. When viewed in the microscope many nuclei showed no trace of cytoplasm adhering to the nuclear membrane. F-actin associated with such nuclei was stained as described above.

**Electron microscopy**

The ultrastructural observations were made during the course of a study concerning the ultrastructural differentiation of centripetally migrating follicle cells in stage 10B follicles. For technical details see Heinrich & Gutzeit (1985).

**Table 1. Effect of cytochalasins on cytoplasmic streaming**

<table>
<thead>
<tr>
<th>Inhibitor concentration (μg ml⁻¹)</th>
<th>NC cytoplasm: complete inhibition of streaming after (min)</th>
<th>Ooplasmic streaming (min)</th>
<th>NC streaming: release of inhibition after wash (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CyB, 10</td>
<td>2-2</td>
<td>n.e.</td>
<td>2-8</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>n.e.</td>
<td>n.d.</td>
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<tr>
<td></td>
<td>3-5</td>
<td>n.e.</td>
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<tr>
<td></td>
<td>2-6</td>
<td>n.e.</td>
<td>n.d.</td>
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<tr>
<td></td>
<td>4-0</td>
<td>n.e.</td>
<td>n.d.</td>
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<tr>
<td></td>
<td>n.d.</td>
<td>n.e. (&gt;103)</td>
<td>n.d.</td>
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<tr>
<td></td>
<td>3-5</td>
<td>n.e.</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>2-0</td>
<td>n.e. (&gt;112, &lt;142)</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

CyB, 2

|                                 | 1-9                                                     | n.e.                     | 5-5                                                |
|                                 | 1-2                                                     | n.e.                     | 3-1                                                |

CyD, 2

|                                 | 3-8                                                     | n.e.                     | 1-8                                                |
|                                 | 7-5*                                                   | n.e.                     | 3-2                                                |
|                                 | 8-3                                                     | n.e.                     | 17-1                                               |
|                                 | 9-0                                                     | n.e.                     | >14-6, <34-6                                       |
|                                 | 2-1                                                     | n.e.                     | n.d.                                               |

CyD, 1

|                                 | 13                                                      | n.e.                     | n.d.                                               |
|                                 | 5-1                                                     | n.e.                     | 13-6                                               |

In each line the measurements on a single follicle are presented. n.e., no effect during analysed period (~15-30 min after cessation of nurse cell streaming); n.d., not determined; CyB, cytochalasin B; CyD, cytochalasin D; NC, nurse cell.

* Drastically reduced streaming but not complete inhibition.
RESULTS

Inhibition of cytoplasmic streaming

The effect of cytoskeletal inhibitors on cytoplasmic streaming in stage 10B/11 follicles was studied by time-lapse film analysis. Using an improved flow-through chamber connected to a two-way valve, the effects of the drugs tested could be studied in follicles whose normal pattern of streaming had previously been recorded.

When follicles were exposed to cytochalasin B or D (1-10 μg ml⁻¹), the cytoplasmic streaming from the nurse cells to the oocyte stopped within a few minutes (for qualitative description, see Fig. 2; for quantitative data, Table 1). Appropriate controls (see Materials and Methods) showed that this inhibitory effect cannot be attributed to the small quantities of ethanol or DMSO in the culture medium, which were used as solvents for the applied inhibitors. From the first noticeable slow-down of cytoplasmic streaming to its complete cessation it usually took less than 1 min, except in some experiments using cytochalasin D when the inhibition was more gradual and it took a few minutes longer for cytoplasmic streaming to cease (Table 1). The time until complete inhibition was achieved seemed to depend partly on the velocity of streaming before the inhibitors were applied: follicles with particularly fast nurse cell streaming of several μm s⁻¹ often took a few minutes longer for inhibition to be complete. However, the inhibitory effect of cytochalasin B and D on nurse cell streaming was clearly observed in all follicles analysed (Table 1). Surprisingly, ooplasmic streaming was not affected by these drugs and continued for up to roughly 2 h (Fig. 2, Table 1). The final cessation of ooplasmic streaming is probably not due to the drug action, since ooplasmic streaming also stops in normally developing follicles at the end of vitellogenesis (Gutzeit & Koppa, 1982).

When cytochalasin-B-treated follicles were incubated subsequently in drug-free culture medium, the nurse cell plasm resumed its streaming (Fig. 2, Table 1) and nurse cell regression continued normally up to stage 12. The reversible inhibition of nurse cell streaming by cytochalasins gives strong evidence that this process depends...
Microfilaments in cytoplasmic streaming

Fig. 3. Transient changes in pattern of cytoplasmic streaming. Drawings from a time-lapse film showing four subsequent phases of streaming in the nurse cell chamber (nc) of a stage 11 follicle. Arrows indicate the direction of cytoplasmic streaming (velocity not quantified). Duration of each phase: 1.5 min (A), 0.2 min (B), 0.7 min (C), 1.9 min (D). ooc, oocyte; fc, follicle cells; •, path of cytoplasm after sudden local contraction in the nurse cell cytoplasm; presumably the cytoplasm is forced to flow around a nurse cell nucleus; Δ, transient reversal in direction of streaming; *, local cytoplasmic motion lasting for less than 15 s and without long-range effect.

on the activity of microfilaments. Colchicine or other microtubule inhibitors had no effect on nurse cell streaming (Gutzeit, unpublished data).

Transient changes in the pattern of cytoplasmic streaming in regressing nurse cells

During stage 10B the pattern of cytoplasmic streaming in the nurse cells is stable and the reduction in size of all nurse cells is roughly to scale. However, in some exceptional cases, late stage 11 and stage 12 follicles showed transient changes in the pattern of cytoplasmic streaming in the nurse cells. These sudden changes appeared to be due to altered intracellular pressure, so that the cytoplasm was forced to flow via an alternative route (and sometimes a longer route) towards the oocyte. An example of this observation is shown in Fig. 3. Cytoplasm in a nurse cell was suddenly forced to reverse its previous direction of streaming and flowed towards the periphery of the follicle (Fig. 3b) from where it moved anteriorly (Fig. 3c) and finally the diverted cytoplasm contributed to the central stream of cytoplasm that flowed towards the oocyte. This process took less than 2 min. The transient changes in intracellular pressure of one cell are likely to affect the pattern of streaming in other sister cells (Fig. 3b, open triangle, for possible example). In some cases minor local contractions had no visible long-range effect (3c, asterisk). In follicles treated with cytochalasins the cytoplasm always became motionless. The observed transient changes in nurse cell streaming are, therefore, likely to be due to the activity of F-actin. Although it was not possible with the available techniques to see directly MF bundles contracting (or possible effects on cell wall or nucleus), the occasionally observed sudden pressure changes in stage 11/12 follicles are compatible with the notion of a contractile mechanism.

At late stage 12 and stage 13 when cytoplasmic streaming has ceased (Gutzeit & Koppa, 1982), regressing nurse cell nuclei were frequently seen to become suddenly deformed, to rotate or to change their location in the degenerate nurse chamber. The same observation has also been made in late vitellogenic follicles of the mutant dicephalic (Bohrmann, 1981).
Microfilament pattern in vitellogenic follicles before cytoplasmic streaming

Since F-actin, the principal constituent of MF, is likely to be the force-generating molecule for cytoplasmic streaming, we studied the F-actin distribution by staining follicles with RH-phalloidin before, during and after the phase of nurse cell streaming as defined by time-lapse films. In young vitellogenic follicles (stages 7—9) the nurse cell membranes stain intensely. The fine network of MF often cannot be resolved into individual strands (see also Frey et al. 1984). In stage 10A follicles, which are characterized by the presence of ooplasmic streaming and absence of nurse cell streaming, MF bundles can be identified in the plane of the membrane (Fig. 4A,c). In median optical sections (Fig. 4b) these bundles do not extend into the nurse cell cytoplasm since only cell membranes stain intensely. The only exceptions

Fig. 4. Changes in microfilament pattern during stage 10. A—C. MF pattern in a stage 10A nurse cell. A. Upper focus (plane of nurse cell membrane); B, median optical section (arrowheads point to MF associated with ring canals); C, lower focus (plane of nurse cell membrane). Bar, 20 μm. D. MF associated with nurse cell membranes of stage 10B/11 follicle. Note brightly fluorescent ring (ring canal). Bar, 20 μm. E. Median optical section of isolated stage 10B nurse cell. MF are seen to extend from the plasma membrane presumably to the nuclear membrane. Bar, 20 μm.
Microfilaments in cytoplasmic streaming

are MF bundles around the ring canals; these bundles extend for some distance into
the nurse cell cytoplasm (Fig. 4B, see also Warn et al. 1985).

Microfilament distribution at the time of nurse cell streaming

At stage 10B/11, when nurse cell streaming is most intense, the MF pattern
changes drastically as compared to stage 10A. Thick MF bundles can be seen to span
the nurse cell cytoplasm. On closer inspection at different focal levels, it becomes
apparent that many MF bundles radiate from the nurse cell membrane into the cell:
when focused on the level of the nurse cell membrane, the contact sites of the MF
bundles with the membrane appear as brightly fluorescent dots (Fig. 4D) from which
the bundles can be seen to extend for some distance into the interior of the nurse cell.
In median optical sections, MF bundles were seen to traverse the cytoplasm in the
plane of focus (Fig. 4E), in contrast to the situation observed in stage 10A follicles
(compare Fig. 4B and E). In many cases MF bundles terminate at or close to the
nuclear membrane (Fig. 4E). We have identified these MF bundles also in the
electron microscope (Fig. 5). Casual reference to these structures had been made
previously (Giorgi, 1976). In the electron microscope we have seen particularly
conspicuous MF bundles in the posterior nurse cells (bordering centripetally mi-
grated follicle cells), an observation that is also corroborated by the RH-phalloidin
staining pattern. These MF bundles are anchored in cell membrane invaginations of
both nurse cell and adjacent follicle cell (Fig. 5), suggesting that mechanical strain
acts on these structures. Some MF bundles could be followed from the nurse
cell/follicle cell border to the region of the nurse cell nucleus.

F-actin bundles are not distributed homogeneously in the nurse cells. Rather, in
some areas of a particular nurse cell, a large number of conspicuous MF bundles may
be present while in other regions of the same cell few if any can be seen (Fig. 4D).

Fig. 5. Ultrastructural identification of microfilament bundles. Electron micrograph
showing MF bundles in a nurse cell that borders a centripetally migrated follicle cell (fc)
between the nurse cell cluster and the oocyte. Note the infolding of nurse cell and follicle
cell plasma membranes at the base of an MF bundle. nc, nurse cell. Bar, 1 μm.
Fig. 6. F-actin in degenerate nurse cells. A. Late stage 12 nurse cell chamber with short and thick MF bundles and aggregates of F-actin. B. Fragment of stage 12 nurse cell chamber. The nuclear membranes of both nuclei are lined with F-actin. Irregular masses of F-actin are associated with one side of each nucleus only. Bars, 20 μm.

Concomitant with the decrease in nurse cell diameter during stages 10B to 12, the length of the MF bundles also decreases but at the same time the thickness of the bundles increases.

Follicles were cultured in cytochalasin-B-containing medium and stained with RH-phalloidin after different incubation times to study the effect of the inhibitor on the MF pattern. The MF bundles did not depolymerize even after 24 h culture. However, the MF pattern became gradually abnormal and after a few hours of incubation, MF bundles were often seen to be arranged irregularly around the nurse cell nuclei without any connection to the cell membranes.

Pattern of microfilament distribution after cessation of cytoplasmic streaming

In late stage 12 follicles large and irregularly shaped masses of F-actin accumulate in the nurse cell chambers (Fig. 6A; Warn et al. 1985). Since nurse cell membranes break down at this stage (Cummings & King, 1970), a connection between MF bundles and the remaining cell membranes can no longer be recognized. Much of the actin appears to be at or in nurse cell nuclei. Often actin associates with nuclei in an asymmetric fashion and only one side of the nuclei is stained brightly (Fig. 6B).

Microfilaments associated with isolated nuclei

In order to verify that MF bundles indeed associate with the nucleus, we broke the nurse cells open with tungsten needles and mechanically isolated nuclei of stage 10B/11 and stage 12 follicles. After staining with RH-phalloidin, MF bundles were always found to be associated with nuclei albeit in varying quantities. In isolated nuclei of stage 10B and stage 11 follicles, MF bundles were found exclusively at the nuclear membrane (Fig. 7A). In median optical sections F-actin forms an (often interrupted) ring of staining material around the circumference of the nucleus but no bundles inside the nucleus are in focus (Fig. 7B). This observation suggests that the
stained F-actin is mainly or exclusively localized at the nuclear membrane but not inside the nucleus.

During the final phase of nurse cell degeneration, however, F-actin filaments also appear to be in focus in median optical sections (Fig. 7c,d). For this reason, aggregates of F-actin may not only be associated with the nuclear membrane but also be present inside the degenerating nuclei (Fig. 7d).

DISCUSSION

Nurse cell regression is a most rapid process in Drosophila follicles: the nurse cell cytoplasm streams into the oocyte ('terminal injection') where, owing to ooplasmic streaming, it becomes rapidly mixed with the ooplasm. Nurse cell streaming and ooplasmic streaming have been postulated to be under different control since onset and cessation of both processes are different (Gutzeit & Koppa, 1982). This has been confirmed by the inhibitor studies, which show that nurse cell streaming and ooplasmic streaming can be uncoupled by cytochalasins.

Transport of molecules from the nurse cells to the oocyte must also occur in the absence of cytoplasmic streaming. Nurse cell streaming in Drosophila has to be seen rather as an adaptation to rapid oogenesis than as a principal mechanism for molecular transport. In some insects with polytrophic ovaries (e.g. Hyalophora), the phase of terminal injection is never observed. It has been suggested that in Hyalophora molecular transport occurs by way of intercellular electrophoresis (Woodruff & Telfer, 1980). However, there is yet no evidence for such a mechanism in Drosophila follicles (Bohrmann, Huebner, Sander & Gutzeit, 1986). Cytoskeletal elements may well be involved in the localization of molecules at specific sites within the egg as has been demonstrated in other species for the differentiation of the germ plasm (Gutzeit, 1985; Strome & Wood, 1983).

Fig. 7. Microfilament bundles attached to isolated nuclei. F-actin associated with isolated nuclei of stage 10B follicles (A,B) and stage 12 follicle (C,D). A,C, top view (focus in plane of nuclear membrane); B,D, median optical section. Arrows in B point to F-actin lining the nuclear membrane. Bar, A–D, 20 μm.
The reversible inhibition of cytoplasmic streaming by cytochalasins gives indirect support for a contractile mechanism. Cytoplasmic streaming and cell locomotion in a number of different cell types have been studied intensively and a role of the MF network in these processes has been clearly demonstrated in some systems (for recent reviews, see Taylor & Condeelis, 1979; Schliwa, 1984). However, the exact mechanisms are still poorly understood. In the myxomycete plasmodium (*Physarum polycephalum*) cytoplasmic streaming has been thoroughly analysed (reviewed by Kamiya, 1981). In principle, local contractions of membrane-bound actin filaments are thought to lead to local differences in the internal pressure, thus resulting in cytoplasmic streaming (pressure flow). When the cytoplasmic strand of *Physarum* is induced to contract isometrically MF bundles appear in the cytoplasm during the contraction phase (Wohlfarth-Bottermann & Fleischer, 1976). MF bundles can actively contract *in situ* as well as after laser micro-dissection (Isenberg, Rathke, Hülsmann, Franke & Wohlfarth-Bottermann, 1976). If the MF bundles seen in *Drosophila* follicles at the time of nurse cell streaming are contractile structures, a similar pressure flow mechanism as in the myxomycete plasmodium may be operating.

The arrangement and anchoring of MF bundles suggest that cell membrane and nuclear membrane transmit the force generated by MF contractions onto the cytoplasm, which, owing to increased pressure, is forced to flow out of the nurse cell through the cytoplasmic bridge(s) into the oocyte. Nurse cell streaming begins in the proximal nurse cells at stage 10B (Gutzeit & Koppa, 1982). If pressure is generated in all nurse cells at the same time, only those cells that are connected directly to the oocyte by ring canals (proximal nurse cells) are expected to show cytoplasmic streaming at the beginning of the contraction phase. As the pressure in these nurse cells decreases owing to the loss of cytoplasm, streaming in more distal nurse cell begins as seen in in time-lapse films (Gutzeit & Koppa, 1982).

The uniform regression of nurse cells during stages 10B–12 implies that the suggested contractile MF system must be highly regulated and dynamic to ensure that all cells function in concert. The simultaneous and uniform regression of the nurse cells is complicated by the fact that all cells are densely packed and consequently, often irregularly shaped. Furthermore, if neighbouring nurse cells belong to different nurse cell families (Frey *et al.* 1984), their cytoplasm must stream into the oocyte via different routes. Because of the inferred dynamic regulatory mechanisms, we are likely to visualize in our preparations only the momentary MF pattern at the time of fixation.

Transient changes in the streaming pattern during stages 11 and 12 (when masses of MF bundles accumulate in the degenerating nurse cells), give further indirect evidence for cytoplasmic streaming by way of a contractile mechanism based on F-actin.

On present evidence, alternative explanations for nurse cell streaming in *Drosophila* are conceivable. Yet the concept of pressure flow, although only supported by circumstantial evidence, appears most attractive.
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REFERENCES


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