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Comparison of microfilament patterns in nurse cells of different insects with polytrophic and telotrophic ovarioles

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SUMMARY

The localization of F-actin (microfilaments) in the nurse cells of ovarian follicles has been studied in 12 different insect species by fluorescence microscopy after specifically staining F-actin with rhodamine-conjugated phalloidin. In the analysed species with polytrophic ovaries (*Apis mellifica, Pimpla turionellae, Bradysia tritici, Ephestia kuehniella, Protophormia terraenovae*) a dense F-actin network was found to be associated with the nurse cell membranes. Only in *Protophormia* were microfilament bundles seen to extend from the cell membrane into the nurse cell cytoplasm and in a few cases appeared to make contact with the nuclear membrane. In the analysed coleopteran species with telotrophic ovarioles (*Strangalia melanura, Leptinotarsa decemlineata, Oryzaephilus surinamensis*) the fluorescence was also concentrated at the nurse cell membranes only. However, in all analysed hemipteran species (*Lygus pratensis, Calocoris affinis, Graphosoma lineatum, Euscelis plebejus*) the microfilament pattern was very different: while the nurse cells stained only weakly, we always found a characteristic (in some species massive) microfilament network surrounding the trophic core, a central area in the germarium from where material is transported through the trophic cords into the oocytes. The observed differences in the microfilament patterns are likely to reflect different mechanisms for transporting macromolecules and organelles within the ovariole.

INTRODUCTION

Meroistic ovaries are characterized by the presence of nurse cells whose sole function during oogenesis is to synthesize large quantities of macromolecules which finally become incorporated into the growing oocytes. Although the function of nurse cells had been recognized more than a century ago (Lubbock, 1859; Korschelt, 1886), the mechanism for transport of trophoplasm from the nurse cells to the oocyte is still far from being understood. Based on ultrastructural evidence microtubules have been suspected to mediate transport in telotrophic ovarioles but conclusive evidence has not been obtained (reviewed by Hyams & Stebbings, 1979).

Key words: microfilaments, oogenesis, nurse cells, polytrophic, telotrophic, insect.

Recently, intercellular electrophoresis has been suggested as a mechanism for molecular transport (Woodruff & Telfer, 1980). Electrical phenomena are well documented both in polytrophic and telotrophic ovaries. However, with present evidence the contribution of intercellular electrophoresis to the observed cytoplasmic transport within the ovariole cannot be assessed. Circumstantial evidence suggests that it may not be the principal transport mechanism in *Drosophila* (Bohrmann, Huebner, Sander & Gutzeit, 1986).

Surprisingly, microfilaments (abbreviated MF) have not been considered as a motor for intrafollicular transport in meroistic ovarioles. In *Drosophila*, indirect evidence for a pressure flow mechanism during late vitellogenesis has recently been obtained (Gutzeit, 1986) and in this species the importance of MF activity during the phase of nurse cell regression was shown by inhibitor studies combined with the analysis of the MF pattern in the nurse cells before, during and after the period when nurse cell cytoplasm streams into the oocyte.

Recently, we have analysed the MF pattern in the telotrophic ovariole of *Rhodnius* and found a massive array of MF bundles surrounding the trophic core of the tropharium (Huebner & Gutzeit, in preparation). The formation of such a massive array of MF bundles must be dictated by some functional necessity. We have analysed a number of other hemipteran species in order to see whether these MF structures are commonly found amongst this group of insects. We have also included telotrophic ovarioles of polyphage Coleoptera and a number of polytrophic species in our analysis to see if there are common features or striking differences amongst these groups. The principal intercellular connections in the three analysed types of ovaries are illustrated in Fig. 1 (for morphological details see King & Büning, 1985). Although polyphage coleopterans (Fig. 1B) and hemipterans (Fig. 1C) both possess telotrophic ovaries they differ in an important morphological feature: in polyphage coleopterans material synthesized in the nurse cells is transported through interconnected nurse cells directly into the oocyte (like in polytrophic species, see Fig. 1A), while in hemipterans the material first collects in a central acellular region (trophic core) from where it is transported into the oocyte.

In this study we have concentrated on those phases of oogenesis when efficient cytoplasmic transport from the nurse cells to the oocyte is known to occur. In polytrophic ovaries we, therefore, mainly studied the MF pattern in midvitellogenic follicles when the nurse cells are particularly large up to the phase of nurse cell regression. In one species with telotrophic ovarioles (*Rhodnius*: Huebner and Gutzeit, in preparation) and one with polytrophic ovarioles (*Drosophila*: Frey, Sander & Gutzeit, 1984: Warn, Gutzeit, Smith & Warn, 1985; Gutzeit, 1986) the MF pattern during different stages of oogenesis has been analysed in great detail.

We undertook this comparative study to see if the results obtained in those two species can be generalized for other insects with the same type of ovarioles. Our results indicate that the MF pattern observed in *Drosophila* is not typically found in species with polytrophic follicles. Furthermore, the drastically different

organization of MF in the germaria of polyphage coleopterans and hemipterans suggests different functions of these cytoskeletal elements during oogenesis.

MATERIALS AND METHODS

Staining of MF (F-actin) was carried out as described previously (Warn *et al.* 1985) using rhodaminylphalloidin which was kindly provided by Prof. Th. Wieland, Heidelberg, FRG. The following species were collected in a forest at Günterstal near Freiburg: *Lygus pratensis* (Heteroptera: Miridae), *Calocoris affinis* (Heteroptera: Miridae); *Strangalia melanura* (Coleoptera: Cerambycidae). *Graphosoma lineatum* (Heteroptera: Pentatomidae) was found close to the Rhein river north of Breisach. *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) was collected by Dr R. Fleig from a field near Freiburg. In all cases several (6 to 12) mature females were analysed. The following species are kept in the Zoology Institute at Freiburg as laboratory cultures: *Euscelis plebejus* (Homoptera: Jassidae); *Oryzaephilus surinamensis* (Coleoptera: Cucujidae); *Ephestia kuehniella* (Lepidoptera: Pyralidae); *Bradysia tritici* (Diptera: Sciaridae); *Protophormia terraenovae* (Diptera: Calliphoridae); *Pimpla turionellae* (Hymenoptera: Ichneumonidae). A honey bee queen (Hymenoptera: Apidae) from a hive with brood of all stages was kindly provided by Dr F. Platz, Freiburg.

RESULTS

(A) *Polytrophic ovaries*

We have analysed five different species (of three different orders) with polytrophic ovaries. In all analysed species (*Ephestia, Apis, Bradysia, Pimpla* and, with qualifications, *Protophormia*) most of the fluorescence was associated with the nurse cell membranes (Figs 2–10). The MF pattern of the single nurse cell of *Bradysia* (*Sciara*) follicles resembled that shown in Figs 2, 4 for *Ephestia* and *Apis*. Generally the staining was diffuse but in *Apis* follicles (particularly during late stages) we observed aggregates of actin associated with the nurse cell membrane which sometimes looked granular or appeared to consist of a network of short, interconnected MF bundles (Fig. 5). Generally, the MF distribution in the species with polytrophic ovaries analysed here (except *Protophormia*) resembles the situation found in *Drosophila* during early vitellogenic stages up to stage 10A (Warn *et al.* 1985; Gutzeit, 1986). Regressing nurse cells stained only weakly, as illustrated in Fig. 3. In *Pimpla* the nurse cell nuclei were always lined with fluorescence: associated with some particularly large nuclei MF bundles could be recognized (Fig. 6), but in most cases staining was weak and diffuse (Fig. 7).

Prominent MF bundles spanning the nurse cell cytoplasm, as seen in *Drosophila* follicles during the phase of nurse cell regression (Gutzeit, 1986), have not been seen in any of the analysed species except in the dipteran *Protophormia*. In this species MF bundles were seen to extend from the nurse cell membrane into the cytoplasm. In most cases the bundles were short (Fig. 8) and failed to make contact with the nuclear membrane, although some exceptionally long MF bundles were also found (Fig. 9); when the surface of a nurse cell was in focus, numerous MF bundles associated with the cell membrane could be identified (Fig. 10).

Generally, the ring canals connecting two germ-line sister cells were brightly fluorescent only at previtellogenic or early vitellogenic stages; at late vitellogenic

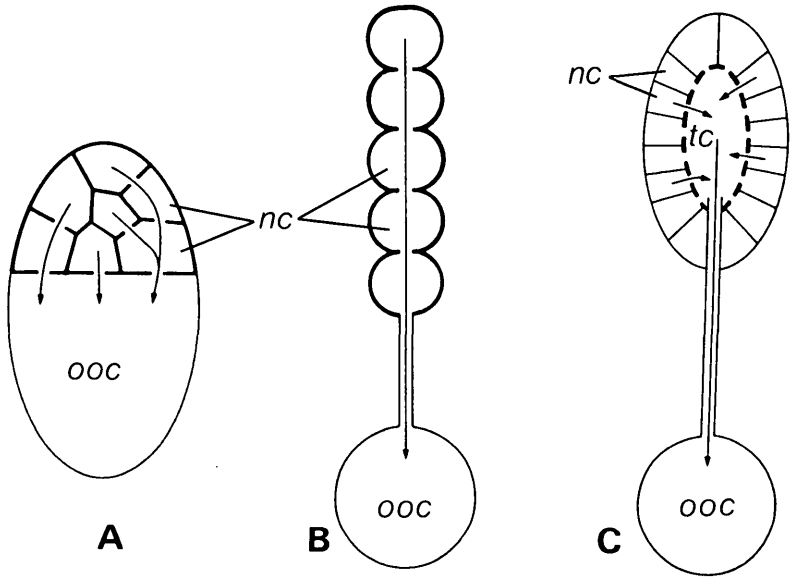


Fig. 1. Schematic drawing of the principal types of oocyte/nurse cell associations analysed here (median sections, follicle cells omitted); (A) follicle of polytrophic ovariole, (B) chain of interconnected germ-line cells in germarium of polyphage coleopteran, (C) germarium of hemipteran, only one of several attached oocytes is shown. Thick lines, locationof particularly dense MF net (diffuse or array of MF bundles, see text for details). Arrows, direction of cytoplasmic transport from nurse cells to oocyte. *nc*, nurse cells;*ooc*, oocyte; *tc*, trophic core; *tcd*, trophic cord.

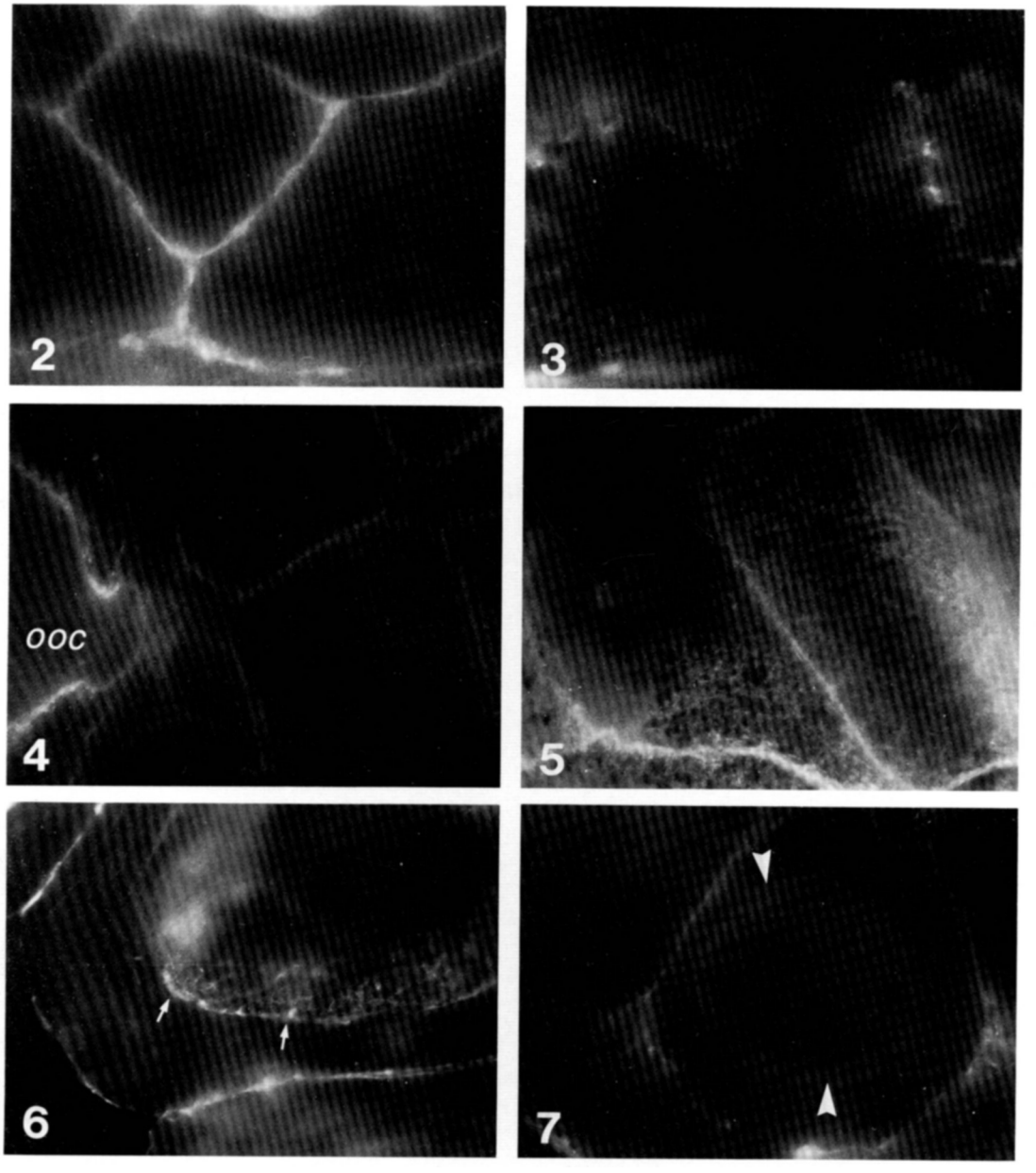
stages, however, they could sometimes no longer be identified by phalloidin-staining (e.g. *Apis*). Further studies with other techniques including electron microscopy must be undertaken to clarify the structure and functional role of the ring canals during late vitellogenesis.

(B) *Telotrophic ovaries*

(1) *Polyphage Coleoptera*

The morphology of telotrophic ovaries in coleopterans has been thoroughly analysed previously (Kloc & Matuszewski, 1977; Büning, 1979). The germ cells form a chain of interconnected cells with the oocyte at the terminal (posterior) position. The nurse cells of a germ cell cluster may be lined up in an almost linear array but branches and fusions of nurse cells are characteristic for some species (Büning, 1979). In principle, this organization is similar to that of polytrophic follicles in which a species-specific number of nurse cells is connected to each oocyte by a defined number of intercellular bridges. The principal difference between polytrophic ovarioles and those of polyphage Coleoptera is that in the latter case the oocyte is distant from its nurse cells and connected with them only by a long tube, the trophic cord (Fig. 1A,B).

The morphological similarities also extend to the MF pattern: the fluorescence was almost exclusively concentrated in the plane of the nurse cell membranes



Figs 2, 3. Lepidoptera: *Ephestia*.

Fig. 2. Fluorescence concentrated at nurse cell membranes (median optical section).

Fig. 3. Degenerating nurse cells do not show any aggregates of MF but stain only weakly.

Figs 4–7. Hymenoptera: Figs 4, 5, *Apis*; Figs 6, 7, *Pimpla*.

Fig. 4. Nurse cell membranes and oocyte cortex stained; *ooc*, oocyte.

Fig. 5. Network of short MF bundles in plane of nurse cell membrane.

Fig. 6. Fluorescence at nurse cell membranes (optical section); note MF bundles associated with nucleus (arrows).

Fig. 7. As in Fig. 5 but fainter and diffuse staining at nuclear envelope (arrowheads). Magnification: ×610.

(Figs 11–16). In most cases the actin network was found to be diffuse. Only in *Leptinotarsa* ovarioles could thin and irregular MF bundles also be seen in the plane of the membrane (Fig. 16).

In all analysed coleopteran species the oocyte cortex showed prominent actin staining (Fig. 13) which was not observed in any of the analysed hemipteran species (see below). The wall of the trophic cords showed no MF staining.

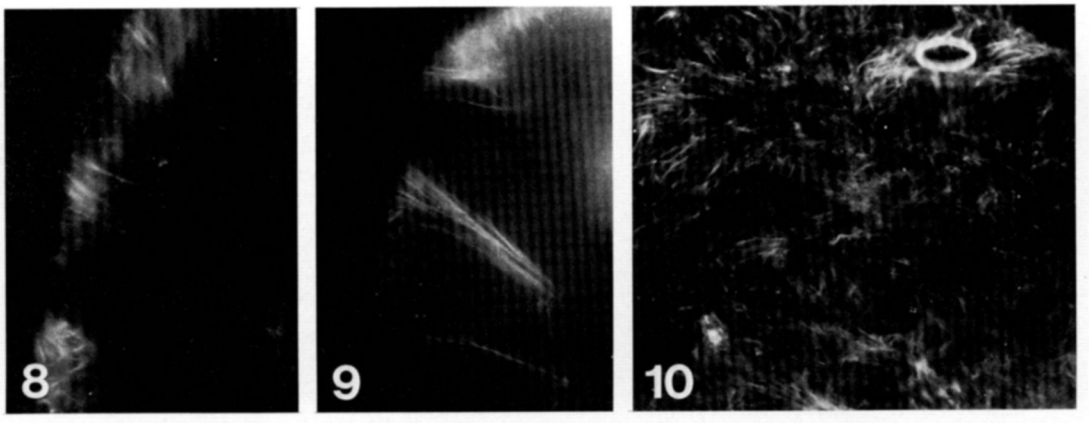
Some follicle cells (interstitial cells) may have contributed to the staining in the germarium. In *Oryzaephilus* follicle cells ('stalk cells') could be seen stained between the oocyte and the nurse cell chamber (Fig. 13).

The organization of nurse cells into strands of interconnected cells was particularly clear in the preparations from *Strangalia* (Fig. 14) and *Leptinotarsa* (Fig. 15).

(2) *Hemiptera (Rhynchota)*

The organization of the hemipteran ovary differs from those of the previously described types of meroistic ovaries in an important aspect: all nurse cells contribute to the growth of all connected oocytes. Newly synthesized material accumulates in a central area of the germarium, the trophic core, from where the material is transported through the trophic cord into the growing oocyte(s) (Fig. 1C; for morphological details see Huebner, 1984; King & Büning, 1985). The trophic core is, therefore, a likely site for the generation of motive force for the transport of nutrients down the trophic cord.

The morphological peculiarities are reflected in a strikingly different MF pattern as compared to the previously described ovariole types. In all analysed hemipteran

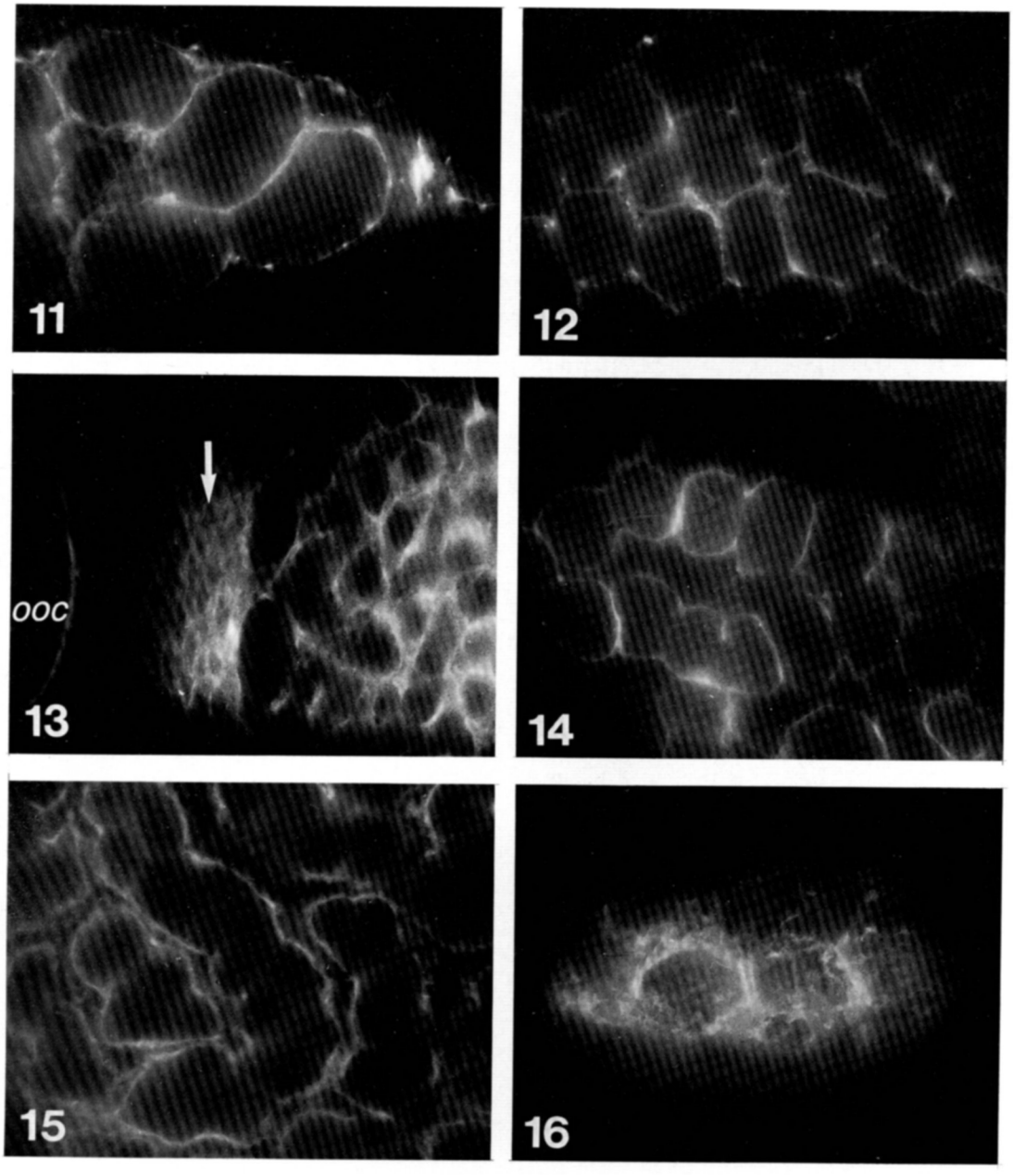


Figs 8–10. Diptera: *Protophormia*.

Fig. 8. Median optical section: MF bundles radiating from nurse cell membrane into nurse cell cytoplasm.

Fig. 9. Same as in Fig. 8 but MF bundles are very long and probably contact the nuclear envelope.

Fig. 10. Focus in plane of membrane; short MF bundles associated with nurse cell membrane (length of bundles approximately as in Fig. 8 but focus differs; note the brightly fluorescent ring canal). Magnification: ×610.



Figs 11–16. Coleoptera: Figs 11, 12, *Dasytes*; Fig. 13, *Oryzaephilus*; Fig. 14, *Strangalia*; Figs 15, 16, *Leptinotarsa*.

Fig. 11. Nurse cells at apex of germarium (optical section).

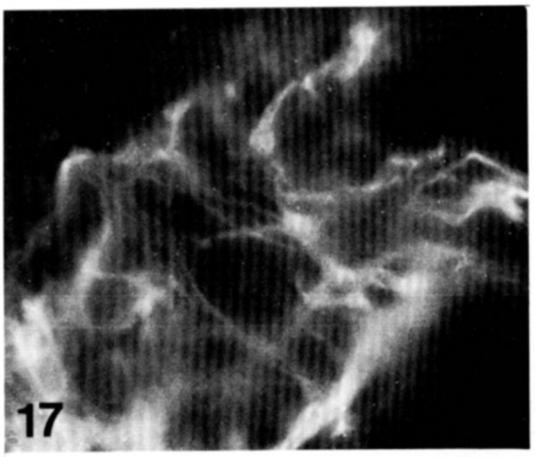
Fig. 12. Nurse cells from central region of germarium (optical section).

Fig. 13. Basal region of germarium with nurse cells (at the right), followed by follicle stalk cells (see arrow) and the anterior part of the oocyte; note the strong fluorescence of the oocyte cortex; *ooc*, oocyte.

Fig. 14. Two nurse cell clusters (optical section).

Fig. 15. Groups of interconnected nurse cells (optical section).

Fig. 16. Focus in plane of nurse cell membrane; MF bundles become distinguishable. Magnification: ×610.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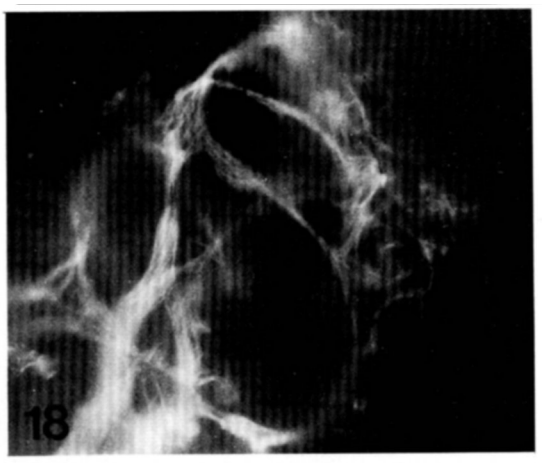
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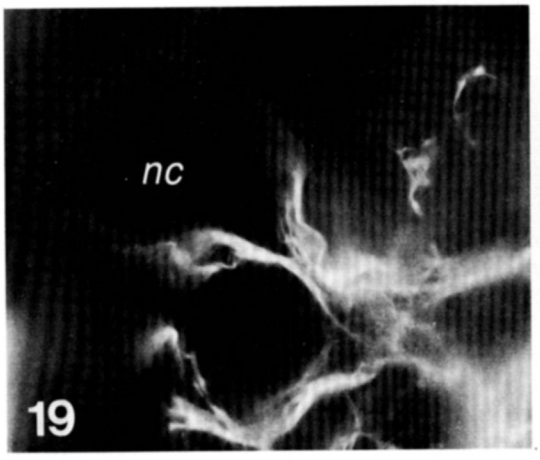

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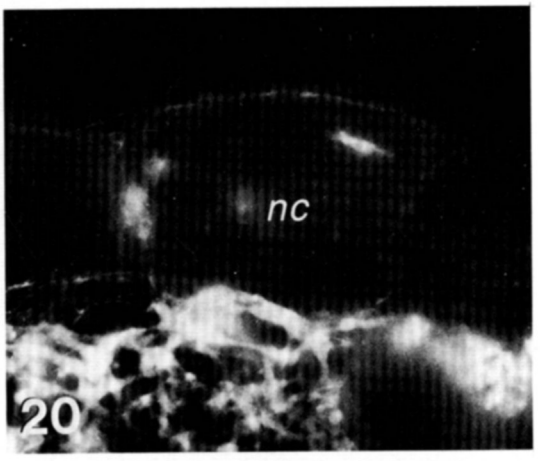

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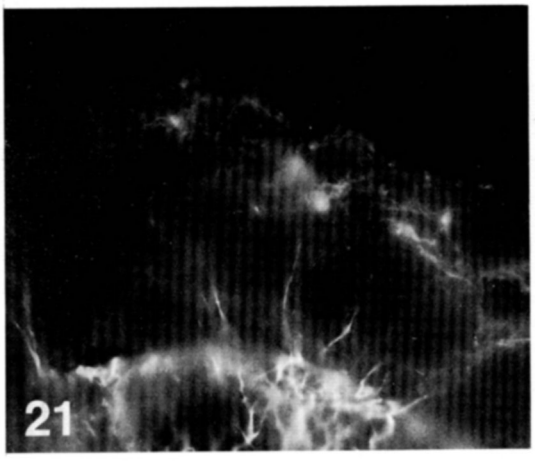
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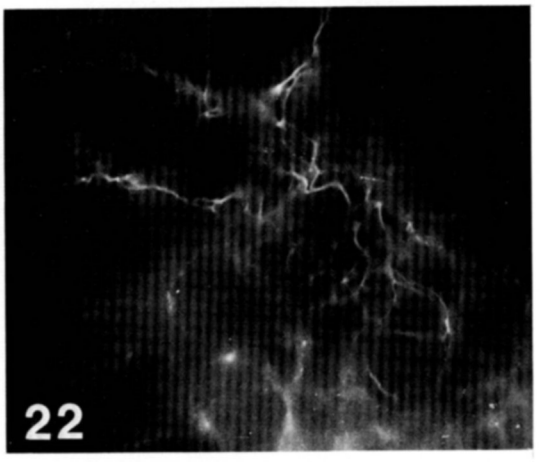
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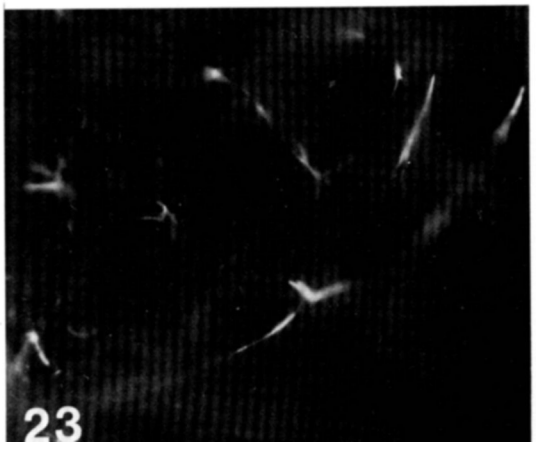
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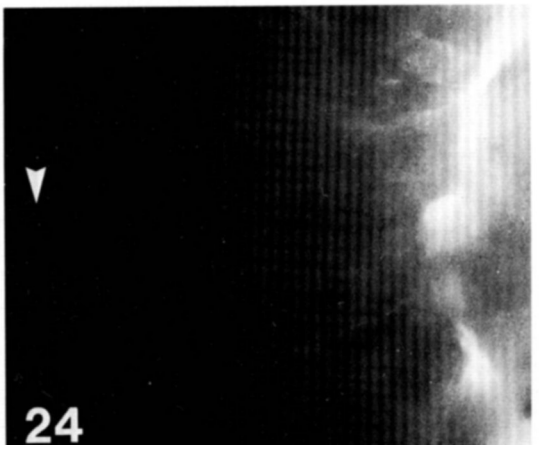
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species the trophic core was surrounded by a network of MF bundles which differs species specifically in its organization. The most dramatic MF network amongst all analysed species was found in *Lygus*: the trophic core was enclosed by a dense net of thick MF bundles (Figs 17, 18). In some cases particularly thick MF bundles could be resolved into sub-bundles (Fig. 18). At the ovariole apex some MF bundles flared out towards the nurse cells but little F-actin was associated with the nurse cell membranes (Fig. 19). This was particularly clear at the laterally located nurse cell lobes (Fig. 20). Here fewer branches of MF bundles were seen to extend from the trophic core into the nurse cell lobes than in other species (see below). A similar but less dramatic MF pattern was observed in *Calocoris*. The nurse cell membranes stained only weakly in both species.

In *Euscelis* the MF scaffold had a rather different appearance: long and thin MF bundles formed an intricate net around the trophic core while the basal portion was lined by a barrel-shaped trabecular network (Fig. 22). Some MF strands extended into the nurse cell lobes (Fig. 21) or flared out at the top of the germarium.

In *Graphosoma* the MF form a delicate, wide-mesh network which was clearly localized at the margin of the core as shown in cross sections (Fig. 24). Short but thick MF bundles formed delicate triradiate junctions (Fig. 23).

DISCUSSION

All analysed species may be classified into two groups according to the MF pattern in the nurse cells: while in all species with polytrophic ovaries and in the polyphage coleopterans F-actin was concentrated at the nurse cell membranes (Fig. 1A,B), the hemipteran ovaries were organized differently (Fig. 1C). Here a basket formed by MF bundles surrounded the trophic core in all analysed species including *Rhodnius prolixus* (Heteroptera: Reduviidae), a species whose MF pattern in the germarium will be described separately (Huebner & Gutzeit, in

Figs 17–24. Hemiptera: Figs 17–20, *Lygus*; Figs 21, 22, *Euscelis*; Figs 23, 24, *Graphosoma*.

Fig. 17. Dense network of massive MF bundles surrounding the trophic core (nurse cell lobes at either side of the core cannot be identified due to their very weak staining).

Fig. 18. Same as in Fig. 17; note that the thick MF strands consist of numerous smaller sized MF bundles.

Fig. 19. Nurse cell lobes at apex of germarium; MF bundles flare out between nurse cells whose cell membranes are only weakly stained (optical section); *nc*, nurse cell.

Fig. 20. Cross section of germarium; trophic core at the bottom, two or three lateral nurse cells above. Massive array of MF in region of trophic core but nurse cell membranes are only weakly stained; *nc*, nurse cell.

Fig. 21. Cross section of germarium; MF bundles flare out from core surface into lobes.

Fig. 22. Fine MF network around trophic core.

Fig. 23. MF net with conspicuous triradiate junctions.

Fig. 24. Cross section through germarium, core at the right; MF network around core is brightly fluorescent but little fluorescence shows in the nurse cell lobes and nurse cell membrane (arrowhead). Magnification: ×610.

preparation). The MF basket varied in complexity between the hemipteran species studied, ranging from thin, wide-mesh filamentous type in *Graphosoma* to massive interconnected MF bundles in *Lygus*. We do not know if these bundles are contractile and provide the moving force for cytoplasmic transport. An alternative possibility must be considered: hydrostatic pressure could build up in the germarium through increased osmolarity which may, for example, be the result of continued activity of ion pumps. Increased internal pressure could result in motive force for transport of diffusible molecules or of organelles. In this situation the MF network (and possibly also the basal lamina surrounding the ovariole) might act as a mechanical strengthening device to limit expansion of the germarium.

In some special cases MF bundles are thought to have lost their contractile properties. Examples are the ring canals, which are heavily lined by MF in *Drosophila* (Warn *et al.* 1985; Frey *et al.* 1984) and have been interpreted as non-contractile rings that aid in the mechanical stability of the ring canals. Some conspicuous MF bundles seen in tissue culture cells ('stress fibers') may also have lost their contractile properties (Herman, Crisona & Pollard, 1981).

Based on inhibitor studies and time-lapse cinematography, indirect evidence for cytoplasmic streaming by pressure flow in the polytrophic follicles of *Drosophila* has been obtained recently (Gutzeit, 1986). At the phase when nurse cell cytoplasm rapidly streams into the oocyte (at stages 10B to 12), MF bundles could be seen to span the nurse cell cytoplasm between nuclear membrane and cell membrane. However, before this stage (from early vitellogenesis up to stage 10A) no streaming of nurse cell cytoplasm could be observed. At these stages F-actin is concentrated mainly at the nurse cell membrane, thus resembling the F-actin distribution in all analysed species with polytrophic ovaries (except *Protophormia*) and in polyphage Coleoptera. In follicles of *Bradysia* during the phase nurse cell regression, MF are also located mainly at the cell membranes and in this case, too, no cytoplasmic streaming could be detected in time-lapse films. Possibly rapid cytoplasmic streaming requires formation of microfilament bundles for force generation. It should be stressed, however, that cytoplasmic transport does not depend on cytoplasmic streaming, which may only be regarded as an adaptation to most rapid oogenesis in the dipterans *Drosophila* and (possibly) *Protophormia* in which radial MF bundles have been found in the nurse cells during the phase of nurse cell regression.

This comparative study surveying the distribution of F-actin in insect ovarioles reveals three different types of MF patterns in nurse tissue as exemplified by *Protophormia, Ephestia* (or *Dasytes*), and *Lygus*. This suggests that F-actin networks, albeit with partly different functions, must be considered in any model of nurse cell/oocyte transport mechanisms.

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