INTRODUCTION

Illumination causes striking photomechanical phenomena in many photoreceptor cells. Such phenomena are well known from lower vertebrates, where the light-dependent extension and retraction of the photoreceptor cells expose either cones or rods to the incident light (reviewed by Burnside and Nagle, 1983). Arthropod photoreceptors exhibit a variety of light-induced modifications in cellular structure, resulting in changes in sensitivity and spatial resolution (reviewed by Autrum, 1981; Walcott, 1975). Among them are organelle translocations perpendicular to the longitudinal axis of the photoreceptor cells within the compound eye of, for example, the locust, the worker honeybee, the cockroach and the ant (Horridge and Bernard, 1965; Kolb and Autrum, 1972; Snyder and Horridge, 1972; Menzel and Lange, 1971; Menzel, 1972). In these systems, sacculi or cisternae of smooth endoplasmic reticulum (ER) are juxtaposed to the bases of the photoreceptive microvilli. Light stimulation causes a translocation of the ER elements towards the main cell body, and an aggregation of mitochondria adjacent to the microvilli. Immunofluorescence studies and electron-microscopic examination of chemically fixed or high-pressure-frozen, freeze-substituted specimens demonstrate a lack of microtubules in the submicrovillar region. However, numerous filament bundles are aligned in close association with mitochondria and ER elements, along the track of their movement. Fluorescent phalloidins and monoclonal antibodies label filament bundles in the submicrovillar region, indicating that they are composed of F-actin. Finally, depolymerization of the submicrovillar actin filaments by incubation with cytochalasin B results in a blockade of the movement of mitochondria and ER cisternae towards the rhabdom. These results suggest that the light-dependent translocation of both ER cisternae and mitochondria occurs along actin filaments.

SUMMARY

Light-dependent changes in the positioning of organelles in photoreceptor cells of arthropods are a well-known phenomenon. In this study, we examine the role of the cytoskeleton in these light-dependent antagonistic movements. In dark-adapted photoreceptor cells of the locust *Schistocerca gregaria*, prominent sacs of smooth endoplasmic reticulum (ER) oppose the bases of the photoreceptive microvilli. Light stimulation causes a translocation of the ER elements towards the main cell body, and an aggregation of mitochondria adjacent to the microvilli. Immunofluorescence studies and electron-microscopic examination of chemically fixed or high-pressure-frozen, freeze-substituted specimens demonstrate a lack of microtubules in the submicrovillar region. However, numerous filament bundles are aligned in close association with mitochondria and ER elements, along the track of their movement. Fluorescent phalloidins and monoclonal antibodies label filament bundles in the submicrovillar region, indicating that they are composed of F-actin. Finally, depolymerization of the submicrovillar actin filaments by incubation with cytochalasin B results in a blockade of the movement of mitochondria and ER cisternae towards the rhabdom. These results suggest that the light-dependent translocation of both ER cisternae and mitochondria occurs along actin filaments.

Key words: organelle translocation, cytoskeleton, actin filament, microtubule, photoreceptor cell, locust (Insecta)

Actin-dependent light-induced translocation of mitochondria and ER cisternae in the photoreceptor cells of the locust *Schistocerca gregaria*

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(Bradley and Satir, 1979; Burnside and Nagle, 1983). (3) Antibodies raised against myosin I, a possible motor for the transport of membrane-bound organelles along actin filaments (Pollard et al., 1991; Coudrier et al., 1992), bind to vesicular structures in the intestinal brushborder (Drenckhahn and Dermitzel, 1988). The presence of myosin I on vesicles within intestinal epithelial cells has been further established by biochemical techniques (Fath and Burgess, 1993). (4) The transport of vesicles along actin filaments can be directly observed by video microscopy of extruded axoplasm of the squid giant axon (Kuznetsov et al., 1992; Bearer et al., 1993).

We have analysed the organization of the cytoskeleton in the photoreceptors of Schistocerca gregaria and the spatial relationship of cytoskeletal elements with respect to mobile organelles by means of cytochemical and electron-microscopic techniques. Our results demonstrate actin filaments, but no microtubules, in close association with the translocated organelles, aligned in the path of their movement. Moreover, we have examined the effect of cytochalasin-induced depolymerization of actin filaments on organelle position and mobility. The results of these experiments indicate that the light-dependent movements of mitochondria and ER cisternae depend on the actin cytoskeleton.

MATERIALS AND METHODS

Animals and preparation

Locusts (Schistocerca gregaria) were obtained from colonies at the Institute. They were kept under a 12-hour:12-hour light-dark rhythm (lights on at 7:00 and off at 19:00). Eyes were prepared at noon. Depending on the adaptational state needed for the experiment, the animals were decapitated under either room light or red light (>610 nm; filter: RG610, Schott, Mainz, Germany) to which the photoreceptor cells of Schistocerca gregaria are insensitive (Vishnevskaya et al., 1986). Subsequently, the eyes were removed from the animals and cut in half with a razor blade. The isolated eye pieces were then transferred to oxygenated Ringer solution (200 mM NaCl, 3.4 mM KCl, 0.5 mM CaCl₂, 10 mM Tris-HCl, 90 mM glucose, pH 7.0) and transferred to oxygenated Ringer solution (200 mM NaCl, 3.4 mM KCl, 0.5 mM CaCl₂, 10 mM Tris-HCl, 90 mM glucose, pH 7.0) and incubated in this solution during the illumination protocol. For studies of the dark-adapted state, specimens were kept in complete darkness. For light adaptation, the eye pieces were illuminated with a 100 W halogen lamp with a heat-absorbing filter (KG1) in the light path. When measured with a calibrated photodiode, the incident light energy was 1.8 mW/cm² at the level of the eyes.

Conventional electron microscopy

Isolated eyes were dark-adapted or illuminated for 1 hour, and then fixed for 4 hours at room temperature (RT) with 4% glutaraldehyde in 0.1 M phosphate buffer (PB), pH 7.4. Subsequently, the samples were washed, post-fixed for 2 hours at 4°C in 2% phosphate-buffered OsO₄, dehydrated in a graded ethanol series and embedded in Epon resin. Ultrathin sections were stained with uranyl acetate and lead acetate, and examined with a Zeiss EM 10 C (Zeiss, Oberkochen, Germany) at 60 or 80 kV.

High-pressure freezing

The compound eyes were dissected as described above and slices of approximately 0.5 mm thickness were cut parallel to the ommatidial axis with a razor blade. Tissue slices were transferred into aluminum plates filled with hexadecane (Fluka, Buchs, Switzerland) and covered with a second platelet (Studer and Müller, 1989; Studer et al., 1989; Wolfrum, 1990). The specimens were then high-pressure frozen in an HPM 010 (Balzers Union, Liechtenstein) at about 2100 bar (Müller and Moor, 1984; Moor, 1987). Subsequently, the frozen samples were freeze-substituted in dry acetone containing 2% OsO₄ (U. Wolfrum, 1990), and embedded in Epon.

Quantitative analysis of the light-dependent movement of organelles

Cross-sections taken at comparable levels within the ommatidia were photographed and enlarged to a final magnification of 13,000. Photoreceptor cells were subdivided by concentric circles around the rhabdom into three different areas, the inner, the middle and the outer area, as illustrated in Fig. 3A, below. To obtain the relative distribution of mitochondria and pigment granules, their number within each of the three areas was counted and divided by their total number within the cell. For the evaluation of the distribution of the ER, a grid lattice (square width 0.5 cm) was laid over the electron micrographs in a random direction. The numbers of intersections within the ER and within the entire cell were counted. The relative area of the ER was calculated by dividing the number of points within the ER by the total number of points within the cell profile. For each adaptational state, 10 cells in each of three animals were analysed.

Immunogold labelling

Specimens were fixed for 2 hours at RT in 3% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M PB, dehydrated in a graded ethanol series and embedded in LR-White. The samples were UV-polymerized for 3 days at 4°C (U. Wolfrum, personal communication). Ultrathin sections were cut and transferred onto Formvar-coated nickel grids. Selected grids were preincubated with 0.01% Tween 20 in phosphate-buffered saline (PBS) for 10 minutes, then treated with 50 mM NaHCl in PBS for 15 minutes to block free aldehyde groups, washed in PBS and incubated with blocking solution (BS; 1% normal goat serum, 0.1% fish gelatine, 0.8% bovine serum albumin in PBS) for 30 minutes. Subsequently, the sections were incubated for 60 hours at 4°C with monoclonal mouse IgM anti-actin antibody (1:750 in BS; Amersham, Buckinghamshire, UK). This antibody has been shown to react specifically with actin in various insect species (Blest et al., 1991; Arikawa et al., 1990). After several washes with PBS and ‘gold buffer’ (0.01 M PB, pH 7.3, with 0.01% Tween 20, 0.1% ovalbumin, 0.5% fish gelatine, 0.5 M NaCl; U. Wolfrum, personal communication), the sections were incubated for 2 hours at RT with 5 nm gold-coupled goat anti-mouse IgG/IgM antibody (BioCell, Cardiff, UK) diluted 1:10 in gold buffer, washed with PBS and fixed with 2.5% glutaraldehyde in PBS. The sections were contrasted with 2% uranyl acetate in ethanol for 2 minutes. On some sections, gold particles were silver-enhanced according to Danscher (1981). Control sections were incubated with normal mouse serum (Sigma, Deisenhofen, Germany) diluted 1:500 in BS.

Immunofluorescence light microscopy

The procedure followed that described by Wolfrum (1990, 1991). Briefly, specimens were fixed for 2 hours at 4°C in 3% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M PB, washed in buffered 10% sucrose, infiltrated overnight at 4°C with buffered 25% sucrose and frozen in melting isopentane. Sections (7-10 µm thick) were cut in a cryostat (2800 Frigocut, Reichert-Jung, Nussloch, Germany) at −18°C to −25°C and collected on poly-L-lysine-coated coverslips. To reveal the distribution of F-actin, sections were incubated for 1 hour with 4 µM TRITC-phalloidin (Sigma) in PBS, washed in PBS, and mounted in Mowiol 4.88 (Farbwerke Hoechst, Frankfurt, Germany) containing 2% n-propyl-gallate as an anti-fading agent. For immunofluorescence labelling of the microtubules, sections were pretreated as described above for immunogold staining, and incubated for 1 hour with either a polyclonal rabbit antibody against sea urchin tubulin (gift from D. B. Murphy; 1:200 in BS) or a monoclonal mouse anti-α-tubulin (clone DM 1A, Sigma; 1:200 in BS). After extensive washing in PBS, sections were incubated with either TRITC-conjugated goat anti-rabbit IgG or FITC-conjugated sheep anti-mouse IgG (Sigma) for
RESULTS

General morphology
The compound eyes of insects consist of up to several thousand individual units, the ommatidia. A detailed description of the morphology of the locust eye is given by Wilson et al. (1978). Fig. 1 shows the general morphology of transversely cut, dark- and light-adapted ommatidia of the compound eye of the locust *Schistocerca gregaria* as seen in the light microscope. Each ommatidium contains 5-6 large and 2 small photoreceptor cells that are arranged in a rosette. The photoreceptive microvilli contain the visual pigment and form a fused-type rhabdom in the centre of the ommatidium. The photoreceptor array is surrounded by pigment cells that shield neighbouring ommatidia optically.

Morphology of dark-adapted photoreceptors
Fig. 2A demonstrates the ultrastructural morphology of the photoreceptor cells in a dark-adapted specimen. Voluminous sacculi of smooth ER reside below the photoreceptive microvilli. This submicrovillar ER forms a zone, approximately 2 μm in width around the rhabdom and separates the transduction compartment, which is composed of the rhabdom surrounded by a rim of cytoplasm ~0.2 μm in width, almost completely from the remaining cell body. Only a few narrow cytoplasmic bridges cross the submicrovillar ER and connect the microvillar/submicrovillar compartment with the bulk cytoplasm. The submicrovillar ER also closely approaches the lateral smooth plasma membrane, leaving only a 25 nm wide gap. The remaining cell body contains numerous other cell organelles, such as mitochondria, pigment granules, rough ER cisternae, smooth ER elements and multivesicular bodies, with no apparent regular arrangement.

Two other structural features of the retina can be seen in Fig. 2; they will reappear in later parts of the paper. Zonulae adherentes link adjacent photoreceptor cells close to the rhabdom. Moreover, the oval profiles sandwiched between the photo-

receptors are cross-sections of crystalline cone processes that extend from the dioptric apparatus down to the basal lamina.

Light-induced structural changes
The light micrograph in Fig. 1B and the electron micrograph in Fig. 2B illustrate the dramatic changes in the organization and positioning of organelles that occur during exposure to light for 1 hour, at an intensity within the range of daylight. The submicrovillar ER, visible as a clear zone around the rhabdom in the light micrograph, disappears (Figs 1B, 2B). A large number of mitochondria aggregates closely around the rhabdom. Numerous smaller saccula ER elements are seen further away from the rhabdom, indicating that the large submicrovillar ER cisternae move in a centrifugal direction (Fig. 2B). We have not been able to determine whether these smaller ER elements represent profiles of a morphologically continuous, loosely spaced network or whether the ER disintegrates into numerous individual sacculi.

The light-dependent structural changes were further analysed quantitatively. For this purpose, cross-sectioned photoreceptors were subdivided by concentric circles around the rhabdom into an inner area (the region next to the rhabdom), a middle area and an outer area lying closest to the pigment cells (Fig. 3A). The distribution of ER, mitochondria and pigment granules over these areas was evaluated by morphometric analysis (see Materials and Methods). The results of these analyses are summarized in Fig. 3B-D.

The most important finding of the quantitative analyses was that the amount of ER and the number of mitochondria, respec-
tively, change only in the inner and the middle zones in response to light. In dark-adapted photoreceptors, most (55% ± 14%; mean ± s.d.) mitochondria are located in the middle area of the cell, whereas in light-adapted photoreceptors, most

Fig. 2. Electron micrographs showing cross-sections through ommatidia. (A) Dark-adapted ommatidium. The smooth ER is mainly located around the rhabdom and builds vacuole-like submicrovillar cisternae (black asterisk). Numerous mitochondria (thin arrows) and pigment granules (white asterisks) are found behind the submicrovillar ER. (B) Light-adapted ommatidium. Mitochondria (thin arrows) surround the rhabdom (r), whereas the submicrovillar cisternae of the ER have disappeared. Smaller elements of smooth ER (arrowheads) are distributed in the cell body. Broad arrows in A indicate zonulae adherentes. Bar, 1 µm.
Actin-dependent organelle motility

Mitochondria are found in the inner area (55% ± 12%). In the outer area, the number of mitochondria does not change in response to light (dark: 25% ± 11%; light: 23% ± 12%). Correspondingly, in dark-adapted cells the largest amount of the ER (54% ± 13%) is located within the inner area, whereas in light-adapted cells, the largest amount (50% ± 9%) resides in the middle area of the cell. These results indicate that the antagonistic movements of ER and mitochondria occur only between the inner and the middle area, and that organelles in the outer area are not involved in the movement of organelles. Fig. 3C demonstrates that pigment granules do not change their relative position within the cell in response to light.

The submicrovillar cytoskeleton

To obtain information about the cytoskeletal components involved with the light-dependent organelle translocations, we have examined the distribution of microtubules and actin filaments in *Schistocerca gregaria* photoreceptors.

Microtubules were visualized by indirect immunofluorescence using a monoclonal anti-α-tubulin antibody (results not shown) or a polyclonal antibody against sea urchin tubulin (Fig. 4). Both antibodies provided the same results. On some sections, the nonmicrovillar plasma membrane of the photoreceptors was visualized by staining with anti-Na,K-ATPase in order to assign anti-tubulin staining to the photoreceptors or the pigment cells (Fig. 4B). Fig. 4A demonstrates intense anti-tubulin staining in both the crystalline cone processes and the pigment cells. There was only weak anti-tubulin staining

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Fig. 3. Quantitative evaluation of the organelle distribution in dark- and light-adapted photoreceptor cells. (A) A single photoreceptor cell was subdivided into an inner, middle and outer area by concentric circles around the rhabdom. (B) Distribution of mitochondria over the three different areas in dark- and light-adapted cells. (C) Distribution of pigment granules. (D) The relative volume of the ER cisternae in the three different areas in relation to the total cellular volume in dark- and light-adapted photoreceptors.

Fig. 4. Immunofluorescence localization of microtubules in the locust retina. (A) Fluorescence image of anti-tubulin staining; (B) superimposed images of anti-tubulin staining (red) and anti-Na,K-ATPase staining (green); and (C) the corresponding Nomarski-contrast image. The anti-Na,K-ATPase was applied to outline the plasma membrane of the photoreceptor cells. Anti-tubulin staining is found at the crystalline cone processes (arrowheads), within the pigment cells (arrows), and in the cell body of the photoreceptors (white asterisks). The rhabdom (black asterisk) and the submicrovillar cell area have no obvious anti-tubulin staining. Bar, 5 μm.
within the photoreceptor cells, all of it localized to the cell body and distant from the area where light-induced organelle movements occur.

However, the absence of anti-tubulin immunofluorescence in the submicrovillar area may have resulted from a fixation artefact. Moreover, we may not have detected individual microtubules in the submicrovillar area by immunofluorescence. To test these possibilities, we examined the distribution of microtubules by electron microscopy of chemically fixed and of high-pressure-frozen, freeze-substituted specimens. With either fixation protocol, no microtubules could be detected in the submicrovillar cytoplasm (Fig. 5A,B,D). Numerous paraxially aligned microtubules were observed in the remaining cell body (Fig. 5C), and within the crystalline cone processes (Fig. 2). In some cases, a single microtubule was associated with the zonulae adherentes (not shown).

Electron microscopic examination of chemically fixed or high-pressure-frozen, freeze-substituted photoreceptors visualized bundles of filaments in the submicrovillar cytoplasm. These filament bundles radiated from the rhabdom into the cell body. In dark-adapted photoreceptors, the filaments extended through the cytoplasmic bridges, that traversed the submicrovillar ER cisternae, into the middle cell area (Fig. 5A,B). Often, the filaments were seen in close association with the mitochondria. Similar filaments were observed in the submicrovillar cytoplasm of light-adapted photoreceptors (Fig. 5D).

Labelling of cryostat sections with fluorescent phalloidin indicated that the submicrovillar filaments that we observed by electron microscopy represented actin filaments. In phalloidin-labelled cryostat sections, intense staining could be assigned to the microvillar rhabdom in the centre of the ommatidium. In addition, fluorescent ‘stripes’ radiated from the rhabdom into the cell body, indicating F-actin in the submicrovillar region (Fig. 6A). We could not observe any difference in staining between dark- and light-adapted photoreceptors.

The distribution of actin was further examined by indirect immunogold labelling with a monoclonal anti-actin antibody (Fig. 7). An intense anti-actin labelling was found on the

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**Fig. 5.** Electron micrographs of cytoskeletal elements in the submicrovillar region of the cell. (A,B) Conventional fixation. (A) Detail of a cross-section through cytoplasmic bridges that cross the submicrovillar cisternae. The section plane is indicated by arrows in B. No microtubules can be detected but electron-dense structures (arrowheads) may represent bundles of filaments in cross-section. (B) Detail of a cross-section through a dark-adapted cell. A bundle of filaments (arrowhead) protrudes from the rhabdom and extends radially through a cytoplasmic bridge into the cell body. (C,D) High-pressure-frozen specimens. (C) Numerous microtubules extend longitudinally through the main cell body (arrows). (D) Microtubules are absent in the submicrovillar region, whereas filamentous material (arrowheads) lies parallel with the direction of movement of mitochondria and ER cisternae. Asterisks, ER cisternae; double arrowhead, zonula adherens; broad arrow, plasma membrane; m, mitochondria. Bars, 0.3 μm.
microvillar rhabdom and in the submicrovillar area. Here, the anti-actin antibody stained the cytoplasmic bridges extending through the submicrovillar ER cisternae of dark-adapted photoreceptors (Fig. 7B). Distinct labelling was also closely associated with mitochondria, whereas the mitochondria themselves remained unlabelled. Moreover, anti-actin labelling was found at the zonulae adherentes, the crystalline cone processes and underneath the plasma membranes. Controls with normal mouse serum did not show labelling other than a low background.

Fig. 6. Fluorescent micrographs of cross-sections through ommatidia. (A) Labelling of a DMSO-incubated control with TRITC-phalloidin. The rhabdom shows intensive staining. Moreover, fluorescent stripes (arrowheads) radiate from the rhabdom into the cell body. F-actin labelling is also visible at the periphery of the ommatidia (arrows). (B) Labelling of cytochalasin-treated cells with TRITC-phalloidin. The rhabdom still shows weak staining, whereas the fluorescent stripes radiating from the rhabdom have completely disappeared (arrowhead). The labelling at the ommatidial periphery remained intense (arrows). Bar, 5 μm.

Fig. 7. Indirect immunogold labelling with a monoclonal antibody raised against actin. (A) Cross-section through an ommatidium. The lateral cell borders of the 6 large and 2 small photoreceptors can be conceived by the position of the zonulae adherentes (arrowheads). Silver-enhanced gold particles are localized in the area of the rhabdomeric microvilli (r) and the zonulae adherentes (arrowheads). Prominent labelling can be found in the cytoplasmic bridges (arrows) that traverse the submicrovillar cisternae (asterisk). (B) Detail of the submicrovillar cell area. The cytoplasmic bridges are labelled with anti-actin (arrows). Note that the ER cisternae (asterisk) and the mitochondria are unlabelled, indicating the specificity of the anti-actin binding. The arrowhead marks a zonula adherens. Bars: 1 μm (A), 0.25 μm (B).
In conclusion, these results suggest that bundles of actin filaments but no microtubules are aligned along the path of mitochondrial and ER movement.

**Experiments with cytoskeletal drugs**

To further examine the role of the cytoskeleton in light-dependent organelle translocations, we have applied cytoskeletal drugs to depolymerize either actin filaments or microtubules.

Cytochalasin B disturbs the actin filament cytoskeleton by blocking the addition of G-actin to the plus ends of the filaments and by cleavage of filaments (Casella et al., 1981). When retinal tissue was incubated for 2 hours with 20 μM cytochalasin B in oxygenated Ringer solution, ultrastructural examination demonstrated that bundles of filaments had disappeared in the submicrovillar area, whereas other F-actin-containing structures, such as the rhabdom and the zonulae adherentes, seemed unchanged. Microtubules in the main cell body and in the cone threads also appeared unaffected. Labelling of cytochalasin-treated retina with fluorescent phallotoxins (Fig. 6B) showed total disappearance of the staining in the submicrovillar region, whereas the rhabdom still showed weak labelling. Intense staining at the periphery of the ommatidia remained even after cytochalasin treatment. In control tissue incubated with 0.2% DMSO, staining of the rhabdom, the ommatidial periphery and the submicrovillar F-actin appeared intact (Fig. 6A). Thus, treatment with cytochalasin led to the depolymerization of mainly the submicrovillar actin filaments.

Four different experiments were set up to study the effects of cytochalasin B on light-dependent organelle translocations. For the first two experimental protocols, dark-adapted eyes were excised and incubated for 2 hours in the dark in oxygenated saline with the addition of cytochalasin in DMSO. The eyes were then incubated for a further hour, either in darkness or in light. For the other two protocols, animals were light-adapted, the eyes were then isolated and incubated with cytochalasin during constant illumination. Subsequently, the eyes where either further light-adapted or dark-adapted. For controls, eyes were incubated with DMSO in oxygenated saline and treated with the same illumination protocols as described above.

In cytochalasin-treated dark-adapted photoreceptors that were then illuminated (Figs 8A, 9D), both submicrovillar ER and mitochondria occupied positions distant from the rhabdom. The ER appeared to have disintegrated into smaller elements.

The vacuolar ER cisternae of a cytochalasin-treated, constantly dark-adapted ommatidium were located further away from the rhabdom (Figs 8B, 9C) than in untreated controls, indicating a movement of the entire submicrovillar ER away from the rhabdom. Moreover, most mitochondria were also located in the middle area of the cell. Incubation of light-adapted photoreceptors with cytochalasin also led to a positioning of ER and mitochondria distant from the rhabdom, independent of whether the eyes remained in the light or were dark-adapted (Fig. 9E,F). Thus mitochondria and ER cisternae resided at some distance from the rhabdom, irrespective of the adaptational state or the sequence of illumination. This indicates that an intact actin filament system is required for the translocation of these organelles towards the rhabdom and for the maintenance of their position at the rhabdom.

Experiments with the microtubule-disrupting agent colchicine were not conclusive. When retinal tissue was incubated with 50 μM colchicine for 3 hours and exposed to the same illumination protocol as described above, mitochondria and ER exhibited similar light-dependent translocations as...
Actin-dependent organelle motility in control tissue (data not shown). However, immunofluorescence and ultrastructural studies showed no obvious differences between colchicine-treated and control tissue in the number and distribution of microtubules (data not shown). One possible interpretation is that the microtubules are stable and resistant to colchicine treatment. Alternatively, colchicine may not diffuse well within the narrow extracellular spaces of the retinal tissue and across the plasma membrane.

DISCUSSION

The involvement of microtubules in the transport of membranous organelles within animal cells has been well established (Kelly, 1990; Schroer and Sheetz, 1991). In contrast, our data provide evidence that the light-induced repositioning of mitochondria and ER elements in the photoreceptor cells of the locust *Schistocerca gregaria* requires the participation of the actin filament system. (1) Actin filaments, but no microtubules, are closely associated with the transported organelles and are aligned in their path of movement. (2) The light-induced shuttling of these organelles is blocked after depolymerization of actin filaments by cytochalasin. These results complement recent work on other systems that also provide evidence for an involvement of actin filaments in the transport of membrane-bound organelles (Adams and Pollard, 1986; Kuznetsov et al., 1992; Bearer et al., 1993; Fath and Burgess, 1993), implying that organelle transport via an acto-myosin system may be a widespread phenomenon in animal cells. However, we can not completely exclude the possibility that, in addition to the actin system, microtubules are somehow involved in the light-induced organelle translocations.

**Organization of the cytoskeleton in photoreceptors of locusts**

Detailed knowledge of the organization of the cytoskeleton is fundamental for understanding the molecular mechanisms of organelle translocation. In the photoreceptor cells of the locust *Schistocerca gregaria*, the architecture of the cytoskeleton is exceptionally clear. The cytoplasm of these cells can be subdivided into distinct zones, the submicrovillar area containing vacuolar ER cisternae during dark adaptation and mitochondria during light adaptation, and the remaining cell body. For our morphometric analysis, we have subdivided the main cell body further into a middle and an outer area.

Immunofluorescence studies with monoclonal and polyclonal antibodies against tubulin demonstrate some microtubules in the main cell body. In contrast, no microtubules are present in the submicrovillar cytoplasm where the light-induced organelle movements occur. These results were confirmed and refined at higher spatial resolution by electron microscopic studies on both chemically fixed specimens and high-pressure-frozen, freeze-substituted retinae. High-pressure freezing and subsequent freeze substitution is supposed to preserve cellular structures in a state that more closely resembles that of living cells than chemical fixation (reviewed by Sitte et al., 1987). Thus the lack of microtubules in the submicrovillar region does not result from a failure of the fixation protocols to preserve microtubules. Ultrastructural studies reveal further that the microtubules are aligned longitudinally in the middle and outer areas of the cell body. This distribution of microtubules parallels that found in the honeybee drone photoreceptor; honeybee and locust photoreceptors are similar in their morphology (Baumann and Lautenschläger, 1994).

Labelling with F-actin-specific fluorescent phalloidin (Faulstich et al., 1988) and immunogold decoration with monoclonal anti-actin demonstrate actin filaments in the submicrovillar cell area. This result is consistent with the observations made on other arthropod photoreceptors. In the fruitfly *Drosophila*, the honeybee drone *Apis* and the crab *Procambarus*, submicrovillar actin filaments have been directly visualized by labelling with myosin fragments (Arikawa et al., 1990; Hafner et al., 1991; Baumann, 1992). These studies have demonstrated that the submicrovillar actin filaments represent elongations of the microvillar actin filaments and are thus comparable to the rootlets within the terminal web of vertebrate microvilli-bearing epithelial cells (Mooseker, 1985; Bretschger, 1991). In *Schistocerca* photoreceptors, the actin filaments traverse the submicrovillar cell area and extend into the middle of the cell body.
area of the main cell body, along the ‘track’ of ER and mito-
ochondrial movements; they lie in close association with these
moving organelles. The site of filament termination in the main
cell body remains unclear.

In conclusion, the cytoskeleton of Schistocerca photorecep-
tors essentially consists of longitudinally aligned microtubules
in the main cell body, and transversely arranged actin filaments
in the microvillar/submicrovillar cell portion. This organi-
fication of the cytoskeleton implies that organelle movements in
the transverse direction between the main cell body and the
microvillar rhabdom, such as the light-dependent shuttling of
ER and mitochondria, occur along actin filaments; membrane
transport in the longitudinal direction, e.g. transport towards
and from the synapses, probably involves the microtubular
system.

The mechanism of organelle translocation

The hypothesis that actin filaments are involved in light-
induced organelle shuttling has been tested by applying
cytochalasin B. This agent leads to a net depolymerization of
actin filaments by capping their fast-growing ends, by severing
the filaments and by binding to actin monomers (Casella et al.,
1981; Schliwa, 1982; reviewed by Cooper, 1987). In Schisto-
cerca photoreceptors, the various F-actin-containing structures
differ in their sensitivity to cytochalasin B. The actin filament
content of the microvilli decreases and the submicrovillar actin
filaments disappear. The cortical bundles of actin filaments at
the periphery of the ommatidia seem to remain unaffected.
These differences in sensitivity could result from interactions
of actin filaments with different actin-binding proteins (Pollard
and Cooper, 1986).

Incubation of the retinal preparation with cytochalasin B
results further in a blockage of light-induced organelle
movements. In other systems, cytochalasin B is known to
inhibit the glucose transporter in addition to its effects on the
actin filament system (Lin and Spudich, 1974). However,
Schistocerca photoreceptors contain glycogen granules, indi-
cating that they are independent of the glucose supply for a
limited time. Moreover, even after cytochalasin treatment,
glycogen granules are still present and, thus, more glycogen
than needed must have been present within the cells, making
a glucose transporter dispensible for the incubation period. The
effects of cytochalasin B on the light-induced organelle
shuttling in Schistocerca photoreceptors can thus be directly
attributed to the depolymerization of actin filaments.

The light-induced movements of organelles can be subdi-
vided into several distinct events. (1) The transport of
organelles towards the rhabdom and away from the rhabdom
during the onset and offset of illumination; and (2) the main-
tenance of organelle position at the rhabdom or in the main cell
body, respectively, during continuous stimulus conditions.
Moreover, two different organelles, the ER and mitochondria,
are subject to these phenomena and move in antagonistic direc-
tions. It is of particular interest whether all these transport and
positioning processes require an intact actin cytoskeleton, and
whether they are mediated by a single mechanism.

The blockage of light-dependent movements of ER and
mitochondria towards the rhabdom in the presence of cytocha-
lasin suggests that the transport of these organelles towards the
microvilli occurs along the submicrovillar actin filaments. In
all arthropod photoreceptors in which the polarity of the
microvillar/submicrovillar actin filaments has been determined
by decoration with myosin fragments, the filaments have a
unipolar orientation with their pointed end being directed away
from the microvillar tips (Arikawa et al., 1990; Hafner et al.,
1991; Baumann, 1992). Myosins are considered as barbed-end-
directed motors (Moosiker and Coleman, 1989; Bearer et al.,
1993) and would thus facilitate transport from the cell centre
towards the rhabdom. The transport of mitochondria at the
onset of illumination and of ER cisternae at the offset of illu-
mination could thus be explained by an acto-myosin system.

In addition to the translocation events, actin filaments may
also be required to maintain the organelles in their character-
istic submicrovillar position. After cytochalasin-induced
depolymerization of submicrovillar actin filaments, the
organelles in the submicrovillar position (either ER or mito-
condria) are displaced in a centrifugal direction, irrespective
of the illumination protocol. This finding is consistent with our
result on the honeybee drone photoreceptor. There, actin
filaments also participate in the maintenance of the localiza-
tion of the submicrovillar ER, which is static in that system
(Baumann and Lautenschläger, 1994). However, although both
the transport towards and the positioning at the rhabdom
require the actin filament system, it is not clear whether these
processes are mediated by the same molecular components.

Finally, the mechanism of the transport of mitochondria and
ER cisternae from the rhabdom towards the main cell body
remains elusive. Cytochalasin treatment leads to a displace-
ment of either organelle in a centrifugal direction, independent
of their adaptational state, indicating that translocation from
the rhabdom towards the main cell body can occur in the
absence of actin filaments. However, we cannot conclude from
these data that this movement is actin-independent in vivo.

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REFERENCES

Acanthamoeba along actin filaments by myosin-I. Nature 322, 754-756.
Arikawa, K., Hicks, J. L. and Williams, D. S. (1990). Identification of actin
filaments in the rhabdomeral microvilli of Drosophila photoreceptors. J. Cell
Sensory Physiology, C: Invertebrate Visual Centers and Behaviour II (ed. H.
71-79.


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