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Distribution of F-actin in the compound eye of the blowfly, *Calliphora erythrocephala* (Diptera, Insecta)

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Summary. Filaments that probably consist of actin have been observed by electron microscopy in various cells and structures of the compound eye of insects and crustaceans. The overall distribution of F-actin in the compound eye of *Calliphora erythrocephala* was studied using fluorescent phalloidins as specific probes for F-actin. F-actin is localized (1) in the rhabdomeres, (2) in the Semper cells, (3) in the basement membrane, and (4) associated with desmosomes and membranes. It is concluded that the actin filaments present in the compound eye are involved in mechanical stabilization (e.g., within microvillar processes of the Semper cells and in association with intercellular contacts) and in motile phenomena (e.g., in the rhabdomeric microvilli).

Key words: Compound eyes – Cytoskeleton – Actin filaments – Cytochemistry – Phalloidin – *Calliphora erythrocephala* (Insecta)

The cytoskeleton in the cells of the ommatidia of the insect compound eye has only partially been examined and characterized (Blest 1988; Blest and Eddey 1984; Blest et al. 1984; Walz 1983). Most studies have focused on the axial filaments within the rhabdomeric microvilli of the photoreceptors (for example Blest et al. 1982a, b; el-Gammalet al. 1987). In contrast to microtubules, microfilaments can be specifically characterized only by immunohisto- or cytochemical methods. Using these techniques, Arikawa and coworkers (1990) have shown in *Drosophila melanogaster*, that the axial filaments of the photoreceptive microvilli are composed of F-actin. However, a systematic study of the distribution of actin filaments within the insect compound eye is still lacking. In the present study it is shown that actin filaments in *Calliphora erythrocephala* occur within (1) the rhabdomeric microvilli, (2) the basement membrane, (3) the microvillar projections of the Semper cells, and are (4) associated with microtubules or with desmosomes or hemidesmosomes.

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Materials and methods

The wild-type and the white-eyed "chalky" mutant of *Calliphora erythrocephala* (Diptera) were obtained from local cultures.

Preparation. Specimens were fixed in a mixture of 0.2% glutaraldehyde and 3% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2, for 3 h at 4°C. They were subsequently washed in cold buffered 10% sucrose for 1 h. followed by infiltration with buffered *25%* sucrose for 12 h at 4° C. The specimens were surrounded by boiled liver, embedded (medium: Reichert-Jung, Nussloch, FRG) and cryofixed in melting iso-pentane. They were sectioned at 6–12 µm thickness in a cryostate (Dittes, Heidelberg, FRG) between – 15°C and -*25*° C, and placed on coverslips precoated with 0.05% aequous poly-L-lysine.

Phalloidin staining. Rhodamine-coupled phalloidin (a generous gift from Dr. H. Faulstich, MPI for Medicine, Heidelberg) and FITC-coupled phalloidin (Sigma, Deisenhofen, FRG) were used as specific probes for filamentous actin (Wulf et al. 1979; Faulstich et al. 1988). Each cryosection was incubated with 7 µl of one of the fluorescent phalloidins in phosphate-buffered saline, pH 7.2, (PBS) (0.01 mg/ml and 0.1 mg/ml. respectively) for 1 h at room temperature and washed 3 times in PBS and one time in H2O to remove unbound dye. The sections were then mounted in Mowiol 4.88 (Farbwerke Hoechst, Frankfurt. FRG) containing *2%* n-propylgallate to retard photobleaching.

Controls. The specifity of the fluorescent phalloidins was tested in insect muscles. Both phalloidins reacted only in the light band of muscle containing F-actin.

For additional controls parallel cryosections were preincubated with unlabeled phalloidin (a generous gift from Dr. H. Faulstich) (1 mg/ml) in PBS for 30 min or 2.5 h at room temperature and washed 3 times in PBS, before treating with the fluorescent phalloidins (see above).

The mounted frozen sections were examined and photographed with a Zeiss Axiophot microscope.

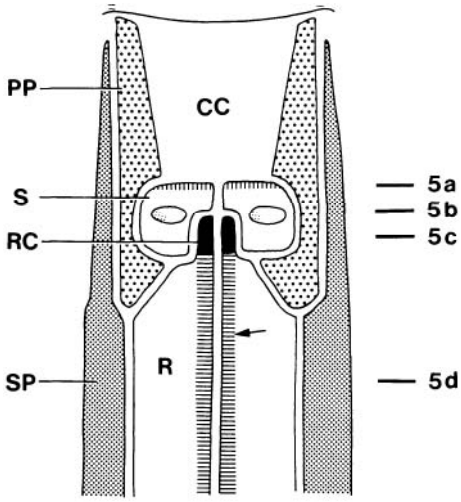

Fig. 1. Diagram of the distal part of an ommatidium in the blowfly compound eye. The Semper cells *(S)* are intercalated between the dioptric apparatus *(C* cornea; CC cristalline cone) and retinula cells (R). PP primary and SP secondary pigment cells; RC rhabdomere caps. Planes of transverse section of Fig. 5a–c are indicated by lines. *Arrow* points to rhabdomeric microvilli

Results and discussion

Specificity of fluorescent phalloidins. Whereas fluorescent NBD-phallacidin binds to the rhabdomeric microvilli of the crayfish photoreceptors nonspecifically (DeCouet et al. 1984), the present preincubation experiments with unlabeled phalloidin show that both fluorescent phalloidins react specifically in all regions of the blowfly compound eye. After 30 min of preincubation with unlabeled phalloidin the phalloidin-fluorescence was notably reduced while after 2.5 h the phalloidin-fluorescence was abolished in all regions of the compound eye usually stained, including the rhabdomeric microvilli.

Phalloidin-staining in the compound eye

The distal part of an ommatidium of the blowfly eye is schematically illustrated in Fig. 1. Both the FITC- and the rhodamine-coupled phalloidin react in the same structures of the compound eye of the wild-type and the white-eyed mutant. In longitudinal sections there is intense fluorescence in a disc-like structure proximal to the dioptric apparatus (Figs. 2, 3a); fluorescent spots are also present in a more proximal layer (Fig. 3). Fluorescent stripes below this layer (Fig. 3) project through the length of the ommatidia down to the brightly stained basement membrane (Fig. 2). The reacting sites can be localized by comparisons between the longitudinal sections, stained transverse sections cut in selected planes, and corresponding Nomarski pictures.

Dioptric apparatus. The fluorescence of the cornea (Figs. 2, 3) is unspecific since it appears also in unstained controls.

Semper cells. Three different planes in the four Semper cells can be distinguished morphologically and by their staining pattern: (1) an apical region, characterized by numerous microvilli containing numerous microfilaments *(7* nm in diameter. Walz 1983). These filaments are most likely the source of the intense staining with both phalloidins (disc-like structure in Figs. 2, 3, 5a) and, hence, they are characterized as actin filaments. (2) A median region at the beginning of the intraommatidial space where the Semper cells and their nuclei are arranged in a typical four-leafed clover structure (Nomarski picture 5b). The nuclei and the extracellular space lack fluorescence (Fig. 5b). An intensely fluorescent band adjoins the extracellular space, but the cytoplasm of the Semper cells is only weakly stained (Fig. 5b). This fluorescent band probably results from actin filaments that lie beneath the plasma membrane facing the interommatidial space (Walz 1983). (3) More proximally, the Semper cells enclose the rhabdomere caps (Nomarski picture in Fig. 5c). These extracellular caps are encircled by a fluorescent band containing fluorescent spots (Fig. 5c). At this plane, microfilaments (7 nm in diameter) are associated with hemidesmosomes connecting the basolateral membrane of the Semper cells with the rhabdomere caps (Walz 1983). Here, these filaments have to be considered F-actin because of the intense phalloidin fluorescence in this region

Retinula cells. In transverse sections of the ommatidia, the rhabdomeres of the seven photoreceptor cells are conspicuous due to their intense staining (Fig. 5d). The fluorescent phalloidins seem to react with the central filaments of the rhabdomeric microvilli (Varela and Porter 1969). Actin filaments have also been characterized within the photoreceptive rhabdomeres of the crayfish (DeCouet et al. 1984), the leech (Blest et al. 1983) and *Drosophila* (Arikawa et al. 1990).

A weak fluorescence was also observed in the subrhabdomeric region of the cytoplasm (Fig. 5d). In this area the staining may be caused by some of the microvillar filaments projecting into the cytoplasm of the photoreceptors (Blest et al. 1984a; Arikawa et al. 1990).

Pigment cells. The primary pigment cells enclose the cones, the secondary pigment cells the ommatidia (Fig. 1). Fluorescence is visible at the sites of contact between the secondary pigment cells (Fig. 4). This is probably due to the actin filaments that are associated

Fig. 2. Fluorescent micrograph of longitudinal section through the compound eye of *Calliphora erythrocephala.* Phalloidin-FITC reacts within a disc-like structure *(arrowhead)* and in stripes projecting to the intensely stained basement membrane *(bm). Bar:* 100 µm; × 125

Fig. 3a, b. Longitudinal section through the distal parts of ommatidia of the compound eye. a Fluorescent micrograph and b Nomarski optics. *Arrowhead* points to disc-like structure; *arrow* indicates a fluorescent spot. c Cornea; cc crystalline cone: s Semper cells. Compare with diagram in Fig. 1. *Bar:* 20 µm: × 500

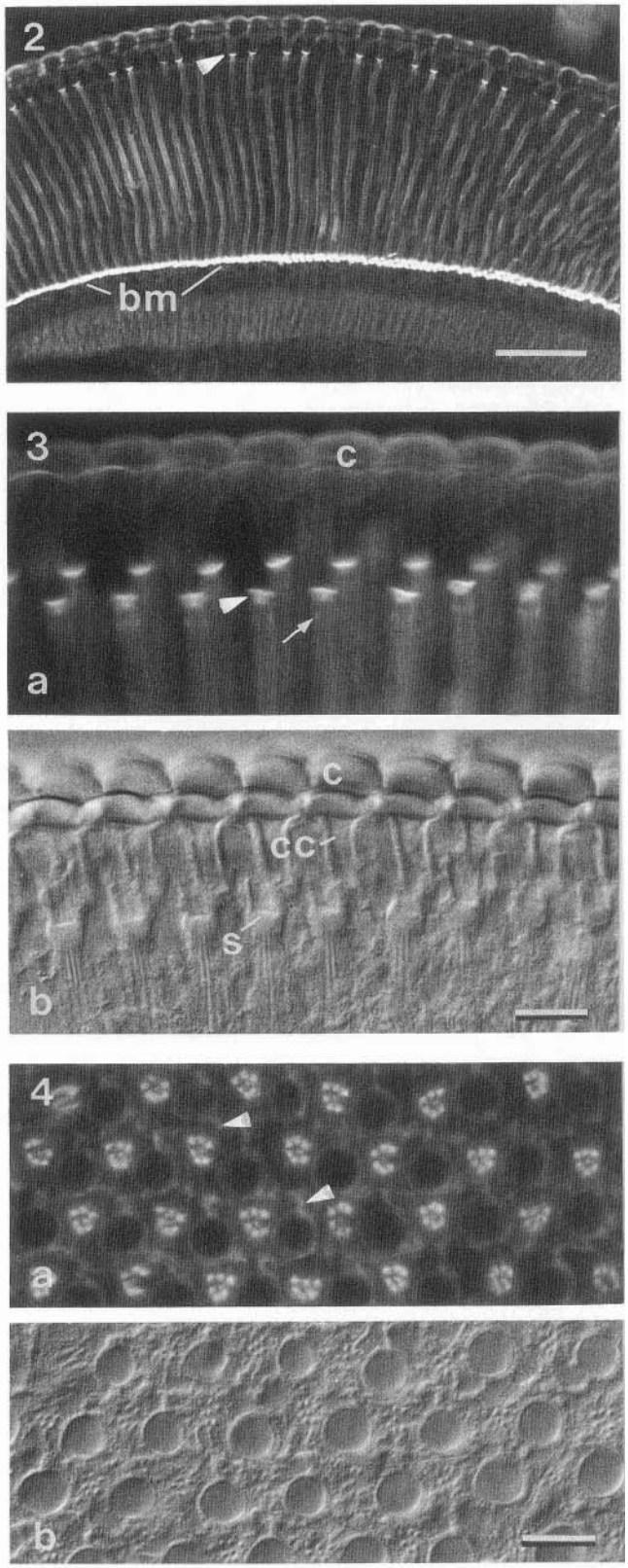

Fig. 4a, h. Transversc section through omniatidia of the compound eye or C. *erythrocephala* at the level of rhabdomeric microvilli. a Fluorescent micrograph and **h** Nomarski picture. Phalloidin-rhodamine stains microvilli of the seven rhabdomeres and reacts in regions facing contacts between the secondary pigment cells *(arrowheads). Bar:* 20 pm; x 500

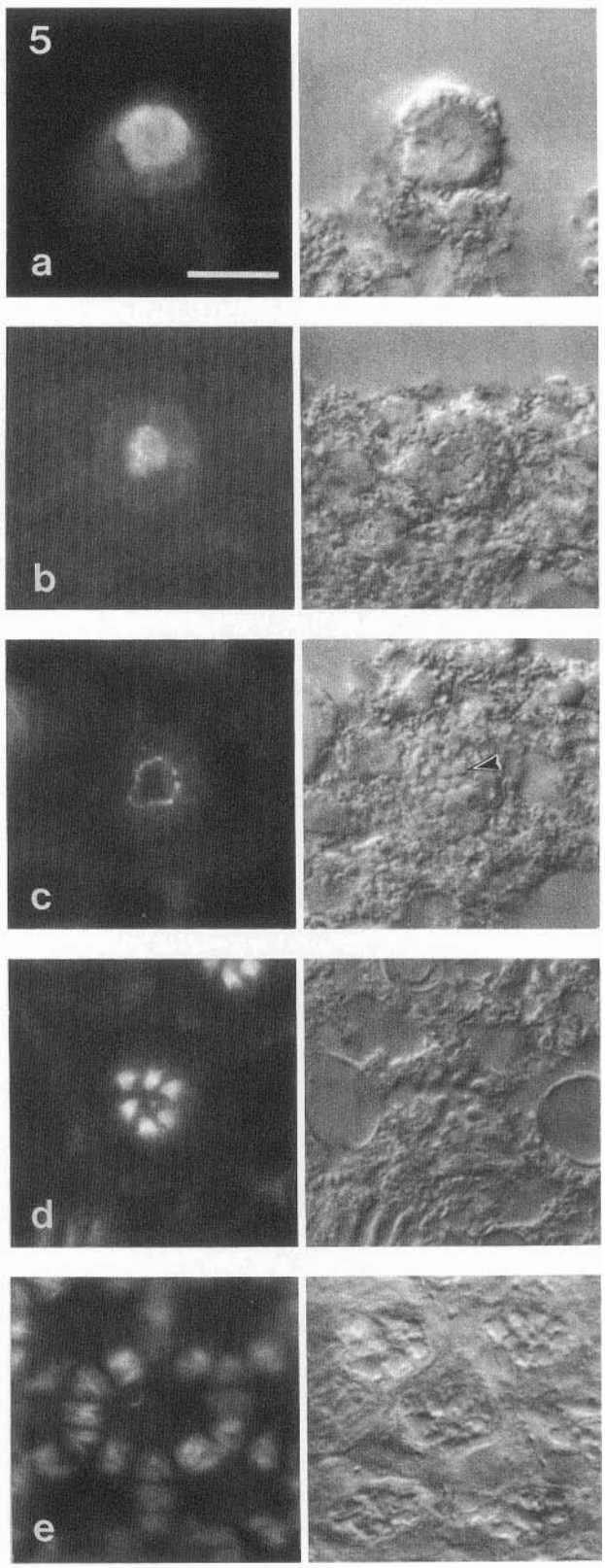

Fig. 5ae. Transverse sections through an ommatidium of the compound eye in selected planes *(on the left,* phalloidin-fluorescence images; *on the right,* Nomarski optics), the levels of a apical region of Semper cells; b median region of Semper cells; c rhabdomere caps *(arrowhead);* d rhabdomeric microvilli; and e basement membrane. (Planes of a–c are indicated in Fig. 1.). *Bar:* 10 pm; x 1250

with desmosomes linking the membranes of neighboring cells (Chi and Carlson 1981). The cytoplasm of both cell types is not stained (Figs. 2–5).

Basement membrane. The cellular components of the basement membrane of the compound eye of the blowfly comprise flattened extensions of both the Semper cells and the secondary pigment cells (Odselius and Elofsson 1981). Intense fluorescence is observed in the pigment cells, which encircle each ommatidium at this level (Fig. 5e; for ultrastructural comparison, see Fig. 20 in Charlson and Chi 1979). Both the projections of the Semper cells and the axons of the photoreceptor cells remain unstained (Fig. 5e).

Blest and DeCouet (1983) previously described actin filaments within the cellular components of the basement membrane of the blowfly *Lucilia cuprina.* However, in the present material the phalloidin-staining pattern of the transverse sections through the basement membrane (Fig. 5e) differs from that published by Blest and DeCouet (1983; Figs. 10, 11). Their NBD-phallacidin-staining pattern does not correspond with the phalloidin-staining pattern in transverse sections, but, in contrast with the staining pattern found in rather oblique sections through the compound eye of *Calliphora.*

Function of actin filaments in the insect compound eye. Actin filaments in non-muscle cells may serve either a contractile or a mechanically stabilizing cytoskeletal function. They are mechanical stabilizers in microvillar projections of cells (for example, in the brush border of vertebrates; see Mooseker 1985) and in cellular elements where actin filaments are associated with microtubules (Slepecky and Chamberlain 1983; Morgenson and Tucker 1988; Wolfrum 1990). In contractile systems, actin is always associated with myosin (Fulton 1984).

The actin filaments within the Semper cells are located in microvillar projections of the apical portion and associated with microtubules in the middle and proximal cell region (Walz 1983). Therefore, they may stabilize the Semper cells, and, thus, support the linkage between the rhabdomeres and the dioptric apparatus. The actin filaments in the cellular components of the basement membrane (see also Blest and DeCouet 1983) and the filaments facing membranes or desmosomal structures within both Semper and pigment cells (Walz 1983) may also have stabilizing functions.

Contraction in the filament systems associated with desmosomes may be possible, since actin, myosin and a-actinin are associated with desmosomes in other tissues (Drenckhahn and Dermietzel 1988). Furthermore, the actin within the rhabdomeric microvilli may differ from the actin within microvilli of other cell types (Stowe and Davis 1990). In *Drosophila*, the axial filaments may be linked to the plasma membrane by myosin I (Montell and Robin 1988; Arikawa et al. 1990). A similar ATPase, the 110-kD protein is localized in the intestinal microvilli (Collins and Borysenko 1984; Mooseker and Coleman 1989), but nothing is known about its function there. Myosin I may function as a spacer in the rhabdomeric microvilli of insect photoreceptors holding the axial filaments centrally and stabilizing the microvilli. It is also possible that actin filaments (axial filaments, cytoplasm filaments) are involved in motile phenomena such as organelle movements or movements of particles (Adams and Pollard 1989). In addition, the axial filaments itself may be involved in movements induced by interaction of actin and myosin.

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References

Adams RJ, Pollard TD (1989) Binding of myosin I to membrane lipids. Nature 340:565–568

Arikawa K, Hicks JL, Williams DS (1990) Identification of actin filaments in the rhabdomeral microvilli of Drosophila photoreceptors. J Cell Biol 110:1993–1998

Blest AD (1988) The turnover of phototransductive membrane in compound eyes and ocelli. In: Evans PD, Wigglesworth VB (eds) Advances in insect physiology, vol 20. Academic Press, London, pp 1–53

Blest AD, DeCouet HG (1983) Actin in cellular components of the basement membrane of the compound eye of a blowfly. Cell Tissue Res 231:325–336

Blest AD, Eddey W (1984) The extrarhabdomeral cytoskeleton in photoreceptors of Diptera. 2. Plasmalemmal undercoats. Proc R Soc Lond [Biol] 220:353–359

Blest AD, Stowe S, Eddey W (1982a) A labile, Ca^{2+}-dependent cytoskeleton in rhabdomeral microvilli of blowflies. Cell Tissue Res 223:553–573

Blest AD, Stowe S, Eddey W, Williams DS (1982b) The local deletion of microvillar cytoskeleton from photoreceptors of tipulid flies during membrane turnover. Proc R Soc Lond [Biol] 215:469-479

Blest AD, DeCouet HG, Sigmund C (1983) The cytoskeleton of microvilli of leech photoreceptors. A stable bundle of actin microfilaments. Cell Tissue Res 234:9-16

Blest AD, DeCouet HG, Howard J, Wilcox M, Sigmund C (1984) The extrarhabdomeral cytoskeleton in photoreceptors in Diptera 1. Labile components in the cytoplasm. Proc R Soc Lond [Biol] 220:338–352

Carlson SD, Chi C (1979) The functional morphology of the insect photoreceptors. Ann Rev Entomol 24:379–416

Chi C, Carlson SD (1981) Lanthanum and freeze fracture studies on the retinular cell junction in the compound eye of the housefly. Cell Tissue Res 214:541–542

Collins JH, Borysenko CW (1984) The 110000-dalton actin- and calmodulin-binding protein from intestinal brush border is a myosin-like ATPase. J Biol Chem 259:14128–14135

DeCouet HG, Stowe S, Blest AD (1984) Membrane-associated actin in the rhabdomeral microvilli of crayfish photoreceptors. J Cell Biol 98:834–846

Drenckhahn D, Dermietzel R (1988) Organization of the actin filament cytoskeleton in the intestinal brush border: a quantitative and qualitative immunoelectron microscope study. J Cell Biol 107:1037–1048

el-Gammal S, Hamdorf K, Henning U (1987) The paracrystalline structure of an insect rhabdomere (Calliphora erythrocephala). Cell Tissue Res 248:511–518

Faulstich H. Zobeley S, Rinnerthaler G, Small JV (1988) Fluorescent phallotoxins as probes for filamentous actin. J Muscle Res Cell Motil 9:370–383

Fulton AB (1984) The cytoskeleton: cellular architecture and choreography. Chapman and Hall, New York

Montell C, Rubin GM (1988) The Drosophila ninaC locus encodes two photoreceptor cell specific proteins with domains homologous to protein kinases and the myosin heavy chain head. Cell 52:757–772

Mooseker MS (1985) Organization, chemistry and assembly of the cytoskeletal apparatus of the intestinal brush border. Ann Rev Cell Biol 1:209–241

Mooseker MS, Coleman TR (1989) The 110-kD protein-calmodulin complex of the intestinal microvillus (brush border myosin I) is a mechanoenzyme. J Cell Biol 108:2395–2400

Morgensen MM, Tucker JB (1988) Intermicrotubular actin filaments in the transalar cytoskeletal arrays of Drosophila. J Cell Sci 91:431-438

Odselius R, Elofsson R (1981) The basement membrane of the insect and crustacean compound eye: definition, fine structure. and comparative morphology. Cell Tissue Res 216:205-214

Slepecky N, Chamberlain SC (1983) Distribution and polarity of actin in inner ear supporting cells. Hearing Res 10:359–370

Stowe S, Davis DT (1990) Anti-actin immunoreactivity is retained in rhabdoms of Drosophila ninaC photoreceptors. Cell Tissue Res 260:431–434

Varela FG, Porter KR (1969) Fine structure of the visual system of the honeybee (Apis mellifera). I. The retina. J Ultrastruct Res 29:236–259

Walz B (1983) Association between cytoskeletal microtubules and Ca^{2+}-sequestering smooth ER in Semper cells of fly ommatidia. Eur J Cell Biol 32:92-98

Wolfrum U (1990) Actin filaments: the main components of the scolopale in insect sensilla. Cell Tissue Res 261:85–96

Wulf E, Deboben A, Bautz FA, Faulstich H, Wieland T (1979) Fluorescent phallotoxin, a tool for the visualization of cellular actin. Proc Natl Acad Sci USA 76:4498–4502