The Distribution of Actin in Cultured Normal and Dystrophic Rat Pigment Epithelial Cells during the Phagocytosis of Rod Outer Segments

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In the previous article the authors reported that the ingestion phase of phagocytosis is defective in cultured dystrophic rat pigment epithelial (PE) cells. When these cells are challenged with isolated rod outer segments (ROS), attachment of ROS to the PE cell surfaces occurs to a normal extent. However, only a small number of these bound ROS are subsequently ingested. This raised the possibility that the contractile protein actin might not function normally in the dystrophic rat PE cells, since actin is intimately involved in the ingestion mechanism in other phagocytic cells. Utilizing actin antibodies and the technique of indirect immunofluorescence, we have studied the distribution of actin in cultured normal and dystrophic rat PE cells. Results show that the arrangement of actin fibers in the dystrophic cells appears normal both before and during the attachment of ROS to the cell surfaces. With the additional use of an ROS antiserum to label externally bound ROS, it is also possible to show that actin is involved with the ingestion of ROS by both normal and dystrophic PE cells. Thus, it appears that actin can function normally in dystrophic PE cells, but that the ingestion mechanism becomes activated at only a few sites of ROS attachment. The results of a scanning electron microscope study support this conclusion and also show the presence of a saucer-shaped elaboration of the PE cell plasma membrane beneath attached ROS. These may correspond to the actin feltworks seen with immunofluorescence microscopy at sites of ROS attachment. Invest Ophthalmol Vis Sci 24:821–831, 1983

Rod outer segments (ROS), the light sensitive organelles of rod photoreceptors, undergo a continuous renewal of their components throughout life. Packets of membranous discs are shed from the apical end of ROS each day, and these disc packets are phagocytized by the adjacent pigment epithelial (PE) cells. Newly synthesized discs are added at the base of the ROS, thus maintaining a relatively constant ROS length. The Royal College of Surgeons (RCS) strain of rat has been studied extensively in recent years because it exhibits a hereditary retinal dystrophy in which there is a defect in the phagocytosis of shed ROS material by the PE cells. Rather than being ingested by the PE, discs reaching the apical end of the ROS build up as a layer of membranous debris. Disruption in the equilibrium of ROS renewal leads to photoreceptor cell death, with resulting blindness. In a previous study, we showed that there is a defect in the ingestion phase of phagocytosis in cultured dystrophic rat PE cells. Isolated rat ROS were able to bind normally to the surfaces of these cells, but only a small percentage of the bound ROS were subsequently ingested.

Phagocytosis represents one form of non-muscle cell motility. It has become apparent in recent years that the motive force required for the various forms of non-muscle cell motility is provided by contractile proteins, the most prominent of which is actin. Studies on macrophages and leukocytes have shown that oriented bundles of actin filaments accumulate beneath the plasma membrane at sites of particle attachment. These filaments are the main components of the villi or pseudopods that extend around a bound particle and, after membrane fusion, finally ingest it. Thus, actin is intimately involved with the ingestion mechanism of phagocytosis.

The apical processes of PE cells that ensheath the ends of rat ROS in situ are ideally positioned to participate in the phagocytosis of shed packets of ROS discs. These PE processes have been shown to contain parallel arrays of axially oriented actin filaments.
Such filament arrangement seems well suited for the task of transporting "newly ingested" ROS disc packets, or phagosomes, up into the PE cell body. A comparison of the filament distribution in normal and dystrophic rat PE cells has shown no apparent difference between the two. However, as mentioned earlier, the defect in dystrophic PE cells has now been shown to involve ingestion. The array of actin filaments seen in the PE processes is not suited for this step of phagocytosis, since the ingestion of ROS material occurs as the disc packets are surrounded by lateral protrusions of the PE processes that extend inward from all sides. This is analogous to the formation and movement of pseudopods that surround a bound particle during its ingestion by macrophages. As such, the ingestion of ROS disc packets would be expected to involve actin, but actin other than that in the filament arrays.

It was recently shown that the neutrophilic polymorphonuclear leukocytes of an infant with repeated bacterial infections contained an abnormally functioning actin that caused an impairment in locomotion, and in the ingestion of particles, by these phagocytic cells. As was shown for ROS binding to PE cells in our previous report, the leukocytes were able to bind particles to their surfaces normally, but they were unable to ingest the particles.

The above evidence from other cell types and the results that show a defect in the ingestion phase of phagocytosis by dystrophic rat PE cells suggested that a dysfunction in actin might underlie the disorder in the dystrophic cells. Utilizing actin antibodies and the technique of indirect immunofluorescence, we have studied the distribution of actin in cultured normal and dystrophic rat PE cells during the phagocytosis of ROS. A scanning electron microscope study was carried out in conjunction with the immunofluorescence work in order to visualize the cell surface structures important for ROS binding and also to gain a better understanding of the ingestion phase of phagocytosis.

Materials and Methods
Preparation of Acetone Dried Muscle

Smooth muscle from fresh chicken gizzards was used in preparing the acetone dried muscle. The procedure followed was that of Carsten and Mommaerts, with modifications. Connective tissue was cut away and the remaining gizzard muscle was minced with two passes through a meat grinder. In the cold room the minced muscle was extracted first with 3 vol of 0.1 M KCl and then with 3 vol of 0.05 M NaHCO₃. Each extraction was accomplished by homogenizing in a Waring blender until smooth (about 20 sec), and the homogenates were then centrifuged at 22,000 × g for 30 min. The residue was washed once with 10 vol of 0.001 M EDTA and twice with 10 vol of deionized water. Each wash was followed by filtration through two layers of cheesecloth. The muscle residue was extracted twice with 2 vol of acetone and once with 1 vol of acetone precooled to 0 C. Each acetone extraction was for 10 min with stirring and was followed by filtration through cheesecloth. The residue was then air dried overnight in the cold room. In a typical preparation, 700 g of cleaned chicken gizzards yielded 12 to 35 g of acetone dried muscle. This was stored under vacuum at −20 C.

Actin Purification

The extraction procedure was basically that of Spudich and Watt, modified for smooth muscle actin. Buffer A contained 2 mM Tris-Cl, 0.2 mM ATP, 0.5 mM β-mercaptoethanol, 0.2 mM CaCl₂, pH 7.3 at room temperature (RT).

Acetone dried muscle (8.5 g) was extracted with 170 ml of Buffer A (0 C) for 3 hours and then filtered through a coarse sintered glass funnel. The residue was washed with 85 ml of Buffer A (0 C) and again filtered. The extracts were combined and cleared by centrifugation at 10,000 × g for 1 hr at 4 C. The supernatant was brought to RT before adding powdered KCl to 50 mM and solid MgCl₂ to 2 mM. The pH of the solution was adjusted to 7.3 with 1 M Tris base and the actin was allowed to polymerize for 2 hr at RT without stirring. Additional powdered KCl was then added to 0.6 M or 0.8 M, and the solution was stirred gently in the cold room for 1.5 hr. The polymerized actin was recovered by centrifugation at 80,000 × g for 3 hr. The translucent, gel-like pellets were dissolved in 10 ml of Buffer A using a glass-glass tissue grinder, and the solution was dialyzed against several changes of Buffer A for 3 days at 4 C. Dialysis resulted in depolymerization of the F-actin, and the resultant G-actin solution was clarified by centrifugation at 80,000 × g for 3 hr. This solution was stored at 4 C with 0.02% sodium azide. Yields were 1.5 to 2.0 mg actin per gram of acetone dried muscle extracted.

Electrophoretic Elution of Actin

In preparation for use as antigen, actin was purified further by electrophoretic elution. About 3 mg of G-actin was run on a 4-mm × 165-mm preparative SDS-polyacrylamide slab gel prepared according to Laemmli. Narrow strips were cut from both sides of the gel, stained, and electrophoretically destained. Using
these strips as guides, the actin-containing band in
the remainder of the slab gel was located and cut out.
This unstained actin-containing band was cut into
pieces and packed into several 6-mm X 100-mm glass
tubes that were tapered at the bottom. The tapered
end contained a plug of 4.6% polyacrylamide in 0.1%
SDS. The tubes were filled with the Tris glycine run-
ning buffer and a Kimwipe tissue plug was inserted
at the top. A dialysis bag filled with Tris glycine buffer
was placed over the tapered bottom of each tube. The
electrophoretic elution of the actin was carried out
overnight at 4 mamps per tube. The actin in Tris
glycine buffer was collected from the dialysis bags
after elution, pooled, and put into a fresh dialysis bag
for overnight dialysis against 0.1% SDS.

Recovery of actin through this electrophoretic elu-
tion procedure was 50% to 70%. This actin in 0.1%
SDS was used immediately in the immunization pro-
cedure, or it could be frozen at —20 C until used.

Preparation and Purification of Actin Antibodies

The procedures followed were those described by
Jockusch et al., wherein alum adsorbed actin was
used for two initial subcutaneous injections, followed
by a series of intravenous booster injections. Actin
in 0.1% SDS, following electrophoretic elution, was
used as the antigen in this study. White New Zealand
rabbits of either sex were used for the immunization.

An actin-sepharose column was prepared accord-
ing to Jockusch et al. In preparation for binding to
sepharose, actin in Buffer A was dialyzed overnight
against the same buffer minus the Tris. This dialysis
was necessary to remove Tris, which would interfere
with the coupling of actin to the sepharose. The re-
sulting actin-sepharose beads were packed into a 10-
cm X 1.5-cm Econocolumn (Bio-Rad Laboratories)
and stored in PBSA (10 mM sodium phosphate,
0.145 M NaCl, 0.02% NaN₃ pH 7.2) at 4 C.

Whole immune serum was applied to the actin-
sepharose column, and the bound antibodies were
eluted with 4 M MgCl₂. The absorbance of each
fraction was read at 280 nm, and the peak fractions
were pooled and dialyzed against several changes of
PBSA at 4 C for 4 days. The actin antibody containing
dialysate was concentrated to a final protein concen-
tration of 0.10 to 0.16 mg/ml. Protein was estimated
by the method of Lowry, with bovine serum albu-
min as a standard.

ROS Antiserum

An ROS antiserum was produced in a white New
Zealand rabbit against intact bovine ROS.

Animals

RCS- p+ rats were derived from breeding pairs origi-
nally supplied by Dr. Matthew M. LaVail. Long
Evans rats were used as controls. Both of these are
pigmented strains. Colonies of each were maintained
in an animal room with a 12-hr light, 12-hr dark
cycle. Food and water were supplied ad libitum.

Tissue Culture of Rat PE Cells

PE cells from normal and dystrophic rats were iso-
lated and cultured according to the procedure of Ed-
wards, modified by the inclusion of 0.05% collagenase
(Worthington, No. 4196 CLS) in the trypsin
digestion step. The cells were seeded onto glass cover
slips. Growth medium was changed after 3 to 4 days
and the PE cells were used 4 to 6 days after seeding.

Isolation of Rat ROS

Rat ROS were isolated as described by Chaitin and
Hall.

Incubation of ROS with Cultured PE Cells

Cover slips on which PE cells were growing were
overlaid with 3 ml of growth medium containing 10
million ROS. Incubation was at 37 C for the desired
time.

Immunofluorescent Labeling of PE Cells with
Actin Antibodies

PE cells were rinsed free of excess growth medium
and unattached ROS by twice shaking the cover slips
vigorously for 5 sec in PBS (10 mM sodium phos-
phate, 0.145 M NaCl, pH 7.2) containing 0.81 mM
MgSO₄ and 1.27 mM CaCl₂ at 37 C. The cells were
then fixed for 30 min at 37 C in 3.5% formaldehyde
in PBS, pH 7.2. Following fixation, the cells were
washed with PBS at RT for at least 30 min with sev-
eral buffer changes. The cells were next put through
a series of graded acetone washes at 4 C as follows:
50% acetone in water for 3 min, absolute acetone for
5 min, 50% acetone for 3 min. The PE cells were then
rehydrated in PBS at RT for at least 30 min with
several buffer changes.

In order to label actin, each cover slip was inverted
onto a 50 µl aliquot of affinity purified actin anti-
bodies in PBSA and incubated at 37 C for 1 hr. After
incubation, the cover slips were washed with PBS at
RT for at least 2 hr, with frequent buffer changes.
Cover slips were then inverted onto a 50 µl aliquot of
a 1:10 dilution of FITC-GARG (fluorescein iso-
thiocyanate conjugated goat anti-rabbit IgG, Miles
Fig. 1. Phase contrast (a, c) and FITC-fluorescence (b, d) micrographs of resting normal (a, b) and dystrophic (c, d) PE cells. a, c stress fibers, such as the ones pointed out by the arrows, are apparent throughout the cells; b, d actin antibodies label the stress fibers, some of which are seen to criss-cross each other. Additional fibers are arranged circumferentially and appear to be situated in the basal portion of the cells. Foci, or dense areas, where actin containing fibers converge, are also seen (arrows). There appears to be an absence of fibers in the perinuclear region of the cells, but several fibers can usually be seen crossing over the nuclei (a, b X435, c, d X450).

Yeda, Ltd.) in PBS and incubated at 37 C for 1 hr. Cells were next rinsed with PBS at RT for at least 1 hr or left overnight in PBS at 4 C before mounting for microscopy. The cover slips were mounted in 50% glycerol in PBS with the cells facing up.

**Double Immunofluorescent Labeling**

When labeling phagocytizing PE cells with two different antisera, and/or fluorescent GARPs, the first was applied prior to the acetone washes. Following fixation and rinsing with PBS, each cover slip was inverted onto a 50 µl aliquot of a 1:50 dilution of ROS antiserum in PBS and incubated at 37 C for 10 min. The PE cells were then washed with PBS at RT for at least 1.5 hr with several buffer changes, after which the cover slips were inverted onto a 50 µl aliquot of a 1:15 dilution of Rh-GARP (tetramethylrhodamine conjugated goat anti-rabbit IgG, Miles Yeda, Ltd.) in PBS and incubated at 37 C for 30 min. After washing with PBS at RT for at least 1.5 hr, or overnight at 4 C, the PE cells were put through the graded acetone washes, rehydrated, and labeled with affinity purified actin antibodies as described above.

When control labeling was carried out, PBS or an appropriate dilution of a preimmune serum was substituted for the ROS antiserum or the actin antibodies. In order to show actin antibody specificity, 80 µl of the affinity purified actin antibodies were mixed with 20 µl of a 3 mg/ml solution of G-actin in Buffer A and left at 4 C overnight. The mixture was then centrifuged at 100,000 X g for 7 min in a Beckman Airfuge, and the supernatant was used for the labeling of PE cells.

**Microscopy**

The PE cells were examined with a Zeiss Photomicroscope III RS using epifluorescent illumination. Photographs were taken on Kodak Tri-X pan and Kodak Ektachrome 400 films using a 25X Neo Fluor phase objective with water immersion. For ROS that were attached to PE cells and labeled with rhodamine, the film exposure time was 10 sec. Exposures of 30
Fig. 2. Phase contrast (a) and FITC-fluorescence (b) micrographs of dystrophic PE cells. a, stress fibers are apparent in these cells, b, no labeling of the stress fibers is seen when using an actin antibody preparation that has undergone an overnight adsorption of the antibodies with G-actin (X435).

Results

The immunization procedure outlined by Jockusch et al.21 was successful when using electrophoretically purified actin in 0.1% SDS as the antigen. Two rabbits were each given two initial subcutaneous injections followed by a series of intravenous boosters, and both produced actin antibodies. Immune serum from one of these rabbits was used throughout the experiments for this study. Because of the difficulty that others have had in producing actin antibodies, we also immunized two rabbits using three subcutaneous injections prior to the intravenous boosters. Two additional rabbits were immunized following the original procedure, but using G-actin in Buffer A rather than electrophoretically purified actin. These four rabbits also produced actin antibodies.

Utilizing actin antibodies and indirect immunofluorescence, it was apparent that the actin fiber distribution in spread rat PE cells is similar to that reported for other cell types in culture.19,25-29 Parallel arrays of fibers and criss-crossing fibers in different focal planes were observed. This was true for normal (Figs. 1a–b), as well as for dystrophic cells (Figs. 1c–d). On occasion, the PE cells exhibited geodesic domes that are believed to be sites of actin fiber organization.19,31 and that may be important for cell shape.32 Few membrane ruffles, characteristic of motile cells, were seen in the spread PE cells, and those that were present did not appear to be very extensive. In confluent PE cells, actin was seen to accumulate along the cell margins, although actin fibers could also be seen to cross the length of many cells. Preimmune serum did not label the actin fibers. An aliquot of the actin antibody solution that had been incubated overnight with G-actin failed to label the stress fibers in spread PE cells (Fig. 2).

The arrangement of actin fibers in both normal and dystrophic PE cells did not appear to change when these cells were challenged with isolated rat ROS. This was true whether ROS were bound to the cell surface, were in the process of being ingested, or had been fully internalized. When these cells were labeled with actin antibodies, the fibers were seen in their typical arrangements (Figs. 3a–b). Additionally, some of the ROS were seen to have accumulations of actin, or actin feltworks, associated with them (Figs. 3c–d). ROS, themselves, exhibited no actin labeling, as shown in Fig. 4.

When using actin antibodies to label PE cells, it was not always possible to determine whether those ROS associated with actin feltworks were still bound to the PE cell surfaces, were in some stage of ingestion, or were already internalized. In order to distinguish externally bound ROS, while still labeling intracellular actin containing structures, a double immunofluorescent labeling procedure was carried out. Cells were fixed, then incubated with an ROS antiserum, followed by Rh-GARG. This resulted in labeling of only the external ROS (Fig. 5b) as previously reported by us.7 Next, the PE cells were permeabilized with acetone and incubated with actin antibodies, followed by FITC-GARG. Using this approach, the typical actin fiber arrangement could be seen once again (Fig. 5c). Because of the reactivity between the antisera and GARGs, ROS externally bound to the...
cells displayed fluorescein, as well as rhodamine, labeling. This prevented us from viewing actin feltworks beneath bound ROS. However, this double labeling technique allowed the visualization of ROS in the process of being ingested and the association of actin feltworks with these ROS (Fig. 6). In such situations, only that portion of the ROS still outside of the PE cell became labeled with the ROS antiserum (Fig. 6b). The portion already inside of the cell could only be labeled with actin antibodies once the cell...
was made permeable with acetone (Fig. 6c). Controls showed that the portion of the ROS already inside of the PE cell did not label with FITC-GARG alone (Fig. 7).

ROS that did not label with ROS antiserum but that did show actin antibody labeling, were also seen. These apparently were newly ingested ROS that still had an actin feltwork surrounding them. ROS that did not label with either the ROS antiserum or with the actin antibodies were seen as well (Fig. 5).

For the scanning electron microscopy study, PE cells were challenged with ROS for 4 hr and then rinsed free of unattached ROS prior to fixation (Fig. 8). A structure that we refer to as an “attachment saucer” is seen to be elaborated beneath ROS that are bound to the surfaces of normal and dystrophic PE cells (Fig. 8b). Following the attachment of ROS to the plasma membrane of normal PE cells, the ROS are engulfed by numerous villi that appear to be elaborated from both the cell surface and from the edge of the “attachment saucer” (Fig. 8c). The final stage of ingestion results in the fusion of villi that completely surround the ROS (Fig. 8d). Rare instances of such ROS ingestion are observed in dystrophic PE cells.

Discussion

With the discovery that it is the ingestion phase of phagocytosis that is defective in dystrophic PE cells, it seemed possible that a defect in the functioning of the contractile protein actin might be involved in this disorder. In other types of phagocytic cells, actin filaments are found to accumulate at sites of particle attachment, as well as in the villi that surround an attached particle during its ingestion. This study was conducted, therefore, to determine if actin is involved in the ingestion of ROS by normal PE cells and, if so, whether this involvement is defective in the dystrophic PE cells.

Actin fibers were initially visualized in cultured normal rat PE cells by immunofluorescent labeling of the cells with actin antibodies. These fibers were seen to be arranged in patterns similar to those exhibited by other types of cultured cells. Interestingly, these actin fiber arrangements did not appear to change when the PE cells were challenged with isolated ROS. This was true whether the ROS were bound to the external cell surface, were in the process of being ingested, or were fully internalized. In phagocytizing leukocytes, the quantity of actin fibers typical of resting cells is much reduced following particle ingestion. It was suggested that this represented a mobilization of actin filaments to the cell periphery and/or the progressive change in cell shape from a

Fig. 5. Phase contrast (a), Rb-fluorescence (b), and FITC-fluorescence (c) micrographs of a dystrophic PE cell which is phagocytizing ROS. a, stress fibers are apparent in the cell, and ROS are seen as phase dense particles (arrow and arrowheads): b, prior to acetone treatment, the ROS antiserum labels only one of the ROS (arrow) that is bound to the external surface of the cell, e, following acetone treatment, the actin antibodies label the stress fibers and the external ROS (arrow). Labeling of this ROS is due to the reactivity between the GARGs and antisera. The other ROS (arrowhead) is inside of the PE cell but is not labeled with the actin antibodies (X440).
Fig. 6. Phase contrast (a), Rh-fluorescence (b), and FITC-fluorescence (c) micrographs of an ROS (arrows) that is in the process of being ingested by a normal PE cell. a, the ROS is seen on the edge of the cell; b, prior to acetone treatment, the ROS antiserum labels only those portions of the ROS that are still outside of the PE cell; c, following acetone treatment, the actin antibodies label an actin feltwork that is associated with the ROS. The actin fibers in the PE cell are out of focus and in a lower focal plane (X560).

Fig. 7. Phase contrast (a) and FITC-fluorescence (b) micrographs of an ROS (arrows) that is in the process of being ingested by a normal PE cell. a, the ROS is seen on the edge of the cell; b, following ROS antiserum labeling of the external portion of the ROS as previously described, the PE cell was made permeable with acetone treatment and then labeled with FITC-GARG. The external top portion of the ROS has become labeled with FITC-GARG because of the reactivity between the GARGs and antiserum. The internal bottom portion of the ROS, however, remains unlabeled, showing the specificity of actin antibodies for the labeling of actin feltworks that are associated with ROS in the process of being ingested (see Fig. 6) (X688).

flattened to a round morphology as phagocytosis proceeded. It may well be that similar changes will occur in those PE cells which must ingest large numbers of ROS. Additional study is required to answer this question.

It has been reported that in an infant with repeated bacterial infections, there was an abnormally functioning actin in the child's neutrophilic polymorphonuclear leukocytes. This caused an impairment in locomotion and in the ingestion of particles by these phagocytic cells, although normal particle binding did
Fig. 8. Scanning electron micrographs of ROS in the process of being ingested by normal PE cells. a, a single cell is shown with numerous ROS attached to its surface (×4700); b, a single ROS is shown with a prominent attachment saucer (arrow) (×6500); c, after attaching to the cell surface, the ROS is progressively engulfed by numerous microvilli elaborated from the cell surface (×3850); d, the ROS are completely engulfed by the villi (arrowheads) and are transported into the body of the cell (×3050). All of the stages of phagocytosis can be seen in the large micrograph (a).

occur. These cells also exhibited a reduced quantity of actin fibers, as compared with normal neutrophils. In the present study, dystrophic rat PE cells in culture were observed to have normal arrangements and quantities of actin fibers. These fiber patterns did not change upon ROS attachment to the cell surface, nor did they undergo a change in those dystrophic cells which exhibited a limited amount of ROS ingestion. Additionally, actin feltworks were seen to be associated with bound ROS in both normal and dystrophic PE cells.

Utilizing a double immunofluorescent labeling procedure, with an ROS antiserum and actin antibodies, it was found that actin is involved with the ingestion of ROS by normal PE cells and that an actin feltwork is associated with both attached and newly ingested ROS. For ROS that are in the process of being ingested, only that portion of the ROS still outside of the PE cell could be labeled with the ROS antiserum. However, once the cell was made permeable with acetone, the internalized portion of the ROS could be labeled with actin antibodies. A similar observation has been reported for macrophages that are ingesting zymosan particles. Many internalized ROS that lacked an actin feltwork were also seen in the PE cells. These apparently represented phago-
somites that had lost their associated actin feltwork as they moved farther into the PE cell body on their way to being concentrated in the perinuclear area of the cell.

Infrequent examples of ROS ingestion by dystrophic PE cells were observed. In these instances, when using the double immunofluorescent labeling procedure, it was apparent that actin does participate in the ingestion of ROS by dystrophic cells, although such ingestion occurs to a very limited extent. Additionally, when labeling the dystrophic cells with actin antibodies alone, large numbers of actin feltworks were seen to be associated with the bound ROS. Thus it appears that actin can function normally in the phagocytosis of ROS by dystrophic rat PE cells. The contractile apparatus necessary for ingestion does not appear to be defective, but apparently becomes activated at few sites of ROS attachment in these cells.

Results of a scanning electron microscopic study supported the above results. ROS bound to the external surfaces of normal and dystrophic PE cells were seen to have an “attachment saucer” underneath them. These may correspond to the actin feltworks seen with immunofluorescence and are probably responsible for the tight attachment of ROS to the PE cell surface. A similar actin containing lamellipodal structure that forms in macrophages at sites of particle attachment was reported recently by Painter et al. During ingestion, villi were observed to surround ROS as they were ingested. Since these villi are known to contain actin filaments, they presumably correspond to the actin feltwork that encases an ROS during its ingestion.

In the experiments reported here, no abnormality was observed with the involvement of actin in the binding of ROS to the surfaces of dystrophic PE cells. Additionally, actin appears to participate in the ingestion of ROS in these cells. However, the mechanism that results in the ingestion of bound ROS appears to be activated at very few sites of ROS attachment in dystrophic PE cells. This may be due to defective cell surface receptors that normally trigger ingestion or to a lack of such receptors. In either case, the transmembrane signal that is necessary to initiate ingestion appears to be transmitted at few sites of ROS attachment. Additional work is currently underway to answer these questions.

Key words: retinal dystrophy, RCS rat, rod outer segments, pigment epithelium, phagocytosis, actin, immunofluorescence

Acknowledgments

The advice of Drs. Ira Herman, Elias Lazarides, and Christine Oriol is greatly appreciated. We thank Dr. Virginia Clark for providing us with the ROS antiserum, Ms. Deborah Wong and Ms. Toshka Abrams for assisting with the culturing of PE cells, and Dr. Izhak Nir and Ms. Sarah Beyder for assistance and advice with scanning electron microscopy. We also thank Ms. Joanne Siniscalchi for typing the manuscript.

References


