Attachment of steroidogenic lipid droplets to intermediate filaments in adrenal cells

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Summary
Light microscopy of living and extracted adrenal cells (Y-1 mouse adrenal tumour cells and cultured bovine fasciculata cells), using Nomarski optics and fluorescence with nile red to stain lipid, revealed in both cell types that lipid droplets remain attached to intermediate filaments when the cells are extracted to prepare these structures.

Electron microscopy of thin sections shows the presence of lipid droplets in both cell types. The droplets differ in appearance but are, in both cases, surrounded by a complete capsule 5nm wide. The droplets in Y-1 cells include those associated with lysosomes and crystalline structures in addition to typical rounded forms. Only the latter type is seen in bovine fasciculata. Intermediate filaments apparently ending in droplets can also be seen. Immunoelectron microscopy with antivimentin and Protein A conjugated to gold particles together with measurement of the diameter of these structures identifies them as intermediate filaments. When adrenal cells are permeabilised and extracted under mild or severe conditions using Triton X-100, thin sections showed that lipid droplets remain associated with the cytoskeleton and in particular intermediate filaments. Extraction under mild and severe conditions cleared the cell contents, revealing attachment of intermediate filaments to lipid droplets with greater clarity than in unextracted cells, i.e. homogenised cells or cells subjected to lysis. Such attachment was unequivocally demonstrated in stereo pairs. These observations support our earlier studies showing attachment of droplets to intermediate filaments, which suggests a role for these filaments in intracellular transport of cholesterol.

Key words: lipid droplets, vimentin filaments, adrenal cells, electron microscopy, steroidogenesis, cholesterol.

Introduction
We have recently reported that the lipid droplets that serve to store the cholesterol ester used for synthesis of steroid hormones in adrenal cells are specifically attached to intermediate filaments (Almahbobi and Hall, 1990). In these studies we used a mouse tumour cell line (Y-1, American type culture). Lipid droplets constitute a conspicuous feature of steroidogenic cells where they occur in the cytoplasm (Rhodin, 1971; Almahbobi et al. 1985; Almahbobi et al. 1988). Steroidogenesis begins in the inner mitochondrial membrane where cholesterol is converted to pregnenolone (Hall, 1987). The transport of cholesterol from the lipid droplets to the inner mitochondrial membrane constitutes a slow step in the synthesis of steroid hormones that is stimulated by ACTH and cyclic AMP (Hall, 1987). This process of transport involves several steps, at least one of which requires actin (Mrotek and Hall, 1977; Hall et al. 1979a; Osawa et al. 1984).

Our recent studies mentioned above (Almahbobi and Hall, 1990), were based upon whole-mount preparations of cells extracted with detergent. This approach involves a procedure that is relatively free of artefacts (i.e. freeze-drying) and also gives three-dimensional information, which is essential for demonstrating physical relationships between intracellular structures such as droplets and cytoskeleton. Because of the importance of the conclusion reached (important for understanding both the regulation of steroidogenesis and the functions of intermediate filaments), our whole-mount studies must be confirmed by conventional transmission electron microscopy of intact Y-1 adrenal cells, which can be used to eliminate possible artefacts resulting from adventitious binding of droplets in broken cells. Moreover, it is important to confirm these findings with those from primary cultures of normal adrenal cells as opposed to tumour cells, since the composition of intermediate filaments is known to differ between normal and tumour cells (Hedberg and Chen, 1986). For this purpose, in the studies to be reported here we have used both Y-1 cells and cultured bovine fasciculata cells. Although transmission electron microscopy employs a fundamentally different approach from the whole-mount method used in the previous studies, it provides little three-dimensional information. We have therefore devised a procedure based on progressive extraction of cultured cells, with
electron microscopy at each step. By using this approach we have demonstrated attachment of lipid droplets to intermediate filaments in both unextracted and extracted cells. We have also studied the appearance of lipid droplets by Nomarski and fluorescence microscopy.

Materials and methods

Cell preparation and culture

Primary bovine fasciculata cells were prepared as previously described with minor modification (Gospodarowicz et al. 1977). Slices of adrenal fasciculata were incubated in serum-free medium (Ham’s F12 plus Dulbecco’s MEM containing 1mg/ml collagenase (Type 1A), 1mg/ml trypsin and 0.1mg/ml deoxyribonuclease I (Sigma)) for 40 minutes with gentle stirring at 37°C. The suspension was filtered through nylon mesh of 297μm and then through nylon mesh of 74μm pore size. The cells were then washed once in the same medium containing 12.5% horse serum and 2.5% foetal calf serum (v/v). Cells were plated at a density of 1 × 10⁶ cells/ml in serum-supplemented medium. Cultures were performed in a humidified CO₂ incubator at 37°C. After 24 hours the medium was changed and culture continued for 3-6 days. Mouse adrenal tumour cells (Y-1 American Type Culture) were cultured for 7-10 days as described for bovine cells. Steroidogenic activity of both cell types was routinely checked by measuring steroid production.

Broken cell preparations

Cells grown on plates and coverslips were ruptured using the hypotonic swelling technique of Beckers and collaborators (1987) referred to here as lysis. The ruptured cells were processed either for electron microscopy or for further extraction. Mild extraction (0.1% Triton X-100, v/v, for one minute) was performed on cells grown on Formvar-coated gold grids using a method described by Small and co-workers (1978), whereas severe extraction using Triton X-100 was performed as previously described on cells grown on plates, coverslips, glass slide chambers and grids (Fey et al. 1984; Almahbobi and Hall, 1990). Some plates of broken cells and cytoskeletons prepared on culture plates were scraped and pelleted in preparation for electron microscopy. Other plates of cells were scraped, homogenised and processed as pellets for electron microscopy.

Light microscopy

Living cells (Y-1 and bovine fasciculata) grown on glass slide chambers were stained with nile red as previously described (Greenspan et al. 1985; Almahbobi and Hall, 1990). To study the effect of extraction on lipid droplets, living cells were photographed and the stage of the microscope was fixed. Cells were extracted in situ to prepare cytoskeleton (Almahbobi and Hall, 1990), stained with nile red and the same field was photographed. Samples were examined with a Zeiss photomicroscope equipped for epi-illumination using a mercury light source and Nomarski objectives. These procedures have been described elsewhere (Almahbobi and Hall, 1990).

Electron microscopy

Conventional transmission electron microscopy

Intact, broken and Triton-extracted cells were processed as monolayers or pellets for transmission electron microscopy. For conventional microscopy samples were fixed in 1/2 Karnovsky fixative for 60 minutes as previously described (Almahbobi et al. 1988) and embedded in Spurr’s resin. Some experiments were performed according to the method of Frank and collaborators using osmium tetroxide/glutaraldehyde fixation (Franke et al. 1976). Thin sections were examined under a Philips 300 and a Hitachi 7000 electron microscope. The procedure used for whole-mount freezedrying has been reported in detail previously (Almahbobi and Hall, 1990).

Immunoelectron microscopy

Pellets of homogenised Y-1 cells were fixed in a mixture of glutaraldehyde (0.25% v/v) and paraformaldehyde (4% v/v) for 2 hours at 4°C. Samples were dehydrated at 4°C and embedded in Lowicryl K4M. Infiltration and polymerisation were performed at -20°C. Immunogold staining was carried out on thin sections as follows: distilled water, 10 minutes; phosphate-buffered saline (PBS) containing bovine serum albumin (BSA) (2% w/v), 20 minutes; glycine (0.2% w/v) in PBS-BSA, 20 minutes; gelatine (0.1% w/v) in PBS-BSA, 20 minutes; rabbit anti-vimentin (Calbiochem, diluted × 5 with PBS), 60 minutes; gelatine in PBS-BSA as above, three times for 20 minutes each; Protein A/gold (5nm) (Janssen), diluted ×40 with PBS, for 30 minutes; PBS/BSA, three times for 20 minutes each; and distilled water, twice for 10 minutes. Other steps were performed as described above for conventional electron microscopy.

Stereo pairs of thin sections of fasciculata cytoskeletons

The same fields of thin sections of fasciculata cytoskeletons were photographed with tilting at +6 and -6°.

Materials

All materials used have been described previously (Almahbobi and Hall, 1990).

Results

Light microscopy of adrenal cells

It is shown for the first time that lipid droplets in fasciculata cells can be displayed with Nomarski optics (Fig. 1A and C). The droplets are scattered randomly throughout the cytoplasm and are shown to be composed of lipid by staining with nile red as previously reported from this laboratory (Fig. 1B and D). When the same cell is extracted with Triton X-100 to give cytoskeleton, many of the droplets remain unaltered in their distribution on the cytoskeleton (Fig. 1A-D, arrows). Some droplets may be damaged by the extraction procedure used and in some cases droplets out of the plane of focus are difficult to examine in detail. It must be concluded that the droplets are tightly attached to the cytoskeleton, since they have resisted the various extraction and washing procedures used to prepare cytoskeleton. Similar findings are seen in Y-1 cells treated in the same way (not shown).

Electron microscopy of adrenal cells

Fig. 2 shows transmission electron microscopy of thin sections of Y-1 (A and B) and bovine fasciculata cells (C and D). Sections were made parallel to the surface of the coverslip (en face). Lipid droplets are readily
Fig. 1. Nomarski and fluorescence microscopy of bovine fasciculata cells. A high-power field was chosen to show one cell under both Nomarski (A) and fluorescence (B) microscopy. The cell was photographed, the stage fixed and the cell was extracted with Triton X-100 (0.5% for 10 minutes). The cell was photographed immediately after extraction under Nomarski (C) and fluorescence (D) optics. Arrows show specific droplets before and after extraction. n, nucleus; Bar, 7μm.
Fig. 2. Electron microscopy of intact adrenal cells. Cultured Y-1 (A and B) and bovine fasciculata (C and D) cells were prepared for electron microscopy by fixation in situ as a monolayer. Arrows show capsule of droplets (B and D). Double arrows show an oblique section through capsules (B). Arrowheads show intermediate filaments (B and D). er, endoplasmic reticulum; g, Golgi apparatus; l, lipid droplet; m, mitochondrion; n, nucleus. Bars, A and C, 220nm; B, 100nm; D, 60nm.
identified in both cell types (A-D) but the appearance of the droplets is quite different in the two cell types. The droplets of Y-1 cells are less electron-opaque and more variable in shape and appearance than in the case of fasciculata cells, which are highly osmophilic and uniform in shape and size (Fig. 2A and C, respectively). The droplet in the top left of Fig. 2A in a Y-1 cell shows the typical appearance of lipid droplets as commonly described in the literature. The droplets are shown at higher magnification in Fig. 2B and D, where arrows show the limiting membrane or capsule of the droplets in both cell types. The capsule stains darkly (arrows) and is too narrow to constitute a typical membrane bilayer, being $5.0 \pm 0.1\text{nm}$ in width. Capsules of droplets are not readily discerned when they surround deeply osmiophilic droplets or when the section cuts the droplet obliquely (double arrows in Fig. 2B). Arrowheads indicate intermediate filaments that can be seen through the cytoplasm and in Fig. 2D some $10\text{nm}$ filaments approach and even appear to end on droplets before leaving the plane of section. However, details of contact points cannot be seen in the presence of cytosol. Other typical cellular structures can be seen in these micrographs including smooth endoplasmic reticulum, which is prominent in steroidogenic cells and is situated close to mitochondria and lipid droplets.

**Electron microscopy of lipid droplets in Y-1 cells**

The lipid droplets of Y-1 cells were examined in greater detail by transmission electron microscopy (Fig. 3). The droplets show a variety of forms in contrast to those of fasciculata cells. Some droplets are seen adjacent to endoplasmic reticulum (Fig. 3A and D, arrows). Other intracellular structures. In A a droplet is associated with endoplasmic reticulum. Cholesterol crystals are seen in B and C and association of a droplet with a lysosome can be seen in C, D and E. Micrograph E is a high magnification of the rectangle in D. Arrows indicate endoplasmic reticulum (A and D), cholesterol crystals (B and C) and endoplasmic reticulum and lysosome (E). Arrowheads, capsule of droplets (A,B,D and E). l, lipid droplet; ly, lysosome; m, mitochondrion; *, residual body; Bars, A, 55nm; B, 250nm; C, 170nm; D, 110nm; E, 45nm.

![Fig. 3. Transmission electron microscopy of lipid droplets in Y-1 cells. Droplets are shown associated with various intracellular structures. In A a droplet is associated with endoplasmic reticulum. Cholesterol crystals are seen in B and C and association of a droplet with a lysosome can be seen in C, D and E. Micrograph E is a high magnification of the rectangle in D. Arrows indicate endoplasmic reticulum (A and D), cholesterol crystals (B and C) and endoplasmic reticulum and lysosome (E). Arrowheads, capsule of droplets (A,B,D and E). l, lipid droplet; ly, lysosome; m, mitochondrion; *, residual body; Bars, A, 55nm; B, 250nm; C, 170nm; D, 110nm; E, 45nm.](image-url)
droplets are close to cholesterol crystals (Fig. 3B and C) and yet others are in intimate contact with structures that can be readily identified as lysosomes from the work of other investigators (Szabo, 1968; Rhodin, 1971, Mattson and Kowal, 1982). Fig. 3E represents high magnification of the rectangle marked on Fig. 3D and shows a droplet that is in contact with such a lysosome. In this case the inset shows the bilayer membrane of the lysosome (arrow) in close proximity to the capsule of the droplet (arrowheads) giving rise to a three-layered structure, i.e. the bilayer membrane of lysosome, the capsule of the droplet and the space in between (Fig. 3E, see the droplet labelled with 1 at the top right-hand corner of the micrograph). Fig. 3C shows a droplet that is closely associated with both a lysosome on one side and a crystal on the other.

Electron microscopy of extracted Y-1 adrenal cells
Progressive extraction of Y-1 cells from simple homogenisation through mild to severe extraction was followed by transmission electron microscopy as shown in Fig. 4. Following homogenisation (Fig. 4A) cytoskeleton, droplets and other structures including mitochondria can be seen. In some fields, droplets are seen to be associated with intermediate filaments (not shown). Fig. 4B shows a whole-mount prepared by mild extraction (0.1% Triton X-100 for 1 minute). Some organelles and membranes have been removed, leaving cytoskeleton and lipid droplets. The affinity of droplets for cytoskeleton must be greater than that of cytoskeleton for other organelles and internal structures. Again droplets can be seen attached to intermediate filaments as in our previous studies (Almahbobi and Hall, 1990). With severe extraction (1% Triton X-100 for 10 minutes) thin sections reveal intermediate filaments associated with droplets (Fig. 4C and D). Droplets appear to be attached to the intermediate filaments. In some cases vesicular structures are seen throughout the cytoskeleton (Fig. 4D). In a whole-mount preparation of intermediate filaments (Fig. 4E) droplets are seen to be attached to the filaments. Such a preparation shows the full thickness of the intermediate filament component of the cytoskeleton. In addition a homogeneous material of unknown composition is seen throughout the preparation.

Electron microscopy of broken fasciculata cells
Fig. 5 shows a bovine fasciculata cell broken in situ on a culture dish by hypotonic swelling. The cells were processed for electron microscopy as monolayers without further disruption of cellular structure. The usual cellular structures are present and cell rupture has produced some changes in the general ultrastructure of the cell. The inset shows high magnification of a lipid droplet in direct contact with intermediate filaments. When studies like those shown for Y-1 cells in Fig. 4 were performed with fasciculata cells similar results were obtained. Two stages in the progressive extraction of these cells are shown in Fig. 6. The appearance of the cells after homogenisation is shown in Fig. 6A and Fig. 6B, which shows a higher magnification of the rectangle marked in Fig. 6A. Intermediate filaments are more readily detected after homogenisation as the result of loss of cytosol, which partly obscures the cytoskeleton. However, the ultrastructure of the membranous organelles is altered to some degree. Intermediate filaments appear to be in contact with lipid droplets (Fig. 6B). Fig. 6C shows a similar association between filaments and a mitochondrion. With severe extraction (0.5% Triton for 10 minutes) (Fig. 6D) lipid droplets remain attached to intermediate filaments. The appearance of droplets closely resembles that seen in the intact cell (Fig. 2C) and reinforces the findings made with light microscopy (Fig. 1). Fig. 6D also show the nuclear skeleton. At higher magnification the association between droplets and filaments is again revealed and some unidentified vesicles are seen (v in Fig. 6E).

Immunoelectron microscopy of adrenal cells
Protein A conjugated to gold particles (5nm) was used to identify specific binding of anti-vimentin to thin sections of Y-1 cells. Although immunolabelling was not strong, gold particles are seen associated in areas where intermediate filaments were visible (Fig. 7A). Gold particles are also seen on the limiting membrane around droplets (Fig. 7B). No gold particles are seen in areas around the droplet where no intermediate filaments are seen. When anti-vimentin was preabsorbed with vimentin no gold particles were seen (Fig. 7C). In this micrograph numerous ribosomes are to be seen and can be distinguished from gold particles by shape, size, density and relation to the plane of section.

The relatively small number of gold particles in Fig. 7A and B is a typical result of the limited exposure of the relevant epitopes in a thin section. Careful measurement of the diameters of the filaments gave values of 10 ± 2nm (mean and range, n=200). We conclude that structures attached to the droplets can indeed be unequivocally identified as vimentin intermediate filaments, in keeping with our earlier observations (Almahbobi and Hall, 1990). In addition, sections treated with Protein A/gold but without first antibody and those treated with normal IgG showed no gold particles (not shown).

Stereo pairs of thin sections of fasciculata cytoskeleton
Stereo pairs of extracted bovine fasciculata cells (0.5% Triton X-100 for 5 minutes) were examined with tilting at +6 and −6° (Fig. 8). Whereas most of the filaments in the vicinity of a given droplet pass above or below the droplet, others can be seen in direct contact with a droplet. In sections passing obliquely through a droplet capsule, filaments that do not appear to be in direct contact with the droplet are in fact touching the capsule at some points above or below the plane of section.

Discussion
Light microscopy of Y-1 cells and cultured bovine fasciculata cells shows that both cell types contain droplets staining with Nile Red. The sizes, distribution...
Fig. 4. Transmission electron microscopy of extracted Y-1 cells. (A) Homogenised cells; (B) whole mount of mildly extracted cell (0.1% Triton X-100 for 1 minute); (C and D) thin sections of severely extracted cell (0.5% Triton X-100 for 10 minutes); (E) whole mount of intermediate filaments. Arrows, intermediate filaments; l, lipid droplet; m, mitochondrion; n, nucleus; *, dilated endoplasmic reticulum; v, unidentified vesicular structure. Bars, A, 170nm; B, 360nm; C, 100nm; D, 110nm; E, 400nm.

and staining of these droplets, together with the results of an earlier study (Almahbobi and Hall, 1990), show that these structures are the well-known lipid droplets in which steroidogenic cholesterol is stored. Moreover, extraction of the cells in situ by means of Triton X-100 and ammonium sulphate leaves the droplets associated with intermediate filaments. We have demonstrated that this method of extraction removes other organised cytoskeletal elements in Y-1 cells (Almahbobi and Hall, 1990). Extraction, with fixed stage, shows that individual droplets resist removal by the various agents and the washing procedures used in this extraction.
Fig. 5. Transmission electron microscopy of bovine fasciculata cells following lysis. Bovine fasciculata cells were subjected to lysis (Materials and methods) and then prepared for transmission electron microscopy of thin sections en face. Inset shows high magnification of the rectangle. Arrows, intermediate filaments attached to lipid droplet; l, lipid droplet; m, mitochondrion; n, nucleus. Bars, 230nm; 80nm (inset).
Fig. 6. Transmission electron microscopy of extracted bovine fasciculata cells. (A, B and C) Cell homogenate; (D and E) severely extracted cells (0.5% Triton X-100 for 10 minutes). Arrows, intermediate filaments. I, lipid droplet; m, mitochondrion; n, nucleus; *, dilated endoplasmic reticulum; v, unidentified vesicular structure. Bars, A, 300nm; B, 100nm; C, 110nm; D, 600nm; E, 60nm.

Obviously the droplets must be closely attached to the intermediate filaments that make up the residue remaining after extraction.

It is well known that lipid droplets are difficult to study by electron microscopy, because the usual procedures required for this method are likely to remove lipid structures (Blanchette-Machie et al. 1989). Moreover, it is very difficult to study the cytoskeletons of cells, because they are obscured by the overlying cytosol, which must be removed if we are to examine these structures (Lin et al. 1990). In this study we have investigated the association between droplets and intermediate filaments by progressive extraction, proceeding from intact cells to those that have been severely extracted. In the intact cell, electron microscopy of Y-1 cells shows that lipid droplets vary considerably in size, shape and osmiophilia, as well as in their association with other cellular structures. Some droplets appear to have fused with lysosomes so that a three-layered structure incorporating the lysosomal bilayer with the single layer of droplet capsule is seen between lysosome and droplet. Since a lipase respon-
Fig. 7. Immunoelectron microscopy of homogenised Y-1 cells. (A and B) Positive immunogold staining of homogenised cells. (C) Negative staining with anti-vimentin preabsorbed with vimentin before use in the immunogold procedure (Materials and methods). I, lipid droplet; n, nucleus; *, extracellular resin. Bar, 80nm.

Lipid droplets and intermediate filaments

...sible for converting cholesterol ester to free cholesterol has been reported to occur in lysosomes (Brown et al. 1979; Boyd et al. 1983), the association between these two organelles may be a step on the pathway from cholesterol ester in droplets to free cholesterol in mitochondria. An association between the lipase activity and lipid droplets was demonstrated by cytochemical localisation in cultured mouse macrophages (McGookey and Anderson, 1983).

In Y-1 cells some droplets are close to smooth endoplasmic reticulum. Since Y-1 cells synthesise little cholesterol from acetate, a process requiring the enzymes of endoplasmic reticulum (Faust et al. 1977), it is not clear why the droplets should be associated with this structure. In this connection it should be pointed out that frequent references in the literature to the relative paucity of lipid droplets in Y-1 cells (Kawaoi et al. 1977; Mattson and Kowal, 1982) may arise from...
overlooking cholesterol crystals and lysosome/droplet complexes that constitute stores of cholesterol ester that may be functionally as important as the more common form of droplets.

The droplets of bovine fasciculata cells are more nearly uniform in size and shape and are intensely and uniformly osmiophilic. In these cells association of droplets with lysosomes is not apparent and crystals of cholesterol are not seen. Although adrenal cells in the bovine adrenal gland synthesize much of their cholesterol from acetate (Fruhling et al. 1973), when the cells are cultured under the conditions used in these studies they use low-density lipoprotein (LDL) as the major source of cholesterol and lipid droplets become more conspicuous (unpublished).

Careful examination of transmission electron micrographs shows numerous filaments of 10 nm in diameter approaching and in contact with lipid droplets. The close association of these two structures can be seen when the cells are broken by such mild procedures as homogenisation and osmotic lysis. Clearly this association cannot be attributed to artefact. The intimate association between these two structures is rendered more obvious by mild extraction, which shows direct contact in thin sections. Severe extraction with Triton and ammonium sulphate leaves droplets closely associated with intermediate filaments under conditions in which we have shown direct contact by tilting the specimens (Almahbobi and Hall, 1990). This process not only clears the cell to make the contact between droplets and filaments more obvious but demonstrates both the tightness of the association, which resists repeated washing, and the protection against attack of the lipid by the detergent, which results from the presence of a capsule around each of the droplets. The identity of the filaments has been demonstrated by determination of their characteristic diameters (10 nm), by immunofluorescence and by immunoelectron microscopy with anti-vimentin and Protein A/gold (Almahbobi and Hall, 1990, and these results). The filaments are not heavily labelled by this procedure but the specificity of the reactions used shows beyond doubt that these intermediate filaments contain vimentin.

This association of droplets with intermediate filaments is reminiscent of that reported in cultured macrophages (McGookey and Anderson, 1983) and in breast tissue (Franke et al. 1987). In those cases, however, the intermediate filaments formed a fenestrated cage-like structure around nonmembrane-bound droplets. In the adrenal cell each droplet is surrounded by a complete capsule of 5 nm in width and via such a capsule they are tightly bound to intermediate filaments. Presumably it is because the capsule is complete that the lipid droplets are so effectively protected from the effects of Triton and agents used in the preparation of thin sections. The immunofluorescent and immunoelectron labelling demonstrate the presence of vimentin around the droplets. But the question of whether vimentin is the only component of the capsule or whether other protein(s) might be associated with this structure is not yet answered. Moreover, possible involvement of other cytoskeletal structures (micro-
of these structures to intermediate filaments that might specifically to mitochondria. Possibly, the direction of false appearance of direct contact. This possibility is excluded by the use of stereo pairs, which show no evidence of separation between droplet and some of the intermediate filaments (Fig. 8). It is clear on examination of the stereo pairs that some of the filaments that are seen in the same plane as a droplet are in direct contact with the capsule of the droplet. The fact that lipid droplets are tightly attached to intermediate filaments suggests a mechanism by which the intermediate filaments might serve to direct the droplets specifically to mitochondria. Possibly, the direction of movement of lipid droplets is regulated by attachment of these structures to intermediate filaments that might be seen as "guide wires". Previous studies from this laboratory showed that stimulation of cholesterol transport to mitochondria under the influence of ACTH requires microfilaments (Mrotek and Hall, 1977; Hall et al. 1979a, b; Osawa et al. 1984). A recent report showed that ATP causes a reorganisation of intermediate filaments in permeabilised fibroblasts (Tint et al. 1991). This reorganisation results from the contraction of peripheral actomyosin. These observations suggest one way in which the two filamentous structures (intermediate filaments and microfilaments) could co-operate in directing intracellular transport of droplets to mitochondria. These and other possibilities are currently being investigated in this laboratory.

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References


