Cytoplasmic filaments: their role in motility and cell shape

Moving cells are fascinating to watch. Today, 300 years after cells and their movements were first observed with the primitive microscopes of van Leeuwenhoek, cell biologists are beginning to understand how cells move. New fluorescence and electron microscopic techniques are now being used in eye research to study cell movements which occur in corneal wound healing, phagocytosis by the retinal pigment epithelium (RPE), morphogenetic cell shape changes which occur at the lens bow, and other biologically important cell shape changes.

Filamentous structures within the cytoplasm are involved in cell motility and cell shape. Three major size classes of cytoplasmic filaments or tubules are present in higher eukaryotic, nonmuscle cells. They are microfilaments (actin filaments), intermediate filaments, and microtubules.

Microfilaments

Microfilaments, the smallest of the three types are approximately 50 to 75 Å in diameter and of variable length and are composed of globular monomers of the protein actin, aligned to form double-helical filaments. The actin of microfilaments is similar to muscle actin, and this protein is present in all eukaryotic cells examined to date. (For reviews see refs. 1 and 2.)

Actin filaments can be localized intracellularly by a specific histochemical technique which uses fragments of myosin, either heavy meromyosin (HMM) or the smaller subfragment-one (S-1). Both HMM and S-1 retain the active ATPase head, and when they penetrate cells whose membranes are made permeable by glycerination, the fragments bind specifically to actin, forming arrowheads, visible by electron microscopy, along the actin filaments. Two light microscopic techniques for actin localization have been developed; both use actin-specific protein ligands labeled with fluorescent dyes. One method employs fluorescent actin antibodies and the other fluorescence-tagged HMM. These methods show that actin filaments are distributed in two basic patterns in nonmuscle cells: (1) cortical networks of actin filaments along plasmalemmata, with individual filaments of the network appearing to insert into the membrane, and (2) parallel bundles of filaments known as sheaths or stress fibers. The latter occur along basal plasma membranes of moving cells, in cleavage furrows of cytokinesing cells, in microvilli of intestinal epithelia, and in the acrosomal process of some invertebrate sperm.

Myosin also appears to be present in nonmuscle cells, although in lower concentration than actin. The properties of specific myosins from different cell types vary considerably, but all have ATPase activity. Antibodies to myosin of platelets and smooth muscle have been prepared, and their distribution studied with immunofluorescence microscopy. Myosin distribution appears to parallel actin filament distribution in fibroblasts.

Since actin filaments are associated with motile cells (amoeba and moving cells in culture) and with motile portions of cells (i.e., cleavage furrows, microvilli of intestinal epithelia) and because myosin is also present in the cytoplasm of these cells, the
general theory has arisen that an acto-myosin system, similar in some respects to that found in striated muscle cells, is involved in generating force for cell motility. The actin networks at cell surfaces are also believed to contribute to cell shape. (For reviews see refs. 1 and 2.)

With the use of S-l labeling, actin filament distribution has been studied in two ocular epithelia: corneal epithelia of rats and RPE of monkeys and humans. In corneal epithelial cells of rats, actin filaments form a cortical network under microvilli of the superficial cells. Perhaps these filaments form a cytoskeleton which holds microvilli in their orderly array. Corneal epithelial cells moving to cover an abrasion have a dramatically different arrangement of actin filaments in their cytoplasm; bundles of parallel actin filaments extend along the base of the cell and out into their leading edges. In addition, inside the very tip of the leading edge of the moving cell there are thick networks of actin filaments. The bundles may play an important role in generating the force required for movement of these cells across the denuded basement membrane.

It has been long known that local anesthetics inhibit corneal epithelial wound healing. Although it was assumed that these drugs act directly at the level of the plasma membrane, recent reports indicate that the tertiary amine local anesthetics (including procaine) disrupt actin filaments along cell membranes of endothelioid 3T3 cells. Filament arrangements within corneal epithelial cells migrating to cover corneal abrasions are also disrupted when corneas are cultured in the presence of 1 mM proparacaine. These findings indicate that the adverse effects of local anesthetics on wound healing are related to disruption of the contractile filament system of the epithelial cells.

In human and monkey RPE, actin filaments are present in ordered arrays in the apical processes which extend to surround the distal disks of the photoreceptors. The filaments appear to be attached or inserted into the cell membrane, and it has been suggested that they form a cytoskeleton which supports the processes or perhaps they generate an active force for the phagocytic event. With the use of actin antibody, cortical networks and large quantities of parallel sheaths of actin filaments have been demonstrated in sheets of cultured bovine lens cells. These filaments may play a role in the cell movements and shape changes which occur at the lens bow.

Recent reports indicate that cytochalasin B reversibly reduces outflow resistance in intact and disinserted monkey eyes. Since cytochalasin B causes disruption of microfilaments in some cell types, it has been suggested that microfilaments play a role in aqueous outflow. It should be noted, however, that cytochalasin B has numerous effects on cells, including disruption of hexose transport across cell membranes (for review see ref. 13), and thus the conclusions of these studies must be viewed with caution. The effects of cytochalasin B on the ultrastructure of the endothelial cells of the trabecular meshwork and Schlemm's canal have not yet been reported, but study of these cells in normal and glaucomatous eyes, with modern techniques for actin localization, may provide new information on the etiology and morphological basis of open-angle glaucoma.

Investigations of the role that defects in actin filaments may play in disease process have only begun. Already two human diseases are known that show defects in microfilament distribution or in proteins associated with actin; they are adenomatosis of the colon and rectum and spherocytosis, respectively. Fibroblasts of patients with the inherited cancer of the colon have an altered actin filament distribution; in spherocytosis, spectrin, a membrane protein which together with actin has a role in regulation of cell shape, is altered.

Intermediate filaments

Intermediate filaments are distinct from actin filaments and microtubules, and they,
too, are ubiquitous in eukaryotic cells. These 100 Å diameter filaments have been called tonofilaments, neurofilaments, glial filaments, and endothelial filaments, depending on the cell of origin. Little is known about the biochemistry of these filaments and even less of their cellular function. Moreover, it is not clear whether these filaments from different tissue sources are related chemically or functionally.

One of the most characteristic features of tonofilaments is their insertion into desmosomes, structures along plasma membranes that form attachments between cells or between cells and basement membranes. Tonofilaments occur in loose networks in epithelial cells and are especially plentiful in the basal cells of the epidermis. Some evidence indicates that they play a role in keratinization in epidermis, and these intermediate filaments have therefore been termed keratin fibers. Proteins of keratin filament-enriched preparations from epidermis separate into four to seven major bands termed α-keratins on sodium dodecyl sulfate (SDS) electrophoresis. Neural and glial filaments have been isolated by cell fractionation, and a major component of both filaments appears to be a protein of about 54,000 to 56,000 m.w. Antibodies to neurofilament protein will cross-react with glial filament protein, and immunofluorescence studies show that neurofilament antibodies will stain 100 Å filaments of endothelial cells. The intermediate filaments of various cell types and species may be related, but biochemical evidence of this is lacking.

Ocular tissues which have an abundance of intermediate filaments are the corneal and conjunctival epithelia, RPE, and all neurons. Little direct evidence is available concerning the function of the intermediate filaments at these sites. Generally, they are considered to be cytoskeletal structures.

**Microtubules**

Largest of the three types of cytoplasmic filaments is the microtubule. It is made up of 13 protofilaments arranged concentrically to form hollow circular tubes 180 to 250 Å in diameter. The protofilaments are made of two electrophoretically separable protein chains (α- and β-tubulin) of identical molecular weight but different amino acid composition (for review see ref. 17).Microtubules are present in most cell types, and they are most obvious in mitotic spindles, cilia, flagella, and neurons.

Microtubules contribute to formation and maintenance of cell shape. Neurotubules of axons maintain the shape of these important long cytoplasmic processes. Microtubules are often found as components of biological motility machines, e.g., sperm, flagella, and mitotic spindles. The drug colchicine specifically depolymerizes tubulin and inhibits microtubule formation. By the criterion of colchicine sensitivity, microtubules are known to contribute to pigment granule movement in a variety of cell types and axonal vesicle transport, as well as chromosome movement.

Morphological evidence suggests that in epithelial cells at the lens bow microtubules elongate to force these cells to form lens fibers. Other ocular cells in which microtubules are prominent are the cilia of photoreceptors, all neurons, and RPE cells. Microtubules are associated with phagocytic vacuoles of RPE, and it has been suggested that they play a role in movement of the vacuoles into the cell. Microtubules are also evident in mitotic spindles of the corneal epithelium and in the cytoplasmic extensions that spread during corneal wound healing. Colchicine inhibits epithelial motility during wound healing.

At least one human disorder is known to be caused by defective microtubules: congenital immotile-cilia syndrome. Defects in the dynein arms along microtubules of cilia result in chronic airway infections, and men have immotile spermatozoa. A deficiency of microtubules has also been implicated in Chediak-Higashi syndrome.

In summary, it is clear that there are distinct differences between the major cytoplasmic filaments, both in their morphology and biochemistry. It is also clear that cell...
biologists are just beginning to sort out the functions of these filamentous structures within cells. Investigations of the role they may play in disease processes have only just begun. We are rather ignorant of disease at the cell organelle level; a sick organelle may be the very basis for a sick organ. Investigating the cell biology of filament systems of eye tissues may prove to be a fruitful avenue of research on normal and abnormal function of cells of the eye.

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REFERENCES