Organizing the Cytoplasm

An intricate, interconnected array of filaments in the cytoplasm gives this part of the cell more order and complexity than was once thought

It is something of an understatement when Keith Porter of the University of Colorado in Boulder says, "The cytoplasm of the cell emerges as highly structured." Over the past several years cell biologists have shown the cytoplasm to be pervaded by an intricate network of filaments that participate in all aspects of a cell's life.

The filaments are needed to shape and maintain cell structure, but they also contribute to more dynamic cellular activities, including cell division and movements ranging from the wanderings of amoeboid cells to the beating of the cilia and flagella that propel many kinds of microorganisms, to the transport of granules and other substances within the cell. In addition, certain cytoplasmic proteins, including enzymes, which were once thought to be freely diffusible, may be bound to components of the network, a finding which suggests new levels of organization where none had been expected.

The cytoplasmic network includes the three major filament systems of the cell skeleton—the microtubules, microfilaments, and intermediate filaments—plus an interconnecting filigree of thin filamentous bridges, which has been named the microtrabecular lattice by Porter and his colleagues, who discovered it. Participants at a recent meeting on "The Cytoplasmic Matrix and the Integration of Cellular Function"* gathered in Bethesda, Maryland, to discuss the workings of these elements. The term "cytoplasmic matrix" encompasses all of the cytoplasmic filaments, plus their associated proteins and water. It implies that the cytoplasm is a tissue woven of ordered and cooperating elements.

Cooperation among the cytoplasmic elements is an emerging area of study in cell biology. Although much has been learned about the structural properties of the cytoskeletal filaments and some of their activities—the beating of cilia and flagella, for example—many problems remain unsolved. Among these are the extent and nature of the interactions among the different filaments of the cytoskeleton. As Porter puts it, "Most investigators are captivated by the separate units. Very few have gotten down to examining the glue that ties them into one structure. But you can't blame people for studying the parts before the whole."

The problems of studying the functions of the individual filament systems are formidable, even without worrying about interactions. For the most part, investigators have been limited to studies by electron and other forms of microscopy, which require stains or fixing procedures that may alter structures, or to test-tube systems that may not fully reflect conditions in the cell. Studying the dynamic activities of the proteins in the living cell is much more difficult. Investigations of the role of the microtubules in mitosis are a good case in point.

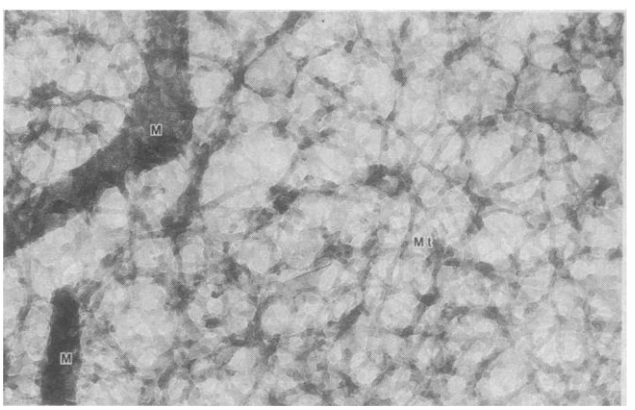
The microtubules, which have a diameter of about 25 nanometers, are the largest of the cytoskeletal filaments. They are polymers composed primarily of subunits of the protein tubulin. Dividing cells undergo profound microtubular rearrangements. Those observed in nondividing cells disassemble as the cell gets ready to divide and the mitotic spindle forms. The spindle consists of long microtubules that extend from both poles of the dividing cell to the equatorial region where they overlap and also shorter ones that extend from the poles to the chromosomes. These latter appear to pull the two halves of the duplicated chromosomes apart before the cell divides.

Despite years of study, the way in which the spindle works is poorly understood and the source of energy for the chromosomal movements has not been identified with any certainty. Two groups of investigators at the Bethesda meeting described their work on the microtubules in mitosis, but the views they presented were quite different.

Thomas Pollard and his colleagues at the Johns Hopkins School of Medicine have been studying the interactions between microtubules and another type of cytoskeletal filament, the microfilaments. These are polymers of the protein actin and, at a diameter of about 7 nanometers, the smallest of the cytoskeletal filaments. "We are part of an intrepid small band interested in the possibility that the three filament systems interact with one another," Pollard notes.

Pollard and his colleagues have shown that microtubules interact with actin filaments in the test tube. The interaction requires the presence of proteins that are normally associated with microtubules (called MAP's for microtubule-associated proteins) in the cell and may serve to cross-link the two types of filaments. But Pollard cautions, "We don't known whether any of the interesting interactions we have been studying in the test tube have anything to do with the cell."

Pollard and other investigators have reported that actin is present in the spindle, although not every study has confirmed this observation. More recently, the Pollard group, using electron microscopy, has identified filaments, with diameters in the microfilament range, in the spindle. However, the investigators have not yet shown that the putative microfilaments, which are much shorter than these structures usually are, actually contain actin. Actin filaments can interact with myosin, an enzyme that supplies energy by splitting adenosine triphosphate, and Pollard hypothesizes that actin filaments, if present in the spindle, might help power the mov

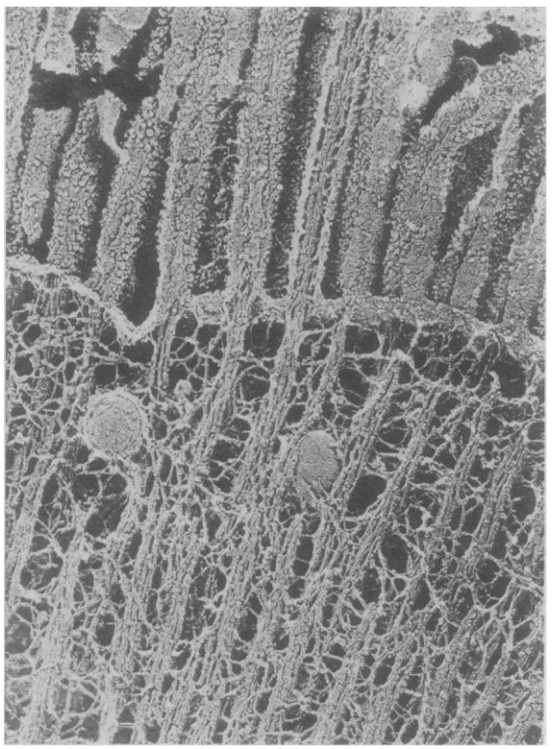


Keith Porter and K. L. Anderson

Microtrabecular lattice

This micrograph shows the complex pattern of filaments in the cytoplasm. The mitochondria (M) are the elongated dark structures. Microtubules (Mt) can also be seen as long filaments running through the micrograph. Interconnecting these structures are the smaller microtrabecular filaments.

*The meeting, which was organized by Keith Porter for the Fogarty International Center of the National Institutes of Health, was held on 17 to 20 October.



Brush border filaments

Bundles of actin filaments run vertically from the finger-like microvilli into the brush border cell (from the chicken intestinal lining). The smaller filaments that cross-link the actin fiber bundles are composed of at least two proteins, brush border myosin and a spectrin-like protein called TW 260/240. [Source: N. Hirokawa of Washington University and T. Keller and M. Mooseker of Yale University]

ments of the chromosomes during mitosis.

Jeremy Pickett-Heaps of the University of Colorado does not see any evidence for actin-microtubular cooperation in the spindle, but proposes instead that the microtubules constitute a relatively passive framework over which the chromosomes may slide. Much of the data on which this theory is based has been obtained with diatoms, unicellular plants that have external silica skeletons. Although there are questions about whether diatoms are representative of other, especially animal, cells, Pickett-Heaps says, "I see no reason to believe that diatoms have evolved a fundamentally different system of achieving mitosis."

Pickett-Heaps and his colleagues have identified in diatoms a diffuse material, which they call the collar and which extends from the two poles of the cell to the regions where the chromosomes attach to the spindle fibers. Pickett-Heaps suggests that the collar, possibly a special form of the microtrabecular lattice, may be the contractile material that pulls the chromosomes over the microtubular framework to the poles. The chromosomal movements may be analogous, he hypothesizes, to the movement of pigment granules, a system described in Bethesda by Mark Stearns of Georgetown University and Mark McNiven of the University of Colorado.

The pigment cell of certain animals carry colored granules that can be dispersed radially throughout the cell or aggregated into the center, thus altering the animal's coloration. According to Stearns, who previously worked with Porter in Boulder, the microtubules of the pigment cells from the fish *Holocentrus* extend radially from the cell center to the periphery. There is also a three-dimensional network of small filaments, part of the microtrabecular lattice, that connects the microtubules to one another and to the pigment granules.

As the pigment granules disperse or aggregate, the lattice material to which they are connected moves with them. The microtrabecular fibers shorten and thicken during aggregation and elongate during dispersion, an observation which suggests that the microtrabecular lattice may be propelling the granules back and forth on the microtubular framework. It also helps to maintain their relative positions in the cell, which do not change as they move.

Stearns and his colleagues have shown that the microtrabecular lattice that connects the microtubules to one another is rich in one of the microtubular-associated proteins (designated MAP-1), whereas the lattice to which the granules are connected is rich in the protein designated MAP-2.

In any event, Pickett-Heaps suggests, albeit cautiously, that chromosome movement during mitosis may be brought about in the same way as that of the pigment granules. He is cautious because other theories of mitosis, which had promising starts, encountered problems when explored in greater detail. "The more we delve into mitosis, the more subtle and complicated it gets," he points out.

Although the question of whether actin filaments are present in the mitotic spindle has not been settled, other aspects of their structure and function are somewhat clearer. They can form connections with the cell membrane and may influence cell shape, locomotion, and the distribution of ion channels and receptors for hormones and other biologically active materials. For example, treatment with concanavalin A (Con A), a plant protein that binds to receptors on cell surfaces, causes cells of the slime mold *Dictyostelium discoideum* to become linearly polarized with a cap of bound Con A on one end and a pseudopod on the other. Actin filaments, in conjunction with myosin, are involved in this capping.

John Condeelis of Albert Einstein College of Medicine presented data suggesting that one consequence of capping is a concentration of ion channels or pumps in the capped end, which could effectively polarize the cells electrically and thus influence the structural polarity of the cells and their direction of movement.

As cells move, their arrangement of actin filaments may change dramatically. Parts of cells that have settled down and made contacts with the surface contain "stress fibers," bundles of actin filaments, that are not present in the forward-moving edges.

Because actin filaments that connect with the cell membrane are thought to be involved in the transmission of signals from the membrane to the cell interior, the way in which they attach to the contact sites has received a great deal of attention. A number of proteins are involved in these connections. Among those that came in for attention at the Bethesda meeting is vinculin. According to Benjamin Geiger of the Weizmann Institute of Science in Rehovot, Israel, this protein forms part of the organizing center for formation of actin filaments at the sites of contact between a cell membrane and the substrate surface.

Vinculin first binds to the inner surface of the cell membrane at the site of contact where the protein helps to organize formation of actin filaments and attach their ends to the membrane. Other proteins are also involved in binding actin ends to the contact sites, however. A newly discovered one, which is called talin, was identified by Keith Burridge and his colleagues at the University of North Carolina in Chapel Hill.

A relatively new development in cell biology is the discovery in several labo-

ratories, including that of Burridge, that many types of cells contain proteins of the spectrin family. Until a year or two ago, spectrin had been thought to be present only in red blood cells where it is a prominent component of the cytoskeleton, serving to cross-link actin filaments.

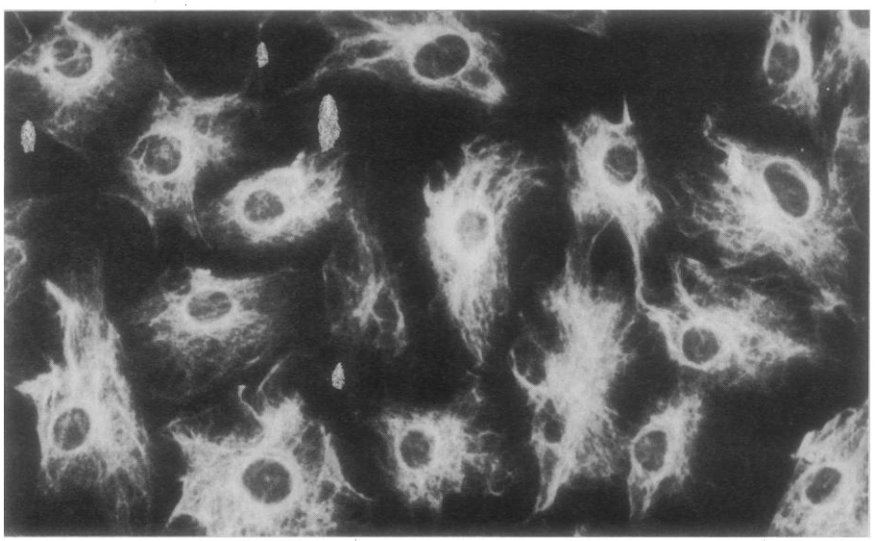
Burridge thought that it might play a similar role in nonerythrocyte cells. However, injection into cells of a monoclonal antibody against spectrin, produced a surprising result. The antibody bound to spectrin and caused it to aggregate, but there was little effect on the microfilament bundles. "Spectrin is not involved in the integrity of these large microfilament bundles," Burridge says. "It is not needed for anchorage [of the microfilaments]." Instead the antibody caused the clumping of the third type of cytoskeletal filament, the intermediate filaments, especially in regions where the spectrin aggregates were located.

The microtubules and microfilaments behave dynamically in that they may disassemble or reaggregate, depending on conditions in the cell. In contrast, the third type of cytoskeletal filament, the intermediate filaments, are more structural in nature. (They are designated intermediate because their diameter—about 10 nanometers—is between the diameters of the other two filament types.)

Sheldon Penman of the Massachusetts Institute of Technology suggests that intermediate filaments, in conjunction with the filaments of the nuclear matrix, may serve as a scaffold for the cytoskeletal framework. Epithelial cells from which 95 percent of the protein had been extracted retain networks of intermediate and nuclear matrix filaments. They also retain their shape.

One function of the intermediate filaments may be to maintain the position of the nucleus. They form a ring around the nucleus with branches extending outward through the cytoplasm. Robert Goldmann of Northwestern University has observed that the filaments extend into the pores of the nuclear membrane. "The inference is," says Peter Steinert of the National Cancer Institute, "that the basket-weave network of the filaments around the nucleus stabilizes the nucleus." In addition, they may also be involved in transport of materials into and out of the nucleus.

There are indications that intermediate filaments can interact with other elements of the cytoskeleton. For example, when certain types of cells are treated with agents that disrupt microtubular structure, the intermediate filaments, which normally extend from a ring around the nucleus throughout the cytoplasm, collapse, too.



Intermediate filaments

Intermediate filaments may be composed of any of 30 or so different proteins, but the filaments of a given cell type usually contain only two or three of these. The filaments shown, in bovine lens-forming cells, contain the protein vimentin. They extend throughout the cell from a ring encircling the nucleus. When the cells are treated with a drug that disrupts the microtubules, vimentin-containing intermediate filaments collapse back into the perinuclear ring. [*Source: Reprinted with permission from T. E. Kreis* et al, Cell *32, 1125 (1983)*]

Raymond Lasek of Case Western Reserve University School of Medicine has been studying the transport of materials from the cell bodies of neurons, the site of all synthetic activities in these cells, along the axon, the long projection that connects a neuron with its target. He finds that there is complete separation of the transport of the components of the membranous portions of cells, which move at the rate of 0.5 meter per day, and the transport of the components of the cytoplasmic matrix, which travel much more slowly.

The proteins of the microtubules and intermediate filaments move together at 0.004 meter per day and the other materials in the slow component move at 0.001 meter per day. The association of the microtubules and intermediate filaments in one transport component suggests an interaction between the two.

In addition, Richard Vallee of the Worchester Foundation for Experimental Biology, presented evidence suggesting that the protein MAP-2 cross-links the two types of filaments. The proteins MAP-1 and MAP-2, he noted, appear as fine filamentous arms on the microtubule surface. The composition of the microtrabecular lattice has been something of a mystery but it is beginning to appear as if the microtubule-associated proteins constitute at least part of some forms of the lattice.

Even the cellular water, which occupies roughly 70 percent of the cell volume, may be organized, although this point is still subject to dispute. James Clegg of the University of Miami has calculated that as much as 20 to 40 percent of the water may be bound to the filaments of the cytoplasmic matrix. "The object of this exercise is to hint that we should expect a large fraction of the water to be influenced by the cytoplasmic matrix," he explains.

In addition, approximately 80 percent of the cytoplasmic proteins cannot diffuse freely, according to Philip Paine of the Michigan Cancer Foundation in Detroit. Some of the proteins may be bound to the cytoskeletal filaments. Colin Masters of Griffith University in Brisbane, Australia, told the Bethesda meeting that certain enzymes in the glycolytic pathway, which produces energy for the cell by breaking down glucose, are bound to the actin filaments.

A great deal more remains to be learned about the cytoplasmic matrix and the filaments it encompasses. Mutant cells that are defective in one or another of the filament proteins might help cell biologists dissect the functions of the various structures. But mutants of the cells of higher organisms cannot as yet be produced at will. Consequently, investigators are turning to monoclonal antibodies that specifically bind the proteins to see what effect this has on cell structure and activity. Meanwhile, the more traditional microscopic and in vitro methods are permitting the acquisition of a vast amount of information about the cytoplasmic matrix, showing it to be highly ordered, yet dynamic and capable of change.—**JEAN L. MARX**