Microtubules: Versatile Organelles

Although the undulating motion of a paramecium and the complex chromosome movements of a dividing sea urchin egg would not appear to be related, both depend on the activity of structures called microtubules. In fact, all nucleated cells, both plant and animal, contain microtubules during at least part of their lives. They are found in such diverse places as cilia and flagella, nerve axons, and the mitotic spindles of dividing cells. However, despite the ubiquitous distribution of microtubules, their functions, especially in mitosis, are not well understood. Now, investigators hope that recent advances will enable them to elucidate the mechanism of microtubule action.

According to these investigators, isolation of microtubules that retain the characteristics they had in living cells is a prerequisite for achieving this goal. Such microtubules could be recovered from cilia and flagella but not—until recently—from other parts of the cell. Because cytoplasmic microtubules are less stable than those of cilia and flagella, they break down when the cell is disrupted during isolation attempts. Last year, however, Richard Weisenberg of Temple University, Philadelphia, Pennsylvania, devised a technique for putting them back together again.

Microtubules are polymers composed of subunits of a protein called tubulin; cell disruption results in recovery of the tubulin subunits instead of intact microtubules. Weisenberg used tubulin that he had extracted from rat brain. He found that the key to in vitro microtubule formation was removal of calcium ions from the medium with a strong chelating agent. Polymerization also required the presence of magnesium ions and either adenosine triphosphate (ATP) or guanosine triphosphate (GTP). The properties of the reassembled tubules resembled those of microtubules in vivo.

In general, microtubules have an outer diameter of approximately 240 Å. Lengths may vary according to function and location. Flagella, for example, contain longer microtubules than cilia. The outer walls of microtubules, which are composed of tubulin subunits, are 50 Å thick. The tubulin appears to be arranged in protofilaments—linear arrays of subunits parallel to the long axis of the tubule. Most investigators agree that there are 13 protofilaments per tubule.

Microtubular proteins isolated from cilia, flagella, nerve tissue, and the mitotic apparatus have similar properties. Tubulin has a molecular weight of 110,000 and is itself a dimer. At least two different proteins, with molecular weights of approximately 55,000, can be identified in tubulin preparations. According to R. E. Stephens of the Marine Biological Laboratory, Woods Hole, Massachusetts, and others who have investigated the properties of tubulin, these proteins are quite similar but have slightly different amino acid compositions. Two such protein molecules form one molecule of tubulin but there is some uncertainty about how they are arranged: some investigators think that the tubulin of a given microtubule contains two different protein subunits while others have proposed that there is only one kind per tubule.

Microtubules and Mitosis

Mitosis is the process by which one somatic cell—plant or animal—divides to form two identical daughter cells. During mitosis, fibers composed of microtubules pull the chromosomes (which have previously been duplicated) to poles located at opposite ends of the dividing cell. This is done in such a way that each daughter cell acquires exactly the same chromosome composition as its parent. The fibers form the mitotic spindle. There are two types of fibers in spindles: those that extend from pole to pole and those that extend from chromosome to pole. The centrioles, cylindrical structures also containing microtubules, are found at the poles.

In order to understand how mitosis works, it is necessary to understand how microtubules assemble to form the spindle at the appropriate time. Colchicine and Colcemid have been valuable tools for such studies because these chemicals specifically disrupt microtubular structure. According to Edward W. Taylor of the University of Chicago and Gary Borisy, now at the University of Wisconsin, Madison, and to Leslie Wilson of Stanford University Medical School, Stanford, California, colchicine binds to the tubulin subunits and thus prevents it from polymerizing.

The mitotic spindle may be investigated in vivo. For example, Shinya Inoué and his colleagues at the University of Pennsylvania, Philadelphia, found that lowering the temperature of cells or treating them with colchicine or Colcemid resulted in spindle breakdown and the cessation of mitosis. The effects were reversible; the spindle reformed when the temperature was raised or the chemical removed. According to Inoué, this recovery did not require new protein synthesis because it was not hindered by inhibitors of protein synthesis. These observations, together with those from several other laboratories, have led to a general acceptance of the theory that microtubules exist in dynamic equilibrium with their subunits. Assembly occurs when the equilibrium shifts in favor of the polymerized form. The as yet unanswered question is: What causes the equilibrium to shift? The roles of calcium ions, which appear to prevent microtubule assembly in vitro, and of GTP, which is required for such assembly, are of special interest; however, their mechanisms of action are still unknown.

Interpretation of results obtained in vivo is complicated by the obvious complexity of living cells. Thus, investigators in several laboratories are attempting to make isolated but still functional microtubule preparations. Borisy and his colleague Joanna Olmsted have defined the conditions needed for in vitro assembly of the microtubules of pig brain. They found that tubules, with properties resembling those of the microtubules of living cells, would form from brain tubulin under conditions similar to those in vivo. Calcium ions prevented microtubule formation, but much higher concentrations were required than those observed by Weisenberg.

Tubulin purified from brain will add to and extend microtubules of flagella. In preliminary experiments, Carol Allen (working in Borisy's laboratory) and also Joel Rosenbaum of Yale University, New Haven, Connecticut, found that, under some but not all conditions, the subunits preferentially added to the distal ends of microtubules. Rosenbaum had previously shown that it was the distal ends of flagella of a unicellular alga that elongate in vivo. Microtubule assembly appears to have directionality regulated by factors now unknown.

A large number of investigators are attempting to reconstruct functional mitotic spindles. Among those pursuing this goal are J. Richard McIntosh of the

University of Colorado, Boulder; Lionel Rebhun of the University of Virginia, Charlottesville; Borisy; Inoué; Rosenbaum; and Weisenberg. One approach to this problem is to add purified tubulin, usually from brain, to the medium in which spindles are isolated; this should prevent spindle depolymerization. The investigators are encouraged because they can obtain spindles; these spindles, however, are not yet completely satisfactory because they lack some of the properties of spindles in vivo.

The present highly competitive nature of microtubule research stems from the belief of most investigators that the ability to make such functional spindles will provide the key for elucidating the mechanism of mitosis. Mitosis, even though it is one of the most fundamental processes of cell biology, is still poorly understood. Theories have been proposed on the basis of results from in vivo experiments, but researchers think that more definitive answers will result if they can test their hypotheses in less complicated systems.

According to the polymerization-depolymerization theory of mitosis, proposed by Inoué, mitotic movements of chromosomes and other organelles are the result of lengthening and shortening of spindle fibers. At the start of mitosis, the two centriole pairs separate and form two mitotic poles at opposite ends of the cell. Inoué suggests that growth of the polar spindle fibers, caused by polymerization of tubulin to form microtubules, begins at orienting centers around the centrioles and pushes them apart. Chromosome kinetochores (also called centromeres) are the regions on the chromosomes where the chromosome-to-pole fibers originate. Tubulin polymerization and elongation of these chromosomal fibers first pushes the chromosomes to the cell center. Then the fibers shorten as the microtubules depolymerize, and the daughter chromosomes are pulled to the poles.

The theory that mitosis occurs as a result of sliding of the filaments (or fibers) of the spindle has been proposed by a number of investigators. Since arms extend from the spindle fibers and form bridges between them, McIntosh, for example, thinks that such sliding may occur because of interactions between the fibers themselves. The bridges could generate enough force to move chromosomes if the fibers are oriented in opposite directions. The mechanism of the sliding is analogous to that of the sliding filaments of muscle and would require ATP or possibly GTP hydrolysis as a source of energy. Thus identification of an adenosine triphosphatase (an enzyme that catalyzes hydrolysis of ATP) in spindles would constitute further evidence for sliding filaments in mitosis.

The key role of centrioles in mitosis has been further delineated by B. R. Brinkley of the University of Texas Medical Branch, Galveston. He found that Colcemid prevents mitosis in cultured Chinese hamster cells by blocking formation of the polar spindle fibers that should push apart the centriole pairs. Thus, they do not separate to form the mitotic poles. Chromosomal fibers are not as susceptible to Colcemid action; microtubules do grow from the kinetochores toward the one pole.

Weisenberg is attempting to isolate and study the orienting center for microtubule growth that he believes surrounds, but is not part of, the centrioles. He is using clam eggs that have been artificially stimulated to divide. In preliminary experiments, Weisenberg found that stimulated eggs contained such organizing centers while unstimulated eggs did not. When he added tubulin to organizing centers from stimulated clam eggs, rays of microtubules formed around the centers. In a similar experiment with material from unstimulated, nondividing eggs, no such rays formed. Tubulin, however, could be isolated from such eggs and did form microtubules when added to organizing centers from stimulated eggs.

Ciliary and Flagellar Movement

The mechanism of ciliary and flagellar movement is better understood than is that of mitosis. Although they differ in length, cilia and flagella have similar internal structures. Microtubules are arranged in the "nine plus two" pattern in both. There is an outer ring consisting of nine tubule doublets (two fused microtubules that look somewhat like a figure eight); inside the ring are two single microtubules. The cilia or flagella whip back and forth presumably as a result of the tubules sliding over one another. Adenosine triphosphate provides energy for their movement.

Ian Gibbons at the University of Hawaii, Honolulu, found that the microtubules of cilia are associated with a protein of high molecular weight. This protein, which Gibbons named "dynein," is an adenosine triphosphatase. Dynein forms arms or projections that are attached to one tubule (the A tubule) of the outer doublets.

When Gibbons removes the membranes of cilia or flagella, the "nine plus two" configuration of the microtubules, including the dynein arms, remains relatively undisturbed. If he adds ATP to such preparations and observes them under the light microscope, they beat like normal flagella. When ATP is added after the preparations are treated with an enzyme that hydrolyzes a protein that connects the fibers, the fibers appear to slide over one another. Gibbons has hypothesized that the flagellar fibers slide in a manner analogous to the sliding of the actin and myosin filaments of muscle, with ATP hydrolysis providing the energy.

Although many problems of microtubule biology remain, microtubule research may yield an unexpected fringe benefit—a new method of fertility control. According to Brinkley, when Colcemid is injected under the skin of hamsters or mice in the vicinity of their testes, the animals no longer produce viable sperm. Colcemid treatment apparently disrupts the microtubules of the Sertoli cells. These cells, which are necessary for sperm maturation, have projections that grip developing sperm. Breakdown of the microtubules in the projections causes premature sperm release. Brinkley said that the animals appeared normal in all respects but fertility and did not suffer an impaired libido. Moreover, the effects of Colcemid were reversible; when the treatment was stopped, the animals regained their fertility and fathered normal offspring.

Mitosis, cell motility, and axonal transport are just some of the processes in which microtubules participate. They have been implicated in a variety of permeability and transport phenomena, including secretion of hormones like insulin and thyroxine. They may also act as skeletons in maintaining cell shape, especially of elongated structures such as axons. Changes in cell shape during development also depend on intact microtubules, since they are blocked by colchicine and other agents that disrupt microtubules. Thus, a better understanding of the regulation of microtubule assembly and function may also clarify these other fundamental processes.—JEAN L. MARX

Additional Readings

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2. S. Inoué and H. Sato, *J. Gen. Physiol.* **50** (Suppl.), 259 (1967).
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