ceiving equipment. Further shakedown of the new system should improve the resolution of the Venus radar maps to 4 to 5 km, according to Don Campbell. By contrast, the Goldstone facility was not intended for mapping at all, but rather for communications and deep-space tracking. The Venus radar maps were the result of a small program, piggybacked along by the Goldstone staff in addition to their primary responsibilities.

The high-resolution maps only cover a small fraction of the area of Venus, and they raise as many questions as they answer. But it is already clear that the topography of Venus is abundant, complex, and fascinating, and worth many more observations.—WILLIAM D. METZ

Cell Biology: Cell Surfaces and the Regulation of Mitosis

Cell membranes are now known to be dynamic structures in which the components can and do move about. Learning what causes and controls the movements has become a favorite occupation of many investigators during the past few years. This is not just a sterile exercise designed to determine how many proteins can dance on the surface of a cell.

Normal cells usually divide in culture only when anchored to a surface; they stop dividing, and also moving, when in contact with other cells. Transformed or cancerous cells, on the other hand, may divide even when floating in the culture medium, and they do not necessarily stop when in contact with other cells. Since cells must communicate with each other and with the environment through their membranes, many investigators think that alterations in the spatial arrangements of membrane components may be signals that are involved in the control of cell division, mobility, and interaction. The research on membranes not only has obvious implications for understanding cancer, but also applies to organ formation in the developing embryo, since this requires migration and interaction of cells.

If surface events do participate in the regulation of these activities, then there must be a mechanism for transmitting signals from the membranes to the internal machinery involved in mitosis and the other functions. There is evidence,* although largely circumstantial and thus somewhat controversial, that certain cytoplasmic structures, the microtubules and microfilaments, participate in regulating signal transmission. The evidence includes demonstrations that the movements of some membrane constituents are restricted or nonrandom, and are apparently controlled by microfilaments or microtubules or both. Moreover, for at least one cell type, the lymphocyte, the capacity to divide appears to be influenced by alterations in the mobility of some of its membrane components.

*Some of this evidence was described at the 1976 ICN-UCLA Winter Symposium on Supramolecular Structure: Cell Shape and Surface Architecture. The symposium was held in Squaw Valley, California, on 7 to 12 March.

Two types of experiments have led to the conclusion that microtubules and microfilaments somehow regulate the distribution and mobility of membrane components. One involves studying how the distribution of various membrane constituents changes during phagocytosis. The other makes use of ligands, agents that bind to receptors on the cell surface, in order to label the receptors and permit observation of their movements. (A receptor is any constituent of the cell surface that combines with another chemical entity. The specific combination of certain receptors with agents such as hormones can alter biological functions.)

Phagocytosis is the process by which cells such as macrophages engulf external particles. The cytoplasmic membrane invaginates and envelops the particles. Eventually the portion of the membrane engulfing the particles pinches off from the surface membrane and forms a vesicle inside the cell. In an actively phagocytizing cell as much as 50 percent of the membrane may end up in the intracellular vesicles. According to Richard Berlin, Janet Oliver, and their colleagues, first at Harvard Medical School and more recently at the University of Connecticut Health Center, certain membrane constituents are redistributed during phagocytosis in such a way that none are lost from the external surface.

The transport of a number of nutrients is known to require the activity of specific carriers located in the membrane. The investigators found that the nutrients were transported just as well after phagocytosis as before, even though a large portion of the surface membrane was removed during vesicle formation. Berlin and his colleagues ruled out the possibility that the cells were synthesizing new carriers and inserting them into the membrane. They concluded that there must be a means of separating the carriers from the portions of the membrane that go into vesicles.

In the presence of colchicine, a drug that disrupts the structure of microtubules, phagocytosis did result in a decrease in the transport rate that was proportional to the amount of membrane lost in the vesicles. This result suggests that the microtubules somehow directed the movements of the transport carriers during phagocytosis.

Lymphocytes are frequently used for the ligand-binding studies because they are readily available and relatively well characterized. One class of lymphocytes, the B (for bone marrow–derived) cells, carries immunoglobulins on their outer surfaces. It is possible to make antibodies, which are themselves immunoglobulins, against the surface immunoglobulins.

Several investigators, including Steffano de Petris of the Basel Institute for Immunology, Martin Raff of the Medical Research Council Neuroimmunology Project at University College in London, Emil Unanue and Morris Karnovsky of Harvard Medical School, and Gerald Edelman and Ichiro Yahara of Rockefeller University, have studied what happens when antibodies bind to the surface immunoglobulins. They find that the antibody-receptor complexes first associate to form a number of aggregates or patches. The patches may then coalesce to form a single cap on the cell surface. The formation of caps, but not of patches, requires energy; it will not occur in the presence of inhibitors of cell metabolism.

A number of agents can interfere with patch and cap formation. One of these is concanavalin A (Con A), a plant protein that also binds to cell surface components. Yahara and Edelman found that antibodies against surface immunoglobulins failed to elicit patching and capping on cells that had first been exposed to Con A. This restriction of the movements of the antibody receptors, which Edelman calls anchorage modulation, was reversible. Con A has four sites that can bind to receptors; in order to produce anchorage modulation it must be in its normal multivalent state. The restriction does not occur with Con A that has been chemically altered so that it has only one or two binding sites.

Edelman thinks that Con A does not act directly on the membrane to restrict receptor movements but, rather, that it

acts indirectly through structures—such as microtubules—that form networks beneath the membrane. This conclusion is based partly on the results of experiments in which Yahara and Edelman found that colchicine and other drugs known to disrupt microtubular structure relieved the restriction. Lumicolchicine, a colchicine derivative that does not alter microtubules but interacts with membranes the way colchicine does, had no effect on anchorage modulation.

The possibility that Con A directly restricts the movements of the surface immunoglobulins by forming cross-links between them instead of acting indirectly through submembranous microtubules also had to be eliminated. The Rockefeller investigators did this by showing that attachment of Con A molecules to a very small fraction of the Con A receptor sites inhibited the mobility of the immunoglobulins. They exposed lymphocytes to Con A bound to platelets, disks that are involved in blood clotting and are much smaller than lymphocytes. Lymphocytes could bind the platelets through the Con A moiety. Attachment of as few as ten platelets to Con A binding sites prevented capping induced by antibody against immunoglobulins even though the platelets were in contact with only a fraction of the cell surface.

Addition of colchicine produced a redistribution of the platelets from a random arrangement on the cell surface to a caplike structure; however, the surface immunoglobulins were not carried into the cap. Since binding of only a small number of Con A molecules produced anchorage modulation, the experiment showed that the phenomenon of restricted movement was propagated over the entire cell surface.

The investigations of Edelman and Yahara, Berlin, and the other researchers generally indicate that microtubules somehow restrict or direct the movements of membrane constituents. Microfilaments also appear to be involved in regulating the movements of membrane components. For example, de Petris has shown that cytochalasin B, a drug that disrupts microfilaments, inhibits capping induced by ligands.

Microfilaments probably consist of actin, one of the major contractile proteins of both muscle and nonmuscle cells (*Science*, 4 July 1975). Thus, some investigators think that a contractile process involving microfilaments could participate in the movements required to achieve capping. The observation that capping requires energy is consistent with this hypothesis because contraction also requires energy. In Edelman's view, microfilaments may serve as connecting links between membrane components and microtubules although there is as yet no direct evidence for interactions between these entities.

Additional experiments on the effects of both colchicine and cytochalasin B on ligand-induced capping in lymphocytes further support the idea that both microfilaments and microtubules participate in the phenomenon. Several investigators, including Unanue and Karnovsky, have found that colchicine alone either has no effect on capping or else slightly enhances it. Cytochalasin B does inhibit it but only by about 20 percent. Both drugs together, however, abolish capping. The investigators found that local anesthetics such as Xylocaine also completely inhibit cap formation. They suggested that this effect could be due to a direct action of the anesthetics on membranes or to disruption by the agents of microtubules.

Effects of Local Anesthetics

Recently, Garth Nicolson of the Salk Institute and the University of California at Irvine and George Poste and Dimitri Papahadjoupoulos of Roswell Park Memorial Institute obtained evidence that the local anesthetics actually do disrupt both microtubules and microfilaments. They found that the agents had the same effect on the mobility of receptors for Con A on mouse B cells as did a combination of colchicine with cytochalasin B. Moreover, they showed by electron microscopy that, after anesthetic treatment, microfilaments could not be observed in the cytoplasm just under the membrane of mouse endothelial cells, although some were present deep within the cell. Few microtubules were observed anywhere after the treatment. Normally, these cells have a dense network of microfilaments under the surface membrane with numerous microtubules deeper in the cell.

How local anesthetics alter the organization of the structures is unclear, but one possibility is that they act through an effect on the distribution of calcium ions in the cell. Both microtubules and microfilaments are dynamic structures whose organization can change with the physiological state of the cell. The factors controlling assembly and disassembly of the structures is not completely understood. However, calcium ions are known to cause disassembly of microtubules. Calcium ions are also well known to control contraction in muscle cells and presumably also the contractile processes in which actin-containing filaments participate in nonmuscle cells.

According to Nicolson and Poste, calcium ionophores (an ionophore is an organic chemical that can complex an ion and carry it through membranes that otherwise would not permit penetration of the ion) had the same effects as colchicine on the mobility and distribution of lymphocyte receptors. The investigators hypothesize that the ionophores cause dissociation of the microtubules by increasing the intracellular concentration of calcium ions. The effects were not seen in the absence of calcium nor were they produced by an ionophore for potassium ions.

Thus, there is evidence from several laboratories indicating that microfilaments and microtubules are important regulators of the movements of cell surface components. However, a number of investigators who are studying the cytoplasmic structures have expressed reservations about the evidence. One important criticism relates to the dependence of the arguments on the pharmacological effects of cytochalasin B and colchicine, both of which have effects in addition to those on microfilaments and microtubules. For example, cytochalasin B alters the transport into cells of a number of substances. This raises the possibility that its effects on the distribution of membrane components could result from an action on the membrane itself or from changes in the transport of nutrients. Nicolson points out, however, that the effects of cytochalasin B and colchicine occur very quickly before the cellular nutrient pools would be depleted.

The demonstration by Yahara and Edelman that colchicine, but not lumicolchicine, abolishes anchorage modulation indicates that colchicine is not simply acting on the membrane. In addition, Berlin has evidence that colchicine does not affect the viscosity of membrane preparations from polymorphonuclear leukocytes. Finally, according to Victor Ling and his colleagues at the University of Toronto, the drug has to penetrate into the cell in order to alter receptor mobility.

A method for studying the function of the cytoplasmic structures without depending almost exclusively on drug effects might help to further answer the objections. There may be such a model for studying microtubular function, according to Oliver. The Chediak-Higashi syndrome is a rare inherited disease that afflicts both humans and animals, including mice. It is characterized by partial albinism, high susceptibility to infection, and defective polymorphonuclear leukocytes. These defective cells resemble cells treated with colchicine in a number of ways—including the mobility of their membrane components, which is greater

than it is in normal cells. This is what would be expected if the cells lacked microtubules, which are thought to restrict receptor mobility. Oliver has found that Chediak-Higashi cells from mice make tubulin, the protein subunit of which microtubules are composed, but that the subunits do not assemble to form the normal complement of microtubules.

Other investigators have shown that adenosine 3′,5′-monophosphate (cyclic AMP) decreases microtubule assembly in normal cells, whereas guanosine 3′,5′-monophosphate (cyclic GMP) enhances it. According to Oliver, cyclic AMP had no effect on the receptor mobility of Chediak-Higashi cells. On the other hand, cyclic GMP or agents that increase its concentration restored receptor mobility to normal. Therefore, she thinks that a defect in the mechanism controlling microtubule assembly that involves cyclic GMP is operating in Chediak-Higashi cells. According to this hypothesis, addition of cyclic GMP to Chediak-Higashi cells should correct the defect, permit assembly of microtubules, and return receptor mobility to normal.

Technique for Studying Interactions

Direct evidence for interactions between microtubules, microfilaments, and membrane components has also been lacking. Berlin and his colleagues have developed a technique that may help to remedy this deficiency. It involves the transfer of resonance energy between different fluorescent materials separately conjugated to the substances whose interaction is under investigation. Resonance energy transfer occurs when a chromophore that is excited by the absorption of energy transmits the energy to an acceptor chromophore. The two chromophores must be relatively close—not more than 100 angstroms apart—for the transfer to occur.

For example, Berlin labeled one batch of tubulin with the dye fluorescein isothiocyanate and another with rhodamine isothiocyanate and then mixed them. Before the tubulin polymerizes to form microtubules there is no resonance energy transfer between the chromophores. With polymerization, however, the chromophores come sufficiently close together for energy transfer to occur. The degree of the transfer depends on the extent of polymerization. Berlin has used this technique to show that tubulin does interact with membrane components. He says that it could also be applied to studying the postulated interaction between microfilaments and microtubules.

One of the reasons for all this interest in whether or not membrane receptors are connected to microtubules and microfilaments is the possibility that these cytoplasmic structures are part of the mechanism for regulating the signals that tell cells when to start or stop dividing and for regulating cellular mobility and interactions. Edelman has evidence that, at least in lymphocytes, restriction of receptor mobility is associated with blockage of the events leading to mitosis.

One of the effects of Con A binding to lymphocytes is the triggering of mitosis. The commitment of cells to mitosis can be determined by measuring DNA synthesis that must occur before cell division. Edelman and his colleagues found that low concentrations of Con A stimulate DNA synthesis but that high concentrations inhibit it. The concentration of Con A that induces inhibition is in the same range as that producing restriction of the mobility of the antibody receptors —a phenomenon that appears to require intact microtubules. The modified Con A's that have only one or two binding sites do not produce either anchorage modulation or inhibition of mitosis. Edelman suggests that in this situation, the microtubules and other components of the system involved in regulating the mobility of surface receptors act to prevent induction of cell division.

At the concentrations of Con A that stimulate mitosis, the opposite appears to be true. The stimulation is blocked by low concentrations of colchicine and other drugs that alter microtubules. The blockage is reversed when colchicine is removed. According to Edelman, kinetic analysis of the process by which cells are committed to mitosis indicates that colchicine acts very early and may block commitment itself. Cells are committed to carry out the biochemical steps needed for cell division several hours before mitosis actually occurs. He hypothesizes that the microtubules are somehow implicated in the regulation of early signals that induce a cell to mature and divide. This would mean that stimulation and inhibition of mitosis are separable events that depend on the induction of different states in the microtubules by different membrane signals.

In any event, several investigators have found changes in microtubules and microfilaments in transformed cells that have acquired malignant characteristics. In general, transformation leads to disorganization of these structures. For example, Keith Porter and his colleagues at the University of Colorado have found this to be true for the microtubules of mouse endothelial cells transformed by viruses. Gerald Fuller and B. R. Brinkley of the University of Texas Medical Branch in Galveston have described similar changes in both dividing and transformed cells.

According to these investigators, normal cultured cells have an extensive cytoplasmic microtubule complex. Many of the tubules, which extend from the cell center to the periphery, appear to terminate just under the outer cell membrane. During mitosis the complex disappears. Fuller and Brinkley say that the 18 transformed cell lines they have studied—some transformed spontaneously and others by viruses—also have greatly diminished microtubule complexes. Porter and the Texas group find that the transformed cells make tubulin but that it does not assemble normally to make microtubules. They hypothesize that the assembly mechanism is defective. Their results are similar to those of Oliver with Chediak-Higashi cells.

Microfilaments in Transformed Cells

Transformed cells are generally round throughout their life cycle whereas normal cells become round only at the time of mitosis. The rest of the time they are flat and spread out on the surface on which they are cultured. Robert Goldman and his colleagues at Carnegie-Mellon University have identified bundles of microfilaments under the membrane of normal cells at the places where the membrane is in contact with the surface. In contrast, virally transformed cells contained few, if any, microfilament bundles.

Whether these changes in microtubules and microfilaments are in fact the causes of the altered properties, including uncontrolled division, of transformed cells is still open to question. Investigators have sought to identify these causes for many years. They have catalogued a long list of differences between normal and transformed cells without achieving a unified theory to explain what is really happening. One prime handicap has been the lack of an explanation of what controls cell division in normal cells. The results and hypotheses described here may or may not provide that explanation. But they do provide a framework for synthesizing research in several areas of cell biology and a stimulus for further research.—JEAN L. MARX

Additional Readings

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2. G. M. Edelman, *Science* **192**, 218 (1976).
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