RECENT DEATHS

Arturo E. Burkart, 69; director, Botanical Institute Darwinion, Argentinian Academy of Sciences; 25 April.

Robert G. Bingham, 36; former professor of astronomy, University of Washington; 13 April.

Leigh E. Chadwick, 70; retired head, entomology department, University of Illinois; 4 February.

William G. Hackler, 52; professor of engineering, Virginia Polytechnic Institute and State University; 8 April.

Howard L. Hunter, 70; professor emeritus of chemistry, Clemson University; 27 March.

Percy L. Julian, 76; former professor of chemistry, Howard University; 19 April.

John S. Kieffer, 70; former president, St. John's College; 29 March.

Joseph N. Knull, 83; professor emeritus of entomology, Ohio State University; 24 April.

Dorothy D. Lee, 69; former professor of anthropology, Vassar College; 18 April.

Samuel H. Lee, 56; professor of chemistry, Texas Tech University; 3 April.

Stuart Mudd, 81; former professor of microbiology, University of Pennsylvania; 6 May.

Charles M. Nevin, 82; professor emeritus of geology, Cornell University; 24 March.

Timothy J. O'Leary, 66; professor emeritus of chemistry, Gonzaga University; 3 May.

William A. Pearl, 81; former professor of engineering, Washington State University; 5 April.

Lyman B. Porter, 81; former professor of chemistry, University of Arkansas; 24 March.

Henry A. Schroeder, 68; professor emeritus of physiology, Dartmouth College; 20 April.

Joseph Solomon, 71; clinical professor of psychiatry, University of California, San Francisco; 29 March.

RESEARCH NEWS

Actin and Myosin: Role in Nonmuscle Cells

Actin and myosin, contractile proteins once considered characteristic of muscle, are now known to occur in numerous cell types ranging from amoebas and cellular slime molds to mammalian fibroblasts, nerve cells, and platelets. Actin, at least, is probably ubiquitous. With the wide distribution of these proteins firmly established, investigators are concentrating on developing a better understanding of their functions. The evidence acquired thus far indicates that cell motility and mitosis may depend on the activities of these contractile proteins.

In striated muscle, actin and myosin are arranged in a highly ordered manner. A muscle fiber consists of a series of contractile subunits called sarcomeres. Two structures called Z-lines constitute the boundaries of each sarcomere. The ends of thin filaments of actin are attached to the Z-lines. Interspersed between the thin filaments are thick filaments composed of myosin. Driven by the energy supplied by hydrolysis of adenosine triphosphate (ATP), the thin and thick filaments interact and slide over one another, thus drawing the Z-lines closer together and producing contraction.

Many investigators think that an analogous interaction occurs between nonmuscle or cytoplasmic actin and myosin, and that the interaction is involved in such processes as amoeboid motion, development of the cleavage furrow during mitosis, cytoplasmic streaming, blood clot retraction, and certain changes in cell shape that occur during embryo development. Studying the postulated interaction between contractile proteins in nonmuscle cells is difficult because in these cells they are not arranged in such neat patterns as they are in striated muscle.

More is known about the localization of cytoplasmic actin in cells than about that of myosin. This is because there is a convenient label for actin. A fragment of myosin called heavy meromyosin (HMM) retains both the adenosine triphosphatase (an enzyme that catalyzes the hydrolysis of ATP) activity and the actin-binding capacity found in the intact molecule. When HMM is added to cells that have been treated with glycerin to increase their permeability, HMM will bind to actin filaments in a distinctive arrowhead pattern. Harunori Ishikawa, now at the University of Tokyo, and Howard Holtzer of the University of Pennsylvania used this technique to show that microfilaments consist of actin.

Fig. 1. Pattern of fluorescent HMM in chick fibroblast during interphase (× 1950). [Source: Joseph Sanger, University of Pennsylvania]

Microfilaments are a distinct class of filaments with a diameter of about 50 Å that can be found in most types of cells. In resting cells in culture they often form bundles (also called stress fibers) that are more or less parallel to one another. They exist in another form, as a network of filaments, in the ruffled or moving edges of motile cells. There appears to be a correlation between the location of these filaments and cell movements. Moreover, several investigators have found that the ends of actin filaments are attached to, or perhaps embedded in, the cell membrane. This is important because generation of contractile force and movement requires anchorage of the actin filaments just as those in muscle are attached to the Z-line.

The cells containing actin "decorated" with HMM must be examined with the electron microscope in order to observe the arrowhead patterns formed. This complicates the determination of the three-dimensional actin patterns in a cell because only thin sections of the cell can be examined.

Two new techniques, both of which permit visualization of entire cells with the light microscope, do not suffer from this handicap. One, used by Joseph Sanger of the University of Pennsylvania School of

Medicine, involves labeling the actin in cells with a fluorescent derivative of HMM.

Sanger is studying the distribution of actin at different stages of the cell cycle with this technique. He has found four distinct patterns of actin distribution in dividing chick fibroblasts. Interphase, the resting stage of the cycle, is characterized by the presence of fluorescent strands, apparently the typical stress fibers, that run the length of the cell (Fig. 1). When the cells become round prior to division, the fluorescent strands are replaced by a diffuse, uniformly distributed fluorescence. During telophase, the last stage of mitosis, the chromosomes move to opposite poles of the cell and a cleavage furrow or contractile ring forms between the poles as the cell pinches in two. The fluorescence becomes concentrated in the furrow during telophase (Fig. 2). This observation agrees with an earlier demonstration by Thomas Schroeder of the University of Washington that the contractile ring of HeLa cells contains actin microfilaments.

Within 5 minutes of the completion of cleavage, most of the fluorescent material disappears from the furrow region and reappears at the opposite poles of the daughter cells where pseudopods are forming. After the cells move apart, they flatten once again and the interphase pattern returns.

Sanger says that these results indicate that cellular actin is associated with the motile regions of the cell. Because dividing cells synthesize little protein, he thinks that the changing patterns of actin distribution during cell division are due to a recycling of actin within the cell.

Rat kangaroo cells do not become round during mitosis and thus permit observation of the mitotic spindle. The mitotic spindle includes the chromosomes, the centrioles located at the two poles of the cell, and three types of spindle fibers: the chromosomal fibers that extend from the chromosomes to the centrioles; the continuous fibers that run from centriole to centriole; and the astral fibers that radiate from the centrioles to the cell periphery. Using fluorescent-labeled HMM, Sanger has identified actin in the chromosomal fibers (Fig. 3). This result is consistent with the hypothesis of Arthur Forer of York University, London, Ontario, that an actin-myosin interaction is involved in chromosomal movements during mitosis.

Spindle fibers also contain microtubules that are known to be necessary for mitosis (*Science*, 28 September 1973, p. 1236). Sanger suggests that actin filaments, together with myosin, could produce the contractile force that shortens the chromosomal fibers and pulls the chromosomes to

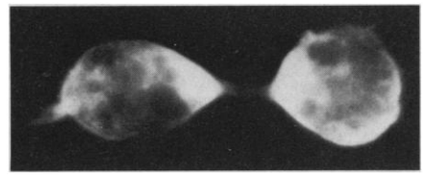

Fig. 2. Chick fibroblast that has just completed cleavage. The fluorescent HMM is still found almost entirely in the region of the cleavage furrow. No fibers like those in Fig. 1 are seen (× 1300). [Source: Joseph Sanger, University of Pennsylvania]

the poles. The microtubules, on the other hand, could act as a cytoskeleton and participate in the lengthening of the continuous fibers that push the poles apart. Continuous fibers probably do not contain actin.

Provided that the antibody specificity can be established, antibody against a particular contractile protein can be used to determine its intracellular arrangement. The antibody, which binds to the protein, is then located by allowing it to react with fluorescent-labeled antibody against the antibody. This technique, indirect immunofluorescence, is another that permits the localization of contractile proteins in intact cells by light microscopy. Moreover, it can be applied to the localization of myosin, for which no previous method was available, and of troponin and tropomyosin, in addition to actin. (Troponin and tropomyosin are two proteins involved in controlling actin-myosin interactions in muscle and probably in some nonmuscle cells.)

A major problem was preparing the antibodies against the contractile proteins, especially against actin. Actin is not very antigenic, possibly because its structure varies so little from species to species that it is not recognized as foreign. However, Elias Lazarides, Klaus Weber (now at the Max Planck Institut für Biophysikalische Chemie, Göttingen, Germany), and their

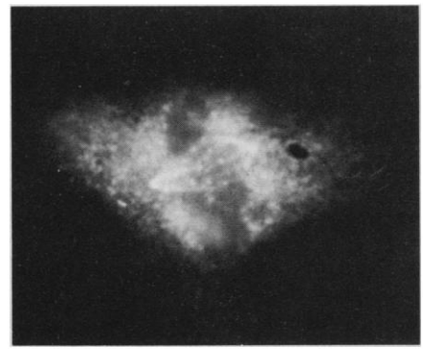

Fig. 3. A rat kangaroo cell in metaphase showing fluorescent HMM in the chromosomal spindle fibers. The chromosomes are in the dark area in the center of the cell (× 1140). [Source: Joseph Sanger, University of Pennsylvania]

colleagues at Cold Spring Harbor Laboratory have prepared antibodies to actin and to myosin and tropomyosin and have used them to locate the three proteins in nonmuscle cells. As expected, the actin is in filamentous structures (Fig. 4). In muscle cells, tropomyosin is complexed with actin in the thin filaments. The results obtained by the Cold Spring Harbor investigators indicate that this holds true for nonmuscle cells, too. At least some of the myosin also appeared to be located in the microfilaments with actin. They conclude, however, that the data do not permit a firm conclusion about the exact arrangement of the myosin.

Although much less is known about the organization of myosin than of actin in nonmuscle cells, most investigators do assume that the two proteins interact in a manner similar to the sliding filaments of muscle to generate contractile forces. It is known that cytoplasmic actins, which are present in a wide variety of nonmuscle cells in concentrations as high as 10 percent of the total cellular protein, greatly resemble one another, and also the actins from skeletal, cardiac, and smooth muscles. The globular, monomeric forms of all these actins have molecular weights of about 45,000 and similar amino acid compositions. The globular form of the cytoplasmic actins polymerizes in vitro to form double-helical filaments that look like the thin filaments of actin in striated muscle.

(The large number of investigators, frequently working in collaboration on their studies of actin and myosin, makes identification of a particular investigator with a given result very difficult. A partial list of those who have contributed information on the structure and biochemistry of the proteins would include Mark Adelman of Duke University; Robert Adelstein and Edward Korn, both at the National Heart and Lung Institute; Dennis Bray of the Medical Research Council Laboratory of Molecular Biology, Cambridge, England; Sadashi Hatano of the University of Nagoya, Japan; Vivianne Nachmias of the University of Pennsylvania; Thomas Pollard of Harvard Medical School; James Spudich of the University of California Medical School, San Francisco; and Robert Weihing of the Worcester Foundation for Experimental Biology.)

Myosin is also present in a wide variety of cell types, although not in concentrations as high as those of actin. Cytoplasmic myosins from various sources may differ significantly from one another and from skeletal muscle myosin. Most of the cytoplasmic myosins, especially those from vertebrates, do closely resemble the smooth muscle protein in structure, however.

Despite these differences, two functions found in muscle myosin and thought to be essential for the development of contractile force are also found in nonmuscle myosin. These are the capacities to bind reversibly to actin filaments and to act as an adenosine triphosphatase. In addition, most cytoplasmic myosins form filaments in vitro.

In vitro experiments indicating that cytoplasmic actin and myosin are capable of carrying out the same reactions as their counterparts in muscle are further evidence for an interaction between the two proteins in nonmuscle cells. For example, cytoplasmic actin stimulates the adenosine triphosphatase activity of cytoplasmic myosin. Finally, all cytoplasmic actins interact with muscle myosin and stimulate its adenosine triphosphatase, and all cytoplasmic myosins interact with muscle actin.

The factors controlling these reactions between cytoplasmic myosin and actin are now receiving a great deal of attention. In muscle, control of contraction is effected by calcium ions and the proteins troponin and tropomyosin. These proteins are located in the actin filaments and confer calcium sensitivity on the contractile process. In the absence of calcium ions, they inhibit the stimulation of adenosine triphosphatase activity by actin. But when calcium is released from its storage reservoirs in response to stimulation of the muscle, the actin and myosin filaments interact with consequent stimulation of the enzyme activity.

Many nonmuscle cells contain tropomyosin, and possibly troponin, and there is evidence that some cellular movements are sensitive to calcium ions. Thus, regulation for some nonmuscle cells may be similar to that in muscle. For others, different or perhaps additional control mechanisms may be operating.

Calcium does not appear to play a physiological role in at least two cell types. One is *Acanthamoeba castellanii* studied by Pollard. And the other is the rabbit macrophage studied by Thomas Stossel of Harvard Medical School. Macrophages are motile, phagocytic cells that form pseudopods as they migrate to and engulf particulate matter.

Each of these investigators has found that a protein cofactor is required for activation of myosin adenosine triphosphatase in his system by actin. In both cases, calcium does not affect the resulting activity, and troponin and tropomyosin inhibit the enzyme in the presence of calcium ions as well as in their absence. Thus the availability of the cofactors may determine the activity of the myosin adenosine triphosphatase in *Acanthamoeba* and macrophages.

Actin filaments are not necessarily permanent structures in the cell. In fact, the evidence indicates that they may form and dissociate as needed. This is true for those in the contractile ring, according to Schroeder and Sanger. Consequently, the formation of microfilaments, or of filament bundles or networks, is another point at which the activities of the contractile proteins may be controlled.

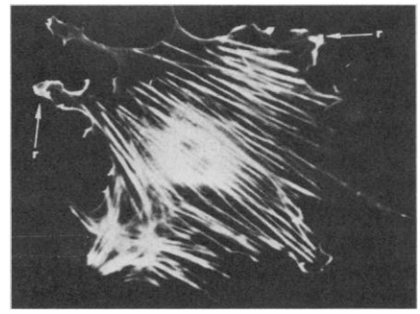

Fig. 4. Immunofluorescent staining of rat embryo fibroblast with actin antibody. The *r*'s indicate areas of membrane ruffling (× 445). [Source: Elias Lazarides, Cold Spring Harbor Laboratory]

Spudich has been studying the in vitro assembly of filaments from actin from the slime mold, *Dictyostelium discoideum*. He says that filament formation from cytoplasmic actin resembles that from muscle actin. The equilibrium between the globular and filamentous actin favors filament formation. Depending on the conditions, more complex structures may also be generated. These include filament nets similar to those seen at the migrating edges of cells and filament bundles similar to stress fibers. The formation of these supramolecular structures may require the presence of additional components because highly purified actin would not form them.

According to Stossel, a macrophage protein, which he calls actin-binding protein, may play such a role in these cells. In the presence of the protein, the globular form of actin polymerizes to form filament networks similar to those observed in macrophage pseudopods. Thus, there should be at least two controls for the activities of contractile proteins in macrophages; one would act on actin filament formation and the other on the interaction between actin and myosin.

In one system, the formation of the acrosomal process of certain sperm, myosin does not appear to play a role and the polymerization of actin produces the motion, according to Hatano and Lewis Tilney of the University of Pennsylvania. The acrosome is a caplike structure at the head of the sperm. When the acrosome contacts an egg, it extends a long projection or process for egg penetration. Sperm from *Thyone briareus* (an echinoderm) generate acrosomal processes up to 90 micrometers long in less than 30 seconds. Tilney and Hatano found that the force for the generating process is the polymerization of actin located in the acrosome to form a bundle of microfilaments. The acrosome does not contain myosin.

Tilney cautions against the tendency of many investigators to think solely in terms of a sliding filament mechanism for producing motility in nonmuscle cells. He points out that in most nonmuscle cells the ratio of myosin to actin is much less than that in muscle, and he suggests—with the acrosomal process as an example—that mechanisms other than sliding filaments are feasible.

Investigators studying the previously described systems emphasized the role of actin in controlling contractile events. But control through myosin is also possible, according to Adelstein. He has found a kinase (an enzyme that transfers a phosphate from ATP to an acceptor) that can catalyze the phosphorylation of platelet myosin. Such phosphorylation increases the actin-activated adenosine triphosphatase activity of the myosin about fivefold. The enzyme will phosphorylate myosins from fibroblasts and from smooth muscle (chicken gizzard), but not from skeletal or cardiac muscle. Adelstein hypothesizes that the kinase acts as a switch that turns on the myosin so that it can interact with actin. He is now looking for an off switch, that is, an enzyme that dephosphorylates myosin.

These experiments indicate that contractile proteins are involved in cell division and cell motility. The apparent presence of the proteins in membranes makes them candidates for involvement in contact phenomena, including contacts between cells. For these reasons, investigators who want to determine the differences between normal and cancerous cells have been turning their attention to the contractile proteins.

Weber and Robert Pollack, also of Cold Spring Harbor Laboratory, have noted changes in the actin and myosin patterns of mouse fibroblasts after transformation with the oncogenic virus SV 40. Generally there was a loss of stress fibers following transformation. In transformed cells that regained certain normal characteristics, the fibers were present in more normal numbers. Adelstein has found a cytoplasmic myosin in a rhabdomyosarcoma, a tumor of muscle cells, in addition to the expected skeletal muscle myosin. He hypothesizes that the cytoplasmic myosin plays a role in cell division. The significance of these findings remains to be established, but investigators of oncogenesis now have something else to look for.

—JEAN L. MARX