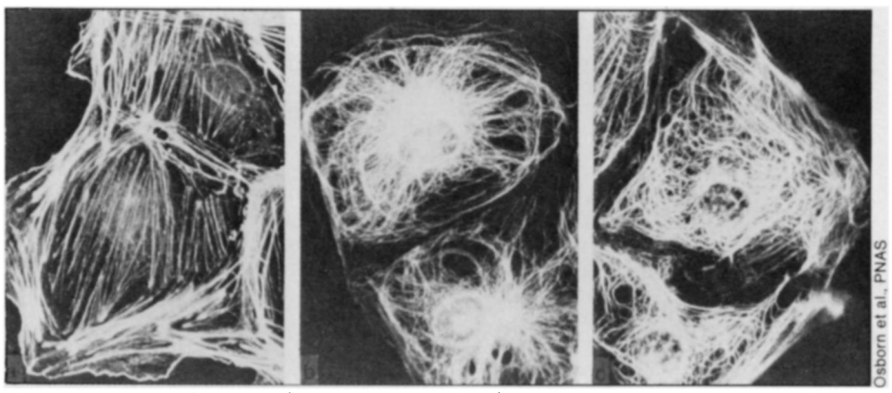
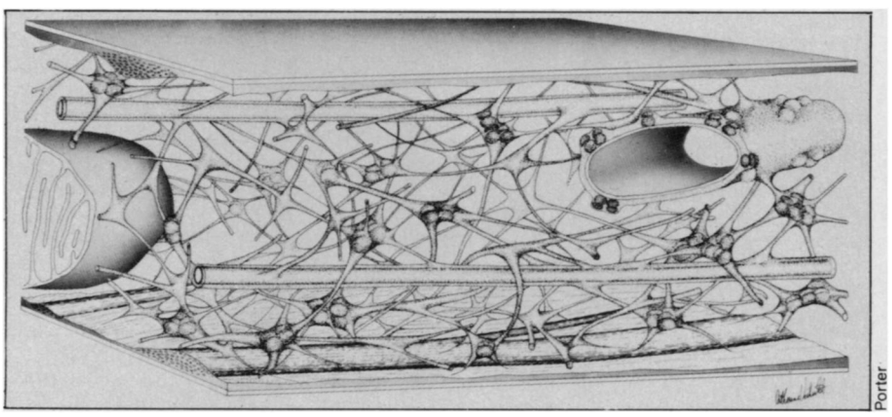
THE BONE AND MUSCLE OF CELLS

Three-dimensional electron microscopic analysis of cells reveals a multi-functional lacy lattice

BY JULIE ANN MILLER



Patterns of microfilaments (right), microtubules (center) and intermediate fibers (left).



In the emerging view, a lattice of thin fibers anchors the many cellular components.

The structure and function of all life, from the simplest bacteria to the most complex animal, depends on small but autonomous entities—cells. The nature of these living units dictates the capabilities of the organism. But what is the nature of a cell? Are we built of solid blocks studded with cell components or of flexible sacks of fluid and freely floating organelles? The answer may change, depending on your point of view. And a new view is emerging.

With the aid of recently developed molecular labels and powerful microscopic techniques, biologists have discovered a complex architecture within the cells: three-dimensional networks of fibers. Most cell components seem firmly anchored to this scaffolding, and the movements of the components, as well as the motion of the cell itself, may rely on the actions of the lattice.

With the most primitive microscopes, the cell seemed composed only of a nucleus suspended in a translucent syrup that was encased by an external membrane. With better optical techniques, researchers discovered within the cytoplasm a remarkable variety of structures, such as mitochondria, ribosomes and the endoplasmic reticulum.

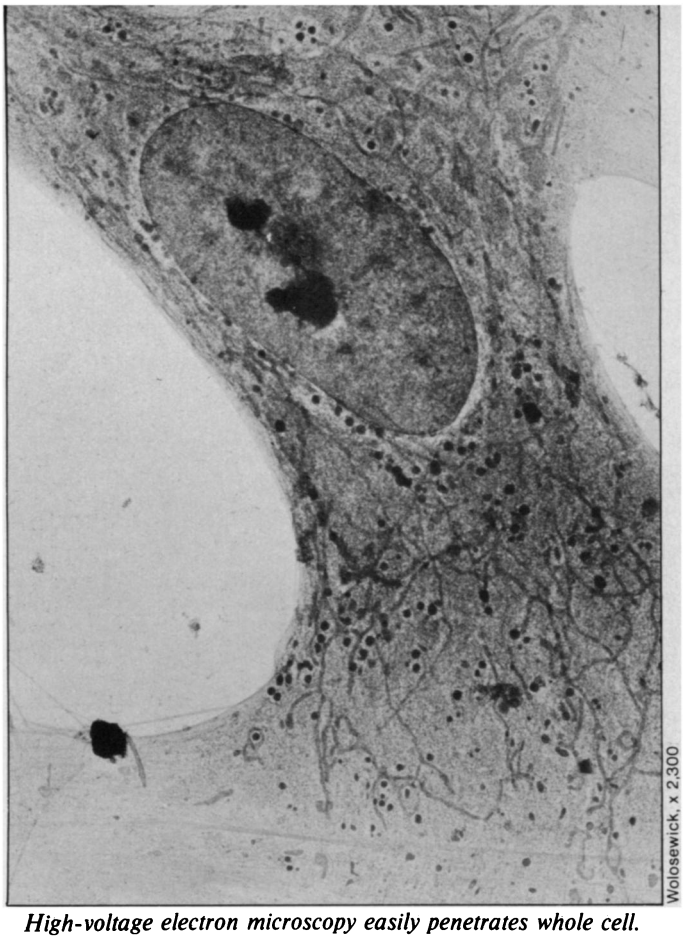
The electron microscope provided the greatest boost in ability to examine the minute subcellular structures. For the first time, microscopists could focus on details as small as the separate layers of membranes. But even the electron microscope has serious limitations. Material must be sliced very thin if electrons are to penetrate the sample. A single cell might have to be sliced into thousands of pieces. Therefore, while getting magnificent detail on a thin slice of a cell, the electron microscope provides much less information on the three-dimensional relationships between different cellular components. It was as if scientists were analyzing a fruitcake by looking at thin slices; they could identify nuts and raisins and cherries, but might easily miss any thin threads holding the ingredients in place.

Biologists are now using two new techniques to study the architecture of whole unsliced cells. What they see are fibrous networks that organize cellular components and perform crucial cell functions.

Three types of webs are seen in experiments using a fluorescent marker to identify single fiber types in whole cells. Mary Osborn and Klaus Weber of the Max Planck Institute in Göttingen and Werner W. Franke of the German Cancer Research Center in Heidelberg, Germany have visualized the separate fiber systems in laboratory grown cells derived from rat kangaroo kidney. The markers are goat antibodies bound to the fluorescent compound fluorescein. These antibodies bind specifically to certain rabbit antibodies, which in turn bind specifically to (or as the microscopists say, decorate) one type of subcellular fiber. At the recent Neuroscience Research Program meeting in Boulder, Colo., Weber described this microscopic work as "protein chemical anatomy."

The three major fiber systems the German biologists have visualized were previously identified with electron microscopy, but their patterns had not been revealed. The thickest fibers are the microtubules, 20 to 25 nanometers wide. These structures contain tubulin as the major protein. Microtubules are thought to participate in a long list of cell functions, such as maintaining cell shape, cell movement and transport of materials within the cell. Osborn and colleagues found that the microtubules tend to extend radially along straight or curved paths from the nucleus and terminate near the cell surface. This elaborate microtubular system disappears at the beginning of cell division. Weber suggests that the tubulin is restructured into the spindle tubules that seem to pull apart the duplicated chromosomes as cells divide.

The microfilaments are thinner fibers, 5 to 6 nanometers wide. These fine threads appear to be important to cell motility. At the edges of a moving cell



High-voltage electron microscopy easily penetrates whole cell.

At ten times greater magnification, the fiber network is clear.

the microfilaments can form either bundles or a mesh, depending on the direction of motion. Microfilaments contain molecules similar to those of muscle, including actin, myosin and tropomyosin. Varying in diameter and length, most microfilaments are arranged parallel and concentrated toward one side of the cell, Osborn found.

Intermediate in size between the microfilaments and microtubules are fibers with a diameter of 7 to 10 nanometers. The function and composition of these fibers is unknown, but the system displays a pattern of wavy, intermingled lines distinct from that of the other fiber systems.

The fluorescent antibody work, however striking in showing patterns, is limited because a light microscope, rather than an electron microscope, must be used. Keith R. Porter and co-workers at the University of Colorado are peering into whole cells with an especially high-voltage electron microscope (one of two one million-volt microscopes being used for biological research in the United States). They have chosen to study laboratory grown derivatives of human fetal lung cells, because those cells are very flat—less than 1.5 microns at the thickest part.

Even a clear picture through a whole cell is difficult to interpret, because the often translucent components are superimposed into a confusing tangle. Investigators have solved this problem by using three-dimensional visualization to discover the relationships of the various cell parts.

The principle of stereo-microscopy is the same as that used in three-dimensional movies. Each eye sees a slightly different picture, and the brain interprets the disparity as depth. In microscopy, researchers take two pictures of the same preparation with the specimen tilted at different angles. In a lecture room the two resulting photographs can be projected with polarized light of perpendicular orientations. Viewers wear special glasses so that each eye receives only light polarized in one direction. The results of stereo-microscopic studies are published in journals as side-by-side images that readers can view with an instrument called a stereoscopic binocular lens. However, experienced microscopists can perceive the pictures as three-dimensional by simply looking at one picture with each eye and mentally fusing the images.

High-voltage three-dimensional microscopy has shown many of the cellular components to be essentially as biologists had predicted from previous studies. Nuclei appear either ovoid or spheroid. Mitochondria are long, slender rods, which may be branched. The dimensions of the microtubules and microfilaments are similar to those seen in thin-section of fluorescent antibody studies. However, stereo-microscopy for the first time clearly shows what is between these various structures in the gel that microscopists have ambigously called "ground substance." There appears to be a system of fibers even finer than microfilaments.

Previous sightings of bridges and networks spanning the more easily identifiable cellular components had strained both the eyes and credibility of col-

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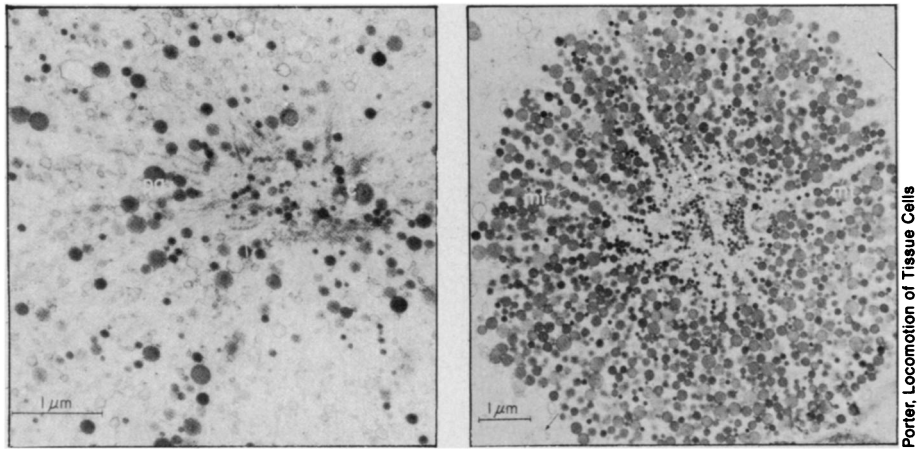

Fish appears red when pigment granules are dispersed in specialized scale cells. Two seconds later, granules are crowded into cell center, and the fish is white.

. . . Cells

leagues, Porter quips. Now Porter and electron microscopist John J. Wolosewick report, "Similar irregular lattices of 3 to 6 nanometer strands can be seen in all micrographs of the cytoplasmic ground substance and seem to represent a continuum that includes (or contains) microtubules, microfilaments, free ribosomes and the membrane-limited vesicles of the endoplasmic reticulum." They suggest that these fine strands were overlooked in conventional microscopic techniques for two reasons. First, the threads were obscured by embedding material, which is not necessary when the cells are prepared simply by drying and are not sliced. Second, many of the strands would lie perpendicular to the plane of the cuts and therefore appear just as small dots.

Porter calls this three-dimensional lattice the microtrabecular system, because its structure reminds him of the trabecular structure of spongy bone scaled down by a factor of 10,000. The microtrabecular system organizes parts of the cell previously thought to float free. Wolosewick and Porter find, for example, "free" ribosomes bound at the lattice intersections. "Functionally the cell requires a structured frame for the non-random distribution of organelles and membraneous systems, Wolosewick and Porter say. Porter adds, "Components cannot perform their functions if they are swimming in cytosol. They need a matrix for structural support."

The microtrabeculae might be significant not only as skeleton for cells, but as "cyto-musculature," too, Porter says. Rearrangement of the lattice could move organelles around inside the cells.

The obvious motion of colored particles within scales of a strange Caribbean fish provides an opportunity for biologists to study intercellular movement. The red squirrel fish, *Holocentrus ascensionis*, pales in response to some stimuli; its red pigment granules aggregate into a tiny, spherical mass at the center of each cell. This movement changes the fish's color—from red to white with a slight pink tinge. The pigment can also be redispersed throughout the cells so the fish again appears red. Pigment aggregation takes 2 to 3 seconds with the granules moving at a uniform velocity of 15 to 20 microns per second. Dispersion takes twice as long and exhibits a jerky motion characteristic of activities where fibers are being restructured.

When these modified nerve cells are removed from the fish scales and maintained in a laboratory they respond to certain chemicals with pigment movement, as in the fish. The laboratory cells also pulsate spontaneously every 10 seconds.

H. Randolph Byers and Porter suspect the microtrabeculae are responsible for the pigment movement. The fiber network disappears as the pigment aggregates and reforms as the granules move out from the center. "Even though only a few granules have had time to leave the central region, the cytoplasm beyond the pigment seems already to have achieved a fine and lacy organization," Byers and Porter say. These fish cells do not have any microfilaments, but the microtubules radiating from the center to the edges appear to guide the pigment granule movements.

Although the researchers conclude that the microtrabecular system is intimately involved with the pigment movements, further experiments must demonstrate whether the fiber rearrangements actually provide the force necessary for motion.

The pigment-containing cell of the red squirrel fish is a special case. But the researchers expect to find microtrabeculae involved with motion in other cells. For example, Porter and co-workers are currently investigating the fiber network in nerve axons where transport of large particles resembles the outward pigment granule movement in the fish.

Porter sees the microtrabecular lattice as essential to the dynamic organization of cells, with their constant redistribution and reorientation of internal elements, including the larger fiber systems. Admitting his prejudice, Porter speculates that the microtrabecular network does just about everything important in a cell. □