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“Porterplasm” and the microtrabecular lattice

K eith Porter was to many the father of biological electron microscopy (EM) and even of cell biology. He helped instigate the founding both of this journal and of the American Society of Cell Biology, and was a key figure in defining the structures of intact and sectioned cells.

The latter stages of Keith Porter’s eminent cell biology career began with the installation of a 1-MeV high voltage electron microscope (HVEM) at the Molecular, Cellular, and Developmental Biology department at the University of Colorado, Boulder, in 1972. Porter had high hopes that the HVEM would allow him to pursue a long-term goal of defining the structure of the cytoplasm by looking at whole cell mounts. Although success did come in imaging whole cells, in the end, the ambitious goal of defining the definitive structure of the cytoplasm was not matched by the technology of the day (Heuser, 2002).

Structure in the void

As early as the mid-1950s, Porter had a strong hunch that there must be some structure to the “optically ‘empty’ parts of the protoplasm” that gave the cell its “elastic framework” (Porter, 1956). With the HVEM he could study a variety of types of cultured cells without the interference of embedding resins. Under a myriad of conditions, he saw a scaffold, or spongework, that encased all the then-known components of the cell. The scaffold consisted of fine, interconnected fibrils, which Porter named microtrabeculae (trabeculae roughly translates from Latin to tiny beams or girders).

The concept of the microtrabecular lattice was first described in 1976 (Wolosewick and Porter, 1976). The lattice backed up the idea that the cytosol was not all liquid, but rather contained a structured, linking framework. Porter also extended the responsibilities of the lattice beyond a mere scaffold, to include directing intracellular movements (Byers and Porter,

1977), giving shape and rigidity to cells, and even perhaps possessing information for cellular organization (Porter, 1978).

Skeptics weigh in

But critics soon voiced concern that the lattice might simply represent a fixation artifact of condensed soluble components of cytosol. Cytoskeletal components had just recently been identified, and many cell biologists still struggled with changing their concept of the cytoplasm from “soup” to “scaffold.” Until the Porter studies, investigators had mostly concentrated on the visible cell structures—membranes, organelles, and filaments—and had ignored any possible cytoplasmic matrix. It was hard to believe that this empty space was highly organized. To address “the artifact controversy,” Wolosewick and Porter used combinations of the best possible, least distorting EM techniques of the day to show relatively unchanged microtrabeculae (Wolosewick and Porter, 1979).

The study explored chemical (glutaraldehyde and osmium tetroxide) and nonchemical (freeze drying and freeze substitution) fixation techniques. It compared cells dehydrated by conventional alcohol/acetone dehydration and those that were critical point dried—a method favored by Porter because it caused less damage to specimens. Finally, they examined two model systems as negative controls: red blood cells—presumably a membrane filled with hemoglobin protein—and a bovine serum albumin solution. They saw no lattice structures in those latter preparations and concluded, “the microtrabecular lattice must exist in a form not too dissimilar” from that depicted in the article.

Glomming but structured

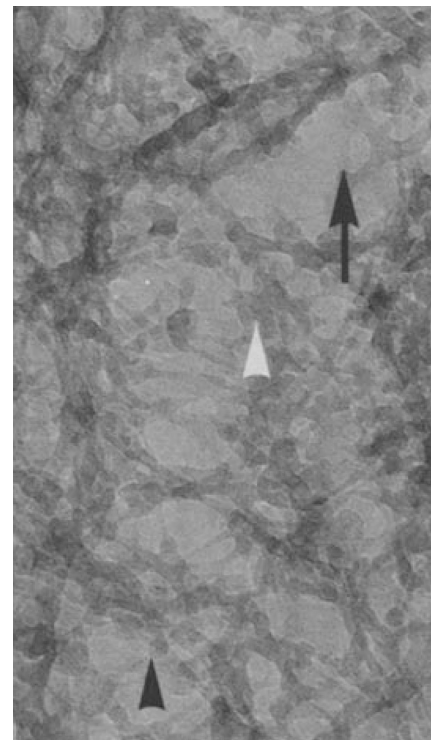
Unfortunately, the best EM techniques of the day were giving what some consider to be a consistent artifact. Two later papers showed that aldehyde fixation and water

contamination in critical point drying caused the soluble, hydrophilic proteins in the cytoplasm to glom onto the insoluble cytoskeletal filaments (Heuser and Kirschner, 1980; Ris, 1985). The resulting cytoskeleton, overdecorated with goopy protein, looked like a microtrabecular meshwork.

But Porter’s basic idea that the cytoplasm is structured still holds true, although what it “looks like” in a live cell is still up for discovery. “If Professor Porter were alive today, he would still defend the concept of a nonrandomly organized cytoplasm,” says John Wolosewick (University of Illinois, Chicago). He says the work sparked an ongoing debate about how cell structures are linked. He contends the lattice was not necessarily an artifact, but rather the best representation at the time of the numerous molecular cytoskeletal cross-linkers that have since been identified.

The latest EM images and tomograms of cytoplasm from the labs of John Heuser and Richard McIntosh (a student

At the end of a long, distinguished career, Keith Porter tangles with a possible EM artifact.



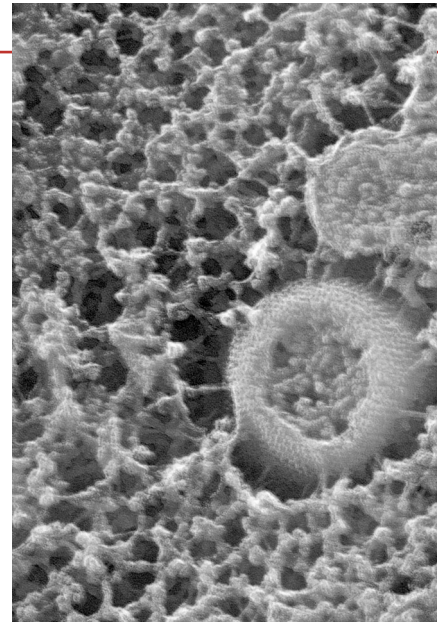
The microtrabecular lattice (arrows and arrowheads) as seen by Wolosewick and Porter.

of Porter's) confirm that Porter was correct in his intuition, if not his details. "The cytoplasm is 'Porterplasm'—a beautiful spongework with organelles suspended in it," says Heuser of his latest freeze-dried, frozen thin sections of cells. However, the soluble components are so densely packed that the overall structure is still difficult to discern.

Porter himself best described the EM conundrum: "In the strictest sense, of course, the content of the images is all artifact where the usual procedures are employed. The question is one of equivalence. To what extent do the images represent what was in the [cytoplasm] when the fixative was applied, and to

what extent may these images be used to investigate the form and function of this part of the cell?" **KP**

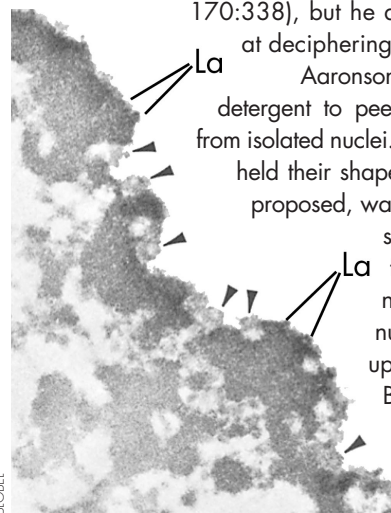
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Heuser's more modern view of cytoplasmic structure.

The isolation of the nuclear lamina

The number of monikers early cell biologists attached to the nuclear lamina reflected their uncertainty about its function and architecture, and whether it was widespread or confined to a few specialized cells. Electron micrographs often disclosed a layer of varying thickness nestled against the backside of the nuclear membrane, which various researchers dubbed the "dense lamella," "fibrous lamina," "zona nucleus limitans," or just plain "lamina." Günter Blobel (Rockefeller University, New York, NY) had his mind on the signal hypothesis, for which he won the Nobel Prize in 1999 (see "Lost in translation: the signal hypothesis" *JCB* 170:338), but he decided to take a crack at deciphering the lamina.



An immunoperoxidase stain that tags one lamina protein doesn't penetrate the nucleus ("La" indicates the lamina; arrows mark nuclear pores).

Aaronson and Blobel (1974) used detergent to peel away the membranes from isolated nuclei. The husks that remained held their shape. This sturdy layer, they proposed, was the lamina, and the results suggested two of its functions—bracing the nucleus and cradling the nuclear pores. Two follow-up studies (Aaronson and Blobel, 1975; Dwyer and Blobel, 1976) provided more evidence that the layer they had identified enclosed the nucleus and wasn't just part of the membrane.

Then Larry Gerace (Scripps Research Institute, La Jolla, CA), Blobel's first Ph.D. student, picked up the analysis. He wanted to definitively describe the lamina in part because of what he viewed as the erroneous conclusions of Berezney and Coffey (1977). They had proposed that proteins not only formed the lamina but also a "nuclear matrix" that extended throughout the nucleus and intermingled with the DNA. "Our localization was a riposte to their conclusions," Gerace says. He characterized three lamina proteins and created antibodies against them. Immunoperoxidase staining showed that the antibodies strongly labeled the rim of the nucleus; they didn't recognize anything in the interior (Gerace et al., 1978). Rather than a mesh that permeated the nucleus, the lamina was a protein polymer that hugged the nuclear membrane, the researchers concluded—and subsequent work has backed them up.

The proteins Gerace identified turned out to be lamins A, B₁, and C, three of the four major components that interweave to form the lamina. "We felt we had made a conclusive argument that lamins are primarily at the nuclear envelope, and the data have held up," says Gerace. Blobel describes this series of studies as one of the first examples of molecular cell biology. Instead of being content to identify new cellular structures, researchers were now breaking down these discoveries into their molecular components to elucidate their workings. **ML**

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