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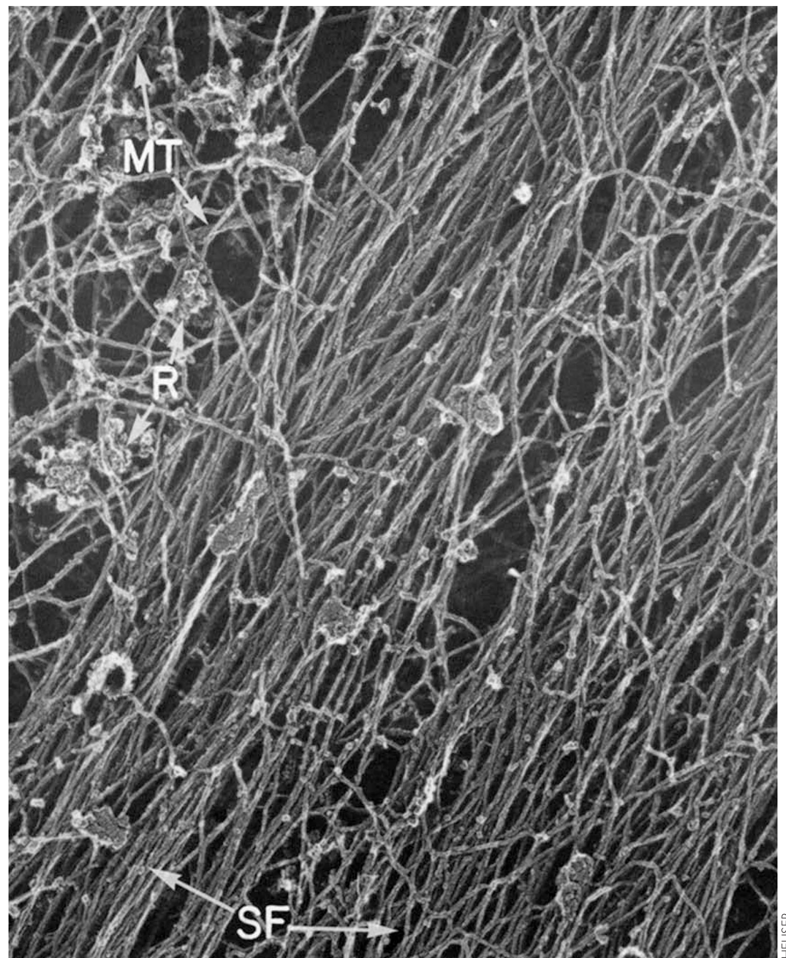
What the cytoskeleton really looks like

The late 1970s brought the discovery that nonionic detergents such as Triton X-100 could extract most cell components and leave behind the insoluble cytoskeleton. "Just making cytoskeletons and naming them was brand new," recalls John Heuser (Washington University in St. Louis, MO). He credits the efforts of James Spudich, Susan Brown, and Klaus Weber for perfecting the structure's isolation.

But the favored EM technique at the time—involving thin sectioning of samples embedded in plastic—was nearly impossible with the gossamer skeletons. And air-drying for negative staining caused them to collapse into a two-dimensional jumble. So Heuser tried a new approach: freeze-drying samples in a vacuum, where the solid water would just evaporate straight to the gas phase, thus removing the surface tension of air-drying.

"Good freeze-drying just requires good, rapid freezing with little time for ice crystals to form," Heuser explains. He had already perfected the use of his "slammer freezing machine"—a technique that quickly froze cell preparations by smashing them against a cold copper block—to capture the rapid kinetics of synaptic vesicle exocytosis (Heuser et al., 1979). And when he and Marc Kirschner decided to try the technique on cytoskeletons, along with coating the dried sample in platinum to make a high-contrast replica, the result was a highly detailed, three-dimensional view of the cytoskeletal filaments (Heuser and Kirschner, 1980).

"In my view, this shows exactly what the cytoskeleton looks like," says Don Cleveland (University of California, San Diego, CA). Cleveland explains that with the advent of indirect immunofluorescence around 1975, the trend was to look at the thinnest, most two-dimensional cells possible. This paper, along with Keith Porter's on the structure of cytoplasm (Wolosewick and Porter, 1979; see "'Porterplasm' and the microtrabecular lattice" *JCB* 170: 864), reminded scientists to think about cells in three dimensions.



A freeze-dried fibroblast cytoskeleton includes stress fibers (SF), microtubules (MT) and polyribosomes (R).

Heuser and Kirschner's study also showed that the major components of the cytoskeleton—microtubules, actin filaments, and intermediate filaments—could each be identified based solely on their ultrastructural appearance. The method proved useful for "seeing" all manner of cellular phenomena, including, notably, clathrin-coated pit formation (Heuser, 1980), the budding of COPI-coated vesicles from Golgi (Weidman et al., 1993), and the dynein arm powerstroke (Goodenough and Heuser, 1982).

The cytoplasmic connections between elements of the cytoskeleton hinted at by the work were further solidified when Gary Borisy's lab added immunogold labeling to the quick-freeze, deep-etch EM technique. The study identified plectin as a cross-linking molecule between intermediate filaments and both microtubules and actin filaments (Svitkina et al., 1996). In hindsight, Heuser says, perhaps the name "cytomuscle" would have been more appropriate than cytoskeleton since "the filaments are responsible for cell movement and are not just the 'bare bones' of a skeleton." JCB

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