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Roll-your-own endothelial tubes

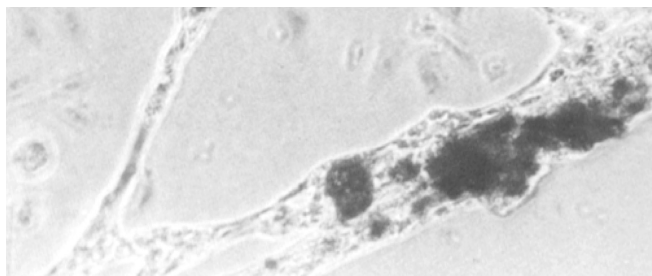
Just one ingredient can make the difference between a delicious meal and an inedible mess even the dog won't touch. Likewise, 20 years ago the recipe for cultivating endothelial cells lacked a vital factor, and many a dish of cells ended up in the trash. The vascular biologists who nabbed the missing ingredient not only simplified the process of growing endothelial cells; their discovery was also a boon for angiogenesis research.

The early to mid-1970s saw a spate of cell-rearing successes, as biologists nurtured primary cultures of endothelial cells (Gimbrone et al., 1974), liver parenchymal cells (Bissell et al., 1973), sympathetic neurons (Mains and Patterson, 1973), and smooth muscle cells (Ross, 1971). Other workers managed to raise secondary cultures of smooth muscle cells (Schubert et al., 1974) and pioneered two- and three-dimensional collagen substrates (Elsdale and Bard, 1972). But as Michael Stemerma (University of California, Riverside) recalls, human endothelial cells remained tricky to cultivate—particularly cells from the most readily available source: the umbilical vein. Researchers tried hard to be good hosts, tempting the cells with sumptuous beds of fibronectin, a tantalizing broth of calf's serum, and a mixture called medium 199. But the ungrateful cells usually died after two or three passages.

Finding what the cells craved was a matter of plying them with one growth factor after another, Stemerma says.

One additive the scientists tried was endothelial cell growth factor (ECGF), which they isolated from the hypothalamuses of cattle. Previous work had suggested that ECGF, now known as fibroblast growth factor 1, stimulated endothelial cells, and indeed it galvanized the cultures. Instead of perishing after three passages, the cultures were vibrant after more than 20 (Maciag et al., 1981). "For the first time, we showed that you could propagate these cells almost indefinitely," Stemerma says. "We were shocked."

The cultures would surprise the team again. Withholding ECGF and fibronectin, the researchers discovered, spurred the cells to roll up into tiny tubes (Maciag et al., 1982). Within a month to six weeks, the tubes would branch into a complex network, creating the beginnings of a capillary tree right there in the culture dish. Following up on the finding, other researchers sought to pin down the conditions that promoted this behavior. Madri and Williams (1983) showed that collagens from the basement membrane, which sheaths the endothelial cells in a capillary, prompted rapid tube formation. Further work indicated that laminin, a basement membrane protein, stimulates cells to roll up (Kubota et al., 1988) and that the stickiness and strength of the extracellular matrix supporting the cells might also determine whether they proliferate or get tubular (Ingber and Folkman, 1989). **JCB**



MACIAG

After 7–8 weeks in culture, endothelial cells form interconnected tubes filled with debris.

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A portrait of the nuclear pore complex

A striking photograph in a cell biology text convinced Ron Milligan (now at the Scripps Research Institute, La Jolla, CA) to take a closer look at the nuclear pore complex, the portal that ships materials back and forth across the nuclear membrane. The shot was the first he'd seen that clearly showed the pore's eightfold symmetry, with structures radiating from the center like petals of a flower, recalls Milligan, then a lab technician with Nigel Unwin's group at the Medical Research Council in Cambridge, UK. Apart from this detail, researchers knew little about the

pore's architecture, except that it was a cylinder that sat in a hole spanning the two nuclear membranes, says Milligan. He and Unwin agreed that sophisticated image processing techniques might sharpen this hazy understanding.

They zoomed in on pores—some still embedded in the nuclear membrane and others that they had broken free—using Fourier analysis to enhance the electron micrographs and eke out more structural detail (Unwin and Milligan, 1982). The pair identified four, previously unrecognized pore components: thin rings resembling washers; spokes

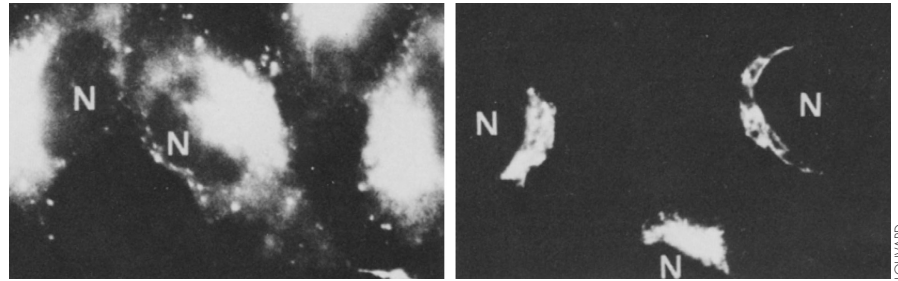
that clustered inside the rings; a central hub, or plug; and large particles that sometimes sat on the pore's cytoplasmic side. These pieces gave the pore its cylindrical shape, the researchers concluded, with the spokes affixing the complex to the membrane.

Flanked by rings on the cytoplasmic and nuclear sides, the spokes extend inward toward the hub. What the large particles were and what they were doing was a mystery. The researchers raised the possibility that they were ribosomes—and took a lot of grief for it, Milligan recalls. He now thinks they

Tagging an organelle

In the early 1980s, cell biologists kept slamming into the same obstacle when they tried deploying antibodies to elucidate the working of the Golgi complex. True, use of antibodies to identify proteins (Bader et al., 1982; de Camilli et al., 1983a,b; Huttner et al., 1983; Weiss et al., 1984; Woodcock-Mitchell et al., 1982; Yen and Fields, 1981), localize cellular structures (Levine and Willard, 1981), and differentiate cell types (Schnitzer et al., 1981; Skene and Willard, 1981) was booming. The difficulty was crafting antibodies to target exclusively proteins from the Golgi complex, recalls Graham Warren (now at Yale University, New Haven, CT). Even the purest mixtures of Golgi membranes contained contaminants, such as shards of cell membrane, and stimulated production of antibodies that labeled non-Golgi structures. Warren and his colleagues Daniel Louvard and Hubert Reggio, all then at the European Molecular Biology Laboratory in Heidelberg, Germany, devised a technique for weeding out the unwanted antibodies.

To the “raw” antibody solution they added debris they had initially separated from the Golgi membranes. This junk was mainly plasma membranes and bits of endoplasmic reticulum. After letting the



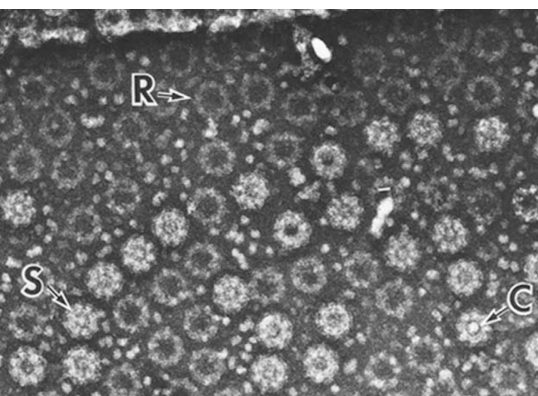
Raw anti-Golgi antibodies (top) are more specific after extraneous antibodies are removed (bottom).

combination incubate, they again removed the membrane gunk—and in the process eliminated many of the unwanted antibodies (Louvard et al., 1982). The researchers then performed the step again with rat plasma, which sopped up antibodies against secretory proteins. Using immunofluorescence, the researchers showed that the leftover mixture labeled only the perinuclear region, where the Golgi complex forms. Meanwhile, cells tagged with the “raw” antibody concoction glowed all over. “This was a dramatic demonstration that you could make high-affinity antibodies to organelles,” says Warren. Cell biologists expressed their approval in the usual way, he says: “They asked us for samples.”

The researchers determined that the antibodies were recognizing one Golgi protein—though they weren’t sure of its identity or location. Subsequent work revealed that it was mannosidase II, a key Golgi enzyme. Louvard and colleagues applied the same technique to uncover four markers for the endoplasmic reticulum.

Their discovery helped researchers better understand the anatomy and activity of the Golgi complex. But it also sparked a controversy—over whether the complex forms spontaneously or requires a template—that hasn’t abated today (Wells, 2001). **JCB**

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Nuclear pore complexes include spokes (S), central plugs (C), and rings (R).

could be structures that collapsed during preparation of the membranes. The central plug was also puzzling—Milligan speculates that it could be material passing through the pore.

Subsequent studies added some

elaborations to this layout. For example, the spokes are much more complex than previously thought, and their inner portions are connected (Akey, 1989; Hinshaw et al., 1992). Jarnik and Aebi (1991) identified filaments emanating from the rings on both sides of the pore, while Goldberg and Allen (1992) spotted basket-like features hanging from the nuclear side. And by analyzing the proteins in the pore complex, Rout et al. (2000) suggested that it contains no more than ~30 components—less than half as many as a ribosome. But the basic arrangement that Unwin and Milligan deduced proved accurate, Milligan says.

“The value of the work,” he says, “is that it provides a realistic and detailed picture that one can have in one’s head” while thinking about possible models of the pore’s transport function. **JCB**

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