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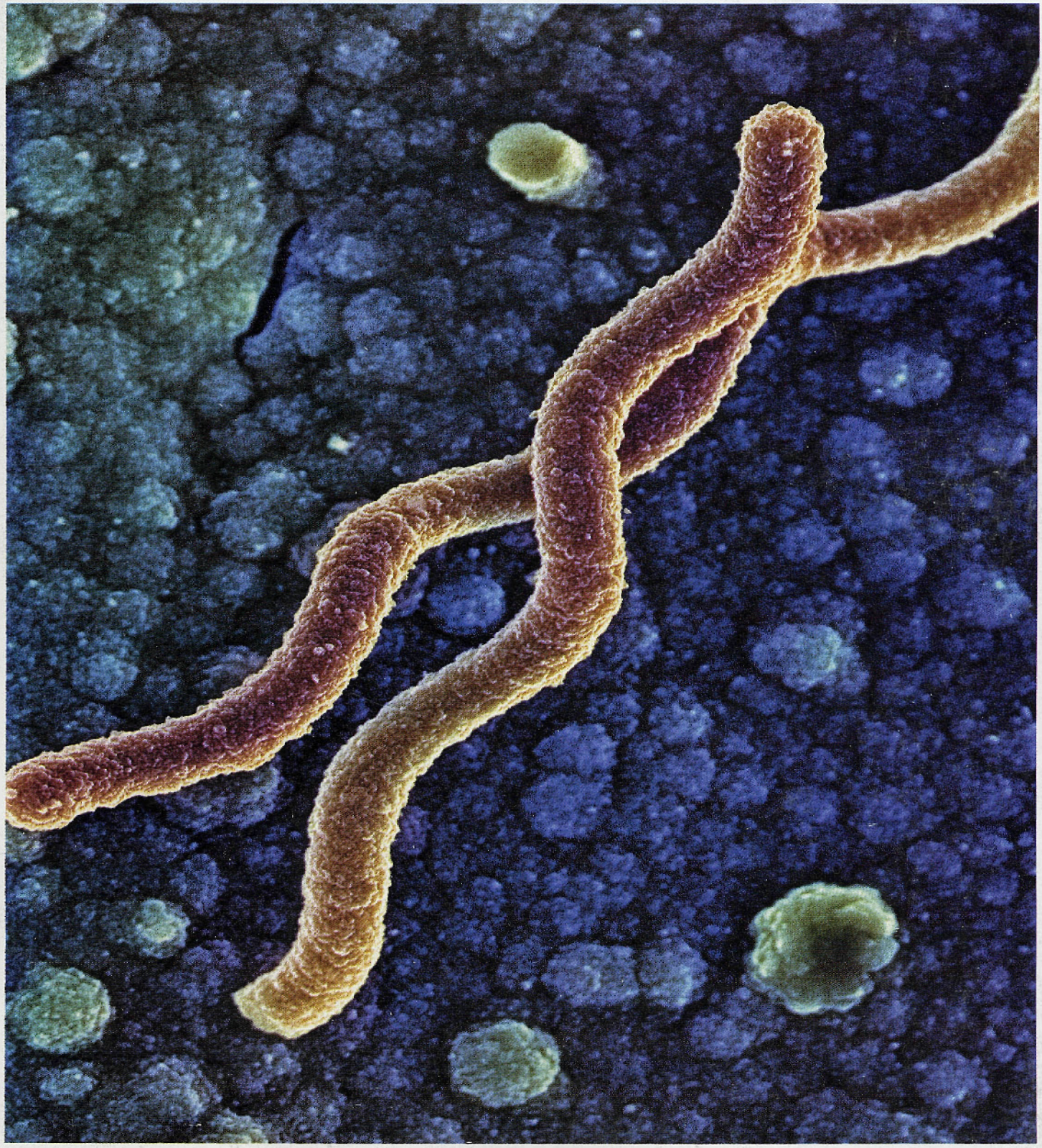

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CELL-FREE SECRETION

Stephen W. Carmichael[1], Mayo Clinic

Regulated secretion occurs when the cell detects a signal, vesicles containing the secretory product fuse with the plasma membrane of the cell, and the contents are released into the extracellular space. This mechanism is called exocytosis. Many laboratories around the world have studied exocytosis for the past couple of decades, and we know a lot about the many steps that are involved. The cytoskeleton appears to play a role in allowing vesicles to approach the plasma membrane, and many other proteins are in the cascade between reception of the signal and the release of the secretory products. But which steps are essential, which ones are regulatory, and which ones modulate secretion? In order to answer these questions, we need a simple model system; intact cells are simply too complicated. While many models have been utilized to unravel the mechanisms of exocytosis, a cell-free system is needed. This "Holy Grail" of secretion has been sought, and it looks like we now have one.

This breakthrough was recently reported by Julia Avery, Darren Ellis, Thorsten Lang, Phillip Holroyd, Dietmar Riedel, Robert Henderson, Michael Edwardson, and Reinhard Jahn. After several preliminary studies, they took advantage of the fact that a fluorescent marker called acridine orange is a weak base. Acridine orange is sequestered within vesicles that have an acidic interior. The cell they began with is called the PC12 cell, derived from a tumor of the rat adrenal medulla. PC12 cells are a type of chromaffin cell that grows particularly well in culture, and chromaffin cells are notable for containing vesicles that are acidic (pH about 5.5). As acridine orange becomes concentrated within these vesicles, they form dimers and larger aggregates, and due to a phenomenon called quenching, these aggregates do not fluoresce. During exocytosis, when the vesicle contents are released into the extracellular medium, the acridine orange is diluted and fluoresces. This is the basis of the assay that Avery *et al.* used in their study.

PC12 cells were loaded with acridine orange, then they were physically disrupted with ultrasonic waves. After just the right amount of physical disruption, Avery *et al.*, could visualize patches of plasma membrane with secretory vesicles attached. Using cytochemical markers for mitochondria and other organelles, they determined that virtually nothing else remained, therefore they had a cell-free system. With a phase contrast microscope, they could visualize patches of membrane. With a fluorescence microscope, they could see flashes of fluorescence when the appropriate amount of calcium was added to the incubation medium. It is known that, under most circumstances, calcium ions are required for exocytosis to occur. When calcium was eliminated from the medium (by a chelating agent) the fluorescent flashes ceased. The flashes were interpreted to represent the release of acridine orange into the incubation medium. If this is the correct interpretation, then we have a cell-free assay for exocytosis.

Several different types of microscopes were used to strengthen the evidence for this cell-free system. Transmission electron microscopy demonstrated chromaffin vesicles attached to pieces of membrane in unstimulated specimens. In specimens treated with calcium, the number of chromaffin vesicles was significantly decreased. The atomic force microscope (AFM) was used to image fixed specimens, both stimulated and unstimulated, with the number of attached particles being dramatically reduced in the stimulated specimens. Finally, the AFM was used to capture "live" images from unfixed specimens in an aqueous environment. When calcium was added to the medium, scans about 4 minutes apart showed a dramatic decrease in the number of vesicles on the patches. This demonstrates the potential for temporal resolution, as well as spatial resolution, of exocytosis.

As another control, Avery *et al.*, also showed that tetanus toxin, which is known to block exocytosis, also blocked secretion in their cell-free system. And interestingly, an extract of cytosol had to be included in the incubation medium for exocytosis to occur. However, we finally have a system where the process of exocytosis can be methodically dissected. Using this system, we should be able to separate steps that are essential, from those that regulate and modulate secretion. An exciting development, indeed! ■

1. The author gratefully acknowledges Dr. Reinhard Jahn for reviewing this article.

2. Avery, J., D.J. Ellis, T. Lang, P. Holroyd, D. Riedel, R.M. Henderson, J.M. Edwardson, and R. Jahn, A cell-free system for regulated exocytosis in PC12 cells, **Journal of Cell Biology**, 148:317-324, 2000.
3. For more information about chromaffin cells, check out the web site: www.chromaffincells.org

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