https://doi.org/10.1017/S1551929500052391

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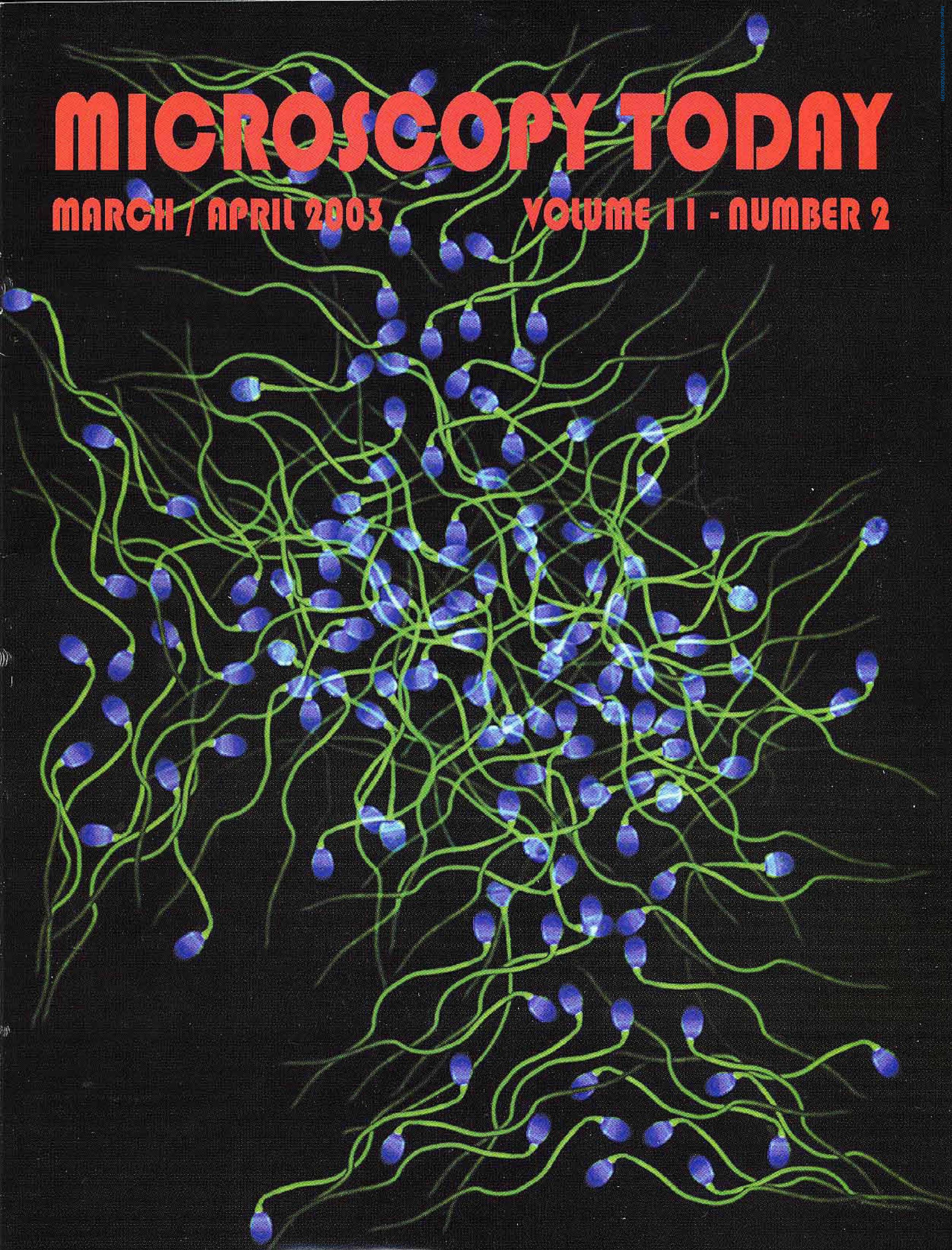
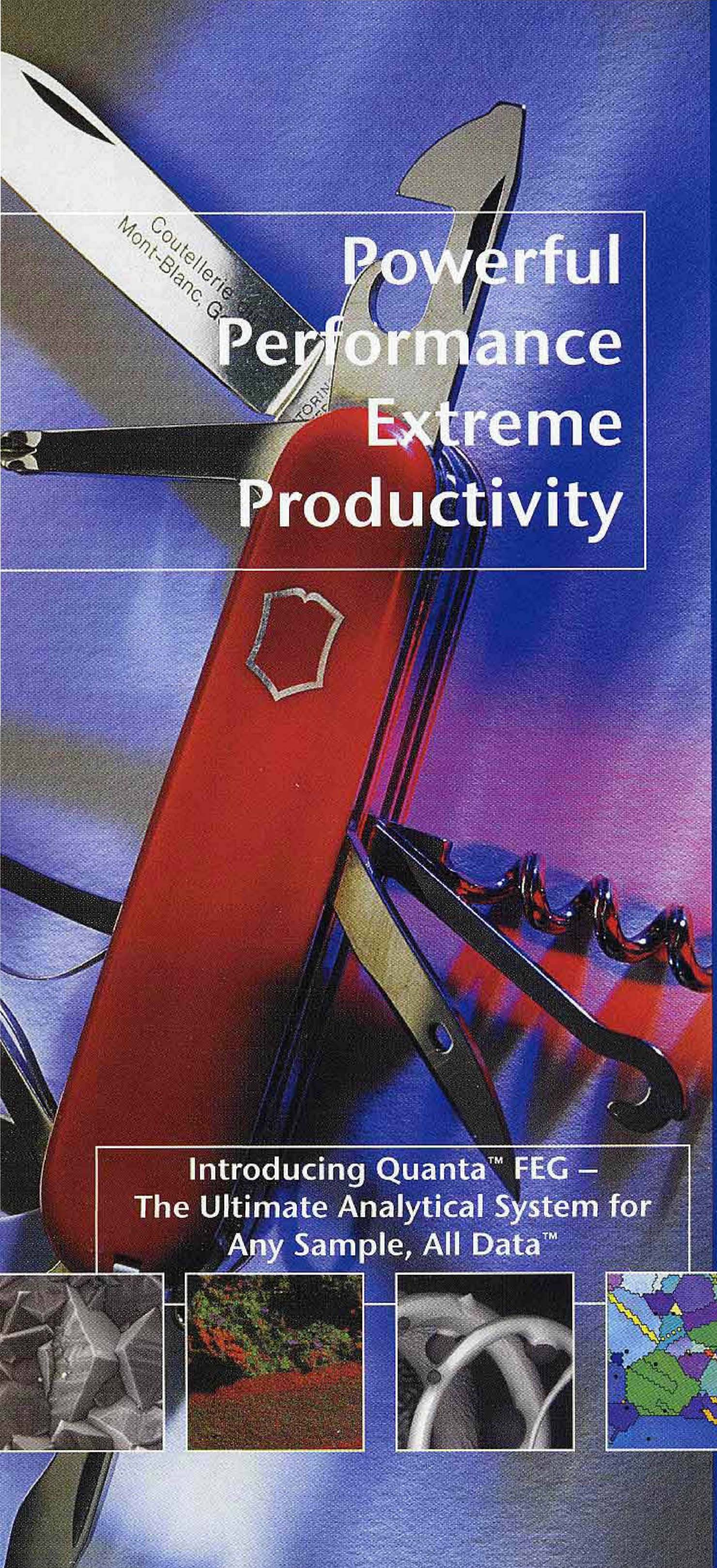
MARCH / APRIL 2003 VOLUME 11 - NUMBER 2





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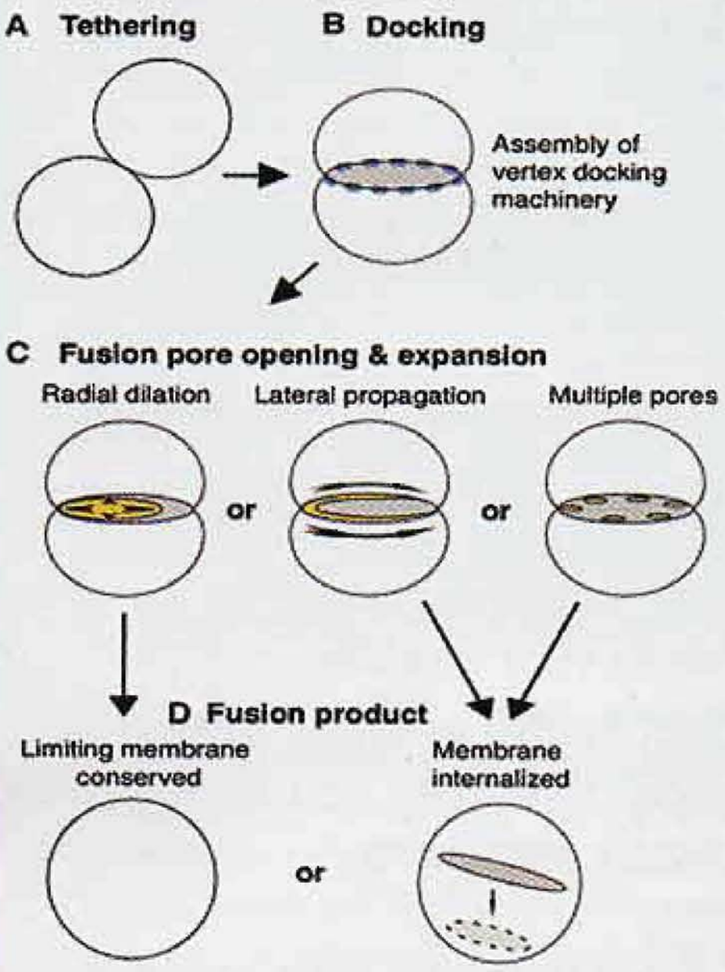
Membrane Leftovers after Fusion of Vacuoles

By Stephen Carmichael and Mark McNiven
Mayo Clinic[1]

In a clever application of time-lapse fluorescence microscopy, Li Wang, Scott Seeley, William Wickner, and Alexey Merz looked to see if there was membrane left over after vacuoles fused within a cell.[2] Specifically, it is not known what happens when two vacuoles come together (this is referred to as "docking") and join together as one larger vacuole ("fusion"). For reference, the surfaces of the vacuoles where they have joined is the "boundary" membrane, the membranes on the outside are the "outside" membranes, and the circular junction of the boundary and outside membranes is the "vertex." A popular theory has a fusion pore opening somewhere near the center of the boundary membrane, expanding radially to the vertices. When this process is complete, there is one larger vacuole with no residual membrane; the boundary membrane has become incorporated into the outside membrane of the new vacuole. But, as pointed out by Wang *et al.*, there have been several observations over the years showing pieces of membranes floating within vacuoles. Could there be another mechanism that would show that this extra membrane was a result of a different fusion process?



Wang *et al.* looked for fusion pores by loading isolated vacuoles with a fluorescent dye and fusing them with unloaded vacuoles. By using specific inhibitors to block all steps after tethering, no fluorescent dye was transferred between vacuoles, and their size did not change, as you might expect if fusion was not allowed to occur. When inhibitors of fusion downstream from docking were used, no exchange of the dye was seen either. This led to the conclusion that although small or transient fusion pores may form during docking, they do not lead to the final fusion event.

Many proteins are known to mediate the docking and fusion events. Wang *et al.* labeled several of these proteins, and certain lipids within the membranes, and demonstrated that the molecules they labeled were functionally intact. They then looked at the ratio of the fluorescent signals to elucidate the relationship between proteins and lipids in the membranes at the site of fusion. After establishing the utility of this assay, they demonstrated that the accumulation of the fusion proteins at the junction vertices is a regulated and selective process. This accumulation was shown to lie on the authentic fusion pathway.

Most interestingly, Wang *et al.* demonstrated that the boundary membrane is released from the vertices, apparently to become incorporated into the resultant vacuole. It is not known what happens to this internalized membrane, but it clearly demonstrates that fusion of these cytoplasmic compartments is the result of events that occur at the vertex of docked vacuoles, rather than a fusion pore event. It will be interesting to see if this fusion mechanism occurs wherever compartments fuse within the cell.

References

1. The authors thank Professor William Wickner for reviewing this article.
2. Wang, L., E.S. Seeley, W. Wickner, and A.J. Merz, Vacuole fusion at a ring of vertex docking sites leaves membrane fragments within the organelle, *Cell* **108**:357-369, 2002.

INDEX OF ARTICLES

ABOUT THE COVER

Isolated Human Sperm Cells

Sperm flagella were highlighted with a novel polypeptide conjugated to green fluorescent protein. This polypeptide binds to a sperm cell surface receptor and may play a role in sperm motility. Sperm heads were counterstained with the blue, nuclear dye, Hoechst 33258. Specimen was processed and imaged by Albert Tousson from the Imaging Facility at the University of Alabama at Birmingham. This fluorescence micrograph is part of a study by Dr. Ramarao Chodavarapu and Dr. Lawrence DeLucas and won 11th place in the 2002 Nikon International Small World Photomicrography contest.