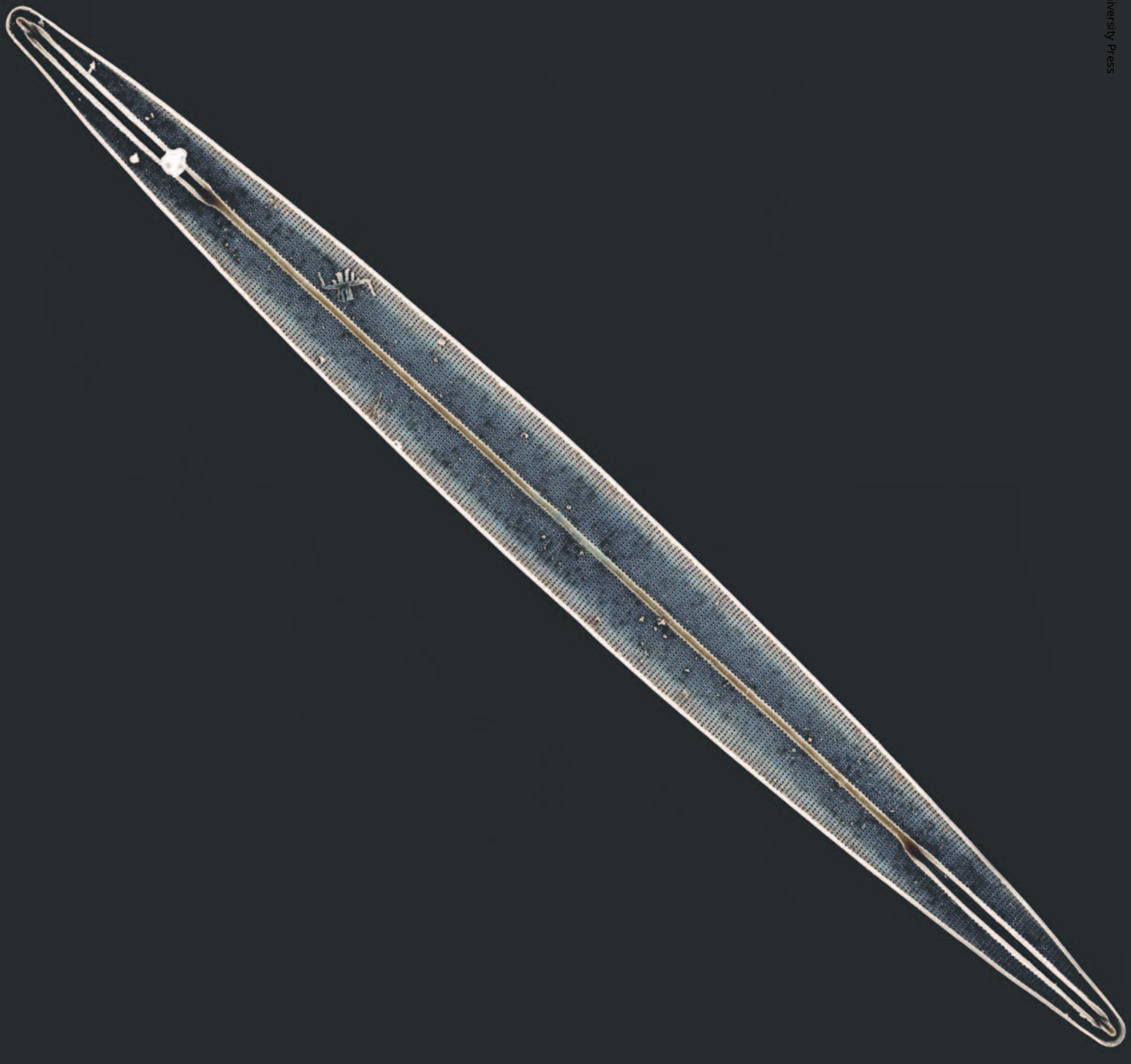
MICROSCOPY TODAY

MAY 2007 VOLUME 15 - NUMBER 3



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Hitachi S-5500

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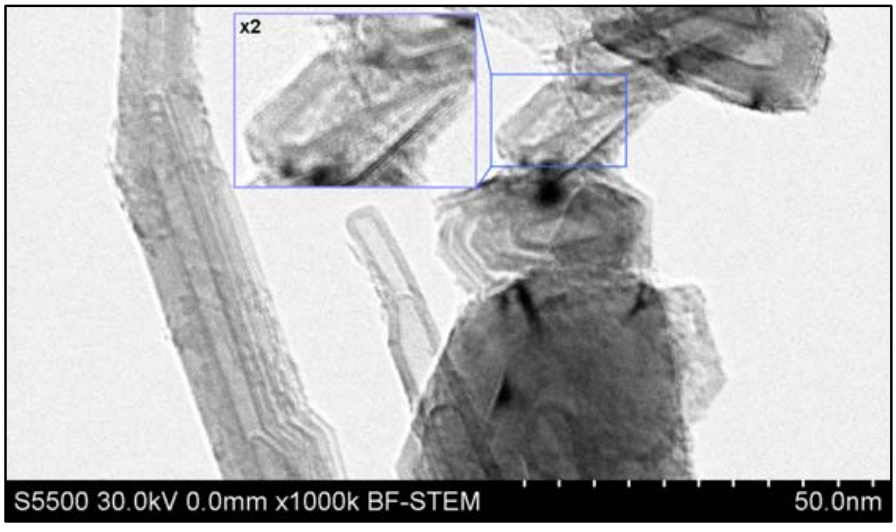
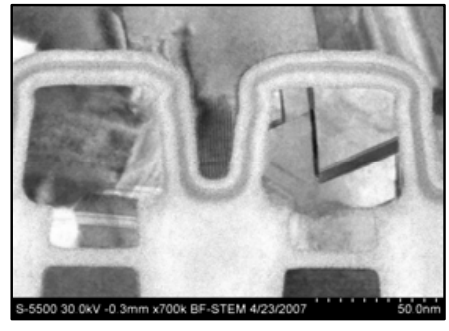
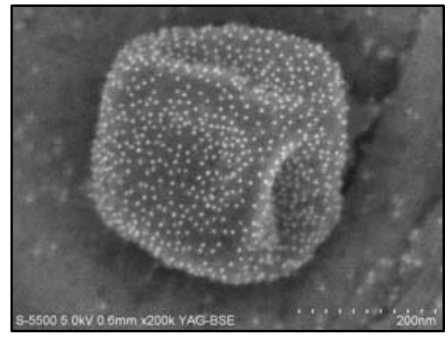


Fringes of MWCNT in STEM



NAND double-gate cross-section in STEM



Immuno Labeling

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Illuminating the Formation of Lumens

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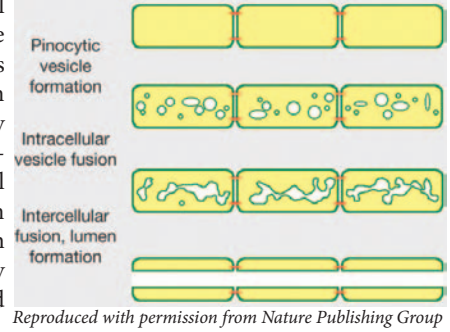
How do lumens form? Two mechanisms that come readily to mind are a wrapping model, similar to the wrapping of the myelin sheath around a neuronal process, and a solid core of cells followed by apoptosis of the central cells. Another obvious mechanism that was suggested over 100 years ago is the fusion of intracellular vacuoles. Whereas several recent studies have supported this latter mechanism, it has not yet been proven. Now, the appropriate animal model (zebrafish), the modern techniques (transgenic chimeras), dyes (green fluorescent protein and monomeric red fluorescent protein) that can be linked to proteins to label vacuoles, and two-photon imaging in real time finally have provided the strongest support yet. In an article by Makoto Kamei, Brian Saunders, Kayla Bayless, Louis Dye, George Davis, and Brant Weinstein[2] the assembly of endothelial tubes from intracellular vacuoles was observed *in vitro* and *in vivo*.

The *in vitro* model was human vascular endothelial cells grown in a three-dimensional collagen gel matrix. Large intracellular vacuoles could be clearly seen by light microscopy when they took up carboxyrhodamine from the medium. To observe the dynamics of endothelial vacuoles and their role in vascular lumen formation *in vivo*, Kamei *et al*. expressed a specific fusion protein that incorporated green fluorescent protein (GFP) or monomeric red fluorescent protein (mRFP1) in endothelial cells of zebrafish. The optical clarity and accessibility of zebrafish embryos and larvae made this an excellent model for this study. They first demonstrated that their transgenic zebrafish ran a normal embryonic course and developed into adults that were indistinguishable from normal controls in viability and fecundity, showing that the fluorescent fusion proteins expressed as transgenes did not have deleterious effects on endothelial cells. They focused on trunk intersegmental vessels composed of three endothelial cells that emerge from the dorsal aorta and migrate as a chain along the boundaries of the myotomes. Two-photon imaging of growing intersegmental vessels in living transgenic embryos showed vacuoles labeled with either GFP and/or mRFP1. Control studies showed that the vacuole formation was not an artefact. Endothelial vacuoles were also observed in transmission electron micrographs of newly-formed blood vessels in non-transgenic zebrafish embryos. These vacuoles were similar in appearance to those in the *in vitro* model. Time-lapse imaging demonstrated that endothelial vacuoles are very dynamic (appearing, disappearing, and fusing to form larger compartments on a timescale of minutes). Later, vacuoles merge into nascent lumenal compartments that remain very dynamic. Next, Kamei *et al.* demonstrated that the enlarged vacuolar compartments underwent fusion to generate multicellular lumenal spaces. *In vitro* studies indicated that the formation of a common lumenal space might occur by the exocytosis of intracellular vacuoles into junctional spaces between adjacent endothelial cells. *In vivo* studies involved injecting red quantum dots into the circulatory system of GFP-tagged transgenic zebrafish embryos and the red fluorescent label could be observed to move from the dorsal aorta into previously unlabeled vacuolar compartments of the proximal intersegmental endothelial cells, and then move to more distal cells. Taken together, these *in vitro* and *in vivo* studies support the mechanism of lumen formation by intracellular and intercellular fusion of endothelial vacuoles. This cartoon from their publication summarizes their theory of lumen formation. And that's the hole story! ■



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1 The author gratefully acknowledges Dr. Brant Weinstein for reviewing this article.

2 Kamei, M., W.B. Saunders, K.J. Bayless, L. Dye, G.E. Davis, and B.M. Weinstein, Endothelial tubes assemble from intracellular vacuoles in vivo, *Nature* 442:453-456, 2006.

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The cover shows a pseudo color SEM image of Amphipleura pellucida from the UK at 1440× relative to 5" × 4". This is a whole view of the concave side of the valve. See the article on page 12.