A finer look at a fine cellular meshwork

The endoplasmic reticulum is imaged at super-resolution

By Mark Terasaki

Students learning about the eukaryotic cell are taught that mitochondria are the powerhouse of the cell, the nucleus is the information storagehouse, and lysosomes are the garbage disposal. Summing up the role of the endoplasmic reticulum (ER) is more problematic, even for professional cell biologists. Classically, the ER is where membrane proteins, secreted proteins, and most lipids are synthesized. It is also the site of calcium regulation. More recent work shows that it is the site of antigen presentation by major histocompatibility complex class I molecules, and the location of the unfolded protein response, now thought to be central to several major diseases. A new frontier is its relationships with other organelles (1). On page 433 of this issue, Nixon-Abell et al. (2) describe how an armamentarium of super-resolution imaging techniques reveals new aspects of the ER’s very heterogeneous morphology. Indeed, the “form follows function” adage may help to make sense of this organelle’s functional issues.

The ER is a single-membrane system distributed throughout the cell. Like the vascular and nervous systems, many ER functions are likely to be involved in coordinating processes. At the ultrastructural level, the ER is a complex system of interconnecting tubules and sheets. The discovery of “reticulons” and related proteins showed that gene products control the shape of the ER at this level of organization (3). ER membranes are now thought to be naturally flat, and these “hairpin” proteins convey curvature to ER membranes such as occurs in ER tubules and edges of ER sheets. Mutations in several of these proteins are directly associated with the human disease hereditary spastic paraplegia (4), though frustratingly, it is still not clear how.

Just as capillaries were discovered in the thin webbing of a frog’s foot by Marcello Malpighi in the 17th century, the ER was discovered in 1945 by Keith Porter in a special situation where it is two-dimensional (2D). Fibroblasts (connective tissue cells) adhere to some substrates in vitro so strongly that the periphery of the cell spreads out in a thin layer. The periphery is thin enough (<1 μm) that Porter could see, by looking completely through this part of the cell with transmission electron microscopy, a “reticulum” that was present mostly in the “endoplasm” (nonmitotic part of the cell) (5). The invention of the ultramicrotome in 1953 opened up detailed microscopic study of all cells of the body by producing 70-nm-thick sections that can be visualized at subnanometer resolution. The first serial section study demonstrated a complex system of ER tubules and sheets (think of pita bread) (6). Sheets are particularly well-established components of secretory cells. The work on fibroblasts was forgotten until years later, when improved optics and fluorescent probes such as dyes and, later, green fluorescent protein tags allowed the ER to be seen “whole” in the cell periphery. The discovery of the cytoskeletal (microtubule) interaction with the ER (7) and the visualization of protein traffic from the ER to the Golgi apparatus (8) were two of many highlights of this approach.

Nixon-Abell et al. subjected this classic cell preparation (10 different cell lines) to several super-resolution techniques. The resolution of conventional light microscopes is determined by diffraction, and is roughly half the wavelength of light (~0.5 μm). Super-resolution is accomplished by single-molecule techniques and by clever new methods of illumination. A live-cell technique, grazing incidence structured illumination microscopy (GI-SIM), provided substantial improvements in spatial and temporal resolution. ER tubules, which have a diameter of 50 to 100 nm, and three-way junctions of tubules were observed to oscillate ~70 nm with a period of ~250 ms. These movements were affected by inhibitors of energy metabolism, so they are not due to random molecular collisions. These observations of Nixon-Abell et al. are of interest for the ER, but also may be relevant to the long-standing question of whether the cytoplasm is like water (a dynamic gel) or has a kind of molecular association unique to living cells.

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When fluorescently labeled and observed by light microscopy, some of the peripheral ER has been thought to be structured as membrane sheets. However, the essentially 2D peripheral ER has always been difficult to capture by thin sectioning, so these “sheets” have never before been looked at by electron microscopy. The super-resolution methods used by Nixon-Abell et al. make it clear that these are, instead, a structure with many openings too small to be resolved previously. The authors observed that motions of the components of this dense network are similar to those seen in ER tubules and three-way junctions. They also found that the structure can rapidly form and dissipate at its edges. Macromolecular motion was stopped by chemical fixation and imaged by a high-density 3D fluorescent localization microscopy called lattice light sheet-point accumulation for imaging in nanoscale toposcopy (LLS-PAINST), which combines novel light illumination with single-molecule imaging of fluorescent lipids. An image of a fine meshwork emerges, very consistent with the live-cell imaging.

Focused ion beam–scanning electron microscopy (FIB-SEM) is a recently developed technique for producing very thin, 8-nm-thick serial section electron microscopic images. Although the peripheral ER was difficult to capture, Nixon-Abell et al. reconstructed an overlapping, dense tubular matrix (together with bona fide sheets) from a thicker part of the cell. This proposed “ER matrix” is reminiscent of a type of ER, sometimes called fenestrated sheets, which has been seen previously in various cell types (9). Nevertheless, Nixon-Abell et al. show that the ER matrix exists in living cells (i.e., is not an artifact of fixation), and document its dynamic properties.

A recent paper examines the involvement of membrane curvature in a loose tubular membrane network in a cell-free system (10). The membrane protein atlastin was required for the formation and maintenance of this network, whereas the ER tubule-forming proteins reticulons and lunapark had complementary roles. Nixon-Abell et al. also investigated the involvement of curvature-shaping proteins in ER morphology and observed atlastin in the ER matrix, with reticulons and cytoskeletal linking membrane protein (CLIMP) occasionally present in the ER matrix, but they did not report on lunapark. This raises the question of whether the ER matrix is a dense tubular network, a membrane sheet with densely packed holes, or something else. An additional issue is whether the ER matrix is confined by its components to be a 2D structure within the generally 3D ER.

The study of Nixon-Abell et al. reveals a dynamic type of densely packed ER (see the image). The authors offer several interesting speculations on its function, such as allowing for rapid changes in calcium regulation, lipid metabolism, or organelle interactions. For more ideas, it may be useful to focus on the nature of the unusual, very thin peripheral cytoplasm, because its particular cellular functions may be supported by this ER. There is no analog for the thin “peripheral cytoplasm” in some cell types, such as a liver hepatocyte. An important aspect of this work is that cells in culture are very amenable to experimental manipulation, so we can look forward to future testing of ideas using super-resolution techniques. The mystery of this ubiquitous changeable organelle endures.

**REFERENCES**


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**QUANTUM PHYSICS**

**Versatile cluster entangled light**

Dark excitons are used to entangle strings of hundreds of photons for quantum information processing

By Hans J. Briegel

Performing a quantum computation may seem complicated, but it can be done with a specially prepared beam of light and good photodetectors. On page 434 of this issue, Schwartz et al. (1) report on a prototype device that uses semiconductor quantum dots that generate long strings of photons in an entangled cluster state of light. Cluster states (2) carry a specific sort of entanglement—that is, the way in which the properties of different photons are correlated. In two dimensions—when the entangling connections form a net or lattice—cluster states become a universal resource; they can be used for all kinds of quantum information processing and are like a fuel for quantum computers. The entanglement can be realized between the polarizations of individual photons—the directions in which their individual electric field vectors point. A quantum calculation (3) can be run by measuring the polarization of each photon, one by one, in a specific order and direction, almost as simple as moving the beads of an abacus.

Although this scheme is simple, actually producing large-scale cluster states of photons has been a formidable challenge. Previous experiments have demonstrated the feasibility of measurement-based quantum computation with single photons (4) and ions (5). Photonic implementations have mainly used parametric down-conversion to generate entangled pairs of photons from a laser beam focused on a nonlinear crystal. A toolbox of beam splitters, phase shifters, and photodetectors then enables photonic quantum information processing. The drawback of this approach is that the generation of entangled pairs and their combination to larger-scale cluster states work only probabilistically (it requires post-selection), which...
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Editor's Summary