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Introduction: Our Changing Views of Mitochondria

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Recent years have seen a renewed interest in things mitochondrial. There are many reasons for this. Card-carrying mitochondriologists see it as a natural consequence of what they have long known—or at least suspected. Whatever their early origins, mitochondria are not merely semiautonomous ATP generators, but highly evolved organelles that are fully integrated into the functioning of the eukaryotic cell. Thus, our view of mitochondria changes with each new discovery of the organelle's involvement in yet another fundamental biological process. Recent issues of the *Journal of Bioenergetics and Biomembranes* reflect a new awareness of mitochondrial participation in cell death and aging (Vol 31:4), clinical disorders (Vol 29:2), and even obesity (Vol 31:5). The current issue of JBB follows in this vein, containing minireviews and original papers derived, for the most part, from presentations at the Second Albany Conference on "Frontiers of Mitochondrial Research" (September, 1998, Rensselaerville, New York). A major focus of this meeting was mitochondrial membrane transport and associated phenomena (*e.g.*, Ca^{2+} regulation, the permeability transition, protein import) and so this general theme was chosen for the papers included in this issue.

MITOCHONDRIAL IMAGING

Our views of mitochondria are changing in another way, one that relates to new structural insights being provided by advances in microscopy. A half-century ago, research into—and controversies about—mitochondrial structure and function played a major role in shaping the field of cell biology (Rasmussen, 1995). The promise of transmission electron microscopy (TEM) to reveal cellular organization was just beginning to be realized, as were its limitations. The conventional picture of mitochondria as discrete, ellipsoidal organelles (consistent with a largely autonomous life style) was reinforced by cross-sectional profiles observed in electron micrographs of thin plastic sections of cells. The development of laser confocal microscopy and novel fluorescent probes (*e.g.*, new dyes and GFP-fusion proteins) has provided cellular views of mitochondria as a dynamic reticulum, operating in close association with the cytoskeleton and another extended membrane system, the endoplasmic (or sarcoplasmic) reticulum (see below). While this understanding of mitochondrial organization is not totally new, its widespread acceptance is the direct result of compelling images produced by increasingly sophisticated (and increasingly available) light microscopic technology (*e.g.*, Johnson *et al.*, 1980; Bereiter-Hahn and Voth, 1994; Rizzuto *et al.*, 1998).

INTERIOR DESIGN OF MITOCHONDRIA

Likewise, the textbook depiction of the internal organization of mitochondria, showing bafflelike cristae with wide openings into the intermembrane compartment, also arose from interpretation of inherently two-dimensional (2-D) TEM images. Advances in three-dimensional (3-D) electron microscopy, in particular, tomography of organelles and cells embedded in thick plastic sections (Frank, 1992), have provided a dramatic new view inside mitochondria. These 3-D images show the mitochondrial cristae (inner membrane involutions) to be pleiomorphic, with narrow, sometimes very long tubular connections to the periph-

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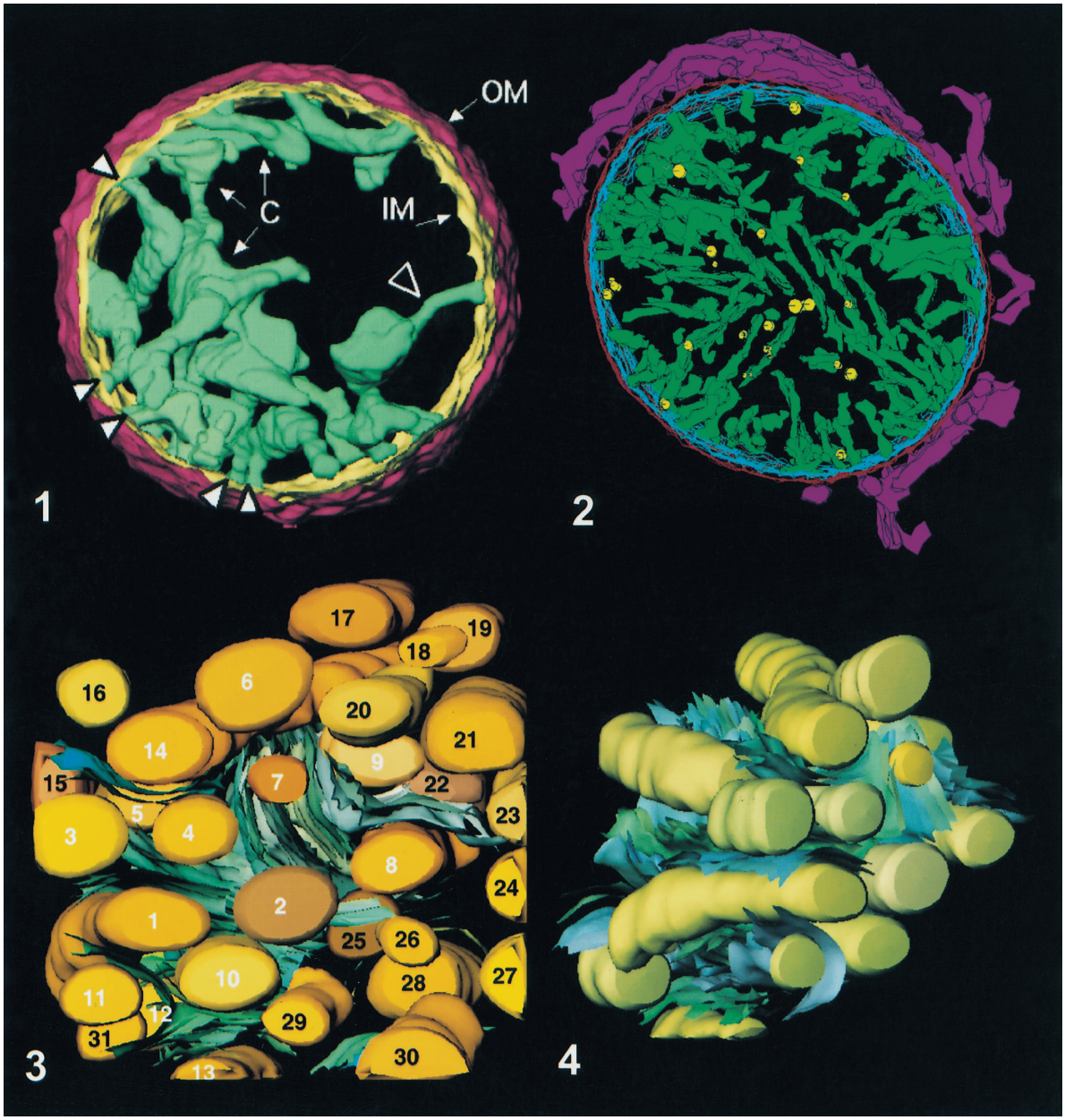

Figs. 1±4. Three-dimensional reconstructions of rat-liver mitochondria obtained by high-voltage (1000 kV) electron microscopic tomography, using two-axis tilting as described by Penczek *et al.* (1995). Fig. 1. Isolated mitochondrion showing outer membrane (OM), inner peripheral membrane (IM), and selected cristae (C). Arrowheads point to narrow tubular regions that connect cristae to periphery and to each other. Mitochondrial diameter: 1.5 mm; section thickness: 0.5 mm; conventional glutaraldehyde fixation, osmication, plastic embedding, and poststaining with lead/uranyl. (Figure modified from Mannella *et al.* 1998.) Fig. 2. Mitochondrion surrounded by endoplasmic reticulum in a rat liver cell. Model shows contours of endoplasmic reticulum (red±violet-filled), and mitochondrial outer membrane (red), inner peripheral membrane (blue), and cristae (green-filled), as well as matrix granules (yellow). Mitochondrial diameter: 1.0 mm; section thickness: 0.25 mm; tissue was perfusion fixed with glutaraldehyde followed by conventional osmication and plastic embedding, and poststained with lead/uranyl. (Figure modified from Mannella *et al.* 1997b.) Fig. 3. Group of 34 mitochondria (brown) in the vicinity of a multilayered stack of endoplasmic reticulum membranes (blue/green) in a rat liver cell. Field: 2.5 3 3 mm; section thickness: 1.5 mm; tissue preserved by high-pressure freezing and cryosubstitution prior to plastic embedding and staining (Monaghan *et al.*, 1998). Fig. 4. Cluster of 14 mitochondria in close apposition with endoplasmic reticulum in the field of Fig. 3 (tilted view).

ery of the inner membrane (Fig. 1) (Mannella *et al.*, 1994, 1997a; Perkins *et al.*, 1997). This design concept is not entirely new, having been inferred earlier, to some extent, from serial-section TEM analysis (Daems and Wisse, 1962) and scanning electron microscopy (Lea and Hollenberg, 1989). The novel (and perhaps more compelling) aspect of tomography is that it provides a full range of 3-D information throughout the reconstructed volume at a resolution approaching 5 nm. Even more faithful determination of the geometry of mitochondrial compartments is being provided by advances in cryoelectron tomography of frozen-hydrated mitochondria, unexposed to chemical fixatives or stains (*e.g.*, Mannella *et al.*, 1999).

The restricted openings of cristae into the peripheral (intermembrane) space raises the possibility that lateral gradients of ions, molecules, and macromolecules may occur between mitochondrial compartments previously assumed to be in diffusional equilibrium. Such microcompartmentation could have important functional consequences, *e.g.*, by influencing the magnitude of local pH gradients generated during chemiosmosis, the internal diffusion of adenine nucleotides, and even the release of cytochrome *c* during apoptosis (Westerhoff *et al.*, 1988; Mannella *et al.*, 1997a, 1998). It is unlikely that a complete description of the regulation of mitochondrial energy metabolism will be possible without taking into account the spatial organization of mitochondrial compartments. Combined with new modeling tools (*e.g.*, Virtual Cell; Schaff *et al.*, 1997), tomography may help to provide quantitative descriptions of such phenomena and formulation of experimentally verifiable hypotheses.

MITOCHONDRIAL INTERACTIONS WITH ENDOPLASMIC RETICULUM

Until recently, the view was widely held that mitochondria did not normally take part in regulation of cellular Ca^{2+} concentration, since mitochondrial Ca^{2+} transporters have K_m's orders of magnitude larger than mean physiological Ca^{2+} levels. The consensus has changed in recent years for a number of reasons, among them the realization that mitochondria are often in close proximity to the main intracellular Ca^{2+} storage compartments, *i.e.*, the endoplasmic and sarcoplasmic reticula (*e.g.*, Sharma *et al.*, 2000). A striking example is the recent demonstration by confocal microscopy of interwoven endoplasmic and mitochondrial reticula in HeLa cells (Rizzuto *et al.*, 1998). This cellular organization suggests that Ca^{2+} release from internal stores may create transient microdomains of high [Ca^{2+}] in the immediate vicinity of mitochondria, with subsequent energy-dependent Ca^{2+} uptake by the organelles. The nature and implications of mitochondrial involvement in cellular Ca^{2+} signaling are the subject of several papers in this issue (Hajnoczky *et al.*, 2000; Huser *et al.*, 2000; Sharma *et al.*, 2000; Smaili *et al.*, 2000; Simpson, 2000).

Conventional thin-section TEM images of a wide variety of cells often show mitochondria to be bordered by membranes of the endoplasmic reticulum (ER). For example, Fig. 2 is a tomographic reconstruction of a single mitochondrion almost totally encircled by ER in a rat liver cell. Not so obvious in routine TEM images is the fact that mitochondria may occur in clusters in rat hepatocytes and that these clusters may have special orientation with respect to the ER. Figure 3 is an axial view of a tomographic reconstruction of a group of 34 mitochondria in a 1.5-μm-thick section of a rat liver cell. The mitochondria are elongated tubes (not ellipsoids) aligned roughly in parallel that surround or are embedded in a multilamellar stack of endoplasmic reticulum. This remarkable subcellular organization raises at least two obvious questions: what is organizing it (the cytoskeleton?) and does it have a function? Within the large group of mitochondria there is a subset of 14, each of which is in close proximity (10–20 nm) to a membrane of ER (Fig. 4). This mitochondrial/ER cluster is about 2 μm across, which is the same size as the so-called elementary units of IP_3-induced Ca^{2+} release in rat lymphocytes (Horne and Meyer, 1997). Whether, in fact, Ca^{2+} transients are initiated or propagated by such clusters of mitochondria and ER remains to be determined (see Simpson *et al.*, 1997).

CONCLUSIONS

Efforts to understand mitochondrial structure in the context of their bioenergetic function helped to shape the field of cell biology in the middle of the twentieth century (Rasmussen, 1995). Fifty years later, the questions being asked about mitochondria reflect a growing awareness of their integration into the biology of the cell. The only certainty is that our views of mitochondria will continue to change as these questions are answered and new ones take their place.

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