Advanced Electron Microscopy in Biology

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The major task of biological electron microscopy (EM) is to provide structural information with which one may correlate structure and function. Since the first biological EM observations made at the early 40's of the last century, EM has remained the only method which can provide information about the complex hierarchical architecture of biological matter from the cellular and sub-cellular levels to the level of macromolecular assemblies, within the context of the living organism rather than in isolated systems.

Towards the 90's of last century it seemed that the potential of EM technique was exhausted, yet, in recent years due to the synergistic advances in different fields, the potential of EM to elucidate structural information, which was heretofore obscured, has greatly increased. Advanced cryo-immobilization techniques, such as cryo-plunging and high-pressure freezing, enable the preservation of biological specimens in a state which better reflects their native structure and better preserves immunological determinants (figure 1 A-C). Hence, comparing to conventional preparation methods, more structural information is retained in the sample, artifacts are greatly reduced and the chance of targeting proteins by immuno-localization techniques is much improved (figure 1A). The 3D structural elucidation of intact cells and organelles (figure 1C) by 3D transmission electron microscopy (TEM), as well as of isolated large macromolecular complexes such as ribosomes, protein-DNA complexes (figure 1D) or viruses, became feasible due to the availability of higher voltage transmission electron microscopes as well as advances in cryo-TEM, low dose techniques, computerized control of the TEM, combined with digital image capturing and algorithms for image analysis.

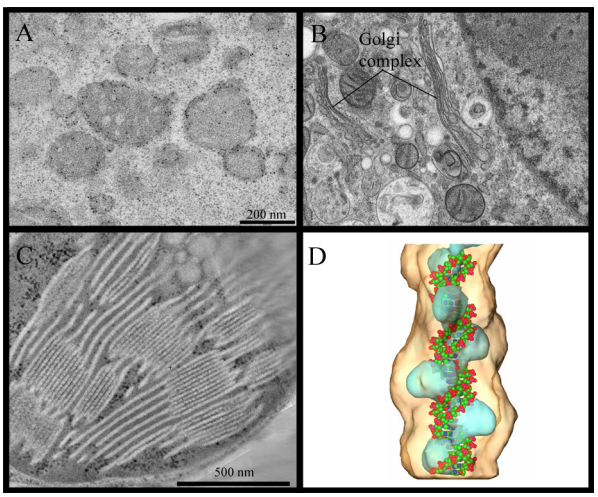

Fig. 1 *A - Mitochondria of high pressure frozen COS-7 cells. A GFP-fusion protein is localized to the membrane of the mitochondria (black dots), utilizing anti-GFP antibodies conjugated to colloidal gold (Dov Zipori's group, Dept. of Molecular Cell Biology). B - Golgi apparatus of high-pressure frozen Hela cells. The excellent preservation quality and the fast cryo-immobilization are optimal conditions for the investigation of a dynamic organell such as the Golgi complex (Sima Lev's group, Dept. of Neurobiology). C - A section through a reconstructed tomographic volume of a lettuce chloroplast. The reconstruction provides the basis for a new model of thylakoid architecture (Ziv Reich's group, Dept. of Biological Chemistry). D - Fiber consisting of DNA coated with the bacterial spore protein SspC. In the spore, the coated chromosome is protected from damage for very long periods of time. The image shows the structure of the coated filament in yellow, with high density regions in blue, and the suggested placement of DNA is shown. The fiber structure was solved by hybrid single-particle helical reconstruction approach from images obtained by cryo-EM (Avi Minsky's group, Dept. of Organic Chemistry).*

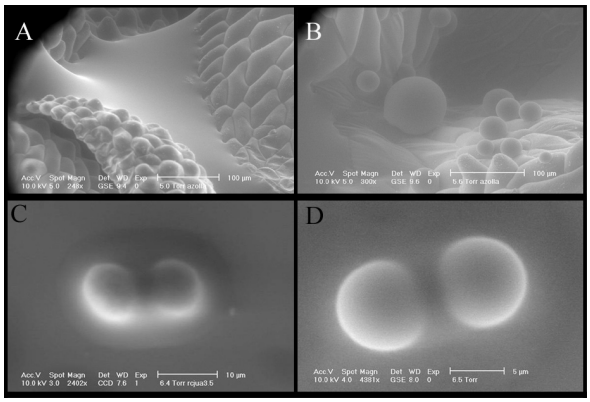

Fig. 2 *A - Azolla leaf with a drop of water, observed in the environmental SEM. B - The same leaf after partial drying. Drops of water can be observed (Eugenia Klein, EM Unit). C - rcj cells - the wet hyaluronan layer around the cells, stained with 2% uranyl acetate, is seen as a hallow in the environmental SEM. D - Polystyrene beads coated with hyaluronan are used as a model for the native rcj cells and show a similar hallow of stained hyaluronan (the groups of Lia Addadi and Benjamin Geiger, Deptartments of Structural Biology and Molecular Cell Biology).*

The introduction of field emission guns and immersion objective lenses lead to the production of a new generation of scanning electron microscopes (SEM) with intense beams, which have cross sections in the order of nanometers. These advances permit the study of small structures on biological surfaces, such as nuclear pore complexes of cells, bone-forming assemblies of crystals and collagens etc., which could not be properly observed before, thus shedding new light on them.

The environmental scanning electron microscope (ESEM) permits control of the humidity and temperature in the specimen chamber, thus allowing to observe, photograph and measure fully hydrated samples, at the dew point (figure 2 A-B). Under these conditions, the artifacts produced during the drying of the specimens are avoided, and various kinds of cell secretions, which are otherwise washed out, can be preserved. Thus, the observation of hydrated hyaluronan gel (figure 2 C-D), which is playing an important role in cell adhesion, helped to confirm the proposed model for the mechanism of cell adhesion. We could also study the formation and stabilization mode of transient phases, such as amorphous calcium-carbonate, which is a precursor in the formation of some biominerals.

The integration of novel structural information obtained by advance EM techniques, may not only be complementary with current biochemical, molecular biological and genetic techniques, but may also set the foundation for whole new research avenues in biology.