
Preface

Cell biology is the science of correlating cell structure and function. The electron microscopist obtains high-resolution pictures of the intricate structures found in cells. Electron tomography and serial sections can be used to reconstruct the three-dimensional structure of the cell and its organelles. Pictures are worth a thousand words, but they are unable to provide information regarding the function of the intricate structures found in cells and their macromolecular composition.

The biochemist and molecular biologist determine the functions of the molecules, macromolecular complexes, and organelles found within cells. They isolate individual cellular constituents and reconstruct vital cellular processes. These *in vitro* experiments provide a detailed understanding of cellular function. Organelle isolation provides a method to place macromolecular functions within a structural context. Understanding structure–function relationships through organelle isolation has restricted utility because organelles cannot be isolated from every organism, not every organelle can be isolated free of contamination by other organelles, suborganellar compartments often cannot be purified for biochemical characterization, and, when a protein is recovered in multiple organelles, it is often difficult to distinguish true localization from contamination artifacts.

Immunoelectron microscopy is the technique that bridges the information gap between biochemistry, molecular biology, and ultrastructural studies placing macromolecular functions within a cellular context. Immunoelectron microscopy can be used on virtually every unicellular and multicellular organism. The only requirements are suitable fixation protocols and the availability of an antibody to the molecule whose structural location is to be determined. Structure–function relationships can be determined even when it is impossible to purify the organelle or suborganellar compartment containing the macromolecules being studied. Most importantly, immunoelectron microscopy is a totally objective procedure that is not dependent on conjectures as to where the protein is localized and thus which organelles to isolate for biochemical studies.

Two examples from our own work demonstrate how immunoelectron microscopy provides unexpected insights into structure–function relationships. Immunoelectron microscopy first demonstrated that the *Euglena* light harvesting chlorophyll a/b binding protein of photosystem II (LHCPII) was found in the Golgi apparatus prior to its presence in the chloroplast. This finding was the impetus for detailed biochemical studies that elucidated a new mechanism for chloroplast protein import: transport from the ER to the Golgi apparatus to the chloroplast. Immunoelectron microscopy identified the pyrenoid as the site of the enzyme ribulose 1–5 biphosphate carboxylase/oxygenase (RUBISCO) showing that the enzyme moves from the stroma to the pyrenoid at cell cycle phases when enzyme activity is high while the pyrenoid disappears and RUBISCO redistributes back to the stroma at cell cycle phases when enzyme activity is low. Biochemical studies identified the activity changes but organelle fractionation experiments never identified this change in suborganellar localization because pyrenoids could not be isolated. At all cell cycle stages, the enzyme was recovered in the soluble chloroplast fraction.

The successful application of immunoelectron microscopy requires combining the tools of the molecular biologist with those of the microscopist. From the molecular biology toolbox, this volume will present methods for antigen production by protein expression in bacterial cells and by expression of epitope tagged proteins in plant and animal cells. Methods for production of anti-peptide, monoclonal, and polyclonal antibodies will be presented. From the microscopy toolbox, this volume will present methods for cryoultramicrotomy and rapid freeze-replacement fixation which have the advantage of retaining protein antigenicity at the expense of ultrastructural integrity as well as chemical fixation methods that maintain structural integrity while sacrificing protein antigenicity. Plants and algae contain cell walls, vacuoles, and other structures which present barriers to antibody penetration and complicate fixation. Due to these problems, separate chapters will discuss fixation and immunolabeling protocols for animals, plants, and algae. Pre- and post-embedding immunogold labeling protocols will be presented. Pre-embedding methods perform immunogold labeling before ultrathin sections are prepared from resin-embedded samples resulting in greater sensitivity and better microstructure preservation. Post-embedding methods perform immunolabeling after ultrathin sections are prepared from resin-embedded samples resulting in decreased antigenicity. The detailed methods and notes will facilitate choosing the best method for the antibody and biological material to be studied. Finally, methods will be presented for immunogold labeling of two antigens for protein colocalization studies, for three-dimensional reconstruction of intracellular antigen distribution, for immunogold labeling of DNA, and for immunogold scanning electron microscopy. It is our hope that the toolbox created by this volume will facilitate an increased understanding of structure–function relationships.

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