2

Electron Tomography of Frozen-hydrated Sections of Cells and Tissues

Michael Marko, Chyong-Ere Hsieh and Carmen A. Mannella

Michael Marko, Chyong-Ere Hsieh and Carmen Mannella • Resource for Visualization of Biological Complexity, Wadsworth Center, Empire State Plaza, PO Box 509, Albany, NY 12201, USA

1. INTRODUCTION

1.1. Cryoelectron Tomography

The technique of cryoelectron tomography of frozen-hydrated biological specimens is opening a new window on cellular structure and organization. This imaging method provides full 3D structural information at much higher resolution (typically 5–10 nm) than is attainable by light microscopy, and can be applied to cells and organelles that are maintained in a state that is as close to native as can be achieved currently in electron microscopy. Not only can cryoelectron tomography be used to visualize directly extended cellular structures, such as membranes and cytoskeleton, but it can also provide 3D maps of the location, orientation and, perhaps, the conformation of large macromolecular complexes, the cell's 'molecular machinery'. This information complements that coming from single-particle cryoelectron microscopy (Frank *et al*., 1996, 2006) and X-ray crystallography, about the subnanometer structure of the same molecular assemblies after isolation. As with studies using single-particle cryoelectron microscopy, specimens smaller than 1 μm in size can be prepared for cryoelectron tomography by plunge-freezing (Dubochet *et al*., 1988). Cells or organelles can be rapidly frozen directly on an electron microscope grid in thin layers of glass-like, amorphous ice, without the formation of ice crystals that would otherwise disrupt fine structure (Kellenberger, 1987). Specimens are imaged directly, without chemical fixation, dehydration or staining with heavy metals. Cryoelectron tomography is made possible by electron microscope automation, which allows the recording of image series from sequentially tilted specimens with a sufficiently low cumulative electron dose, such that damage to high-resolution fine structure is avoided (Dierksen *et al*., 1993, 1995; Koster *et al*., 1997; Mastronarde, 2005; Rath *et al*., 1997; Chapter 4 of this volume).

Examples of specimens studied by cryotomography since the year 2000 include (i) isolated organelles such as mitochondria (Mannella, 2005; Mannella *et al*., 2001; Nicastro *et al*., 2000), axonemes (McEwen *et al*., 2002)