From the nuclear periphery to cell adhesion by cryo-electron tomography

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Visualization of the three-dimensional (3-D) organization of a eukaryotic cell, with its dynamic organelles, cytoskeletal structures, and distinct protein complexes in their native context, requires a non-invasive imaging technique of high resolution combined with a method of arresting cellular elements in their momentary state of function.

Vitrification of cells ensures close-to-life preservation of the molecular architecture of actin networks and organelles. With the advent of automated electron tomography it has becomes possible to obtain tomographic data sets of frozen hydrated specimen. By electron tomography 3-D information from large pleomorphous structures, as cell organelles or whole cells can be retrieved with 'molecular resolution'. At that resolution it becomes possible to detect and identify specific macromolecular complexes on the basis of their structural signature.

Here we employed cryo-electron tomography to eukaryotic cells grown directly on an EM grid. We have recorded several single axes tilt series of intact ice-embedded cells using a 300kV TEM operated in zero-loss mode of the energy filter. After alignment of the projection images, 3-D reconstructions were calculated by using weighted backprojection. We have analyzed unlabeled cellular structures within intact eukaryotic cells by cryo-electron tomography. I will discuss our analysis of the nuclear periphery and the cell adhesion machinery.

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