Three dimensional analysis of the Golgi apparatus: Reorganizations in response to ATP-depletion and replenishment

E.Wollmann, M. Vetterlein, A. Ellinger, J. Neumüller, and M. Pavelka

Department of Cell Biology and Ultrastructure Research, Center for Anatomy and Cell Biology, Medical University Vienna, Schwarzspanierstrasse 17, A-1090, Vienna, Austria.

eva.wollmann@gmail.com

The Golgi apparatus in HepG2 hepatoma cells is a very sensitive and reactive organelle. It rapidly responds to changes of its environment. Depletion of the cellular adenosintriphosphate (ATP) pool leads to massive but reversible Golgi apparatus alterations [1]. By examination of thin sections in the electron microscope, a dissociation of the Golgi apparatus stacks is visible; it appears as if the stacks of cisternae would be fragmented into vesicle-like small pieces. Since examination of thin sections provides only a very limited insight into the interior of cells, we used for further exploration electron tomography from semithin sections, and studied the 3D-architecture of the Golgi apparatus in response to modulations of the cellular ATP-concentration. The results revealed that in response to ATPdepletion Golgi cisternae are not fragmented but transformed in reticulo-glomerular organelles; Golgi stacks are rapidly reorganized during ATP-replenishment.

Human HepG2 hepatoma cells were grown on sapphire disks and on glass coverslips. For ATP-depletion, the cells were incubated in glucose-pyruvate-free Dulbecco's medium containing 50mM 2-deoxy-D-glucose (DOG medium) for different times up to 60min. For ATP-replenishment, incubation was performed in a glucose and pyruvate free medium containing 1M D-glucose (GLUC medium) for 15-45min. Both chemical fixation and high pressure freezing (HPF) were employed. Conventional chemical fixation was performed using 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer, and 1% veronal acetatebuffered OsO4 for postfixation. High pressure freezing was carried out in a BAL-Tec HPM 010. Following freeze substitution, carried out in acetone, with 1% OsO4 and 0.4% uranyl acetate, in a Leica AFS system (Leica Microsystems, Vienna, Austria), the cells were embedded in Epon. After a primary examination of ultrathin sections, semithin sections of 200-300nm were prepared for electron tomography. Single and dual axis tomography was performed in a 200kV transmission electron microscope (Tecnai-20, FEI), using a high tilt rotation holder (Gatan) under control of the Xplore3D software (FEI) for acquisition of tilt series; for reconstructions, the Inspect 3D software (FEI), as well as the "tom_toolbox" software, kindly provided by W.Baumeister, J.Plitzko, MPIB, Martinsried, were used; 3Dmodels were constructed with the help of Amira 4.1 software (Mercury Computer Systems, Merignac Cedex, France).

The 3D-analyses showed that in response to ATP-depletion Golgi cisternae detach from each other, loose their regular organization, and form branches and tubules; later, reticulo-glomerular organelles are built. Replenishment of the ATP-pool leads to a restoration of the regular Golgi apparatus organization, and Golgi apparatus stacks are reformed. Comparable results were obtained with chemically fixed and high pressure frozen cells. An early stage of restoration is demonstrated in figures 1-4, which show different aspects of a 3D-model of a transformed Golgi apparatus during its reorganization. The organelle already shows a well organized stack of 3 cisternae in parallel orientation (asterisks) surrounded by

more reticular regions. Particularly prominent is a group of interconnected columns (circles, and curved arrows; Figs.2, 3) considered as possibly being involved in the new formation of Golgi cisternae.

1.   M. del Valle et al. J.Cell Sci. **112** (1999) p4017.
2.   The authors gratefully acknowledge the valuable help of Mrs. Beatrix Mallinger, Mrs. Barbara Kornprat, Mrs. Elfriede Scherzer, Mr. Peter Auinger, Mr. Ulrich Kaindl, and Mr.Matthias Eibauer (MPIB Martinsried).
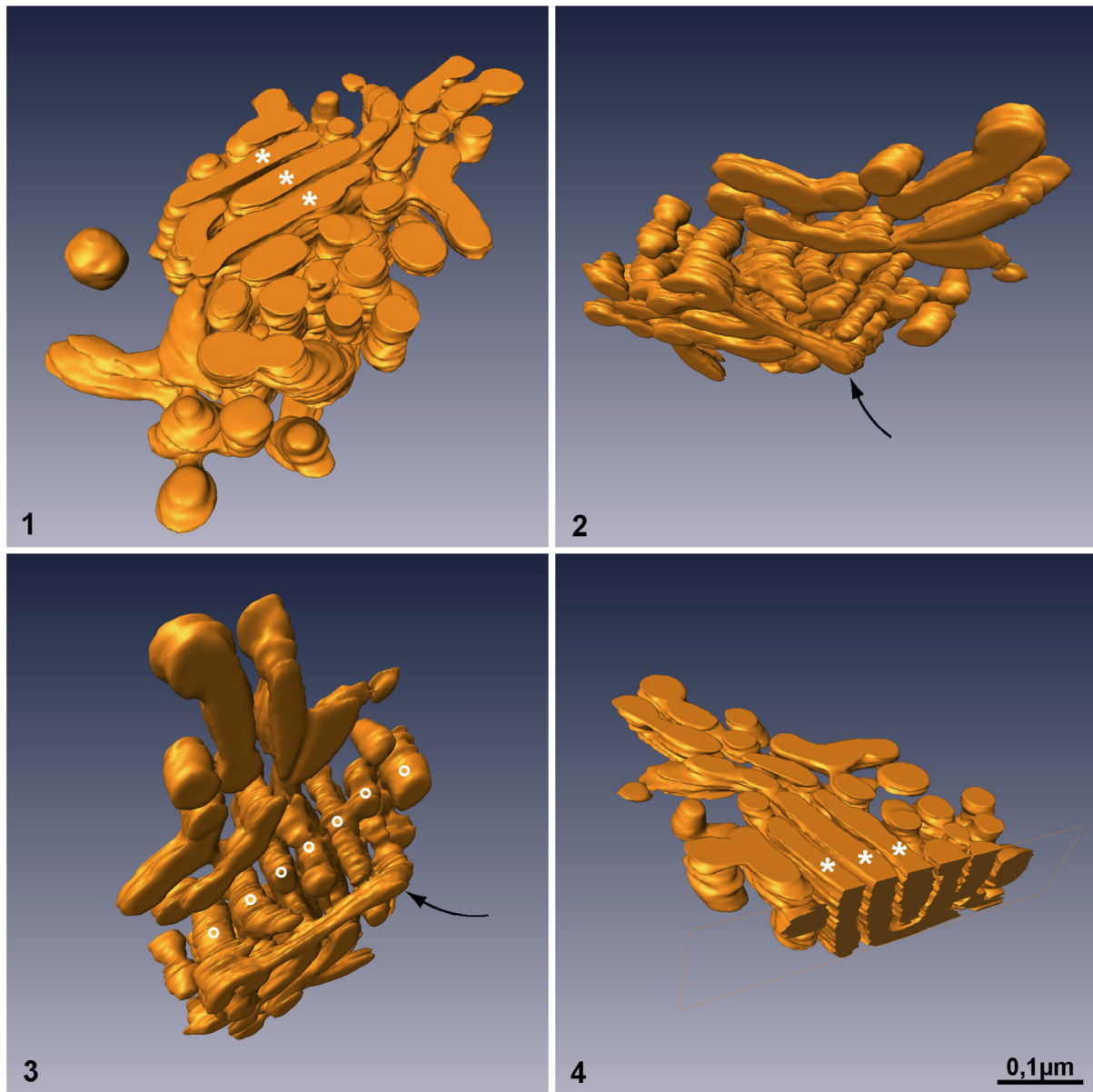
Figs.1-4. Different aspects of a 3D-model, which shows a Golgi apparatus in reorganization during ATP replenishment.