Membrane dynamics during formation of the endocytic TGN, visualized by 3D-electron tomography

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Endocytosis of wheat germ agglutinin (WGA) in human HepG2 hepatoma cells leads to reorganizations of the Golgi apparatus at its *trans* side, resulting in the formation of an extended endocytic *trans* Golgi network (endocytic TGN, 1,2). It is not known up to now, how the endocytic TGN is formed, and how it is related to the TGN, which is well known as playing an important role in the secretory system (for review 3). Here, we show the results of 3D-electron microscopic analyses of the endocytic TGN during its formation at early and later times after onset of WGA-endocytosis.

The cell system used (human HepG2 hepatoma cells) is well established for studies of WGA-endocytosis (1,2),. The cells were grown on glass coverslips, or on sapphire disks. For WGA-endocytosis, the cells were incubated in media containing 33µg/100µl peroxidase-labeled WGA, and were studied at early and late time points (2, 5, 10, 15, 30, 60min) after onset of endocytosis. For electron microscopic examination, the peroxidase activity was visualized by means of diaminobenzidine oxidation performed either prior to or after fixation. Cells grown on glass coverslips were chemically fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer; cells grown on sapphire disks were rapidly immobilized by high pressure freezing (HPF). The latter was carried out in a BAL-Tec HPM 010. Following freeze substitution in acetone with 1% OsO_4 and 0.4% uranyl acetate in a Leica AFS system, the cells were embedded in Epon. After electron microscopic examination of ultrathin sections, 200-300nm sections 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. The Inspect 3D software (FEI), as well as the "tom_toolbox" software, kindly provided by W.Baumeister, J.Plitzko, MPIB, Martinsried, were used for reconstructions; 3D-models were made with the help of Amira 4.1 software (Mercury Computer Systems, Merignac Cedex, France).

Following internalization, WGA is rapidly transported to a cell region close to the Golgi apparatus. In the early stage I after 10-15min of WGA-internalization, WGA-containing endosomes accumulate at the *trans* Golgi side. In stage II after 15-30min of WGA-internalization, endocytic *trans* Golgi networks appear, and increase with increasing duration of WGA-uptake. The 3D-analyses performed in stages I and II revealed that at early times, the WGA-containing endosomal compartments mainly exist as individual organelles, though frequently contacting each other and exhibiting interconnections by fine rod-like structures. At later times, such patterns are still visible (e.g. central area in Fig.1a) but mainly are surrounded by extended interconnected networks. The figure shows 3D-models of endocytic *trans* Golgi networks. They consist of interconnected globular, cylindrical, and columnar elements (white curved arrows in Fig.1a), which in shapes and dimensions resemble earlier individual endosomes. Interconnections between endocytic compartments (coloured in red), and non-endocytic compartments (coloured in yellow) are evident. Contacts are formed by

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fine, rod-like structures (arrows in 1a, b), which can be observed in all specimens irrespective of the preparation technique used. In the high pressure frozen cells however, a much more differentiated analysis was possible, and different shapes of these connecting structures could be discriminated (Fig.1b).
The results of this work indicate that early WGA-containing endosomes accumulated at the *trans* Golgi side in early phases of endocytosis may be precursors of compartments of the endocytic TGN, and further suggest that non-endocytic compartments contribute to the formation of the endocytic TGN as well. It is tempting to consider that the fine rod-like connecting structures observed during all phases of TGN-formation have a role in the *trans* Golgi membrane dynamics, possibly corresponding to tethering molecules.

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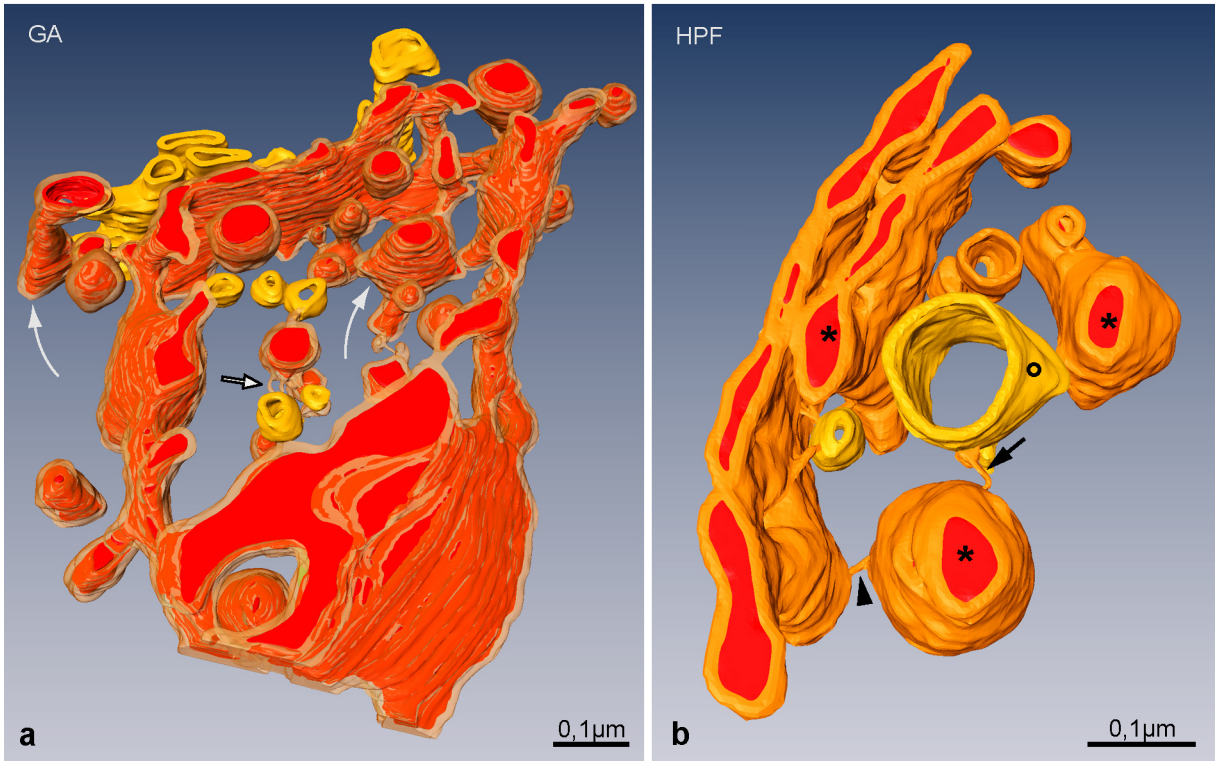

Fig.1a,b Models of the endocytic TGN after 30min WGA-endocytosis. a. Chemical fixation in glutaraldehyde (GA). b. High pressure freezing (HPF). The lumina of WGA-containing elements are coloured in red, WGA-negative organelles appear in yellow. Curved arrows in a) indicate columnar and cylindrical TGN elements. Arrows point to differently shaped interconnecting structures; *endocytic, °non-endocytic compartments

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