

# Correlative 3D-electron microscopic and biochemical analyses of cellular reorganizations in response to 2-deoxy-D-glucose treatment

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To visualize the dynamic reorganizations of cellular compartments is an ongoing challenge in cell biology. Our attention is particularly drawn to the highly complex and dynamic fine structure of the Golgi apparatus, which is a central organelle of the secretory and endocytic systems and continuously traversed by a flow of membranes and contents; both secretory and endocytic traffic and pathologic conditions, such as cellular stress, lead to rapid reorganizations of its complex architecture [1-3]. The regular structure of the Golgi apparatus in mammalian cells is characterized by a highly ordered stacked organization of flat *cis*, medial and *trans* cisternae, frequently accompanied by *trans*-Golgi networks (TGN) and *trans*-Golgi endoplasmic reticulum. This organization changes function-dependently, for example during endocytosis [2] or in response to environmental changes, such as reduction of the cells' ATP-levels [3, 4]. Our work in this study is focused on the reorganizations of the Golgi apparatus in response to treatment of the cells with 2-deoxy-D-glucose, which is a non-hydrolyzable analogue of D-glucose [5]. Treatment of cells that are cultured in a glucose- and pyruvate-free medium containing 50 mM 2-deoxy-D-glucose leads to a considerable but non-lethal reduction of the cellular ATP-contents [3, 4]; furthermore, because of its similarity to mannose, 2-deoxy-D-glucose interferes with early steps of N-linked glycosylation, induces an endoplasmic reticulum-stress, and activates autophagy; it is under clinical evaluation for targeting cancer cells [5].

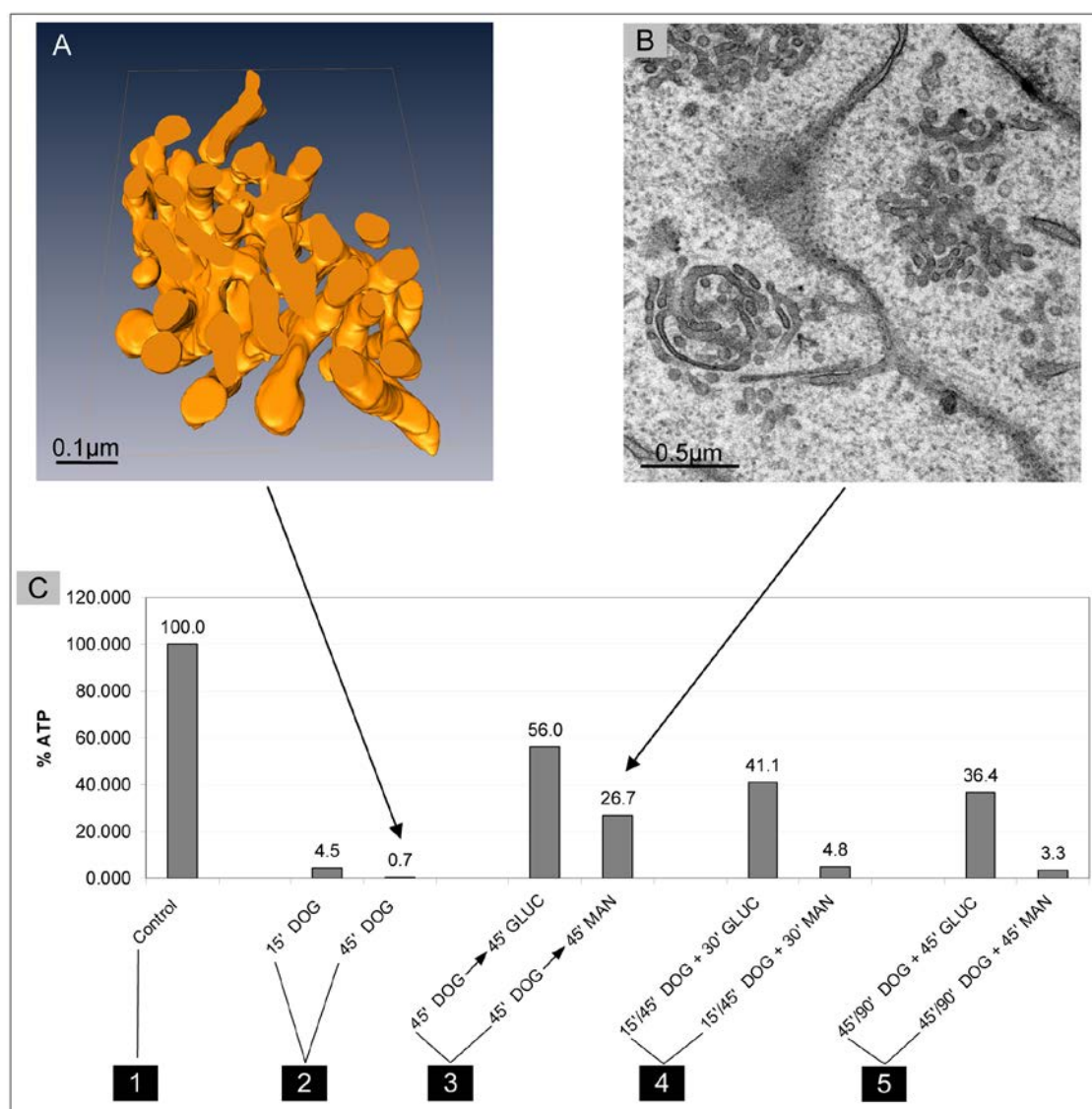
In previous studies using cultured hepatoma cells, we have shown that replacement of glucose in the culture media by 2-deoxy-D-glucose induces reorganizations of the Golgi apparatus and the appearance of tubulo-glomerular bodies and networks [3]. The present work focuses on the question, whether the changes of the cytoplasmic ATP-levels correlate with the Golgi apparatus reorganizations and by which treatments the reorganizations can be reversed. We used either chemically fixed or high pressure-frozen cultured HepG2 hepatoma cells embedded in Epon. Ultrastructural analyses and electron tomography were done using a 200kV transmission electron microscope (Tecnai20, FEI). ATP measurements were performed with the ATPlite Luminescence ATP Detection Assay System from Perkin Elmer.

Treatment of the cell cultures with 50 mM 2-deoxy-D-glucose in a glucose- and pyruvate-free medium (DOG) resulted in a rapid decrease of the cells' ATP levels to less than 10% of the original value within 10-15min (Fig 1C 1,2). The low ATP-contents remained during the entire time of treatment with the DOG medium up to 180 min and were reversed rapidly after removal of 2-deoxy-D-glucose and glucose-replenishment by incubation in a glucose-enriched medium (GLUC, Fig.1C3). Being in accordance with the rapid ATP reduction under the influence of 2-deoxy-D-glucose, initial changes of the Golgi apparatus were apparent as soon as 10 min after start of the experiment; three-dimensional analyses of the Golgi apparatus at this early time showed the appearance of reticular regions within the stacks of cisternae, which became more prominent after 45 and 60 min of treatment. At this time, regular Golgi stacks were rare, whereas tubulo-glomerular Golgi-bodies and networks dominated. In Fig. 1A, a 3D-model of a glomerular Golgi body apparent after 45 min treatment with 2-deoxy-D-glucose is shown. All the changes were reversed concomitantly with glucose- and ATP-replenishment. Both the formation of tubulo-glomerular bodies, and the reconstitution of regular Golgi apparatus stacks could well be correlated with ATP-depletion and replenishment. Since 2-deoxy-D-glucose due to its similarity to mannose interferes with early steps of N-linked glycosylation [5], the findings of the combined studies with mannose are particularly interesting and significant. Preliminary results indicate that mannose fails to replenish the cells'

ATP-contents (Fig. 1C3) and to reconstitute the regular Golgi apparatus stacks (Fig.1B). Addition of mannose to the DOG-medium and subsequent combined incubation did neither influence the reduced ATP contents (Fig. 1C4-5), nor reverse the Golgi apparatus reorganizations.

## References

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**Figure 1.** The fine structures of Golgi apparatus reorganizations in response to 2-deoxy-D-glucose treatments are shown in correlation with the results of the ATP-measurements. A: 3D model, B: thin section, C: diagram. DOG – 2-deoxy-D-glucose, GLUC – D-glucose; MAN - mannose