Subcellular Processes in Plants and Animal Cells

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Controlled disassembly and new formation of Golgi apparatus stacks in cultured hepatoma cells

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The non-metabolizable glucose-analogue 2-deoxy-D-glucose (2DG) has become an important target of interest during the last decade due to its medical and basic-biological effects. 2DG is under clinical evaluation for cancer therapy [1] and other diseases like epilepsy, Morbus Alzheimer and malignant astrocytoma; on the other hand, it is a highly interesting tool for studying the dynamic organization of the Golgi apparatus [2, 3].

Previous research in our laboratory on the effect of 2DG on human hepatoma cells (HepG2) revealed that replacement of D-glucose by 2DG (50mM) leads to transformations of the well-structured Golgi cisternae in tubulo-glomerular bodies and networks [3]. Moreover, the intracellular ATP-content decreases within 10min to 15-30% of the control (Fig.1A and 1B). Experiments mimicking a simple starvation process by abolishing D-glucose from the growth medium do not gain the same results (Fig.1C). HepG2 cells grown in glucose- and pyruvate-free (GPF) medium showed moderately reduced ATP-levels (90% ATP after 3 hours) and no alterations of the Golgi apparatus structure (Fig. 2C).

The cultured HepG2 cells rapidly recover from ATP-depletion and rebuild their well organized Golgi apparatus stacks after removal of 2DG and replenishment of D-glucose (50mM) in the growth medium (GLUC). The ATP-levels arise continuously (Fig.1D) and stacks of cisternae reappear. Detailed correlative biochemical and ultrastructural analyses revealed that both the disassembly of the Golgi apparatus and new formation of the well structured stacks of cisternae correlate well with the changes of the ATP-levels (Fig.2A-E) and 2DG-treatment can be used for exploration of Golgi apparatus dynamics under controlled conditions.

Experiments employing an ATP-replenishment protocol without glucose proved to be particularly helpful. When cells are grown in GPF medium after removal of 2DG, the ATP-level of the cells increases only slowly (Fig.1E). By comparison of the 2 protocols (Fig.1D and 1E), it becomes apparent that a total recovery of the cells' ATP-content in D-glucose can be observed within 2-3 hours, while after even 4 hours incubation in GPF medium the ATP-level remains clearly under 100%. More precisely, cells grown in medium containing D-glucose reach 80% ATP-amount of untreated controls within 60min (Fig.1D), whereas it takes cells in GPF medium four times longer. Morphologically, a similar delay is evident concerning the new formation of the Golgi apparatus stacks. Regularly organized stacks are detectable at 120min after 2DG elimination, while these can already be observed within 30-45min during D-glucose replenishment. The delayed reassembly of the Golgi apparatus allows studying the subsequent steps of reformation much more precisely and helps to define intermediate stages during Golgi apparatus new formation (Fig.2E).

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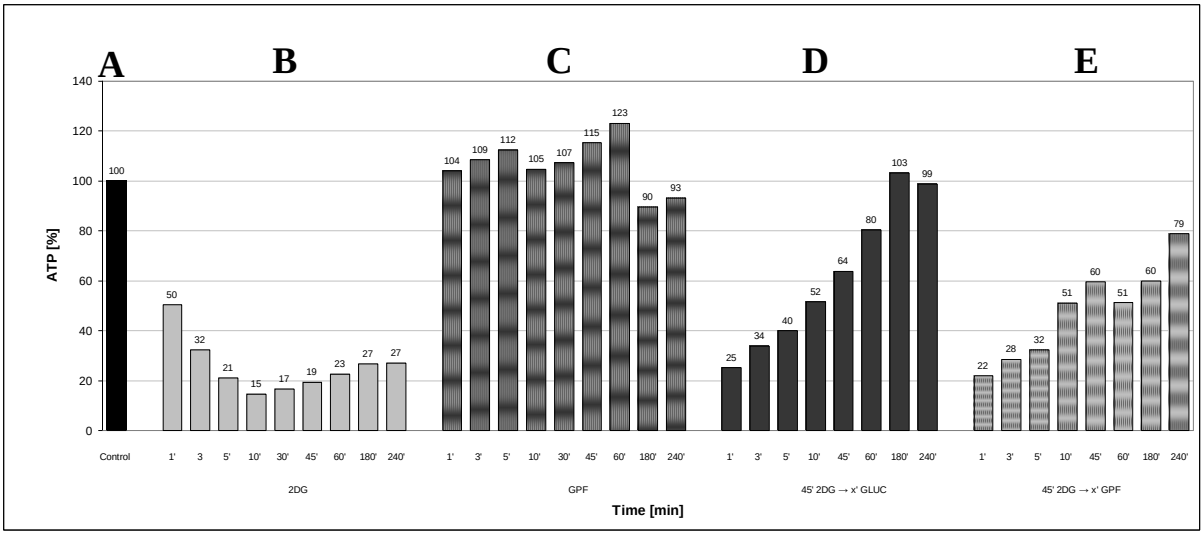

Figure 1. Cellular ATP-levels in response to the various treatments.

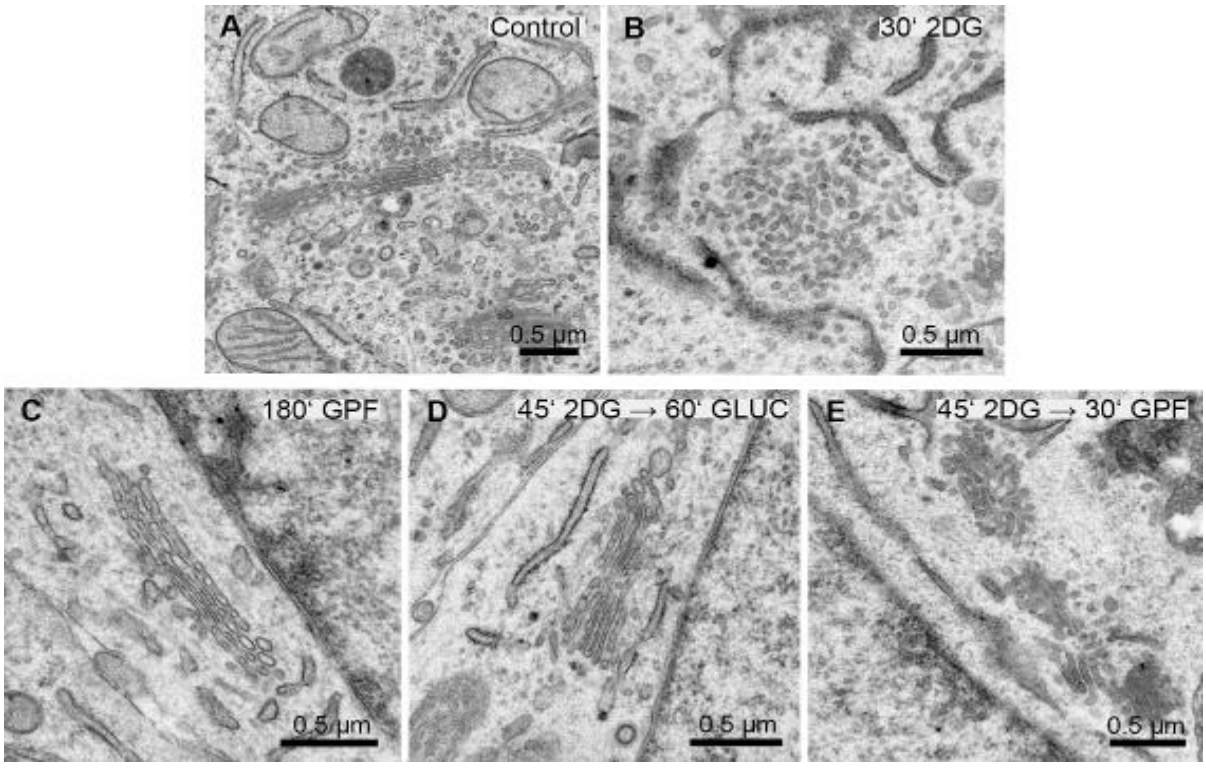

Figure 2. Golgi apparatus morphologies correlating with the ATP-contents shown in Fig.1