appropriate signal peptides. These N-terminal domains changed the subcellular distribution of the resulting protein chimeras, targeting them to specific cell membranes [2]. Our *in vivo* studies have shown that functional complementation of native V-ATPase is better achieved by a plant H^+-PPase directed to endomembranes, and that only a plasma membrane-targeted archaeal Na^+-PPase confers enhanced sodium tolerance to a hypersensitive Ena1-4 knockout strain.

We determined the cellular consequences of a deficiency in yeast Ipp1p [3]. This essential sPPase shows a nucleocytosolic localization, but the biochemical significance of this scenario is not known. We engineered strains expressing this sPPase in the nucleus or in the cytosol only by merging the *IPP1* ORF to a nuclear localization signal or to a nuclear exclusion signal, respectively. Protein and activity levels were much higher/smaller when Ipp1p was excluded from/confined into the nuclear compartment, resulting in the last scenario in slower growth rates and reduced chronological life-span.

On the whole, our results indicate that subcellular localization significantly affects *in vivo* functionality of PPases, a diverse protein group that catalyzes an ancestral and essential biochemical reaction.

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21P19

Specific effects of oxidizer copper (II) ions on *Enterococcus hirae* and *Escherichia coli* cells growth and the F_oF_1 ATPase activity

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Enterococcus hirae and *Escherichia coli* grow well under anaerobic conditions at alkaline pH. It is known that various oxidizers and reducers affect bacterial growth [1]. The purpose of this study was to examine the effects of oxidizer copper (II) ions on bacterial growth. In small concentrations copper ions are required for bacterial normal activity as they are contained in redox active enzymes, such as superoxide dismutase. At the same time in high concentrations they are lethal for cells. It was previously shown in our laboratory [2, 3] that Cu^{2+} ions inhibit *E. coli* and *E. hirae* growth by increasing lag phase duration and decreasing specific growth rate. Similar effects are observed in our study with *E. hirae*. It is also revealed that ATPase activity and proton-coupled transport of these bacteria are inhibited in the presence of Cu^{2+} with or without *N,N′* -dicyclohexyl-carbodiimide (DCCD), specific inhibitor of the F_oF_1 ATPase, though stronger effects are observed with DCCD. Such effect may be resulted by action of this reagent on E_h which in turn regulates F_oF_1 or by direct effect on membrane protein thus changing its activity. Moreover such effects are specific for Cu^{2+}, as other divalent oxidizer metal cobalt (II) ions, within the same concentration range, have no effects on bacterial growth and ATPase activity. Changes in the number of accessible SH-groups are detected in membrane vesicles where in case of *E. hirae* Cu^{2+} markedly decrease while in case of *E. coli* increase the number of accessible SH-groups [4]. In both cases the addition of ATP increases the amount of SH-groups and Cu^{2+} blocks this ATP-stimulated increase of these groups. In these bacteria Cu^{2+} may have different action mechanisms due to the differences between membrane structures.

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Bioenergetic analysis of FDCP-mix cell line subpopulations

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Background

Murine non-leukemic factor – dependent cell Paterson (FDCP) – mix is normal hematopoietic cell line of progenitors, strictly dependant on IL-3 for survival and proliferation. Recently it was shown that culture of FDCP-mix enriched with cytokines induces their differentiation, revealing two functionally different cell subsets: differentiated GR-1^+ (granulocytic differentiation marker) quiescent cells and undifferentiated GR-1^- self-renewing actively cycling cells [1]. In this study our aim was to characterise the energetic profile of those subpopulations.

Methods

We measured O_2 consumption rate (OCR) and extracellular acidification rate (ECAR) using the non invasive Seahorse XF24 extracellular flux analyzer. In order to estimate mitochondrial contribution to the energetic metabolism, we have used a pharmacological profiling approach, Mito stress test, by combining the use of three mitochondrial inhibitors (oligomycin, DNP and rotenone). The ECAR response was analysed in the glycolysis stress test in order to estimate the main glycolytic cellular parameters. ATP content was determined by the bioluminescence assay, in the basal state without the inhibitors (total ATP), in the presence of the glycolytic inhibitor, iodoacetate, or mitochondrial respiration inhibitor, and antimycin.

Results

Analysis of mitochondrial activity showed that basal and the amount oxygen consumption, that is linked to ATP synthesis in the mitochondria, is higher in GR-1^+ cell subset than in GR-1^- cells (the latter was estimated as a response to olygomicin), whereas both populations reached the same maximal respiration capacity (response to uncoupling agent, DNP). Analysis of glycolysis stress test revealed that undifferentiated GR-1^- cells were more glycolytic and had higher glycolytic capacity compared to the differentiated GR-1^+