P10-031
Hypoxia/reperfusion injury evaluated in a cardiac cell model: protection by antioxidant plant extracts

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Ischemic heart disease is one of the main causes of death worldwide and its relationship with ROS has been well established.

We have implemented an i*n vitro* model of cardiac ischemia, using H9c2 cells, based on GOX/Cat activities that are described as rapidly generate a stable oxygen atmosphere of about 2%. With this enzymatic system we intend to clarify the role of ROS in hypoxia-induced cardiomyocyte cell death and evaluate the effects of promising novel antioxidant plant extracts in the prevention of ischemia/reperfusion cardiac damage.

Incubation with the enzymatic system results in cell death, depending on the ratio of GOX to Cat and on the time incubation period. For 24 h incubation we achieved more than 50% of cell death evaluated by the SRB assay. This is a much more effective result than that obtained with $CoCl_2$ incubation, as oxygen depleting agent. This hypoxia-induced cell death was characterized by Hoechst staining, which confirmed more than 40% of apoptotic cells, after 16 h incubation. Mitochondrial membrane depolarization was also affected by the enzymatic system, as accessed by TMRM fluorescence. Trolox was shown to protect H9c2 cells from GOX/Cat system-induced hypoxia, reverting the decrease in cell proliferation and the number of apoptotic nuclei. We also present evidence that these hypoxic cells are able to recovery when incubated for 24 h more, with fresh media. The effect of antioxidant plant extracts, are being evaluated as potential new agents useful in the overcoming of cardiac infarction.

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P10-033
Expression of cellobiose dehydrogenase from *Phanerochaete chrysosporium* in yeast *Saccharomyces cerevisiae* for directed evolution

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Cellobiose dehydrogenase (CDH) gene from *Phanerochaete chrysosporium* has been cloned in yeast *Saccharomyces cerevisiae* for extracellular expression. Recombinant CDH produced in yeast had lower specific activity of 0.6 U/mg of pure protein than native CDH produced in *P. chrysosporium*. Recombinant enzyme showed similar substrate specificity for cellobiose and lactose. Optimal temperature and pH stability was slightly different compared to native CDH. The molecular weight of recombinant CDH was higher than molecular weight of native CDH (90 kDa) with a broad band on SDS electrophoresis gel at 120 kDa that was result of hyperglycosylation. Results showed that CDH can be expressed in yeast *S. cerevisiae* which can be used in directed evolution experiments. CDH gene library was generated using error-prone PCR to create random mutations and obtained mutants were tested in microtiter plates for improved activity using adapted DCIP assay. Several mutants with increased activity were detected in microtiter plates.

P10-034
Molecular dissection of Mia40 functions in *Saccharomyces cerevisiae*

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The majority of mitochondrial proteins are encoded in the nucleus, synthesized in cytosol and imported into the mitochondria. Proteins targeted to the mitochondrial matrix carry an N-terminal targeting sequence which allows the translocation across the outer and the inner mitochondrial membranes. Many proteins of the mitochondrial intermembrane space (IMS) lack the presequence but contain conserved cysteine residues that are organized in so-called Cx_3C or Cx_9C motifs. In addition, IMS proteins contain special hydrophobic binding sequences named MISS regions. The import of these proteins into the IMS is mediated by the mitochondrial oxidoreductase Mia40. Mia40 possesses two characteristics which are combined within a single protein – the ability to oxidize incoming substrates via a redox-active CPC motif and a chaperone-like activity via a hydrophobic binding cleft. In this study, we addressed the possibility to separate both Mia40 functions on molecular level. Our preliminary results show that both functions have to be present within a single protein. However, different Mia40 substrates differ considerably in their dependence on the chaperone and oxidase activities of Mia40.

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P10-035
Molecular dissection of the mitochondrial protein import machinery

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Nuclear encoded mitochondrial proteins are synthesized in the cytosol and are translocated to their respective destinations within the mitochondria. The TIM23 complex mediates the translocation of precursor proteins that are targeted to the mitochondrial matrix or to the mitochondrial inner membrane. Tim17 is an integral component of the TIM23 translocase containing four transmembrane domains. The highly conserved Tim17 protein contains two conserved cysteines that are located directly adjacent to the first and second transmembrane domains facing the intermembrane space (IMS). These cysteines are oxidized at steady state under *in vivo* and *in vitro* conditions, whereas the other two cysteines are found to be in a reduced state within the transmembrane domain. The cysteines at position 10 and 77 are involved in a disulfide bond formation. The disulfide bond in Tim17 presumably has a stability rather than a regulatory function and can only be released upon treatment with unphysiologically high concentration of reductants. Severely affected import kinetics of radiolabelled Tim17 into Mia40 down regulated mitochondria indicates a role of Mia40 for the import. However, it is not clear whether the disulfide bond of Tim17 is formed directly by Mia40 or by another IMS protein that accumulates in the IMS in a Mia40-dependent manner. The implications for the role of Tim17 and its cysteine motif in regulatory and mechanistic function remain yet to be examined.

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