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Identification of a novel mitochondrial protein that is essential for peroxisomal membrane synthesis in the yeast Hansenula polymorpha

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Abstracts

cycle depend on their formation/decomposition rates ratio. Therefore, an increase in the observed concentrations of individual products, for instance, nitrotyrosine or quinone diazide forms of a particular protein, might be a consequence of both their synthesis acceleration and decomposition deceleration. Hundreds of proteins are known to undergo nitration and nitrosation *in vivo*. Their diazo forms might be likewise participants of cyclic NO-dependent conversions of these proteins. Their distribution and functional significance remain to be elucidated.

V1-011P

Glycan differences between healthy and pancreatic adenocarcinoma serum ribonuclease 1.

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Pancreatic adenocarcinoma has a poor prognosis because of its high death : incidence ratio, close to 1. Its diagnosis is still difficult due to its low symptomatology and the absence of a specific marker. One of the general features of tumour cells consists of changes in their cell surface, which can be also reflected in the glycosylation pattern of their secreted glycoproteins. Ribonuclease 1 is a glycoprotein expressed mainly by the pancreas and it is also found in serum. RNase 1 from human healthy pancreas presents a different glycosylation pattern when secreted by adenocarcinoma cell lines Capan-1 and MDA-Panc-3. One of the most significative differences is the presence of sialic acid on RNase 1 from tumour cells. In order to elucidate whether similar differences in the glycosylation pattern can be found between serum RNase 1 from healthy and pancreatic cancer patients, two-dimensional electrophoresis studies were performed. Three healthy patients sera and five pancreatic adenocarcinoma patients sera samples were pretreated by two different chromatographic methods in order to enrich RNase ratio, and analyzed by two-dimensional electrophoresis. Some differences due to changes in pI were attributed to different glycosylation patterns. In order to get a further insight in these differences, a sandwich Glycosylation Immuno-Sorbent Assay (GISA), determining the sialylation potential by Sialyl-Transferase activity, was carried out. With the aim to fully characterize the glycan structure of healthy and pancreatic adenocarcinoma serum RNase 1, glycan sequencing is in progress. The results presented show differences in glycosylation structures of serum RNase 1, which indicate a possible way for characterize pancreatic adenocarcinoma and could be useful to improve the pancreatic cancer diagnostic.

V1-012P

Yellow head virus infection induces a modulation of cytoskeletal-related proteins in hemocyte from black tiger shrimp

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Yellow head virus (YHV), a rod shaped, single-stranded RNA virus, is one of the most severe infectious agents to black tiger shrimp (*Penaeus monodon*). Rather than lymphoid organ and gills, the hemocytes were previously reported to be infecting by YHV. Light microscopy of hemocytes from forty-eight hours post-infection has shown a clear morphological change. Proteomic profiles

of hemocytes from YHV-infected shrimps by two-dimensional gel electrophoresis were elucidated showing up and down regulated proteins. These proteins were *in gel* digested and analyzed by mass spectrometry. Some of the down-regulated proteins were identified as tropomyosin and actin. A vast modulation of tropomyosin and actin could clearly explain the transformation of hemocytes morphology observed in an infected shrimp. The possible relation of the cytoskeletal-related proteins in mechanism of YHV infection was also investigated and discussed.

V1-013P

Identification of a novel mitochondrial protein that is essential for peroxisomal membrane synthesis in the yeast *Hansenula polymorpha*

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Peroxisomes are important subcellular organelles, which can function in a variety of metabolic processes, dependent on environmental conditions and the organism in which they occur. Yeasts are attractive model organisms to study peroxisome biogenesis and function. In recent years, vast studies have aimed at the elucidation of the mechanisms involved in these processes. Various genes (PEX genes) have been identified which are essential for peroxisome biogenesis. Here we report on the isolation of a mitochondrial protein that functions in the formation/stability of the peroxisomal membrane. This protein of yet unknown function has been identified within a novel collection of conditional temperature sensitive (ts) mutants of the yeast Hansenula polymorpha, affected in the utilization of methanol at restrictive temperatures. This growth defect appeared to be associated with the destabilization of peroxisomes in the cells due to the disintegration of the peroxisomal membrane. Total peroxisome desintegration occurred in a time interval of 2-4 h after the shift of cells from permissive (37 °C) to restrictive (44 °C) temperature. The mutant gene was sequenced and appeared to contain a point mutation that changed D^{407} into N. The D^{407} -N mutation was subsequently introduced into a WT H. polymorpha strain to confirm the mutant phenotype. Fusion of the gene to GFP localized the protein to mitochondria. Hence, this protein represents the first known mitochondrial protein involved in peroxisome membrane assembly. Mass spec lipid analysis was performed to analyze the phospholipid components affected in the mutant strain.

V1-014P

Phthalocyanine conjugates of oligonucleotides as new reagents for sensitized and catalytic modification of DNA and DNA-binding proteins

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Phthalocyanines (Ptc) is now of interest as a drugs for photodynamic therapy of malignant tumors. Their therapeutic effect is based on the ability to sensitize the generation of singlet molecular oxygen ${}^{1}O_{2}$ under irradiation. Besides, some metal-Ptc can catalyze in dark conditions the formation of other reactive oxygen species ${}^{\bullet}O_{2}$, H₂O₂, ${}^{\bullet}OH$. These properties of Ptc make them very attractive as a reactive group to be linked to antisense oligonucle-