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P29 **Overexpression and Characterization of wild type and mutant forms of *Desulfovibrio gigas* Flavoredoxin**

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Flavoredoxin (Flr), a FMN-containing protein from the sulfate-reducing bacteria *Desulfovibrio gigas*, is thought to be one of the redox carriers of the bisulfite reduction from molecular hydrogen, together with a membrane-bound cytochrome, flavodoxin and ferredoxin.
Flr was found to be a member of the Flavin-Reductase family, showing homologies with proteins from these family (a). Although members of this family do not have canonical FMN binding motifs, they have residues that establish potentially strong interactions with FMN. Using comparative modeling techniques, it was possible to derive a model for Flr based on the known X-ray structures of similar proteins of *Archaeoglobus fulgidus* and *Methanobacterium thermoautotrophicum*. This model for D. gigas Flr led to the identification of some potential residues involved in the FMN binding.
In order to confirm the involvement of these residues in the *D. gigas* Flr FMN binding, substitution of some of them was performed by site directed mutagenesis, contributing to the structure-function characterization of Flr.

(a)Agostinho, M, Oliveira, S., Broco, M. and Rodrigues-Pousada, C. (2000), BBRC, 272(3), 653-656.
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P30 **A functional genomics approach to the evolution of alternative genetic codes**

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The genetic code was thought to be immutable and universal. Such dogma led to interpret the discovery of alternative codes as aberrations of nature without evolutionary consequences. However, recent studies indicate that some genetic code alterations evolve through non-neutral mechanisms and represent novel mechanisms for "fast-track" evolution of phenotypic traits.
One such alternative code is the reassignment of the CUG codon from leucine to serine, which occurs in several *Candida* species. This unique genetic code change is mediated by a novel transfer RNA (ser-tRNA$_{CAG}$), with non-standard decoding and aminoacylation properties.
In order to elucidate how and why *Candida spp.* evolved an alternative genetic code, we have reconstructed the CUG reassignment pathway in *Saccharomyces cerevisiae* by expressing the *C.albicans* ser-tRNA$_{CAG}$ in *S.cerevisiae*. The latter triggers an adaptive response to adverse growth conditions suggesting that *S.cerevisiae* expressing CUG ambiguity mimics the natural resistance to stress characteristic of *C.albicans*.
Analysis of the *S.cerevisiae* proteome shows that the induction of the general stress response counteracts the negative impact of ambiguous mRNA translation, creating a pre-adaptation potential to face adverse conditions and develop new phenotypes. A detailed study of the full impact of genetic code ambiguity on cell physiology is being carried out by combining transcriptomics and proteomics methodologies.

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P31 **Isoenzymatic variability in populations of *Lupinus* from Beira Interior**

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Isoenzymatic studies were performed in order to study the variability of *Lupinus* spp. populations from Beira Interior region.
Thirty accessions of *Lupinus* (*L. luteus, L. albus, L. angustifolius* and *L. Hispanicus*) from Beira Interior region were studied. The zymograms were obtained by poliacrilamide gel electrophoresis (PAGE). Extracts were prepared from young leaf and cotyledons tissues. Four enzyme systems were studied: aspartate aminotransferase (AAT), esterase (EST), acid phosphatase (ACP) and malate desidrogenase (MDH).
Isoenzyme polymorphisms were found for all the enzyme systems studied. The comparative analysis of AAT and MDH zymograms show interspecific variation and made it possible to identify the four species studied. Significant intraspecific variation was found in EST and ACP zymograms; however intrapopulational variation was also detected and the different populations could not be distinguished inside each species.
The existance of a greater genetic proximity between *L. luteus* and *L. hispanicus* was detected.

P32 **Co-localization of Matrix Gla protein gene expression and protein accumulation with cartilage distribution in the teleost fish *Sparus aurata***

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Matrix Gla protein (MGP) is an extracellular mineral-binding protein, expressed in several tissues with high accumulation in bone and cartilage. Although its molecular mechanism of action remains unknown, all evidence indicates that MGP acts as an inhibitor of mineralization. We investigated the sites of gene expression and protein accumulation of MGP throughout development of the bony fish *Sparus aurata*, by in situ hybridization, Northern and RT-PCR Southern hybridization and immunohistochemistry. The results were compared with the patterns of developmental appearance of cartilaginous and mineralized structures, and with mRNA presence and protein accumulation of osteocalcin, a marker for osteoblasts. MGP mRNA was first detected at 2 days post-hatching (dph) and thereafter continuously detected at various levels of intensity, until 130 dph. Until circa 45 dph the MGP gene was highly expressed in a variety of tissues, including heart and kidney, whereas at 85 dph MGP gene expression and protein accumulation were restricted to the remaining cartilaginous structures. Osteocalcin gene expression and protein accumulation were localized in most mineralized structures. Our results agree with the available data from higher vertebrates, strengthening the hypothesis of a conserved function for MGP from fish to man, throughout more than 200 million years of evolution.

P33 **Poly(ADP-ribose) polymerase inhibition in mice increases gamma-ray induced mutant Frequency**

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The catalytic activation of poly(ADP-ribose) polymerase (PARP; EC2.4.2.30) represents one of the earliest cellular responses to DNA damage and is particularly stimulated by the presence of DNA strand breaks. PARP is known to participate in the base excision repair pathway and in apoptosis. However, the mechanisms underlying PARP activity are still largely unknown. The objective of this work was to evaluate the effect of PARP inhibition in the repair of gamma radiation-induced DNA lesions *in vivo*.

A group of mice was injected with the PARP inhibitor 3-aminobenzamide (3-AB, 20mg/kg) and irradiated with gamma-rays (3 Gy) three hours later. Irradiated and unirradiated controls were similarly injected with saline. From days 2-6, mice were daily treated with 3-AB or saline and were sacrificed on day 7. Genomic DNA was extracted from livers and testis for *lacZ* mutation analysis. It was observed that the 3-AB treated and irradiated mice displayed a significant increase in mutant frequency in both organs as compared to irradiated or unirradiated controls. These results indicate that PARP activity inhibition increases the sensitivity to gamma radiation-induced DNA damage in mice and, to our knowledge, provide the first evidence of a mutagenic effect of PARP inhibition *in vivo*.

P34 **Involvement of the Nuclear Receptors PXR, RXRα, COUP-TFI and CAR in the Development-Dependent Induction of the CYP3A1 by Synthetic Glucocorticoids**

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The orphan nuclear receptor PXR has been identified as the mediator of the CYP3A1 gene induction by synthetic glucocorticoids (GCs). Nevertheless, differences between immature and adult rat liver have been observed, which suggest the existence of alternative pathways involved in the up-regulation of the hepatic CYP3A1 gene expression by dexamethasone (DEX).

We have investigated the participation of several nuclear receptors in the CYP3A1 developmental-dependent induction by DEX.

Our results demonstrate that induction of CYP3A1 mRNA is preceded by significant changes in PXR and RXRα expression levels in the adult animals, while development-dependent differences were found in CAR basal expression.

We further demonstrate that the CYP3A1 natural promoter is slightly activated by PXR, RXRα and COUP-TFI, but not by CAR. Addition of GC-ligands results in a net transcriptional activation by PXR when co-transfected with COUP-TFI. Activation triggered by the GC-ligand is impaired by RXRα co-transfection even at low concentration. Site-directed mutagenesis of the CYP3A1 natural promoter have revealed that both the previously characterized DEX-responsive sites are important for the inductive response.

Our results suggest that CYP3A1 is under development-dependent regulation through a mechanism that reflects differential physiological balances of dynamic heterodimers PXR:RXRα, PXR:COUP-TFI and CAR:RXRα.

P35 **Expression of Human Transacetylase Phenotypes in Transfected COS-7 Cells with NAT2 Allele Recombinants *NAT2*4* and *NAT2*5B***

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Epidemiological studies evidence the role of environment aggressions in the etiology of multifactorial diseases including Alzheimer's Disease (AD). Effects of xenobiotics depend on polymorphic drug-metabolizing-enzymes (DME) that control the inactivation/ potentiation of their cytotoxicity/ genotoxicity. We have previously demonstrated a putative role of N-Acetyltransferase (NAT2) as a low-penetrance gene in sporadic AD (Pharmacogenetics, 1999). Here we constructed two *NAT2* recombinants in the eukariotic vector pTargetT^{TM} starting from homozygotic *NAT2*4* and *NAT2*5B* subjects - representative of the rapid (wild-type) and slow acetylator phenotype. After PCR- RFLP diagnose the respective 870bp *NAT2* ORF was PCR amplified from the single *NAT2* DNA exon. The PCR products were subsequently ligated to the pTargetT^{TM}, and expressed in COS-7 cells by transfection. Evaluation of the Human recombinant heterologous expression of both mRNA and proteins were performed by RT-PCR and by Western Blot analysis. N-acetylation activity of the transfected proteins was further determined by spectrophotometry. We confirmed the specificity of the pTargeT-*NAT2*4* recombinant that conferred the acetylator phenotype to the transfected COS-7 cells. The present model will be used for a direct evaluation of the putative role of NAT2 alleles in the potentiation of environmental injury leading to neuronal cell degeneration.

P36 **Localization of sites of expression of the BAT-1 gene encoding a RNA Helicase dependent protein, during the different stages of development of the oyster C. gigas.**

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BAT1 is a member of the DEAD-box family of ATP-dependent RNA helicases involved in a number of cellular functions including initiation of translation, RNA splicing, and ribosome assembly. The BAT-1 gene from the oyster *Crassostreia gigas* was previously identified by subtractive hybridization analysis as a gene over expressed during oyster development. To study the role of this protein, tissues of expression and localization of its mRNA were analysed by Northern analysis and in situ hybridization throughout *C.gigas* development. Two different gene transcripts with different levels of expression at different developmental stages were detected. An increase of up to 12 fold was observed throughout the main differentiation steps until the D-veligera stage. In adult tissues, expression of the two BAT-1 gene transcripts was observed in all tissues analysed, albeit with different patterns of expression. In gonads, an additional third transcript was observed that could result from transfer of maternal mRNA to the embryo. To elucidate the relationship between the sites of BAT-1 gene expression and the stage of larvae development, *in situ* hybridization in currently being conducted to identify specific sites of expression and detect associated cell structures The identification of these structures should contribute to clarify the role and importance of this protein, well conserved throughout evolution from invertebrates to man.